Serum Ferritin Level in Controlled and Uncontrolled Type-2 Diabetes Mellitus Patients, *Aldaraga* Health Centre, Wad Medani, Gezira State, Sudan (2016)

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الاهداء

هانحن اليوم والحمد لله نطوي سمر الليالي وتعب الإياو وخلاصة مشوارنا بين ثنايا هذا العمل المتواضع و اهديه الي :

إلى منارة العلم والامام المصطفي إلى سيد الخلق إلى رسولنا الكريم سيدنا محمد صلى الله عليه وسلم.

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مستوي مصل الفيريتين لدي مرضي السكري المنظمين وغير المنظمين من النوع الثاني في مركزالدرجة ودمدني، ولاية الجزيره، السودان 2016

ريان عبد الرحمن حمد عبد الرحمن

ملخص الدراسة

مرض السكري من النوع 2 يعتبر من اشيع الامراض الناتجه من اختلال الغدد ويصيب اكثر من 285 مليون شخص علي نطاق العالم المسبب الرئيسي له لم يفهم بصوره كامله بعد . ولكن حديثا اعتبر تخضب الدم الغير مشخص كاحد الاشياء التي يمكن ان تتسبب في مرض السكري.اجريت هده الدراسه لاختبار العلاقه بين فيريتين المصل كاحد المرقمات لزيادة حديد الدم مع مرض السكري من النوع 2 في مرضي السكري المنظمين مع غير المنظمين .اجريت هذه الدراسه علي 60 مريض بمرض السكري من النوع الثاني الذين يراجعون مركز الدرجه الصحي بمدينة ودمدني ,تم قياس فيريتين المصل وسكر الدم الصائم والسكر التراكمي وذلك بسحب ثلاث عينات دم مقدره ب3مل في كل سحبه ووضعها في حاويه خاليه من مانع التجلط, حاوية فلوريد الصوديوم وحاوية ايثيلين ثنائي الامين رباعي حمض الخليك (ايديتا) علي التوالي وتم فحصها باستخدام جهاز A25 (رقمه التسلسلي 331054841 بايوسيستم ,برشلونه ,اسبانيا) وجهاز اي كروما لقياس السكر التراكمي تم تحليل النتائج احصائيا باستخدام برنامج الحزم الاحصائيه للعلوم الاجتماعيه (اختبار مربع كاي).تم اختبار 15 ذكر و45 انثي وكان هنالك فرق احصائي ملحوظ ما بين تراكيز فيريتين المصل في المرضي المنظمين(35.1 ± 63.) والمرضي غير المنظمين (بقيمه افتراضيه 0.03) كما كان هنالك فرق احصائي معنوي ما بين تراكيز السكر الصائم في المرضي المنظمين والمرضي غير المنظمين بقيمه افتراضيه (0. 3) وكذلك لم يكن هنالك فرق احصائي معنوي في العمر بين مرضي السكري المنظمين وغير المنظمين (بقيمه افتراضيه 0.6). تراكيز الفيريتين كانت مرتبط بصوره ايجابيه مع الفتره الزمنيه لمرض السكري لدي مجموعة الدراسه بينما لم يكن هنالك علاقه احصائيه معنويه بين الفترة الزمنيه والسكر التراكمي او سكر الدم الصائم, كما لم توجد علاقه احصائيه بين مصل الفيريتين والنوع (بقيمه افتراضيه 0.6),توصلت الدراسه الي ان مصل الفيريتين يزيد لدي مرضي السكري غير المنظمين وانه ربما لمصل الفيريتين دور في امراضية مرض السكري من النوع 2. اوصت الدراسه باجراء المزيد من الدراسات علي حجم مجموعه اوسع مع اجراء مع ضبط و مساواة توزيع المتغيرات كما اوصت ايضا بان يتم قياس الفيريتين علي الاقل مره واحده لكل مريض بمرض السكري من النوع 2.

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Abstract

Diabetes mellitus (DM) type 2 is one of the most common endocrine disorders that affect more than 285 million people in the world. The etiology of the disease is not fully understood, but recently subclinical hemochromatosis has been considered as one of the probable causes of diabetes mellitus. This study was carried out to examine the relationship between serum ferritin as a marker of iron overload with diabetes mellitus among controlled and uncontrolled diabetics. This study was conducted on 60 patients with type 2 diabetes mellitus who were referred to Al-Darage health center in wad Medani town. Fasting serum ferritin, heamolobinA1c, and fasting blood glucose were measured inblood samples by collecting 3 ml of venous blood into ethylene di-amine tetra acetic acid (EDTA), sodium fluoride and plain containers and processed usingbiosystem A25 chemistry analyzer, serial No. 331054841 (Bio-system, Barcelona-Spain) and I Chroma instrument for heamolobinA1c measurement. Results were analyzed statistically by SPSS (Chi-square test) program. (15) Males and (45) female were tested, there was a statically significant difference between serum ferritin levels of controlled (M ± SD= 63. ± 35.1) and uncontrolled diabetics (M ± SD = 117.9 ± 95) (P value = 0.03). And there was no significant difference between fasting blood glucose levels of controlled diabetics group and uncontrolled diabetics group (*P* value = 0. 3). Mean Serum ferritin of males was (99.9 ng/ml) while Mean Serum ferritin of females was (111.6 ng/ml) with no significant difference between the two means (*P* value = 0. 7).The study concluded that serum ferritin concentrations were increased in uncontrolled diabetics when compared to un controlled diabetics, the study recommended that also serum ferritin should be monitored for diabetics to reduce the complications of diabetes, alsomore studies including a larger population size and equally distributed variables should be done to confirm these results.

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List of abbreviations:

BMI	Basal metabolic index
CT	Computed tomography
DM	Diabetes mellitus
DPP4	Non-steroidal Anti-inflammatory Drugs
EDITA	Messenger ribonucleic acid
FBG	Fasting blood glucose
GIP	Glucose dependent insulinotropic poly peptide
GIPP	Gastric inhibitory polypeptide
GLP	Glucagon like peptide
HDL	High density lipoprotein
IDDM	Insulin dependent diabetes mellitus
IFG	Impaired fasting glucose
IGF	Insulin like growth factor
IGM	Impaired glucose tolerance
LDL	Low density lipoprotein
M+SD	Mean + standard deviation.
ROS	Reactive oxygen species
SGLT2	Sodium glucose co transporter
SNPs	Single nucleotide polymorphism
SPSS	Statistical package for social science
T1DM	Type1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
WHO	World health organization

CHAPTER ONE
INTRODUCTION

1.1 General Introduction:

The explosive increase of Diabetic population worldwide is a major public health concern both in developing and developed countries. Metabolic syndrome is also on an increasing trend. The metabolic syndrome is closely linked to insulin resistance and numerous studies indicate a link to iron overload. Increased serum ferritin, reflecting body iron overload, is often associated with measures of insulin resistance, such as elevated blood glucose and insulin levels.1 In addition, two prospective studies have identified an independent association between baseline elevations in iron stores and the incidence of diabetes (Thomas *et.al*, 2004).

Elevated iron stores may induce diabetes through a variety of mechanisms, including oxidative damage to pancreatic beta cells, impairment of hepatic insulin extraction by the liver, and interference with insulin's ability to suppress hepatic glucose production (Ford ES *et.al*, 1999)

Raised Serum Ferritin may possibly be related to the occurrence of long term complications of diabetes, both micro vascular and macro vascular (Kim NH 2000).

Type 2 Diabetes mellitus is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. Obesity, particularly visceral or central (as evidenced by the hip-waist ratio), is very common in type 2 DM (80% or more are obese). In the early stages of the disorder, glucose tolerance remains near-normal, despite insulin resistance, because the pancreatic beta cells compensate by increasing insulin output. As islets in certain individuals are unable to sustain the hyperinsulinemic state. (Alvin CP 2008).

Impaired Glucose Tolerance (IGT), characterized by elevations in postprandial glucose, then develops. A further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia. Ultimately, beta cell failure ensues (Alvin CP 2008).

The regulation of blood-iron levels is mediated by the protein ferritin. Ferritin can release iron if the blood has a low iron concentration, and it can help to store excess iron if the blood and tissues have a high iron concentration. Hence, ferritin functions as a "buffer" against irondeficiency and iron overload. Ferritin has the shape of a hollow sphere. Inside the sphere, ironis stored in the Fe (III) oxidation state. It is incorporated in the mineral ferrihydrite, [FeO (OH)] 8[FeO (H2PO4)], which is attached to the inner wall of the sphere. To release iron when the body needs it, the iron must be changed from the Fe3+ to the Fe+2 oxidation state. Then, the iron leaves through channels in the spherical structure. Although the exact mechanism of iron-induced diabetes is uncertain; it is likely to be mediated by three key mechanisms: 1) insulin deficiency, 2) insulin resistance, and 3) hepatic dysfunction. An understanding of the pathogenic pathways of iron-induced diabetes is derived mainly from studies on animal models of hemochromatosis (Sundararaman S *et.al*,2008).Hence this study was carried out to examine the relationship between serum ferritin and type 2 diabetes mellitus and metabolic syndrome and to establish a correlation between S. ferritin, FBS and HbA1c in type II diabetics.HbA1c is the most commonly glycosylated hemoglobin and it is the reliable method in monitoring long term diabetes control rather than FBG ,two factors determine the Glycosylated hemoglobin level; the red blood cell life span and the average glucose concentration (Bishop 2005).

1.2 Justification:

Serum ferritin has some association with increasing the insulin resistance in type II diabetics through interfering with hepatic insulin, elevation in oxidative stress through the increased formation of hydroxyl radicals catalyzed by iron, which may lead to systemic insulin resistance and hyperglycemia so it's important to measure its level.

1.3 Objectives:

1.3.1 General objective:

To measure serum ferritin level in control and non-control type-2 diabetes mellitus patients in al gezira state.

1.3.2 Specific objectives:

1. To measure fasting blood sugar.
2. To measure fasting serum ferritin.
3. To measure HA1c.
4. To correlated between fasting serum ferritin, HbA1c and fasting blood sugar in control and non-control diabetic type 2.

CHAPTER TWO

LITERATURE REVIEW

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and relative insulin deficiency, resistance or both. It affects more than120 million people world-wide, and it is estimated that it will affect 220 million people by the year 2020.diabetes is usually irreversible, although patients can have a reasonably normal lifestyle (Kumar *et.al*, 2002).

2.1Insulin structure and secretion:

Insulin is a key hormone involved in the storage and controlled release within the body of the chemical energy available from food. It is coded for in the chromosome 11 and synthesized in the beta cells of the pancreatic islets. It is manufactured in the ribosomes as pre- pro-insulin by insulin m RNA. The hydrophobic ' pre' portion of re insulin allows it to transfer to Golgi apparatus, and is subsequently enzymatically cleaved ,pro insulin is parceled into secretory granule in the Golgi apparatus .these mature and pass towards the cell membrane where they are stored before release . the pro insulin molecules fold back on itself and is stabilized by disulphide bond , the biochemically inert peptide fragment known as connecting (c) peptide split off from pro insulin molecules in the secretory process, leaving insulin as acomplex of two linked peptide chains .equimolar quantities of insulin and c-peptide arereleased into the circulation.(Kumar *et.al*,2002).Beta cells in the islets of Langerhans release insulin in two phases; the first phase release is rapidly triggered in response to increased blood glucose levels. The second phase is a sustained, slow release of newly formed vesicles triggered independently of sugar. Synthesized insulin is stored in secretory vesicles (Cawston EE*et.al*, 2010).

2.2glucose metabolism:

Blood glucose levels are closely regulated in health and rarely stray outside the range 3.5-8mmol/l (63-144mg/dl) ,despite the varying demands of food, fasting and exercise. The principle organ of glucose homeostasis is the liver which absorbs and stores glucose (as glycogen) in the post absorptive state and releases it into the circulation between meals to match the rate of glucose utilization by the peripheral tissues (Kumar *et.al*,2002).

2.2.1Glucose production and utilization:

About 200g of glucose is produced and utilized each day. More than 90% is derived from liver glycogen and hepatic gluconeogenesis, and the remainder from renal gluconeogenesis. The brain is the major consumer of glucose; its requirement is 1mg/kg man. Glucose uptake by the brain is obligatory and is not dependent on insulin, and the glucose is oxidized to carbon dioxide and water. Other tissue such as muscle and fat are facultative glucose consumers. The effect of insulin peaks associated with meals is to lower the threshold for glucose entry to the cells, at other time, energy requirements are largely met by fatty acid oxidation. Glucose taken up by muscle is stored as glycogen or broken-down to lactate Whichre-enter the circulation and becomes a major substance for hepatic gluconeogenesis .glucose is used by fat tissue as a source of energy and as a substrate for triglyceride synthesis; lipolysis release fatty acids from triglyceride together with glycerol(Kumar *et.al*,2002).

2.2.2 Hormonal regulation:

Insulin is the major regulator of intermediary metabolism, although its action is modified in many respects by other hormones. Insulin concentration in the fasting state is low and it acts mainly as a hepatic hormone, modulating glucose production (via ……. glycogenolysis and gluconeogenesis) from the liver, hepatic glucose production rises as insulin levels fall. In the post prandial state insulin concentration are high and it then suppresses glucose production from the liver and promote entry of glucose into the peripheral tissues (increased glucose utilization)(Kumar *et.al*,2002).

2.2.3 Glucose transport:

Cell membranes are not inherently permeable to glucose. A family of specialized glucose transport Proteins (GLUT) carries glucose through the membrane into cell. (Kumar *et.al*, 2002).

2.3 The insulin receptor:

This is a glycoprotein (400KDa), coded for on the short arm of chromosome 19, which straddles the cell membrane of many cells. It consists of dimer with two alpha-subunits, which include the binding sites for insulin, and two beta-subunits, which traverse the cell membrane. When insulin binds to the alpha subunits it induces a conformational change in the beta-subunits results in activation of tyrosine kinase and initiation of a cascade response involving a host of other intracellular substrates. One consequence of this is migration of GLUT glucose transporter to the cell surface and increased transport of glucose into the cell. The insulin –receptor complex is then internalized by the cell, Insulin is degraded, and the receptor is recycled to the cell surface (Kumar *et.al*, 2002).

2.4 Types of diabetes mellitus:

2.4.1 Type I diabetes mellitus:

Most pediatric patients with diabetes have type 1 diabetes mellitus (T1DM) and a lifetime dependence on exogenous insulin.T1DM reaches a peak incidence around the time of puberty, but can present at any age. And the absence, destruction, or other loss of these cells results in type 1 diabetes (insulin-dependent diabetes mellitus IDDM). Healthy individual, blood glucose levels usually do not rise above 180 mg/dL (9 mmol/L). In a child with diabetes, blood sugar levels rise if insulin is insufficient for a given glucose load. The renal threshold for glucose reabsorption is exceeded when blood glucose levels Exceed 180 mg/dL(10 mmol/L), causing glycosuria with the typical symptoms of polyuria and polydipsia(Gloyn AL*et.al*,2004).

2.4.2 Type II diabetes mellitus:

Type 2 diabetes mellitus consists of an array of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion. Poorly controlled type 2 diabetes is associated with an array of micro vascular, macro vascular, and neuropathic complications.Microvascular complications of diabetes include retinal, renal, and possibly neuropathic disease. Macro vascular complications include coronary artery and peripheral vascular disease. Diabetic neuropathy affects autonomic and peripheral nerves. Unlike patients with type 1 diabetes mellitus, patients with type 2 are not absolutely dependent on insulin for life. This distinction was the basis for the older terms for types 1 and 2, insulin dependent and non–insulin dependent diabetes. However, many patients with type 2 diabetes are ultimately treated with insulin. Because they retain the ability to secrete some endogenous insulin, they are considered to require insulin but not to depend on insulin. Nevertheless, given the potential for confusion due to classification based on treatment rather than etiology, the older terms have been abandoned (Philippe MF*et.al,* 2011). Another older term for type 2 diabetes mellitus was adult-onset diabetes. Currently, because of the epidemic of obesity and inactivity in children, type 2 diabetes mellitus is occurring at younger and younger ages. Although type 2 diabetes mellitus typically affects individuals older than 40 years, it has been diagnosed in children as young as 2 years of age who have a family history of diabetes. In many communities, type 2 diabetes now outnumbers type 1 among children with newly diagnosed diabetes. Diabetes mellitus is a chronic disease that requires long-term medical attention to limit the development of its devastating complications and to manage them when they do occur.(Bacha F*et.al*,2010).It is a disproportionately expensive disease; in the United States in 2007, the direct medical costs of diabetes were $116 billion, and the total costs were $174 billion; people with diabetes had average medical expenditures 2.3 times those of people without diabetes. The emergency department utilization rate by people with diabetes is twice that of the unaffected population. (Bacha F*et.al*, 2010).

2.4.2.1Pathophysiology of Type II diabetes mellitus:

Type 2 diabetes is characterized by a combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Insulin resistance, which has been attributed to elevated levels of free fatty acids and pro-inflammatory cytokines in plasma, leads to decreased glucose transport into muscle cells, elevated hepatic glucose production, and increased breakdown of fat. A role for excess glucagon cannot be underestimated; indeed, type 2 diabetes is an islet paracrinopathy in which the reciprocal relationship between the glucagon-secreting alpha cell and the insulin-secreting beta cell is lost, leading to hyperglucagonemia and hence the consequent hyperglycemia (Bacha F*et.al,*2010).For type 2 diabetes mellitus to occur, both insulin resistance and inadequate insulin secretion must exist. For example, all overweight individuals have insulin resistance, but diabetes develops only in those who cannot increase insulin secretion sufficiently to compensate for their insulin resistance. Their insulin concentrations may be high, yet inappropriately low for the level of glycaemia. A study by Philippe et al used computed tomography (CT) scan findings, glucagon stimulation test results, and fecal elastase-1 measurements to confirm reduced pancreatic volume in individuals with a median 15-year history of diabetes mellitus (range, 5-26 years).(Wheeler E*et.al,*2011).

2.4.2.2 Beta-cell dysfunction

Beta-cell dysfunction is a major factor across the spectrum of prediabetes to diabetes. A study of obese adolescents by Bacha etal, confirms what is increasingly being stressed in adults as well: Beta-cell dysfunction develops early in the pathologic process and does not necessarily follow the stage of insulin resistance (Billings LK*et.al, 2010).* Singular focus on insulin resistance as the "be all and end all" is gradually shifting, and hopefully better treatment options that address the beta-cell pathology will emerge for early therapy. In the progression from normal to abnormal glucose tolerance, postprandial blood glucose levels increase first. Eventually, fasting hyperglycemia develops as suppression of hepatic gluconeogenesis fails. (Nielsen E M *et.al*, 2003).

During the induction of insulin resistance (such as occurs with a high-calorie diet, steroid administration, or physical inactivity), increased glucagon levels and increased glucose-dependent insulinotropic polypeptide (GIP) levels accompany glucose intolerance. However, the postprandial glucagon-like peptide-1 (GLP-1) response is unaltered (Nielsen EM*et.al*,2003).

2.4.2.3Genomic factors:

Genome-wide association studies of single-nucleotide polymorphisms (SNPs) have identified a number of genetic variants that are associated with beta-cell function and insulin resistance. Some of these SNPs appear to increase the risk for type 2 diabetes. Over 40 independent loci demonstrating an association with an increased risk for type2 diabetes has been shown(Ukkola O*et.al*, 2001).A subset of the most potent are shared below.(Lindgren CM*et.al*, 2008).Decreased beta-cell responsiveness, leading to impaired insulin processing and decreased insulin secretion (TCF7L2)Lowered early glucose-stimulated insulin release (MTNR1B, FADS1, DGKB, GCK)Altered metabolism of unsaturated fatty acids (FSADS1)Dysregulation of fat metabolism (PPARG)Inhibition of serum glucose release (KCNJ11).Increased adiposity and insulin resistance (FTO and IGF2BP2) (Sandhu MS*et.al, 2007).*Control of the development of pancreatic structures, including beta-islet cells (HHEX).Transport of zinc into the beta-islet cells, which influences the production and secretion of insulin (SLC30A8) (Chiefari E*et.al, 2011).*

Survival and function of beta-islet cells (WFS1) (Wang TJ*et.al*,2011).Susceptibility to type 2 diabetes may also be affected by genetic variants involving incretin hormones, which are released from endocrine cells in the gut and stimulate insulin secretion in response to digestion of food. For example, reduced beta-cell function has been associated with a variant in the gene that codes for the receptor of gastric inhibitory polypeptide (GIPR) (Testa R*et.al, 2011).*The high mobility group A1 (HMGA1) protein is a key regulator of the insulin receptor gene (INSR).(Krssak M*et.al*,2011).

2.5 Insulin resistance pathophysiology in diabetes:

In insulin resistance, various clinical entities of this state are evident. The clinical heterogeneity can be explained, at least in part, on a biochemical basis. Insulin binds and acts mainly through the insulin receptor and also acts via the insulin-like growth factor–1 (IGF-1) receptor; cellular actions of insulin involve a wide variety of effects on postreceptor signaling pathways within target cells. The b subunit of the insulin receptor is a tyrosine kinase, which is activated when insulin binds to the a subunit; the kinase activity auto phosphorylates and mediates multiple actions of insulin. Ambient insulin levels, various physiological and disease states, and drugs regulate insulin receptor concentration or affinity. Insulin sensitivity and secretion are reciprocally related; thus, insulin resistance results in increased insulin secretion to maintain normal glucose and lipid homeostasis (Ahrén B*et.al, 2005).*The mathematical relation between sensitivity and secretion is curvilinear or hyperbolic. Several mediators are thought to signal the pancreatic B cells to respond to insulin resistance; failure of the signals or of the B cells to adapt adequately in relation to insulin sensitivity results in inappropriate insulin levels, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and type 2 diabetes. These potential signaling mediators include glucose, free fatty acids, autonomic nerves, fat-derived hormones (eg, adiponectin), and the gut hormone glucagonlike peptide 1 (GLP-1).(Reaven GM1995).GLP-1 is an incretin hormone that stimulates insulin secretion, causes B-cell mitosis while inhibiting apoptosis, inhibits glucagon secretion, and delays gastric emptying with overall antidiabeticeffects.The mechanisms responsible for insulin resistance syndromes include genetic or primary target cell defects, autoantibodies to insulin, and accelerated insulin degradation(Reaven GM1995).Given that glucose and lipid metabolism largely depend on mitochondria to generate energy in cells, mitochondrial dysfunction may play an important role in the development of insulin resistance and associated complications(Kim JA *et.al*, *2008*). Obesity, the most common cause of insulin resistance, is associated with a decreased number of receptors and with post-receptor failure to activate tyrosine kinase. (Lee SH *et.al*, 2010).

2.6 Diabetes complications:

Although the pathophysiology of the disease differs between the types of diabetes, most of the complications, includingmicrovascular, macrovascular, and neuropathic, are similar regardless of the type of diabetes. Hyperglycemia appears to be the determinant of micro-vascular and metabolic complications. Macro-vascular disease may be less related to glycemia. Telomere attrition may be a marker associated with presence and the number of diabetic complications. Whether it is a cause or a consequence of diabetes remains to be seen (Stern MPM *et.al,*1996).Cardiovascular risk in people with diabetes is related in part to insulin resistance, with the following concomitant lipid abnormalities: Elevated levels of small, dense low-density lipoprotein (LDL) cholesterol particles Low levels of high-density lipoprotein (HDL) cholesterol Elevated levels of triglyceride-rich remnant lipoproteins Thrombotic abnormalities (i.e. elevated type-1 plasminogen activator inhibitor [PAI-1], elevated fibrinogen) and hypertension are also involved. Other conventional atherosclerotic risk factors (e.g., family history, smoking, elevated LDL cholesterol) also affect cardiovascular risk. Insulin resistance is associated with increased lipid accumulation in liver and smooth muscle, but not with increased myocardial lipid accumulation (Haffner SM *et.al*, 1999).

2.7Iron homeostasis:

Iron is an essential element for many living organisms In humans it is required for oxygen transportation (in hemoglobin and myoglobin) and electron transfer reactions. Approximately two-thirds of the body's iron is found in erythrocytes, and a further 15% is in muscle and cellular enzymes. (PADMAJA P, *et.al* 2015).

The remaining iron is excess to needs and stored primarily as ferritin or hemosiderin in the liver and within macrophages in the reticulo-endothelial system. Iron recycling through the mobilization of these stores means that most human diets can account for minor daily losses from the sloughing of epithelial cells or insignificant blood losses, Absorption of iron from the small intestine and its release from macrophages is tightly controlled, as free iron has the potential to cause tissue damage through the production of reactive oxygen species. Maintaining low levels of free iron also aids resistance to infection, as bacteria constantly scavenge for iron from their environment for growth. Hepcidin, a 25 amino acid peptide produced by the liver, is the principal iron-regulatory hormone providing the link between iron metabolism and innateimmunity.Hepcidin production Hepcidin production is stimulated by both iron loading, infection/inflammation — conditions where the body aims to limit the uptake of iron and its availability to invading organisms.Hepcidin acts by binding ferroportin, a trans membrane Protein involved in exporting iron from macrophages, erythrocytes and enterocytes. This interaction leads to ferroportin degradation. (PADMAJA P, *et.al* 2015).

2.7.1 Ferritin:

Ferritin is an intra-cellular storage protein with the capacity to store up to 4000 iron atoms. The concentration of ferritin in serum correlates well with the amount of storage iron as proven by phlebotomy trials. Hence, serum ferritin is a good marker of total body iron stores. A low serum ferritin is almost only seen in iron deficiency. In the presence of conditions such as inflammation, infection, malignancy (hematological and solid tumors), or liver or kidney disease, serum ferritin concentrations do not reflect iron stores alone and are typically higher than otherwise expected.(WHO.2001). Ferritin serves to store iron in a non-toxic form, to deposit it in a safe form, and to transport it to areas where it is required. The function and structure of the expressed ferritin protein varies in different cell types. This is controlled primarily by the amount and stability of mRNA. mRNA concentration is further tweaked by changes to how it is stored and how efficiently it is transcribed. The presence of iron itself is a major trigger for the production of ferritin, with some exceptions (such as the yolk ferritin of the gastro-podLymnaea, which lacks an iron-responsive (PADMAJA P, *et.al* 2015).

The concentration of ferritin has been shown to increase in response to stresses such as anoxia; this implies that it is an acute phase protein (PADMAJA P, *et.al* 2015).

2.7.2 Serum ferritin and insulin resistance:

Previously, Zumin Shi et al. conducted a prospective study which showed that higher body iron stores measured by serum ferritin were associated with an increased risk of hyperglycemia in a Chinese population. However, they included subjects with anemia, which might affect the serum ferritin levels. Furthermore, the association became marginally significant when other confounding variables such as BMI were included. (Shi Z*et.al*2010) .One possible explanation is that iron deposition in the liver can result in hepatic insulin resistance and increase hepatic glucose production.(Fernandez-Real JM,*et.al*1998).

Furthermore, other studies have suggested a link between serum ferritin and nonalcoholic fatty liver disease, currently regarded as one of the independent risk factors for incident type II diabetes. (Williams KH*et.al*2012).Another explanation for the relationship between elevated ferritin levels and incident type II diabetes involves an elevation in oxidative stress through the increased formation of hydroxyl radicals catalyzed by iron, which may lead to systemic insulin resistance and hyperglycemia. (Oberley LW 1988). In addition, iron excess probably contributes initially to insulin resistance and subsequently to decreased insulin secretion. (Wilson JG*et.al*2003).Considering the positive correlations described above, an increase in ferritin-related insulin resistance, rather than a decrease in insulin secretion, is likely to prevail during this early stage of type 2 diabetes. Lastly, elevated ferritin concentrations might reflect systemic inflammation besides elevated body iron stores.(Gabay C*et.al1999).*It is well known that inflammation is an important mediator of type 2 diabetes. This might implicate that ferritin increases the risk of type 2 diabetes through another various mechanisms besides systemic inflammation. Chronic inflammation with insulin resistance is also known to be involved in the development of metabolic syndrome, a well-known risk factor for type 2 diabetes and cardiovascular diseases. (Haffner SM 2006).

Recently, Park et al. reported that elevated serum ferritin levels were independently associated with future development of metabolic syndrome during the 5 year follow-up period in Koreans. (Park SK*et.al 2012).*oxidative stress have been implicated in the pathogenesis of T. 2 DM. (Radoi v.*et.al 2012*). "oxidative stress " is defined as oxidation of cellular component thereby introducing harm to that cellular tissue oxidation reaction insure that molecular oxygen is reduced to water, but the products of partial reduction of oxygen are highly and create havoc in living system, hence they are also called reactive oxygen species (ROS). Oxygen normally accepts four electrons and is converted directly to water however partial reduction of oxygen can and does occur in biological systems. Thus the sequential reduction of oxygen along the univalent pathway leads to the generation of the superoxide anion, hydrogen peroxide, hydroxyl radicals and water. Superoxide and hydrogen peroxide appear to be the primary generated species. These species may then play a role in the generation of more and additional reactive oxidants, including the highly reactive hydroxyl radicals (or a related highly oxidizing species) in which iron salts play catalytic role in a reaction. This reaction commonly referred as the metal catalyzed Haber-weiss reaction. (haliwell B*et.al*1990).

$$Fe^{+3} + O_2 \rightarrow Fe^{+2} + O_2$$

$$. Fe^{+3} + H_2O_2 \rightarrow Fe^{+3}OH + OH^-$$

$$O_2^- + H_2O_2 \rightarrow OH + OH^-$$

Iron is the most abundant trace element in the body and almost all iron occur bound to protein. It is an essential element in all cells metabolism, but it is toxic when unleashed. (Herbert v.1992).

Because of its ability to switch back and forth between ferrous and ferric oxidation state, iron is both strong biological oxidant and reluctant. Although the exact mechanism of iron induced diabetes is uncertain, it is likely to be mediated by key mechanisms: insulin deficiency, insulin resistance and hepatic dysfunction. (swaminathan s*et.al,*2007). The central importance of iron in the pathophysiology of the disease is derived from the ease with which iron is reversibly oxidized and reduced, this property while essential for its metabolic function make iron potentially hazardous because of its ability to participate in the generation of powerful oxidants species such as hydroxyl radicals. free radicals damage is primarily produced by the hydroxyl radicals (OH).most of the OH generated in vivo come from iron-dependent reduction ofH_2O_2 when ferrous iron reduces H_2O to generate OH vitamin C (ascorbic acid) convert ferric iron back to ferrous iron, it self-becoming oxidized ascorbic acid, thus allowing another cycle of OH generation from renewed ferrous iron. Another endogenous source of catalytic free iron is the iron released when the harming is opened by hemeoxygenase (Herbert v.1992). The intracellular generation of Apo ferritin is cytoprotective antioxidant stratagem of endothelial cells. (Balla G.*et.al*1992).since serum ferritin is elevated in T2DM.under normal condition quantitative relationship is exist between the level of plasma ferritin and the amount of storage iron in condition of iron overload there is generally an increase in the expression of L-subunits rich ferritins, paralleled by an increase in this ferritin in the plasma. Ferritin is considered a positive acute phase protein and is up regulated intracellularly in many cells types, and extracellulary, in the plasma as a result of an increase in cellular secretion.an important role for ferritin during the acute phase response restrict the availability of iron by sequestration into the cavity of the ferritin protein shell. Oxygen radicals i.e. molecules containing unpaired electrons are generated in large amounts during infectious and inflammatory conditions. They react with proteins, lipids and nucleic acids resulting in degradation of phagocytosed materials in the confinement of the phagosome in the neutrophil and macrophage. (Sharifi F*et.al*2004).

However large amounts of these toxic metabolites leak to the fluids and tissues in the area of inflammatory reaction and by reacting with cellular constituents can result in substantial damage .Iron, due to its role in fenton-type chemistry can result in exacerbation of oxygen radical production. In general a reduction in the bioavailability of iron will offer protection against cell injury by hydroxyl radicals that are generated from neutrophil and macrophage derived superoxide. Iron sequestration by cells in the zone of inflammation may therefore provide protection against the free radicals assault. This role of host cell protection against an increase in the free radicals onslaught is consistent with observations that a reduction in ferritin sensitizes cell to pro-oxidant cytotoxicity, and that overexpression of ferritin reduces reactive oxidant species (ROS) in cells challenged by oxidants and by implication reduces the oxidative e toxicity. The role of iron in the pathogenesis of diabetes is suggested by n increased incidence of type 2 diabetes n diverse causes of iron overload and reversal or improvement in diabetes (glycemic control) with reduction in iron load achieved using either phlebotomy or iron chelating therapy. The importance of protein glycation is well known in the in the pathogenesis of diabetic vascular complication of diabetes. Different theories regarding the ferritin role in DM have been suggested. Ferritin has been referred as a marker for insulin resistance possibly due to iron deposition in the liver leading to hepatic insulin resistance and increased hepatic glucose production. Other has determined ferritin just as a marker of pancreatic damage due to some degree of subclinical hemochromatosis has been considered in some cases of diabetes. (Sharifi F*et.al*2004)

2.8 Laboratory diagnosis of diabetes mellitus:

2.8.1 Random blood glucose:

A random blood sugar level of 200 milligrams per deciliter (mg/dL) — 11.1 mill-moles per liter (mmol/L) — or higher suggests diabetes.(Selvin E*et.al*,2011).

2.8.2Fasting plasma glucose (FPG)

The measurement of fasting glucose yields information on the capability of basal levels of insulin to control glycogenolysis and gluconeogenesis. When basal production (or action) of insulin is insufficient, fasting hyperglycemia develops. Determination of fasting plasma glucose is the very first procedure to be performed when entertaining a diagnosis of diabetes. The patient is kept on an average usual diet and, after an overnight fast (no food or sweetened drinks after midnight), blood is drawn in the morning.

2.8.2.1 Interpretation :(Selvin E*et.al*,2011)

Range	Interpretation
<100 mg/dL	Normal
100 – 125 mg/dL	May be indicative of IGT (perform glucose tolerance test)
≥ 126 mg/dL	On more than one occasion is indicative of diabetes mellitus; there is no need to perform a glucose tolerance test.

2.8.3 HbA1C test:

A1C, or Hb1c; sometimes also referred to as being HbA1c or HGBA1C) is a form of hemoglobin 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 three months. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. HbA1c is a measure of the beta-N-1-deoxy fructosyl component of hemoglobin (Peterson KPE*et.al, 1998).*

Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous three months before the measurement as this is the lifespan of red blood cells.In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy. Monitoring HbA1c in type 1 diabetic patients, for the purpose of assessing glycemic control and modifying therapy, may improve outcomes. (Huisman TH*et.al* 1958)

- Reference Range: 3.8-6.3%; Target for therapy is < 7% (Skyler JSE*et.al* 2009).

2.9 Diabetes mellitus treatment:

Metformin is the most widely used oral anti-hyperglycemic drug and reduces the amount of glucose released by the liver into the bloodstream.Oral anti-hyperglycemic drugs have three modes of action to reduce blood glucose levels:3Secretagogues enhance insulin secretion by the pancreasSensitizers increase the sensitivity of the peripheral tissues to insulin Inhibitors impair gastrointestinal absorption of glucose. Each class of anti-hyperglycemic drug has a different adverse event or safety profile, and side effects are the main consideration when it comes to choosing a medication. Possible side effects range from weight gain, through gastrointestinal ones such as diarrhea, to pancreatitis and more serious problems. Hypoglycemia is also a possible adverse event.2, Metformin is usually the first treatment offered, however, and it is the most widely used oral anti-hyperglycemic. Metformin is a sensitizer in the class known as biguanides; it works by reducing the amount of glucose released by the liver into the bloodstream and increasing cellular response to insulin. A metformin pill is usually taken twice a day.This drug is a low-cost anti-hyperglycemic with mild side effects that can include diarrhea and abdominal cramping.(silivio et al 2012)

Metformin is not associated with weight gain or hypoglycemia.Sulphonylureas are secretagogues that increase pancreatic insulin secretion. There are several drug names in this class, including:Chlorpropamide,GlimepirideGlipizide,Glyburide.Again, the choice of drug is an individual one. In the case of sulphonylureas, the choice depends on daily dosing and the level of side effects. These drugs are associated with weight gain and hypoglycemia. Glitazones (also known as thiazolidinediones)are sensitizers - they increase the effect of insulin in the muscle and fat and reduce glucose production by the liver. (silivio et al 2012).

Two glitazones are available: pioglitazone and rosiglitazone. These drugs can have the side effects of weight gain or swelling and are associated with increased risks of heart disease and stroke, bladder cancer and fractures..Alpha-glucosidase inhibitors are intestinal enzyme inhibitors that block the breakdown of carbohydrates into glucose, reducing the amount absorbed in the gut.1,3,4Available as acarbose and miglitol, they are not usually tried as first-line drugs because of common side effects of flatulence, diarrhea and bloating, although these may reduce over time. Dipeptidyl peptidase-4 (DPP4) inhibitors include alogliptin, linagliptin, saxagliptin and sitagliptin.1Also known as gliptins, DPP4 inhibitors have a number of effects, including stimulating pancreatic insulin (by preventing the breakdown of the hormone GLP-1). They may also help with weight loss through an effect on appetite.1-4These drugs do not increase the risk of hypoglycemia. Mild possible side effects are nausea and vomiting.1-4Sodium-glucose co-transporter 2 (SGLT2) inhibitors include canagliflozin and dapagliflozin. They work by inhibiting the reabsorption of glucose in the kidneys, causing glucose to be excreted in the urine (glycosuria). SGLT2s may also cause modest weight loss. Side effects include urinary infection.Meglitinides include repaglinide and nateglinide. They stimulate the release of insulin by the pancreas. Meglitinides are associated with a higher chance of hypoglycemia and must be taken with meals three times a day. As a result, these drugs are less commonly used. (silivio et al 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area:

Gezira state Wad Medani town, Aldaraga health center.

3.2. Study design:

Retrospective Cross sectional study.

3.3 Sample size:

60 blood samples were collected from patients with diabetes mellitus (non-insulin dependent).

3.4 Data collection and analysis:

Data was collected by using a questionnaire. A sufficient copy of the questionnaire was produced. Questionnaires were then filled by the investigator during each time when blood samples collected. Completed questionnaires from selected study areas (60) were collected. Data was then analyzed and tabulated using statistical package for social sciences (SPSS) program version 20, T test, a crosstabs and correlation were performed.

3.5 Study variables:

3.5.1 Dependent variables:

- Fasting blood glucose, HbA1c and Serum ferritin.

3.5.2 Independent variables:

- Age, gender, disease duration, (appendix).

3.6 Materials and equipment:

The following materials and equipment's were utilized in this study:

- A25 analyzer, serial No. 331054841 (Biosystem, Barcelona-Spain).
- Centrifuge, D. 87532 (Germany Hettich), and Automatic pipette.
- Syringes, gloves, alcohol (70% ethanol), plain containers, EDITA container and sodium fluoride containers.
- I chroma HbA1c instrument.

3.7 Blood sampling and Collection:

About 3ml of venous blood were collected from the participants by using a sterile needle and syringe into a labeled plain container, each sample was stood until complete clot occurs. Clotted blood sample was then centrifuged to obtain the serum for ferritin test and other 3 ml of venous blood centrifuged in sodium fluoride to obtain plasma for fasting blood glucose test, 2 ml of whole blood in EDITA containers was used for HbA1C testing.

3.8Inclusion criteria:

Diagnosed type 2 diabetes mellitus patients (non-insulin dependent).

3.9 Exclusion criteria:

- Overt thyroid dysfunction.
- Chronic kidney disease.
- Chronic liver disease.
- On insulin therapy patients.
- Anemic patients.

3.10Methods:

3.10.1 Serum ferritin:

3.10.1.1Principle

Serum ferritin causes agglutination of latex particles coated with anti-human ferritin antibodies. The agglutination of the latex particles is proportional to the ferritin concentration and can be measured by turbidmetry.

3.10.1.2 Procedure:

Volumes	Sample	8µL
	Reagent 1	240µL
	Washing	1.2 µL
Times		
Filter	Main	535 nm

3.10.1.3 Quality control:

It is recommended that to use the protein control serum level I (cod. 312110) and II (cod 31212) to verify the performance of the measurement procedure.

.Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

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 defined limits.

3.10.1.4 Reference values for serum Ferritin:

Reference values of ferritin in females (12 - 160) ng/dl.

Reference values for ferritin in males (18 - 250) ng/dl.

3.10.2 HbA1C:

3.10.2.1 Principle:

Ichroma HbA1c is based on the fluorescence immunoassay technology specifically the sandwich immune-detection method

3.10.2.2 Procedure:

Whole blood (5µL) is added using blood tube to the mixture of hemolysis buffer and detection buffer, which result in hemolysis of red blood cells, such that by mixing detector buffer with blood specimen. (75 µL) of the sample mixture isloaded and migrate on the matrix of test cartridge; the complexes of detector Antibody and HbA1c are captured to the anti-HbA1c sandwich pair antibody that has been immobilized on test matrix.as a result the higher concentration of HbA1c produces a higher fluorescence signal from HbA1c. the signal is interpreted and the result displayed on Ichroma reader in units of mmol/mol and mg/dl.

3.10.2.3 Quality control:

A quality test should be performed at regular intervals and before using a new kit with patient specimens. To perform QC of ichromaHbA1C, we recommend using Boditech Med Inc.'s ichroma HbA1c control.

3.10.2.4 Reference values forHbA1c:

Reference value forHbA1c is (4.5 -6.5) %

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results:

This study was carried out on 60patients with type II diabetes mellitus (non-insulin dependent) at different ages and sex to determine correlation between serum ferritin, fasting blood sugar (FBG) and HbA1C on those patients. The age range of the patients was 27-80 years with mean of 57.31 years, 15 (25%) were male and 45(75%) were female, and the disease duration range was 1-13 years with mean of 4.7(Table 4.1).

Table 4.1: General characteristics of patients:

Characteristic	M±SD
Age	57.31 ± 10.86
Duration of disease	4.76± 3.51
HbA1c	9.66 ± 2.26
FBG	188.08 ± 69.85
Serum ferritin	108± 90.04

After conducting the appropriate tests the following results were obtained:

Serum ferritin:

The distribution of the serum ferritin concentrations relative to the reference range was: (18%) of patients was found to have a serum ferritin concentration within the reference range, while 49 (72%) of patients was found to have a serum ferritin concentration above the reference range.

Fasting Blood Glucose

The distribution of the fasting blood glucose concentrations according to the (WHO) was: 16 (27%) of diabetics was found to have a normal fasting blood glucose levels (<126 mg/dl) while, 44 (73%) of diabetics was found to have abnormal fasting blood glucose levels (>126 mg/dl).

HbA1C:

The distribution of the HbA1C levels in cases relative to the reference range was: 10 (17%) of diabetics were found to have anHbA1C 7% or less than 7% (control) diabetics (M±SD = 6.5 ± 0.5 %), while 50 (83%) of diabetics was found to have HbA1C levels that exceeded the 7% (non-control) diabetics (M±SD = 10.3 ± 1.9 %).

Age distribution:

Table (4.2): age distribution of the total study population

Age groups	Number	Percent
27 – 50 years	13	22%
> 50 years	47	78%
Total	60	100%

Disease duration:

Table (4.3): show disease duration of the distribution of the total study population

Disease duration	Number	Percent
1 – 6 years	39	65%
7 – 13 years	21	35%
Total	60	100%

Table (4.4): show the male and female description among control and non-control diabetic of the total study population.

	Gender	Control		Non-control	
		Number	Percent %	Number	Percent %
	Male	2	20%		
	Female	8	80%		
	Male			13	26%
	Female			37	74%
Total		10	100%	50	100%

Table (4.5): show distribution of normal and abnormal serum ferritin within non-control and total population

	Serum ferritin	Non -control		Total	
		Number	Percent %	Number	Percent %
	Normal ferritin	39	78%	49	81.7%
	Abnormal ferritin	11	22%	11	18.3%
Total		50	100%	60	100%

The means of FBG and serum ferritin were increased in non-control diabetic group compared to control diabetics group; there is a statically significant difference between serum ferritin concentrations of control (M ± SD= 63. ± 35.1) and non-control diabetics (M ± SD = 117.9 ± 95) (P value = 0.03) while there was no statically significant difference of FBG levels between the two groups (P value = 0. 3).

Table (4.6): show the means and stander deviation of FBG, and serum ferritin among control and non-control diabetics.

Parameter	Control diabetics (N=10)	Non –control diabetics(N=50)	P value
Serum ferritin	(M ± SD) 63. ± 35.1	(M ± SD) 117.9 ± 95	0.03
FBG	(M ± SD) 142 ± 54.9	(M ± SD) 197.3±69.3	0.32

The mean and stander deviation of Hb A1C was increased in abnormal FBG group while the mean and stander deviation serum ferritin was increased in normal FBG group, and there is astatically significant difference between serum ferritin of normal and abnormal FBG groups.

Table (4.7): The means and stander deviation of Hb A1C, and serum ferritin among normal FBG group (<126 mg/dl) and abnormal FBG group (>126 mg/dl).

Parameter	Normal FBG N=16)	abnormal FBG diabetics(N=44)	P value
Serum ferritin	142 ± 123.6	96.9 ± 72.3	0.004
Hb A1C	8.1 ± 2.1	10.2 ± 2.1	0.88

There was no statically significant difference between age group of control and non-control diabetic groups. (P value =0.6).

Table (4.8): show the distribution of age groups number and percentage among control and non-control diabetic groups.

	Age	Control		Non-control		P value
		Number	Percent %	Number	Percent %	
	27 – 50 year	3	30%	10	20%	0.6
	> 50 year	7	70%	40	70%	
Total		10	100%	50	100%	

The means of FBG, Hba1C were increased with long duration group of diabetes while the mean of serum ferritin was increased among short duration group of diabetes compared to long duration group; there was statically significant correlation between serum ferritin and long and short duration of diabetes group (*P* value= 0.001). While there was no statically significant difference of FBG and HbA1C between the two groups (*P* value = 0.8), (*P* value = 0.7) respectively.

Table (4.8): show the correlation between serum ferritin, HbA 1C, FBG and disease duration groups.

parameter	Duration groups	Number of subject	Mean ± STD	P value
Serum ferritin	1 - 6 years	39	131.3 ± 101.8	0.001
	7 - 13 years	21	66.8 ± 37.1	
Hb A1C	1 - 6 years	39	9.5 ± 2.3	0.7
	7 - 13 years	21	10 ± 2.3	
FBG	1 - 6 years	39	178 ± 70.2	0.8
	7 - 13 years	21	206 ± 66	

There was no statistical significant difference in serum ferritin levels between diabetic males and females in the study population (*P*> 0.05), (Table 4.3).

Table 4.9: Mean of serum ferritin level in diabetic males and females:

	Study groups	Number	Mean	SD	*P*. value
Serum Ferritin	Males	15	99.93	92.625	0.756
	Females	45	111.67	90.040	

4.2 Discussion:

This study was carried out on 60 infected non-insulin dependent (Type II DM cases) and at different ages and sex to assess the correlation between serum ferritin, fasting blood sugar and HbA1C. The age range of the patients was 27-80 years with mean of 57.31 years, 15 (25%) were male and 45(75%) were female, and the disease duration range was 1-13 years with mean of 4.7 years.

The relationship between elevated ferritin levels and incidence type II diabetes involves an elevation in oxidative stress through the increased formation of hydroxyl radicals catalyzed by iron, which may lead to systemic insulin resistance and hyperglycemia in addition; iron excess probably contributes initially to insulin resistance and subsequently to decreased insulin secretion also iron deposition in the liver leading to hepatic insulin resistance and increased hepatic glucose production.

There was a statically significant difference between serum ferritin concentrations of control diabetic group and non-control diabetics group (*P* value = 0.03) opposing the results of a study done by F. Sharifi and Sh. Sazandeh at Zanjan, Iran.

And there were no statically significant difference between FBG concentrations of control diabetic group and non-control diabetics group (*P* value = 0. 3).

The mean serum ferritin of abnormal FBG group' was lower than normal FBG group' mean serum ferritin and this was due to the younger ages of this group since serum ferritin is normally increased in younger people and get decreased with age and there was astatically significant difference between serum ferritin of normal and abnormal FBG groups (*P* value = 0. 004) and this opposing the results of a study done by PSIMS &RF at India.

There was no statically significant difference between the age of control and non-control diabetics (*P* value = 0. 6). Serum ferritin concentrations were positively correlated disease duration while HbA1c and FBG were not correlated disease duration serum ferritin was also low positively correlated with gender (*P* value = 0. 7) and this agreed with the results of a study done by PSIMS &RF at India.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion:

This study concluded that serum ferritin concentrations were increased in non-control diabetics. Higher positive correlation of serum ferritin with HbA1c shows that metabolic control or dysglyceima affect ferritin levels possibly due to inflammation or oxidative stress. High serum ferritin levels were reported in 11 (18%) of the diabetic patients, while the rest 49(72%) were found to have normal serum ferritin levels. Oxidative stress caused by serum ferritin may play a role in the pathogenesis of T2DM and that serum ferritin increase may cause T2DM.

5.2 Recommendations:

- More studies including a larger population size and equally distributed variables should be done to confirm these results.
- Serum ferritin should be measured at least once for each T2DM patient.
- Each diabetic patient with high serum ferritin should undergo chelators' treatment and monitor its insulin resistance status after treatment.

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Questionnaire

S. No. ☐

Name: ☐ **Tel** ☐

Age: ☐

Gender: ♀ ☐ ♂ ☐

Address: ☐

Duration of the disease

.....

Rcent blood transfusion ?

Yes ☐ **No** ☐

Liver disease?

yes ☐ **No** ☐

Smoking?

yes ☐ **No** ☐

Any history of chronic disease?

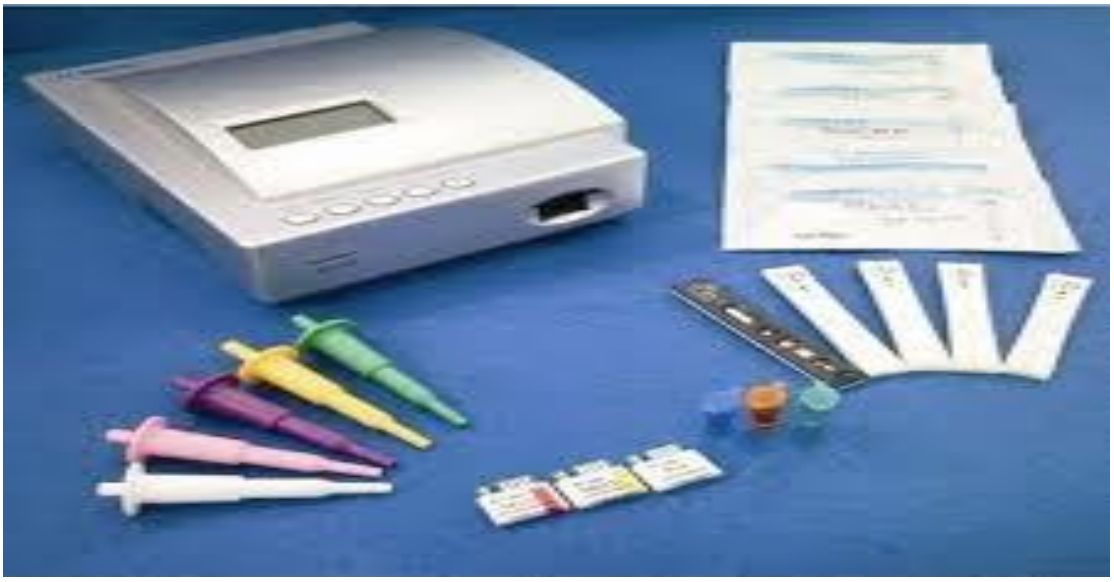
Yes ☐ **No** ☐

If yes mention?.....

The dose of treatment?

One pill ☐ **tow pills** ☐ **half pill** ☐

I Chroma instrument for HbA1c:



A25 bio system instrument