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Assessment of Serum Zinc and Copper Levels among Sudanese Patients with Diabetes Mellitus Type 2 in Khartoum state

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Dedication

To my life.....

My Mother that

Her prayers are secrete of my success

To my lovely.....

Brothers and Sisters

To my friends.....

For their patience

Acknowledgments

All thanks to Allah who blessed us with courage for preparation and completion of this projects.

First and foremost, I would to my supervisor ***Dr. Abd Elwahab Abdien Saeed*** *for his help throughout our time in this research.*

Gratefully thanks for my teachers who teach us in different education stages.

All my pure thanks send to all those who helped and supported my and I not mentioned them.

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List of Abbreviation

Abbreviation	Mean
DM	Diabetes Mellitus
GAD	Glutamic Acid Decarboxylase
GMD	Gestational Diabetes Mellitus
FPG	Fasting Plasma Glucose
HBA1C	Glyclated Haemoglobin A1C
HLA	Human Leukocyte Antigen
HNF	Hepatocyte Nuclear Factors
IA2	Islet Antigen 2
MELAS	Mitochondrial Encephalopathy ,Lactic Acidosis, and Stroke-Like Episodes
MODY	Maturity Onset Diabetes of the Young
NGSP	National Glycohemoglobin Standardization Program
NHANES	National Health and Nutrition Examination Survey
OGTT	Oral Glucose Tolerance Test
PG	Plasma Glucose
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
ZAC/HYMAI	ZAC(ZINC FINGER PROTEIN Associated with Apoptosis and Cell Cycle arrest) HYMAI(Imprinted in Hydatidiform Mole)

المستخلص:

مرض السكر هو اضطراب في التمثيل الغذائي للكربوهيرات ،يتميز بارتفاع السكر في الدم . السكر مرض وبائي في معظم البلدان ، في جميع انحاء العالم قدرت نسبة الناس بمرض السكر 150 مليون شخص ز تعداد مرض السكر في السودان حوالي مليون شخص 90% منهم مصابين بمرض السكر النوع الثاني.

قد اجريت هذه الدراسة في ولاية الخرطوم لتقييم العلاقة بين مرض السكري النوع الثاني والعناصر الشحيحة بقياس مستوي الزنك والنحاس في مصل الدم بين السودانين المصابيين بمرض السكر النوع الثاني خلال الفترة من ابريل الي يوليو 2018.

تضمنت الدراسة 40 مريض بالسكري النوع الثاني (25 انثي و15 ذكر) مرضي السكري المصابين بالسرطان، المدخنين، الحوامل، الفشل الكلوي، مدمني الكحول والذين يستخدمون ادوية تحتوي علي الزنك والنحاس تم استبعادهم من الدراسة. مرضي السكري قسموا الي مجموعتين حسب قياس نسبة السكر التراكمي . 30 شخص اصحاء تضمنوا كمجموعة ضابطة.

تم قياس نسبة الزنك والنحاس باستخدام جهاز الامتصاص الذري. وقد تم تحليل النتائج التي تم الحصول عليها باستخدام الحزمة الاحصائية للعلوم الاجتماعية .

واظهرت هذه الدراسة نقصان ضئيل في اجمالي الزنك في مصل الدم بين مرضي السكري النوع الثاني بالمقارنة مع المجموعة الضابطة ، ومن جهة اخري اظهرت الدراسة زيادة كبيرة في النحاس في مصل الدم في كل مجموعة مقارنة مع المجموعة الضابطة.

توصلنا الي ان الزنك في مجموعتي مرضي السكري اقل من المجموعة الضابطة ، وان النحاس في مجموعتي مرضي السكر اعلي من المجموعة الضابطة وكمية الزنك والنحاس تتأثر بنوع المرض وتوجد علاقة ايجابية ضعيفة بين الزنك والسكر التراكمي وعلاقة سلبية ضعيفة بين النحاس والسكر التراكمي.

نحن نقترح باعطاء كمية كافية من هذه المواد في وجبات مرضي السكري فهي مفيدة جدا في ضبط لمرض السكري على المدى الطويل.

Abstract:

Diabetes Mellitus is disorder of carbohydrate metabolism characterized by hyperglycaemia , diabetes is an epidemic disease in most countries ,world wide an estimated 150 million people are affected by diabetes. Diabetes population in sudan is around one million 90% have type 2 diabetes.

This study was conducted at Khartoum state to assess the relationship between diabetes mellitus type 2 and trace elements by measured serum zinc and serum copper levels among Sudanese diabetes mellitus type 2 during period from April to July 2018.

Forty patients with diabetes mellitus type 2 were included for this study (25 female and 15 male). Patients with carcinoma, smokers, pregnancy, renal disease, alcoholism, taking drugs contain zinc and copper were excluded. Diabetes patients were divided into controlled and uncontrolled groups by measured HBA1C. 30 healthy subjects were considered as control group.

Serum zinc and copper levels were analysis using Atomic Absorption Spectroscopy; the obtained results were analyzed using social science software package.

This study showed significant lower of serum zinc in diabetes type 2 patients comparison with control group and on another hand showed significant increase of serum copper diabetes type 2 patients.

We concluded serum zinc significant lower in both diabetes group than healthy group , copper significant high in both diabetes group than healthy group ,gender have significant effect in serum zinc and copper

,weak positive correlation between zinc and HbA1C and weak negative correlation between copper and HbA1C.

We suggested that an adequate supply of these substances in diet of diabetic patients can be beneficial in the long term management.

1. Introduction

Diabetes Mellitus: Is disorder of carbohydrate metabolism, leading to both metabolic and oxidative stress, characterized by hyperglycemia .[1]commonly referred to as diabetes, is group of metabolic diseases in which there are high blood sugar levels over a prolonged period.[2]Symptom of high blood sugar include frequent urination, increased thirst, increased hunger. If left untreated, diabetes can cause much complication. Acute complication can include diabetic ketoacidosis, nonketotic hyperosmolar coma, or death serious long term complication include heart diseases, stork, chronic kidney failure ,foot ulcers and damage of eye .[3]

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced .[4] there are three main type of diabetes mellitus: type 1 DM, type 2DM, gestatinonal diabetes. Prevention and treatment involve maintaining a healthy diet, regular physical exercise, a normal body weight . [5]

As of 2015, an estimated 415 million people had diabetes worldwide[6] with type 2 DM making up about 90% of the case[7] this represents 8.3% of the adult population[8] with equal rates in both women and men as of 2014, trends suggested the rate would continue to rise . [9]

Diabetes at least doubles a person risk of early death [5] From 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from diabetes .[10]

Is an epidemic disease in most countries, worldwide an estimated 150 million people are affected by diabetes, and this number is likely to reach 300 million by the year 2025.

Diabetes type 2: is characterized by insulin resistance with relative insulin deficiency, which may be combined with relatively reduced insulin

secretion.[11] The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor .However the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 DM is common type of diabetes mellitus. This type account for 90%of all diabetic cases and commonly appear in adult, so they are at increased risk of developing macro vascular and micro vascular complication .[12]

In the early of type2, the predominant abnormality is reduced insulin sensitivity, at this stage, high blood sugar can be reversed by a variety of measures and medication that improve insulin sensitivity or reduce the liver glucose production.[13]

Type 2 DM is due primarily to lifestyle factors and genetics a number of lifestyle factors are known to be important to the development of type 2 DM, including obesity, lack of physical activity, poor diet , stress .[7]

In Sudan diabetes is an increasingly important problem, being responsible for 10% of hospital admissions and mortality.[14]

Recently increase in incidence of diabetes mellitus has been observed especially among population indicating that diabetes mellitus is emerging as important health problem .[15]The result of study carried out indicated that diabetes population in Sudan is at around one million, 90%of them have type2diabetes.it also showed a prevalence of 3.4%of type 2 diabetes mellitus.[16]

Zinc is essential for the correct processing, storage, secretion, and action of insulin in beta (β)-cells. Insulin is stored inside secretory vesicles or granules, where two Zn++ ions coordinate six insulin monomers to form the hexameric-structure on which maturated insulin crystals are based. It is also known that like, most other chronic disorders, diabetes increase the excretion of minerals. [16]

The increase in Cu ion levels in patients with diabetes mellitus may be attributed to hyperglycaemia that may stimulate glycation and release of copper ions and this accelerates the oxidative stress, so that, Advanced Glycation End products are formed, that are involved in the pathogenesis of diabetic complications. Also copper have antioxidant function in diabetic patients. Ceruloplasmin and serum albumin are the main Cu binding proteins in plasma and there is some evidence that chronic hyperglycemia can damage the Cu binding properties of both.[16]

1.2. Rationale

Diabetes mellitus is major cause of morbidity in developing countries.

In Sudan diabetes is as increasing problem, being responsible for 10% of hospital admission and mortality.

Measurement of zinc and copper is an important in decrease development of complication of diabetes mellitus, any disturbance in level of both zinc and copper have role in pathogenesis, prognosis of T2DM.

1.3. Objectives

1.3.1. General objective:

To determine Serum zinc and copper level among Sudanese patients with Diabetes mellitus type 2 in Khartoum state.

1.3.2. Specific Objectives:

1. To measure glycated haemoglobin (HbA_{1c}) in diabetes mellitus type 2 patients.
2. To estimate the level of serum zinc and copper in healthy individuals , controlled diabetic type 2 patients and uncontrolled diabetic type 2 patient.
3. To Compare between the Means of serum Zinc ,Copper in controlled Diabetic Patients and in healthy individuals..
4. To Compare between the Means of serum Zinc ,Copper in uncontrolled Diabetic Patients and the in healthy individuals.
5. To compare between the Means of serum copper, zinc in control Diabetic Patients and in uncontrolled Diabetic Patients.
6. To Compare between the Means of serum zinc, copper in Female &Male Patients
7. To correlate between HbA_{1c} with serum Copper in case group.
8. To correlate between HbA_{1c} with serum Zinc in case group.

2. Literature Review

2.1Diabetes Mellitus

2.1.1Definition and Description of Diabetes Mellitus

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the pancreatic β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia .[17]

2.1.2. Symptoms

Marked hyperglycemia includes polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia.[17]

2.1.3. Complications of diabetes

1. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome.

2. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes.[17]

2.1.4. Classification of Diabetes Mellitus and Other Categories of Glucose Regulation

Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class. For example, a person diagnosed with gestational diabetes mellitus (GDM) may continue to be hyperglycemic after delivery and may be determined to have, in fact, type 2 diabetes. Alternatively, a person who acquires diabetes because of large doses of exogenous steroids may become normoglycemic once the glucocorticoids are discontinued, but then may develop diabetes many years later after recurrent episodes of pancreatitis. Another example would be a person treated with thiazides who develops diabetes years later. Because thiazides in themselves seldom cause severe hyperglycemia, such individuals probably have type 2 diabetes that is exacerbated by the drug. Thus, for the clinician and patient, it is less important to label the particular type of diabetes than it is to understand the pathogenesis of the hyperglycemia and to treat it effectively.[6]

2.1.4.1. Type 1 Diabetes (β-Cell Destruction, Usually Leading to Absolute Insulin Deficiency)

a. Immune-Mediated Diabetes

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to GAD (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β. One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. Also, the disease has strong HLA associations, with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective.

In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Immune-mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life.

Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia.

b. Idiopathic Diabetes

Some forms of type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian ancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go.[6]

2.1.4.2. Type 2 Diabetes (Ranging From Predominantly Insulin Resistance with Relative Insulin Deficiency to Predominantly an Insulin Secretory Defect with Insulin Resistance)

This form of diabetes, which accounts for ~90–95% of those with diabetes, previously referred to as non–insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not

occur, and patients do not have any of the other causes of diabetes listed above or below.

Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal.[6]

2.1.4.3. The Risk of Diabetes type 2:

The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type 1 diabetes. However, the genetics of this form of diabetes are complex and not fully defined.[8]

2.1.5 Other Specific Types of Diabetes

2.1.5.1. Gestational diabetes Mellitus

For many years, GDM has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Although most cases resolve with delivery, the definition applied whether or not the condition persisted after pregnancy and did not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. This definition facilitated a uniform strategy for detection and classification of GDM, but its limitations were recognized for many years. As the ongoing epidemic of obesity and diabetes has led to more type 2 diabetes in women of childbearing age, the number of pregnant women with undiagnosed type 2 diabetes has increased.[17]

2.1.5.2. Genetic Defects of the β-Cell

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an autosomal dominant pattern. Abnormalities at six genetic loci on different chromosomes have been identified to date. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1α. A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the β-cell. Thus, glucokinase serves as the “glucose sensor” for the β-cell. Because of defects in the glucokinase gene, increased plasma levels

of glucose are necessary to elicit normal levels of insulin secretion. The less common forms result from mutations in other transcription factors, including HNF-4α, HNF-1β, insulin promoter factor (IPF)-1, and NeuroD1.

Diabetes diagnosed in the first 6 months of life has been shown not to be typical autoimmune type 1 diabetes. This so-called neonatal diabetes can either be transient or permanent. The most common genetic defect causing transient disease is a defect on ZAC/HYAMI imprinting, whereas permanent neonatal diabetes is most commonly a defect in the gene encoding the Kir6.2 subunit of the β-cell K_{ATP} channel. Diagnosing the latter has implications, since such children can be well managed with sulfonylureas.

Point mutations in mitochondrial DNA have been found to be associated with diabetes and deafness. The most common mutation occurs at position 3,243 in the tRNA leucine gene, leading to an A-to-G transition. An identical lesion occurs in the MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like syndrome); however, diabetes is not part of this syndrome, suggesting different phenotypic expressions of this genetic lesion.

Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families, and such traits are inherited in an autosomal dominant pattern. The resultant glucose intolerance is mild. Similarly, the production of mutant insulin molecules with resultant impaired receptor binding has also been identified in a few families and is associated with an autosomal inheritance and only mildly impaired or even normal glucose metabolism .[17]

2.1.5.3. Genetic Defects in Insulin Action

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities

associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Some individuals with these mutations may have acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries. In the past, this syndrome was termed type A insulin resistance. Leprechaunism and the Rabson-Mendenhall syndrome are two paediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance. The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia.

Alterations in the structure and function of the insulin receptor cannot be demonstrated in patients with insulin-resistant lipoatrophic diabetes. Therefore, it is assumed that the lesion(s) must reside in the postreceptor signal transduction pathways.[17]

2.1.5.4. Diseases of the Exocrine Pancreas

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adenocarcinoma that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in β-cell mass. If extensive enough, cystic fibrosis and hemochromatosis will also damage β-cells and impair insulin secretion. Fibrocalculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications identified on X-ray examination. Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy.[17]

2.1.5.5. Endocrinopathies

Several hormones (e.g., growth hormone, cortisol, glucagon, and epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g.,acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved. Somatostatinomas and aldosteronoma-induced hypokalemia can cause diabetes, at least in part, by inhibiting insulin secretion. Hyperglycemia generally resolves after successful removal of the tumor.[17]

2.1.5.6. Drug- or Chemical-Induced Diabetes

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance. In such cases, the classification is unclear because the sequence or relative importance of β-cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells. Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids. Patients receiving α-interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency. is not all-inclusive, but reflects the more commonly recognized drug-, hormone-, or toxin-induced forms of diabetes.[17]

2.1.5.7. Infections

Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In

addition, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease.[17]

2.1.5.8. Uncommon Forms of Immune-Mediated Diabetes

In this category, there are two known conditions, and others are likely to occur. The stiff-man syndrome is an autoimmune disorder of the central nervous system characterized by stiffness of the axial muscles with painful spasms. Patients usually have high titers of the GAD autoantibodies, and approximately one-third will develop diabetes.

Anti-insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby blocking the binding of insulin to its receptor in target tissues. However, in some cases, these antibodies can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia. Anti-insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases. As in other states of extreme insulin resistance, patients with anti-insulin receptor antibodies often have acanthosis nigricans. In the past, this syndrome was termed type B insulin resistance.[17]

2.1.5.9. Other Genetic Syndromes Sometimes Associated With Diabetes

Many genetic syndromes are accompanied by an increased incidence of diabetes. These include the chromosomal abnormalities of Down syndrome, Klinefelter syndrome, and Turner syndrome. Wolfram syndrome is an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of β-cells at autopsy. Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy, and neural deafness.[17]

2.1.6 Diagnostic Criteria for Diabetes Mellitus

For decades, the diagnosis of diabetes has been based on glucose criteria, either the FPG or the 75-g OGTT. In 1997, the first Expert Committee on

the Diagnosis and Classification of Diabetes Mellitus revised the diagnostic criteria, using the observed association between FPG levels and presence of retinopathy as the key factor with which to identify threshold glucose level. The Committee examined data from three cross-sectional epidemiological studies that assessed retinopathy with fundus photography or direct ophthalmoscopy and measured glycemia as FPG, 2-h PG, and A1C. These studies demonstrated glycemic levels below which there was little prevalent retinopathy and above which the prevalence of retinopathy increased in an apparently linear fashion. The deciles of the three measures at which retinopathy began to increase were the same for each measure within each population. Moreover, the glycemic values above which retinopathy increased were similar among the populations. These analyses confirmed the long-standing diagnostic 2-h PG value of ≥200 mg/dL (11.1 mmol/L). However, the older FPG diagnostic cut point of 140 mg/dL (7.8 mmol/L) was noted to identify far fewer individuals with diabetes than the 2-h PG cut point. The FPG diagnostic cut point was reduced to ≥126 mg/dL (7.0 mmol/L) .[9]

2.2. Glycated haemoglobin (haemoglobin A_{1c}):

Is a form of haemoglobin that is measured primarily to identify the three month average plasma glucose concentration. The test is limited to a three month average because the lifespan of a red blood cell is four month (120 days). But RBCs do not all undergo lysis at the same time, so HbA1C is taken as a limited measure of 3 months. it is formed in non enzymatic glycation pathway by haemoglobin exposure to plasma glucose. HbA_{1c} is a measure of the beta –N-1- deoxy fructosyl component of haemoglobin.[18]

Normal level of glucose produce a normal amount of glycated haemoglobin. As the average amount of plasma glucose increases, the fraction of glycated haemoglobin increase in a predictable way.

In diabetes mellitus, higher amount of glycated haemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuropathy, retinopathy.

Hba1c led to change in diabetes treatment and improvement of metabolic control compared to monitoring only of blood glucose. [19]

HbA1C is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a 2- to 3-month period of time. The test plays a critical role in the management of the patient with diabetes, since it correlates well with both microvascular and, to a lesser extent, macrovascular complications and is widely used as the standard biomarker for the adequacy of glycemic management. Prior Expert Committees have not recommended use of the A1C for diagnosis of diabetes, in part due to lack of standardization of the assay. However, A1C assays are now highly standardized so that their results can be uniformly applied both temporally and across populations. In their recent report, an International Expert Committee, after an extensive review of both established and emerging epidemiological evidence, recommended the use of the A1C test to diagnose diabetes, with a threshold of ≥6.5%, and ADA affirms this decision. The diagnostic A1C cut point of 6.5% is associated with an inflection point for retinopathy prevalence, as are the diagnostic thresholds for FPG and 2-h PG. The diagnostic test should be performed using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial reference assay. Point-of-care A1C assays are not sufficiently accurate at this time to use for diagnostic purposes.

There is an inherent logic to using a more chronic versus an acute marker of dysglycemia, particularly since the A1C is already widely familiar to clinicians as a marker of glycemic control. Moreover, the A1C has several

advantages to the FPG, including greater convenience, since fasting is not required, evidence to suggest greater preanalytical stability, and less day-to-day perturbations during periods of stress and illness. These advantages, however, must be balanced by greater cost, the limited availability of A1C testing in certain regions of the developing world, and the incomplete correlation between A1C and average glucose in certain individuals. In addition, the A1C can be misleading in patients with certain forms of anemia and hemoglobinopathies, which may also have unique ethnic or geographic distributions. For patients with a hemoglobinopathy but normal red cell turnover, such as sickle cell trait, an A1C assay without interference from abnormal haemoglobins should be used. For conditions with abnormal red cell turnover, such as anemias from hemolysis and iron deficiency, the diagnosis of diabetes must employ glucose criteria exclusively.

The established glucose criteria for the diagnosis of diabetes remain valid. These include the FPG and 2-h PG. Additionally, patients with severe hyperglycemia such as those who present with severe classic hyperglycemic symptoms or hyperglycemic crisis can continue to be diagnosed when random (or casual) plasma glucose of ≥200 mg/dL (11.1 mmol/L) is found. It is likely that in such cases the health care professional would also measure an A1C test as part of the initial assessment of the severity of the diabetes and that it would (in most cases) be above the diagnostic cut point for diabetes. However, in rapidly evolving diabetes, such as the development of type 1 diabetes in some children, A1C may not be significantly elevated despite frank diabetes.

Just as there is less than 100% concordance between the FPG and 2-h PG tests, there is not full concordance between A1C and either glucose-based test. Analyses of NHANES data indicate that, assuming universal screening of the undiagnosed, the A1C cut point of ≥6.5% identifies one-

third fewer cases of undiagnosed diabetes than a fasting glucose cut point of ≥126 mg/dL (7.0 mmol/L) (However, in practice, a large portion of the population with type 2 diabetes remains unaware of their condition. Thus, it is conceivable that the lower sensitivity of A1C at the designated cut point will be offset by the test's greater practicality, and that wider application of a more convenient test (A1C) may actually increase the number of diagnoses made.[17]

Further research is needed to better characterize those patients whose glycemic status might be categorized differently by two different tests (e.g., FPG and A1C), obtained in close temporal approximation. Such discordance may arise from measurement variability, change over time, or because A1C, FPG, and post challenge glucose each measure different physiological processes. In the setting of an elevated A1C but "non diabetic" FPG, the likelihood of greater postprandial glucose levels or increased glycation rates for a given degree of hyperglycemia may be present. In the opposite scenario (high FPG yet A1C below the diabetes cut point), augmented hepatic glucose production or reduced glycation rates may be present .[10]

2.3 Trace Elements

The metals Mn, Fe, Cu, and Zn and the non-metal Se are considered "trace elements" (TE) because of their essentiality and very limited quantity in humans. The biological activities of Cu, Fe, Mn, and Se are strongly associated with the presence of unpaired electrons that allow their participation in redox reactions. In biological systems these metals are mostly bound to proteins, forming metalloproteins. Many of the metals in metalloproteins are part of enzymatic systems, have structural and storage functions, or use the protein to be transported to their target site in the organism. In humans Mn, Fe, Cu, Zn, and Se accomplish decisive functions to maintain human health. Deficiency in any of these TE leads

to undesirable pathological conditions that can be prevented or reversed by adequate supplementation. In sufficiently nourished persons, supplementation should be carefully controlled, given the toxic effects ascribed to TE when present in quantities exceeding those required for accomplishing their biological functions. The dietary reference intakes provided by national regulatory agencies are guides to define intake, supplementation and toxicity of Mn, Fe, Cu, Zn, and Se, as well other elements considered micronutrients for humans .[20]

2.3.1. Association between diabetes and alteration in metabolism of trace elements:

A number of studies have reported correlation between diabetes and trace elements such as zinc, copper. Scott and Fisher (1938) first recognized the relationship between zinc and insulin .[21] Zinc affects antigenic properties of insulin which leads to hyperglycemia, the copper levels in patients with diabetes mellitus (DM)may be attributed to hyperglycemia that may stimulate glycation and release of copper and these accelerate the oxidative stress .[22]

2.3.2. Oxidative stress and diabetes:

Recently, some evidences suggest that oxidative stress may play an important role in the aetiology of diabetes and diabetes complication .[23]

Oxidative stress is defining 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 complication of diabetes mellitus (DM) has also been attributed to oxidative stress .[24]

The body defence against oxidative stress is accomplished by interconnecting system of antioxidant micronutrients (vitamin and minerals) and enzyme .[25]

While the vitamin acts as donors and acceptor of ROS, minerals regulate activity of the enzyme .[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 peroxide which inactivate SOD.[27] The primary catalytic cellular defence that protects cells and tissue against potentially destructive reaction of superoxide radicals and their derivatives is Cu/Zn –SOD.[28] Decrease activity of the antioxidant enzyme such as (Cu/Zn –SOD) may increase the susceptibility of diabetic patients to oxidative injury. Appropriate support for enhancing antioxidant supplies may help to prevent clinical complication of diabetes mellitus. In view of low activities of SOD in diabetes it was concluded that supplementary trace element such as copper and zinc, the essential component of the enzyme structure may be useful in preventing the development of diabetic complication .[29]

2.3.3. Oxidative stress and chronic complication of type2 diabetes:

Hyperglycemia causes oxidative stress, which increase glycosylation and oxidation of protein involved in the pathogenesis of the complication of diabetes.[30]Development of diabetic complications has been hypothesized to be accelerated by generation of free radical in the cell and tissue.[31]

In diabetes, oxidative stress is due in part to an increased production of plasma free radical concentration and a sharp reduction in antioxidant defences, it has been postulated that free radical production could result in hyperglycemia, hyperinsulinemia and /or insulin resistance, and it was postulated that oxidative stress represents that common pathway through which hyperglycemia and insulin resistance induce depressed insulin action.[32]

Oxidative stress in person with diabetes is also related to decreased antioxidant defences; oxidative stress contributes to impairment of islet

function.[33]Insulin resistance, and micro vascular and macro vascular disease. Diabetic patient with uncontrolled hyperglycemia are at risk for oxidative stress and complication, and oxidative stress may increase their requirement for vitamins with antioxidant effects. Damage tissue may have altered response to vitamin 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 a muscle cells.[34]

2.3.4 Zinc in human physiology and pathology:

Zinc is one of the most abundant nutritionally essential elements in the human body. It is found in all body tissues with 85% of the whole body zinc in muscle and bone, 11% in the skin and the liver and the remaining in all the other tissues. In multi cellular organisms, virtually all zinc is intracellular; 30–40% is located in the nucleus, 50% in the cytoplasm, organelles and specialized vesicles (for digestive enzymes or hormone storage) and the remainder in the cell membrane. Zinc intake ranges from 107 to 231 μmol/d depending on the source, and human zinc requirement is estimated at 15 mg/d. Zinc has been shown to be essential to the structure and function of a large number of macromolecules and for over 300 enzymatic reactions. It has both catalytic and structural roles in enzymes, while in zinc finger motifs; it provides a scaffold that organizes protein sub-domains for the interaction with either DNA or other proteins. It is critical for the function of a number of metalloproteins, inducing members of oxido-reductase, hydrolase ligase, and lyase family and has co-activating functions with copper in superoxide dismutase or phospholipase C. The zinc ion (Zn^{++}) does not participate in redox reactions, which makes it a stable ion in a biological medium whose potential is in constant flux. Zinc ions are hydrophilic and do not cross cell membranes by passive diffusion. In general, transport has been

described as having both saturable and non-saturable components, depending on the Zn (II) concentrations involved. Zinc ions exist primarily in the form of complexes with proteins and nucleic acids and participate in all aspects of intermediary metabolism, transmission and regulation of the expression of genetic information, storage, synthesis and action of peptide hormones and structural maintenance of chromatin and biomembranes .[35]

2.3.5. Zinc and diabetes mellitus:

Zinc serves an essential role as cofactor for more than 200metal enzyme, many of which regulate the metabolism of carbohydrate, lipids, protein. Insulin itself is stored in the presence of zinc .[36]

Zinc ions in the secretary granules of beta cells are known to insulin molecules, when the secretary granules open to the surface, the zinc ion pressure decrease rapidly and pH level change from acid to physiology levels, which result in free insulin monomer and zinc ion will be release from the pancreas .[37] Thus zinc is require for insulin synthesis and storage there is accumulating evidence that the metabolism of zinc is altered in insulin dependent diabetes mellitus and that zinc might have specific role in the pathogenesis and progress of this disease, increased urinary loss of zinc is a commonly encountered feature of diabetes .[38]

About 70% of the Zn is bound to albumin and any pathological alteration of albumin affects the serum Zn levels.[39]Zn malabsorption results in various types of disorders including the dermal, gastrointestinal, neurological and immunological abnormalities.[40]

2.3.6. Copper in human physiology and pathology

Copper is a trace element, important for the function of many cellular enzymes. Copper ions can adopt distinct redox states oxidized Cu(II) or reduced (I), allowing the metal to play a pivotal role in cell physiology as a catalytic cofactor in the redox chemistry of enzymes, mitochondrial

respiration, iron absorption, free radical scavenging and elastin cross-linking. If present in excess, free copper ions can cause damage to cellular components and a delicate balance between the uptake and efflux of copper ions determines the amount of cellular copper. In biological systems, copper homeostasis has been characterized at the molecular level. It is coordinated by several proteins such as glutathione, metallothionein, Cu-transporting P-type ATPases, Menkes and Wilson proteins and by cytoplasmic transport proteins called copper chaperones to ensure that it is delivered to specific subcellular compartments and thereby to copper-requiring proteins .[41]

2.3.7. Copper and diabetes Mellitus:

Copper is the third most abundant essential trace mineral in the body, copper is present in the body combined with enzymes to form metalloenzymes such as ceruloplasmin and superoxide dismutase (SOD), these enzyme play major role in redox reaction, and antioxidant defence. it has been postulated that copper possesses insulin –like activity and promotes lipogenesis.[21] Human studies demonstrate that diabetic patients may have abnormal levels of serum copper 13 in facts copper has an important role in the body and it most important antioxidant. Cu imbalance is implicated in cholesterol elevation by disrupting normal high density lipoproteins (HDL) and low density lipoproteins (LDL) balance. Cu also activates cytochrome oxidase which is involved in the electron transport chain of the mitochondria .[42]

In case of copper deficiency, cytochrome oxidase reduces its activity which might lead to the distortion of mitochondria in metabolically active tissues such as pancreatic a cinar cells, hepatocytes etc.[43]Published data show that Cu deficiency is one of the reasons for the development of cardiovascular diseases. Other reports suggest that Cu is also beneficial to prevent arthritis associated inflammation. More recently, it has been

reported that disturbances in copper levels in various bio fluids and tissues are associated with abnormalities implicated in metabolic pathways of diabetes and its complications. Copper as well as zinc metals play roles in order to protect oxidative damage of body tissues.[44]

2.4 Previous studies

Thiyam Romola Devi, Davina Hijam, Abhishek Dubey., et al. these author shown that level of trace elements like copper and zinc have some role in progression of this disease .purpose of the study was to estimate serum zinc and copper levels in type2 diabetes mellitus patients compare with that of healthy individuals and to identify the relationship among these. Serum Zn levels were lower for T2DM cases with complication (89.65 ± 4.21) than cases without complications (92.32 ± 5.15) and controls (95.40 ± 3.90), while serum Copper was highest among cases with complication (164.05 ± 9.32) than cases without complication (161.40 ± 6.43) and controls (131.85 ± 7.92). highly significant ($p < 0.001$) statistically, altered level of zinc & copper are found to be important factor in diabetic patients for developing complication, because of important role of trace elements like zinc and copper in DM, it is suggested that an adequate supply of these substance in diet of diabetic patients can be beneficial in the long term management.[45]

Other study had done by Olaniyan, M A M A wonuga, A F Ajetunmobi. et al these author also show that fifty three diabetic patients and non diabetic control subjects, serum zinc and copper were measured and the association of trace elements compare with glycaemic status, The serum zinc level was significantly lower (11.9±2.9mmol/L) in diabetic patients as compared with control subjects (14.6±2.5mmol/L, $p<0.001$), A significantly higher difference was observed in serum copper levels with a mean of 23.3±4.3mmol/L in diabetic patients as compared with

19.9±3.9mmol/L ($p<0.001$) in controls., There was no association with age, gender, glycaemic status, and duration of diabetes with the serum concentration of these trace elements in the type-2 diabetic patients studied further studies need to be carried out to determine the molecular role of copper and zinc in the development of diabetic complication in a larger population , also glycated haemoglobin (HbA1c) would be useful to measure in such studies.[38]

Other study conducted by A. H. Zargar, N. A. Shah, S. R. Masoodi, B. A. Laway, F. A. Dar, A. R. Khan, F. A. Sofi, A. I. Wani. This study evaluated the role of such a relationship in 83 patients with non-insulin dependent diabetes mellitus (40 men and 43 women), with a mean duration of diabetes of 3.9 +/- 3.6 years. Patients with nephropathy were excluded. Thirty healthy non-diabetic subjects were studied for comparative analysis. Subjects were subdivided into obese and non-obese. Diabetic subjects were also subdivided into controlled and uncontrolled groups; control was based on fasting blood glucose and serum fructose amine levels. Plasma copper, zinc and magnesium levels were analysed using a GBC 902 double beam atomic absorption spectrophotometer. Plasma zinc and magnesium levels were comparable between diabetic and non-diabetic subjects, while copper levels were significantly elevated ($p < 0.01$) in diabetic patients. Age, sex, duration and control of diabetes did not influence copper, zinc, or magnesium concentrations. We conclude that zinc and magnesium levels are not altered in diabetes mellitus, but the increased copper levels found in diabetics in our study may merit further investigation of the relationship between copper and non-insulin dependent diabetes mellitus.[46]

Study conducted by; M. Basaki Email author, M. Saeb, S. Nazifi, H. A. Shamsaei. The aim of present study was to compare the concentration of

essential trace elements, zinc, copper, iron, and chromium in serum of patients who have type 2 diabetes mellitus ($n = 20$) with those of non diabetic control subjects ($n = 20$). The serum concentrations of zinc, copper, iron, and chromium were measured by means of an atomic absorption spectrophotometer (Shimadzu AA 670, Kyoto, Japan) after acid digestion. The results of this study showed that the mean values of zinc, copper, and chromium were significantly lower in the serum of patients with diabetes as compared to the control subjects ($P < 0.05$). Our results show that deficiency of some essential trace elements may play a role in the development of diabetes mellitus .[47]

Another study conducted by: Tasneem GulKazi Email author Hassan Imran Afridi, NaveedKazi, Mohammad Khan Jamali, Mohammad Bilal Arain, NussaratJalbani, Ghulam Abbas Kandhro The aim of present study was to compare the level of essential trace elements, chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn) in biological samples (whole blood, urine, and scalp hair) of patients who have diabetes mellitus type 2 ($n = 257$), with those of nondiabetic control subjects ($n = 166$), age ranged (45–75) of both genders. . The results of this study showed that the mean values of Zn, Mn, and Cr were significantly reduced in blood and scalp-hair samples of diabetic patients as compared to control subjects of both genders ($p < 0.001$). The urinary levels of these elements were found to be higher in the diabetic patients than in the age-matched healthy controls. In contrast, high mean values of Cu and Fe were detected in scalp hair and blood from patients versus the nondiabetic subjects, but the differences found in blood samples was not significant ($p < 0.05$). These results are consistent with those obtained in other studies, confirming that deficiency and efficiency of some essential trace metals may play a role in the development of diabetes mellitus.[48]

3. Materials & Methods

3.1. Study design:

A descriptive, case control study .

3.2.Study area:

Khartoum state.

3.3Study Duration:

period April to July 2018

3.4. Study population and sample size:

Forty Sudanese patients with diabetes mellitus (20 controlled patients with diadetes,20 uncontrolled patients with diabetes) (female25% and male 15%) , and participants with different age. were selected as case and 30 apparently healthy individuals were selected as control group.

3.5. Inclusion criteria:

-Patients were diagnosed with DM type2

-Healthy individuals not know to have DM

3.6. Exclusion criteria:

Patient suffering from carcinoma, smoker, pregnancy, renal disease, alcoholism, take drugs contain zinc or copper were excluded using questionnaire .

3.7. Sampling:

Blood samples were collected from peripheral Vein(3ml) from each subject in Plain container and centrifuged at 4000r pm for 5 minutes to obtain serum that were stored at -20^0c

3.8. Statistical analysis:

All data was performed using the Statistical Package for the Social Sciences software package (SPSS), Values with normal distribution was presented as mean and standard deviation. The student t-test was used for two group comparison and level of significant was set as $p < 0.05$.

3.9. Ethical consideration:

After explaining the details and utility of the study, informed consent was taken from both cases and controls; ethical clearance was taken from the ethical committee of Sudan.

3.10. Materials required: Syringe, plain container, alcohol swabs, cotton and marker pen, centrifuge, reagent, sample.

3.11. Measurement of serum Zinc and Copper: By using Atomic absorption spectroscopy.

Principle:

A much larger number of gaseous metal atoms will normally remain in ground state. These ground state atoms are capable of absorbing radiant energy of their own specific wavelength , if light is passes through aflame containing the atoms the part of the light will be absorbed , the extend of absorption will be proportional to number of atoms in ground state.

3.11.1. Estimation of zinc and copper:

Principle:

Metal atom absorb strongly at discrete characteristic wave length which coincide with the emission spectra of the metal ion. A solution of the sample is dissolved into an aerosol which is injected into aflame which the converts the sample into an atomic and molecular vapour. The atomic vapour absorbs radiation from a hallow cathode lamp at specific wavelength .The beam then travels the flame and is focused onto monochromatic, which is set to read the intensity of the spectral length .Light with specific wavelength is absorbed by the metal in the flame and degree of absorption is function of the concentration of the metal in the sample.

Chemicals and reagents:

atomic absorption was used to estimate the concentration of zinc and copper, analytical grade reagent (Biosystem) was used, in zinc and copper, and demonized water was also used ,disposable plain container was used for collect of sample, automated pipettes was used to take samples and reagents, tips injector were used with pipette.

3.11.2. Zinc estimation:

Procedure:

1ml of serum or standard was pipetted in separate test tube, 9ml water was added in to each tube, each tube was mixed, and the result was read.

Calculation:

The instrument was adjusted with standard solution for zinc and then the concentration of samples was calculated.

Preparation of calibration solutions:

0,10,20 ml of zinc stock solution were pipetted into three 100 ml volumetric flasks, 10 ml of sodium stock solution were added to reach flask, these solutions contain 0, 10, 20 µg 100 ml in the sample solution (see appendix 1).

Linearity range:

This method is linear up to 300µg/dl.

3.11.3. Copper estimation:

Procedure:

1ml of serum or standard was pipetted in separate test tube, 9ml water was added in to each tube, each tube was mixed, and the result was read .

Calculation:

The instrument was adjusted with standard solution for copper, and then the concentration of sample was calculated.

Preparation of calibration solutions:

10, 20 ml of copper stock solution were pipetted into three 100 ml volumetric flasks. These solution contain 10, 20 µg of copper, 100 ml corresponding to 100 and 200 µg in the sample solution. (See appendix 1)

Linearity range: This method is linear up to 300µg/dl.

3.12.4. Estimation of HbA1c:

Principle of the assay:

HbA1c is a boronate affinity assay. The kit contains test devices with a porous membrane filter, test tubes prefilled with reagent and reagent and washing solution. The reagent contains agents that lyse erythrocytes and precipitate haemoglobin specifically, as well as a blue boronic acid conjugate that binds cis-diol of glycated haemoglobin .when blood id added to the reagent, the erythrocytes immediately lyse. All haemoglobin precipitates. The boronic acid conjugate binds to the cis-diol configuration of glycated haemoglobin .An aliquot of the reaction mixture is added to the test device, and all the precipitated haemoglobin, conjugate bound and unbound, remains on top of the filter. Any excess of coloured conjugate is removed with the washing solution. The precipitate is evaluated by measuring the blue (glycated haemoglobin) and the red (total haemoglobin) colour intensity with NycoCard reader, the ratio between them being proportional to the percentage of HbA1c in the sample.

Kit contents:

TD/Test Device, plastic device with membrane filter.

R1/Reagent, Glycinamide buffer containing dye - bound boronic acid and detergents.

R2/Washing solution, morpholine buffered NaCl solution and detergents.

Specimen collection:

Blood sample with anticoagulant (EDTA) can be stored at up to 10 days at 2-8ºc before analysis. Do not freeze.

Test procedure:

1. Preparing the sample, 5µl whole blood was added to the test tube with R1/reagent and mixed well. The tube leaved for 2 minutes.
2. Applying the sample, the sample remixed to obtain a homogenous suspension. 25µl of mixture applied to a TD/test devise. The pipette approx. 0.5 cm hold above the test well and the pipette emptied quickly in the middle of the test well. The mixture allowed soaking completely into the membrane and waited for 10 seconds.
3. Applying R2/washing solution, 25 µL R2/washing solutions was applied to TD/test devise. The reagent allowed to soak completely into the membrane and waited for 10 seconds.
4. Read of the test result, read after 5 minutes. (See appendix 2)

4. Results

Shows Significant different between the mean of zinc in control group of diabetic case (n=20) and healthy individual (n=30), Mean of case is 85.05, SD is 5.6 and healthy individual Mean is 95.4, SD is 3.8 and mean of copper in control group of diabetic mean of case is (162.4), SD is 6.4 and mean of healthy individual (129.2).SD 8.3. Table (4.1)

Shows Significant different between the mean of zinc in uncontrolled group of diabetic case (n=20) and healthy individual (n=30) mean of case is 91.2, SD is 5.7 and healthy individual mean is 95.4, SD is 3.8. Mean of copper in uncontrolled group of diabetic case (n=20) and healthy individual (n=30) mean of case is (155.6); SD is 11.1 and mean of healthy individual (129.2).SD 8.3. Table (4.2)

Shows significant different between Mean & Standard Deviation of serum copper (mean162.4, SD6.4), zinc (mean 85.05, SD5.6) for control and copper (mean155.6, SD 11.1), zinc (mean 91.2, SD5.7) for uncontrolled diabetic patient. Table (4.3)

Comparison of Mean & Standard Deviation of serum zinc (mean 91, SD 6.1), copper (mean 158.6, SD 11) between the gender Female,

And zinc (mean 83.0, SD1.9), copper (mean 159, SD4.7) between the gender Male, show significant different between zinc and copper in gender Female& male. In table (4.4)

Shows the correlation between the HbA1c and copper (r= -0.3, p value=0.002) show significant different between copper and Hba1c in figure (4.1)

Shows the correlation between HbA1c and zinc(r=0.5, p value = 0.001) significant different between zinc and HbA1c. In figure (4.2)

Table (4.1) Comparison between the Means of serum Zinc ,Copper in controlled Diabetic Patients and in healthy individuals

Variables	Controlled diabetes (Mean±SD) (n=20)	Healthy individual (Mean±SD) (n=30)	P- value
Zinc	85.05ug/L ±5.6	95.4ug/L±3.8	0.001*
Copper	162.4ug/L±6.4	129.2ug/L±8.3	0.001*

*Significance of p.value = < 0.05

Table (4.2) Comparison between the Means of serum Zinc ,Copper in uncontrolled Diabetic Patients and in healthy individuals

Variables	Uncontrolled diabetes (Mean±SD) (n=20)	Healthy individual (Mean±SD) (n=30)	P- value
Zinc	91.2ug/L ±5.7	95.4ug/L±3.8	0.010*
Copper	155.6ug/L±11.1	129.2ug/L±8.3	0.001*

*Significance of p.value = < 0.05

Table (4.3) comparison between the Means of serum copper, zinc in control Diabetic Patients and in uncontrolled Diabetic Patients

Variables	Controlled diabetes(Mean±SD) (n=20)	Uncontrolled diabetes(Mean±SD) (n=20)	P- value
Zinc	85.05ug/L ±5.6	91.2ug/L±5.7	0.01*
Copper	162.4ug/L±6.4	155.6ug/L±11.1	0.02*

*Significance of p.value = < 0.05

Table (4.4) Comparison between the Means of serum zinc, copper in Female &Male Patients

Variables	Female (Mean±SD) (No=25)	Male (Mean±SD) (No=31)	P- value
Zinc	91ug/L ±6.1	83.0ug/L ±1.9	0.008*
Copper	158.6ug/L±11	159ug/L±4.7	0.001*

*Significance of p.value = < 0.05

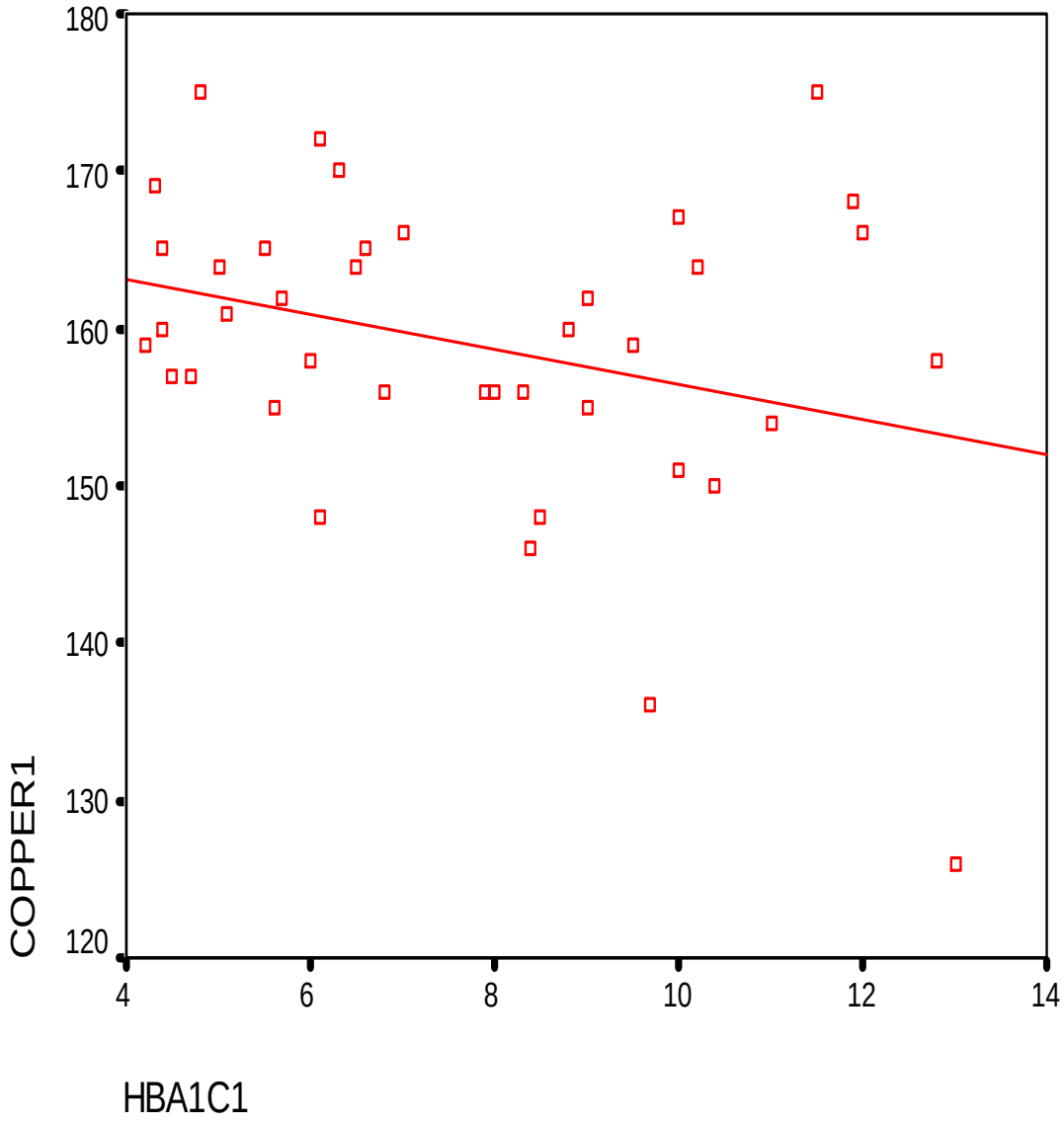

Figure (4.1) Relationship between HbA1c with serum copper in case group

R= - 0.3, r^2 =0.002.

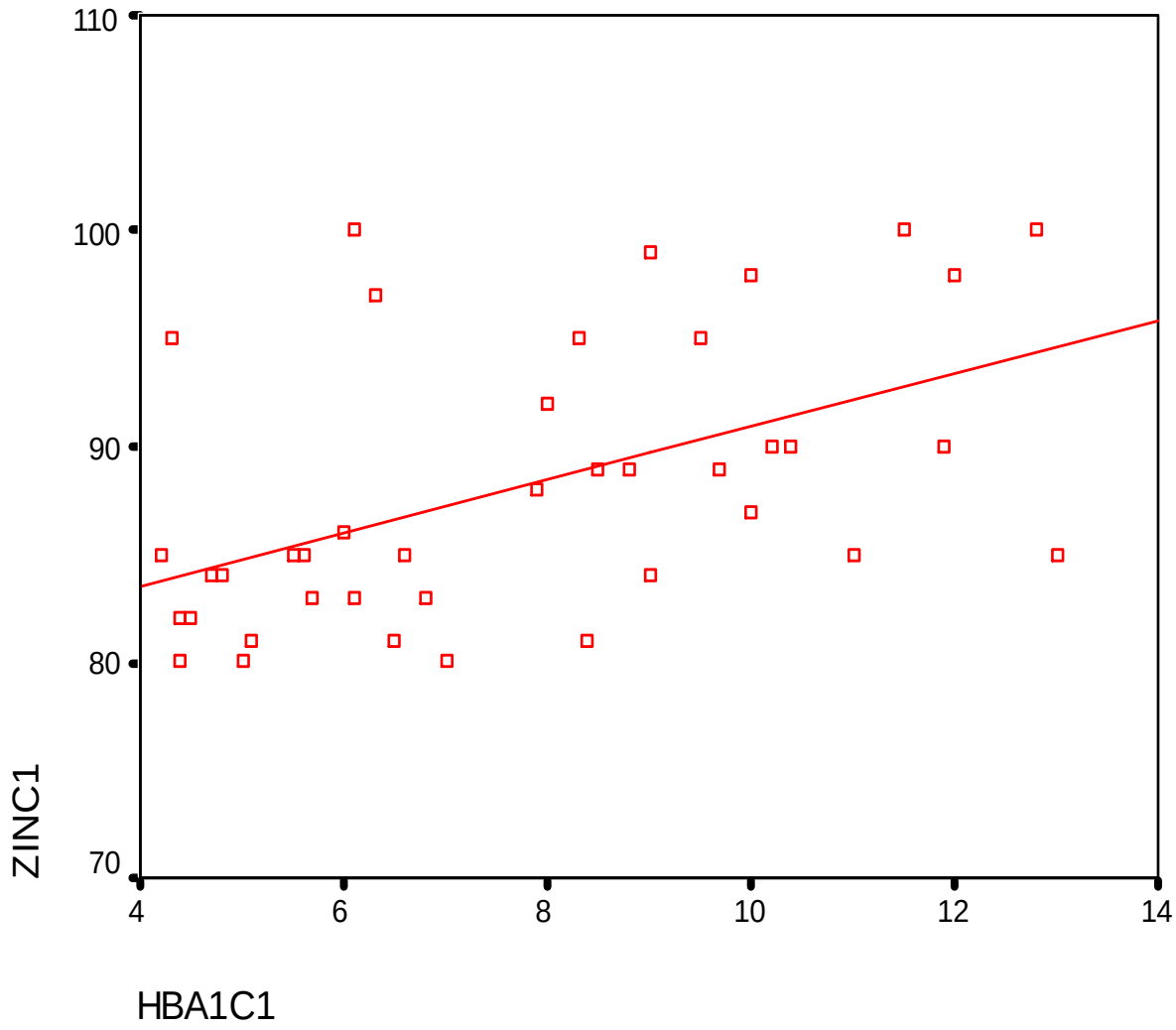

Figure (4.2) Relationship between HbA1c with serum zinc in case group

r=0.5, r^2 =0.001

5.1 Discussion

This case control study conducted in Khartoum state from period of April to August 2018, including 40 diabetic patients (20 patients controlled diabetes, 20 patients uncontrolled of diabetes) 30 Healthy individual.

The serum levels of zinc and copper in type 2 diabetes Healthy groups were determined in this study and related to the, gender, glycaemic status of diabetes.

- In this study, zinc levels in controlled diabetes patient (Mean 85.05 ,SD 5.6) and uncontrolled diabetes (Mean 91.2, SD 5.7) were lower than the healthy group with P-value <0.001 , 0.01 respectively, that mean there was statistically lower significant effect of diabetes on serum Zn by decreasing it.

  This finding was in agreement with the findings of Olaniyan, et al (The serum zinc level was significantly lower (11.9±2.9) in diabetic patients as compared with control subjects (14.6±2.5, p<0.001) (38) and agree with study conducted by Devi, et al [(45)], and disagree with study conducted by A.H. Zargar, et al [(46)] that they found there was no significant difference between test and control and also agree with study conducted by M. Basaki, et al. [(47)] That they found significant decrease in Zn.

  The mean of Zn in male was (83.0±1.9) when compared with female was (91±6.1) with P-value 0.008.

  There was weak positive correlation between Zn and HbA1c, r=0.5

  Zinc is essential for the correct processing, storage, secretion, and action of insulin in beta (β)-cells. Insulin is stored inside secretory vesicles or granules, where two Zn++ ions coordinate six insulin monomers to form the hexameric-structure on which maturated insulin crystals are based. It is also known that like, most other chronic disorders, diabetes increase the excretion of minerals. Hyperglycemia in diabetes is usually associated

with hyperzincuria and increased urinary loss of Zn++, which is responsible for decreases in total body Zn++. Zinc has antioxidant properties; thus it can stabilize macromolecules against radical induced oxidation. Zinc is a component of the important antioxidant enzyme superoxide dismutase (Cu-ZnSOD). Thus the protection of this antioxidant against free radicals generated in the disease will be diminished. It is also very important to note that Zn concentration regulates the metabolism of other very important members of the antioxidant defence system. Zinc deficiency produces high lipoprotein oxidation.

Present study has shown increased Cu levels in diabetic patients- both with controlled (mean 162.4 SD 6.4) and uncontrolled diabetes (mean 155.6 SD 11.1) than the healthy with P-value <0.001 in both, that mean there was statistically highly significant effect of diabetes on serum Cu by increasing it.

Similar finding has been observed by other studies as Thiyam Romola Devi, Davina Hijam, Abhishek Dubey .,et al(serum copper levels with a mean of 23.3±4.3 in diabetic patients as compared with 19.9±3.9 ($p<0.001$) in controls (38) and agree with other studies conducted by Olaniyan, et al, (45) and A.H. Zargar, et al (46) and disagree with study conducted by M.Basaki, et al (47) that they found significant decrease in copper in test group than control group.

The mean of Cu in male was (159±4.7) when compared with female was (158.6 ±11) with P-value 0.001>.

There was negative correlation between Cu and HBA1C, r=-0.3.

Copper is toxic in its unbound form, causes redox imbalance due to highly redox active nature, which leads to activation of stress sensitive intracellular signaling pathways.

The increase in Cu ion levels in patients with diabetes mellitus may be attributed to hyperglycaemia that may stimulate glycation and release of copper ions and this accelerates the oxidative stress, so that, Advanced Glycation End products are formed, that are involved in the pathogenesis of diabetic complications. Also copper have antioxidant function in diabetic patients. Ceruloplasmin and serum albumin are the main Cu binding proteins in plasma and there is some evidence that chronic hyperglycemia can damage the Cu binding properties of both.

In present study there exists antagonistic relationship in the levels of Cu and Zn in diabetes. Thus the role of trace elements in diabetes mellitus becomes important.

5.2. Conclusion

Upon our study, we conclude that:

- Serum levels of Zinc significant lower in type 2 diabetes than healthy individuals.
- Serum levels of Copper significant increase in type 2 diabetes than healthy individuals as have been found in present study.
- Gender have significant effect in serum Zn and Cu in diabetic patients
- Weak Positive correlation between serum Zinc with HbA1c and weak negative correlation between copper with HbA1c.

5.3. Recommendations:

By the end of this study; we recommended that:

- Oral Zn replacements can be beneficial in the long term management of diabetic patient.
- Can utilize zinc and copper for the screening, diagnosis and management of diabetes mellitus.
- Recommended that regular assessment of trace element like zinc and copper and further studies in this field are recommended.
- Other study must be conducted with large sample size and all trace elements must be evaluated.

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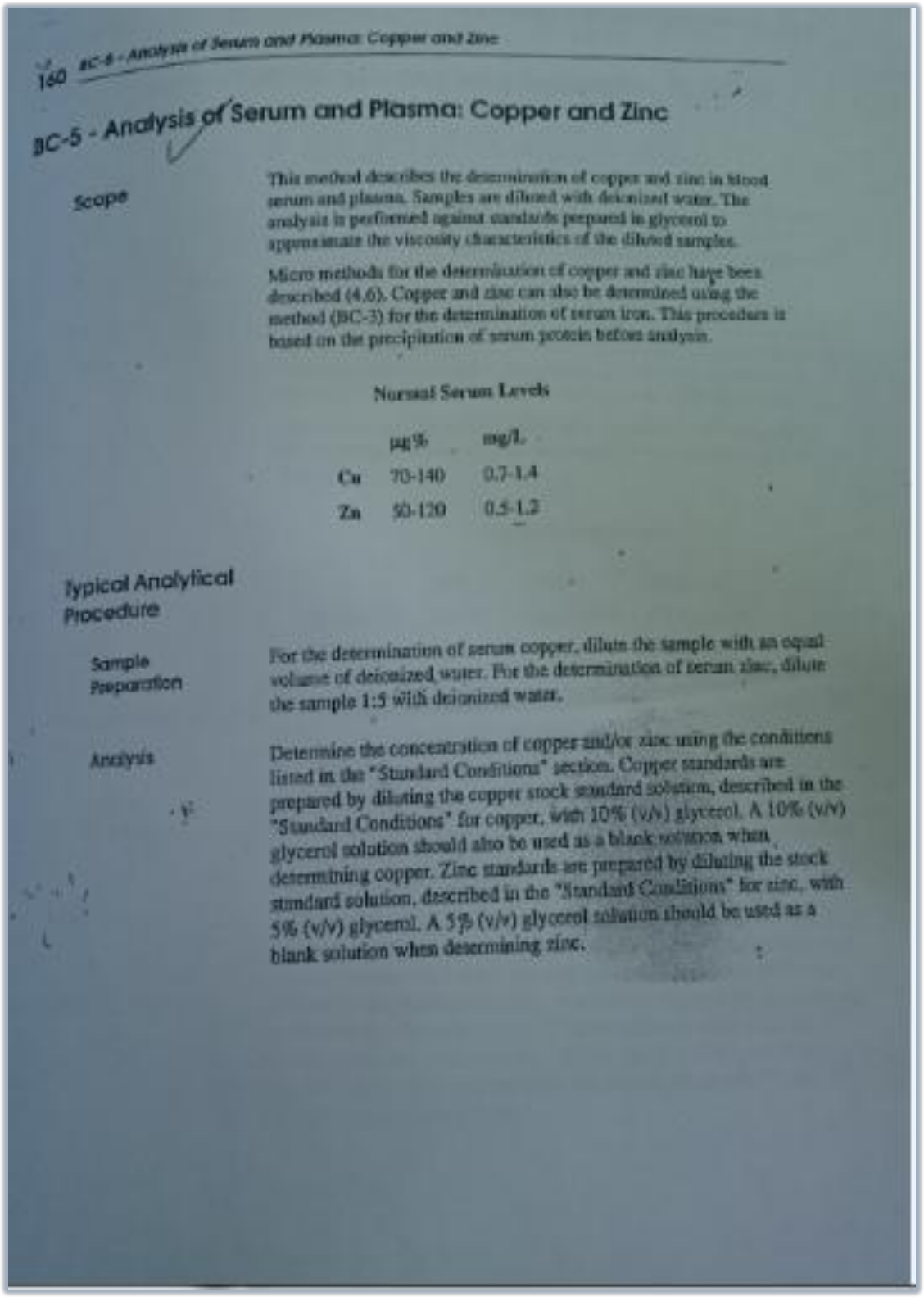
Appendix 1:



BC-5 - Analysis of Serum and Plasma: Copper and Zinc

Scope

This method describes the determination of copper and zinc in blood serum and plasma. Samples are diluted with deionized water. The analysis is performed against standards prepared in glycerol to approximate the viscosity characteristics of the diluted samples.

Micro methods for the determination of copper and zinc have been described (4,6). Copper and zinc can also be determined using the method (BC-3) for the determination of serum iron. This procedure is based on the precipitation of serum protein before analysis.

Normal Serum Levels

	µg%	mg/L
Cu	70-140	0.7-1.4
Zn	50-120	0.5-1.2

Typical Analytical Procedure

Sample Preparation

For the determination of serum copper, dilute the sample with an equal volume of deionized water. For the determination of serum zinc, dilute the sample 1:5 with deionized water.

Analysis

Determine the concentration of copper and/or zinc using the conditions listed in the "Standard Conditions" section. Copper standards are prepared by diluting the copper stock standard solution, described in the "Standard Conditions" for copper, with 10% (v/v) glycerol. A 10% (v/v) glycerol solution should also be used as a blank solution when determining copper. Zinc standards are prepared by diluting the stock standard solution, described in the "Standard Conditions" for zinc, with 5% (v/v) glycerol. A 5% (v/v) glycerol solution should be used as a blank solution when determining zinc.

Appendix 2:

LabonaCheck™ A1c HbA1c Test Kit

CE IVD

Read this entire insert thoroughly before using the LabonaCheck™ A1c HbA1c Test Kit. Only use the LabonaCheck™ A1c HbA1c Test Kit with the LabonaCheck™ A1c HbA1c Analyzer. Keep this insert for future reference. If you have any inquiry or question, please contact your local distributor.

Product description

• Intended use

The LabonaCheck™ A1c HbA1c Test kit is intended for the quantitative determination of glycated hemoglobin in human blood.

• Test principle

The LabonaCheck™ A1c is a boronate affinity assay. The LabonaCheck™ A1c HbA1c Test kit consists of the cartridges, the R1/Reagent and the R2/Reagent. The R1/Reagent contains the agents that lyse erythrocytes and precipitate hemoglobin specifically, as well as a blue boronic acid conjugate that binds cis-diol of glycated hemoglobin. When blood is added to the R1/Reagent, the erythrocytes are lysed and all hemoglobin precipitates, as well as the boronic acid conjugate binds to the cis-diol configuration of glycated hemoglobin. An aliquot of the reaction mixture is added to the cartridge and all the precipitated hemoglobin, conjugate-bound and unbound, remains on top of the filter. Any unbound boronate is removed with the R2/Reagent. The precipitate is evaluated by measuring the blue (glycated hemoglobin) and the red (total hemoglobin) color intensity respectively with the LabonaCheck™ A1c HbA1c Analyzer, the ratio between them being proportional to the percentage of glycated hemoglobin in the sample.

• Test Kit contents

- Cartridge(with the membrane filter) ..... 1 x 24 units
- R1/Reagent ..... 1 x 24 x 0.2 ml
- R2/Reagent ..... 1 x 2.0 ml
- Insert Paper

• Reagent composition

R1/Reagent
- Boronate derivative ..... 0.04 mg
- Organic solvent ..... 6.2 %
- Lysing agent ..... 0.15 %

R2/Reagent
- Detergent ..... 0.5 %

Cartridge
- Filter(Glass Fiber)
- Membrane(Nylon)
- Absorption pad(Glass Fiber)

• Material needed (not supplied with the kit)

- Capillary tubes
- Capillary tube holder
- Volume fixed pipette and pipette tips
- LabonaCheck™ A1c HbA1c Analyzer

• Warnings and precautions

- For in vitro diagnostic use only.
- Do not transfer components from or to any different kit lots.
- Do not use the kit after the expiration date.
- The R1/Reagent and R2/Reagent contain a toxic agent(0.05%). Avoid direct contacts to the skin.
- Do not drink the R1/Reagent and R2/Reagent.
- The R1/Reagent and cartridge are single use only.
- Dispose of used reagents and cartridges according to the local guidelines.
- Exercise the normal precautions required for handling all laboratory reagents.
- Blood specimens, used reagents, pipette tips and tubes should be considered potentially infectious.
- This LabonaCheck™ A1c HbA1c Test Kit shall be used with the Labona Check™ A1c HbA1cAnalyzer only. Do not use it with other brands' analyzers.
- Change the pipette tip between each pipetting step.
- The test will be applied on a routine basis and not in emergency situation.

Test characteristics

• Measuring range

- Measuring range: 4.0~15.0 % or 20 ~ 140 mmol/mol
- Measuring interval: 0.1 % or 1 mmol/mol

• Reference range[1]

	NGSP	IFCC
Prediabetes	5.7~6.4 %	39 ~ 46 mmol/mol
Presence of diabetes	≥6.5 %	≥48 mmol/mol
Target in diabetes	<7.0 %	<53 mmol/mol

• Accuracy

The Accuracy of the LabonaCheck™ A1c HbA1c system was evaluated at three clinical sites from 120 Patients with replicate measurement.
The correlation obtained between LabonaCheck™ A1c HbA1c system results and the reference method was :
N=120, y=0.991x+0.083; R^2=0.979

• Precision

The precision of the LabonaCheck™ A1c HbA1c system was estimated with venous blood samples and control solution in the laboratory. Readings obtained with the LabonaCheck™ A1c HbA1c system were compared to those obtained using Tosoh HLC-723 GHb G7 (Tosoh Bioscience).

Within Run Precision(venous blood)

HbA1c concentration(%)	5.3	8.7	11.1
Mean	5.4	8.7	11.2
STD	0.14	0.25	0.25
CV(%)	2.5	2.3	2.2

Day to Day Precision(control solution)

HbA1c concentration(%)	5.6	8.6	11.5
Mean	5.7	8.7	11.4
STD	0.16	0.24	0.31
CV(%)	2.9	2.8	2.7

• Limitations of the test

- Operation temperature and humidity
Temperature Range: 15~35℃(59~95°F) the recommened is 20~25℃ (68~77°F)
Humidity Range: 15~75% RH
- The reagent must be stored in the designated temperature range (2~8℃). If the reagents are stored in the temperature out of the designated temperature range(2~8℃), the test result can be inaccurate.
- Do not keep the reagents for more than 3 hours in room temperature.
- Use only fresh capillary whole blood or venous blood. Do not use serum or plasma.
- Interference substances
1) The venous blood collected with an anticoagulant (e.g.: K3 EDTA, Heparin, NaF) using aseptic technique is available for testing.
2) The Hb-concentration lower than 10g/dL or higher than 20g/dL can cause inaccurate test results.
3) The test results are not affected by albumin, ascorbic acid, bilirubin, glucose, lipid.

Questionnaire:

No ( )

Name ……

Age : ……Sex: male female

Duration of diabetes: ……

Type of diabetes: ……

Family history of diabetes : No Yes

Regular follow up: No Yes

Renal disase: No Yes

Smoking : No Yes

Eating fish or sea food Large quantity : No Yes

Take drugs contain Zinc or Copper : No yes

History of diabetic complication : No Yes

Laboratory Examinations:

S. Zinc .....

S. Copper .....

HeamoglobinA1c .....

Informed consent

هذه دعوه مني الباحثة / ثويبة الهادي عمر ماجستير مختبرات طيبيه قسم الكيمياء السريريه – شندي و لمشاركتك في برنامج بحث هدفه معرفه وتشخيص تقيم مرض السكري النوع الثاني.

إذا رغبت في ألمشاركه و إنجاح هذا العمل فاني وفريق البحث سنقوم:

- ملء استمارة بمعلومات تخصك لها علاقة بي موضوع البحث.
- أي معلومة تخصك في الاستمارة سوف تكون سريه.
- اخذ عينك من دمك لقياس مستوي الخارصين والنحاس ومعرفه السكر التراكمي.
- بمشاركتك في البرنامج يمكنك الحصول علي نتائج الفحوصات في عينه دمك.

لك كامل الحرية في المشاركة أو عدم المشاركة هو الانسحاب من البرنامج في آي وقت رغبت فيه.

يمكنك الحصول علي آي اجابه لآي سؤال عن برنامج البحث.

التاريخ:.....

المتبرع توقيع:.....