Comparison of serum alpha amylase level between type one and type two diabetes mellitus among Sudanese in Khartoum state in 2015.

A thesis submitted for the partial fulfillment of the degree
Of the M.S.C in clinical chemistry

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

وفي أنفسكم أفلا تبصرون

صدق الله العظيم

سورة الذاريات الآية (21)
Dedication

To my partner- in- life

For his love, support and

Encouragement

To my family members

Who have been our constant source of inspiration. They have given me the drive and discipline to tackle any task with enthusiasm

And determination

I dedicate this work... ... ...
Acknowledgment

- Heartily thankful to my supervisor Dr. Abde Alkarim A. Abdabo for his fruitful guidance, unlimited assistance, encourage and sustained interest, throughout the course of this work.

- I wish to extend my warmest thanks to the staff of the clinical chemistry department Sudan Universal, for their continuous support and encouragement.

- Also I am grateful to all people from whom samples were taken.

- Lastly, we offer our regards and blessings to all of those who supported me in any respect during the complication of the project.
Abstract

Diabetes Mellitus result from insufficiency of insulin secretion or concomitant resistance to the metabolic action of insulin on target tissue, then hyperglycemia as developed. Alpha amylase is enzyme which hydrolyses starch molecules to give smaller polymers composed of glucose unit which cause increase blood glucose. Therefore analytical, case control and hospital base study conduct in Ahmed Qasum hospital in Bahri in Khartoum state (Sudan) during the period from March to Jun 2015 to compare serum alpha amylase level in diabetic mellitus type one and type two.

Thirty-three diabetic patients in type one and thirty-three diabetic patients in type two were selected from Ahmed Qasum hospital as test groups, thirty-three healthy person as control groups. Three millimeter of venous blood was collected from each person; blood was separated into plain container for measurement of Alpha amylase.

Alpha amylase activity was estimated by using commercial reagent Kitts from Bio System Company, statistical package for social science (SPSS version 16) computer software was used for data analysis.

There was significant decrease alpha amylase activity in diabetes mellitus type one compared to type two (P value 0.034) and control (P value 0.001). It was found that there is negative correlation of alpha amylase with duration of disease and age among type one Diabetes mellitus.

Also show insignificant decrease alpha amylase activity in diabetes type two compared to control (P value 0.150)
It was found not correlation of alpha amylase with duration of disease and age among type two Diabetes mellitus.

From this study it is concluded that; alpha amylase activity in diabetes type one decrease than type two. Due to diabetes type one has insulin insufficient more than type two that effect on AMY.
المستخلص

أن مرض السكري ينتج عن نقص إفراز هرمون الأنسولين أو مقاومة الفاعلية الاستقلابية للأنسولين على الأنسجة مما يسبب ارتفاع جلوكوز الدم. إنزيمات الأميليز من الإنزيمات التي تعمل على تكسير النشويات إلى وحدات سكريات عديدة وجلوكوز مما يعمل على زيادة جلوكوز الدم.

لذا فقد أجريت هذه الدراسة الوصفية والتحليلة بمجمع احمد قاسم ببحري بولاية الخرطوم (السودان)، من الفترة من مارس إلى يوليوز 2015 لمقارنه مستوى نشاط إنزيم الأميليز في السودانيين المرضى المصابين بمرض السكري من النوع الأول والثاني (33 من النوع الأول و33 من النوع الثاني و33 من المتطوعين الأصحاء كمجموعة ضبط) وقد تم جمع عدد ثلاث مللي لتر من الدم من كل شخص وتم فصل مصل الدم في حاوية بليين.

تم قياس إنزيم الأميليز بواسطة جهاز قياس الطيف الضوئي. وتم تحليل البيانات باستخدام حزمة برامج التحليل الإحصائي. ووجد نقصان في متوسط إنزيم الأميليز في المرضى المصابين بمرض السكري من النوع الأول مقارنة مع النوع الثاني ومجموعة الضبط ذا دلاله معنوية في القيمة الاحتمالية مقارنة مع النوع الثاني (ب 0.034) والقيم الاحتمالية مقارنة مع مجموعة الطبطط (ب 0.01). ووجد أن نقصان نشاط الإنزيم لها ارتباط بالعمر ومدة المرض في النوع الأول لمرضى السكري.
وإيضاً لوحظ من هذه الدراسة عدم وجود اختلاف في متوسط نشاط إنزيم الأميليز في المرضى المصابين بمرض السكري من النوع الثاني مقارنة مع مجموعة الضبط فالقيمة الإحتمالية (ب=0.150) ووجد أيضاً ان نشاط الإنزيم ليس له ارتباط بالعمر ومدة المرض في النوع الثاني لمرضى السكري.

ومن هذه الدراسة نلخص ان مستوى نشاط إنزيم الأميليز في المرضى المصابين بمرض السكري من النوع الأول اقل من النوع الثاني. نتيجة ان مرضى السكري من النوع الأول لديهم انسولين اقل من مرضى السكري من النوع الثاني مما أثر على نشاط إنزيم الأميليز.
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<tr>
<td>ADP</td>
<td>Adenine Di Phosphate</td>
</tr>
<tr>
<td>AMY</td>
<td>Amylase enzyme</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenine Tri Phosphate</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>G6P</td>
<td>Glucose six Phosphate</td>
</tr>
<tr>
<td>IDDM</td>
<td>Insulin- Dependent Diabetes Mellitus</td>
</tr>
<tr>
<td>NADP</td>
<td>Nicotine Di Phosphate</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non Insulin- Dependent Diabetes Mellitus</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
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Chapter One
Introduction and Literature Review
1.1 Introduction

Diabetes is worldwide in distribution and incidence of both type one and two diabetes are rising, it is estimated that in the years 2000,150 million people worldwide had diabetes, this is became to double by 2010(Christopher et al,2002).

Type one and two will be definitely life threatening if left untreated over time; diabetes can lead to blindness, kidney failure, nerve damage and coronary heart disease (Lawrence et al, 2008) Diabetes is the third lead cause of death in united state after heart disease and cancer (Ahmed, 2001). Death may result from acute metabolic decomposition (Christopher et al, 2002).

Knowledge of the diabetes epidemic in Sudan is limited and the most recent data come from small scale study indicated a prevalence of 3.4%but recent research estimates about four millions and around 95%of whole have type two diabetes mellitus(Abrahamson et al ,1990) .

For many years, low serum amylase was thought to reflect diffuse pancreatic destruction secondary to advanced Pancreatic diseases, such as chronic pancreatitis. Recently several large clinical studies have shown that low serum amylase is also associated with metabolic syndrome and diabetes (Jeong, 2011) (Chiolero et al, 2008). Disturbance of serum amylase is associated with insulin deficiency in patients with type1diabetes and less commonly with type 2 diabetes (Lee et al, 2011) (Martin et al, 2008)
In previous studies showed amylase activity was low in diabetic patient compared with Control and there was negative correlation between amylase activity and hyperglycemia and duration (Mufeed et al, 2014). Other previous studies showed low serum amylase levels may reflect impaired exocrine-endocrine relationship in the pancreas. However, few clinical studies have addressed this issue. Therefore, in this epidemiological study, we investigated whether low serum amylase was associated with the pathogenesis of impaired insulin action: metabolic syndrome and diabetes (Nakajima et al, 2011).
1.2 literature review

1.2.1 DIABETES MELLITUS

Diabetes mellitus is a metabolic disorder characterized by the presence of abnormally high blood glucose levels (hyperglycemia). It is caused by defective in insulin secretion, defective action or both (Martha, 2010). Individuals with uncontrolled diabetes mellitus are unable to transport glucose into fat and muscle cells and are thus said to demonstrate glucose intolerance (Bechmann et al., 2012). The classic symptoms of diabetes are excessive urination, thirst and weight loss (Funnell et al., 2003). Diabetes is now ranked among one of the most common non-communicable diseases in the world. It falls within 4th–5th leading cause of death in most developed countries (Helaine and Resnick, 2013)

1.2.1.1 Classification of diabetes mellitus

1.2.1.1.1 Type one diabetes mellitus (formerly insulin-dependent diabetes mellitus (IDDM), type1 or juvenile onset diabetes (Wendy, 2008)

Type 1 diabetes mellitus is a result of cellular-mediate autoimmune destruction of the β- cells of the pancreas, causing an absolute deficiency of insulin secretion. Upper limit of 110 mg/dL on the fasting plasma glucose is designated as the upper limit of normal blood glucose. Type 1 constitutes only 10% to 20% of all cases of diabetes and commonly occurs in childhood and adolescence. This disease is usually initiated by an environmental factor or infection (usually a virus) in individuals with a genetic predisposition and causes the immune destruction of the β-cells of the pancreas and, therefore, a decreased production of insulin. Characteristics of type 1 diabetes include abrupt onset, insulin dependence, and ketosis tendency. This diabetic type is
genetically related. One or more of the following markers are found in 85% to 90% of individuals with fasting hyperglycemia. Complications include microvascular problems such as nephropathy, neuropathy, and retinopathy. Increased heart disease is also found in patients with diabetes. Idiopathic type 1 diabetes is a form of type 1 diabetes that has no known etiology, is strongly inherited, and does not have β-cell autoimmunity. Individuals with this form of diabetes have episodic requirements for insulin replacement (Micheal et al, 2005)

**1.2.1.1.2 Type two diabetes mellitus (formerly non insulin- dependent diabetes mellitus (NIDDM), type2 or adult onset diabetes** (Wendy, 2008).

Type 2 diabetes mellitus is characterized by hyperglycemia as a result of an individual’s resistance to insulin with an insulin secretory defect. This resistance results in a relative, not an absolute, insulin deficiency. Type 2 constitutes the majority of the diabetes cases. Most patients in this type are obese or have an increased percentage of body fat distribution in the abdominal region. This type of diabetes often goes undiagnosed for many years and is associated with a strong genetic predisposition, with patients at increased risk with an increase in age, obesity, and lack of physical exercise. Characteristics usually include adult onset of the disease and milder symptoms than in type 1, with ketoacidosis seldom occurring. However, these patients are more likely to go into a hyperosmolar coma and are at an increased risk of developing macrovascular and microvascular complications (Micheal et al, 2005)

**1.2.1.1.3 Gestational diabetes:**

Is similar in etiology to type 2 diabetes; however, it is defined as diabetes that is diagnosed in pregnancy. Pregnancy is associated with increased tissue
cell resistance to insulin. Most pregnant women will compensate with increased secretion of insulin; those individuals who are unable to compensate may develop gestational diabetes. The hyperglycemia of gestational diabetes diminishes after delivery; however, the individual who has developed gestational diabetes is at higher risk for the development of type 2 diabetes thereafter.

1.2.1.1.4 Other specific causes of diabetes:

This form of hyperglycemia may be the secondary result of non–insulin-related events. Blood glucose levels are increased in endocrine disorders, such as Cushing’s syndrome; in exocrine disorders, such as cystic fibrosis; and as a response to specific drugs, such as protease inhibitors and glucocorticoids. Other causes of this form of diabetes are the result of genetic defects that affect pancreatic beta cells or the action of insulin (Wendy, 2008).
Table 1-1 Classification of Diabetes Mellitus (Micheal et al, 2005).

<table>
<thead>
<tr>
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<td>Type 1</td>
<td>_-Cell destruction</td>
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<tr>
<td></td>
<td>Absolute insulin deficiency</td>
</tr>
<tr>
<td></td>
<td>Autoantibodies</td>
</tr>
<tr>
<td></td>
<td>• Islet cell autoantibodies</td>
</tr>
<tr>
<td></td>
<td>• Insulin autoantibodies</td>
</tr>
<tr>
<td></td>
<td>• Glutamic acid decarboxylase autoantibodies</td>
</tr>
<tr>
<td></td>
<td>• Tyrosine phosphatase IA-2 and IA-2B autoantibodies</td>
</tr>
<tr>
<td>Type 2</td>
<td>Insulin resistance with an insulin secretory defect</td>
</tr>
<tr>
<td></td>
<td>Relative insulin deficiency</td>
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<tr>
<td>Other</td>
<td>Associated with secondary conditions</td>
</tr>
<tr>
<td></td>
<td>• Genetic defects of _-cell function</td>
</tr>
<tr>
<td></td>
<td>• Pancreatic disease</td>
</tr>
<tr>
<td></td>
<td>• Endocrine disease</td>
</tr>
<tr>
<td></td>
<td>• Drug or chemical induced</td>
</tr>
<tr>
<td></td>
<td>• Insulin receptor abnormalities</td>
</tr>
<tr>
<td></td>
<td>• Other genetic syndromes</td>
</tr>
<tr>
<td>Gestational</td>
<td>Glucose intolerance during pregnancy</td>
</tr>
<tr>
<td></td>
<td>Due to metabolic and hormonal changes</td>
</tr>
</tbody>
</table>

1.2.1.2 Signs and symptoms of diabetes Mellitus;

The classic symptoms of untreated diabetes are weight loss, polyuria (frequent 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 signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape,
resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes (Wendy, 2008).

1.2.1.3 Pathophysiology of diabetes Mellitus;

Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, muscle, and adipose tissue. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus (Brain Jensen, 2014). The body obtains glucose from three main places: the intestinal absorption of food, the breakdown of glycogen, the storage form of glucose found in the liver, and gluconeogenesis, the generation of glucose from non-carbohydrate substrates in the body. Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen (Shoback et al, 2011). Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Lower glucose levels result in decreased insulin release from the beta cells and in the breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin (Kim et al, 2012). If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and
muscles. The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis (Rebert et al, 2012). When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria) (Bechmann et al, 2012). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (polydipsia).

1.2.1.4 Diagnosis of diabetes Mellitus;

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following (Gautron, 1999);

- 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 (HbA1C) ≥ 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. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test according to the current definition,
two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) is considered diagnostic for diabetes mellitus. Per the World Health Organization (WHO) 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 (Drucker, 2010). 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 (Santaguida et al, 2008). The American Diabetes Association since 2003 uses a slightly different range for impaired fasting glucose of 5.6 to 6.9 mmol/l (100 to 125 mg/dl) (Bartoli et al, 2011). Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Selvin et al, 2010).

**Table (1-2) WHO diabetes diagnostic criteria**

<table>
<thead>
<tr>
<th>Condition</th>
<th>2 hour glucose</th>
<th>Fasting glucose</th>
<th>HbA$_{1c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/l (mg/dl)</td>
<td>mmol/l (mg/dl)</td>
<td>mmol/mol</td>
</tr>
<tr>
<td>Normal</td>
<td>&lt;7.8 (&lt;140)</td>
<td>&lt;6.1 (&lt;110)</td>
<td>&lt;42</td>
</tr>
<tr>
<td>Impaired fasting glycaemia</td>
<td>&lt;7.8 (&lt;140)</td>
<td>≥ 6.1 (≥110) &amp;</td>
<td>42-46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;7.0 (&lt;126)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>≥11.1 (≥200)</td>
<td>≥7.0 (≥126)</td>
<td>≥48</td>
</tr>
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</table>
1.2.1.5 Methods of Glucose Measurement

The Specimen
Glucose in serum, plasma, and cerebrospinal fluid (CSF) may be measured by these methods. Glucose levels in serum or plasma are 10% to 15% higher than those in whole blood. Serum or plasma must be separated within 1 hour to prevent degradation by glycolysis. Glucose is stable for 24 hours in whole blood when preserved with sodium fluoride.

The Reaction: Enzymatic Method
Three enzymatic approaches to the measurement of glucose have been explored. Enzymatic methods are specific for glucose.

**Hexokinase method** is the reference method for glucose. The method involves two coupled reactions:

\[
\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase+mg}} \text{G6PO}_4 + \text{ADP}
\]

\[
\text{G6PO}_4 + \text{NADP} \xrightarrow{\text{G6PD}} 6\text{-phosphogluconate} + \text{NADPH} + \text{H}^+
\]

The increase in absorbance of NADPH at 340 nm is measured as directly proportional to glucose. The hexokinase reaction may also be coupled to an indicator reaction and measured through the development of a colored product.

**Glucose oxidase method** is specific for beta-D-glucose. The initial reaction:

\[
\beta\text{-D-Glucose} + \text{O}_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + \text{H}_2\text{O}_2
\]

may be coupled with a peroxidase indicator reaction:

\[
\text{H}_2\text{O}_2 + \text{reduced chromagen} \xrightarrow{\text{peroxidase}} \text{oxidized chromagen} + \text{H}_2\text{O}
\]

or may be assessed by measuring oxygen consumption, using an oxygen electrode.
Glucose dehydrogenase method involves the measurement of NADH production:

\[
\text{Glucose} + \text{NAD} \xrightarrow{\text{glucose dehydrogenase}} \text{D-gluconolactone} + \text{NADH} + \text{H}^+ 
\]

(Micheal et al, 2005).

1.2.1.6 Glucose Tolerance and 2-Hour Postprandial Tests:

Guidelines for the performance and interpretation of the 2-hour postprandial test were set by the Expert Committee. A variation of this test is to use a standardized load of glucose. A solution containing 75 g of glucose is administered, and a specimen for plasma glucose measurement is drawn 2 hours later. If that level is \( \geq 200 \) mg/dL and is confirmed on a subsequent day by either an increased random or fasting glucose level, the patient is diagnosed with diabetes. The oral glucose tolerance test (OGTT) is not recommended for routine use under the ADA guidelines.

This procedure is inconvenient to patients and is not being used by physicians for diagnosing diabetes. However, if the OGTT is used, it is important that proper patient preparation be given before this test is performed. The patient should be ambulatory and on a normal carbohydrate intake for 3 days before the test. The patient should be fasting for at least 10 hours and not longer than 16 hours, and the test should be performed in the morning because of the hormonal diurnal effect on glucose. Just before tolerance and while the test is in progress, patients should refrain from exercise, eating, drinking (except that the patient may drink water), and smoking. Factors that affect the tolerance results include medications such as large doses of salicylates, diuretics, anticonvulsants, oral contraceptives, and corticosteroids. Also,
gastrointestinal problems, including malabsorption problems, gastrointestinal surgery, and vomiting and endocrine dysfunctions can affect the OGTT results. The guidelines recommend that only the fasting and the 2-hour sample be measured, except when the patient is pregnant. The adult dose of glucose solution (glucola) is 75 g; children receive 1.75 g/kg of glucose to a maximum dose of 75 g.

1.2.1.7 Glycated Hemoglobin (HbA1C)

Hb A1C, glycated hemoglobin, is an indicator of long-term glycemic control. The term glycated hemoglobin describes a chemically stable conjugate of any of the forms of hemoglobin with glucose. Glycated forms of hemoglobin are formed slowly, nonenzymatically, and irreversibly at a rate that is proportional to the concentration of glucose in the blood. The level of glycated hemoglobin in a blood sample provides a glycemic history of hemoglobin. Glycation over the life span of the erythrocyte, the cell that contains the hemoglobin. The average lifespan of the erythrocyte is 120 days, and glycated hemoglobin describes the average glucose levels in the blood over that life span. Clinically, glycated hemoglobin is used to reflect glycemic control over the previous 90 to 120 days. Glycated hemoglobin measurements are unaffected by daily variation of glucose from diet and exercise. Glycated hemoglobin measurements are, however, influenced by conditions that affect the life span of the hemoglobin molecule, such as sickle cell disease and hemolytic disease, which can falsely decrease glycated hemoglobin results (Micheal et al, 2005).
1.2.2 Enzymes

Enzymes are specific biologic proteins that catalyze biochemical reactions without altering the equilibrium point of the reaction or being consumed or changed in composition. The other substances in the reaction are converted to products. The catalyzed reactions are frequently specific and essential to physiologic functions, such as the hydration of carbon dioxide, nerve conduction, muscle contraction, nutrient degradation, and energy use. Found in all body tissue, enzymes frequently appear in the serum following cellular injury or, sometimes, in smaller amounts, from degraded cells. Certain enzymes, such as those that facilitate coagulation, are specific to plasma and, therefore, are present in significant concentrations in plasma. Plasma or serum enzyme levels are often useful in the diagnosis of particular diseases or physiologic abnormalities.

1.2.2.1 Pancreatic enzymes;

Assay of serum amylase (AMY), lipase (LPS), trypsin (TRY), chymotrypsin (CHY) and elastase1 (E1) are applied to investigation of pancreatic disease, pancreatic function and pathology.

1.2.2.1.1 Amylase enzyme:

Amylase is a hydrolase that catalyzes the breakdown of starch, glycogen, and some oligosaccharides. Calcium is a necessary cofactor in the reaction.

Biochemistry:

AMYs normally occurring in human plasma are small molecules with molecular weights varying from 54 to 62 kDa. The enzyme is thus small enough to pass through the glomeruli of the kidney, and AMY is the only plasma enzyme physiologically found in urine. AMY is present in a number of organs and tissue. The greatest concentration is present in the silvery
gland, which secret a potent AMY (S-type) to initiate hydrolysis of starches while the food is still in the mouth and esophagus, in the pancreas, the enzyme (p-type) is synthesized by the acinar cells and then secreted into the intestinal tract by way of the pancreatic duct system. AMY activity is also found in extract from (1) semen, (2) testes, (3) ovaries, (4) fallopian tubes, (5) striated muscle, (6) lung, (7) adipose tissue, and (8) milk.

The AMY activity present in normal serum and urine is of pancreatic (p-AMY) and silvery gland (S-AMY) organ. These isoenzyme are products of two closely linked loci on chromosome1, AMY isoenzyme also undergo posttranslational modification of deamidation, glycosylation and deglycosylation to form a number of isoforms. These isoform have been separation in bothe serum and urine using isoelectric focusing or electrophoresis (Wendy, 2008).

1.2.2.1.1 α-Amylase:

The α-amylases (EC3.2.1.1) (alternative names: 1, 4-α-D-glucan glucanohydrolase; glycogenase) are calcium metalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase. In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0 (Yegneswaran, 2010). In human physiology, both the salivary and pancreatic amylases are α-amylases. The α-amylases form is also found in plants, fungi (ascomycetes and basidiomycetes) and bacteria (Bacillus).
1.2.2.1.1.2 β-Amylase:

Another form of amylase, β-amylase (EC3.2.1.2) (alternative names: 1, 4-α-D-glucan maltohydrolase; glycogenase; saccharogen amylase) is also synthesized by bacteria, fungi, and plants. Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1, 4 glycosidic bond, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. Both α-amylase and β-amylase are present in seeds; β-amylase is present in an inactive form prior to germination, whereas α-amylase and proteases appear once germination has begun. Many microbes also produce amylase to degrade extracellular starches. Animal tissues do not contain β-amylase, although it may be present in microorganisms contained within the digestive tract. The optimum pH for β-amylase is 4.0–5.0

1.2.2.1.1.3. γ-Amylase:

γ-Amylase (3.2.1.3) (alternative names: Glucan 1,4-α-glucosidase; amyloglucosidase; Exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucan glucohydrolase) will cleave α(1–6) glycosidic linkages, as well as the last α(1–4)glycosidic linkages at the nonreducing end of amylase and amylopectin, yielding glucose. The γ-amylase has most acidic optimum pH of all amylases because it is most active around pH 3.

1.2.2.1.2 Hyperamylasemia

Blood serum amylase may be measured for purposes of medical diagnosis. A higher than normal concentration may reflect one of several medical conditions, including acute inflammation of the pancreas (it may be measured concurrently with the more specific lipase) (Aughesteen, et al.)
but also perforated peptic ulcer, torsion of an ovarian cyst, strangulation ileus, mesenteric ischemia, macroamylasemia and mumps. Amylase may be measured in other body fluids, including urine and peritoneal fluid.

A January 2007 study from Washington University in St. Louis suggests that saliva tests of the enzyme could be used to indicate sleep deficits, as the enzyme increases its activity in correlation with the length of time a subject has been deprived of sleep (Louis, 2007).

1.2.2.1.3 **Hypoamylasemia:**

Low levels of serum amylase may indicate pancreatic insufficiency such as found in cystic fibrosis (Micheal et ale 2005).
Chapter Two
Rationale and Objectives
2.1 Rationale:

Prevalence rate of diabetes mellitus in Sudanese is increasing especially in elder people.

The majority of diabetic research has been focused on normalization of hyperglycemia and abnormal lipid profile to the other hand, very little concern on pancreatic exocrine function in diabetes has been paid.

This study will be conducted to assess the defect insulin to enzyme of pancreas.

It knows that alpha amylase is interacting with carbohydrate metabolism through hydrolysis of starch. In recent studies pancreatic dysfunction may lead to diabetes mellitus. Also previous studies showed a significant difference in amylase level in diabetic patients and control group no published work according to my knowledge has been done in Sudanese diabetes mellitus. So that the study could be applied in Sudan.
2.2 Objectives

2.2.1 General objective:

To Compare of serum alpha amylase level between type one and type two diabetes mellitus among Sudanese in Khartoum state.

2.2.2 Specific objective:

1- To measure and compare serum level of alpha amylase in type one and type two diabetic patients in comparison to amylase level in control group.

2- To correlate between age, duration of disease and serum alpha amylase level in diabetic mellitus in type one and type two.
Chapter Three
Materials and Methods
3. Materials and methods

3.1 study approach

Quantitative research approach

3.2 study designs

Analytical, case control and hospital base study.

3.3 study area

This study was done in Khartoum state in Ahmad Gasem hospital.

3.4 study period

This study was carried during the period from February to Jun2015

3.5 study population

This study was conducted on diabetic Sudanese type one and two as test group and apparently healthy non diabetic as control group.

3.6 sample size

Thirty three Sudanese diabetic type one and Thirty three Sudanese diabetic type two as test group and Thirty three healthy Sudanese non diabetic as control group, so ninety nine volunteers was included in this study.

3.7 inclusion criteria

Test group: Sudanese diabetic type one and two.
Control group: apparently healthy Sudanese non diabetic.

3.8 Exclusion criteria

Individual complaining from any disorder such renal failure, acute or chronic pancreatitis, liver disease, cancer in pancreas, abdominal disease was excluded from this study.
3.9 Ethical consideration

The volunteers on this study were notified well about the objectives and the need of this study and must accept to donate the blood sample before the collection process.

3.10 Data collection

Interviewed and collected samples from volunteers (99 samples)

3.11 Sampling technique

3.11.1 Sampling

Local antiseptic use for cleaning the skin (70% ethanol), 3ml of venous blood was collected in plain container tubes from each participant. Serum was separated directly from the plain tube by centrifugation at (300 rpm) for 5 minutes. Serum levels of alpha amylase were measured using spectrophotometer by Kits from bio system Company.

3.12. Method of detection

3.12.1 Instrument.

Spectrophotometer was used for chemical analysis to alpha amylase.

3.12.2 Material requirement

Syringes, alcohol, marker, cotton, centrifuge, plain tubes, alpha amylase reagente, disposable cuvettes, automatic pipette, stop watch and tips.

3.12.3 Measurement of serum alpha amylase

Principle

The alpha amylase liquicolor colorimetric test comprises a new substrate 2-choloro-4-nitrophenyle-maltotrioside (CNPG3). This substrate reacts directly with alpha amylase and does not require the presence of ancillary enzymes. The release of 2-chloro-4-nitrophenol (CNP) from the substrate
and the resulting absorbance increase per minute is directly related to the alpha amylase activity in the sample.

**Reference Ranges**

Serum, plasma = 12-45 U/l at 25°C
Serum, plasma = 22-80 U/l at 37°C

**Quality control**

The precision and accuracy of all methods will use in this study will check each time a batch was analyzed by including commercially prepared control sera.

**3.13. Data analysis**

The data was collected in this study was analyzer using (SPSS Vr.16) computer analysis program. The means and stander deviation of the serum levels of alpha amylase was obtain for the test and control groups and the one sample t-test.

To compared statistical differences between three groups by using ANOVA test will use as appropriate. The relationships between variables was determine used correlation coefficient test (p-value less than 0.05 was considered to be significant).

**3.14. Data presentation**

Data was presented inform of tables and figures.
Chapter Four

Results
4- Results

This study was conducted on 33 healthy volunteers as a control group and 66 volunteers diabetic patient as test group. In this study the test group was composed of 33 diabetic type one and 33 diabetic type two.

Table (4-1) shows a significant difference between the means of serum alpha amylase in test group and control group. (35.4±21U /L) versus (48.1±20.6 U/L) p-value = 0.005(p<0.05).

Table (4-2) shows a significant difference between the means of serum alpha amylase in diabetic type one and two. (30 ±18.5 U /L) versus (41±22 U /L) p-value =0.034 (p<0.05).

Table (4-3) shows a significant difference between the means of serum alpha amylase in diabetic type one, two and control. The mean level of serum alpha amylase in control group (48±20.6 U /L) versus (30±18.5 U /L) in type one versus (41±22.06 U /L) in type two p-value = 0.002 (p<0.05)

Figure (4-3) shows a significant correlation between serum alpha amylase U /L and age (year) in diabetic type one (r=-0.439, p= 0.011).

Figure (4-4) shows an insignificant correlation between serum alpha amylase U /L and age (year) in diabetic type two (r=120, p=0.507)

Figure (4-5) shows an insignificant correlation between Serum alpha amylase U /L and age (year) in control group (r=157, p=0.383)

Figure (4-6) shows a significant correlation between serum alpha amylase U /L and duration of disease among type one(r=-0.431, p=0.012)
Figure (4-7) shows an insignificant correlation between serum alpha amylase U/L and duration of disease among type two (r=0.047, p=0.797)
Table (4-1) shows the statistics and mean difference of α amylase U/L and age between test and control groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>α amylase U/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test group</td>
<td>66</td>
<td>35.4244</td>
<td>20.96400</td>
<td>0.005</td>
</tr>
<tr>
<td>Control group</td>
<td>33</td>
<td>48.1667</td>
<td>20.69294</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test group</td>
<td>66</td>
<td>29.15</td>
<td>18.732</td>
<td>0.518</td>
</tr>
<tr>
<td>Control group</td>
<td>33</td>
<td>31.42</td>
<td>10.344</td>
<td></td>
</tr>
</tbody>
</table>

- t-test was used to calculate P value
- P value less than 0.05 considered significant.
Figure (4-1) shows mean of α amylase U/L in test and control groups
Table (4-2) shows the statistics and mean difference of α amylase U/L between type I and type II of study group.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>α amylase</td>
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<td>33</td>
<td>29.9973</td>
<td>18.57373</td>
</tr>
<tr>
<td>U/L</td>
<td>Type II</td>
<td>33</td>
<td>40.8515</td>
<td>22.06765</td>
</tr>
</tbody>
</table>

- t-test was used to calculate P value
- P value less than 0.05 considered significant
Table (4-3) shows the statistics and mean difference of α amylase U/L between type I, type II and control group.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>18.57373</td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>33</td>
<td>40.8515</td>
<td>22.06765</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>48.1667</td>
<td>20.69294</td>
<td></td>
</tr>
</tbody>
</table>

- ANOVA test was used to calculate P value
- P value less than 0.05 considered significant.
Figure (4-2) shows mean of α amylase U/L in type I, type II and control group.
Figure (4-3) correlation of age and α amylase among type I diabetic group.

P value=0.011

R=- - 0.439
Figure (4-4) correlation of age and α amylase among type II diabetic group.

P value=0.507

R=-0.120
Figure (4-5) correlation of age and α amylase among control group.

P value=0.383

R=-0.157
Figure (4-6) correlation of duration of disease and α amylase among type I diabetic group.

P value=0.012

R=- - 0.431
Figure (4-7) correlation of duration of disease and α amylase among type II diabetic group.

P value=0.797

R=-0.047
Chapter Five
Discussion & Conclusion &
Recommendation
5.1 Discussion

The human exocrine pancreatic secretions are affected in the diseases which affect the pancreas. The low serum amylase levels in diabetes may reflect the impaired exocrine-endocrine interactions of the pancreas. Several animal and cellular studies have tried to find out the pathogenic features and the underlying mechanisms which link the islet cells and the acinar cells (Rakhee et al, 2013). The results was showed that, there was significant difference in serum alpha amylase between healthy controls and diabetic mellitus (P value 0.005) may be due to derangement in the endocrine-exocrine axis of the pancreas, as a disease which affected any portion of an organ would affect the adjoining area of that organ functionally. This finding is consistent with other previous studies (Nakajima et ale, 2011) showed significantly low serum alpha amylase levels were found in the diabetic patients as compared to those in the healthy controls (p value <0.001). Serum alpha amylase in diabetic mellitus between type one and two showed significant different (P value 0.034) due to type one have insulin insufficient more than type2 that effect on exocrine. This finding is consistent with other previous studies (Gretchen Becker 2012) the results from the people with low amylase were not as severe as those in people with type 2 diabetes. Also showed from this study ,there was insignificant difference serum alpha amylase between type two and controls ,this finding inconsistent with other previous study done in India( Reddy and Shankarah ,2011) showed significant increase in alpha amylase level type 2 diabetic patients.

It was found that there is negative correlation of serum alpha amylase in diabetic type one and age (P value 0.011) (r=-0.439) this is not consist with
other previous studies (Nakajima et al., 2011) showed no correlation between serum alpha amylase in diabetic type one and age.

It was found that there is negative correlation of serum alpha amylase in diabetic type one and duration of disease (P value 0.012) ($r=-0.431$).

It was found not correlation of serum alpha amylase diabetes type two with age (P value 0.5) ($r=0.120$) and duration of disease (p value 0.7) ($r=0.4$)

There is evidence that the pancreatic hormones, insulin and glucagon, influence the enzyme synthesis and release in the exocrine pancreas. Insulin has a trophic effect on the acinar cells, whereas glucagon has been found to have an inhibitory influence on the exocrine secretions and moreover, there is a decrease in the sensitivity of the diabetic pancreatic acini to secretagogues. So, the insulin deficiency and the glucagon excess in diabetes affect the normal milieu of the pancreas, thereby decreasing the total volume, the amylase secretions and the bicarbonate content of its exocrine secretions. Our results suggest that the low serum amylase levels in diabetes are associated with an impaired insulin action due to insulin resistance and/or inadequate insulin secretion, as was indicated by the raised blood glucose levels in our study. Moreover about 50% of the diabetics have been found to have pancreatic fibrosis and pathological findings such as atrophy, fatty infiltration and loss of the exocrine acinar cells. The deferent result may be due to dietary status, ethnic group, and genetic variation in deferent area or degree of complication of diseases (Nakajima et al., 2011).
5.2 Conclusion

From the present study, the following could be concluded:

1- There was significant difference in serum alpha amylase between healthy controls and diabetic mellitus.

2- Alpha amylase activity in diabetes type one lower than type two.

3- It was found that there is negative correlation of serum alpha amylase in diabetic type one with age and duration of disease.

4- It was found not correlation of serum alpha amylase diabetes type two with age and duration of disease.
5.3 Recommendation;

1. Specific iso enzyme (pancreatic amylase) should be measured in the further studies.

2. Further studies should be done including more sample size.
References
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Appendices
اذن باخذ عينه دم من متطوع او مريض
(بسم الله الرحمن الرحيم)

انا مني حسين داود طالب دراسات عليا، ماجستير مختبرات طبيه جامعه السودان للعلوم والتكنولوجيا، ارغب في اخذ عينه خاصتك (حوالي ثلاث سي سي) لغرض البحث العلمي الطبي في مرض السكري حيث اقيس فيها مستوى نشاط انزيم الانيليزي واحتفظ بالعينه حيث يمكن الحوجه لها مره اخرى.

- لك الحق في المشاركة في البحث او الانسحاب في اي وقت تشاء
- والحق في الاطلاع على نتائج البحث
- البحث يتم بدون ذكر اسماء للاشخاص فقط الاستقاده من بيانات البحث

اقرار بالموافقة

انا _______________________________ ليس لدي مانع من اخذ عينه دم خاصتي لغرض البحث العلمي بعد ان علمت ما ذكراعلاه.

توقيع المريض او المشارك ________________ التاريخ __ /__ / __

توقيع الباحث ______________________