Islet Amyloid and Selected Trace Elements among Type 2 Diabetes Mellitus Patients in Gaza City

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Abstract

Background: Diabetes mellitus is one of the most worldwide spread chronic diseases, and its complications are very serious if it is untreated. Amyloid deposits derived from a peptide called amylin are found in the pancreas in the vast majority of cases of type 2 diabetes mellitus (T2DM). Free Fe and Cu ions are the most redox-active metals in mammalian tissues, where they may contribute to tissue damage by generation of reactive oxygen species (ROS) such as hydroxyl radicals. It has not been established if altered transition metal metabolism, play a role in the forms of heart disease that complicate the major classes of diabetes or if altered transition metal ion metabolism plays a role in islet amyloid or β-cell toxicity. The hypothesis that T2DM individuals have high levels of islet amyloid polypeptide (amylin) and impaired levels of trace elements which may be involved in the toxicity of the amylin peptide among T2DM patients have been investigated.

Aim of the study: To investigate the concentration of selected trace elements and islet amyloid level among T2DM in comparison with control.

Materials and methods: For this project, ELISA (Enzyme Linked Immunosorbent Assay) technique was used to detect amylin level. Blood sugar and metal ion level such as Copper, Zinc, Iron, and Magnesium were detected by colorimetric methods in the serum of T2DM patients and control. Variations in the concentration of trace elements and amylin levels in T2DM and non-diabetic control were statistically analysed and evaluated. SPSS software was used to analyse obtained data.

RESULTS: There was statistically significant difference between patients and controls in the blood sugar level, Zn, Mg and amylin (p<0.05) with negative correlation for Zn and Mg and positive correlation for amylin. There was statistically significant differences for patients who suffer from diabetic coma for blood sugar, and Zn (p<0.05). Patients with neuropathy have statistical significant value with the level of Zn (p<0.05), While, Copper and iron didn't show any difference (p>0.05). There was significant negative correlation between blood sugar and Zn (p=0.006) also there
were significant positive correlation between Cu and both of Zn \((p=0.026)\) and Mg \((p=0.014)\).

**Conclusion:** There were significant differences between healthy group and diabetic group with regard to the level of trace elements (Zn, Mg) and amylin hormone. Diet and food restriction was a helpful tool in managing and controlling of diabetes and then minimizing the risk of diabetes complications. Blood sugar level, amylin hormone and Fe were influenced with age while, Fe was only influenced with sex. Mg level was influenced with smoking in patient's group. Blood sugar and Zn levels were altered in diabetic patients with diabetic coma. In diabetic neuropathy patient's Zn level was significantly low compared with patients who don't have neuropathy.

**Keywords:** Type 2 diabetes mellitus, Trace elements, Copper, Zinc, Amylin, Iron, Magnesium.
الملخص

يعتبر داء السكرى (النوع الثاني) من الأمراض المزمنة المنتشرة في أنحاء العالم كافة، وتعتبر مضاعفاته خطيرة إذا لم يتم علاجها. تهدف الدراسة إثبات فرضية أن مرضى السكرى يعانون من اعتلال في مستوى بعض العناصر النادرة (النحاس، الزنك، المغنيسيوم، الحديد و هرمون الاميلين). تم فحص عينات لمرضى يعانون من داء السكرى (النوع الثاني) ومقارنتها بعينات ضابطة، استخدم خلالها الباحث فحوصات لقياس نسبة العناصر النادرة في دم المرضى وعينات الضابطة وواستخدام الطرق الكيميائية كما تم قياس هرمون الاميلين في كلا المجموعتين باستخدام الطرق المناعية المعروفة بالأليرزا.

أظهرت النتائج وجود فروقات ذات دلالة إحصائية بين العينة المصابة وعينة الضابطة فيما يخص نسبة السكر، الزنك و المغنيسيوم و هرمون الاميلين حيث كانت هذه الفروقات ضمن المدى $P<0.05$، واظهرت الدراسة وجود فروقات أخرى ذات دلالة إحصائية فيا يتعلق بعض المضاعفات الناتجة عن مرض السكر. مثل الغيبوبة الناتجة عن ارتفاع السكر حيث كان هناك فروقات ذات دلالة إحصائية بين المرضى الذين يعانون من هذه المضاعفات و الذين لا يعانون فيما يخص مستوى كل من السكر و الزنك حيث كانت هذه الفروقات بدرجة $P<0.005$ أيضا كان هناك فروقات $>0.05$ بين المرضى الذين يعانون من اعتلال الاعصاب الناتج عن داء السكرى فيما يخص مستوى الزنك.

أظهرت الدراسة كذلك وجود، ارتباط إيجابي بين مستوى النحاس و الزنك و مستوى النحاس و المغنيسيوم وارتباط سلبي بين نسبة السكر و مستوى الزنك في دم المرضى والذي يفسره أهمية الزنك في تصنيع هرمون الأنسولين من خلاله البيتا الموجودة في غدة البنكرياس.

الكلمات المفتاحية: داء السكرى (النوع الثاني)، العناصر النادرة، النحاس، الزنك، المغنيسيوم، الحديد، هرمون الاميلين.
Declaration

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where due acknowledgment has been made in the text".

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I would like to express my gratitude to all people who have contributed to this work. In particular, I would like to thank:

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Finally, special thanks to my family, wife, and my sons and daughter. Without their guidance, help and patience, I would have never been able to accomplish this work.

- Also I would like to thank all participants in this study specially patients and healthy people.
DEDICATION

To my family who supported me all the way since the beginning of this study.
# List of contents

Abstract English ........................................................................................................ iii  
Abstract Arabic .......................................................................................................... v  
Declaration ................................................................................................................ vi  
Acknowledgments ...................................................................................................... vii  
Dedication .................................................................................................................. viii  
List of tables ............................................................................................................... xv  
List of figures ............................................................................................................. xvii  
List of abbreviations ................................................................................................. xviii  
List of annexes ......................................................................................................... xx  

## CHAPTER I: INTRODUCTION

1.1 Overview ............................................................................................................... 1  
1.2 Islet amyloids and Type 2 diabetes mellitus ....................................................... 2  
1.3 General objectives of the study ........................................................................ 3  
1.4 Specific objectives of the study ........................................................................ 3  
1.5 Significance of the study .................................................................................. 3  

## CHAPTER II: LITERATURE REVIEW

2.1 Diabetes mellitus .................................................................................................. 5  
2.2 Normal physiology of glucose in the body ....................................................... 5  
2.3 Diabetes pathophysiology ................................................................................ 6  
2.4 Types of diabetes ............................................................................................... 7  
2.4.1 Type 1 diabetes mellitus ................................................................................ 7  
2.4.1.1 Genetic causes of Type 1 diabetes mellitus .......................................... 7  
2.4.1.1.1 HLA genes ......................................................................................... 7  
2.4.1.1.2 The insulin gene ............................................................................. 8  
2.4.1.2 Viral infection ....................................................................................... 8  
2.4.1.2.1 Entero virus ................................................................................... 8  
2.4.1.2.2 Bacteria ......................................................................................... 8  
2.5 Type 2 diabetes mellitus .................................................................................... 8  
2.6 Insulin resistance, obesity and type 2 diabetes ............................................... 9
2.7 Maturity onset diabetes of the young................................................. 9
2.8 Gestational diabetes................................................................. 10
2.9 Idiopathic................................................................................. 10
2.10 Complications of diabetes mellitus......................................... 11
  2.10.1 Diabetic nephropathy....................................................... 11
  2.10.2 Diabetic neuropathy....................................................... 11
  2.10.3 Diabetic retinopathy....................................................... 11
  2.10.4 Diabetic cardiopathy...................................................... 12
2.11 Anti-oxidant effect of trace elements........................................ 12
2.12 Role of oxidative stress in diabetes......................................... 13
  2.12.1 Mechanisms for oxidative stress in diabetes...................... 13
    2.12.1.1 The glycoxidation pathway.................................... 13
    2.12.1.2 Reactive nitrogen pathway.................................... 14
2.13 Role of anti-oxidants in preventing oxidative stress............... 14
2.14 Oxidative stress and β- cell dysfunction.............................. 15
2.15 Metabolic staging of type 2 diabetes ................................... 15
2.16 Trace elements and type 2 diabetes mellitus......................... 16
  2.16.1 Effect of magnesium consumption on glucose concentration 16
  2.16.2 Magnesium homeostasis................................................ 16
  2.16.3 Intestinal absorption...................................................... 18
2.17 Role of zinc in Type 2 diabetes mellitus............................... 19
  2.17.1 Zinc and insulin interactions........................................ 21
  2.17.2 Effect of zinc on diabetes.............................................. 22
  2.17.3 Zinc and coronary heart disease in type 2 diabetes mellitus 22
2.18 Role of iron in diabetes mellitus.......................................... 23
  2.18.1 Iron overload and diabetes............................................. 24
  2.18.2 The role of iron in diabetes without overt iron overload....... 24
2.19 Copper, oxidative stress and diabetes.................................... 25
2.20 Copper and inflammation..................................................... 26
2.21 Copper and metabolic abnormalities...................................... 27
2.22 Metals, Fenton reaction and oxidative stress............................ 28
2.23 Reactive oxygen species and neurodegenerative disease .................. 29
2.24 Amylin ................................................................. 29
2.25 Role of amylin in diabetes mellitus ......................................... 30
2.25.1 Role of fatty acids in induction of amylin expression and release .... 31
2.25.2 Role of intracellular amyloid β in Alzheimer's disease ............... 31
2.25.3 The role of amylin in osteoporosis .................................... 32
2.25.3.1 Amylin's effect on osteoblasts .................................. 33
2.25.3.2 Amylin's effect on osteoclasts .................................. 33

CHAPTER III: MATERIAL AND METHODS

3.1 Study design ................................................................. 34
3.2 Target population .......................................................... 34
3.3 Sample size ................................................................. 34
3.4 Inclusion criteria .......................................................... 34
3.5 Exclusion criteria .......................................................... 34
3.6 Ethical consideration ....................................................... 34
3.7 Data collection ............................................................. 35
3.7.1 Questionnaire interview ............................................... 35
3.7.2 Sampling ................................................................. 35
3.7.3 Sampling process ....................................................... 35
3.8 Biochemical analysis ...................................................... 36
3.8.1 Glucose determination ................................................ 36
3.8.1.1 Principle of the method ........................................... 36
3.8.1.2 Method procedure ................................................ 36
3.8.1.3 Calculation ......................................................... 37
3.8.2 Magnesium determination ............................................. 37
3.8.2.1 Principle of the method ........................................... 37
3.8.2.2 Method procedure ................................................ 38
3.8.2.3 Calculation ......................................................... 38
3.8.3 Iron determination ..................................................... 38
3.8.3.1 Principle of the method ........................................... 38
3.8.3.2 Method procedure ................................................ 39
CHAPTER IV: RESULTS

4.1 General characteristics of the study population................. 44
4.1.1 Distribution of the population according to sex................. 44
4.1.2 Distribution of the population according to the age........... 45
4.1.3 Distribution of the age match of the study population according to the sex................................................................. 45
4.2 Association between F.B.S and family history among the case group....... 45
4.3 Association between F.B.S, gender and smoking among the case group................................................................. 46
4.4 F.B.S and sport among the case group........................................ 46
4.5 Diabetes and type of treatment in case group.............................. 47
4.6 Association between diet and F.B.S among the case group............. 48
4.7 Association between F.B.S and hypertension among the case group
........................................................................................................ 48
4.8 Distribution of cases according to the complications of diabetes........ 49
4.9 Association between biochemical parameters levels among the studied population according to matched age ................................. 49
4.10 Association between gender and biochemical parameters among the case group.......................... 50
4.11 Association between biochemical parameters and age among the case group........................................ 51
4.12 Correlation of blood sugar with selected trace elements and amylin in the case group....................................................... 52
4.13 Association between treatment and the studied biochemical parameters among the case group.................. 52
4.14 Association between smoking and biochemical parameters among the case group.............................. 53
4.15 Association between hypertension and the studied biochemical parameters among the case group........ 54
4.16 Association between diabetic coma and the level of biochemical parameters among the case group........ 54
4.17 Association between cardiopathy and the level of biochemical parameters among the case group........... 55
4.18 Association between nephropathy and the level of biochemical parameters among the case group........... 56
4.19 Association between retinopathy and the level of biochemical parameters among the case group.............. 57
4.20 Association between neuropathy and the level of biochemical parameters among the case group............... 57
4.21 Correlation between tested biochemical parameters.............................................................. 58

CHAPTER V: DISCUSSION
5.1 Diabetes, gender and age................................................................. 60
5.2 Family history and diabetes mellitus.................................................. 61
5.3 Diet and diabetes mellitus................................................................. 61
5.4 Smoking and diabetes mellitus.......................................................... 62
5.5 Magnesium and smoking................................................................. 62
5.6 Sport and diabetes mellitus............................................................... 63
5.7 Diabetes mellitus and hypertension.................................................... 63
List of tables

Table (4.1): Distribution of the age of the age match of the study population according to sex ................................................................. 45
Table (4.2): Association between F.B.S and family history among the case group ................................................................................................. 46
Table (4.3): F.B.S and gender among the case group ................................................................................................................................. 46
Table (4.4): F.B.S and smoking among the case group ................................................................................................................................. 46
Table (4.5): F.B.S and sport among the case group ................................................................................................................................. 47
Table (4.6): Association between diet and F.B.S among the case group ........ 48
Table (4.7): The relationship between diabetes and hypertension .................. 48
Table (4.8): Association between biochemical parameters among the age match study population ........................................................................ 50
Table (4.9): Association between gender and biochemical parameters among the case group ........................................................................ 51
Table (4.10): Association between biochemical parameters and age among the case group ........................................................................ 51
Table (4.11): Correlation of F. blood sugar with trace elements and amylin in the case group ..................................................................................... 52
Table (4.12): Association between treatment and biochemical parameters among the case group ................................................................. 52
Table (4.13): Mean concentration of each test according to treatment .............. 53
Table (4.14): Association between smoking and the studied biochemical parameters among the case group ........................................................................ 53
Table (4.15): Association between hypertension and the tested biochemical parameters among the case group ................................................................. 54
Table (4.16): Association between diabetic coma and the level of biochemical parameters among the case group ................................................................. 55
Table (4.17): Association between cardiopathy and the level of biochemical parameters among the case group ................................................................. 56
Table (4.18): Association between nephropathy and the level of biochemical parameters among the case group .................................................. 56
Table (4.19): Association between retinopathy and the level of biochemical parameters among the case group.................................................. 57
Table (4.20): Association between neuropathy and the level of biochemical parameters among the case group.................................................. 58
Table (4.21): Correlation between tested biochemical .................................. 59
List of figures

Figure (2.1): Magnesium homeostasis................................................................. 17
Figure (4.1): Distribution of the study population according to sex…………… 44
Figure (4.2): Types of treatments that used by patients…………………………… 47
Figure (4.3): Distribution of case group according to the complications of diabetes 49
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ADA</td>
<td>The American Diabetes Association</td>
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<tr>
<td>Aβ</td>
<td>Amyloid β-peptide</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ANOVA</td>
<td>One-Way Analysis of Variance</td>
</tr>
<tr>
<td>Anti–GAD</td>
<td>Glutamic Acid Decarboxylase anti-bodies</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>Cu++</td>
<td>Divalent Copper</td>
</tr>
<tr>
<td>CuZnSOD</td>
<td>Copper-zinc superoxide dismutase</td>
</tr>
<tr>
<td>D.M.</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>DR</td>
<td>Diabetic retinopathy</td>
</tr>
<tr>
<td>ESRD</td>
<td>End Stage Renal Disease</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immune sorbent assay</td>
</tr>
<tr>
<td>Fe++</td>
<td>Ferrous</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acids</td>
</tr>
<tr>
<td>hIAPP</td>
<td>Human Islet Amyloid Polypeptide</td>
</tr>
<tr>
<td>HH</td>
<td>Hereditary Hemochromatosis</td>
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<tr>
<td>HHS</td>
<td>Hyperosmolar hyperglycemic state</td>
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<tr>
<td>HbA1c</td>
<td>glycosylated hemoglobin</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
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<tr>
<td>IAPP</td>
<td>Islet amyloid polypeptide</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>GIP</td>
<td>Glucose-dependent Insulinotropic Peptide</td>
</tr>
<tr>
<td>Mg++</td>
<td>Divalent Magnesium</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>MODY</td>
<td>Maturity Onset Diabetes of the Young</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Noninsulin dependent diabetes mellitus</td>
</tr>
<tr>
<td>NTBI</td>
<td>Non– transferrin-bound iron</td>
</tr>
<tr>
<td>PN</td>
<td>Peripheral neuropathy</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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<tr>
<td>T.E.</td>
<td>Trace Elements</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<tr>
<td>T1DM</td>
<td>Type 1 Diabetes Mellitus</td>
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<tr>
<td>Zn++</td>
<td>Divalent Zinc</td>
</tr>
</tbody>
</table>
List of Annexes

Annex 1  A letter from the Islamic university of Gaza to Helsinki committee Gaza .......................... 94
Annex 2  Approval from IUG to conduct the research........... 95
Annex 3  Approval from the directorate of human resource development to conduct the research ................. 96
Annex 4  Approval from Helsinki committee....................... 97
Annex 5  Questionnaires................................................. 98
1.1 Overview

The term diabetes mellitus (DM) describes a metabolic disorder of multiple aetiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. The effects of DM include long– term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss [1].

Human body is composed of three kinds of elements, abundant elements, semi major elements and trace elements (T.E) [2]. Abundant elements are involved in the formation of covalent bond; these elements (Oxygen, Carbon, Hydrogen, Nitrogen, etc) are the major constituent of tissues, while semi major elements are involved in the maintenance of osmotic pressure and membrane potential [2]. Essential T.E include Zinc (Zn++), Copper (Cu++), Selenium (Se), Chromium (Cr), Cobalt (Co), Manganese (Mn) and Molybdenum (Mo). Trace elements (except for Iodine) serve as an active centre of enzymes [2]. So deficiency of one of these T.E may affect the activity of these enzymes, and may have an effect on the pathogenesis of several diseases.

Thus, knowledge of the clinical aspects of T.E is becoming indispensable for front line clinicians; meanwhile, epidemiological surveys and animal studies have suggested the possibility that some T.E deficiencies are associated with reduced antioxidant potential, which is believed to be linked to accelerate aging, developmental retardation in children, increased abnormal pregnancies and lifestyle- related diseases [2]. Diabetes Mellitus (DM) is one of these diseases, it is linked to perturbations in mineral metabolism, it is not clear if diabetes affect mineral metabolism or alteration in mineral homeostasis [3]. Type-2 diabetes mellitus (T2DM) results from defect in insulin action in hepatic and peripheral tissues, especially muscle tissues and
adipocytes [4]. The specific etiologic factors are not known but genetic input is much stronger in T2DM than type 1 diabetes mellitus (T1DM) [4].

Type-2 diabetes mellitus is reported to be caused by obesity and sedentary lifestyle [5]. Diabetes can lead to heart diseases, nerve changes, kidney disease and vision loss [4]. DM plays a role in accelerating the hardening and the narrowing of the arteries [6]. T.E such as Zn has an insulin-like effect on the manifestations of diabetes [4], and it's reported that Zn supplantations have lowered blood sugar level in people with T1DM [7], but in T2DM it has not the same effect [8]. Correction of Zn deficiency in patients with T1DM led to decrease lipid peroxidation and improvement of glucose homeostasis [9]. Trace elements such as chromium have been shown to improve glucose and related variables in people with glucose intolerance [10]. Cu depletion doubled glucose in the blood of diabetic rats that were fed with glucose, and higher for sucrose. It's also reported that rats fed with copper-deficient diet have high blood sugar due to glycation of haemoglobin consequence concluded that both the early and advanced stages of protein glycation increased significantly in rats fed with copper-deficient diet [4].

Magnesium is an essential ion involved in glucose homeostasis at multiple levels [11]. Hypomagnesaemia has been reported in both T1DM and T2DM patients [9]. Mg plays an important role in the activities of various enzymes involved in the glucose oxidation and may play a role in the release of insulin [11]. Iron (Fe) plays an important role in the pathophysiology of DM, which is derived from the ease with which iron is reversibly oxidized and reduced. This property, is essential for its metabolic functions, it makes Fe potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical [12].

1.2 Islet amyloids and type 2 Diabetes

Human islet amyloid polypeptide (hIAPP) and named as amylin is 37 amino acid, it is co-secreted with insulin from pancreatic islet β cells. This peptide when accumulates and aggregates forms fibrils, Amyloid deposits is associated with β cells degeneration which considered a hallmark of noninsulin dependent diabetes mellitus.
(NIDDM) [13]. It is not clear why soluble IAPP molecules aggregate to form toxic amyloid deposits in T2DM although several mechanisms have been proposed. Presence of an amyloidogenic sequence in the midportion of the human IAPP molecule and an increase in IAPP production and secretion in T2DM are thought to be important factors but do not appear to be sufficient for amyloid formation [14].

1.3 General objectives

To investigate the concentration of selected trace elements and islet amyloid level among T2DM patients in comparison with control.

1.4 Specific objectives

1- To determine the level of amylin in the blood of T2DM.
2- To determine the level of selected T.E such as (Cu++, Mg++, Zn++ and Fe++) in the blood of T2DM patients.
3- To investigate the levels of trace elements with the level of islet amyloid in both patients and control.

1.5 Significance of the study

Diabetes is one of the most common non-communicable diseases affecting mankind, and is recognized as one of the leading causes of morbidity and mortality in the world. Many factors may play a role in the pathogenesis of T2DM, such as islet cell amyloidosis and impairment of trace elements level. Islet amyloid has been recognized as a feature of the disease for over one hundred years, and is now considered to be a significant factor in the loss of β-cells in the pathogenesis of the disease. Free Fe and Cu ions are the most redox-active in mammalian tissues, where they may contribute to tissue damage by generation of ROS such as hydroxyl radicals. The generation of various free radicals is closely linked with the participation of redox-active metals. Aluminium, copper, iron and zinc ions have all been reported to induce the aggregation of Aβ and it is now becoming clear that one of the major factors involved appears to be the direct binding of certain metal ions to the aggregating peptide. The results of this study may draw the attention of the
importance of islet amyloid evaluation and trace elements among T2DM patients which may modify treatment protocols for such patients. This study may help diabetic patients to improve their life style through monitoring the level of T.E to avoid any undesired complications of diabetes. In addition, his study is the first to be conducted in Gaza.
Chapter 2
Literature Review

2.1 Diabetes Mellitus

Diabetes mellitus is one of the most common metabolic diseases in the world, and its prevalence is increasing rapidly, it's one of the most expensive diseases with regards to total health care costs per patient [15], it's a syndrome characterized by a loss of glucose homeostasis [16].

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and expected to be 4.4% in 2030 [17]. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than women. The urban population in developing countries is projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people 65 years of age [17]. The American Diabetes Association (ADA) estimated the national costs of diabetes in the USA for 2002 to be $US 132 billion, increasing to USD 192 billion in 2020 [18].

2.2 Normal physiology of glucose in the body

Plasma glucose concentration is a function of the rate of glucose entering the circulation balanced by the rate of glucose removal from the circulation. Circulating glucose is derived from three sources: intestinal absorption during the fed state, glycogenolysis, and gluconeogenesis. The major determinant of how quickly glucose appears in the circulation during the fed state is the rate of gastric emptying. Other sources of circulating glucose are derived chiefly from hepatic processes: glycogenolysis, (the breakdown of glycogen, the polymerized storage form of glucose); and gluconeogenesis, (the formation of glucose primarily from lactate and amino acids) during the fasting state [19].

Glycogenolysis and gluconeogenesis are partly under the control of glucagon, a hormone produced from the α-cells of the pancreas. During the first 8–12 hours of
fasting, glycogenolysis is the primary mechanism by which glucose is made available. Glucagon facilitates this process and thus promotes glucose appearance in the circulation. Over longer periods of fasting, glucose, produced by gluconeogenesis, is released from the liver [19].

Glucoregulatory hormones include insulin, glucagon, amylin, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), epinephrine, cortisol, and growth hormone. Of these, insulin and amylin are derived from the β-cells, glucagon from the α-cells of the pancreas, and GLP-1 and GIP from the L-cells of the intestine [19]. The glucoregulatory hormones of the body are designed to maintain circulating glucose concentrations in a relatively narrow range. In the fasting state, glucose leaves the circulation at a constant rate. To keep pace with glucose disappearance, endogenous glucose production is necessary. For all practical purposes, the sole source of endogenous glucose production is the liver [19].

Renal gluconeogenesis contributes substantially to the systemic glucose pool only during periods of extreme starvation. Although most tissues have the ability to hydrolyze glycogen, only the liver and kidneys contain glucose-6-phosphatase, the enzyme necessary for the release of glucose into the circulation. In the bi-hormonal model of glucose homeostasis, insulin is the key regulatory hormone of glucose disappearance, and glucagon is a major regulator of glucose appearance. After reaching a post-meal peak, blood glucose slowly decreases during the next several hours, eventually returning to fasting levels. In the immediate post-feeding state, glucose removal into skeletal muscle and adipose tissue is driven mainly by insulin. At the same time, endogenous glucose production is suppressed by the direct action of insulin, delivered via the portal vein, on the liver, and the paracrine effect or direct communication within the pancreas between the α- and β-cells, which results in glucagon suppression [19].

2.3 Diabetes pathophysiology

Our understanding of the pathophysiology of diabetes is evolving. Type 1 diabetes has been characterized by an autoimmune-mediated destruction of pancreatic β-cells [20]. The resulting deficiency in insulin also means a deficiency in
the other co-secreted and co-located β-cell hormone, amylin [21]. As a result, postprandial glucose concentrations rise due to lack of insulin-stimulated glucose disappearance, poorly regulated hepatic glucose production, and increased or abnormal gastric emptying following a meal [21]. Early in the course of type 2 diabetes, postprandial β-cell action becomes abnormal, as evidenced by the loss of immediate insulin response to a meal [22]. Peripheral insulin resistance coupled with progressive β-cell failure and decreased availability of insulin, amylin, and GLP-1 contribute to the clinical picture of hyperglycemia in diabetes [23].

Abnormal gastric emptying is common to both T1DM and T2DM. The rate of gastric emptying is a key determinant of postprandial glucose concentrations [24]. If gastric emptying is accelerated, then the presentation of meal-derived glucose to the circulation is poorly timed with insulin delivery. In individuals with diabetes, the absent or delayed secretion of insulin further exacerbates postprandial hyperglycemia. Both amylin and GLP-1 regulate gastric emptying by slowing the delivery of nutrients from the stomach to the small intestine [24].

2.4 Types of Diabetes

2.4.1 Type 1 diabetes mellitus (T1DM)

Type 1 diabetes mellitus usually starts in people younger than 30 and is therefore also termed juvenile-onset diabetes, even though it can occur at any age. T1DM is a chronic autoimmune disorder that precipitates in genetically susceptible individuals by environmental factors [25]. The body’s own immune system attacks the beta-cells in the islets of Langerhans of the pancreas, destroying or damaging them sufficiently to reduce and eventually eliminate insulin production [25].

2.4.1.1 Genetic causes of type 1 diabetes Mellitus

2.4.1.1.1 HLA genes

Early studies indicated that the human leukocyte region (HLA region) on chromosome 6p21 (commonly termed IDDM1, for insulin-dependent diabetes mellitus locus) is a critical susceptibility locus for many human autoimmune diseases, including T1DM the class II genes remain the strongest genetic contributor. There is accumulating evidence for the presence and functionality of HLA-A*02-restricted CD8 T cells reacting against beta-cell antigens such as insulin,
glutamate decarboxylase (GAD), and IAPP in T1DM patients and islet transplant recipients [25].

2.4.1.1.2 The insulin gene

A lesser genetic predisposition to T1D is conferred by the IDDM2 locus on chromosome 11 containing the insulin gene region. A polymorphic region located 5’ of the insulin gene was first reported in 1984 to be associated with T1DM in caucasoids [26].

2.4.1.2 Viral infections

2.4.1.2.1 Enteroviruses

Extensive circumstantial data designate enteroviruses, and more specifically coxsackieviruses, as prime viral candidates that can cause precipitation of T1D [27]. This was based on the finding of higher neutralizing antibody titers in serum from recent-onset patients versus healthy controls [28]. These data were later confirmed using PCR technology [29].

2.4.1.2.2 Bacteria

The bacterial composition of the intestine has long been acknowledged as an important variable affecting T1D development. Direct evidence exists in rodents, as diabetes is aggravated under specific pathogen-free conditions or upon administration of antibiotics [30].

2.5 Type 2 diabetes mellitus

Type 2 diabetes is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically manifest. By definition, the specific reasons for the development of these abnormalities are not yet known [1]. T2DM is a heterogeneous disease, involving both genetic and environmental factors. Probably there is not one major T2DM gene which is responsible for this disease in the majority of the patients. An exception is MODY (Maturity Onset Diabetes of the Young), for which several predisposing genes have been identified in affected families [15].
2.6 Insulin resistance, obesity and type 2 diabetes

Insulin resistance and diabetes mellitus type 2 (T2DM) are strongly associated with excess lipid accumulation in non-adipose tissues like skeletal muscle, most likely by interference of the accumulated lipid metabolites diacylglycerol (DAG), ceramides and long chain fatty acyl-CoA with insulin signaling [31]. The dynamics of lipid oxidation and fine tuning with fatty acid uptake and intramyocellular triacylglycerol (IMTG) turnover may be very important to limit the accumulation of lipid intermediates. This may be particularly relevant in situations when energy demand does not challenge the fat oxidative capacity of skeletal muscle, for example during fasting or after a meal [32]. Recently, it has become more and more clear that the obese, insulin resistant and T2D phenotype is associated with an impaired fat oxidation during fasting, with an impaired switch from fat to glucose oxidation after a meal [33], and an impaired rise in fat oxidation after beta-adrenergic stimulation or during exercise [34].

An increase in free fatty acid (FFA) availability would lead to an increased FFA oxidation, inhibiting pyruvate dehydrogenase and phosphofructokinase. A subsequent accumulation of glucose-6-phosphate inhibits hexokinase activity, and the rise in intracellular glucose concentrations would then result in a negative feedback to glucose uptake. Dysfunction of other organs may play a role via changes in the neuro-endocrine environment of skeletal muscle, of which the effect will depend on intrinsic muscle characteristics [32].

2.7 Maturity Onset Diabetes of the Young

The mutated MODY gene appears to be the prime determinant for disease development by impairing insulin production. In the vast majority of ‘common’ T2DM patients, the disease seems to be the result of a combination of several, different genetic determinants (that may affect insulin production and/or insulin sensitivity) and environmental factors, notably excess food intake and a sedentary lifestyle (lack of physical exercise), which promote development of insulin resistance [15].
2.8 Gestational diabetes

Gestational diabetes is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy but has been previously unrecognized. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy. Women who become pregnant and who are known to have diabetes mellitus which antedates pregnancy do not have gestational diabetes but have “diabetes mellitus and pregnancy” and should be treated accordingly before, during, and after the pregnancy[1]. In the early part of pregnancy (e.g. first trimester and first half of second trimester) fasting and postprandial glucose concentrations are normally lower than in normal, non-pregnant women. Elevated fasting or postprandial plasma glucose levels at this time in pregnancy may well reflect the presence of diabetes which has antedated pregnancy, but criteria for designating abnormally high glucose concentrations at this time have not yet been established [1].

The occurrence of higher than usual plasma glucose levels at this time in pregnancy mandates careful management and may be an indication for carrying out an OGTT. Individuals at high risk for gestational diabetes include older women, those with previous history of glucose intolerance, those with a history of large for gestational age babies, women from certain high-risk ethnic groups, and any pregnant woman who has elevated fasting blood glucose level [1].

2.9 Idiopathic

There are some forms of T1DM which have no known etiology. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. This form of diabetes is more common among individuals of African and Asian origin. In another form found in Africans an absolute requirement for insulin replacement therapy in affected patients may come and go, and patients periodically develop ketoacidosis [1].
2.10 Complications of diabetes mellitus

2.10.1 Diabetic nephropathy

Increased urinary protein excretion in patients with diabetes has long been known to predict increased mortality, and its absence is associated with near-normal life expectancy [35]. 45% of 592 patients who had T1DM and participated in a study progressed to ESRD or died before onset of end stage renal disease (ESRD) during a median follow-up of just under 10 years [35]. In Joslin clinic in the USA, they found the rate of ESRD was the predominant among patients with T1DM [35]. In a another research it's mentioned that patients with type 1 diabetes face a 20–50% probability of developing end stage renal disease (ESRD) requiring dialysis or renal transplantation [36].

2.10.2 Diabetic neuropathy

Peripheral neuropathy is a common complication of diabetes, Autonomic symptoms were present more commonly in type 1 than in T2D, with symptoms of orthostatic intolerance, secretomotor, urinary control, diarrhea, and sleep disturbance and pupillomotor, vasomotor, and erectile dysfunction significantly increased over healthy control subjects in type 2 diabetic patients [37]. It's reported that neuropathy prevalence of 66% in type 1 and 59% in type 2 diabetes in the Rochester, Minnesota, population [37].

2.10.3 Diabetic retinopathy

Proliferative retinopathy is a severe microvascular complication in patients with T1D. After 20 years of diabetes, almost all patients with type 1 diabetes and 58% of patients with type 2 diabetes show signs of retinopathy. When retinopathy worsens, severe visual loss eventually threatens 5–10% of the patients [38]. The most severe form of retinopathy is proliferative retinopathy, and most of the patients with this complication will become blind after 5–10 years without treatment. The prevalence of proliferative retinopathy varies between and 50% after 15–25 years of diabetes in patients who need insulin [38].
2.10.4 Diabetic cardiopathy

Although the increased risk of premature heart disease in type 1 diabetes has been recognized for some time, the underlying pathogenesis is still poorly understood [39]. A high occurrence of and mortality from, CHD in type 1 diabetes has been documented since the late 1970s [39]. It's reported that those with T1D by 55 years of age experienced a six fold greater cumulative CHD mortality compared with the rate expected using Framingham study data [39].

2.11 Antioxidant effect of trace elements

Oxidative damage due to free radicals is associated with vascular disease in people with T2DM; there are several potential sources of increased free radical production in diabetes including auto oxidation of plasma glucose, activation of leukocytes, increased transition metal bioavailability [9] and presence of islet amyloid [40]. Increased oxidative stress, in relation with glucose auto-oxidation, is well documented too [9]. In Tunisia, the incidence of T2DM is approximately 10%, with a high incidence of oxidative complications such as retinopathies, nephropathies and vascular complications [9].

In a study of the antioxidant effect of zinc and chromium supplementation in people with T2DM carried out in Tunisia, this study suggested the potential beneficial antioxidant effects of the individual and combined supplementation of Zn and Cr in people with T2DM [9]. In Nigeria, a study discussed the level of cadmium, lead, arsenic and selenium in patients with T2DM, the serum concentration of Se was significantly lower in diabetic patients than healthy control, in addition, depression in antioxidant concentration (especially, Se) may further aggravate the development and pathogenesis of diabetes mellitus [41]. Also there is an inverse relationship between fasting blood sugar and selenium [41]. In Izmir, Turkey a research titled with correlation of serum Cu, Zn, Mg, and HbA1c there was slightly negative correlation between Cu and Mg, also the same but positive correlation was found between serum Cu and glucose levels in non-obese T2DM [42]. Serum copper level was increased in all patients groups, respectively non-obese T2DM, obese T2DM and T1DM group. The meaningful decrease in serum Mg level
was only found in T2DM group, there were no significant alterations in levels of serum Zn [42]. The determination of serum zinc level is not enough to assess the oxidative stress in DM, because the probable lacking of serum Zn/Cu antagonism [42].

2.12 Role of oxidative stress in diabetes

There are many potential mechanisms whereby excess glucose metabolites travelling along these pathways might cause beta cell damage. However, all these pathways have in common the formation of reactive oxygen species that, in excess and over time, cause chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion as well as increased apoptosis [43]. Abnormalities in the regulation of peroxide and transition metal metabolism are postulated to result in establishment of the disease as well as its longer-term complications. Diabetes mellitus is associated with oxidative reactions, particularly those which are catalysed by decompartmentalized transition metals, but their causative significance in diabetic tissue damage remains to be established [16].

2.12.1 Mechanisms for oxidative stress in diabetes

2.12.1.1 The glycoxidation pathway

Because an elevated level of glucose is one of the metabolic hallmarks of diabetes, much attention has focused on the sugar’s oxidative chemistry. One widely studied mechanism involves autoxidation of glucose itself, which generates reactive oxygen species such as hydroxyl radical and also cross-links proteins [44]. Glucose also reacts non-enzymatically with proteins to form the reversible Schiff base adduct, which subsequently can rearrange to form the stable Amadori product and AGE products. These protein-bound forms of glucose and their oxidized, cleaved, and dehydrated derivatives can produce reactive intermediates [44]. AGEs can damage tissues through a number of mechanisms, including generation of oxidizing intermediates, formation of immune complexes, interaction with a cellular receptor called RAGE (receptor for AGE), and promotion of cytokine release[44].
2.12.1.2 The reactive nitrogen pathway

Another pathway for generating oxidants involves nitric oxide (NO), which is produced by endothelial cells to regulate vascular tone. NO reacts with superoxide to produce peroxynitrite (ONOO\(^-\)), a potent oxidant that converts tyrosine residues to 3-nitrotyrosine. Thus, 3-nitrotyrosine is a marker for the reactive nitrogen pathway [44]. Its detection in low density lipoprotein isolated from human atherosclerotic lesions suggests that production of reactive nitrogen species increases in diseased vascular tissue [44]. Because acute hyperglycemia promotes vasodilation in humans, glucose might directly or indirectly enhance NO release and oxidant generation [44]. NO is also produced during inflammation by macrophages, which are early components of atherosclerotic lesions and persist at all stages of the atherosclerotic process. NO production can also be increased in inflammatory states by induction of inducible nitric oxide synthase [44].

2.13 Role of anti-oxidants in preventing oxidative stress

If generalized or localized oxidative stress proved to be a major contributor to diabetic complications, effective antioxidant regimens would be important therapies. Over the past decade, there has been an explosion of interest by scientists and the public in the possibility that dietary and supplemental antioxidant vitamins, such as beta-carotene, vitamin E, and vitamin A, might prevent human disease [44]. However, trials of antioxidants and carbonyl trapping agents in humans suffering from diabetes have yielded mixed results, despite impressive findings from rat studies. Chronic treatment with vitamin E failed to decrease cardiovascular events in a large study that included a high percentage of diabetic patients [45].

Also, trials of lipoic acid—a proposed antioxidant—for treating diabetic neuropathy have been equivocal [46]. One possible reason for these discouraging results is that antioxidant therapy might benefit only subjects who exhibit increased oxidative stress. Indeed, the renal failure patients who benefited from vitamin E therapy [47]. Might have been a subset with greatly increased carbonyl and oxidative
stress [48]. However, there is remarkably little evidence that compounds such as vitamin E and vitamin C actually inhibit oxidative reactions in humans [44].

2.14 Oxidative stress and β-cell dysfunction

Glucotoxicity and lipotoxicity are generally considered as the major contributors of beta-cell failure in the developing stage of T2DM [49]. Abundant evidences demonstrate that chronic exposure to high circulating glucose or free fatty acids (FFA) increases reactive oxygen species (ROS) production and decreases insulin content and glucose-stimulated insulin secretion of beta-cells both in vivo and in vitro [50]. Antioxidant treatment or over-expression of glutathione peroxidase or catalase can reverse these effects [51]. Beta-cells express low levels of anti-oxidative enzyme including catalase and glutathione peroxidase, which make them particularly susceptible to oxidative stress [52]. Also, it’s reported that islet amyloid contributes to loss of beta-cell mass and function in type 2 diabetes. It is poorly understood how the building block of amyloid, islet amyloid polypeptide (IAPP), misfolds and accumulates within the islet to contribute to cellular dysfunction [53].

The Aβ peptide can generate H₂O₂ directly from molecular oxygen, apparently via electron transfer reactions involving bound redox-active transition metal ions (Fe and Cu). H₂O₂ is itself toxic to cells, but, if formed in the vicinity of redox-active metal ions, such as Fe (II), is readily converted into hydroxyl radicals, via Fenton’s reaction. These highly reactive radicals can induce damaging oxidation, cell toxicity and cell death [40].

2.15 Metabolic staging of type 2 diabetes

Type 2 diabetes is a progressive disorder that begins with peripheral insulin resistance and ends with failure of pancreatic beta-cells. In most cases, peripheral insulin resistance, defined as the attenuated response to insulin in fat tissue, liver, and skeletal muscle, appears long before the development of hyperglycemia [54]. Resistance to insulin in skeletal muscle results in reduced glucose uptake. Resistance to the insulin-mediated suppression of hepatic - gluconeogenesis and glycogenolysis
increases glucose output from the liver. Resistance to the anti-lipolytic action of insulin in fat tissue causes increased lipolysis and increased FFA flux into circulation. Chronically elevated FFA can induce pancreatic beta-cell death (“lipotoxicity”) [54]. To compensate peripheral insulin resistance, pancreatic beta-cells increase in mass and secrete more insulin, however, at some point, beta-cells can no longer compensate peripheral insulin resistance and plasma glucose levels start to rise. Elevated glucose levels can further damage pancreatic beta-cells, leading to progressive loss of pancreatic islet beta cells and finally the development of frank hyperglycemia [54].

2.16 Trace elements and type 2 diabetes mellitus

2.16.1 Effect of magnesium consumption on glucose concentration

Magnesium is mainly an intracellular cation, with less than 1% of total body content present in the extracellular fluids. The Mg concentration in serum represents not more than 0.3% of total body Mg [55].

2.16.2 Magnesium homeostasis

Mg is absorbed in the gastrointestinal tract (Figure 2.1) and eliminated primarily through urinary excretion, to a smaller extent through gastrointestinal excretions and to a negligible part through sweat, menstrual losses and other corporal secretions [56].
During periods of Mg deprivation, Mg homeostasis is maintained by increased fractional absorption of Mg in the kidney and the gastrointestinal tract, and by release from internal Mg stores such as bone and skeletal muscle [56]. About one third of bone Mg resides on the surface of bone either within the hydration shell or on the crystal surface [56]. This fraction is surface exchangeable and is thought to serve as a reservoir to maintain extracellular Mg concentration [56]. Bone provides the highest buffering potential, but skeletal muscle Mg contributes to the buffering and can lose an average of 15 % of its total Mg. Together, the bone and skeletal muscle pools can provide an average of 1.7 mmol/kg body weight equivalents to 15 % of total body Mg to maintain Mg homeostasis [56].
2.16.3 Intestinal absorption

The mechanisms of intestinal Mg absorption are not entirely clear. The major sites of Mg absorption are probably the distal small intestine and the colon [57]. There is data to support the existence of both gradient-driven (passive) and saturable Mg absorption. It is not clear, however, which process predominates under normal conditions. A direct correlation between Mg absorption and the luminal or dietary Mg load has been shown in several studies [57].

Nevertheless, serum or plasma Mg measurement is the most readily available and widely used test of Mg status. In human studies, instituting a diet low in Mg produces a predictable decline in serum Mg [58]. However, there are a number of reports of low Mg values in various blood cells and tissues associated with normal serum/plasma Mg concentrations [55]. It appears therefore that plasma Mg concentration is an insensitive, but highly specific indicator of low Mg status. Of the total Mg in serum, around 55% is present as free ionized Mg2+, 15% is complexed to anions (e.g. bicarbonate, citrate, and sulfate) and 30% is bound to proteins, mainly albumin [60]. It could therefore be argued that in diabetics with microalbuminuria, serum Mg might be reduced because of lower serum albumin concentration [61].

Magnesium is an essential metal in carbohydrate metabolism. It causes activation and release of insulin. Increasing of blood sugar in diabetic patients, results in a decrease of magnesium in the serum and its low concentration in urine. Measurement of glucose concentration in diabetic rats after treatment with magnesium was done in 80 rats. They were categorized in ten groups including control, diabetic without treatment of magnesium, diabetic with treatment of magnesium in one (1), two (2), three (3) and four(4) week(s). The other 4 normal groups were treated with magnesium in same timescales [62].

In the diabetic groups, diabetes was induced with injection of 60mg/kg streptozotocin. Besides weight measurements, glucose concentrations of animals were measured with enzymatic-calorimetric method. Statistical analysis was carried out by ANOVA and Tukey test. The results showed that the difference in animal
weight and glucose concentrations between control and diabetic groups was significant ($P<0.0001$). Glucose concentrations of magnesium treated diabetic groups significantly differed between 1 and 2 ($P<0.005$); 1 and 3 or 4 groups ($P<0.0001$); 2 and 3 ($P<0.001$) and 2 and 4 ($P<0.01$). Statistical differences among control and magnesium treated diabetic groups were significant between control and 1 ($P<0.0001$); control and 2 ($P<0.05$). In the weight data there was no significant difference between diabetic in one side and 1 and 2 groups in other side [62].

But difference between diabetic and the other 3 and 4 groups showed significance with $P<0.001$ and $P<0.0001$, respectively. In this study results from flinching and licking responses have been evoked by formalin in biphasic model of formalin test. Magnesium consumption in diabetic groups resulted in an increase of animal weight and decrease of glucose concentration and such effects show time dependency. Although, such changes was not reached to the control measurements [44]. A deficiency of magnesium is significantly more common in T2DM than in the general population. Magnesium deficiency has been associated with complications of diabetes, retinopathy in particular. One study found patients with the most severe retinopathy were also lowest in magnesium [63].

In another study based on the effect of administration of oral magnesium on the patients of T2DM, it was concluded that oral magnesium supplementation for 4–16 weeks may be effective in reducing plasma fasting glucose levels and raising HDL cholesterol in patients with T2DM, although the long-term benefits and safety of magnesium treatment on glycaemic control remain to be determined [64]. On contrary, in a study dealed with fiber and magnesium intake and incidence of T2DM, neither magnesium nor total fiber intake was associated with risk of developing T2DM [65].

2.17 **Role of zinc in typ2 diabetes mellitus**

Zinc is involved in the synthesis, storage, secretion and conformational integrity of insulin [66]. In the presence of Zn, insulin monomers assemble to a dimeric form for storage and secretion as crystalline insulin. In vitro, dimeric insulin
assembles further into a hexamer in the presence of Zn. This form of insulin is relatively stable, this hexameric crystal which is the commonly used pharmacological form. Conformational changes of this form may also affect the receptor binding of insulin [66]. Patients with T2DM were found to have decreased plasma Zn and intracellular Zn concentrations, and increased urinary Zn excretion compared to nondiabetic subjects [67]. Decreased serum Zn concentrations in diabetes reflect impaired Zn status, which is caused by hyperzincuria and maybe impaired intestinal absorption. Hyperzincuria appears to be due to hyperglycemia rather than any specific effect of insulin on renal excretion [66].

There is a positive correlation between metabolic control (HbA1c) and urinary Zn excretion in a study with 175 diabetic patients [68]. Decreased intestinal Zn absorption has been proposed to contribute to impaired Zn status. In a study with T1DM using a stable isotope technique, absorption of Zn tended to be lower in diabetics, but did not reach the level of statistical significance [69]. In contrast, there is no difference in Zn metabolism or absorption between T2DM and healthy controls using a stable isotope technique, although urinary Zn excretion was increased in diabetic males [56].

Supplementation with Zn may be associated with an improvement in glucose homeostasis and a decrease in lipid peroxidation [56]. The antioxidant function of Zn is probably due to the protection of sulfhydryl groups in proteins against oxidation and to the inhibition of the production of ROS by Fe and Cu [56]. Moreover, smaller studies in older subjects with diabetes have suggested some benefit from Zn supplementation in healing skin ulcerations [56]. Low dietary Zn intake appears to be related to a higher risk for developing diabetes. In a cross-sectional survey in 3575 Indian subjects, lower dietary Zn intake was associated with a higher prevalence of diabetes, glucose intolerance and coronary artery disease [70].
2.17.1 Zinc and Insulin interactions

Insulin is secreted by the beta cell both tonically (at a constant low level release rate) and as a high level spike in response to an immediate glucose load such as a meal. Since the discovery of insulin in the late 1920s by Banting and Best, attempts to mimic this physiologic response with injected insulin have been central to the treatment of diabetes [66]. Insulin is produced by the beta cell of the pancreatic islets as a single chain peptide that is bent around itself and linked by two inter-chain disulfide bonds [66]. This proinsulin is cleaved by the removal of an intracellular chain fragment known as the “C-peptide” to form two peptide chain (alpha and beta) molecules of 51 amino acids cross-linked to each other by inter-chain disulfide bonds. This is the insulin monomer. In the presence of zinc within the cell, insulin monomers assemble to a dimeric form for storage and secretion as the zinc crystal. It is showed in 1994 that high concentrations of glucose and other secretagogues decrease the islet cell labile Zn and video fluorescence analysis showed Zn concentrated in the islet cells was related to the synthesis, storage and secretion of insulin [71].

In vitro, in the presence of zinc and at neutral pH, dimeric insulin assembles further into a hexamer consisting of three dimeric units. This form of insulin is relatively stable and it is this hexameric crystal which is the commonly used pharmacologic form. The size of the crystal is, at least in large part, the determinant of dissolution rate. Changes in the tertiary conformation of the hexamer may also result in significant biologically important changes relating to release rate and consequent biologic activity [72]. This hexamer is capable of adopting at least three conformations, the physiologic consequences of which are not known [73]. Beyond the physical chemical effects of conformation, there are data to suggest that the conformational changes also affect the receptor binding and antigenic properties of insulin. In vitro data suggest that insulin binds to isolated liver membranes to a greater extent and that there is less degradation when Zn is co-administered with insulin [74].

While it is not clear if there is any relationship to degradation or alterations in binding, it has also been suggested that antigenic determinants are altered by the removal of Zn from insulin by changing the conformation of the molecule. In one
series of studies, Zn-free insulin was much less immunologically active than Zn insulin in immune hemolysis inhibition assays while there was little difference in radio immune assay determinations. This suggests that there are several binding and activation mechanisms and at least one of them is conformational dependent [75]. With the development of genetic “engineering,” it has become possible to develop analogues of insulin which do not form Zn-insulin hexamers which result in a more rapid absorption from the injection site [76].

2.17.2 Effect of Zinc on diabetes

There is increasing evidence supporting the role of zinc as an antioxidant that could protect insulin and cells from being attacked by free radicals [77]. Despite the evidence from animal studies that zinc intake may have protective effects against type 2 diabetes; few studies in humans have been conducted to examine this relationship. In obese Brazilian women, 4 weeks of zinc supplementation (30 mg/day) significantly improved insulin sensitivity [78]. In a cross-sectional analysis, higher dietary zinc intake was associated with a lower prevalence of diabetes and metabolic syndrome in an Indian population [70]. Several human studies already demonstrated that both inorganic iron and heme iron can inhibit the absorption of Zn [79].

2.17.3 Zinc and coronary heart disease in type-2 diabetes mellitus

Coronary heart disease (CHD) is a major cause of death in T2DM, and the risk of CHD is two to fourfold higher among type-2 diabetic patients than in non-diabetic subjects [80]. This risk is enhanced by other factors such as smoking, obesity, increased plasma lipids and hypertension [80]. Because serum zinc is a micronutrient with known antioxidant activity, it might be relevant to assess its role in the atherogenesis in T2DM. In Finland, during follow-up of 156 patients with T2DM died from CHD and 254 patients had fatal or nonfatal myocardial infarction (MI) patients with serum Zn concentration ≤ 14.1 µmol/l had a higher risk for death from CHD than patients with serum zinc level > 14.1 µmol/l so, low serum zinc concentration was significantly associated with CHD mortality [80].

In Egypt biochemical changes of trace elements were evaluated in diabetic patients. Plasma Cu, Zn and Mg, were evaluated in the two types of diabetes in
comparison to control group, the plasma level of Cu was increased, Zn and Mg were significantly diminished in diabetic patients as compared to control group [81]. While on contrary in a study in Osogbo, Nigeria mean serum concentration of Mg were non-significantly lowered in T2DM than in control group [82].

In Gorgan city, (South East of Caspian Sea-Iran) Zn and Mg were significantly decreased in T2DM when compared with control group. Patients with T2DM have an increased mortality and morbidity compared with control groups and more likely to CHD; the possible explanation for this situation is that there is loss of large amount of Zinc from the body via urine. The source of the Zinc that is excreted remains incompletely resolved there is a concurrent hypozincemia and a decrease in tissue zinc stores, but it's not clear if this is a result of the hyperzincemia [83]. The reasons why Mg decreasing occurs in diabetes are not clear but may include higher urinary losses of impaired absorption of Mg compared with healthy persons [83]. In pregnant women complicated with diabetes mellitus impacts negatively on plasma Zn status, but lacks effect on plasma copper [84]. Zinc in the urine is found with both T1DM and T2DM [85].

2.18 Role of Iron in Diabetes mellitus

Excess iron has been implicated in the pathogenesis of diabetes and its complications [86]. Free iron serves as a catalyst for lipid and protein oxidation and the formation of ROS. In addition, iron indices are correlated with obesity and insulin sensitivity [87]. These factors have led some to promote iron chelation as a possible adjunctive therapy in diabetes [88]. Not only Iron is a transitional metal but also a potential catalyst in many cellular reactions that produce ROS [89]. Such reactions contribute to tissue damage and increase oxidative stress, thereby potentially altering the risk of T2DM [89]. Several studies have suggested a possible link between high body iron stores and metabolic parameters such as insulin and glucose as well as hypertension, dyslipidemia, and obesity [90].

In addition, epidemiological studies have reported an association between high iron stores and increased risk of cardiovascular disease, metabolic syndrome,
gestational diabetes, and T2DM [89]. The major source of body iron is derived from the diet, which exist as either heme (derived from meat and meat products) or nonheme iron. In two recent prospective cohort studies, intake of total or nonheme iron was not associated with the risk of T2DM, but heme iron was associated with elevated risk. The aim of this study was to evaluate the association between iron intake and the risk of T2DM [89].

2.18.1 Iron overload and diabetes

Evidence that systemic iron overload could contribute to abnormal glucose metabolism was first derived from the observation that the frequency of diabetes is increased in classic hereditary hemochromatosis [12]. However, with the discovery of novel genetic disorders of iron metabolism, it is obvious that iron overload, irrespective of the cause or the gene involved, results in an increased incidence of T2DM [12]. The role of iron in the pathogenesis of diabetes is suggested by:

1. An increased incidence of T2DM in diverse causes of iron overload.
2. Reversal or improvement in diabetes (glycemic control) with a reduction in iron load achieved using either phlebotomy or iron chelation therapy.

Recently, a link has been established between increased dietary iron intake, particularly eating red meat and increased body iron stores, and the development of diabetes [91]. A causative link with iron overload is suggested by of the improvement in insulin sensitivity and insulin secretion with frequent blood donation and decreased iron stores [91].

2.18.2 The role of iron in diabetes without overt iron overload

Numerous studies have confirmed that, the relationship between iron and diabetes is related to the high heme content of meat and increased dietary heme intake [92]. Similarly, high body iron stores have been linked to insulin resistance [93], metabolic syndrome [94], and gestational diabetes [95]. In a cohort study, the mean concentration of serum ferritin was significantly higher compared with control subjects, and the mean ratio of transferrin receptors to ferritin was significantly lower. This relationship with markers of body iron stores persisted after correction for various other risk factors for diabetes, including markers of obesity and inflammation [96].
Others suggested that the modest elevations in ferritin levels observed in diabetes may be a consequence or marker rather than the cause of impending insulin resistance and that elevated ferritin may not reflect elevated body iron stores or an intracellular labile iron pool that participates in oxidant injury [97]. However, the common presence of non–transferrin-bound iron (NTBI) in (59–92% of patients), is a form of iron most susceptible to redox activity, in excess amounts in type 2 diabetes with a strong gradient for severity [98], and the preliminary evidence that reduction in body iron stores with bloodletting in T2DM results in improvement in glycemic control and insulin resistance suggests a pathogenic role of iron in type 2 diabetes [99].

2.19 Copper, oxidative stress and diabetes mellitus

There are conflicting data on the associations between copper and glycemia, plasma lipids, and atherosclerotic diseases. Copper has both pro-oxidant and antioxidant effects [100]. Copper is an essential nutrient in humans and acts as a critical cofactor when incorporated into specific cupro-enzymes that catalyze electron transfer reactions required for cellular respiration, iron oxidation, pigment formation, neurotransmitter biosynthesis, antioxidant defense, peptide amidation, and connective tissue formation [101].

Both overt copper deficiency and excess are associated with specific clinical manifestations [101]. Copper deficiency causes an accumulation of arterial lipid peroxides, possibly due to the decreased activity of the copper-zinc superoxide dismutase (CuZnSOD). Many of the pathological effects of copper overload are consistent, however, with oxidative damage to membranes or macromolecules [102]. Copper intake in vivo has shown both pro-oxidant and antioxidant effects; ceruloplasmin, the major copper-containing plasma protein, may act as either an antioxidant or pro-oxidant, depending on ambient conditions [102].

Thus, the definitive role of copper in oxidative processes is still a matter of debate. Furthermore, experimental and epidemiological data regarding the possible role of copper on metabolic abnormalities and atherogenesis are conflicting. Increased concentrations of plasma copper were observed in diabetic patients with
chronic complications or macro vascular diseases [103]. On the other hand, copper supplementation exerted beneficial effects in diabetic rats by reducing glucose levels [104]. Both inverse and direct correlations between serum and dietary copper and total cholesterol concentrations have been reported [102].

The zinc/copper hypothesis proposed an increased atherosclerotic risk in copper deficiency due to hypercholesterolemia [105]. In contrast, epidemiological studies showed that higher serum copper concentrations may promote coronary artery diseases [105]. Copper supplementation in healthy volunteers showed an antioxidant effect, because it protected RBC from oxidation [100]. However, this effect did not result from increased CuZnSOD activity and was evident for supra-physiological doses of copper only (7 mg/d). On the other hand, copper ions participate in radical reactions such as the conversion of superoxide to hydrogen peroxide and hydroxyl radicals, and catalyze the oxidative modification of LDL in vitro and in the arterial wall; copper excess can induce oxidative damage to DNA [106]. Increased concentrations of lipid peroxides were found in women using oral contraceptives; estrogen treatment resulted in increased plasma copper and there was a strong relationship between plasma copper and lipid peroxides in these patients [107].

2. 20 Copper and inflammation

Ceruloplasmin responds as an acute-phase reactive protein to stress and trauma and increased copper concentration was reported in response to inflammation, infection, and various chronic diseases, such as arthritis and neoplasia [108]. Serum copper concentration is higher than normal in various inflammatory diseases in humans and laboratory animals [100]. The rise of ceruloplasmin is probably responsible for the increased serum copper. The increased mortality from cardiovascular disease in subjects with higher serum copper reported by epidemiological studies [109] clearly emphasizes the duplicitous nature of copper. Indeed, copper seems to be associated with a favorable metabolic pattern and there is now some interest in its potential insulin-mimetic effect [110]. Although marginal copper deficiency may pose problems, copper supplementation might not be
desirable due to its association with inflammation, markers of oxidative stress, and increased cardiovascular risk [100].

2. 21 Copper and metabolic abnormalities

Currently, data regarding dietary intake and blood concentrations of copper and glucose are conflicting. In rats, glycation was enhanced in dietary copper deficiency [111], whereas copper supplementation reduced glucose levels in diabetic mice [104]; in rat isolated adipocytes, copper sulfate inhibited free fatty acid release and enhanced glucose uptake [110]. Other studies found that in diabetic patients, circulating copper concentrations were not different [100], or were greater in patients with chronic complications or macro vascular diseases [103]. Indeed, in the presence of overt diabetes with chronic complications, the associated chronic low-grade inflammatory state might be responsible for an increase in blood copper concentrations. There are several reports of an inverse correlation between serum and dietary copper and total and LDL-cholesterol in experimental studies in humans and rats. In contrast, a positive association between serum copper concentrations and cholesterol has been reported [112].

The mechanisms by which copper deficiency may act on LDL-cholesterol may include: decreased heparin-releasable lipoprotein-lipase activity [113], increased clearance of cholesterol esters (with newly synthesized cholesterol esters entering the serum pool at an increased rate) [114], enhanced hepatic apolipoprotein B synthesis, and increased hydroxymethylglutaryl CoA reductase activity [115]. A relationship between copper deficiency and hypertension was reported, perhaps from impaired vasodilatation in response to acetylcholine [111]. There is an inverse association between diastolic blood pressure and dietary copper intake in the entire cohort but not for serum copper concentrations in the subsample of men, even though systolic and diastolic blood pressures were higher in subjects in the lowest serum copper tertile than in subjects in the other 2 tertiles [100].
2. 22 Metals, Fenton reaction and oxidative stress

Metal-induced toxicity and carcinogenicity, with an emphasis on the generation and role of reactive oxygen and nitrogen species, is reviewed. Metal-mediated formation of free radicals causes various modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulfhydryl homeostasis. Lipid peroxides, formed by the attack of radicals on polyunsaturated fatty acid residues of phospholipids, can further react with redox metals finally producing mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal and other exocyclic DNA adducts (etheno and/or propano adducts). Whilst iron (Fe), copper (Cu), chromium (Cr), vanadium (V) and cobalt (Co) undergo redox-cycling reactions, for a second group of metals, mercury (Hg) [116]. Cadmium (Cd) and nickel (Ni), the primary route for their toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins. Arsenic (As) is thought to bind directly to critical thiols, however, other mechanisms, involving formation of hydrogen peroxide under physiological conditions, have been proposed [116].

The unifying factor in determining toxicity and carcinogenicity for all these metals is the generation of reactive oxygen and nitrogen species. Common mechanisms involving the Fenton reaction, generation of the superoxide radical and the hydroxyl radical appear to be involved for iron, copper, chromium, vanadium and cobalt primarily associated with mitochondria, microsomes and peroxisomes. However, a recent discovery that the upper limit of “free pools” of copper is far less than a single atom per cell casts serious doubt on the in vivo role of copper in Fenton-like generation of free radicals. Nitric oxide (NO) seems to be involved in arsenite-induced DNA damage and pyrimidine excision inhibition. Various studies have confirmed that metals activate signalling pathways and the carcinogenic effect of metals has been related to activation of mainly redox-sensitive transcription factors, involving NF-kappaB, AP-1 and p53. Antioxidants (both enzymatic and nonenzymatic) provide protection against deleterious metal-mediated free radical attacks [116].
2.23 Reactive oxygen species and neurodegenerative disease

There is mounting evidence for a major contribution played by oxidative stress in the pathology of most, if not all, of the neurodegenerative diseases. Oxidative stress is due to an imbalance between the production of ROS and the ability of antioxidant defences to cope with ROS production [117]. Evidence for oxidative stress covers features such as increased levels of redox-active transition-metal ions and the detection in the brain of products of lipid peroxidation and DNA, RNA and protein oxidation. In the case of Alzheimer disease (AD), some researchers have stressed the fact that this type of oxidative damage could precede and even precipitate the aggregation of Aβ (amyloid β-peptide). However, Aβ has been shown to generate H₂O₂, a key ROS, directly from molecular oxygen, through electron-transfer interactions involving bound redox-active copper and/or iron ions. H₂O₂ is readily converted into the very aggressive hydroxyl radical, and this highly reactive free radical could be responsible for much of the early oxidative damage seen in AD [117].

2.24 Amylin

Amylin, a naturally occurring hormone, also termed islet amyloid polypeptide; (IAPP) consists of 37 amino acids, amidated at the C-terminal [118].

Amylin is produced through gene expression on chromosome 12. It is transcribed as an 89-aminoacid prepolypeptide, which is cleaved to form the mature peptide in the β cells of the pancreas, where it is stored along with insulin and C-peptide in the same granules [119]. Amylin is a normal product of β cells and is co-released with insulin in a molar ratio of ≈1 to 100 in healthy non-diabetic subjects in response to nutrient stimuli (carbohydrate- and protein-containing meals) [120]. The amylin to insulin molar ratio is alterable as seen in dexamethasone treated rats that exhibit an increased ratio. Amylin residence time in the plasma is longer than insulin and similar to C-peptide, although amylin has a faster clearance rate than insulin by the kidneys. Because body weight and adiposity influence kinetic parameters and overall levels of insulin and amylin, the molar ratio of amylin to insulin is a good indicator of relative amylin deficiency [118].
2.25 Role of Amylin in diabetes mellitus

Amyloid deposits derived from the amylin peptide accumulate within pancreatic islet β-cells in most cases of type-2 diabetes mellitus (T2DM). Human amylin ‘oligomers’ are toxic to these cells. Using two different experimental techniques, H$_2$O$_2$ was generated during the aggregation of human amylin into amyloid fibrils. This process was greatly stimulated by Cu (II) ions, and human amylin was retained on a copper affinity column. In contrast, rodent amylin, which is not toxic, failed to generate any H$_2$O$_2$ and did not interact with copper. The conclusion is the formation of H$_2$O$_2$ from amylin could contribute to the progressive degeneration of islet cells in T2DM [40]. Islet amyloid deposits are found in >90% of patients with type 2 diabetes at autopsy [121]. Evidence from an increasing number of both in vitro and in vivo studies suggests that islet amyloidosis is a diabetogenic factor and has shown that there is a close relationship between islet amyloid deposition and the development and progression of T2D [14].

Studies performed on diabetic nonhuman primates have shown that formation of islet amyloid precedes the development of hyperglycemia [122]; supporting the idea that islet amyloid might have an important role in the pathogenesis of type 2 diabetes. The finding that the degree of islet amyloid deposition correlates with severity of the disease in humans [123] and that formation of islet amyloid is associated with a decrease in beta cell mass in both humans and nonhuman primates further supports this hypothesis. In humans, it has been hypothesized that islet amyloid develops even in pre-diabetic stages, when individuals have impaired glucose tolerance [14]. Furthermore, it's found that formation of islet amyloid deposits in transgenic mice over expressing fibrillogenic human islet amyloid polypeptide (IAPP) is closely associated with the development of hyperglycemia; In vitro studies have shown that exposure to human IAPP fibrils induces death of both human and rat beta cells [124]. However, despite these supportive studies, it is still debated whether islet amyloid deposits are a major cause of beta cell failure in type 2 diabetes or whether they are simply a marker of beta cell death caused by other factors [124].
It's believed that the evidence, however correlative, points strongly to islet amyloid as a major contributor to the decline in beta cell function and ultimate failure of insulin secretion in this disease. Whether islet amyloid may also play a role in promoting early beta cell dysfunction remains an open question. Proof of a role for islet amyloid in the pathogenesis and progression of type 2 diabetes awaits studies using specific inhibitors of islet amyloidosis in appropriate animal models to test whether inhibition of this process can slow disease progression [14].

2.25.1 Role of fatty acids in induction of amylin expression and release

In pancreatic β-cells, amylin is co-stored with insulin in the secretory granules and co-secreted in a regulated manner following stimulation with glucose and a variety of other secretagogues [125]. Glucose stimulates amylin mRNA expression and protein release by rodent and human pancreatic islets [126]. The plasma level of amylin increased in normal mice fed a high-fat diet for long term [127]. In human amylin transgenic mice, amyloid does not appear spontaneously but appears after long-term, high-fat dietary intake [128]. These results suggest that fatty acids (FAs) may stimulate amylin release and enhance islet amyloid formation. But the effect of FAs on amylin expression and release by pancreatic β-cells is not clear [125].

2.25.2 Role of intracellular amyloid β in Alzheimer’s disease

Extracellular amyloid β (Aβ) that confers neurotoxicity and modulates synaptic plasticity and memory function has been central to the amyloid hypothesis of Alzheimer’s disease (AD) pathology. Like many other misfolded proteins identified in neurodegenerative disorders, Aβ also accumulates inside the AD neurons. This intracellular Aβ affects a variety of cellular physiology from protein degradation, axonal transport autophagy to apoptosis, further documenting the role of Ab in AD [109]. Alzheimer’s disease is a progressive neurodegenerative disorder characterized by cognition and memory impairment, it's the most common neurodegenerative disorder worldwide, in united states there are about 4.5 million people suffering from this disorder [129].
Alzheimer’s disease brains are characterized by the presence of (1) extracellular senile plaques composed primarily of a ≈4 kDa polypeptide, amyloid β peptide (Aβ), (2) intracellular neurofibrillary tangles (NFTs), (3) dystrophic neurites, (4) degenerating neurons, and (5) activated astrocytes and microglia especially around the senile plaques [130].

In AD, neurons of the hippocampus and cerebral cortex are selectively lost, amyloid plaques contain small cleavage products denote (Aβ 40 and Aβ 42) of the amyloid precursor protein [129]. The Apo E4 (Apolipoprotein E4) genotype is a risk factor for developing AD, and it may affect Aβ deposition and neurofibrillary tangle formation [129]. Mutations in three genes that are inherited in an autosomal dominant have been linked to rare familial, early onset form of AD. These genes are APP, presenilin 1 (PS1) and presenilin 2 (PS2), one common event in both familial and sporadic types of AD is the increase production and accumulation of the toxic Aβ [129].

2.25.3 The role of amylin in osteoporosis

Osteoporosis is a systemic skeletal disorder that remains a major public health problem due to significant fracture-associated morbidity and mortality. Because it has been shown that individuals having type I diabetes mellitus also suffer from osteopenia or osteoporosis, there is probably a pathophysiological mechanism that links pancreatic beta cell insufficiency with inappropriate bone formation. Many factors have been suggested, including amylin [131].

Amylin has many important functions in the organism:

1. It participates with insulin in the control of glucose homeostasis by slowing of gastric motility, modulation of insulin sensitivity of skeletal muscles and mediation of signals of thirst and satiety in the CNS [132].

2. In the bone, it acts as a growth factor stimulating proliferation of osteoblasts. On the contrary, amylin was shown to have an osteoclast-inhibitory effect.

3. Amylin also regulates blood pressure, causes vasodilatation [133] and acts as a growth factor in renal proximal tubular cells and islet beta cells [134]. Amylin influences behavior, memory and motor activity through its action in the CNS, [135] and has a potential to form amyloid deposits
in the pancreatic islets, thereby contributing to beta cell destruction, resulting in overt DM [136].

2.25.3.1 Amylin's effect on osteoblasts

In fetal rat osteoblasts, the intact amylin and amylin residuesn - (1–8) stimulate cell proliferation. Approximately 10-fold higher concentration of the fragment is needed to achieve the effect of the intact peptide [137]. Amylin residues- (8–37), COOH terminally deamidated amylin and reduced amylin (the disulfide bond is cleaved) act in an antagonistic manner. In osteoblasts, amylin acts through increase of Cyclic AMP and activation of mitogen-activated protein kinase and protein kinase C [137].

2.25.3.2 Amylin's effect on osteoclasts

In contrast, inhibition of bone resorption only occurs with the intact amylin molecule and is dependent on the presence of the carboxyl-terminus amide group of the peptide [138]. Amylin inhibits osteoclast activity through increase of cyclic AMP and reduces osteoclast development [139]. Several studies confirmed amylin being able to reduce both basal and stimulated bone resorption through stimulation of Cyclic AMP production [140]. The dissociation of the actions on bone formation and resorption suggests that amylin acts through two separate groups of receptors, the first one on the osteoclast and the second on the osteoblast [131].
Chapter 3

Material and Methods

3.1 Study Design
The present study is a cross-sectional.

3.2 Target population
The target population of this study was type 2 diabetes mellitus patients, diagnosed and under treatment in El- Rimal clinic in Gaza city, who regularly visit the clinic of diabetic patients.

3.3 Sample size
According to previous studies, the prevalence of DM in Palestine is about 9-10%. The sample size of the study is calculated by using the formula of WHO/WFP/UNHCR/IFRC with 95% confidence interval, 3% margin of error and 10% prevalence of diabetes and based on studies that carried out in Palestine. Accordingly a total of 100 samples of (41 male and 59 female) patients suffer from T2DM and 33 healthy control individuals (13 male and 20 female) were collected.

3.4 Inclusion criteria
Patients who are suffering and diagnosed as T2DM patients.

3.5 Exclusion criteria
1) Pregnant women.
2) Patients with liver diseases
3) Type 1 Diabetes mellitus patients.

3.6 Ethical Consideration
All necessary approvals have been obtained from the Islamic university of Gaza on the 6th of April 2011 (Annex1, 2), Approval from the directorate general of human resources development obtained on the 20th of April 2011(Annex 3) and an approval from Helsinki committee obtained on the 6th of June 2011 (Annex 4). Helsinki committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area. The participants were given a full explanation about the purpose of the study and assurance about the confidentiality of the information and that the participation was optional.
3.7 Data Collection

3.7.1 Questionnaire Interview

A meeting interview used for filling in the questionnaire which was designated for matching the study need. All interviews were conducted face to face by the researcher himself. The questionnaire (Annex 5) was similar to diabetic clinic questions at Al Rimal Medical Center with some modifications. During the study the researcher explained to the participants any of the confused questions that were not clear for them. A questionnaire was piloted with 10 patients in order to check reagents, procedures and discuss the primary results. The questionnaire includes questions on the personal data (name, age, age at diagnosis, sex, smoking, family history of diabetes), clinical data (duration of DM) only for patients, and the most important complications of diabetes (retinopathy, cardiovascular disease (CVD), hypertension (HTN) and neuropathy.

3.7.2 Sampling

One hundred blood samples were collected from T2DM patients from El-Rimal clinic, diagnosed according to the WHO protocol. Patients were selected randomly way after making sure that they meet the inclusion criteria, also a thirty three control sample were collected from a healthy individual who don't suffer from diabetes, hypertension, cardiovascular disease, any other chronic diseases, under cortisol treatment or suffer from any autoimmune disease.

3.7.3 Sampling process

All blood samples were collected at the out-patient clinic in El-Rimal clinic. Blood samples were collected in a vacutainer tubes for trace elements and blood sugar testing, and in an EDTA- tubes for amylin testing. All patients were fasting for at least eight hours and not to exceed 12 hours. After collection, samples were centrifuged, and serum were separated and stored in a deep freeze in the central lab at (-20.0 °C). Hemolysis avoided during serum separation.
3.8 Biochemical analysis

Patients' serum was tested for fasting blood sugar, Cu++, Zn++, Mg++, Fe++ and amylin. Available and approved commercial kits were used for the determination of the selected trace elements, blood sugar and EIA kits for amylin quantitation.

3.8.1 Glucose determination

The kit was composed of the following reagents

**Reagent A:** Composed of the following:
- Phosphate buffer pH 7.4 25g/l
- Phenol < 0.9%
- 4-Aminoantipirine 0.4mmol/l
- Glucose Oxidase (GOD) ≥ 30kU/l/l
- Peroxidase (POD) ≥ 1Ku/l
- NaN₃ 0.95 g/l

**Standard:** Composed of the following
- D-Glucose
- Benzoic acid 100mg/dl

3.8.1.1 Principle of the method

Glucose is transformed by glucose oxidase (GOD) in gluconic acid and hydrogen peroxide, which, in presence of peroxidase (POD), reacts with phenol and 4-aminoantipirine to form a red complex, whose intensity at 505 nm is proportional to the glucose concentration in the sample.

\[
\begin{align*}
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} & \xrightarrow{\text{GOD}} \text{Gluconic acid} + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + \text{Phenol} + 4\text{-Aminoantipirine} & \xrightarrow{\text{POD}} \text{Red complex} + \text{H}_2\text{O}
\end{align*}
\]

All reagents are ready to use

3.8.1.2 Method Procedure

This test and the following chemical tests were done by using chemistry auto analyzer Rayto model 240 serial number301218001 EZ. The reaction volume of serum, standard and reagent were proportionally changed with accepted range according to the kit Limitations. For all tests and before testing, serum was thawed at room temperature (R.T) for 30 minutes (min), and then thawed at 37°C for 10.0 min,
mixing by vortex for 10.0 seconds (sec). Double distill water (D.D.W) was used in all tests; completely new cells were used during running tests and a universal quality control material with two levels (normal and abnormal) from Humatrol was used.

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<td>Reagent A</td>
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<td>D.D.W</td>
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<tr>
<td>Standard</td>
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<tr>
<td>Sample</td>
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<td>3.0 µl</td>
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Test was read at 510 n.m.

### 3.8.1.3 Calculation

Serum, plasma:

\[
\text{Glucose (mg/dl)} = \frac{\text{Abs. sample}}{\text{Abs. standard}} \times 100
\]

**Normal range of glucose** for adults is 70-115 mg/dl.

### 3.8.2 Magnesium determination

The kit was composed of

**Reagent A**: Composed of the following

- Ethanolamine pH 11.0 1 mol/l
- GEDTA 60 µmol/l
- Xylidyl Blue 110 µmol/l

**Standard**: 1x5 ml Mg++ 2.43 mg/dl

#### 3.8.2.1 Principle of the method

Magnesium in alkaline solution reacts with the chromogen Xylidyl Blue to form a purple complex. The presence in the reagent of glycoletherdiaminotetracetic acid (GEDTA) that binds calcium ions allows the reaction to be specific. OHXylidyl Blue. Purple color intensity at 520 nm is proportional to the concentration of magnesium in the sample.
Xylidyl blue + Mg++ → Mg++Xylidyl Blue complex

3.8.2.2 Method procedure

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<tr>
<td>Sample</td>
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<td>3.0 µl</td>
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</table>

3.8.2.3 Calculation

Serum, plasma

\[
\text{Magnesium, mg/dl} = \frac{\text{Abs. Sample}}{\text{Abs. Standard}} \times 2.43
\]

Normal range of Mg

- Women 1.9-2.5 mg/dl
- Men 1.8-2.6 mg/dl

3.8.3 Iron determination The kit was composed of

Reagent A: Composed of the following:
- Acetate buffer, pH 4.7 0.2 mol/l
- CTMA bromide 0.7 mmol/l
- Cromazurol B 2 mmol/l

Standard: Iron 200µg/dl

3.8.3.1 Principle of the method

Fe++ reacts with Cromazurol B yielding at room temperature a coloured complex whose intensity is proportional to the Iron concentration present in the Sample. This complex is measured at 630 n.m
### 3.8.3.2 Method Procedure

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<td>Reagent A</td>
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<tr>
<td>D.D.W</td>
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<td>Sample</td>
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<td>10.0 µl</td>
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### 3.8.3.3 Calculation

\[
\text{Iron, } \mu\text{g/dl} = \frac{\text{Abs. Sample}}{\text{Abs. Standard}} \times 200
\]

Normal range of iron

- Men: 59 - 158 µg/dl
- Women: 37 - 145 µg/dl

### 3.8.4 Copper determination

The kit was composed of

**Reagent A**: Composed of the following

- Acetate buffer, pH 4.9, 100 mmol/l
- Reducing agents and preservatives

Reagent A at temperature < 18.0 °C forms a particulate. In this case dissolve it warming the reagent at around 25.0 °C for 5 minutes.

**Reagent B**: 3, 5 Di-Br-PAESA, 0.02 g/l

Preservatives

**Standard**: Copper Sulphate, 200 µg/dl as Cu++ ion

### 3.8.4.1 Principle of the method

Copper (Cu++) reacts with the chromogen Di-Br-PAESA at room temperature yielding a colored complex which intensity is proportional to the Copper concentration present in the sample. The method does not require serum deproteinisation either sample blank. This complex is read at 580 n.m.
### 3.8.4.2 Method Procedure

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<td>250µl</td>
<td>250µl</td>
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<tr>
<td>D.D.W</td>
<td>11.0.0 µl</td>
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<tr>
<td>Standard</td>
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<td>11.0.0 µl</td>
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<tr>
<td>Sample</td>
<td>-</td>
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<td>11.0 µl</td>
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### 3.8.4.3 Calculation

\[
\text{Copper (µg/dl)} = \frac{\text{Abs. Sample}}{\text{Abs. Standard}} \times 200
\]

**Normal range of copper**
- **Men:** 80 - 140 µg/dl
- **Women:** 80 - 155 µg/dl

### 3.8.5 Zinc determination

The kit was composed of

**Reagent A:** Composed of the following:

- Borate buffer pH 8.2 370 mmol/l
- Salicylaldoxime 12.5 mmol/l
- Dimethylgluoxime 1.25 mmol/l
- Surfactants and preservatives

**Reagent B:** NITRO-PAPS 0.4 mmol/l

**Preservatives**

**Standard:** Nitrate zinc 200 µg/dl as Zn++ ion

### 3.8.5.1 Principle of the method

Zn++ reacts with NITRO-PAPS yielding at room temperature a colored complex which intensity is proportional to the Zinc concentration present in the sample. The method does not require sample deproteinization either sample blank. This complex is measured at 578 nm.
3.8.5.2 Method Procedure

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<tr>
<td>Reagent A</td>
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<td>250µl</td>
<td>250µl</td>
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<tr>
<td>D.D.W</td>
<td>13.00 µl</td>
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<tr>
<td>Standard</td>
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<td>13.00 µl</td>
<td>-</td>
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<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>13.0 µl</td>
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</tbody>
</table>

3.8.5.3 Calculation

\[
\text{Zinc, } \mu g/dl = \frac{\text{Abs. Sample}}{\text{Abs. Standard}} \times 200
\]

Normal range of Zn : Adult 70-115 µg/dl

3.8.6 Amylin EIA Kit

Two enzyme immune assay (EIA) kits were used to measure total human amylin in either plasma or serum, but serum was used in the procedure to prevent any cross reactivity with any undesired material.

The kits were from Peninsula laboratories member of Bachem group, USA Cat. No. S-1420.

3.8.6.1 Kit Principle

The principle of the kit is based on EIA method in which the antiserum is captured by antibodies coated on a 96-well plate. A constant concentration of Bt-tracer (biotinylated tracer) and varying concentrations of unlabeled standard or sample peptide compete for binding specifically to the antiserum. Captured Bt-tracer is subsequently bound by SA-HRP (streptavidin-conjugated horseradish peroxidase), which produces a soluble colored after a substrate is added.
3.8.6.2 Kit procedure

The procedure of the test was followed exactly as the manufacturer instructions, as the following steps:

a. Into each well of the immunoplate 25 µl antiserum (in EIA buffer) was added, beside 25 µl EIA buffer to blank wells.

b. The plate was incubated at room temperature for 1 hour.

c. Into each well 50 µl of standard or sample (in diluents) were added, then 50 µl of diluents was added to blank wells.

d. The plate was incubated at room temperature for 2 hours. Shorter preincubations may result in lower sensitivity.

e. Bt-tracer (in EIA buffer) was rehydrated then 25 µl/well was added.

f. The plate was incubated at 4°C overnight. For best results re-equilibrate to RT before proceeding

g. The plate was washed 5 times with 300 µl/well of EIA buffer.

h. Into each well 100 µl of streptavidin-HRP (diluted with EIA buffer 1/200) was added.

i. The plate was incubated at room temperature for 1 hour, then washed 5 times

j. Into each well 100 µl/well of TMB solution was added.

The plate was incubated at room temperature (30 - 60 minutes).

k. Terminate reactions by adding 100 µl 2 N HCl per well.

l. The plate was read at 450 n.m
3.9 Normal range of amylin

According to kit's instructions every lab should establish his normal range according to the population features however; it's reported that circulating amylin concentrations in normal healthy adults range from ~4–8 pmol/L in the fasting state and from ~15–25 pmol/L in the fed state [141].

3.10 Data Analysis

Statistical Package for Social Sciences (SPSS) software version 19 was used in summarizing, tabulation and analyzing the data. Several statistical procedures have been used for that purpose such as: Pearson correlation coefficient to find relationship between quantitative variables, frequency and descriptive analysis, parametric tests like independent samples T-test and analysis of variance. The independent samples T-test was used to examine if there is statistical significant difference between two means among the respondents. The One-Way Analysis of Variance (ANOVA) was used to examine if there is statistical significant difference between several means among the respondents. Results were presented in tables and graphs.
Chapter 4

Results

4.1 General characteristics of the study population

The study population of was 133 subjects, 100 of them were patients suffering from T2DM while the other 33 subjects were normal group (don’t suffer from any kind of chronic diseases such as diabetes, hypertension or cardiovascular disease).

4.1.1 Distribution of the population according to sex

The study group was categorized as shown in figure 4.1 according to sex, there were 41 males, represented 41% of the total patient group and 59 females, represented 59% of the total patient group. The control group was distributed according to sex as shown in table 4.1, there were 13 males represented 39.4% of the control group and 20 females represented 60.6% of the same group.

Figure 4.1: Distribution of the study population according to sex
4.1.2 Distribution of the study population according to Age

The mean age for patients was 57.9 years; the minimum age was 34 years while the maximum age was 84 years. The mean age for control group was 39.4 years with a minimum age of 23 years, while the maximum was 84 years.

4.1.3 Distribution of the age match of the study population according to sex

The mean age of the males in age match case group was 44.8 ±9.5 years as shown in table 4.1; the minimum age was 35.0 years while the maximum age was 65.0 years. The mean age of the females in the age match cases was 47.95 ±10.64 years of minimum age 34.0 years and of maximum age of 84.0 years. In the control group males have mean age of 42.38 ±15.0 years with minimum age of 28.0 years and maximum age of 84.0 years and females have mean age of 37.23 ±12.00 years with minimum age of 23 years and a maximum age of 65 years.

Table 4.1: Distribution of the age match study population according to sex

<table>
<thead>
<tr>
<th></th>
<th>Case</th>
<th></th>
<th>Control</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>No.</td>
<td>12</td>
<td>21</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Mean(Year)</td>
<td>44.8 ±9.5</td>
<td>47.95 ±10.6</td>
<td>42.38 ±15.0</td>
<td>37.4 ±12.0</td>
</tr>
<tr>
<td>Minimum(Year)</td>
<td>35.0</td>
<td>34.0</td>
<td>28.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Maximum(Year)</td>
<td>75.0</td>
<td>84.0</td>
<td>84.0</td>
<td>65.0</td>
</tr>
</tbody>
</table>

4.2 Association between F.B.S and family history among case group

Table 4.2 revealed that 75 patients of the case group answered with yes for the question, if there is anyone in the family suffer from DM (positive family history F.H +) which represent 75% of the case group, while 25 patients answered with no for the same question (F.H- ) which represent 25% of the same population, p-value was 0.034.
Table 4.2: Association between F.B.S and family history among the case group

<table>
<thead>
<tr>
<th>Patients</th>
<th>No.</th>
<th>%</th>
<th>Mean mg/dl</th>
<th>S.D mg/dl</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.H (+)</td>
<td>75</td>
<td>75%</td>
<td>186.3</td>
<td>55.5</td>
<td>1.8</td>
<td>0.034*</td>
</tr>
<tr>
<td>F.H (-)</td>
<td>25</td>
<td>25%</td>
<td>163.4</td>
<td>47.6</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

4.3 Association between F.B.S, gender and smoking among the case group

There was no significant statistically relationship between DM and both gender and smoking in the case group, the mean blood sugar for males was 186.4, S.D ±61.6 mg/dl while the mean blood sugar for females 176.5, S.D ±48.7 mg/dl as shown in table 4.3. Table 4.4 shows the relationship between smoking and the level of fasting blood sugar in patients group. In case group 90% of the patients are nonsmokers while, 10% were smokers.

Table 4.3: F.B.S and gender among the case group

<table>
<thead>
<tr>
<th>Sex</th>
<th>No.</th>
<th>%</th>
<th>Mean(mg/dl)</th>
<th>S.D</th>
<th>t value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>41</td>
<td>41%</td>
<td>186.4</td>
<td>±61.6</td>
<td>0.893</td>
<td>0.374</td>
</tr>
<tr>
<td>Female</td>
<td>59</td>
<td>59%</td>
<td>176.56</td>
<td>±48..7</td>
<td>0.856</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4: F.B.S and smoking among the case group

<table>
<thead>
<tr>
<th>Case</th>
<th>Response</th>
<th>No</th>
<th>%</th>
<th>Mean(mg/dl)</th>
<th>S.D</th>
<th>t value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>10</td>
<td>10%</td>
<td></td>
<td>165.9</td>
<td>±46.9</td>
<td>-0.9</td>
<td>0.370</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>90</td>
<td>90%</td>
<td></td>
<td>182.2</td>
<td>±55.0</td>
<td>-1.0</td>
<td></td>
</tr>
</tbody>
</table>

4.4 F.B.S and sport among the case group

Table 4.5 revealed that 74% of the patients answered with no for the question (if anyone is involved in any physical activities), while 26 % of the same group answered with yes for the same question.
Table 4.5: F.B.S and sport among the case group

<table>
<thead>
<tr>
<th>Case</th>
<th>Response</th>
<th>No.</th>
<th>%</th>
<th>Mean(mg/dl)</th>
<th>S.D</th>
<th>t-value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>26</td>
<td>26%</td>
<td>169.7</td>
<td>48.4</td>
<td>-1.91</td>
<td>0.236</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>74</td>
<td>74%</td>
<td>184.4</td>
<td>56.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5 Diabetes and type of treatment in case group

The treatment which was used by the patients group was either tablets, or insulin, or a combination of tablets and insulin, diet and insulin or all of them. The highest percent of patients which represented 42% was under tablets as diabetes drug and diet as nutritional system, while 4% were dependent on insulin. Most of T2DM patients were dependent on oral hypoglycemic agents more than insulin treatment, as shown in Figure 4.2

Figure 4.2: Types of treatments that used by patients
4.6 Association between diet and F. blood sugar among case group

Table 4.6 reveals that 43 patients were committed with special diet while, 57 patients were not. The mean of blood sugar for committed patients was lower than the mean concentration of patients who don't commit with diet (166.9 ±43 Vs. 190.9 ±59.8 mg/dl, *p* =0.028)

**Table 4.6: Association between diet and F. blood sugar in the case group**

<table>
<thead>
<tr>
<th>Type</th>
<th>Response</th>
<th>No.</th>
<th>%</th>
<th>Mean (mg/dl)</th>
<th>S.D mg/dl</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Yes</td>
<td>43</td>
<td>43%</td>
<td>166.9</td>
<td>±43.0</td>
<td>-2.231</td>
<td>0.028*</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>57</td>
<td>57%</td>
<td>190.9</td>
<td>±59.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level

4.7 Association between F.B.S and hypertension among case group

Table 4.7 revealed that 60% of diabetic patients were suffering from hypertension while 40% of diabetic patients were not; the mean of blood sugar for hypertensive patients was lower than the mean of blood sugar for normotensive patients ( *p*-value= 0.062).

**Table 4.7: The relationship between diabetes and hypertension**

<table>
<thead>
<tr>
<th>Type</th>
<th>Response</th>
<th>No</th>
<th>%</th>
<th>Mean (mg/dl)</th>
<th>S.D</th>
<th>t-value</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Hypertensive</td>
<td>60</td>
<td>60.0%</td>
<td>172.3</td>
<td>±47.1</td>
<td>-1.889</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>Normotensive</td>
<td>40</td>
<td>40.0%</td>
<td>193.0</td>
<td>±62.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation is significant at *p*≤ 0.05 level
4.8 Distribution of cases according to the complications of diabetes

Figure 4.3 shows that patients who are suffering from diabetic coma were 25% of the cases, with cardiopathy were 22%, nephropathic patients in the same group were 8% and retinopathy, cases represented 49% of the patients. Neuropathy has the highest rate of complications among diabetic cases in which 62% of the cases group were complaining from neuropathic signs.

**Figure 4.3: Distribution of case group according to the complications of diabetes**

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
<th>No</th>
<th>Yes</th>
<th>No</th>
<th>Yes</th>
<th>No</th>
<th>Yes</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropathy</td>
<td>Retinopathy</td>
<td>Nephropathy</td>
<td>Cardiopathy</td>
<td>Diabetic coma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.9 Association between biochemical parameters levels among the studied population according to matched age

Table 4.8 shows the difference between the cases and control group in terms of the mean concentrations of biochemical tests. 33 cases were chosen to match in age 33 controls. As indicated in this table the mean concentration of blood sugar for case group (33 subjects) was 199.6 ±38.3 mg/dl while the mean concentration of control group was 98.7 ± 8.8 mg/dl (p=0.000). The mean concentration for Zn in case group was 90.8 ± 4.6 µg/dl while control group 95.2 ±4.9 µg/dl (p= 0.000), Mg has a mean concentration of 1.9 ±0.11 mg/dl in case group Vs. 2.0 ± 0.14 mg/dl for control group (p= 0.029). In the case group amylin mean concentration was 2.3 ±4.6 ng/dl Vs. 0.5 ±0.7 ng/dl in the control group (p= 0.039). The mean concentration for Cu in case group was 115.1±27.1 µg/dl while the mean concentration in control group was 110.5 ±17.1 µg/dl, finally Fe has a
mean concentration of 91.81 ±8.6 µg/dl in cases and 88.77 ±11.8 µg/dl in the control group (p-value was> 0.05 for both of Cu and Fe).

Table 4.8: Association between biochemical parameters among the age matched studied population

<table>
<thead>
<tr>
<th>Tests</th>
<th>Mean Case</th>
<th>S.D Case</th>
<th>Mean Control</th>
<th>S.D Control</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.B.S (mg/dl)</td>
<td>199.6</td>
<td>±38.3</td>
<td>98.7</td>
<td>±8.8</td>
<td>14.7</td>
<td>0.000*</td>
</tr>
<tr>
<td>Cu (µg/dl)</td>
<td>115.1</td>
<td>±27.1</td>
<td>110.5</td>
<td>±17.1</td>
<td>0.829</td>
<td>0.410</td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>90.8</td>
<td>±4.6</td>
<td>95.2</td>
<td>±4.9</td>
<td>-3.7</td>
<td>0.000*</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>1.9</td>
<td>±0.11</td>
<td>2.0</td>
<td>±0.14</td>
<td>-2.2</td>
<td>0.029*</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>89.3</td>
<td>±7.3</td>
<td>88.7</td>
<td>±11.8</td>
<td>0.25</td>
<td>0.801</td>
</tr>
<tr>
<td>Amylin(ng/ml)</td>
<td>2.3</td>
<td>±4.6</td>
<td>0.5</td>
<td>±0.7</td>
<td>2.1</td>
<td>0.039*</td>
</tr>
</tbody>
</table>

*Correlation is significant at p≤ 0.05 level

4.10 Association between gender and biochemical parameters among the case group

As shown in table 4.9 there is no statistically significant differences between, Cu, Zn, Mg and amylin levels among both males and females in the case group. The mean concentration of Cu in the serum of the males was 107.1 ± 23.6 µg/dl Vs113.7 ± 23.5 µg/dl for females, the mean Zn concentration for males was 91.3 ± 7.5 µg/dl, while the mean concentration of the same element for females was 90.6 ±5.3 µg/dl, Mg has the mean concentration of 1.9 ± 0.14 mg/dl for males and 1.9 ± 0.15 mg/dl for females. Amylin mean concentration was 1.0 ± 1.0 ng/ml for males and 1.3 ± 2.6 ng/ml for females. Only serum iron has a significant difference (p-value 0.001) in the mean concentration for males was 95. ± 8.4 1µg/dl and for females 89.5 ± 8.1 µg/dl.
Table 4.9: Association between gender and biochemical parameters among the case group

<table>
<thead>
<tr>
<th>Test</th>
<th>Sex</th>
<th>Mean</th>
<th>S.D</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (µg/dl)</td>
<td>M.</td>
<td>107.1 ±23.6</td>
<td></td>
<td>-1.37</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>F.</td>
<td>113.7 ±23.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>M.</td>
<td>91.3 ±7.5</td>
<td></td>
<td>0.560</td>
<td>0.576</td>
</tr>
<tr>
<td></td>
<td>F.</td>
<td>90.6 ±5.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>M.</td>
<td>1.9 ±0.14</td>
<td></td>
<td>0.136</td>
<td>0.892</td>
</tr>
<tr>
<td></td>
<td>F.</td>
<td>1.9 ±0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>M.</td>
<td>95.1 ±8.4</td>
<td></td>
<td>3.33</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>F.</td>
<td>89.5 ±8.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylin(ng/ml)</td>
<td>M.</td>
<td>1.0 ±1.0</td>
<td></td>
<td>0.851</td>
<td>0.397</td>
</tr>
<tr>
<td></td>
<td>F.</td>
<td>1.3 ±2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level

4.11 Association between biochemical parameters and age among the case group

Patients and group was categorized into four age groups. Age was strongly correlated with blood sugar, Fe and amylin. The other tests that have been done were not correlated with age. Table 4.10 shows the mean concentration of every test in the different age groups, and the significant statistical value for every test.

Table 4.10: Association between the studied biochemical parameters and age among the case group

<table>
<thead>
<tr>
<th>Tests</th>
<th>Means/ age group</th>
<th>Test value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 40</td>
<td>40 – &lt;50</td>
<td>50-&lt; 60</td>
</tr>
<tr>
<td>F.B.S(mg/dl)</td>
<td>188.17</td>
<td>198.11</td>
<td>195.79</td>
</tr>
<tr>
<td>Cu(µg/dl)</td>
<td>122.83</td>
<td>103.84</td>
<td>111.00</td>
</tr>
<tr>
<td>Zn(µg/dl)</td>
<td>92.33</td>
<td>89.95</td>
<td>90.58</td>
</tr>
<tr>
<td>Mg(mg/dl)</td>
<td>2.03</td>
<td>1.90</td>
<td>1.93</td>
</tr>
<tr>
<td>Fe(µg/dl)</td>
<td>90.50</td>
<td>89.05</td>
<td>96.18</td>
</tr>
<tr>
<td>Amylin(ng/ml)</td>
<td>4.35</td>
<td>0.96</td>
<td>1.09</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level
4.12 Correlation of F.B.S with selected trace elements and amylin among the case group

Table 4.11 revealed that there was a significant statistical correlation between the level of blood sugar and Zn level in patients group, in contrast Mg, Cu, Fe and amylin in the same population were not significantly correlated to the level of blood sugar.

**Table 4.11: Correlation of F. blood sugar with selected trace elements and amylin among the case group**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Correlation Coefficient</th>
<th>P-Value (Sig.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>-0.160</td>
<td>0.112</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.271</td>
<td>0.006*</td>
</tr>
<tr>
<td>Mg</td>
<td>-0.145</td>
<td>0.151</td>
</tr>
<tr>
<td>Fe</td>
<td>0.089</td>
<td>0.378</td>
</tr>
<tr>
<td>Amylin</td>
<td>0.151</td>
<td>0.134</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level

4.13 Association between treatment and the studied biochemical parameters among the case group

Table 4.12 shows the correlation of treatment and each of F.B.S, Cu, Zn, Mg, Fe and amylin levels in the case group. None of the tests that have been done were correlated to the type of treatment which was used by the patients.

**Table 4.12: Association between treatment and the studied biochemical parameters among the case group**

<table>
<thead>
<tr>
<th>Tests</th>
<th>t-value</th>
<th>P-value(Sig.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.B.S</td>
<td>1.42</td>
<td>0.213</td>
</tr>
<tr>
<td>Cu</td>
<td>1.19</td>
<td>0.314</td>
</tr>
<tr>
<td>Zn</td>
<td>1.13</td>
<td>0.351</td>
</tr>
<tr>
<td>Mg</td>
<td>1.36</td>
<td>0.236</td>
</tr>
<tr>
<td>Fe</td>
<td>0.296</td>
<td>0.937</td>
</tr>
<tr>
<td>Amylin</td>
<td>0.556</td>
<td>0.764</td>
</tr>
</tbody>
</table>

Table 4.13 shows the mean concentration for every test according to the type of treatment which is used by diabetic patients in the case group.
Table 4.13: Mean concentration of each test according to treatment used by the patients

<table>
<thead>
<tr>
<th>Tests</th>
<th>Tablet</th>
<th>Insulin</th>
<th>All</th>
<th>Tablet+ Insulin</th>
<th>Diet +Tablet</th>
<th>Insulin+ Diet</th>
<th>Nothing</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.B.S (mg/dl)</td>
<td>179.09</td>
<td>218.50</td>
<td>196.53</td>
<td>169.60</td>
<td>175.50</td>
<td>144.33</td>
<td>103.12</td>
</tr>
<tr>
<td>Cu (µg/dl)</td>
<td>111.78</td>
<td>114.00</td>
<td>108.11</td>
<td>136.40</td>
<td>108.69</td>
<td>113.83</td>
<td>110.03</td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>90.13</td>
<td>87.50</td>
<td>90.00</td>
<td>93.40</td>
<td>91.17</td>
<td>95.67</td>
<td>94.99</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>1.92</td>
<td>2.00</td>
<td>1.90</td>
<td>2.00</td>
<td>1.94</td>
<td>2.03</td>
<td>2.02</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>90.52</td>
<td>91.25</td>
<td>93.74</td>
<td>92.00</td>
<td>91.95</td>
<td>89.67</td>
<td>88.90</td>
</tr>
<tr>
<td>Amylin (ng/ml)</td>
<td>1.16</td>
<td>0.93</td>
<td>2.04</td>
<td>1.14</td>
<td>0.97</td>
<td>1.10</td>
<td>0.60</td>
</tr>
</tbody>
</table>

4.14 Association between smoking and the studied biochemical tests among the case group

As shown in table 4.14 smoking has no significant effect on the level of all of the blood sugar, Cu, Zn, Fe and amylin. There was no statistical significant relationship for all of them. Only Mg has a significant positive correlation with smoking where the mean of smoker patients was 2.0 mg/dl ±0.15 vs. 1.9 mg/dl ±0.14 for non-smokers.

Table 4.14: Association between smoking and the studied biochemical tests among the case group

<table>
<thead>
<tr>
<th>Tests</th>
<th>Response</th>
<th>Mean</th>
<th>S.D</th>
<th>t-value</th>
<th>P-value(Sig.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.B.S (mg/dl)</td>
<td>Yes</td>
<td>165.9</td>
<td>±46.9</td>
<td>-0.900</td>
<td>0.370</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>182</td>
<td>±55.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu (µg/dl)</td>
<td>Yes</td>
<td>104.7</td>
<td>±24.6</td>
<td>-0.892</td>
<td>0.375</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1117</td>
<td>±23.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>Yes</td>
<td>90.97</td>
<td>±4.4</td>
<td>0.005</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>90.87</td>
<td>±6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>Yes</td>
<td>2.0</td>
<td>±0.15</td>
<td>2.137</td>
<td>0.035*</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1.9</td>
<td>±0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>Yes</td>
<td>92.77</td>
<td>±4.7</td>
<td>0.340</td>
<td>0.735</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>91.77</td>
<td>±9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylin (ng/ml)</td>
<td>Yes</td>
<td>1.3</td>
<td>±1.5</td>
<td>0.164</td>
<td>0.870</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1.2</td>
<td>±2.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level
4.15 Association between hypertension and the tested biochemical parameters among the case group

As indicated in table 4.15 there is no significant association between hypertension and the tested parameters in the case group where the p-value was greater than 0.05.

Also shows that the mean of blood sugar concentration of hypertensive patients in the diabetic group was less than the mean concentration of normotensive diabetic patients. The other tests which have been done also were not associated with hypertension.

Table 4.15: Association between hypertension and the tested biochemical parameters among the case group

<table>
<thead>
<tr>
<th>Tests</th>
<th>Means</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypertensive</td>
<td>S.D</td>
<td>Normotensive</td>
</tr>
<tr>
<td>F.B.S (mg/dl)</td>
<td>172.3 ± 47.1</td>
<td>193.0</td>
<td>±62.2</td>
</tr>
<tr>
<td>Cu (µg/ml)</td>
<td>111.4 ±24.3</td>
<td>110.5</td>
<td>±24.9</td>
</tr>
<tr>
<td>Zn (µg/ml)</td>
<td>91.5 ±7.2</td>
<td>90.0</td>
<td>±4.6</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>1.9 ±0.15</td>
<td>1.9</td>
<td>±0.14</td>
</tr>
<tr>
<td>Fe (µg/ml)</td>
<td>91.6 ±9.4</td>
<td>92.1</td>
<td>±7.5</td>
</tr>
<tr>
<td>Amylin (ng/ml)</td>
<td>1.0 ±0.94</td>
<td>1.4</td>
<td>±3.2</td>
</tr>
</tbody>
</table>

4.16 Association between diabetic coma and the level of biochemical parameters among the case group

Table 4.16 reveals that there is a significant correlation between the onset of diabetic coma and the level of blood sugar in the patients who answered with yes for suffering from diabetic coma the p-value was less than 0.05. The mean concentration of F.B.S in the affected patients with diabetic coma is 199.5 ±65.3 mg/dl while the mean concentration of F.B.S in the people who don't suffer from diabetic coma is 174.3 ±49.1 mg/dl . The mean concentration equal to 103.3 ± 19.9 µg/dl, while the mean concentration of Cu in the patients who don't suffer from diabetic coma is 113.6 ±24.4 µg/dl. Also there is a significant correlation between
complaining from diabetic coma and the level of Zn, where the mean concentration of zinc in diabetic patients who are complaining from diabetic coma is 88.0 ±5.1 µg/dl Vs. 91.8 ±6.4 µg/dl for patients who don't suffer from such diabetic complication. Magnesium, Fe and amylin were not correlated to diabetic coma where the p-value was > 0.05.

Table 4.16: Association between diabetic coma and the level of biochemical parameters among the case group

<table>
<thead>
<tr>
<th>Tests</th>
<th>Means</th>
<th>Test value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>S.D</td>
<td>No</td>
</tr>
<tr>
<td>F.B.S (mg/dl)</td>
<td>199.5 ±65.3</td>
<td>174.3 ±49.1</td>
<td>2.039</td>
</tr>
<tr>
<td>Cu (µg/dl)</td>
<td>103.32 ±19.9</td>
<td>113.67 ±24.4</td>
<td>- 1.9</td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>88.08 ±5.1</td>
<td>91.82 ±6.4</td>
<td>- 2.63</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>1.92 ±0.15</td>
<td>1.93 ±0.14</td>
<td>- 0.445</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>91.4 ±7.7</td>
<td>91.9 ±9.0</td>
<td>- 0.245</td>
</tr>
<tr>
<td>Amylin (ng/ml)</td>
<td>1.3 ±1.4</td>
<td>1.1 ±2.3</td>
<td>0.317</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level

4.17 Association between cardiopathy and the level of biochemical parameters among the case group

As shown in table 4.17 there is no significant difference between the suffering of the subjects from any signs of cardiopathy and the levels of the biochemical parameters since the p-value was greater than 0.05.
Table 4.17: Association between cardiopathy and the level of biochemical parameters among the case group

<table>
<thead>
<tr>
<th>Tests</th>
<th>Means</th>
<th>Test value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>S.D</td>
<td>No</td>
</tr>
<tr>
<td>F.B.S (mg/dl)</td>
<td>186.8</td>
<td>±62.5</td>
<td>178.8</td>
</tr>
<tr>
<td>Cu (µg/dl)</td>
<td>107.6</td>
<td>±18.7</td>
<td>112.0</td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>90.1</td>
<td>±5.1</td>
<td>91.1</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>1.92</td>
<td>±0.13</td>
<td>1.93</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>88.8</td>
<td>±7.7</td>
<td>92.6</td>
</tr>
<tr>
<td>Amylin (ng/ml)</td>
<td>1.16</td>
<td>±1.04</td>
<td>1.25</td>
</tr>
</tbody>
</table>

4.18 Association between nephropathy and the level of biochemical parameters among the case group

As shown in table 4.18 there is no significant difference between patients suffering from nephropathy and the level of blood sugar, Cu, Zn, Mg, Fe and amylin since the p-value in all of them were greater than 0.05.

Table 4.18: Association between nephropathy and the level of biochemical parameters among the case group

<table>
<thead>
<tr>
<th>Tests</th>
<th>Means</th>
<th>Test value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>S.D</td>
<td>No</td>
</tr>
<tr>
<td>F.B.S (mg/dl)</td>
<td>199.6</td>
<td>±81.0</td>
<td>178.9</td>
</tr>
<tr>
<td>Cu (µg/dl)</td>
<td>110.3</td>
<td>±22.1</td>
<td>111.1</td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>88.7</td>
<td>±5.1</td>
<td>91.0</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>1.92</td>
<td>±0.12</td>
<td>1.93</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>89.2</td>
<td>±3.9</td>
<td>92.03</td>
</tr>
<tr>
<td>Amylin (ng/ml)</td>
<td>1.35</td>
<td>±1.41</td>
<td>1.22</td>
</tr>
</tbody>
</table>
4.19 Association between retinopathy and the level of biochemical parameters among the case group

There is no significant difference found between complaining from retinopathy and the level of blood sugar, Cu, Zn, Mg, Fe and amylin in patients, where the *p*-value was greater than 0.05 for all of them, as shown in table 4.19

<table>
<thead>
<tr>
<th>Tests</th>
<th>Means</th>
<th>Test value</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>S.D</td>
<td>No</td>
</tr>
<tr>
<td>F.B.S (mg/dl)</td>
<td>183.4</td>
<td>±60.5</td>
<td>177.8</td>
</tr>
<tr>
<td>Cu (µg/dl)</td>
<td>110.6</td>
<td>±25.2</td>
<td>111.4</td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>90.0</td>
<td>±6.8</td>
<td>91.0</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>1.92</td>
<td>±0.14</td>
<td>1.94</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>90.6</td>
<td>±8.9</td>
<td>92.9</td>
</tr>
<tr>
<td>Amylin (ng/ml)</td>
<td>1.0</td>
<td>±0.86</td>
<td>1.4</td>
</tr>
</tbody>
</table>

4.20 Association between neuropathy and the level of biochemical parameters among the case group

Table 4.20 which show the correlation between neuropathy and tested parameters. There is a significant difference between suffering from neuropathy as a complication of diabetes and the level of Zn where the *p*-value was less than 0.05. However Cu shows a correlation with *p*-value 0.06 and other tests such as (Mg, Fe, and amylin) didn't show any significant difference.
Table 4.20 Association between neuropathy and the level of biochemical parameters among the case group

<table>
<thead>
<tr>
<th>Tests</th>
<th>Means</th>
<th>Test value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>S.D</td>
<td>No</td>
</tr>
<tr>
<td>F.B.S (mg/dl)</td>
<td>184.6 ± 56.2</td>
<td>174.0 ± 51.1</td>
<td>0.949</td>
</tr>
<tr>
<td>Cu (µg/dl)</td>
<td>107.6 ± 22.7</td>
<td>116.6 ± 24.4</td>
<td>-1.85</td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>89.3 ± 4.9</td>
<td>93.3 ± 7.4</td>
<td>-3.24</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>1.91 ± 0.15</td>
<td>1.96 ± 0.14</td>
<td>-1.39</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>92.0 ± 9.4</td>
<td>91.4 ± 7.3</td>
<td>0.302</td>
</tr>
<tr>
<td>Amylin (ng/ml)</td>
<td>1.19 ± 1.18</td>
<td>1.3 ± 3.18</td>
<td>-0.242</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level

4.21 The correlation between the tested biochemical parameters

The table below 4.21 shows a significant negative correlation between blood sugar and Zn where the p-value was <0.05 and a significant positive correlation between Cu and both of Zn and Mg (p-value = 0.026, 0.000 respectively). Iron and amylin were not correlated to any of the tests.
### Table 4.21 Correlation between tested biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>bloodsugar</th>
<th>Cu</th>
<th>Zn</th>
<th>Mg</th>
<th>Fe</th>
<th>Amy lin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Pearson Correlation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bloodsugar</td>
<td>1</td>
<td>-.160</td>
<td>-.271**</td>
<td>-.145</td>
<td>.089</td>
<td>.151</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.112</td>
<td>.006</td>
<td>.151</td>
<td>.378</td>
<td>.134</td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cu</td>
<td>-.160</td>
<td>1</td>
<td>.222*</td>
<td>.245*</td>
<td>-.055</td>
<td>.045</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.112</td>
<td>.026</td>
<td>.014</td>
<td>.588</td>
<td>.655</td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Zn</td>
<td>-.271**</td>
<td>.222*</td>
<td>1</td>
<td>.363**</td>
<td>.185</td>
<td>-.029</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.006</td>
<td>.026</td>
<td>.000</td>
<td>.066</td>
<td>.776</td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mg</td>
<td>-.145</td>
<td>.245*</td>
<td>.363**</td>
<td>1</td>
<td>.014</td>
<td>.169</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.151</td>
<td>.014</td>
<td>.000</td>
<td>.893</td>
<td>.094</td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fe</td>
<td>.089</td>
<td>-.055</td>
<td>.185</td>
<td>.014</td>
<td>1</td>
<td>-.031</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.378</td>
<td>.588</td>
<td>.066</td>
<td>.893</td>
<td>.759</td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Amy lin</td>
<td>.151</td>
<td>.045</td>
<td>-.029</td>
<td>.169</td>
<td>-.031</td>
<td>1</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.134</td>
<td>.655</td>
<td>.776</td>
<td>.094</td>
<td>.759</td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).**

* Correlation is significant at the 0.05 level (2-tailed).
Chapter 5
Discussion

Diabetes mellitus is one of the most common metabolic diseases worldwide, characterized by inappropriately elevated glucose levels in the blood [25]. The concentration of several trace elements have been reported to be altered in diabetes mellitus and these elements might have specific roles in the pathogenesis and progress of this disease [3]. Amylin is a 37-amino acid and is produced from the β islet cells of the pancreas [13]. Amyloid deposits have been shown to be toxic to β cells and may have a progressive role in the onset of T2DM [13].

This study was focused on the hypothesis that T2DM patients have an impairment level of trace elements and high level of islet amyloid which could be related to T2DM complications; which considered as the first to be conducted in Gaza city. Different factors were investigated and analyzed such as the general characteristics of the population in term of age, sex, duration of the disease, some habits like smoking, sport, diseases such as hypertension and any other complications due to diabetes. Blood biochemical analysis, such as F.B.S, Cu++, Zn++, Fe++, Mg++ and amylin were measured for both cases and control groups.

5.1 Diabetes, gender and age

In this study no significant relationship was found between diabetes and gender where the p-value was 0.374 and the mean blood sugar of males was higher than females, however 59% of the patients were females and 41% males which may indicate that females are more susceptible to diabetes more than males. Type 2 diabetes showed a pronounced female excess in the first half of the last century but is now equally prevalent among men and women in most populations [142]. Also this finding may be is due to the fact that females in our society are more susceptible to sedentary and obesity than males. Although it's reported that the mortality between females suffered from diabetes is higher than males in the palestinian society, from 85 dead cases in 2009 there was 45.9% males and 54.1% females [143].
The mean age at diagnosis for patients was 46.8 years ±9.3 while the mean age for diabetic female is 47.95 ±10.6 year and 44.8 ± 9.5 year for males, it is reported that the onset of diabetes is usually after age 40 years [144]. Also contrary to T1DM, T2DM clearly is an age- related disease, with prevalence in the population increasing with age [15]. On contrary in another study age, sex, duration and control of diabetes did not influence copper, zinc, or magnesium concentrations among T2DM patients [11].

5.2 Family history and diabetes mellitus

There was a significant relationship between family history of the studied patients and diabetes where the p-value was 0.034. The mean blood sugar of the patients who have positive family history with diabetes was higher than patients who did not had family history. This result is argued with previous study which found that family history is not correlated to diabetes [145]. However it was reported that in the U.S. population, family history of diabetes has a significant, independent, and graded association with the prevalence of diabetes. This association not only highlights the importance of shared genes and environment in diabetes but also opens the possibility of formally adding family history to public health strategies aimed at detecting and preventing the disease [146]. The presence of a family history of diabetes resulted to an early onset of the disease to the offspring [147].

5.3 Diet and diabetes mellitus

There was a significant correlation between diabetes and diet, where the p-value was 0.028. The mean of blood sugar in the patients committed with diet was lower than that people who don’t commit with diet (166.9 mg/dl ±43.0 Vs. 190.9 mg/dl ±59.8). This in agreement with studies which had indicated that diets with high saturated fat content and low fiber content may increase the risk of insulin resistance and lead to development of type 2 diabetes, however several clinical studies showed a decrease in insulin sensitivity with high fat diets [148].

5.4 Smoking and diabetes mellitus

The majority of the case group were nonsmokers, only 10% of them were smokers, therefore the relationship between smoking and diabetes was not statistically significant (p-value 0.370) however several researchers suggested a significant relationship between smoking and diabetes, since smoking is considered
as an enhancer for oxidative stress and a leading reason for hypertension, there is also substantial evidence that endothelium-dependent vasodilatation is impaired in smokers, type II diabetics, and in subjects with essential hypertension [149]. However, smoking has been shown to cause elevations in blood glucose concentration and may increase insulin resistance [150].

Current smokers also tend to have higher blood concentrations of glycosylated hemoglobin (HbA1c) than do nonsmokers [151]. One recent population-based cross-sectional study showed an independent positive association between cigarette smoking and HbA1c concentration in men and women [151]. Among 1,266 Japanese men from 35 to 59 years of age, the number of cigarettes smoked daily and pack-year history of smoking were positively associated with development of impaired fasting glucose and type 2 diabetes mellitus [152]. Not every study shows this association [153], but overall, it's suggested that cigarette smoking may be an independent modifiable risk factor for development of diabetes [148]. Mg shown significant statistical difference with smoking since the p-value was less than 0.05.

5.5 Magnesium and smoking

Magnesium is significantly correlated with smoking among the case group with p-value 0.035. However, the mean level of magnesium in diabetic smokers was slightly higher than the mean concentration of magnesium in diabetic nonsmokers. This result in argue with different studies based on the relationship between smoking and Mg level. One study mentioned that tobacco smokers had a decreased content of Ca, Mg, Fe, Zn and copper [154]. Other researchers found that smoking didn't significantly influence the level of magnesium where smoking considers as a risk factor for diabetes and cardiomyopathy [155]. However, Magnesium is an essential ion involved in glucose homeostasis at multiple levels [11]. Hypomagnesaemia has been reported in both T1DM and T2DM patients [9]. Mg plays an important role in the activities of various enzymes involved in the glucose oxidation and may play a role in the release of insulin [11] Hypomagnesaemia is common in hospitalized patients, especially in the elderly with coronary artery disease (CAD) and/or those with chronic heart failure [156].
5.6 Sport and diabetes mellitus

Exercise has been well known to benefit patients with (T2DM), and is a cornerstone of diabetes management [157]. Several long-term studies have demonstrated a consistent beneficial effect of regular physical activity training on carbohydrate metabolism and insulin sensitivity, which can be maintained for at least 5 years [157]. Since the vast number of the participants in this study (case and control) were not involved in any physical activities like sport or anything else there was no significant correlation between blood sugar level and sport in either in case group or control group or the whole study population.

This may be referred to the absence of sport from our culture or traditions or facilities for such activities and patients awareness of the importance of sport in their disease management. Other studies suggested that adherence to exercise training is low among DM patients [158], which may be due to factors such as time, venue, transportation, weather, equipment, cost, physical capability, priority, and interest [159].

5.7 Diabetes mellitus and hypertension

Hypertension is an extremely common co-morbid condition in diabetes, affecting 20–60% of patients with diabetes, depending on obesity, ethnicity, and age. In T2DM hypertension is often present as part of the metabolic syndrome complication of insulin resistance also, obesity and dyslipidemia [160]. Patients with diabetes have a much higher rate of hypertension than would be expected in the general population. Regardless of the antihypertensive agent used, a reduction in blood pressure helps to prevent diabetic complications [161]. The overlap between hypertension and diabetes substantially increases the risk of ischemic cerebrovascular disease, retinopathy, and sexual dysfunction. Diabetes mellitus is an independent risk factor for coronary artery disease, and the risk is markedly increased when hypertension is present [162]. The association between hypertension, FBS, trace elements and amylin in this study was not significant in the study group since the \( p \)-value > 0.05; the mean of blood sugar for diabetic hypertensive patients was lower than the mean of blood sugar for diabetic normotensive patients.
5.8 Gender and biochemical parameters in case group

There was no significant difference between males and females in case group in term of the level of trace elements where the \( p \)-value was > 0.05 only iron shown a significant difference between males and females (\( p \)-value 0.001), it's reported that reproductive-age women are particularly vulnerable to iron deficiency, reflecting iron loss through menstruation and pregnancy [163].

5.9 Age and biochemical parameters levels

There was significant positive correlation between age and the level of blood sugar, Fe and amylin and \( p \)- value was \((0.010; 0.004; 0.003)\) respectively. Previous studies reported that older adults and weight gain during middle age and after age of 65 years are associated with risk of diabetes [164]. Amylin was found to be increased with age and the islet amyloid polypeptide has been associated with \( \beta \)-cell apoptosis [165]. As a consequence of increasing age, the accumulation of highly modified proteins together with decrease regenerative potential, might lead to increasing rates of apoptosis [165].

5.10 The correlation between biochemical parameters within the study group

As previously mentioned in the results chapter there was a significant difference with statistical value between the case group and the control group in the level of blood sugar where the mean of blood sugar in diabetic group was higher than that in healthy one \((199.6 \pm 38.3 \text{ mg/dl} \text{ Vs. } 98.7 \pm 8.8 \text{ mg/dl}; \text{ } p\text{-value} \text{ 0.000 and } t=14.7)\). This consider as a confirmatory result shows that the selection of the healthy subject was correct against diabetic patients.

5.10.1 Copper (Cu++) and study population

There was no significant statistical difference between healthy group and patients group in Cu concentration \((p\text{-value}= 0.410)\) where the mean concentration of copper in patients was \(115.1 \pm 27.1 \text{ µg/dl}\) while the mean concentration of the same element in control group was \(110.5 \pm 17.17 \text{ µg/dl}\); our results are in agreement with some studies and contradict with others, in one study it's reported that the mean of blood copper level in the diabetic male and female patients were not significantly different from those found in the control male and female subjects [4]. Other study
shown that plasma copper concentration was significantly higher in T2DM patients than non-diabetic subjects [11] and another study found that the mean copper levels of cases showed no significant difference with controls [166].

It's reported that diabetic patients with microalbuminuria have increased urinary copper excretion however, doesn't exclude the potential toxic effects of this high copper excretion on the progression of diabetic nephropathy [167]. Also serum copper levels were increased in non-obese T2DM and T1DM patients significantly [42]. Additional study confirm the same result that plasma Cu was increased in diabetic patients more than healthy individuals, the elevation in Cu is attributed in hyperglycemic patients is due to increase glycation- because of hyperglycemia- and this will stimulate release of copper from copper rich compounds [81]. These results confirm that T2DM have impaired Cu++ metabolism.

### 5.10.2 Zinc (Zn++) and study population

Zinc shown a significant difference between case group and control group (p-value= 0.000) where the mean concentration in the case group was lower than the control group, also there was a negative correlation between Zn and the level of blood sugar (t=-3.7) which means that Zn level is inversely proportional to blood sugar concentration (when blood sugar is high Zn level is low). Our results are in agreement with previous researches who mentioned that Zn level was significantly diminished in diabetic patients as compared to controls [81]. Many other researchers confirm decrease level of Zn in patients with T2DM compare to control group [83].This hypozincemia is attributed to loss of zinc in urine [83]. The loss of zinc in urine due to hyperglycemia in both T1DM and T2DM is caused by interference with active transport of Zn into the renal tubular cells [66]. There is increasing evidence supporting the role of zinc as an antioxidant that could protect insulin and cells from being attacked by free radicals [77]. In obese Brazilian women, 4 weeks of zinc supplementation (30 mg/day) significantly improved insulin sensitivity [78]. In a cross-sectional analysis, higher dietary zinc intake was associated with a lower prevalence of diabetes and metabolic syndrome in an Indian population [70].
5.10.3 Magnesium (Mg++) and study population

Magnesium is a cofactor in the glucose transporting mechanism of the cell membrane and various enzymes in carbohydrate oxidation [168]. The mean serum Mg level was higher in control group than case group, where the $p$-value was statistically significant (0.029) with negative correlation between Mg and blood sugar ($t = -2.2$). This result in agreement with other studies in which Mg level was found to be decreased in patients with diabetes compared with control group and there were no significant alteration in serum zinc level [43 and 3]. It is reported that hypomagnesaemia is frequently present in diabetic patients [169].

Hypomagnesaemia among patients with T2DM presumably multifactorial [3] which is related to insulin metabolism, poor glycemic control and osmotic diuresis [170].

Another study shows that serum Mg level was decreased just in T2DM and there were no significant alteration in serum zinc level [43]. The homeostatic regulation of Mg is increased by the action of parathormone (PTH), calcitonin, vitamin D, glucagon, anti-diuretic hormone, aldosterone and sexual steroids [169]. In a review article discussing the relationship between Mg, diabetes and Mg ingestion dose as prevention supplement it is reported that hypomagnesaemia is frequently present in diabetic patients [169]

5.10.4 Iron (Fe++) and study population

In this study serum iron in cases was not significantly different from control group; the $p$-value was 0.801. It's reported that iron overload is not a prerequisite for iron to mediate either diabetes or its complications. Important in its pathophysiology is the availability of so-called catalytic iron or iron that is available to participate in free radical reactions [12]. Nevertheless a link has been established between increased dietary iron intake, particularly eating red meat and increased body iron stores, and the development of diabetes. A causative link with iron overload is suggested by of the improvement in insulin sensitivity and insulin secretion with frequent blood donation and decreased iron stores [12].

Emerging scientific evidence has disclosed unsuspected influences between iron metabolism and T2DM. The relationship is bi-directional—iron affects glucose
metabolism, and glucose metabolism impinges on several iron metabolic pathways. Oxidative stress and inflammatory cytokines influence these relationships, amplifying and potentiating the initiated events [87]. In a study discussed bloodletting in high ferritine patients with T2DM suggested that bloodletting might contribute as an adjuvant treatment in patients who have T2DM with increased serum ferritin concentrations [88].

5.10.5 Amylin (IAPP) and study population

Amylin and islet amyloid polypeptide (IAPP) are currently interchangeable terms for the 37 amino acid. Amylin is co-synthesized, co-packaged within the Golgi apparatus, and co-secreted within the secretory granule by the islet beta cell in response to elevations of plasma glucose, it leaves an indelible footprint in greater than 70% of the patients with T2DM [171]. In T2DM, the levels of amylin are raised in parallel with the increased demand for insulin, and this is thought to induce concentration-dependent amylin aggregation [15]. Islet amyloid formation is associated with reduced β-cell mass [172] and human amylin ‘oligomers’ (small, soluble aggregates) are toxic to cultured islet cells [173], suggesting that they could contribute to progressive islet β-cell failure. Amylin oligomers can disrupt membranes [174] and inflict oxidative damage to cells [175]. In this study amylin was measured and compared in both cases and control, there was significant statistical difference between case group and control group where the p-value was 0.039 with a positive correlation between glucose and amylin (t= 2.1) this mean that amylin concentration increase whenever blood glucose increased. It's reported that amylin levels are elevated in the T2DM patient, the insulin resistant obese patient, and the patient with impaired glucose tolerance [176]. Researchers mentioned that islet amyloid deposits are found in >90% of patients with T2DM at autopsy [121]. High concentrations of IAPP have been shown to inhibit insulin secretion [177], and action [178], while lower concentrations of IAPP are found to induce a modest increase in basal insulin secretion [179]
5.11 Type 2 diabetes and its complications

5.11.1 Diabetic coma

Hyperosmolar hyperglycemic state (HHS) represents one of the two most serious acute metabolic complications of diabetes mellitus and is a life-threatening emergency. HHS is the end result of a sustained osmotic diuresis, and is characterized by severe hyperglycemia, hyperosmolarity, and dehydration, but without significant ketoacidosis. Less common than the other critical hyperglycemic diabetic emergency, diabetic ketoacidosis (DKA), HHS carries a higher mortality rate, associated with serious concurrent illness. It is usually seen in older age type 2 diabetics, but can present at any age and in patients with type 1 diabetes mellitus [180].

In this study, there were 25 out of 100 diabetic patients suffered from diabetic coma, the mean age for patients with diabetic coma was 59.3 years ±10.7; it's reported that the mean age of patients with HHS in U.S.A is 60 year [180]. In 30% to 40% of cases, HHS is the initial presentation of a patient’s diabetes [181].

There was a positive significant relationship between blood sugar concentration and diabetic coma where the $p$-value is 0.04 and $t=3.03$ this means that the relationship between blood sugar and having a diabetic coma is directly proportional. The mean concentration of blood sugar for patients who had diabetic coma was higher than patients who didn't have. It's reported that patients with diabetic coma may have blood sugar > 600 mg/dl [181, 180].

Also Zn shown a significant relationship with involvement with diabetic coma, ( the $p$-value was 0.005 and $t=-2.63$) The mean concentration of Zn in people with diabetic coma was less than patients who didn't suffer from coma. It's known that people who have low concentration of Zn have higher blood glucose more than people who have normal or higher level of Zn. Therefore low Zn level correlate with increase blood sugar level and diabetic coma more than patients with normal level of Zn [3, 11 and 182].

Copper was not associated with diabetic coma but the $p$-value was 0.059 and $t=-1.9$, the mean concentration of Cu in patients who suffer from diabetic coma
where lower than who don't, which is argue with other studies reported that copper increased in T2DM [82], and Cu overload may be a significant causative factor for diabetic complications [92]. Magnesium, Fe and amylin were not significantly correlated to diabetic coma where the $p$-value $>0.05$.

### 5.11.2 Cardiomyopathy and the level of biochemical tests among the case group

Twenty two patients of the case group have had different cardiac problems such as ischemic heart disease, angina pectoris and myocardial infarction. In this study there was no significant statistical difference between suffering from any kind of cardiomyopathy and the level of biochemical tests which have been done, where the ($p$-value $>0.05$) for all tests. However, it's reported that CHD is the major cause of death in T2DM, and its risk is two- to four higher among patients with T2DM than normal population [80].

T2DM is associated with an increased risk of stroke, silent infarctions; a higher prevalence of stroke is found in patients with both diagnosed and undiagnosed diabetes and glucose intolerance [183]. Untreated type 2 diabetes and type 2 diabetes with complications are associated with subcortical infarctions [184]. Also it's mentioned that Diabetic cardiomyopathy can occur clinically without major vascular disease, suggesting a primary role for direct effects of diabetes on cardiac myocytes [185]. Also it's reported that Zn has a critical antioxidant action in protecting the heart from various oxidative stresses [186].

### 5.11.3 Nephropathy and the level of biochemical tests among the case group

Only eight patient of the case group have had different signs of problems with their kidneys such as impairment in the kidney function. The results of this study argue with other studies where, there was no statistically significant correlation between any signs of nephropathy and the level of blood sugar, Cu, Zn, Mg, Fe and amylin. (the $p$-value for all of them was greater than 0.05).

Previous study mentioned that one third of T2DM patients requiring renal replacement therapy in western countries [187]. However, in a previous study it's
concluded that the mean magnesium levels of cases were significantly lower than controls. But the mean copper levels of cases, shows no significant difference with controls. The findings in that study suggested that hypomagnesaemia may be linked with development of diabetic nephropathy [188].

Diabetic patients with microalbuminuria have increased urinary copper excretion, which doesn't exclude the potential toxic effects of this high copper excretion on the progression of diabetic nephropathy [189].

5.11.4 Retinopathy and the level of biochemical tests in the case group

Diabetic retinopathy (DR) can be defined as a damage to microvascular system in the retina due to prolonged hyperglycemia [190]. Increased Cu content of the lens presumably has a greater association with the development of lens opacification in diabetics than Zn and Fe content [191]. Oxidative stress has been widely regarded as the key factor for the emergence of ocular disease and has been involved in increased vascular permeability, disruption of blood-retinal barrier, apoptotic loss of retinal capillary cells, microvascular abnormalities and retinal neovascularization [192].

In this study 49% of cases were found to have diabetic retinopathy. Most of them were under laser intervention and have an annual visit for optic hospital. In the present study there is no significant statistical differences between retinopathy as a complication of diabetes and the level of blood sugar, Zn and Mg were the $p$-value was $>$0.05.

5.11.5 Neuropathy and the level of biochemical tests among the case group

Peripheral neuropathy (PN) characterized by pain, numbness, and tingling in the extremities and slow nerve conduction – affects significant percentage of the world population and can be extremely debilitating [193]. In the present study a significant statistical difference was found between the level of Zn and patients with neuropathy,($p$-value = 0.002) . It’s reported that the incidence of PN is significantly high in the subset with diabetes which reached (62%) [193] and in this study, 62% of the diabetic cases were suffering from one or more symptom of neuropathy.
Moreover, prolonged hyperglycemia results in the complications associated with diabetes, including neuropathy. A recent study found that PN can be manifested even in individuals with abnormal glucose tolerance and a pre-diabetic condition [165]. Significant improvements in fasting and postprandial blood sugar were found by Zn supplements compared with diabetics taking placebo [194]. Other study reported that 6 weeks of Zn supplementation (660 mg/d) to diabetic patients was shown to significantly reduce the severity of peripheral neuropathy as assessed by motor nerve conduction velocity [85].

Magnesium was not significantly correlated to PN (p-value was greater than 0.05). Magnesium deficiency has been proposed as a factor in the pathogenesis of diabetes-related complications, including neuropathy [195]. Magnesium plays a role in the pathophysiology of diabetic neuropathy [59]. In other study among T2DM, zinc, magnesium and selenium showed high significant decrease (P < 0.01) in both uncomplicated and complicated diabetic groups than the control group [196]. However, in this study Copper, Iron and amylin were not correlated with PN (p-value was > 0.05). Copper has no statistical significant differences in its serum level among the complicated diabetic, uncomplicated and control group [196].

5.12 Blood sugar and Zinc

In this study there is a negative significant correlation between the level of zinc and blood sugar in patients group (p-value was 0.006). Researches demonstrated that Diabetes causes a significant systemic oxidative stress and also often is accompanied by Zn deficiency [186]. So Zn deficiency may cause the onset of diabetes in part [92]. Diabetes caused significant decrease in hepatic Cu level, and Zn supplementation [185]. There is a modest inverse association between zinc intake and risk of type 2 diabetes in U.S. women after adjustment of established and potential confounders. In addition, a higher zinc–to–heme iron ratio was associated with a significantly lower risk of type 2 diabetes [77].
5.13 Copper, Zinc and Magnesium among the case group

In this study a positive significant correlation was found between Cu and Zn \((p\text{-value}= 0.026)\), Cu and Mg \((p\text{-value} = 0.014)\) and Zn and Mg were in a positive correlation with each other \((p\text{-value} = 0.000)\). These results are in agreement with previous studies which mentioned that Cu, Zn and Mg are decreased in the majority of cases of T2DM \([4, 11, \text{ and } 9]\).
Chapter 6
CONCLUSIONS AND RECOMENDATIONS

6.1 Conclusions
The findings suggested that amyloid formation and trace elements disturbance may have an important role in the pathogenesis of T2DM, and ultimately in the degeneration and death of pancreatic islet cells. These findings provided a new rationale and opening up additional avenues of research into the aetiology, pathogenesis and in the treatment of T2DM. The findings of this investigation may prove significant in future research, especially if they are implemented in further research in vivo. Understanding the potential involvement of copper ions in amylin-induced β-cell toxicity is identified as an important area for further research.

However, several conclusions could be drawn from our study:

1. T2DM patients have impairment in trace elements especially Cu, Zn, Mg and amylin hormone.
2. There were significant differences between healthy group and diabetic group with regard to the level of trace elements (Zn, Mg) and amylin hormone.
3. Diet and food restriction was a helpful tool in managing and controlling of diabetes and then minimizing the risk of diabetes complications.
4. Diabetic retinopathy and neuropathy were the most frequent complications of diabetes among diabetic patients in this study.
5. Amylin hormone and Fe were influenced with age while, Fe was influenced with sex.
6. Smoking as a bad behavior has an effect of Mg level in patients group.
7. Blood sugar, and Zn levels were altered in diabetic patients with diabetic coma.
8. In diabetic neuropathy patients Zn level was significantly lower than healthy individuals.

Nevertheless T2DM is a serious problem that is now reaching epidemic proportions; further understanding of the factors involved in the aetiology and pathogenesis of this disease may aid in the identification of novel therapeutic targets and strategies.
6.2 Recommendations

In the light of these findings the following recommendations may considered to improve the management of diabetic patients and to minimize the risks diabetes complications:

- Increase the awareness of the importance of physical exercise and food restriction among diabetic patients to avoid unnecessary medication or complications.
- Physicians could take in their considerations the importance of monitoring trace elements level in the blood of diabetic patients which could be helpful in improving the diabetic patient’s general health conditions, controlling blood sugar level and reduce the risk of diabetic complications.
- This study needs to be expanded in term of data which should be collected, additional tests should be done, and more precise techniques could be used.

6.3 Limitations

In this study one or more obstacle was faced in carrying out assays.

- Atomic absorption was supposed to be used in measuring the level of trace elements in both patients and control groups but the instrument was not working during the whole period in all universities and research centers in Gaza Strip. Usage of such instrument could give more sensitivity in measuring trace elements. However, all precautions and quality control roles were carried out through all assays done in our study and approved and well established techniques were chosen according to other studies.
References


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السادة أعضاء هيئة هندسكي للبحوث
وزارة الصحة - غزة - فلسطين
السلام عليكم ورحمة الله وبركاته ...

نرجو من مديركما الموافقة على البحث المقدم من قبل الباحث / جهاد سالم محمد شهاب، وذلك ضمن برنامج ماجستير العلوم البيئية - تخصص تحليلات طبية لل_nanوث لكلية العلوم - الجامعة الإسلامية.

والذي يعنوان:
قباس نسبة هرمون الأميلين وبعض النافس المعدية في مرضى السكري (النوع الثاني).

ولكم منا جزيل الشكر والامتنان ...

مرفق بكم نسخة من البحث.

[ลงサيز، منديل برنامج ماجستير العلوم الحياتية]
الجامعة الإسلامية - غزة
The Islamic University - Gaza
كلية العلوم
FACULTY OF SCIENCE

الأخ الفاضل / د. ناصر أبو شعبان
مدير عام تنمية القوى البشرية بوزارة الصحة
السلام عليكم ورحمة الله وبركاته

حفظه الله...

 ينبغي تهديمك عادة كليّة العلوم بالجامعة الإسلامية بغزة أطيب ثوابها.
وترجم الكرم بأن طالب الماجستير / جهاد سالم محمد شعبان بحاجة إلى جميع
عياد من مرضى السكر من النوع الثاني، وكذلك عيادات من أشخاص غير
مصابين بهذا المرض، وذلك من مراكز الرعاية الأولية والمستشفيات الحكومية.
و بذلكipher إجراء دراسة حول تأثير نسب الأمان والمعانى لدى مرضى السكر من
النوع الثاني.

لذا ترجو من سيادتي الكرم تسهيل مبادرة localize أعلاه.

ثكره الكريم مشهور...

عميد كلية العلوم

د. نظام محمود الأشقر

.DATE

10/12/2011

11/12/2011

12/12/2011

التاريخ

ANNEXE 3

The Palestinian National Authority
Ministry of Health
Directorate General of Human Resources Development

السلطة الوطنية الفلسطينية
وزارة الصحة
إدارة العامة لتنمية القوى البشرية

الدلاء:

الاخ / د. فؤاد العيسوي
مدير عام الرعاية الأولية
تحية طيبة وبعد...

الموضوع

تسهيل مهمة بحث

بخصوص الموضوع أعلاه، برجى تسهيل مهمة الباحث / جهاز بال wzglęده

والمتاح في برامج الدراسات كلية العلوم الجامعية 

والدراسات الإسلامية في إجراء بحث بعنوان:

"Islet Amyloid and Selected Trace Elements among Type 2 Diabetes Mellitus patients in Gaza city"

حيث سيقوم الباحث بتجربة استخدام وأخذ جزء من عينات دم سبحة لأفراد الشهداء من مرضى

السكر النوع الثاني المرافعين لعيادات الرعاية الأولية.

كما نأمل توجيهكم لنشر البحوث بالموضوع بالتنسيق إلا بعد الحصول على الموافقة

المستمرة من المشاركين في البحث وفق النموذج المرفق، كما لا يتعارض مع مصلحة العمل وضمن

أخلاقيات البحث العلمي، ودون تحمل الوزارة أي أعباء.

ويضمن تبليغ النتيجة والمقدمة:

مرفق علمي:

* نموذج طلب تأسيس بحث

* نموذج الموافقة المستمرة

* صورة رسم العلاج

م. ناصر رأفت أي شهبان
مدير عام تطعيمات الداء الشهري

Gaza 131/08-2827298 Fax / 08-2868103 Email / gdrui@moh.gov.ps
ANNEXE 4

Palestinian National Authority
Ministry of Health
Helsinki Committee

Name:
I would like to inform you that the committee has discussed your application about:
"Islet Amyloid and Selected Trace Elements Among Type 2 Diabetes Mellitus patients in Gaza City."

In its meeting on June 2011 and decided the Following:-
To approve the above mention research study.

Signature

Member

Member

Chairperson

Conditions:-
✦ Valid for 2 years from the date of approval to start.
✦ It is necessary to notify the committee in any change in the admitted study protocol.
✦ The committee appreciate receiving one copy of your final research when it is completed.
ANNEXE 5

بسم الله الرحمن الرحيم

أنا الباحث / جهاد شع (طالب ماجستير بالجامعة الإسلامية). أرجو المساعدة في اتمام هذه الدراسة وذلك من خلال تعبئة هذا الاستبيان. أتمنى أن تكون هذه الخدمة من خلاله بعض الفحوصات من أجل عمل بحث حول الكشف عن نسبة المعادن والاملين وعلامات أخرى بين مرضى السكري من النوع الثاني في قطاع غزة. ولكل جزيل الشكر مع تمنياتنا لكم بالصحة والعافية.

رقم الاستبانة: ________________________________

الاسم: ________________________________

الجنس: أنتي ذكران

العمر: ________________________________

ضغط الدم: ________________________________

الإنسولين: ________________________________

في أي عام تم تشخيص السكري لأول مرة لديك؟

كم كان عمرك وقت تشخيص المرض؟

هل يعاني أحد أفراد عائلتك من مرض السكري من النوع الثاني؟

هل تقوم بفحص السكر في الدم لديك بشكل دوري؟

يجب أن يشمل ذلك: ________________________________

فيما هي درجة القرابة؟

إذا كانت الإجابة نعم: ما هي علاجك؟

هل تقوم بفحص السكر في الدم لديك بشكل دوري؟

إذا كانت الإجابة نعم: ما هو الرغبة الغذائية؟

أ. متى آخر مرة قمت بفحص السكر؟

ب. ما هو معدل السكر في آخر فحص قمت به؟

ما هو نوع العلاج الذي تستخدمه في الوقت الحالي؟

1. حمية غذائية

2. أقراص دواء عن طريق الفم

3. أنسولين

4. جميعهم

إذا كنت تتبع الحمية الغذائية فهل أنت ملتزم بها تماماً؟

إذا كنت تستخدم أقراص دواء عن طريق الفم: فهل تتناول هذه الأقراص بالانتظام؟

ما هو اسم الأقراص التي تستخدمها؟

إذا كنت تستخدم الأنسولين كعلاج فهل أنت ملتزم بأخذ العلاج بالجرعة المحددة التي وصفها لك الطبيب وفي الوقت المحدد لها؟

هل حدث تغيير في كمية الأنسولين المستخدم خلال الخمس سنوات الأخيرة؟

98
11) هل تمارس أي نوع من أنواع الرياضة؟

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10) هل تغيير في نوع العلاج المستخدم خلال الخمس سنوات الأخيرة؟

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9) هل تم تمارس عادة التدخين؟

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8) هل تعاني من ارتفاع ضغط الدم؟

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أ. منذ متى وانت تناولت علاج ارتفاع ضغط الدم؟

ب. ما هو اسم العلاج الذي تستخدمه لعلاج ارتفاع ضغط الدم؟

11) هل تعاني من أي مضاعفات لمرض السكري؟

أ. غيبيوة السكري

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ب. أمراض القلب

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<th></th>
<th>نعم</th>
<th>لا</th>
</tr>
</thead>
</table>

ج. أمراض الكلى

<table>
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<tr>
<th></th>
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<th>لا</th>
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</thead>
</table>

د. مشاكل في العين

<table>
<thead>
<tr>
<th></th>
<th>نعم</th>
<th>لا</th>
</tr>
</thead>
</table>

ه. مشاكل في الأعصاب

<table>
<thead>
<tr>
<th></th>
<th>نعم</th>
<th>لا</th>
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</thead>
</table>

12) هل تعاني من أي أمراض أخرى غير مرض السكري؟

<table>
<thead>
<tr>
<th></th>
<th>نعم</th>
<th>لا</th>
</tr>
</thead>
</table>

إذا كانت الإجابة نعم : ما هو نوع المرض؟