بسم الله الرحمن الرحيم

Sudan University of Sciences and Technology

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Evaluation of Serum Chromium Level among Type 2 Diabetes Mellitus Patients in Khartoum State

تقدير مستوى الكروم في مصل الدم لدي مرضى السكري من النوع الثاني في ولاية الخرطوم

A dissertation submitted for partial fulfillment for the requirement of MS.c degree in Medical Laboratory Sciences - Clinical Chemistry

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الآيـة

قال تعالى:

هُوَ الَّذِي خَلَقَكُم مِّن تُرَابٍ ثُمَّ مِن نُّطْفَةٍ ثُمَّ مِنْ عَلَقَةٍ ثُمَّ يُخْرِجُكُمْ طِفْلاً ثُمَّ لِتَبْلُغُوا أَشُدَّكُمْ ثُمَّ لِتَكُونُوا شُيُوخًا وَمِنكُم مَّن يُتَوَفَّى مِن قَبْلُ وَلِتَبْلُغُوا أَجَلاً مُّسَمًّى وَلَعَلَّكُمْ تَعْقِلُونَ67)

صدق الله العظيم
سورة غافر(الاية 67)

Dedication

to

My mother’s soul

She always be there every time I fall
she was to me the greatest love of all

Acknowledgment

Thank you my God, for giving me the ability and courage to bring this research to light.

My greater thanks to my supervisor: **Amar** whom started with me this research from zero level, he was very kind with me and greater leader, so I am really grateful to him.

My greater thank to my Classmate friend **Shiemaa Ali Fellah** because she helped me to achieve this work.

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List of abbreviations

DM	Diabetes mellitus
CVD	Cardiovascular diseases
HDL	High density lipoprotein
GDM	Gestational diabetes mellitus
LADA	Latent autoimmune diabetes of adult
HbA1c	Hemoglobin A1c
DCCT	Diabetes Control and Complication Trial
ACEIs	Angiotensin converting enzyme inhibitors
ARBs	Angiotensin receptor blockers
NPH	Neutral protamine Hagedorn
DRIs	Dietary Reference Intakes
RDAs	Recommended Dietary Allowance
AIs	Adequate intake
LMWcr	Low molecular weight chromium
ATP	Adenosine Tri Phosphate
LDL	Low density lipoprotein
NSAIDs	Non-steroidal inflammatory drugs
TPN	Total parenteral nutrition
MI	Myocardial infraction
GTF	Glucose tolerance factor
BMI	Body mass index
EDTA	Ethylene diamine tetra acetic acid
SD	Standard deviation
Cr	Chromium
NIDDM	Non-insulin Dependent diabetes mellitus

Abstract

This is a descriptive analytical study conducted in Khartoum State during the period from the January to September 2015. The aim of this study is to evaluate serum chromium level of type 2 DM patients and its correlation with study variables (BMI, HbA1c, duration of disease, age, and gender).

One hundred and twenty subjects were enrolled in this study; they were classified into 60 type 2 DM patients as case and 60 healthy apparently as control group. Serum chromium and HbA1c level were measured using atomic absorption spectrometry and ICROMA^{TM} .

The results showed significant decrease in chromium level in type 2 DM patients in comparison with healthy group (p-value 0.000). In addition

There was significant increase in chromium level between of good control versus poor control DM patients with (P-value 0.001).Also the study revealed negative correlation between chromium level and duration of DM (R-value -0.354) and (P-value 0.005).

Study concludes that, chromium level is significant decrease in type 2 DM, especially uncontrolled DM patients, therefore may exaggerate the complication of DM especially CVD due to, deficiency of chromium causes insulin resistance and decrease in HDL level.

مستخلص الدراسة

هذه دراسة وصفية تحليلية أجريت في ولاية الخرطوم خلال الفترة من يناير حتي سبتمبر 2015. هدف الدراسة هو تقييم مستوى الكروم في مصل الدم بين مرضى السكري من النوع الثاني وعلاقته مع متغيرات الدراسة(العمر,النوع,مدة المرض, الهيموقلوبين السكري,ومعامل كتلة الجسم).

تم اختيار مائة وعشرون من الأشخاص في هذه الدراسة، تم تصنيفها إلى 60 من المرضى السكري من النوع الثاني لدراسة حالتهم و 60 أشخاص أصحاء ظاهريا كمجموعة تحكم . تم قياس الكروم في مصل الدم ومستوى نسبة HbA1c باستخدام مطياف الامتصاص الذري و^{TM} ICROMA.

أظهرت النتائج وجود انخفاض ذا دلالة معنوية في مستوى الكروم في نوع الثاني من مرضي السكري بالمقارنة مع لمجموعة صحية مع ذات القيمة المعنوية (0.000) . بالإضافة الي انه هنالك زيادة في مستوي الكروم لدى مرضي السكري المنضبط مقارنة بمرضي السكري غير المنضبط والقيمة المعنوية (0.001). كما كشفت الدراسة علاقة عكسية بين مستوى الكروم ومدة مرض السكري مع R ذات القيمة (-0.354) والقيمة المعنوية (0.005).

خلصت الدراسة إلى أن مستوى الكروم أقل في النوع الثاني من مرضي السكري ، خاصة مرضى السكري غير المنضبط ،هذا قد يودي الي زيادة مضاعفات مرض السكري خاصة الأمراض القلبية الوعائية ،اذ ان نقص الكروم يسبب مقاومة الأنسولين وانخفاض في مستوى الكوليسترول عالي الكثافة.

Chapter one

Introduction and literature review

Introduction and Literature Review

1.1 Introduction

Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers and damage to the eyes(Kitabchi *et al.*, 2009).

Diabetes mellitus was classified as type 1,type 2, gestational diabetes mellitus, and other types, prevented by a person being a normal body weight, physical exercise, and following a healthy diet.Diabetes mellitus treated by Metformin, Routine use of aspirin and insulin (Ripsin *et al.*, 2009).

Chromium is a mineral that humans require in trace amounts,chromium was found to correct glucose intolerance and insulin resistance in deficient animals, two indicators that the body is failing to properly control blood-sugar levels and which are precursors of type 2 diabetes (Mertz, 1969).

1.2 Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. If left untreated, diabetes can cause many acute and long term complications (Kitabchi*et al.*, 2009).

1.2.1 Signs and symptoms

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger)Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes.Several other not

specific signs and symptoms includes blurry vision, headache, fatigue, slow healing of cuts, and itchy skin, skin rashes known as diabetic dermatomes(Cooke and Plotnick, 2008).

1.2.2 Diabetic emergencies

People (usually with type 1 diabetes) may also experience episodes of diabetic ketoacidosis, a type of metabolic problems characterized by nausea, vomiting and abdominal pain, the smell of acetone on the breath, deep breathing known as Kussmaul breathing, and in severe cases a decreased level of consciousness. A rare but equally severe possibility is hyperosmolar nonketotic state, which is more common in type 2 diabetes and is mainly the result of dehydration (Kitabchi *et al*., 2009).

1.2.3 Complications

All forms of diabetes increase the risk of long-term complications.The major complications related to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease (Sarwar*et al*., 2010) and 75% of deaths are due to coronary artery disease (Gara*et al*., 2013),stroke and peripheral vascular disease.Diabetic retinopathy is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness.Diabeticnephropathyleads to tissue scarring, urine protein loss, and eventually chronic kidney disease. Diabetic neuropathy results from damage to the nerves of the body(World Health Organization, 2014).

The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin.Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation(Cukierman, 2005).

1.2.4 Classification of Diabetes mellitus:

1.2.4.1 Type 1

Type 1 is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic (Rother, 2007). It causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children.

Type 1 diabetes can be accompanied by irregular and unpredictable hyperglycemia, frequently with ketosis, and sometimes with serious hypoglycemia. Other complications include an impaired counterregulatory response to hypoglycemia, infection, gastroparesis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (e.g., Addison's disease). These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1diabetes (Dorner *et al*., 1977).

1.2.4.2 Type 2

Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (David and Dolores, 2011). 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 diabetes is the most common type. In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. Type 2 diabetes is due primarily to lifestyle factors and

genetics (Riserus*et al.*, 2009). A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than thirty), lack of physical activity, poor diet, stress, and urbanization (Pakhale,1998).Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60–80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders. Those who are not obese often have a high waist–hip ratio. Dietary factors also influence the risk of developing type 2diabetes. Consumption of sugar-sweetened drinks in excess is associated with an increased risk (Malik, 2010). The type of fats in the diet is also important, with saturated fats and trans fatty acids increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk (Riserus *et al.*, 2009). Eating lots of white rice appears to also play a role in increasing risk (Hu, 2012). A lack of exercise is believed to cause 7% of cases (Lee *et al.*, 2012).

1.2.4.3 Gestational diabetes

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery. However, after pregnancy approximately 5–10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases insulin may be required (Saydah *et al.*, 2001).

1.2.4.4 Prediabetes

Indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM. Many people destined to develop type 2 DM spend many years in a state of prediabetes.

1.2.4.5 Latent autoimmune diabetes of adults (LADA)

Is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, based on age rather than etiology. Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases.

Any disease that causes extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells (Saydah*et al*., 2001).

1.2.4.6Malnutrition-related diabetes mellitus

Was deprecated by the World Health Organization when the current taxonomy was introduced in 1999 (Saydah *et al*., 2001)

1.2.4.7Congenital diabetes

This type is due to genetic defects of insulin secretion,

1.2.4.8 Steroid diabetes

Induced by high doses of glucocorticoids

1.2.5 Diagnosis:

Diabetes mellitus is diagnosed by:

Table 1:Diagnosis of diabetes mellitus.(Santaguida*et al*., 2008).

Condition	**2 hour glucose**	**Fasting glucose**	**HbA_{1c}**	
Unit	**mmol/l(mg/dl)**	**mmol/l(mg/dl)**	**mmol/mol**	**DCCT %**
Normal	<7.8 (<140)	<6.1 (<110)	<42	<6.0
Impaired fasting glycaemia	<7.8 (<140)	≥ 6.1(≥110) &<7.0(<126)	42-46	6.0–6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	42-46	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)	≥48	≥6.5

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following (Saydah*et al*., 2001).

Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)

Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test

Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)

Glycated hemoglobin (HbA_{1C}) ≥ 48 mmol/mol (≥ 6.5 DCCT %).A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above methods on a different day. (Saydah *et al*., 2001). According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) are considered diagnostic for diabetes mellitus. Per the Organization people with fasting glucose levels from 6.1 to 6.9 mmol/l (110 to 125 mg/dl) are considered to have impaired fasting glucose. People with plasma glucose at or

above 7.8 mmol/l (140 mg/dl), but not over 11.1 mmol/l (200 mg/dl), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease.

Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Santaguida*et al*., 2008).

1.2.6 Prevention

There is no known preventive measure for type 1 diabetes. Type 2 diabetes millets can often be prevented by a person being a normal body weight, physical exercise, and following a healthy diet. Active smoking is also associated with an increased risk of diabetes, so smoking cessation can be an important preventive measure as well (Willi*et al*., 2007).

1.2.7 Management

Management of diabetes mellitus concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing hypoglycemia. This can usually be accomplished with diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes(Nathan *et al*.,2005).

Learning about the disease and actively participating in the treatment is vital for people with diabetes, since the complications of diabetes are far less common and less severe in people who have well-managed blood sugar levels(Nathan *et al*.,2005). The goal of treatment is an HbA_{1C} level of 6.5%, but should not be lower than that, and may be set higher. Attention is also paid to other health problems that may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise.Specialized footwear is widely used to reduce the risk of ulceration (Cavanagh, 2004).

1.2.8 Medications

Metformin is generally recommended as a first line treatment for type 2 diabetes millets, as there is good evidence that it decreases mortality (Ripsin *et al*., 2009). Routine use of aspirin, however, has not been found to improve outcomes in uncomplicated diabetes (Pignone *et al.*, 2010).Angiotensin converting enzyme inhibitors (ACEIs) improve outcomes in those with DM while the similar medications angiotensin receptor blockers (ARBs) do not(Cheng *et al.*, 2014).Type 1 diabetes is typically treated with a combinations of regular and NPH insulin, or synthetic insulin analogs (Ripsin*et al.*, 2009).

1.3 Chromium

Chromium is a mineral that humans require in trace amounts, although its mechanisms of action in the body and the amounts needed for optimal health are not well defined. It is found primarily in two forms: 1) trivalent (chromium 3+), which is biologically active and found in food, and 2) hexavalent (chromium 6+), a toxic form that results from industrial pollution.

Chromium is known to enhance the action of insulin (Mertz, 1969; Mertz, 1993), a hormone critical to the metabolism and storage of carbohydrate, fat, and protein in the body (Porte *etal*.,2003). In 1957, a compound in brewers' yeast was found to prevent an age-related decline in the ability of rats to maintain normal levels of sugar (glucose) in their blood(Mertz, 1993). Chromium was identified as the active ingredient in this so-called "glucose tolerance factor" in 1959(Schwarz and Mertz,1959).Chromium also appears to be directly involved in carbohydrate, fat, and protein metabolism (Mertz,1969; Mertz, 1993) (Hopkins *etal*., 1968; Vincent, 2003), but more research is needed to determine the full range of its roles in the body.

1.3.1 Food sources of chromium

Chromium is widely distributed in the food supply, but most foods provide only small amounts (less than 2 micrograms [mcg] per serving). Meat and whole-grain products, as well as some fruits, vegetables, and spices are relatively good sources(Anderson *et al.,* 1992)In contrast, foods high in simple sugars (like sucrose and fructose) are low in chromium(Kozlovsky *et al*., 1986).

Dietary intakes of chromium cannot be reliably determined because the content of the mineral in foods is substantially affected by agricultural and manufacturing processes and perhaps by contamination with chromium when the foods are analyzed(Stoecker, 2001; Anderson *et al*., 1992). Therefore, Table 2, and food-composition databases generally, provide approximate values of chromium in foods that should only serve as a guide.

Table 2: **Selected food sources of chromium** (Anderson *et al.,* 1992; Cabrera *et al*., 1997; Dattilo and Miguel, 2003)

Foods	**Chromium (mcg)**
Broccoli, ½ cup	11
Grape juice, 1 cup	8
Potatoes, mashed, 1 cup	3
Garlic, dried, 1 teaspoon	3
Basil, dried, 1 tablespoon	2
Apple, Banana, 1 medium	1
Orange juice, 1 cup	2
Whole wheat bread, 2 slices	2

1.3.2 Dietary and Adequate intake

In 1989, the National Academy of Sciences established an "estimated safe and adequate daily dietary intake" range for chromium. For adults and adolescents that range was 50 to 200 mcg.In 2001,Dietary Reference Intakes(DRIs) for chromium were established. The research base was insufficient to establish Recommended Dietary Allowance (RDAs), so Adequate Intake (AIs) were developed based on average intakes of chromium from food as found in several studies.Chromium AIs are provided in Table 3.

Table 3 Adequate Intakes of chromium in different subjects:(Cocho*et al.*, 1992)

Age	**Infants and children (mcg/day)**	**Males (mcg/day)**	**Femels (mcg/day)**	**Pregnancy (mcg/day)**	**Lactation (mcg/day)**
0 to 6 months	0.2				
7 to 12 months	5.5				
1 to 3 years	11				
4 to 8 years	15				
9 to 13 years		25	21		
14 to 18 years		35	24	29	44
19 to 50 years		35	25	30	45
>50 years		30	20		

*** mcg = micrograms**

Adult women in the United States consume about 23 to 29 mcg of chromium per day from food, which meets their AIs unless they're pregnant or lactating. In contrast, adult men average 39 to 54 mcg per day, which exceeds their AIs.

The average amount of chromium in the breast milk of healthy, well-nourished mothers is 0.24 mcg per quart, so infants exclusively fed breast milk obtain about 0.2 mcg (based on an estimated consumption of 0.82 quarts per day) Infant formula provides about 0.5 mcg of chromium per quart (Cocho*et al* ., 1992)]. No studies have compared how well infants absorb and utilize chromium from human milk and formula (Stoecker, 2001).

1.3.3 Biochemical characteristic of chromium

Very little chromium (<2%) in the form of inorganic compounds is absorbed but may be higher with certain organic formulations .Once absorbed, chromium is distributed to various tissues of the body, but appears to be most concentrated in the kidney, muscle, and liver (Hepburn and Vincent,2003) The principal carrier protein for chromium is transferrin, which also plays a critical role in the movement of chromium from blood to low molecular weight chromium (LMWCr). It has been suggested that migration of transferrin receptors to the plasma membranes of insulin-insensitive cells after insulin stimulation is the initial step in this process. Transferrin containing the plasma-bound chromium is postulated to bind to the transferrin receptors and is internalized by endocytosis The pH of the internalized vesicle is reduced by ATP-driven proton pumps, chromium is released from transferrin, and the resulting free chromium is postulated to be sequestered by LMWCr (Vincent,2000)- (Clodfelder*etal.*,2001), with this step, chromium is transferred from transferrin to LMWCr, which normally exists in insulin-dependent cells in the apo, or inactive, form. Binding with chromium ions converts inactive LMWCr to its holo, or active, form. It is proposed that LMWCr then participates as part of an insulin signal amplification systemas it binds to insulin-

activated insulin receptors and results in stimulating its tyrosine kinase activity. The result of this process is the activation of insulin receptor kinase and potentiation of the actions of insulin (Vincent, 2000; Vincent, 2000; Vincent, 1999) Importantly, LMWCr without bound chromium or in the presence of other metal ions is ineffective in activating insulin-dependent kinase activity and thus enhancing the actions of insulin (Vincent, 1999).

Chromium has also been demonstrated to inhibit phosphotyrosine phosphatase, the enzyme that cleaves phosphate from the insulin receptor, leading to decreases in insulin sensitivity. Activation of insulin receptor kinase and inhibition of insulin receptor phosphatase would lead to increased phosphorylation of the insulin receptor and increased insulin sensitivity,the balance between kinase and phosphatase activity may facilitate the role of insulin in rapidly moving glucose into cells. In addition, it has been suggested that chromium enhances insulin binding, insulin receptor number, insulin internalization, and β-cell sensitivity. (Davis *et al*., 1996)

1.3.4 Factors which affects chromium levels in the body

Absorption of chromium from the intestinal tract is low, ranging from less than 0.4% to 2.5% of the amount consumes (Doisy *et al*., 1971; Anderson *et al*., 1993), and the remainder is excreted in the feces (Offenbacher*et al*., 1986). Enhancing the mineral's absorption are vitamin C and the B vitamin niacin (Offenbacher, 1994) Absorbed chromium is stored in the liver, spleen, soft tissue, and bone (Lim *et al*., 1983).

The body's chromium content may be reduced under several conditions. Diets high in simple sugars (comprising more than 35% of calories) can increase chromium excretion in the urine (Kozlovsky *et al*., 1986). Infection, acute exercise, pregnancy and lactation, and stressful states (such as physical trauma) increase

chromium losses and can lead to deficiency, especially if chromium intakes are already low (Anderson, 1994; Lukaski *et al.*, 1996).

1.3.5 Chromium Deficiency

In the 1960s, chromium was found to correct glucose intolerance and insulin resistance in deficient animals, two indicators that the body is failing to properly control blood-sugar levels and which are precursors of type 2 diabetes (Mertz, 1969).However, reports of actual chromium deficiency in humans are rare. Three hospitalized patients who were fed intravenously showed signs of diabetes (including weight loss, neuropathy, and impaired glucose tolerance) until chromium were added to their feeding solution. The chromium, added at doses of 150 to 250 mg/day for up to two weeks, corrected their diabetes symptoms (Jeejeebhoy*et al.*, 1977; Freund *et al.*, 1979; Brown *et al.*, 1986). Chromium is now routinely added to intravenous solutions.There are reports of significant age-related decreases in the chromium concentrations of hair, sweat and blood (Davies *et al.*, 1997), which might suggest that older people are more vulnerable to chromium depletion than younger adults. One cannot be sure and however, as chromium status is difficult to determine (Gibson, 2005). That's because blood, urine, and hair levels do not necessarily reflect body stores (Lukaski, 1999) Furthermore, no chromium-specific enzyme or other biochemical marker has been found to reliably assess a person's chromium status (Lukaski, 1999; Stoecker, 1999).

There is considerable interest in the possibility that supplemental chromium may help to treat impaired glucose tolerance and type 2 diabetes millets, but the research to date is inconclusive. No large, randomized, controlled clinical trials testing this hypothesis have been reported in the United States .Nevertheless, this is an active area of research.

1.3.6 Chromium Functions

Chromium has long been of interest for its possible connection to various health conditions. Among the most active areas of chromium research are their uses in supplement form to treat diabetes, lower blood lipid levels, promote weight loss, and improve body composition(Althuis *et al.*, 2002).

1.3.6.1 Type 2 diabetes and glucose intolerance

Chromium deficiency impairs the body's ability to use glucose to meet its energy needs and raises insulin requirements. It has therefore been suggested that chromium supplements might help to control type 2 diabetes or the glucose and insulin responses in persons at high risk of developing the disease. A review of randomized controlled clinical trials evaluated this hypothesis(Althuis *et al.*, 2002). This meta-analysis assessed the effects of chromium supplements on three markers of diabetes in the blood: glucose, insulin, and glycatedhemoglobin.It summarized data from 15 trials on 618 participants, of which 425 were in good health or had impaired glucose tolerance and 193 had type 2 diabetes. Chromium supplementation had no effect on glucose or insulin concentrations in subjects without diabetes nor did it reduce these levels in subjects with diabetes, except in one study. However, that study, conducted in China (in which 155 subjects with diabetes were given either 200 or 1,000 mcg/day of chromium or a placebo) might simply show the benefits of supplementation in a chromium-deficient population.

Overall, the value of chromium supplements for diabetes is inconclusive and controversial (Cefalu and Hu, 2004).Randomized controlled clinical trials in well-defined, at-risk populations where dietary intakes are known are necessary to determine the effects of chromium on markers of diabetes(Althuis *et al.*, 2002). The American Diabetes Association states that there is insufficient evidence to support the routine use of chromium to improve glycemic control in people with diabetes.It further notes that there is no clear scientific evidence that vitamin and

mineral supplementation benefits people with diabetes who do not have underlying nutritional deficiencies (Evert *et al*., 2013).

1.3.6.2 Lipids metabolism

The effects of chromium supplementation on blood lipid levels in humans are also inconclusive (Offenbacherand Pi-Sunyer, 1997). In some studies, 150 to 1,000 mcg/day has decreased total and low-density-lipoprotein (LDL or "bad") cholesterol and triglyceride levels and increased concentrations of apolipoprotein A (a component of high-density-lipoprotein cholesterol known as HDL or "good" cholesterol) in subjects with atherosclerosis or elevated cholesterol or among those taking a beta-blocker drug (Roebacr *et al*., 1991)These findings are consistent with the results of earlier studies (Doisy *et al*., 1976; Mossop, 1983).

However, chromium supplements have shown no favorable effects on blood lipids in other studies (Anderson *et al*., 1983; Uusitupa *et al.*, 1992). The mixed research findings may be due to difficulties in determining the chromium status of subjects at the start of the trials and the researchers' failure to control for dietary factors that influence blood lipid levels (Stoecker, 2001).

1.2.6.3 Body weight and composition

Chromium supplements are sometimes claimed to reduce body fat and increase lean (muscle) mass. Yet a recent review of 24 studies that examined the effects of 200 to 1,000 mcg/day of chromium (in the form of chromium picolinate) on body mass or composition found no significant benefits (Vincent, 2003) Another recent review of randomized, controlled clinical trials did find supplements of chromium picolinate to help with weight loss when compared with placebos, but the differences were small and of debatable clinical relevance (Mykkanen*et al.*, 1992)In several studies, chromium's effects on body weight and composition may be called into question because the researchers failed to adequately control for the

participants' food intakes. Furthermore, most studies included only a small number of subjects and were of short duration (Cefalu and Hu, 2004).

1.3.7 Risks of Excessiveness

Toxicity from dietary chromium has not been reported, and not very likely to occur. Studies where researchers use chromium like a drug—at doses close to 50 times more than seen with average diets did not lead to significant risk of adverse effects.In stark contrast to food intake and diets, there are industrial workplace settings where risk of excessive chromium exposure (in the form of hexavalent chromium, or chromium-6) can be significant (Thor *et al.,* 2011).

1.3.8 Chromium and medication interactions

Certain medications may interact with chromium, especially when taken on a regular basis (see Table 3). Before taking dietary supplements, check with your doctor or other qualified healthcare provider, especially if you take prescription or over the-counter medications (Davis *et al.*, 1995).

Table 4: Interactions between chromium and medications, (Davis *et al*., 1995)**.**

Medications	Nature of interaction
Antacids Corticosteroids H2 blockers (such as cimetidine, famotidine, nizatidine, and rantidine) Proton-pump inhibitors (such as omeprazole, lansoprazole, rabeprazole, pantoprazole, and esomeprazole)	These medications alter stomach acidity and may impair chromium absorption or enhance excretion
Beta-blockers (such as atenolol or propanolol) Corticosteroids	These medications may have their effects enhanced if taken together

Insulin Nicotinic acid Nonsteroidal anti-inflammatory drugs (NSAIDS) Prostaglandin inhibitors (such as ibuprofen, indomethacin, naproxen, piroxicam, and aspirin)	with chromium or they may increase chromium absorption

1.3.9 Supplemental sources of chromium

Chromium is a widely used supplement, representing 5.6% of the total mineral-supplement market (Davis*et al.,* 1995), particularly those marketed for weight loss and performance enhancement. Supplement doses typically range from 50 to 200 mcg.

The safety and efficacy of chromium supplements need more investigation.Chromium supplements are available as chromium chloride, chromiumnicotinate, chromium picolinate, high-chromium yeast, and chromium citrate. Chromium chloride in particular appears to have poor bioavailability (Cefaluand Hu, 2004). However, given the limited data on chromium absorption in humans, it is not clear which forms are best to take.

Previous report found that, in both experimental animals and humans indicates that chromium is an essential element involved in the action of insulin as demonstrated in the studies of chromium deficiency,although chromium deficiency has not been defined beyond that in patients receiving TPN, epidemiologic studies suggest that tissue levels of chromium are reduced among diabetic individuals, especially in those with existing CVD, compared with healthy control subjects. Two case-control studies have also found that lower toenail chromium levels predict risk of MI in apparently healthy subjects. However, further epidemiologic studies are

needed to confirm these associations in different populations, and clinical trials are needed to prove the causal relationship (Cefalu and Hu, 2004).

Another study found that when sufficient levels of chromium are present much lower amounts of insulin are required. Diabetes has been shown to develop as a consequence of chromium deficiency in experimental animals and in humans sustained by prolonged total parenteral nutrition. Chromium deficiency is relatively common in patients with Type II diabetes and may impair the function of GTF, causing the uptake of glucose into cells to become less efficient. Impaired chromium metabolism may also play a role in diabetes of pregnancy. High insulin levels also seem to increase chromium excretion (Michael *et al*., 1980).

1.4 Rationale

Diabetes mellitus is the most common non-communicable disease in Sudan, is having an increasing impact on rates of morbidity and mortality in Sudan. The reason

Because the highest prevalence of diabetes in Sudanese communities and the ignorance of its victims in terms of prevention and its attendant complications I do this research. Chromium is trace element and has important role in insulin action.Diabetes mellitus has been shown to develop as a consequence of chromium deficiency in experimental animals and humans sustained by prolonged total parenteral nutrition. Therefore chromium uses as supplement for treated DM, lower blood lipid levels, promote weight loss, and improve body composition. Accordingly the present study conducted to evaluate chromium level among type 2 DM patients and its correlation with study variables.

1.5 Objectives:

1.5.1 General objective:

To evaluate Chromium level among type2 Diabetes mellitus patients

1.5.2 Specific objective:

- To estimateChromium level and HbA1c in study group.
- To compare mean concentration of Chromium in case and control.
- To compare mean concentrations ofchromium level in control and uncontrol diabetic patients.
- To correlate between chromium level and study variable (Age, six, diabetic control, BMI).

Chapter two

Materials and methods

2.1 Materials

2.1.1 Study design

This is a descriptive analytical cross-sectional study carried out during the period from the January 2015 to September 2015.

2.1.2 Study area

The study was conducted in Khartoum state in Almotkamel hospital and Zenam hospital.

2.1.3 Study population

One hundred and twenty subjects were enrolled in the present study, and then classified as 60 DM patients as case group and 60 healthy apparently as control group.

2.1.4 Sampling

Vein puncture Blood (5ml) was collected by standard procedure, from the study groups, divided (2.5ml) into sterile containers without anticoagulant and measured for chromium and (2.5ml) into sterile containers with EDITA as anticoagulant and measured for HbA1c.

2.1.5 Ethical consideration

All participants were told about the research importance during interview and all of them were agreed to participate.

2.1.6 Data analysis

Data was analyzed by using the computer (SPSS) programme.

2.2 Methods

2.2.1 Estimation of chromium

Chromium was estimated by atomic absorption spectrometry.

Principle of method for chromium:

The technique makes use of absorption spectrometry to assess the concentration of analyte in a sample .it requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the beer-lamber law.

In short, the electrons of the atoms in the atomizer can be promoted to higher orbital (excited state) for short period of time by absorbing a defined quantity of energy (radiation of given wavelength).this amount of energy, wavelength, is specific to a particular electron element. In general each wavelength corresponds to only one element, .the radiation flux without a sample and with a sample in the atomizer is measured using detector, and the ratio between the two values (the absorbance) is converted to analyst concentration or mass using the Beer-Lamber Law

2.2.2 Estimation of HbA1c

HbA1c was estimated using ichroma^{TM}HbA1c.

Principle for HbA1c

Ichroma^{TM}HbA1c is based on the fluorescence immunoassay technology , specially the sandwich immune-detection method , whole blood hemolysed by hemolysis buffer and detection buffer, the fluorescence labeled detector anti-HbA1c Antibody in buffer bind to HbA1cAntigene in blood specimen ,this complex capture to anti-HbA1c sandwich antibody, as the result higher concentration ofHbA1c produce higher fluorescence signal from the complex , the signal is interpreted and the result displayed on ichroma^{TM}Reader .

2.2.3 Quality control

For quality control, use control materials as listed in the (Materials required) section. Other suitable control material can be used in addition.

The control intervals and limits should be adapted to each laboratory`s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

2.2.4 Calculation of body mass index:

BMI=Weight (kg) ÷height (m) ^{2}

Chapter three

Results

3. Results

A hundred and twenty randomly samples were collected to evaluate the level of chromium , HbA1c and BMI among study groups, then classified as 60 healthy apparently as control group and 60 type II DM as case, males account 23 (38.33%) and female 37 (61.67%) with ratio of 1:1.6, and participants average age is (51±11SD) years.

Figure (3.1): showsfrequencies of gender among diabetic patients, results expressed as percentage (%).

Table (3.1): Shows mean concentration of chromium in case (0.016 ± 0.009) versus control group (0.263 ± 0.109), with P-value = 0.000

Table (3.2): shows percentages of BMI, classified as normal weight (BMI ≤ 26.5 kg/m2) and over weight (BMI > 26.5 kg/m2) among gender (male and female).

Table (3.3): shows mean concentration of HbA1c in males (9.90 ± 3.85) versus females (9.20 ± 3.93), with P-value = 0.466

Table (3.4): Shows mean concentration of chromium in males (0.014 ± 0.008) versus females (0.017 ± 0.010), with P-value =0.239

Figure (3.2):Shows mean concentration of chromium in HbA1c good control (0.025 ± 0.007) and poor control groups (0.014 ± 0.009), result expressed as (mean ± SD) and significance deference (P-value 0.001).

Figure (3.3):Shows no personal correlation results between chromium and age with R-value -0.109 and P-value 0.238

Figure (3.4)personal correlation results between chromium and duration of type II DM with R-value- 0.354 and p-value0.005

Figure (3.5)shows personal correlation results negative correlation between chromium and BMI with R-value- 0.180 and P-value 0.004

Figure (3.6)personal correlation results shows negative correlation between chromium and HbA1c with R-value -0.27 and P-value 0.011

3.1 Frequencies of gender among diabetic patients

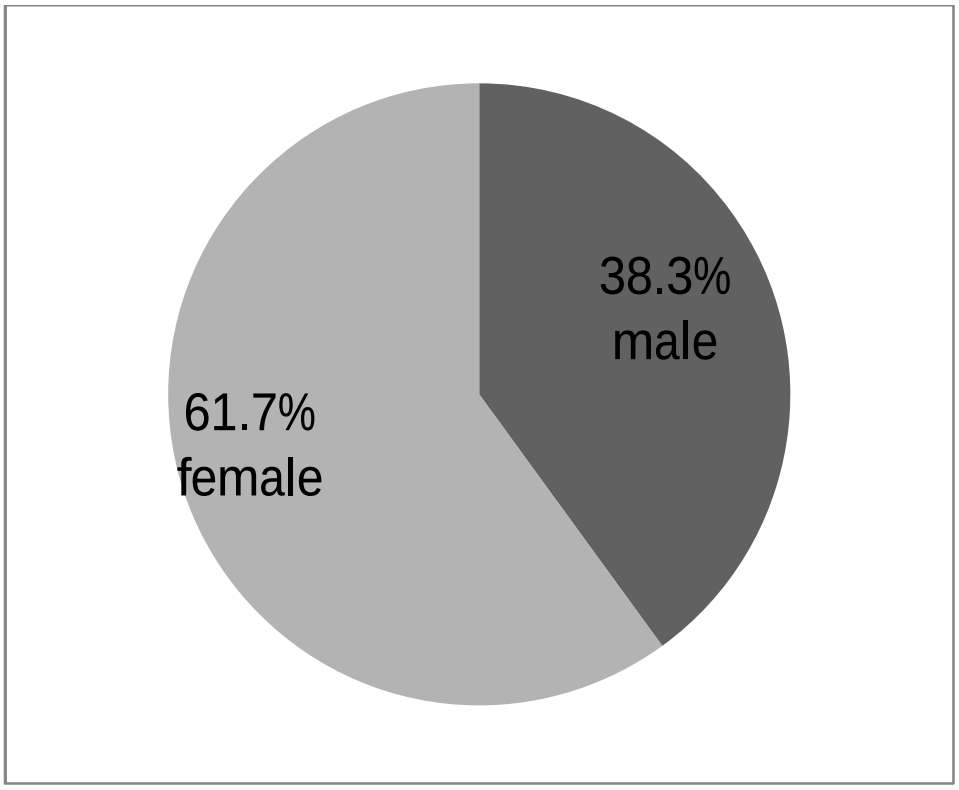

Fig. 3.1 Shows frequencies of gender among diabetic patients, results expressed as percentage (%).

3.2 Mean concentration of chromium in case and control groups:

Variable		Mean ± SD	P-value
Chromium (mg/l)	**Case**	0.016 ± 0.009	0.000**
	Control	0.263 ± 0.109	

Table 3.1 Shows mean concentration of chromium in case versus control group, the result show as(mean±SD) and significant difference consider, *P*-value < 0.05.**indicate high significant.

3.3Frequencies of normal and overweight among gender variation

BMI	Gender	
	Male	**Female**
Normal weight	64.6%	58.3%
Over weight	35.4%	41.7%
Total (%)	100%	100%

Table.3.2 shows percentages of BMI, classified as normal weight (BMI $\leq$ 26.5 kg/m^2) and over weight (BMI > 26.5 kg/m^2) among gender (male and female).

3.4 Mean concentration of HbA1camong gender variation

Variable		Mean ± SD	P-value
HbA1c (%)	**Males**	9.90 ± 3.85	0.466
	Females	9.20 ± 3.93	

Table.3.3 Shows mean concentration of HbA1c in males versus females,the results show as Mean±SD, and significant difference onsider, P-value < 0.05

3.5 Mean concentration of chromium among gender variation with diabetic patient:

Variable		Mean ± SD	P-value
Chromium (mg/l)	**Males**	0.014 ± 0.008	0.239
	Females	0.017 ± 0.010	

Table 3.4 Shows mean concentration of chromium in males versus femalesthe result show as mean±SD and significant difference consider, P-value <0.05

3.6 Mean concentration of chromium in HbA1c % good and poor control groups

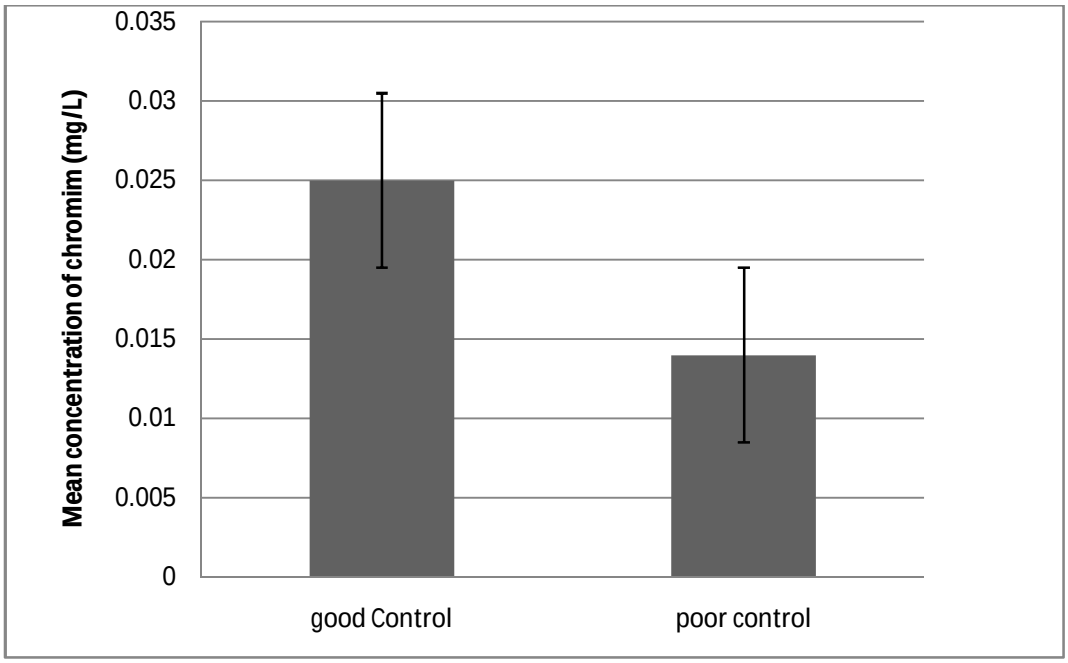

Figure 3.2 Shows mean concentration of chromium in HbA1c good control (0.025 ± 0.007) and poor control groups (0.014 ± 0.009), result expressed as (mean ± SD) and significance deference (P-value 0.001).

3.7 Correlation between chromium and age

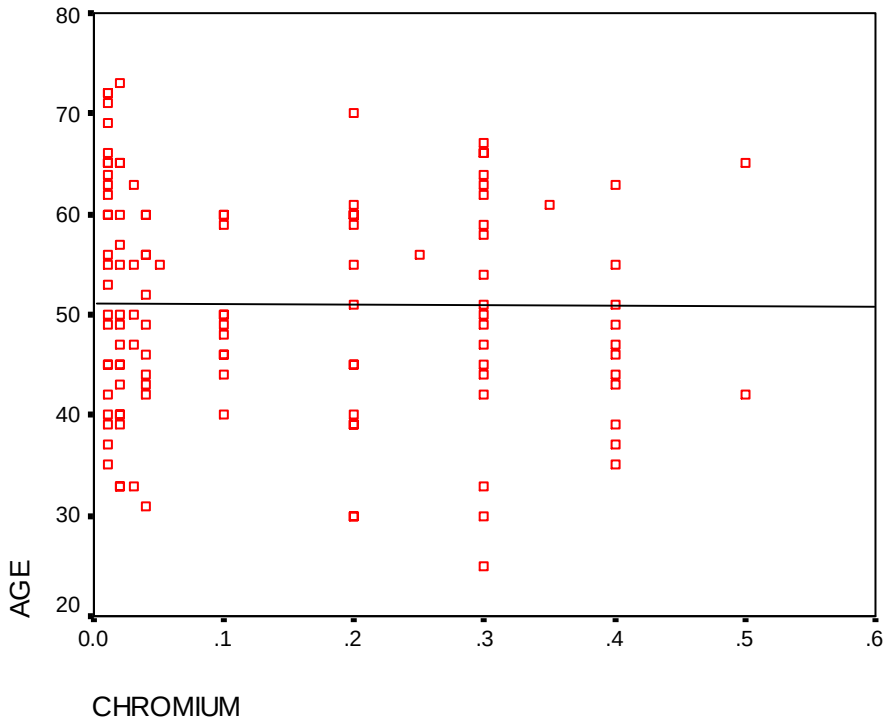

Figure 3.3 Shows Personal correlation results insignificant correlation between chromium and age with R-value -0.109 and P-value 0.238.

3.8 Correlation of chromium and duration of diabetes mellitus

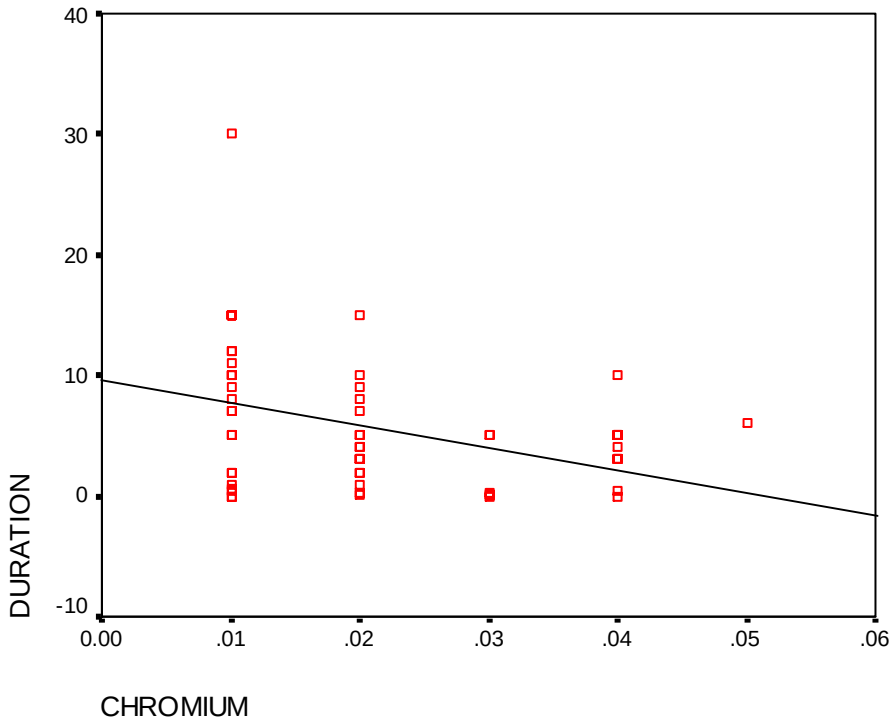

Figure 3.4 Personal value insignificant correlation results between chromium and duration of type II DM with R-value -0.354 and P-value 0.005

3.9 Correlation of chromium and BMI

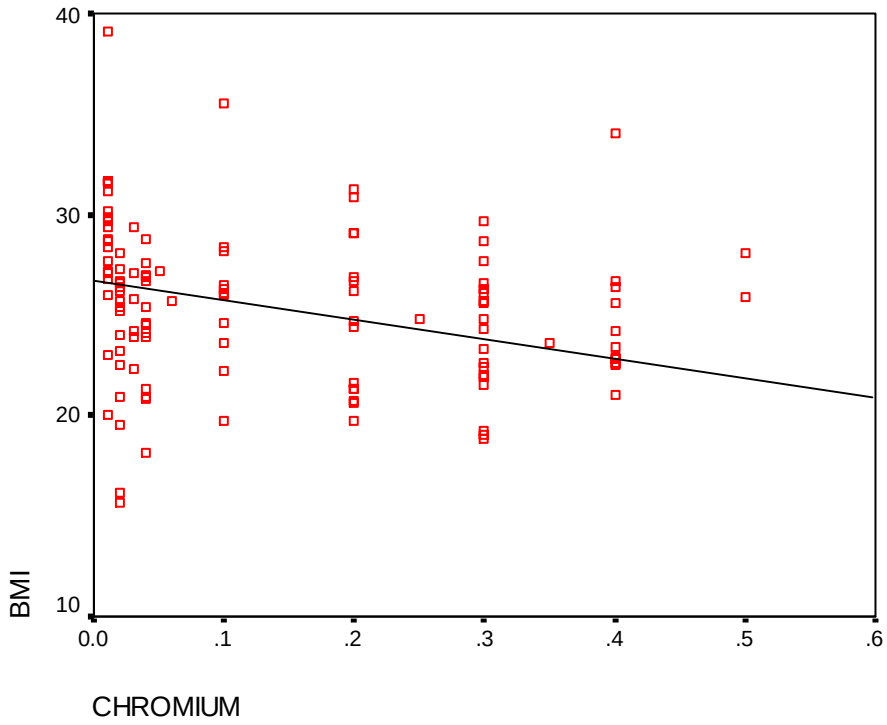

Figure 3.5 Shows personal correlation results significant negative correlation between chromium and BMI with R-value- 0.180 and P-value 0.004

3.10 Correlation of chromium and HbA1C

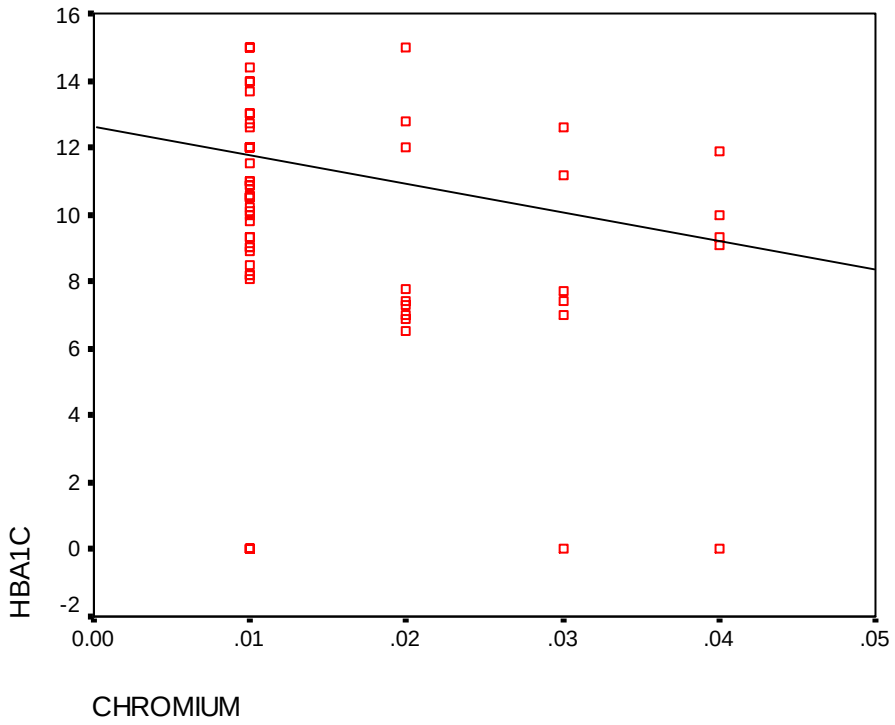

Figure 3.6 personal correlation results shows significant negative correlation between chromium and HbA1c with R-value -0.27 and P-value 0.011

Chapter four

Discussion, conclusion and recommendation

4.1 Discussion

Diabetes mellitus is the most common non-communicable disease in Sudan, is having an increasing impact on rates of morbidity and mortality in Sudan,

researchers link between chromium level and complications of type 2 DM. The present study aims to evaluate chromium level among type 2 DM patients and its correlation with study variables.

The results of frequencies revealed that, type 2 DM is common in females 61.7% than males which account of 38.3%with ratio of 1.6:1The findings was similar to previous reported in America as (39.3)% male and(60.7) for female (Fatima *et al*., 2010),and contradict with other study which state that male more affected by DM than female in Japan(62)%male and (38)% female (Atarue*t al.*,2002),which justified by community gender variation.

The percentage of type 2 DM overweight females was 41.7% compared with 35.4% males, accordingly type 2 DM females are more susceptible to gain weight than males.

In the current study there was significant decrease in mean chromium level of type 2 DM when compared with healthy control subjects (*P*-value 0.000). the findings agreed with previous study which reported that, diabetes mellitus has been shown to develop as a consequence of chromium deficiency in experimental animals and humans sustained by prolonged total parenteral nutrition(Michael *et al.*,1980).

Another study carried on fasting blood and second morning void urine samples from 93 NIDDM patients and 33 healthy volunteers. Significant differences in chromium were seen between patients and controls. NIDDM patients had mean levels of plasma chromium around 33% lower and urine values almost 100% higher than those found in health (Morris *et al*, 1999). In fact that chromium deficiency may exaggerate the complications of type 2 DM patients therefore, there is a considerable interest in the possibility that supplemental chromium may help to treat impaired glucose tolerance and type 2 diabetes mellitus. Nevertheless,

this is an active area of research, the value of chromium supplements for diabetes is inconclusive and controversial (Cefalu and Hu, 2004).

The study showed no significant difference in mean concentration of HbA1c in males compare to females ,with(*P*-value 0.466), these findings agreed with previous study conducted in America which found insignificant impact with HbA1c value in both male and female (Laurie, 2009).

Moreover, the present study showed that, there insignificant difference in mean concentration of chromium in type 2 DM females compared to males group with (*P*-value 0.239). The findings confirmed by a study done in USA that no gender differences in Cr level for adults with type 2 DM (Joanna, 1995).

A study conducted in China in 188 patients with Type II DM who received chromium supplementation for 2 months, the results showed good control of HbA1c as it reduced fasting and postprandial blood glucose and HbA_{1c} without any corresponding change in plasma insulin (Chen, 1997). Interestingly the current study revealed that, there was asignificant decrease in mean concentration of chromium of poor controlled type 2 DM in comparison with good control with *P*-value 0.001. In fact that chromium deficiency leads to insulin resistance and deceased HDL, consequently uncontrolled type 2 DM more vulnerable to develop cardiovascular disease.

The results of correlation revealed that, there was no correlation between chromium level and age of type 2 DM patients with *p*-value 0.238 that was similar to previous study carried on USA which concluded to insignificant correlation between age and Cr level (Joanna, 1995), and also contradicted with many reports showed significant age-related decreases in the chromium concentrations of hair, sweat and blood (Davies *et al*., 1997).

In addition the results of study gives evidence that, there was a negative correlation between chromium level and duration of type II DM with R-value -0.354 and *p*-value0.005, in fact that previous finding showed a significant negative correlation between fasting levels of plasma chromium and insulin in Healthy volunteers, also plasma chromium values were inversely correlated with plasma glucose. This was lost in patients with diabetes of more than 2 years duration. We suggest large losses of chromium over many years may exacerbate an already compromised chromium status in NIDDM patients and might contribute to the developing insulin resistance seen in patients with type 2 diabetes (Morris *et al*, 1999).

The study Showed significant negative correlation between chromium and BMI with R-value -0.180 and P-value 0.004, one study of patients with diabetes indicated no significant effects on either body weight or BMI (Aderson*et al*., 1997), while another study in elderly subjects with impaired glucose tolerance demonstrated significant reductions in BMI (Uusitupa *et al*., 1992). Of the eight double-blind, placebo-controlled trials in individuals without diabetes, chromium supplementation showed decreases in weight and fat in three larger studies (Kaats *et al*., 1998).

These results generally support the view that chromium supplementation has at best modest effects on body weight or composition in individuals with diabetes and perhaps more consistent positive effects in healthy volunteers. However, it must be noted that most of the studies addressing this question included only small numbers of subjects and were of relatively short duration. Personal correlation results show negative correlation between chromium and HbA1c with R-value -0.27 and P-value 0.011.

4.2 Conclusion

The study concludes that:

- Chromium is significantly decreased in patients with diabetic mellitus compared to healthy individuals.
- There is insignificant difference between the chromium levels in male compared to female patients with type2 DM

- There significant difference in chromium serum level in good control compare to poor control
- There no correlation between chromium serum level and ageof patients
- There is correlation between duration of DM and chromium serum level.
- There is significant negative correlation between chromium and BMI of patients with type2 DM.
- There is negative correlation between chromium and HbA1c of diabetic patients.

4.3 Recommendations

From the result of this study it is recommended that:

- The chromium supplementation must be as routine use to improve glycemic control in patients with diabetes
- Further studies should be done to assess Cr dose, duration and the side effects if found.
- For most studies concerning chromium serum level and blood glucose level are inconclusive so further studies should be done.

- to determine the exact mechanism by which Cr affects insulin sensitivity require more researches
- Due to unclear mechanism of how Cr reduces body weight, further studies should be carried.

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Appendixes

Appendix

(1)

Sudan University of Science and Technology

College of Graduate studies

MSc of medical laboratory

Questionnaire

Date: ……. ID NO: …….

Name:

……

Sex: ……

Age: ……

Phone Number: ……

Weight (kg)……. Height (m)……..

Body Mass Index (BMI): …… Kg/m^2

Duration of diabetic mellitus type 2: ……

Other diseases: ……..

Serum chromium level: ……. mg/L

HbA1c: ……. %