Significance of serum levels of copper and zinc in Type II diabetic, hypertensive, and diabetic hypertensive patients in Gaza City

أهمية النحاس والخارصين في مصل مرضى السكري (نوع 2)، مرضى الضغط ومرضى ضغط الدم السكري في مدينة غزة

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DECLARATION

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Abstract

Significance of serum levels of copper and zinc in type II diabetic, hypertensive, and diabetic hypertensive patients

Objective: To evaluate copper and zinc serum levels in diabetic patients, hypertensive patients and diabetic hypertensive patients and investigate existing correlation between trace elements (Zinc and copper) and serum glucose, cholesterol, triglycerides, HDL, and LDL levels in these groups.

Research design and methods: Convenience sample of 52 type II diabetes mellitus, 52 hypertensive patients, 52 diabetic hypertensive patients, and 52 normal subjects considered as apparently healthy by clinical examination and with no history of any disease were included in the study. Fasting blood samples were collected from all subjects and appropriately processed for analysis of glucose, cholesterol, and triglycerides, HDL, LDL by using chemical procedure, serum Cu and Zn were analyzed by atomic absorption spectrophotometry.

Results: In diabetic patients the serum levels of glucose (Mean±SD =213±66.3), serum levels of triglycerides (161±23.3), serum levels of LDL (95.3±36.3), serum levels of Cu (68.8±37.9) were found to be higher than normal group serum levels of glucose (Mean±SD=93.5±12.1), serum levels of triglycerides (151±29.1), LDL levels(73.6±36.2), serum Cu levels (45.3±18.1).

In diabetic group there was no correlation between zinc and glucose, triglycerides and HDL of study group (p > 0.05). While there was an inverse correlation found with cholesterol (P<0.01) and LDL (P<0.01), there exists also positive correlation with copper (p<0.001).

In diabetic group there was a strong positive correlation exists between copper and glucose level (P<0.001), and triglycerides (P<0.05), while there was weak negative correlation found with LDL (p<0.05). Copper also correlates well with zinc (p<0.001). In diabetic group there was an inverse correlation exists between zinc and Cholesterol level (P<0.01), and LDL level (P<0.01).

In hypertensive patients serum levels of glucose (99.5±21.1), serum levels of cholesterol (183±36.6), serum levels of triglycerides (167±58), serum LDL levels (103±35.5), and HDL serum levels in hypertensive (45.6±9.2) were not significantly different from serum control levels. Serum Cu level (52.4±23.5),
serum zinc level (25.3±8.25) were found to be higher than normal group serum levels.

In hypertensive patients there was no correlation between copper and glucose level, cholesterol, triglycerides, HDL, and LDL (p>0.05). Also there were no correlation between zinc and glucose level, cholesterol, triglycerides, HDL, also no correlation found with LDL (p>0.05).

In diabetic hypertensive patients serum levels of glucose (213±87.4), serum Cu levels (61.1 ±28.6), and serum Zn level (23.4±9.99 were found to be higher than normal group. However HDL serum levels (49.2±16.3) was found lower than normal group (50.6±11.9).

In diabetic hypertensive group there was a positive correlation between serum copper levels and serum HDL levels (p<0.05). Zinc also correlated positively with LDL (p<0.05).

Conclusion: Throughout this study we have focused on the role of zinc and copper in diabetes and hypertension. The levels of zinc and copper are differentially changed in the studied groups. Each diseased group demonstrates specific correlations with glucose and lipid profile parameters which implies different mechanisms of etiology.

Key Words: Diabetes, Diabetic hypertensive patient, Hypertension, Copper, Zinc, Lipid Profile
مستخلص

أهمية النحاس و الخارضين في مصل مرضى السكري (نوع 2)، مرضى الضغط، ومرضى ضغط الدم السكري

الهدف: تقييم مستويات مصل النحاس والخارضي لدى مرضى السكري، ومرضى الضغط ومرضى ضغط الدم السكري، وإيجاد علاقات مع مصل الجلوكوز والكولسترول والتراي جليسيريد والدهون عالية الكثافة والدهون قليلة الكثافة في هذه المجموعات.

تصميم البحث: Essay من 52 مريضاً مختارة من 52 مريضاً مريضاً (النوع 2)، من مرضى الضغط و 52 من مرضى السكري، 52 شخص سليم بناءً على النحوصات الإكلينيكية و عدم وجود تاريخ مرضى لأي مرض. وقد تم أخذ عينات الدم من جميع الأشخاص وهم صائمون. تم معالجة الدماء بشكل ملائم لتحليل الجلوكوز، والكولسترول والتراي جليسيريد والدهون عالية الكثافة HDL والدهون قليلة الكثافة في هذه الدراسة.

النتائج: لدى مرضى السكري، ظهرت مستويات مصل الجلوكوز (GLU) مرتفعة عند الأشخاص الذين يعانون من مرض السكري. وجد أن الدهون مرتفعه في جميع المجموعات، ولكنها كانت أعلى في المرضى الذين يعانون من مرض السكري.

لدى مجموعة مرضى السكري، لم تكن هناك علاقة ما بين الخارضي والجلوكوز والتراي جليسيريد والدهون عالية الكثافة HDL والمجموعات الدموية (p>0.05). بينما هناك علاقة عكسية بين الكولسترول (p<0.01).

و في حالة المرضى الذين يعانون من مرض السكري، لم تكن هناك علاقة ما بين الدهون والكولسترول والدهون عالية الكثافة HDL والمجموعات الدموية (p>0.05). بينما هناك علاقة عكسية ضعيفة بين الدهون والكولسترول والدهون عالية الكثافة HDL والمجموعات الدموية (p<0.05).

ملاحظات: يرجى القول إن النتائج التي تم الحصول عليها أوضحت أن اليومن الحسابي ل줄ولة الجلوكوز والتراي جليسيريد والدهون قليلة الكثافة كانت أكبر في مجموعة الدراسة سببًا في العينة الضيقة، ولكن نجد أن تركيز الدهون عالية الكثافة HDL لدى مرضى ضغط الدم (9.2±45.6) كانت بمثابة أقل من العينة الضيقة (11.9±50.6).

IV
لدى مجموعة مرضى الضغط، لم تكن هناك علاقة ما بين مستويات النحاس في البول والكولسترول والتراي HDL، والدهون عالية الكثافة. ولم يتم العثور أيضًا على علاقة مع الدهون قليلة الكثافة (p<0.05). لا توجد علاقة بين مستويات النحاس والكولسترول والتراي جليسيريد والدهون عالية الكثافة، و لم يتم العثور على علاقة مع الدهون قليلة الكثافة HDL (p>0.05).

لدى مرضى ضغط الدم السكري وجد مستوى مصل الجلوكوز (213±87.4) و مستوى مصل النحاس (28.6±61.1) و مستوى الدهون عالية الكثافة (73,6±36.2).

توجد أن النتائج التي تم الحصول عليها أوضحت أن الوسط الحسابي لمعدل الجلوكوز والتراي جليسيريد والدهون قليلة الكثافة كانت أكبر في مجموعة الدراسة عنها في العينة الضابطة ولكن نجد أن تركيز الدهون عالية الكثافة HDL كانت أعلى في مجموعة مرضى ضغط الدم السكري (49,2±16,3) و كانت بمثابة أقل من العينة الضابطة.

لدى مجموعة مرضى ضغط الدم السكري، تم إيجاد علاقة طردية بين النحاس والدهون عالية الكثافة HDL (p<0.05). وجدنا أيضا علاقة ما بين النحاس والخارصين (p<0.05).

أخيراً، ما وجدناه يؤكد على مدى أهمية قياسات المعادن والدهون في كل فئات الدراسة وكذلك يدل على أهمية حفظ مستويات النحاس والخارصين في مرضى السكري ومرض ضغط الدم. وقد وجد حدوث تغيرات في مستويات النحاس والخارصين في فئات الدراسة. كل مجموعة مرضية تظهر علاقات مع السكر والدهون، والتي تفسر ميكانيكيات مختلفة لأسباب حدوث المرض.}

كلمات مفتاحية: مرض السكري، مرض ضغط الدم السكري، مرض الضغط، النحاس، الخارصين، والدهون.
DEDICATION

*I dedicate my research to my loving parents*
I thank Allah first for helping me every moment.

Thanks To my supervisor Dr. Abdalla Abed, who had been a good source of motivation, inspiration and challenge throughout this research.

Thanks to my second supervisor Dr. Nizam El -Ashgar for his help during my research and testing the samples in chemistry department in Islamic University.

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TABLE OF CONTENTS

1. Introduction

1.1 Diabetes Mellitus ........................................................................................................1
   1.1.1 Association between diabetes mellitus and alterations in metabolism of trace elements 1
   1.1.2 The Zinc and diabetes mellitus ........................................................................2
   1.1.3 Copper and diabetes .......................................................................................2

1.2 Blood pressure ..........................................................................................................3
   1.2.1 The Hypertension ..........................................................................................3
   1.2.2 Trace elements and hypertension ..................................................................3
   1.2.3 Zinc and hypertension .................................................................................4
   1.2.4 Copper and hypertension ..............................................................................4

1.3 Aims of the study .....................................................................................................4

2. Literature Review

2.1 Diabetes mellitus ......................................................................................................5
   2.2 Diabetes and trace elements .............................................................................5
   2.2.1 Oxidative stress and diabetes .....................................................................6
   2.2.2 Oxidative Stress and the Chronic Complications of Type II ....................6
   2.2.3 Association between diabetes mellitus and alterations in metabolism of trace elements .........7
       2.2.3.1 Zinc and diabetes .............................................................................8
       2.2.3.2 Copper and diabetes .....................................................................9

2.3 Hypertension ..........................................................................................................10
   2.3.1 Types of hypertension .................................................................................10
       2.3.1.1 Essential hypertension ..................................................................10
       2.3.1.2 Secondary hypertension ................................................................11
   2.3.2 Oxidative stress and mechanism of hypertension .....................................11
   2.3.3 Association between hypertension and alterations in metabolism of several trace elements ..........12
       2.3.3.2 Zinc and hypertension .................................................................12
       2.3.3.1 Copper and hypertension ............................................................13
3. Materials and Methods

3.1 Study design and selection of subjects ........................................... 16
3.2 Setting ................................................................................................................. 17
3.3 Ethical considerations ......................................................................................... 17
3.4 Materials .............................................................................................................. 17
   3.4.1 Equipment ..................................................................................................... 17
   3.4.2 Kits .................................................................................................................. 17
   3.4.3 Reagent ......................................................................................................... 17
3.5 Methods .............................................................................................................. 18
   3.5.1 Sample preparation ...................................................................................... 18
   3.5.2 Glucose Test .................................................................................................. 18
   3.5.3 Cholesterol Test ........................................................................................... 19
   3.5.4 HDL Cholesterol Measurement .................................................................. 21
   3.5.5 Triglycerides Test ......................................................................................... 22
   3.5.6 LDL levels calculation .................................................................................. 23
   3.5.7 Trace Elements Analysis by Atomic Absorption .......................................... 24
3.6 Statistical Analysis ............................................................................................. 27

4. Results

4.1 Diabetes Group ................................................................................................. 28
4.2 Hypertensive Group ........................................................................................... 35
4.3 Diabetic Hypertensive Group ............................................................................ 40

5. Discussion

5.1. Copper in diabetic patients ............................................................................. 46
   5.1.1 Relation between copper and lipid profile in diabetics ............................... 47
5.2. Zinc levels in diabetics ..................................................................................... 49
   5.2.1. Zinc and lipid profile in diabetics ............................................................... 49
5.3 Hypertension ..................................................................................................... 50
   5.3.1 Cu and Zn in hypertensive patients ............................................................ 50
   5.3.2 Oxidative stress and hypertension .............................................................. 51
   5.3.3 Lipid profile in hypertensive patient ......................................................... 51
   5.3.4 Copper and cardiovascular disease ........................................................... 51
5.4 Diabetic hypertensive patients ........................................................................... 53
   5.4.1 Cu and Zn in Diabetic hypertensive patients ............................................. 53
   5.4.2 Lipid profile in Diabetic hypertensive patients ........................................... 54
6. Conclusions and Recommendations ............................................................55
   6.1. Conclusion.......................................................................................55
   6.2. Recommendations.................................................................56

References

Appendices
   Appendix 1: UNRWA agreement
   Appendix 2: Consent form
   Appendix 3: Questionnaire
LIST OF TABLES

Table 4.1 Comparison between the age of diabetic group with the age of control group .............................................................................................................................................28

Table 4.2 Serum glucose and lipid profile levels in both diabetic group and control group ..........................................................................................................................................................29

Table 4.3 Serum trace elements levels in both diabetic group and control group .................................................................................................................................................................................30

Table 4.4 Comparison between copper in diabetic group and age, glucose and lipid profile and zinc....................................................................................................................................................................31

Table 4.5 Comparison between zinc in diabetic group and age, glucose and lipid profile and copper...................................................................................................................................................................33

Table 4.6 Comparison between the age of hypertensive group with the age of control group .............................................................................................................................................................................35

Table 4.7 Comparison between glucose and lipid profile of hypertensive group with control group ........................................................................................................................................................................36

Table 4.8 Serum trace elements in both hypertensive group and control group ...................................................................................................................................................................................................37

Table 4.9 Comparison between copper in hypertensive group with age, glucose, lipid profile and zinc........................................................................................................................................................................38

Table 4.10 Comparison between zinc in hypertensive group with age, glucose, lipid profile and zinc...........................................................................................................................................................................38

Table 4.11 Comparison between the age of diabetic hypertensive group with the age of control group ........................................................................................................................................................................39

Table 4.12 Comparison between glucose and lipid profile of diabetic hypertensive group with control group ........................................................................................................................................................................41

Table 4.13 Comparison between serum trace elements of diabetic hypertensive group with control group ...................................................................................................................................................................41

Table 4.14 Comparison between copper in diabetic hypertensive group with age, glucose, lipid profile and zinc.........................................................................................................................................................................42

Table 4.15 Comparison between zinc in diabetic hypertensive group with age, glucose, lipid profile and zinc...........................................................................................................................................................................44
LIST OF FIGURES

Figure 4.1 Correlation between copper levels and glucose levels in diabetics.31

Figure 4.2 Correlation between copper levels and LDL levels in diabetics .....32

Figure 4.3 Correlation between copper levels and triglyceride in diabetics.......32

Figure 4.4 Correlation between zinc and copper in diabetic group ...............33

Figure 4.5 Correlation between zinc and LDL in diabetic group .................. 34

Figure 4.6 Correlation between zinc and cholesterol in diabetic group ...........34

Figure 4.7 Correlation between zinc and copper in diabetic hypertensive group ........................................................................................................43

Figure 4.8 Correlation between copper and HDL in diabetic hypertensive group ........................................................................................................43

Figure 4.9 Correlation between zinc and cholesterol in diabetic hypertensive group........................................................................................................45
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<td>Atomic absorption Spectrophotometry</td>
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<tr>
<td>AP</td>
<td>Atrial Pressure</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
</tr>
<tr>
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<td>Cholesterol</td>
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<td>Copper</td>
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<td>Diabetes Mellitus</td>
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<td>Gram</td>
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Chapter-1
Introduction

1.1 Diabetes Mellitus

Diabetes is an epidemic disease in most countries. World wide, an estimated 150 million people are affected by diabetes, and this number is likely to reach 300 million by the year 2025 if successful strategies are not implemented for its prevention and control (1). There are three types of diabetes; type I, type II and gestational diabetes.

Diabetes Type I result from pancreatic β-cell destruction. This type constitutes only 10% of diabetic cases and commonly occurs in childhood and adolescence (1).

Diabetes type II is characterized by insulin resistance with relative insulin deficiency. This type accounts for 90% of all diabetic cases and commonly appears in adults so its called adult-onset diabetes. In this type of diabetes, insulin is present in little amounts. Fatty acids are incorporated into triglycerides for release of very low-density lipoproteins. So they are at increased risk of developing macro vascular and micro vascular complications (2).

Recently, some evidences suggested that oxidative stress may play an important role in the etiology of diabetes and its complications such as atherosclerosis, arteriosclerosis, hypertension and chronic renal failure (3). Oxidative stress results from imbalance between free radical and anti-oxidants enzymes such as super-oxide dismutase (4, 5).

Gestational diabetes occurs temporarily during pregnancy in women with an inherited predisposition, over weight; family history of diabetes (6).

1.1.1 Association between diabetes mellitus and alterations in metabolism of trace elements

A number of studies have reported correlation between diabetes and trace elements such as zinc, copper. Scott and Fischer (1938) first recognized the relationship between zinc and insulin (7). Zinc affects antigenic properties of insulin which leads to hyperglycemia. Increase in the copper ion levels in patients with diabetes mellitus (DM) may be attributed to hyperglycemia that may
stimulate glycation and release of copper ion and this accelerate the oxidative stress (8).

1.1.2 Zinc and diabetes mellitus

Zinc serves an essential role as a cofactor for more than 200 metal enzymes, many of which regulate the metabolism of carbohydrates, lipids and proteins. Insulin itself is stored in an inactive form in the presence of zinc (9).

Zinc ions in the secretary granules of β cells are known to glue insulin molecules, creating somatically stable hexamers. When the secretary granules open to the surface, the zinc ions pressure decreases rapidly and pH levels change from acid to physiological level, which results in free insulin monomers and zinc ions will be released from pancreas (10).

The increased levels of zinc in DM has been suggested also to be due to either inability to transport appropriate amount of zinc in the crucial cell types that require relatively higher amount of zinc than the other cell types, because there is presence of genetic and environmental link between human zinc transporters and their differential expressions in the islet beta cells, or may be due to low degree of expressions of the critical zinc transporters in the beta cells (11).

Zinc is considered as an integral component of Cu-Zn SOD which acts as anti oxidant and protects from free radicals. Free radicals when accumulate, this leads to oxidative stress which play important role in the etiology of diabetes and diabetic complication (9).

1.1.3 Copper and diabetes

Copper is the third most abundant essential trace mineral in the body. In fact, copper has an important role in the body, being component of two of our most important anti-oxidant enzymes; Cu-Zn SOD and ceruloplasmin (12).

The intestine plays an important role in regulation of copper. Zinc competes with copper for intestinal absorption, so increased intake of zinc leads to copper deficiency (13).

Both increased and decreased Cu levels were found in diabetic patients (14,15). A deficiency of copper has been shown to result in glucose intolerance,
decreased insulin response; increased glucose response, which lead to depression the activity of Cu-Zn SOD. This leads to increase in the amount of free radicals which result in the increase in the oxidative damage (14). In another study however, in patients with diabetes mellitus it was found an increase in the level of copper may stimulate glycation and release of copper ions and this accelerates the oxidative stress (15).

1.2 Blood pressure

Blood pressure is the force of the blood pushing against the walls of the arteries (1). There are two types of blood pressure; systolic and diastolic. Any increase of blood pressure leads to hypertension which is the major risk factor of atherosclerosis and renal failure (16).

1.2.1 Hypertension

There are two types of hypertension, essential (primary) and secondary forms. Primary hypertension is the most common accounting for more than 95% of all cases. This type of hypertension has no known cause, and could be due to the interplay among many genetic and environmental factors. Secondary hypertension have an identified source i.e. renal parenchyma disease, use of oral corticosteroids and endocrinal disorders (17).

1.2.2 Trace elements and hypertension

Trace elements regulate blood pressure, any imbalance of dietary intake of these elements such as (copper, zinc) will affect the blood pressure and lead to the development of hypertension and vascular disease, including alternation in serum cholesterol and triglyceride levels (18). The role of zinc and copper in hypertension has been implicated in several studies. For example in one study elevated levels of serum copper have been shown to be independent risk factor for heart disease and hypertension, however decrease in the amount of zinc leads to hypertension (19).
1.2.3 Zinc and hypertension
Zinc serves an essential role as cofactor for more than 200 metalloenzymes. Zinc acts as integral components of Cu-Zn SOD, which is essential for the strength and the integrity of the heart and blood vessels, so decrease the amount of zinc will lead to decrease in the SOD activity which leads to impair heart functions (20).

1.2.4 Copper and hypertension
Copper is bound to proteins such as Cu-Zn SOD and ceruloplasmin, which are anti-oxidant enzymes. Insufficient dietary copper were found to elevate blood lipids levels and impair heart function (18). Copper is essential both for its role in anti oxidant enzyme, like Cu-Zn SOD, ceruloplasmin as well as its role lysyl oxide, which is essential for the strength and the integrity of the heart and blood vessels (21). Copper deficiency depress Cu-Zn SOD activity and prostacyclin in the aorta, as well increases the susceptibility of lipoproteins and heart tissue to peroxidation, Therefore it is thought that copper plays a vital role in the protection of the cardiovascular disease from free radical damage (22).

1.3 Aims of the study
The overall aim of the present studies to investigate significance of serum levels of copper and zinc in Type II diabetes, hypertensive, and diabetic hypertensive patients in Gaza City with specific objectives :

1. Determination of serum Cu and Zn in three patient groups (diabetics, hypertensive, diabetic hypertensive groups).
2. Measurement of both sugar and lipid profile (cholesterol, triglycerides, HDL, and LDL) in the three groups.
3. Comparison between the levels of both trace elements (Copper and zinc) and lipid profile in the studied groups.
4. Investigation of the existence of any correlations between the serum levels of copper or zinc with the levels of glucose and lipid parameters in the different studied groups.
Chapter 2
Literature Review

2.1 Diabetes mellitus

Diabetes mellitus is a syndrome characterized by raised glucose concentration in the blood, due to destructions of beta cells of the islet of langerhans (23). The disease is chronic and also affects the metabolism of fat and proteins (6). There are many causes of diabetes hereditary, sometimes by increasing the susceptibility of beta cells to viruses, or by autoimmune antibodies against the beta cells (3).

2.2 Diabetes and trace elements

2.2.1 Oxidative stress and diabetes

Recently, some evidences suggest that oxidative stress may play an important role in the etiology of diabetes and diabetes complications (24). Oxidative stress is defined as excessive production of reactive oxygen species (ROS) in the presence of diminished antioxidant substances. It has been shown that oxidative stress has an adverse effect on glucose metabolism. Development of the disabling chronic complications of diabetes mellitus (DM) has also been attributed to oxidative stress (25). The body's defense against oxidative stress is accomplished by interconnecting systems of antioxidant micronutrients (vitamins and minerals) and enzymes (6). While the vitamins act as donors and acceptors of ROS, minerals regulate activity of the enzymes (26).

In diabetic patients, the persistence of hyperglycemia has been reported as a cause of increased production of oxygen free radicals through non enzymatic glycation, which results in the formation of hydrogen peroxide which inactivate SOD (27). The primary catalytic cellular defense that protects cells and tissues against potentially destructive reactions of superoxide radicals and their derivatives is Cu/ Zn -SOD (28).

Decreased activity of the antioxidant enzymes such as (Cu-Zn SOD) may increase the susceptibility of diabetic patients to oxidative injury (29). Appropriate
support for enhancing antioxidant supplies may help to prevent clinical complications of diabetes mellitus (30). In view of low activities of SOD in diabetes it was concluded that supplementary trace elements such as copper and zinc, the essential components of the enzyme structure may be useful in preventing the development of diabetic complications (31)

2.2.2 Oxidative stress and the chronic complications of type II diabetes

Hyperglycemia causes oxidative stress, which increases glycosylation and oxidation of proteins involved in the pathogenesis of the complications of diabetes (32, 33).

Development of diabetic complications has been hypothesized to be accelerated by generation of free radicals in cells and tissues (34,35). In diabetes, oxidative stress is due in part to an increased production of plasma free radical concentrations and a sharp reduction in antioxidant defenses (36). It has been postulated that free radical production could result in hyperglycemia (37), hyperinsulinemia and/or insulin resistance (38), and it was postulated that oxidative stress represents the common pathway through which hyperglycemia and insulin resistance induce depressed insulin action (38–39).

Oxidative stress in persons with diabetes is also related to decreased antioxidant defenses. Oxidative stress contributes to impairment of islet function (40,41,42), insulin resistance ,and micro vascular and macro vascular disease(43,44). Diabetic patients with uncontrolled hyperglycemia are at risk for oxidative stress and complications, and oxidative stress may increase their requirement for vitamins with antioxidant effects (45, 34, 46). Damaged tissues may have altered responses to vitamins and differing requirements. Reduction of hyperglycemia and improvement of blood sugar control reduces oxidative stress, and reduction of free radical levels should improve metabolic function of beta cells, vascular endothelial cells, fat and muscle cells, and platelets (33, 34, 41, 46, 47). Decreased glycosylation and oxidation of proteins was found to reduce atherosclerosis, retinopathy, nephropathy, and neuropathy attributable to these processes (34)
2.2.3 Association between diabetes mellitus and alterations in metabolism of trace elements

A number of studies have reported an association between diabetes mellitus (DM) and alterations in the metabolism of several trace minerals (48). Impaired insulin release, insulin resistance and glucose intolerance in experimental animals and humans with DM have been linked to a compromised status of copper and zinc (49). Although the emphasis is on macronutrients intakes, there is strong evidence that there is an abnormal metabolism of several micronutrients in diabetic individuals (50). Diabetes seems to be associated with numerous abnormalities of plasma trace elements and magnesium (51), but the mechanism of these abnormalities has not yet been elucidated. A decrease in zinc and selenium concentrations and an increase in copper concentrations might be additional factors of atherogenicity (52).

The amount of selenium, zinc, and copper in diabetic patients have led to contradictory finding as possible relationship between the degree of diabetic control and changes in mineral contents (53).

Zn and Cu serum levels in type 2 and type I DM patients were decreased. The Zn/Cu ratio was higher in both groups of diabetic patients (54).

Copper and zinc are the major components of antioxidant enzyme SOD (55). This enzyme inhibit oxidative stress which results from the accumulation of free radicals oxidative stress and plays an important role in the pathological processes ongoing in the diabetic patient (56). Excessive oxidative stress has adverse effects on islet survival and function and accelerates complications in target organs and tissues (55).
2.2.3.1 Zinc and diabetes

Zinc is an important essential mineral in human nutrition with a wide range of biological functions. Zinc fulfills catalytic, structural, or regulatory roles in more than 200 zinc-requiring metalloenzymes (57). The interaction of zinc with insulin induces conformational changes and enhances binding to the insulin receptor (58,59). With regard to glucose metabolism, zinc is a co-factor of several key enzymes. Zinc is an activator of fructose-1-6-bisphosphate aldolase, and an inhibitor of fructose-1-6-biphosphatase (59). Zinc can also exert antioxidant activity (60), and is a cofactor in Cu-Zn SOD, a major antioxidant enzyme (61).

There is accumulating evidence that the metabolism of zinc is altered in insulin-dependent diabetes mellitus and that zinc might have specific roles in the pathogenesis and progress of this disease. Increased urinary loss of zinc is a commonly encountered feature of diabetes (62).

Some studies have reported zinc deficiency along with alterations in zinc metabolism in patients with diabetes (60, 61).

In another study it was found that diabetes can alter copper, zinc and lipid peroxidation. Plasma copper was higher and plasma zinc and plasma peroxide concentrations were lower in diabetic than in control subjects (52). Consequently, considering the possible modulating effects of zinc on insulin sensitivity and its antioxidant functions, it was postulated that a restored Zn status in individuals with type 2 DM might counteract the deleterious effects of oxidative stress and help to prevent complications associated with diabetes (63).

In patients with type II diabetes mellitus, these patients had decreased serum zinc concentrations, because there presence of male absorption of zinc which leads to hyperzincuria (64).

In diabetes, zinc is decreased ,copper excretion increased and SOD activity decreased (7). Therefore it was postulated that elevated levels of Cu-Zn SOD, elicit a protective effect against diabetes (56).
Another study it was found that the amounts of copper was increased but there were no significant alternation in levels of serum zinc in DM (65).

The amounts of Cu-Zn SOD activity was found reduced in diabetic patient, the copper and zinc status of these diabetic patients was reduced, providing further evidence of a role for these antioxidant trace elements in this disease (66).

Because zinc can exert a number of indirect antioxidant functions, a hypothesis in humans that increased zinc intake will protect against oxidant stress in persons with tendencies for both moderate zinc deficiency and high oxidant stress (60).

2.2.3.2 Copper and diabetes

Copper is the third most abundant essential trace elements in the body. Copper is present in the body combined with enzymes to form metalloenzymes such as ceruloplasmin, SOD (65). These enzymes play major role in redox reactions, such as superoxide dismutase which plays key role in antioxidant defense (2). It has been postulated that copper possesses insulin–like activity and promotes lipogenesis; serum copper is elevated in adult onset diabetes (7). Human studies demonstrate that diabetic patients have abnormal circulation of copper (64).

In another study it was found that diabetes can alter copper. Plasma copper was higher and plasma zinc was lower in diabetic than in control subjects (66). However in one study which measured the SOD levels in erythrocytes there were no significant differences between control and diabetic subjects (53).

Increase in the Cu ion levels in patients with diabetes might be attributed to hyperglycemia that may stimulate glycation and release of copper ions and this accelerate oxidative stress (7).
2.3 Hypertension

Blood pressure is determined by the amount of blood which heart pumps and the amount of resistance to blood flow in the arteries (75).

2.3.1 Types of hypertension

2.3.1.1 Essential hypertension

Hypertension is the blood pressure that is consistently higher than normal when no cause for the high blood pressure can be found. Most experts believe that essential hypertension is caused by several undiscovered factors, which may be why certain treatments lower blood pressure in some peoples but not others (67).

Risk factors for essential hypertension

About 95 percent of people with high blood pressure have essential hypertension. There are no identifiable causes of essential hypertension, but there are several factors that can increase blood pressure, such as the amount of blood pumped by the heart, size and condition of the arteries, water and salt content of the body, condition of the kidneys, nervous system or blood vessels, and hormone levels in the body. Other factors can include stress, being overweight, smoking, alcohol use, a diet high in salt, heredity, gender, age and race (62).

In recent years, considerable evidence has suggested that changes in vascular endothelial function may cause the increase in vascular tone. For example, in hypertensive patients, the vascular endothelium produces less nitric oxide and the vascular smooth muscle is less sensitive to the actions of this powerful vasodilator. There is also an increase in endothelin production, which can enhance vasoconstrictor tone. There is compelling evidence that hypoinsulinemia and hyperglycemia in type 2 diabetes (NIDD) causes endothelial dysfunction by enhanced oxygen free radical mediated damage and decreased nitric oxide bioavailability (68).
2.3.1.2 Secondary Hypertension

Secondary hypertension have an identified sources, accounts for approximately 5-10% of all cases of hypertension, with the remaining being primary hypertension. There are many known conditions that can cause secondary hypertension (69). Regardless of the cause, arterial pressure becomes elevated either due to an increase in cardiac output, an increase in systemic vascular resistance, or both. When cardiac output is elevated, it is generally due to either increased neurohumoral activation of the heart or increased blood volume (68).

Causes of secondary hypertension include renal disease, used of certain medication, including oral corticosteroids, Non steroidal Anti Inflammatory Agents (NSAIDS). Also endocrine disordered such as pheochromocytoma and acromegaly (67).

2.3.2 Oxidative stress and mechanism of hypertension

During the last decade, the terms 'free radicals', 'oxidative stress', and 'antioxidants' have become commonly used to discuss the cellular mechanisms of hypertension (70). A free radical is any molecule that has an odd number of electrons. Free radicals, which can occur in both organic (i.e., quinones) and inorganic molecules (i.e., O(2)), are highly reactive(71).

Free radicals are formed from partial reduction of molecular oxygen which also can generate reactive oxygen species (ROS). ROS are constantly formed in the human body and removed by endogenous antioxidants which constitute a primary means of detoxifying ROS and preventing ROS-induced cellular damage. Most of the ROS formed within cells are highly reactive and they are able to oxidize most of the biomolecules within the cell, leading to tissue injury. Overproduction of free radicals is considered as a common feature of a large broad of diseases and oxidative stress is generally thought to make a significant contribution to hypertension (72).
Recent studies have provided irrefutable evidence that oxidative stress can cause hypertension and hypertension can cause oxidative stress (71).

In mammalian cells, there are two different SODs. These enzymes are metallo-proteins and their metal center is essential for their catalytic activity. Manganese-centered SOD (Mn-SOD) is located in mitochondria, and copper-zinc-centered SOD (Cu/Zn-SOD) in the cytosol. Hydrogen peroxide is a strong oxidant which is able to interact slowly with most organic substrates. In presence of transition metals such as iron or copper, hydrogen peroxide can oxidize superoxide radical at rapid rates (Fenton reaction) to produce hydroxyl radical $^\cdot$OH, the most highly reactive oxidant, which, unlike superoxide radical or hydrogen peroxide, is reactive with most biological substrates (73).

This antioxidant enzyme is responsible for the dismutaion of super oxide radical-free radical initiator. A fine balance between free radicals and a variety of endogenous anti oxidant is believed to exist. Any disturbance in this equilibrium leads to oxidative stress and initiates sub cellular changes leading to heart failure and hypertension (74).

2.3.3 Association between hypertension and alterations in metabolism of several trace elements

Many studies have suggested the presence of correlation between zinc and copper metabolism and increase the risk factor of hypertension (75). Inverse correlations between blood pressures and serum Zn were observed, higher levels of serum copper were associated with increased risk of hypertension (76).

2.3.3.1 Zinc and hypertension

Zinc deficiency severely impairs endothelial cell function, an effect reversed by zinc supplementation. Tumor Necrosis Factor (TNF) signals a cascade of events leading to the synthesis of proteins that regulate oxidative stress and promote free radicals. Zinc supplementation reversed the activation by TNF in these cells, zinc inhibited activation of oxidative stress transcription
factors and the synthesis of Interleukin 8 and promoted a return to homeostatic balance (84).

High zinc intake was found to lower LDL and block the absorption of copper, creating a copper deficiency. Copper deficiency in turn can increase blood cholesterol and lower HDL, thus increasing the risk of cardiovascular disease (85).

Arteries are vulnerable to ongoing oxidative stress due to exposure to oxidizing agents such as drugs and free radicals, including superoxide and lipid peroxyl radicals. Blood contains an elaborate array of antioxidants, including ascorbic acid, uric acid and defensive serum proteins to limit this damage. However, antioxidant defenses are not 100% efficient. Zinc participates in these defenses by serving as a cofactor for the antioxidant enzyme, superoxide dismutase (86).

Higher levels of serum copper were associated with increased risk of hypertension. Inverse correlations between blood pressures and serum Zn were observed. Furthermore, blood pressure was inversely related to lysyl oxidase activity. These findings give further support to the hypothesis that an imbalance of Zn and Cu bioavailability may be associated with hypertensive condition (87).

Another study have detected lower levels of copper and higher levels of zinc in hypertensive patients (88).

2.3.3.2 Copper and hypertension

Copper can act as both an antioxidant and a pro-oxidant. As an antioxidant, it scavenges damaging particles in the body known as free radicals. Free radicals occur naturally in the body and can damage cell walls, interact with genetic material, and possibly contribute to the aging process as well as the development of a number of health conditions. Antioxidants can neutralize free radicals and may reduce or even help to prevent some of the damage caused by free radicals (77).
Because Copper is a cofactor for Cu-Zn SOD and ceruloplasmin, two important antioxidant enzymes, and the possible antioxidant activity of copper may be accounted for, at least in part, by its role in these enzymes (78). So any decrease of copper and zinc leads to decrease the activity of Cu-Zn SOD which leads to oxidative stress, that causes hypertension (79).

Copper is an essential element necessary for optimal absorption and metabolism of iron, normal erythropoiesis and bone collagen formation. Numerous authors report increased serum copper levels in patients and animals with arteriosclerosis, hypertension or myocardial infarction. Harman and his colleagues have also found that experimental animals that have higher intakes of copper have more arteriosclerosis. They have postulated that individuals prone to coronary artery disease may be identified by a high serum copper level (57). It has been hypothesized that an imbalance in zinc and copper metabolism may play a role in coronary heart disease (80).

Studies in the microcirculation demonstrate that copper is important in mechanisms of macromolecular leakage, and vascular smooth muscle reactivity. Nitric oxide (NO)-mediated arteriole vasodilatation is also compromised in copper-deficient rats. This functional deficit to NO can be reversed by the addition of Cu-Zn SOD, suggesting that degradation of NO by superoxide anion occurs during copper deprivation. These observations demonstrate that dietary copper is necessary for several microvascular control mechanisms affecting inflammation, microhemostasis and regulation of peripheral blood flow. So any decrease in the amounts of copper leads to decreased NO concentration which causes vasoconstriction that leads to hypertension (81).

Dietary copper is known to be essential for the normal functioning of the cardiovascular system in both humans and experimental animals. The cardiovascular defects in copper deficiency are often associated with impaired activity of some of the ~30 copper-dependent enzyme systems in living organisms. Structurally, weakened heart and blood vessel walls have been attributed to reduced activity of the copper-dependent enzyme lysyl oxidase (82).
The development of hypertension in copper-deficient rats may be the result of impaired vasoactive functions in the small resistance blood vessels, thus suggesting a role for copper in microvascular control mechanisms (83).

Copper deficiency prolongs homeostasis, inhibits NO-dependent dilation and increases mast cell-mediated macromolecular leakage. Thus, dietary copper is involved in a variety of micro vascular functions involving many different stimuli and responses. The responses studied involve the endothelial interface between the blood and tissues, and suggest that the alterations that occur in the presence of copper deficiency are pathway specific and do not involve any generalized phenomenon such as a loss of endothelial function (75).
Chapter-3
Materials and Methods

3.1 Study design and selection of subjects

The present study included 208 individuals, categorized into 4 groups.

The first group: includes 52 normal subjects considered as apparently healthy by clinical examination and with no history of any disease such as diabetes or hypertension. Their age ranged between 40-60 years. Interviews were performed with a protested and validated questionnaire (Appendix 3) by a physician who obtained detailed information on family history of hypertension and diabetes. In all subjects, blood pressures were recorded by the same physician in the right arm after a 5-minute rest in a sitting position by a single mercury sphygmomanometer.

The second group: includes 52 patients with type II diabetes. Their age ranged between 40-60 years. The criteria for the diagnosis of diabetes were based on World Health Organization. Diabetes mellitus was diagnosed by a positive glucose tolerance test showing fasting blood glucose >110 mg/dl and postprandial blood glucose >140 mg/dl, 2 hours after 75 g of oral glucose.

The third group: includes 52 patients with hypertension. Their age ranged between 40-60 years. The criteria for the diagnosis of risk factors and hypertension were based on World Health Organization. Hypertension was diagnosed in presence of blood pressures >140/90 mmHg, patient has risk factors such as smoker, obese and hypercholesterolemia.

The fourth group: includes 52 diabetic hypertensive patients. Their age range is from 40-60 years. Patients selection was based on fasting glucose levels was greater than 110 mg /dL ,and blood pressure greater than 140/90 mmHg, patient has risk factors such as smoker , obese and hypercholesterolemia.
3.2 Setting
All patients groups consecutively were admitted to UNRWA Zaitoun and Rimal health centers.

3.3 Ethical considerations
An authorization to carry out this research was obtained from UNRWA (appendix 1), where our samples where collected. The research was also conducted after obtaining a letter of agreement from the Islamic University of Gaza (appendix 1).

3.4 Materials

3.4.1 Equipment

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuge</td>
<td>Labofuge 200 Heraeus SEPATECH</td>
</tr>
<tr>
<td>Atomic absorption spectroscopy</td>
<td>Perkin Elmer, A Analyst 100-Germany</td>
</tr>
<tr>
<td>Spectrophotometer</td>
<td>Unico 1100 RS spectrophotometer</td>
</tr>
</tbody>
</table>

3.4.2 Kits

<table>
<thead>
<tr>
<th>Kit</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose kits</td>
<td>Linear Chemicals, S.l</td>
</tr>
<tr>
<td>Cholesterol kits</td>
<td>Human for biochemical and diagnostic test</td>
</tr>
<tr>
<td>Triglycerides kits</td>
<td>Human for biochemical and diagnostic test</td>
</tr>
<tr>
<td>HDL kits</td>
<td>Human for biochemical and diagnostic test</td>
</tr>
</tbody>
</table>

3.4.3 Reagent
- Nitric Acid (0.1N ) for serum dilution for measurement of trace elements.
- Concentrated Hydrochloric acid .
3.5 Methods

3.5.1 Sample Preparation
Venous blood samples (5ml) were obtained from all patients and controls. Patients had fasted from 8 p.m to 8 a.m. Specimens were collected at standardized time to minimize any effect of diurnal variation. Sterile disposable plastic syringes were used. The sample left to clot and the serum was separated by centrifugation. The serum was used for determination of copper and zinc by using atomic absorption spectroscopy; also serum samples were used for determination of serum glucose, cholesterol, triglycerides and HDL-cholesterol.

3.5.2 Glucose Test
Glucose was measured in the samples using commercially available kit (Linear chemicals, s.l) according to Trinder method.

Reaction Principle
In the reaction, the glucose is oxidized to D-gluconate by the glucose oxidase with the formation of hydrogen peroxide. In the presence of peroxidase, a mixture of phenol and 4-aminoantipyrine is oxidized by hydrogen peroxide to form a red quinoneimine dye, which is proportional to the concentration of glucose in the sample.

Content of the kit

Buffer
Phosphate buffer 100 mmol/L pH7.5, glucose oxidase greater than 10ku/L, peroxidase greater than b 2 ku/L,
4-aminoantipyrine 0.5mmol/L, phenol 5mmol/L

Calibrators
The concentration of glucose standard 100mg/dL

Assay Procedure
All reagents, samples and controls were brought to room temperature (20-25°C) before starting the test.
Serum samples free of hemolysis were used because any hemolysis will give false low result because the enzymes released will cause consumption of glucose. Also, catalase liberated from RBCs will compete with peroxidase for Hydrogen peroxide, giving untrue results.

The test was carried out as follows:
1. Reagents and samples were brought to room temperature.
2. The monoreagent buffer (1.0 mL) was pipetted in each labeled tube.
3. Serum was pipetted (0.01 mL) into sample labeled tube.
4. Standard was pipetted (0.01 mL) into standard labeled tube.
5. Tubes were incubated for exactly 10 minutes at room temperature.
6. The absorbance (A) of the samples and the standard was read at 500 nm against the reagent blank by using spectrophotometer.

**Glucose levels calculation**

The concentration was calculated according to the following formula:

\[
\frac{A \text{ sample}}{A \text{ standard}} \times \text{C standard} = \text{mg/dL}
\]

**Normal range values**

- **Adult**: 70-105 mg/dL
- **Children**: 60-110 mg/dL
- **Newborn**: 40-60 mg/dL

**Quality Control**

The use of standard to calculate results to obtain accuracy independent of the system and instrument used. To ensure adequate quality control (QC), each run included a set of controls.

**3.5.3 Cholesterol Test**

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. According to Friedewald method.
**Reaction Principle**

Cholesterol reacts with oxygen to form cholesterol-3-one and hydrogen peroxide which react with 4-aminophenazone and phenol to form quinoneimine and water.

**Content of the kit**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer</td>
<td>100 m mole /L</td>
</tr>
<tr>
<td>4-aminophenazone</td>
<td>0.3 m mole /L</td>
</tr>
<tr>
<td>Phenol</td>
<td>5 m mole /L</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>&gt; 5 K U / L</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>&gt;150 U / L</td>
</tr>
<tr>
<td>Cholesterol oxidase</td>
<td>&gt; 100 U / L</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.05 %</td>
</tr>
</tbody>
</table>

Calibrators: The concentration of Cholesterol 200 mg/dL

**Assay Procedure**

It is important to bring all reagents, samples and controls to room temperature (20-25°C) before starting the test.

The test was carried out as follows:

1- Reagents and samples were brought to room temperature.
2- Cholesterol reagent (1.0 mL) was pipetted in each labeled tubes
3- Serum (0.01 mL) was pipetted into sample labeled tube.
4- Standard (0.01 mL) was pipetted into standard labeled tube.
5- Tubes were incubated for exactly 10 minutes at room temperature.
6- The absorbance (A) of the samples and the standard was read at 500 nm against the reagent blank by using spectrophotometer.

**Cholesterol Levels Calculation**

The concentration was calculated using the following formula

\[
A_{\text{sample}} / A_{\text{standard}} \times C_{\text{standard}} (200) = \text{mg/dL}
\]

**Quality Control**

The use of standard to calculate results to obtain accuracy independent of the system and instrument used. To ensure adequate quality control (QC), each run included a set of controls.
3.5.4 HDL Cholesterol measurement

**Reaction Principle**

The assay combines two specific steps: in the first step chylomicrons, VLDL and LDL cholesterol are specifically eliminated and destroyed by enzymatic reactions. In the second step remaining cholesterol from the HDL fractions is determined by well established specific enzymatic reactions in the presence of specific surfactants for HDL. According to Gordon method.

1\(^{st}\) step:

LDL, VLDL and chylomicrons \(\xrightarrow{\text{specific condition}}\) choletenone+H\(_2\)O\(_2\)

\[2H\text{O}_2 \text{ catalase} \rightarrow HDL+2H\text{O}_2\]

2\(^{nd}\) step:

HDL \(\xrightarrow{\text{CHE+CHO, Specific condition}}\) Choletenone+H\(_2\)O\(_2\)

\[H_2\text{O}_2 + \text{chromogen} \xrightarrow{\text{peroxidase}} \text{quinine pigment}\]

**Content of the kit**

**Enzymes**

Good buffer pH7.0
Cholesterol esterase (CHE)
Cholesterol oxidase (CHO)
Catalase
N-(2-hydroxy-3-sulfopropyle)-3,5-dimethxyaniline

**Substrates**

Peroxidase
4-aminoantipyrin
Good buffer
Sodium azide
Detergent

**Calibrator**

Cholesterol  52 mg/dL
**Assay Procedure**

1- We brought reagents and the cuvette to 37°C.
2- Enzyme reagent (0.75 mL) was pipetted in each labeled tube.
3- Serum (0.01 mL) was pipetted in sample tube.
4- Standard (0.01 mL) was pipetted in standard tube.
5- The tubes were mixed gently and incubated for 5 minutes at 37°C.
6- After 5 minutes we added 0.25 mL of substrate and incubate it for 5 minutes at 37°C.
7- After incubation we read the absorbance of calibrator and samples against reagent blank at 578 nm by using spectrophotometer.

**HDL levels calculation**

The concentration was measured using the following formula

\[
\frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} (52\text{mg/dL}) = \text{mg/dL}
\]

**Normal range values**

35-60 mg/dL

**Quality Control**

All human serum based control sera with HDL values determined by this method can be employed.

**3.5.5 Triglycerides Test**

**Reaction Principle**

The test used is an enzymatic colorimetric test for triglyceride with Lipid Clearing Factor (LCF). Triglycerides determination depends on the hydrolysis by lipase. The indicator quinoneimine is formed from hydrogen peroxide, 4-aminoantipirina and 4-chlorophenol. According to Szasz method.

\[
\text{Triglycerides} \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acid}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}
\]

\[
\text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-aminoantipirina} \xrightarrow{\text{POD}} \text{quinoneimine} + \text{HCL} + 2\text{H}_2\text{O} + 4\text{-chlorophenol}
\]
**Content of the kit**

Tampon pipes pH 7.5 50 m mole /L  
4-chlorophenol 5 m mole /L  
4-aminoantipirina 0.25 m mole /L  
Magnesium 4.5 m mole /L  
ATP 2 m mole /L  
Lipase \( \geq 1.3 \text{ U/mL} \)  
Peroxidase (POD) \( \geq 0.5 \text{ U/mL} \)  
Glycerol kinase (GK) \( \geq 0.4 \text{ U/mL} \)  
Glycerol-3-phosphate oxidase (GPO) \( \geq 1.5 \text{ U/mL} \)

**Standard**

Triglycerides 200 mg/dL

**3.9.3 Assay Procedure**

1- Reagents and samples were brought to room temperature.
2- Triglyceride reagent (1.0 mL) was pipetted in each labeled tube.
3- Serum (0.01mL) was pipetted into sample labeled tube.
4- Standard (0.01mL) was pipetted into standard labeled tube.
5- Tubes were incubated for exactly 10 minutes at room temperature.
6- The absorbance (A) of the samples and the standard was read at 546 nm against the reagent blank by using spectrophotometer.

**Triglycerides levels calculation**

The concentration was calculated according to the following formula

\[
\frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} \times (200) = \text{mg/dL}
\]

**Quality Control**

The use of standard to calculate results to obtain accuracy independent of the system and instrument used. To ensure adequate quality control (QC), each run should include a set of controls

**3.5.6 LDL levels calculation**

The concentration was calculated according to the following formula

\[
\text{Cholesterol} - \text{Triglycerides}/5 - \text{HDL} = \text{mg/dL}
\]
3.5.7 Trace Elements Analysis by Atomic Absorption

To measure the serum levels for trace elements, serum samples were thawed and directly diluted for the determination of the trace elements. For zinc and copper, serum samples were diluted 50 times in 0.1N nitric acid. Levels were determined by an atomic absorption spectrophotometry (Perkin Elmer, A Analyst 100-Germany).

Atomic absorption spectrophotometry (AAS)

Atomic absorption spectrophotometry is commonly used in many analytical laboratories for determination of trace elements in serum sample, water samples and in acid digests of sediment or biological tissues. Zinc and copper, were measured in serum samples.

Principle

While a sample is being aspirated into a flame, a light-beam is directed through the flame into a monochromator and onto a detector that measures the amount of light absorbed by the atomized element in the flame. A source lamp composed of the element of interest is used because each element has its own characteristic wavelength. This makes the method relatively free from spectral or radiation interferences. The amount of energy at the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample over a limited concentration range. Most atomic absorption instruments are also equipped for operation in an emission mode.

Reagents

Metal-free water was essentially used for the preparation of all reagents. Hydrochloric acid and nitric acid, HNO₃, (analytical grade) was used for standard preparation and for digestion methods.

Preparation of standards

Standard solutions of known metal concentrations were prepared in water with a matrix similar to the tested samples. Standards were bracket the expected sample concentration and fall within the method's working range. Very dilute standards having concentrations of at least 0.05 mg L⁻¹ were prepared daily from fresh standard stock solutions. If sample digestion is used, standards should be
carried through the same digestion procedures. The standard stock solutions described below have a concentration of 1000 mg L\(^{-1}\).

**Zinc**: 1.000 g zinc metal was dissolved in 20 ml 1:1 HCl and diluted up to 1,000 ml with water.

**Copper**: 0.100 g copper metal was dissolved in 2 ml concentrated HNO\(_3\), then 10 ml of concentrated HNO\(_3\) was added and the solution mixture was diluted up to 1,000 ml with water.

**Procedure**

It is not possible to provide an operating procedure that would be correct for all atomic absorption spectrophotometers because of differences between models of instrument. The manufacturer’s operating manual should be followed. A general procedure contains three components as described below.

**Adjustment of the Instrument:**
1. A hollow cathode lamp for the desired element was installed in the instrument and the wavelength dial was set to the appropriate setting for the element.
2. The slit width was set according to the manufacturers suggested value for the element being measured.
3. The instrument then turned on and the lamp current was adjusted to the level suggested by the manufacturer.
4. The instrument then warmed up, 10 - 20 minutes, and current readjusted as necessary.
5. The wavelength dial was adjusted until optimum energy gain is obtained.
6. The lamp was aligned in accordance with the directions in the operating manual.
7. The suitable burner head was installed and its position was adjusted.
8. The air was then turned on and its flow was adjusted to the rate recommended to give maximum sensitivity for the metal being measured.
9. Acetylene then turned on and its flow was adjusted to recommended rate, then ignited and the flame was allowed a few minutes to stabilize.
10. A blank of deionized water that has been given the same treatment and acid concentration as the standards and samples was aspirated and the reading adjusted to zero.

11. A Standard solution was aspirated and the aspiration rate was adjusted to obtain maximum sensitivity.

12. The burner was adjusted horizontally and vertically to obtain maximum response.

**Preparation of the calibration curve**

1. At least five concentrations of each metal ion standard solutions were selected to perform a calibration curve. There should be one concentration greater and one less than that expected in the sample(s).

2. A blank was aspirated and adjusted to the zero value.

3. Each standard was aspirated in turn into the flame and the absorbance was recorded.

4. A calibration curve was performed by plotting the absorbance of the standards against their concentrations. This step is not necessary for instruments with direct concentration readout.

**Analysis of samples**

1. The nebulizer was rinsed by aspirating with water containing 1.5 ml HNO₃ per liter. The blank was atomized and set to the zero value.

2. Samples were atomized and there absorbance were determined.

2. Lamps were changed and the procedure repeated for each element.

**Calculations**

Determination appropriate of the concentration of each metal ion, were based on the calibration curves. Results for trace elements were calculated in µg L⁻¹ while, in mg L⁻¹ for the more common metals. Concentrations may be read directly from instruments with a direct readout capability. If a sample has been diluted, appropriate dilution factor were applied. The recommended wavelengths for the metal ions determined are given in the following table:

<table>
<thead>
<tr>
<th>Metal</th>
<th>Selected wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>213.8 nm</td>
</tr>
<tr>
<td>Copper</td>
<td>324.8 nm</td>
</tr>
</tbody>
</table>
3.6 Statistical Analysis

All experimental data were expressed in mean and standard deviation and data obtained in the study and control group were compared by two – tailed t-test for unpaired data. Trace elements in the study group as a whole analyzed by means of SPSS (Statistical Package for Social Science) version 8 for windows software package for multiple comparisons and Pearson's coefficient (r) calculated to determine associations between these parameters. P (probability) < 0.05 was considered significant.
4.1 Diabetes Group
Fifty two diabetic patients and fifty two healthy persons defined by clinical examination and with no history of any disease were compared for glucose level, trace elements "copper and zinc" and lipid profile. The age of each group was between 40 and 60 years.

Age
The mean age and standard deviation of the control group was (46.7±5.6) years, while the mean age and standard deviation of the diabetic group was (49.9±6.2) years. Despite that the mean age of the diabetic group is greater than that of the normal group; the mean age difference was significant (p < 0.05) (Table 4.1).

Table 4.1 Comparison between the age of diabetic group with the age of control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group N=52 Mean±SD</th>
<th>diabetic group N=52 Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.7±5.6</td>
<td>49.9±6.2</td>
<td>&lt; 0.05 *</td>
</tr>
</tbody>
</table>

All values were expressed as mean ±SD
* mean difference is significance at p < 0.05

Glucose analysis
Glucose levels were measured in the diabetic group and the control group. The mean and SD of glucose in control group was (93.5±12.1) , which was lower than that of the diabetic group (213±66.3). The mean glucose difference was found to be significant (p<0.05) (Table 4.2).

Lipid profile analysis

Cholesterol
Cholesterol level was measured in the diabetic group and the control group. The mean and SD of cholesterol in control group was (156±35) and was found to be
lower than that of the diabetic group (170±33.3) however the mean cholesterol difference was significant (p < 0.05) (Table 4.2).

**Triglycerides**

Triglyceride levels were measured in the diabetic group and the control group. The mean and SD of triglyceride in control group was (151±29.1) which was lower than that of the diabetic group (161±23.3). The mean triglycerides difference was significant (p<0.05) (Table 4.2)

**High Density Lipoprotein (HDL)**

HDL level was measured in the diabetic study group and the control group. The mean and SD of HDL in control group was (50.6±11.9) which was higher than that of the diabetic group (42.41±8.25). The mean HDL difference was significant (p<0.05) (Table 4.2).

**Low Density Lipoprotein LDL**

LDL was measured in the diabetic group and the control group. The mean and SD of LDL in normal group was (73.6±36.2) which was lower than that of the diabetic group (95.3±36.3). The mean LDL difference was significant (p <0.05) (Table 4.2).

**Table 4.2** Comparison between glucose and lipid profile of diabetic group with control group

<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>Control group N=52 Mean±SD</th>
<th>diabetic group N=52 Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td>93.5±12.1</td>
<td>213±66.3</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>156±35</td>
<td>170±33.3</td>
<td>&lt;0.05 *</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>151±29.1</td>
<td>161±23.3</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>HDL</td>
<td>50.6±11.9</td>
<td>42.41±8.3</td>
<td>&lt;0.05 *</td>
</tr>
<tr>
<td>LDL</td>
<td>73.6±36.2</td>
<td>95.3±36.3</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

All values were expressed as mean ±SD

* mean difference is significance at p < 0.05
Trace Eléments

Serum levels of the trace elements, copper and zinc, were measured in the control group and diabetic group. Copper (Cu) mean concentration was significantly higher than control group (P < 0.01), also zinc (Zn) mean concentration was significantly higher than control group (P < 0.01) (Table 4.3).

**Table 4.3** Comparison between serum trace elements of diabetic group with control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group N=52 Mean±SD</th>
<th>N=52 diabetic group Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (µg/L)</td>
<td>45.3±18.1</td>
<td>68.8±37.9</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Zinc (Mg/L)</td>
<td>21.8±6.6</td>
<td>28.3±15.2</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

All values were expressed as mean±SD

** Highly significance at P<0.01

Corrélation Analysis

Correlation analysis in the diabetic group was done between trace elements, glucose and lipid profile.

**Copper Correlation**

As shown in table 4.4, in diabetic group there was no correlation between copper and cholesterol or HDL (p > 0.05), however a strong positive correlation exists with glucose level (P < 0.001) (Figure 4.1) and triglycerides (P<0.05) (Figure 4.2), while there is a weak inverse correlation found with LDL (p < 0.05) (Figure 4.3). Copper correlates well with zinc (p<0.001) (Figure 4.4).
Table 4.4 Correlation between copper levels and glucose, lipid profile and zinc in diabetic group.

<table>
<thead>
<tr>
<th>Diabetic group</th>
<th>Pearson correlation</th>
<th>P value</th>
<th>Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age year</td>
<td>0.135</td>
<td>0.339</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.435**</td>
<td>0.001</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>-0.254</td>
<td>0.069</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.339*</td>
<td>0.014</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.039</td>
<td>0.782</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>-0.289*</td>
<td>0.038</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>0.657**</td>
<td>0.000</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

**Correlation is highly significant at P<0.001 level
*Correlation is significant at P<0.05 level

Figure 4.1 Correlation between copper levels and glucose levels in diabetic
Figure 4.2 Correlation between copper levels and triglyceride levels in diabetics

Figure 4.3 Correlation between copper levels and LDL levels in diabetics
**Zinc Correlation**

As shown in table 4.5 in diabetic group there was no correlation between zinc and glucose, triglycerides and HDL of diabetic group (p > 0.05), while there was an inverse correlation was found with cholesterol (P<0.01) *(Fig 4.5)* and LDL (P<0.01) *(Fig 4.6)*. There exists also good correlation with copper (p <0.001) *(Fig 4.4)*.

**Table 4.5** Correlation between zinc in diabetic group with age, glucose, lipid profile and copper.

<table>
<thead>
<tr>
<th>Diabetic group</th>
<th>Pearson correlation</th>
<th>P value</th>
<th>Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>0.116</td>
<td>0.414</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.089</td>
<td>0.529</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>-0.432**</td>
<td>0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.222</td>
<td>0.114</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.025</td>
<td>0.860</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>-0.443**</td>
<td>0.001</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Copper (µg/L)</td>
<td>0.657**</td>
<td>0.000</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

*Figure 4.4* Correlation between zinc and copper in diabetic group
Figure 4.5 Correlation between zinc and cholesterol in diabetic group

Figure 4.6 Correlation between zinc and LDL in diabetic group
4.2 Hypertensive Group

Fifty two hypertension patients and fifty two healthy persons defined by clinical examination and with no history of any disease were compared by glucose level, trace elements "copper, zinc" and lipid profile. The age of each group was between 40 and 60 years.

**Age**

The mean age and standard deviation of the control group was (46.7±5.6) years, and the mean age and standard deviation of the hypertensive group was (49.9±6.2) years. However the mean age difference was significant (p < 0.05) (Table 4.6).

**Table 4.6** Comparison between the ages of hypertensive group with the age of control group

<table>
<thead>
<tr>
<th>Parameter (years)</th>
<th>Control group N=52 Mean±SD</th>
<th>hypertensive group N=52 Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46.7±5.6</td>
<td>49.9±6.2</td>
<td>&lt; 0.05 *</td>
</tr>
</tbody>
</table>

All values were expressed as mean±SD

* mean difference is significance at p < 0.05

**Glucose Analysis**

Glucose levels were measured in the hypertensive group and the control group. The mean and SD of glucose in normal group was (93.5±12.1) which was lower than hypertensive group (99.5±21.1). The mean glucose difference was not significant (p > 0.05) (Table 4.7).

**Lipid profile analysis**

**Cholesterol**

Cholesterol levels were measured in the hypertensive group and the control group. The mean and SD of cholesterol in normal group was (156±35) which was lower than hypertensive group (183±36.6). The mean cholesterol difference was significant (p < 0.05) (Table 4.7).
**Triglycerides**
Triglyceride levels were measured in the hypertensive group and the control group. The mean and SD of triglyceride in normal group was (151±29.1) which was lower than hypertensive group (167±58.2). The mean triglycerides difference was not significant (p>0.05) *(Table 4.7).*

**High Density Lipoprotein HDL**
HDL level were measured in the hypertensive group and the control group. The mean and SD of HDL in normal group was (50.6±11.9) which was higher than hypertensive group (45.6±9.2). The mean HDL difference was significant (p>0.05) *(Table 4.7).*

**Low Density Lipoprotein LDL**
LDL level was measured in the hypertensive group and the control group. The mean and SD of LDL in normal group was (73.6±36.2) which was lower than study group (103±35.5). The mean LDL difference was significant (p < 0.05) *(Table 4.7)*

*Table 4.7* Comparison between glucose and lipid profile of hypertensive group with control group

<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>Control group N=52 Mean±SD</th>
<th>hypertensive group N=52 Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>93.5±12.1</td>
<td>99.5±21.1</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>156±35</td>
<td>183±36.6</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>151±29.1</td>
<td>167±58.2</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>HDL</td>
<td>50.6±11.9</td>
<td>45.6±9.2</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>LDL</td>
<td>73.6±36.2</td>
<td>103±35.5</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

All values were expressed as mean±S

* mean difference is significance at p < 0.05
Trace elements
Trace elements (copper and zinc) were measured in the serum of control group and hypertensive group. The mean levels of copper and zinc in hypertensive group was greater than normal group as shown in the table 4.8. Copper (Cu) mean concentration was higher than control group and not significant, (P > 0.05). Zinc (Zn) mean concentration was higher than control group and significant (P< 0.05).

Table 4.8 Comparison between serum trace elements of hypertensive group with control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group N=52 Mean±SD</th>
<th>hypertensive group N=52 Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper(µg/L)</td>
<td>45.3±18.1</td>
<td>52.4±23.5</td>
<td>&gt; 0.05 NS</td>
</tr>
<tr>
<td>Zinc(mg/L)</td>
<td>21.8±6.6</td>
<td>25.3±8.3</td>
<td>&lt; 0.05 *</td>
</tr>
</tbody>
</table>

All values were expressed as mean±SD

NS (non significant)  P > 0.05.

* mean difference is significance at p < 0.05

Correlation Analysis
Correlation analysis of hypertensive group done between trace elements, age, glucose and lipid profile.

Copper Correlation
As shown in Table 4.9 there was no correlation between copper in hypertensive group and glucose level, cholesterol ,triglycerides ,HDL in hypertensive group , also no  correlation found with LDL(p>0.05).There was no correlation with zinc(p >0.05)
Table 4.9 Correlation between copper in hypertensive group with glucose, lipid profile and zinc.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson correlation</th>
<th>P value</th>
<th>Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>-0.004</td>
<td>0.977</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.207</td>
<td>0.144</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.010-</td>
<td>0.944</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>-0.0109</td>
<td>0.447</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>-0.196</td>
<td>0.167</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>0.079</td>
<td>0.581</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>0.121</td>
<td>0.398</td>
<td>&gt;0.05 NS</td>
</tr>
</tbody>
</table>

NS (non significant) P > 0.05.

Zinc Correlation
As shown in table 4.10 there were no correlation between zinc in hypertensive group and glucose level (P>0.05), no relation found with cholesterol (P <0.05), no relation with triglycerides (P> 0.05), no relation with HDL, also no correlation found with LDL (p>0.05). There was no correlation with copper (p >0.05) (Table 4.10).

Table 4.10 Correlation between zinc in hypertensive group with age, glucose and lipid profile and zinc.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson correlation</th>
<th>P value</th>
<th>Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>0.162</td>
<td>0.260</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.114</td>
<td>0.424</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.041-</td>
<td>0.773</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.019</td>
<td>0.893</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.154-</td>
<td>0.280</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>-0.009</td>
<td>0.952</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Copper (µg/L)</td>
<td>0.121</td>
<td>0.398</td>
<td>&gt;0.05 NS</td>
</tr>
</tbody>
</table>
4.3 Diabetic Hypertensive Group

Fifty two diabetic hypertensive patients and fifty two healthy people defined by clinical examination and with no history of any disease were compared by glucose level, trace elements (copper, zinc) and lipid profile. The age of each group between 40 and 60 years.

**Age**

The mean age and standard deviation of the control group was (46.7±5.7) years. The mean age and standard deviation of the diabetic hypertensive group (52.6±6.3) years. The mean age of study group was higher than normal group, the mean age difference was significant (p <0.05) (Table 4.11).

Table 4.11 Comparison between the age of diabetic hypertensive group with the age of control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group N=52 Mean±SD</th>
<th>Diabetic hypertensive group N=52 Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.7±5.6</td>
<td>52.6±6.3</td>
<td>&lt;0.05 *</td>
</tr>
</tbody>
</table>

All values were expressed as mean±

* mean difference is significance at p < 0.05

**Glucose analysis**

Glucose level was measured in the diabetic hypertensive group and the control group. The mean and SD of glucose in control group was (93.5±12.1), which was lower than study group (213±87.4). The mean glucose difference was found to be significant (p<.05) (Table 4.12).

**Lipid profile analysis**

**Cholesterol**

Cholesterol level was measured in the diabetic hypertensive group and the control group. The mean and SD of cholesterol in control group (156±35) was found to be lower than diabetic hypertensive group (225±75.5) and the mean cholesterol difference was significant (p < 0.05) (Table 4.12).
**Triglycerides**

Triglyceride levels were measured in the diabetic hypertensive group and the control group. The mean and SD of triglyceride in control group was (151±29.1), which was lower than diabetic hypertensive group (183±16.5). The mean triglycerides difference was not significant (p > 0.05) (Table 4.12)

**High Density Lipoprotein (HDL)**

HDL level was measured in the study group and the control group. The mean and SD of HDL in control group was (50.6±11.9) which was higher than study group (49.2±16.3). The mean HDL difference was not significant (p>0.05) (Table 4.12).

**Low Density Lipoprotein LDL**

LDL was measured in the diabetic hypertensive group and the control group. The mean and SD of LDL in normal group was (73.6±36.2) which was lower than diabetic hypertensive group (133±53.2). The mean LDL difference was significant (p < 0.05) (Table 4.12).

**Table 4.12** Comparison between glucose and lipid profile of diabetic hypertensive group with control group

<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>Control group N=52 Mean±SD</th>
<th>diabetic hypertensive group N=52 Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>93.5±12.1</td>
<td>213±87.4</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>156±35</td>
<td>225±75.5</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>151±29.1</td>
<td>183±16.5</td>
<td>&gt; 0.05 NS</td>
</tr>
<tr>
<td>HDL</td>
<td>50.6±11.9</td>
<td>49.2±16.3</td>
<td>&gt; 0.05 NS</td>
</tr>
<tr>
<td>LDL</td>
<td>73.6±36.2</td>
<td>133±53.2</td>
<td>&lt; 0.05*</td>
</tr>
</tbody>
</table>
Trace Elements

Serum levels of the trace elements, copper and zinc, were measured in the control group and diabetic hypertensive group. Copper (Cu) mean concentration was significantly higher than control group (P < 0.05) but zinc (Zn) mean concentration was higher than control group. The mean Zn difference was not significant (P > 0.05) (Table 4.13) (Figure 4.7).

Table 4.13 Comparison between serum trace elements of diabetic hypertensive group with control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group N=52 Mean±SD</th>
<th>diabetic hypertensive group N=52 Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (µg/L)</td>
<td>45.3±18.1</td>
<td>61.1 ±28.6</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>21.8±6.64</td>
<td>23.4±10.0</td>
<td>&gt;0.05 NS</td>
</tr>
</tbody>
</table>

All values were expressed as mean±SD

mean difference is significance at p < 0.05
NS (non significant) P > 0.05

Correlation Analysis

Correlation analysis of study group was done between trace elements, age, and glucose and lipid profile.

Copper Correlation

As shown in Table 4.14. In diabetic hypertensive group there was no correlation between copper, glucose level, cholesterol, triglycerides and LDL (P > 0.05), but presence of positive correlation with HDL (p<0.05) (Figure 4.8). Copper also correlates well with zinc (p<0.05) (Figure 4.7).
Table 4.14 Correlation between copper in diabetic hypertensive with age, glucose, lipid profile and zinc.

<table>
<thead>
<tr>
<th>Diabetic hypertensive group</th>
<th>Pearson correlation</th>
<th>P value</th>
<th>Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>-0.023</td>
<td>0.871</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.154</td>
<td>0.274</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.243</td>
<td>0.083</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>-0.059</td>
<td>0.678</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.278*</td>
<td>0.048</td>
<td>&lt;0.05 *</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>-0.094</td>
<td>0.507</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>0.320*</td>
<td>0.021</td>
<td>&lt; 0.05 *</td>
</tr>
</tbody>
</table>

Figure 4.7 Correlation between zinc and copper in diabetic hypertensive group
Figure 4.8 Correlation between copper and HDL in diabetic hypertensive group
**Zinc Correlation**

As shown in Table 4.15 In diabetic hypertensive group there was no correlation between zinc and glucose of diabetic hypertensive group level, triglycerides ,HDL and LDL, while there was positive correlation with cholesterol (P < 0.05) (Figure 4.9) . Also good correlation with copper (p < 0.05) was existed (Figure 4.7).

**Table 4.15** Comparison between zinc in diabetic hypertensive with age, glucose, lipid profile and zinc.

<table>
<thead>
<tr>
<th>diabetic hypertensive group</th>
<th>Pearson correlation</th>
<th>P value</th>
<th>Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>-0.080</td>
<td>0.574</td>
<td>&gt;.05 NS</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.125</td>
<td>0.377</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.368**</td>
<td>0.007</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.111</td>
<td>0.431</td>
<td>&gt;0.05 NS</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.181</td>
<td>0.200</td>
<td>&gt;.05 NS</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>-0.051</td>
<td>0.719</td>
<td>Other &gt;0.05 NS</td>
</tr>
<tr>
<td>Copper(µg/L)</td>
<td>0.320*</td>
<td>0.021</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

All values were expressed as mean ±SD

NS (non significant) P>0.05.

**Correlation is good significant at P<0.01 level**

- Correlation is significant at P<0.05 level
Figure 4.9 Correlation between zinc and cholesterol in diabetic hypertensive group
Chapter-5
Discussion

In recent years, chronic diseases such as diabetes and hypertension have been shown to be a major cause of death worldwide (1). The prevalence of these diseases in developed countries has reached immense proportions which represent a major problem. Both diabetes and hypertension are considered multifactorial and the physiological role of copper and zinc has been implicated in both diabetes and hypertension (89,76).

The present study was designed to evaluate serum Cu and Zn in type II diabetes, hypertension and diabetic hypertensive patients. Also correlations have been made between the serum levels of copper and zinc in these groups and serum cholesterol, triglycerides, HDL, LDL and glucose levels.

5.1 Copper in diabetic patients

The results have shown that copper levels are increased in diabetic patients. The increased level of copper in the diabetic patients agrees with other studies (52, 89,90).

The increase in glucose levels in diabetics (Hyperglycemia) was suggested to enhance oxidative stress in cells and tissues. This is thought to occur either by loss of anti-oxidant mechanisms or due to an increase in the oxidants (such as free radicals) (60).

Oxidative stress is thought to impair the function of islet and could lead to insulin resistance and vascular diseases (40,41,42). High levels of glucose in diabetic patients could lead to increased levels of oxidative stress and cell damage(44). Therefore oxidative stress increases the need for antioxidant co-factors such as vitamins. The control of blood glucose and reduction of hyperglycemia, therefore reduce the levels of oxidative stress and improve the
function of beta cells, vascular endothelial cells, fat and muscle cells, and platelets (34,35).

The increase in the Cu ion levels in patients with DM might be attributed to hyperglycemia that may stimulate glycation which result in the formation of hydrogen peroxide. The formation of hydrogen peroxide could cause the oxidation of the superoxide radical at higher rates to produce hydroxide radical. This free radical is the most highly reactive oxidant, leading to tissue damage (74).

The increase of hydrogen peroxide could also lead to decreased activity of Cu-Zn SOD, and release of copper ions, therefore acceleration also of oxidative stress. The SOD activity has not been studied in our research, however the SOD activity has been found to consistently increase in diabetic patients (61).

5.1.1 Relation between copper and lipid profile in diabetics

Our results have shown that the levels of cholesterol, triglycerides and LDL in diabetics are increased, while the levels of HDL decreased. These results are typical diagnostic findings of type II diabetics (91). Copper also has been reported to have insulin-like activity and promotes lipogenesis (86).

The results agreed with other studies that have shown total cholesterol and triglyceride levels were moderately elevated and serum (HDL-Ch) levels where lower than normal person and (LDL-Ch) were increased (92).

The increased cholesterol levels may be attributed partly to alternations in the genetic characters; also high intakes of total fat have increased serum cholesterol. Since most of cholesterol in the plasma is carried by LDL, an increase in LDL level directly may lead to an increase in total cholesterol (93).

The increase of LDL seems to be atherogenic and may occur in the absence of hyperlipidemia. In addition, a portion of the plasma LDL undergoes glycosylation, which may increase susceptibility to oxidative stress (91).
Part of the correlations made in our study has focused on the relation between the levels of trace elements (Cu and Zn) with the glucose and lipid profile in the diabetics.

We have found a positive correlation between Cu and triglycerides and a weak negative correlation with LDL; however no correlation exists with cholesterol and HDL levels. This contradicts other finding which has shown positive correlation between the levels of LDL and copper levels in diabetics (92). This positive correlation has been attributed to the lipid peroxidation in the presence of high levels of copper which results in the increase in the LDL and triglycerides and decrease in the HDL (94). Despite the finding in our study that both copper and LDL are increased in diabetics, the presence of a negative correlation between LDL and copper levels seems to be almost insignificant as p value is 0.038.

The absence of any correlation between copper and LDL explains the absence of any correlation with cholesterol.

In the present study strong correlation between glucose and Cu in diabetes patient (p<0.001). Because glucose levels rise with the poor control of diabetes mellitus, and increase levels of Cu supports the view that Cu is an important marker for oxidative stress (7,93).

5.2 Zinc levels in diabetics

Zinc levels in diabetics in our study seem to be higher than control group. It is worth mentioning that despite the finding in many studies that zinc is decreased (57), also our result agree with other studies that shown increased Zn in diabetes mellitus (94,95,96).

The increase in zinc levels in diabetics could be explained by the finding that oxidative stress in diabetics could lead to destruction of β-cells, therefore to
the release of high amounts of zinc from the cells into blood stream, therefore increase in zinc levels in serum occurs (7, 57), the increase of plasma zinc can also reflect a deficient storage or a chronic hyper secretion of insulin in hyperglycemic patients (96).

Despite the increase in the zinc levels in diabetics, there was no correlation between Zn and glucose. The absence of any correlations between zinc and glucose is not restricted to studies which show an increase in the levels of glucose (94), but also in studies which have shown a decrease in the levels of glucose (37).

5.2.1 Zinc and lipid profile in diabetics

Correlations made between zinc and lipid profile in diabetics, indicated the existence of a strong negative correlation between Zn and cholesterol and LDL (p<0.01). Despite the absence of correlations made between zinc and lipid profile in diabetics in some studies (94), in one study which have investigated the correlation between zinc and lipid profile in acute myocardial infarction patients, it was found that zinc correlates well with LDL and cholesterol (92).

Our results demonstrate the importance of correlation studies rather than mean values to investigate the significance of trace elements in diabetes and other diseases. The negative correlation between zinc and both cholesterol, and LDL signifies the importance of zinc in oxidative stress in cases of diabetes. Zinc is thought here to improve the antioxidant activities and therefore to reduce LDL and cholesterol levels.

Therefore serum levels of zinc in diabetes are not of any value to evaluate the oxidative stress in diabetics.

Despite the findings that copper and zinc antagonize each other, intestinal absorption in diabetics it was found that Cu and Zn are correlated well with each
other. This implies that the physiological control of the levels of these elements is not only occurs in the intestine (94).

5.3 Hypertension

5.3.1 Cu and Zn in hypertensive patients

Our results have shown that hypertensive patients have higher levels of Cu and Zn than control group. The results are compatible with other results that shown no significant difference between hypertensive and normotensives in the mean levels of Zn and Cu as well as in Zn- or Cu-dependent enzymes, though higher levels of serum copper were associated with increased risk of hypertension (87). Inverse correlations between blood pressures and serum Zn were observed. These findings give further support to the hypothesis that an imbalance of Zn and Cu bioavailability may be associated to hypertensive condition (91).

Our results therefore confirm the important of the trace elements i.e. Cu and Zn and the etiology and prognosis of hypertension. Despite the great evidence which supports this work, in many studies which have found that essential hypertension patients had higher Zn concentrations (P < 0.001) and Cu concentrations (P < 0.005) (97,98).

Copper deficiency depresses Cu-Zn SOD activity and prostacyclin synthesis in the aorta (92), as well as increases the susceptibility of lipoproteins and heart tissue to peroxidation, providing strong evidence that copper plays a vital role in the protection of the cardiovascular system from free radical mediated damage and disease (99). Thus, it appears clear that adequate copper is vital for optimal functioning of many antioxidant enzymes, both copper dependent and otherwise, in varied organs and tissues (82).

5.3.2 Oxidative stress and hypertension

Recent studies have provided irrefutable evidence that oxidative stress can cause hypertension and hypertension can cause oxidative stress. Essential hypertension is associated with greater than normal lipo-peroxidation and an
imbalance in anti-oxidant status, suggesting that oxidative stress is important in the pathogenesis of essential hypertension or in arterial damage related to essential hypertension (87). Oxidative stress result from accumulation of free radicals (79). These free radicals cause defect to the tissues so presence of antioxidant enzyme such as SOD inhibit the effect of free radicals (74).

It is now well established that several trace elements are essential because of their involvement in the catalytic activity and spatial conformation of antioxidant enzymes. Copper is essential both for its role in antioxidant enzymes, like Cu-Zn SOD and ceruloplasmin, as well as its role in lysyl oxidase, essential for the strength and integrity of the heart and blood vessels (78).

### 5.3.3 Lipid profile in hypertensive patient

In our study the amount of cholesterol and the amount of triglyceride increased, also LDL increased but the amount of HDL have decreased. These results agree with many previous research studied that have implicated that cholesterol, triglyceride and LDL-Ch were significantly higher than corresponding values of control (22). When plasma conc. of LDL-Ch elevates this leads to accumulation of cholesterol and therefore formation of atherosclerotic lesion take place of the wall at the vessels (92).

### 5.3.4 Copper and cardiovascular disease

Many studies discuss the relationship between nutrition and cardiovascular disease almost twenty years ago, it was postulated that there is a direct relationship between the level of copper in the human diet and the incidence of cardiovascular disease. Copper has been known to be associated with lipid metabolism since 1973, and research in numerous animal models, including humans, has shown that copper deficiency can significantly increase the plasma cholesterol concentration. Additionally, this increase in cholesterol results in an increase in LDL-Ch and a decrease in HDL-Ch, resulting in an increase in cardiovascular disease risk (80,83).
It has also been demonstrated that copper deficiency significantly increases the susceptibility of lipoproteins and cardiovascular tissues to lipid peroxidation, thus increasing the risk of cardiovascular disease (92).

Recent research has helped to explain this paradox. It has been suggested, for instance, that an elevated serum copper level is an independent risk factor for heart disease (80). Many researchers have considered this elevation of serum copper to play a role in the pathogenesis of cardiovascular disease, although other researchers have strongly disagreed with this hypothesis (77).

A recent animal study, however, seems to have explained this relationship between copper levels and cardiovascular disease. Administration of additional copper resulted in a further increase in serum copper, a significant decrease in serum cholesterol, and an increase and normalization in aorta and liver copper levels. However, instead of increasing the incidence of atherosclerosis, additional copper significantly decreased the incidence of atherosclerosis in the aorta and coronary arteries. Further, it has been shown that excess dietary cholesterol causes cardiovascular disease by lowering the absorption of copper, an effect that is preventable by increasing the copper level in the diet (83).

5.4 Diabetic hypertensive patients

It is well believed that diabetic hypertensive cases could result from hypertension and diabetes and vice versa. In both cases it is expected that progressed cases of oxidative stress could cause damage of both vascular system and the pancreas (100).
5.4.1 Cu and Zn in Diabetic hypertensive patient

Our results have shown higher levels of both Cu and Zn like hypertensive cases but not like diabetes which showed increased levels of Cu but not Zn. This could give us some hint about the prognosis of such cases. Our results also indicate that β-cell are more sensitive to changes in levels of Cu and Zn than vascular cell and therefore to oxidative damage.

Despite the importance of Cu and Zn in protection of cells against oxidative stress (76), it is likely that copper catalyses the production of ROS and therefore initiates oxidative stress and cell damage (61). Increased in the amounts of zinc as explained earlier is thought to result from the destruction of β-cells in the pancreas, therefore release of zinc from its stores.

5.4.2 Lipid profile in Diabetic hypertensive patient

Serum copper levels were found to correlate with serum HDL levels. HDL is known to lower the levels of cholesterol by transporting it back to the liver, also HDL acts to lower the copper induced oxidation of LDL (97). No previous studies has considered the correlation between copper and HDL in diabetic hypertensive patients. No correlation was found between HDL and copper in hypertensive acute myocardial patients (92). The absence of any agrees with the findings in the diabetic group and the hypertensive group.

Serum cholesterol levels were also found to correlate positively with serum zinc levels in this group. A previous study has postulated that increased levels of zinc lowers the levels of HDL and increase the levels of LDL, and therefore cholesterol (98). It has been assumed that high levels of zinc could lower the absorption of copper, which increases the levels of HDL. However this does not agree with our findings in our studied group, where we found a positive correlation between the serum levels of copper and zinc but not an inverse one. This however could be explained by the difference in the studied population, which indicates that the correlation between trace elements and serum parameters depends on the pathological state of the patient, which means on other fact.
Chapter-6
Conclusions and Recommendations

6.1 Conclusions

Throughout this research we focused on the changes in the levels of serum levels of copper and zinc as well as sugar and lipid profile in three different groups (diabetic, hypertensive, and diabetic hypertensive groups).

In diabetic group we have found that the serum levels of zinc and copper are increased, in addition to LDL, triglycerides and cholesterol.

Correlation analysis has shown that increased levels of glucose are accompanied by increased levels of copper but not zinc. Consistently there was also a positive correlation between copper and triglycerides and inversely correlated with LDL.

Zinc serum levels seem to correlate with both cholesterol and LDL. This signifies the importance of zinc in diabetics as anti-oxidant in lowering the levels of LDL and cholesterol.

In hypertensive group we found that the serum levels of both copper and zinc are increased, also lipid profile was significantly different from control lipid profile.

Unlike the diabetic group both zinc and copper did not show any correlation with any of the lipid parameters, which signifies the difference in the pathological changes in diabetes and hypertension.

In diabetic hypertensive group, like the hypertensive group and unlike the diabetic group, there is a significant increase in the levels of both copper and zinc.

In diabetic hypertensive group, while copper correlates positively with HDL, zinc correlates also positively with cholesterol.

The presence of different correlations between the studied groups implies different roles might copper and zinc play in diabetes and hypertension.
6.2 Recommendations

The suggestive role of zinc in type II diabetes assist in the importance of using zinc as an antioxidant for protecting against type 2 diabetes. Therefore we suggest the supplementation of patients of type 2 diabetes with zinc rich food such as (ginger roots, meat, beef, liver, milk products, whole grains, nuts and cabbage) or a vial of zinc, as explained by the finding that

1. Zinc decreases the amount of glucose in the blood, because zinc inhibits the activity of fructose -1, 6- biphosphate which increases the break down of the glucose (63).
2. Zinc was found to decrease the amount of LDL and cholesterol so this decreases the complication of atherosclerosis.
3. Zinc acts as antioxidant for protection from oxidative stress (47).

Also we suggest to supplement hypertensive patients with zinc and copper since:
1- Zinc inhibits oxidative stress transcription factors and synthesis of interleukin (8).
2- Copper acts as components of Cu-Zn SOD and lysyle oxides which is necessary for strength of blood vessels.

Finally we encourage the diabetic hypertensive patients to be supplemented with copper or supplied their foods by copper such as (chocolate, colas, coffee, tea, seeds, nuts and soy products) because copper correlates well with HDL which carry the cholesterol from the tissues to the liver so decrease the atherogenecity.

In fact, I wish that this study will be followed by further studies such as measurements of Cu -Zn SOD and correlate it with Cu and Zn.
Chapter 7
References


http://womenshealth.about.com/cs/highbloodpressur/a/hypertentionhbp.htm


[68] Richard E.Klabunde,Ph.D.,2004-" Cardiovascular Physiology Concepts "Published by Lippincott Williams & Wilkins.


[94] Osman E., Vliyaoğlu., Levent K., Nuriye U., Nazife K., Baysal K., Ruhan K., Naciye Y., 2004- "Correlations of Serum Cu$^{+2}$, Zn$^{+2}$, Mg$^{+2}$ and HbA$_1c$ in Type 2 and Type 2 Diabetes Mellitus ". Turk. J. of Endo. and Metab. 8(3) Page(s) 75-79.


Appendix 1: UNRWA agreement
Appendix 2: Consent Form

السيد/ السيدة : ..........................................................

بعد التحية,

تقوم باحثة بالجامعة الإسلامية تحت إشراف الطاقم الطبي لعيادة الزيتون في وكالة الغوث بعمل دراسة حول تقييم مستويات مصل النحاس و الخارصين لديكم وقد قامت بجمع عينات دم عشوائية لهذا الغرض لذلك نرجو التكرم منكم بالموافقة على سحب عينة دم (4-3) مل لهذا الغرض.

علما بأنه في حالة الرفض أو القبول لن تتأثر الخدمة الطبية المقدمة إليكم.

شكرى حسن تعاونكم

التوقيع

الباحثة بالجامعة الإسلامية
Appendix 3: Questionnaire

For control group selection the following questions were answered after carrying out blood pressure measurement and blood analysis.

<table>
<thead>
<tr>
<th>No.</th>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Are you smoker?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Are you practice any sports?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Are there any person in your family suffer from hypertension /diabetes mellitus?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Is measurement of the blood pressure greater than 90/140?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Is the person obese? (Body mass &gt;32)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Is the concentration of blood glucose &gt; 110 mg/dl?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Is the concentration of blood cholesterol &gt; 250 mg/dl?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Is the concentration of blood triglyceride &gt; 200 mg/dl?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Is the concentration of blood HDL &gt;50 mg/dl?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Is the concentration of blood LDL &gt; 132 mg/dl?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note:

The criteria of healthy individual are:

Neither smoker or obese
The blood pressure is less than 90/140.
The blood glucose level is less than 110 mg/dl.
The blood cholesterol level is between (150-250) mg/dl.
The blood triglyceride is less than 200 mg/dl.
The blood HDL level is between (35-50) mg/dl.
The blood LDL is less than 132 mg/dl.