DISSERTATION SUBMITTED FOR THE MASTER'S DEGREE IN MEDICAL BIOCHEMISTRY

TITLE

"SERUM CREATINE KINASE AND SERUM LACTATE DEHYDROGENASE IN TYPE II DIABETES MELLITUS: A CASE-CONTROL STUDY"

SUBMITTED

BY

FATMA KHATOON

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DEPARTMENT OF BIOCHEMISTRY

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In partial fulfillment of the requirement for the award of a degree of

Master of Science
In
Medical Biochemistry

By

FATMA KHATOON

Enrollment No: 1900103828

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CERTIFICATE

This is to certify that **Fatma Khatoon,** student of **M.Sc. Medical Biochemistry**, Integral University has completed her dissertation titled **"Serum Creatine Kinase and Serum Lactate Dehydrogenase in Type II Diabetes Mellitus: A Case-Control Study"** successfully. She has completed this work in the Department of Biochemistry, Integral University under the guidance of Dr. Priyanka Thapa Manger. The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and a bright future.

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CERTIFICATE

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I wish her good luck and a bright future.

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DECLARATION BY THE CANDIDATE

I hereby declare that Integral Institute of Medical Sciences and Research, Integral University, Lucknow shall have the right to preserve, use and disseminate this dissertation in print or electronic format for academic/research purposes.

I will publish the research paper related to my dissertation only with the consent of my guide.

Date:

Place: **Fatma Khatoon**

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Date:

Place: Lucknow Fatma Khatoon

CONTENTS

LIST OF ABBREVIATIONS

ADA = American Diabetes Association

WHO= World Health Organization

IDF = International Diabetes Federation

T1DM = Type I Diabetes Mellitus

T2DM = Type II Diabetes Mellitus

GDM = Gestational Diabetes Mellitus

DCCT = Diabetes Control and Complications Trial

CK = Creatine Kinase

LDH = Lactate Dehydrogenases

ICMR = Indian Council of Medical Research

FPG = Fasting Plasma Glucose

IGT = Impaired Glucose Tolerance

IFG = Impaired Fasting Glucose

OGTT = Oral Glucose Tolerance Test

INTRODUCTION

Diabetes is a metabolic disorder characterized by hyperglycemia either caused by insulin synthesis, insulin action, or both. The term “chronic hyperglycemia” of diabetes has been related to long-term effects, disorders, and failure of organs such as the heart, blood vessels, kidneys, nerves, and eyes. (American Diabetes Association, 2009).

Developed nations have been suffering from diabetes, and it has been the fourth leading cause of death. (Burtis et al., 2016) As stated by "The International Diabetes Federation," 537 million adult population of 20-79 age groups have diabetes mellitus. And the population is predicted to increase by 643 million by 2030 with a further increase to 783 million by 2045. Aside from this, it is predicted that nearly 541 million individuals will be affected by impaired glucose tolerance in 2021. About (6.7 million) people worldwide in the age group (20 to 79) are expected to die from diabetes-related diseases in 2021. (International Diabetes Federation,2021)

Diabetes is rapidly approaching epidemic levels in a country like India, with approx. of 62 million diagnosed cases. In the year 2000, India has the highest prevalence (31.7 million individuals) of diabetes cases globally, then came China with a number of 20.8 million and the United States with a number of 17.7 million. As stated by Wild et al, the worldwide diabetes prevalence can be anticipated to be increasing from (171 million) in 2000 to (366 million) by 2030, with India having greater growth. Diabetes mellitus is anticipated to impact 79.4 million Indian citizens in 2030, with significant increases in the number of people living in both the United States (42.1 million) and China (30.3 million). (Kaveeshwar et al., 2014)

Type I Diabetes Mellitus is triggered by an autoimmune response that occurs when the immune system targe β-cells of the pancreas which secret insulin. This type of diabetes can affect anyone at any age, although it most commonly affects children and young people. One of the most

common chronic disorders in adolescents is Diabetes Mellitus Type I. The common symptoms can be considered as excessive thirst, frequent urination (polyuria), lack of energy or fatigue, blurred vision, and diabetic ketoacidosis. (International Diabetes Federation,2021)

T2DM is the most common widespread type of diabetes in the world, with 90 percent of cases. (Shlomo et al., 2016) Hyperglycemia is a complication of type II diabetes mellitus where the body's cells are unable to react and respond properly to insulin, a term known as insulin resistance (IR). Insulin resistance reduces hormone effectiveness, which leads to an increase in the production of insulin. (International Diabetes Federation,2021)

Insulin release and activity must be precise to fulfill the metabolic requirement; as a result, the molecular mechanisms involved in insulin secretion, release, & tissue insulin action must be closely controlled. The defects in the systems can cause metabolic imbalance, leading to T2DM pathogenesis. (Garcia et al., 2020) T2DM lowers life expectancy because it increases the chance of renal disease, peripheral neuropathy, heart failure, stroke, blindness, and amputation. (Smushkin et al.,2010) Both types of diabetes mellitus (DM) cause a decline in pancreatic β-cell function and mass, which is accompanied by a deterioration in glycemic control. Due to their significant overlap, it can be challenging to distinguish between T1DM and T2DM in daily practice. (Snehalatha et al., 2004)

Creatine kinase is an enzyme commonly known as Creatine phosphokinase (CPK) that is located inside cells in high concentrations in skeletal muscle, the brain, and the myocardium, with lower levels found in other visceral tissues. CK is a dimeric molecule made of the M and B subunits. The isoenzymes (CK-MM), (CK-MB), and (CK-BB) are mainly formed by combining these subunits. (Cabaniss et al.,1990)

All three isoenzyme species can be found in the cell's cytosol. However, a fourth variety does exist and that is distinct from others in the terms of electrophoretic mobility & immunology The isoenzyme (CK-Mt) is found between both the inner and outer membranes of mitochondria which accounts for 15 % of the total CK activity in the heart. The CK-Mt gene can be found on chromosome 15. (Burtis et al.,2016 7th edition)

An enzyme called creatine kinase catalyzes the (ATP-dependent phosphorylation) of creatine, which can be essential for energy buffering in tissues with different energy requirements, especially cardiac muscle, and skeletal muscle. The increase in the level of creatine kinase activity may indicate tissue injury and is seen in a variety of clinical diseases, also including myopathy. (Kristjansson et al.,2016)

A standard technique of determining the level of CK activity involves the rate of the preceding reaction using spectrophotometry. These results can differ greatly due to variations in analytical methodology, as well as differences in age, gender, race, and physical activity level. Electrophoresis, column chromatography, or radioimmunoassay are methods for separating CK into its isoenzymes. The majority of clinical laboratories employ cellulose acetate electrophoresis or agarose gel along with band quantification using fluorometric or spectrophotometric techniques. (Cabaniss et al.,1990)

The CKMB/CK level is a promising candidate which is used to determine the cardiovascular disorders associated with uncontrolled diabetes. These results supported the risk of cardiovascular disease-causing death and morbidity related to both macrovascular and microvascular diabetic complications. (Evliyaoğlu et al.,2010)

Lactate dehydrogenase (LDH) is a widely expressed enzyme. It is in charge of catalyzing the anaerobic, NADPH-dependent conversion of pyruvate to lactate, which can be required when muscle activity is elevated. (Kristjansson et al.,2016) The enzyme lactate dehydrogenase (LDH) is found in almost all cells of the body. It is worth noting that LDH is primarily found in the cytoplasm of the cell and becomes extracellular when the cell dies. It has been proposed that LDH concentrations vary depending on the energy requirements of different tissues. (Dmour H et al., 2020)

Lactate dehydrogenase is made up of 4 peptide chains of 2 types: Muscle(M) and Heart(H), which are genetically controlled separately. The structures (LD-M & LD-H) can be primarily determined by genes on human chromosomes no. 11 and 12, separately. (Burtis et al.,2016 7th edition)

Most tissues contain five LDH isoenzymes, which have multiple molecular forms while catalyzing the same reaction. Isoenzymes are made up of two separate types of subunits, H and M, that are randomly combined in a tetrameric structure. M4, M3H, M2H2, MH3, and H4 are the compositions of the five major isoenzymes, with M subunits predominating in skeletal muscle and the liver and H subunits predominating in the heart. (Nagler et al., 2001)

The activity of LDH can be altered with normal glucose metabolism and insulin release through the beta cells of the pancreas, and it may be responsible for insulin secretory abnormalities in type II diabetes mellitus. The above findings also agree with the concept of "that glucose-derived pyruvate metabolism in the mitochondrion is required for glucose-stimulated insulin secretion in the beta cell." (Ainscow et al., 2000)

The appearance of LDH extracellularly is used to detect cell damage or death. Increased levels have been seen in cardiac, hepatic, skeletal muscle, and renal diseases, as well as a variety of hematological and neoplastic conditions. (Klein et al., 2020)

As a result, determining serum creatine kinase and serum lactate dehydrogenase levels is critical when developing a diabetes treatment strategy.

REVIEW

Of

LITERATURE

Diabetes mellitus also referred to as diabetes, becomes a serious, long-term chronic disease in which blood glucose levels rise as a result of the body's inability to synthesize enough insulin or to adequately utilize the insulin that it does produce. (International Diabetes Federation,2021)

Diabetes, a disease characterized by "excessive urine emptying," can be dated back to 1500 B.C. in Egyptian writings. Indian doctors called it "honey urine" or "madhumeha" because of the fact that it attracted ants. In 400–500 A.D. An Ancient Indian physicians Sushruta and Charaka were the first to differentiate between the two kinds of diabetes, which were later referred to as Type I and Type II. (Lakhtakia, 2013) Aratus of Cappodocia (81-133AD) coined the term "Diabetes"(Greek; siphon). Thomas Willis of Britain coined the word "Mellitus" after re-discovering the sweetness of patients' urine and blood." (Latin; honey-sweet) in 1675. (Ahmed, 2002)

Diabetes is a serious lifestyle illness with a rising prevalence in the world. As the prevalence of diabetes rises in these nations, Asia accounts for more than 60% of the world's diabetic population. Similar to this, by the year 2030 (438 million) more persons aged 20 to 70 have type 2 diabetes than there were in 2010. (Indian Council of Medical Research, 2018) According to estimates, in 2021, 536.6 million people would have diabetes worldwide. And an increase of 12.2% will bring the total number up to (783.2 million) population in the year 2045. The prevalence of diabetes was similar among males and females and was highest among people age groups (75 to 79). In the year 2021, the prevalence was estimated to be greater in cities (12.1 percent) than in remote areas (8.3 percent) & in high-income countries (11.1 percent) rather than in low-income countries (5.5 percent). In comparison to high-income countries (12.2 percent) and low-income (11.9 percent) countries, it is predicted that middle-income nations will have the most rapid rise in diabetes cases between 2021 and 2045 (21.1%). In 2021, the total expenses of treating

diabetes-related illnesses worldwide were expected to be 966 billion USD, with a rising to 1,054 billion USD by 2045. (Sun et al., 2021)

Diabetes is a potentially epidemic health problem that is quickly spreading throughout low-income and middle-income nations like India. According to projections, 69.9 million people are expected to be diagnosed with diabetes in India by 2025, the great majority of which would go untreated. This is primarily caused by food changes and inadequate or no physical activity, which alters the physiological environment and causes overweight or obesity as well as diabetes. (Mathur et al., 2022)

PREVALENCE OF DIABETES WORLDWIDE

At Glance	2021	2030	2045
Total worldwide population	7.9 billion	8.6 billion	9.5 billion
Adult demographic age (20 -70yrs)	5.1 billion	5.7 billion	6.4 billion
Children population (0-14 years)	1.2 million	_	_
Diabetes (20-79 years)			
Global prevalence	10.5%	11.3%	12.2%
No. of individuals who have diabetes	536.6 million	642.7 million	783.2 million
No. of people dying due to diabetes	6.7 million	_	_

Table:1. Prevalence of Diabetes worldwide (International Diabetes Federation,2021)

CLASSIFICATION OF DIABETES (Colledge et al.,2021)

1. TYPE I DIABETES MELLITUS
2. TYPE II DIABETES MELLITUS
3. Other specific types
    - Genetic defects in the islet of β-cells function
    - Genetic defects of insulin action
    - Pancreatic disorders
    - Drug-induced
    - Infections with viruses
    - Uncommon types of immune-mediated diabetes
    - Associated with genetic disorders
4. Gestational diabetes mellitus

TYPE I DIABETES MELLITUS

Type I diabetes is an autoimmune disorder caused due the destruction of pancreatic beta cells that produce insulins. The anabolic hormone insulin is required for the regulation of glucose, lipid, protein, and mineral metabolism along with growth. Moreover, the insulin activates the liver to store the glucose as glycogen and synthesize fatty acids, which enables glucose to enter the muscles and adipose tissues inhibiting the breakdown of fat in adipose tissue and stimulating the uptake of potassium into cells. Insulin replacement therapy is essential for type 1 diabetes mellitus patients for the rest of their lives as part of the treatment. In absence of insulin diabetic ketoacidosis (DKA) can be developed which has a high risk of fatal. (Lucier J et al.,2022)

One of the most common chronic disorders among adolescents is T1DM, however, it can start at any age. The rate and prevalence of type 1 DM have been gradually rising and affecting a total of 5% to 10% of all diabetics. (Lucier J et al.,2022) This type of disease develops slowly, the triggering of the environmental event occurs years before the cell mass is sufficiently reduced to cause symptoms. Following diagnosis and early treatment, adolescents frequently experience a good recovery period. This kind of diabetes normally has a shorter course than any other. This youngster may have other autoimmune disorders such as thyroid disorder, hypoadrenalism, vitamin B12 deficiency anemia, alopecia areata, vitiligo, and other diseases of the thyroid-gastric complex. (Guthrie et al., 2004)

TYPE 2 DIABETES MELLITUS

90% of people have T2DM, a condition caused by inadequate insulin production by beta cells and tissue insulin resistance. (IR), and an insufficient compensatory insulin secretory response**.** Patients with this disease are typically overweight or have higher a percentage of body fat, which is mainly present in the abdomen regions. Under this situation, adipose tissue encourages insulin resistance through several inflammatory pathways, along with the increased synthesis of free fatty acids and deregulated adipokines. (Garcia et al.,2020)

The main causes of the T2DM epidemic include population aging, sedentary lifestyles, high-calorie meals, and global increases in obesity, which caused the increases in the rate and prevalence of T2DM to increase by four times. (Garcia et al.,2020) T2DM is most widespread among adults, however, it is also affecting children and adolescents (WHO, 2019)T2DM symptoms are comparable to Type 1 DM symptoms, despite the fact, that symptoms are much less

severe and the disease may even have no symptoms at all. Furthermore, identifying the precise time of development of T2DM is usually difficult. (IDF, 2021)

Risk Factors of T2DM (Dennis et al., 2019)

- Obesity (termed as a BMI of 25 kg/m2 or appropriate criteria of overweight)
- Family history of diabetes
- Lack of physical exercise
- Racial or ethnicity
- Earlier diagnosed with Impaired Fasting Glucose, Impaired Glucose Tolerance, or an HbA1c of 5.7–6.4%
- History with GDM or a new-born weighing more than 4 kg (9 lb)
- Hypertension
- PCOD or acanthosis nigricans
- Cardiovascular disease history

PATHOGENESIS OF T2DM (Shlomo et al., 2016)

T2DM pathogenesis is complicated, involving the combination of hereditary and environmental factors. In particular, a sedentary lifestyle and high-calorie intake that results in obesity have been found to play a significant impact in the development of the condition. The clinical features vary widely in terms of onset of age with severity -related to hyperglycaemia, and the degree of obese people. From a pathophysiological perspective, people having T2DM frequently exhibit these 3 primary defects:

• Resistance to insulin action in peripheral tissues, especially muscle and fat, and also in the liver

• Insufficient insulin production, particularly in response to a glucose stimulus

• Liver's rise in the ability to produce glucose

DIABETES MELLITUS COMPLICATION

Diabetes complications can affect numerous organ systems and this disease has a greater majority of morbidity and deaths. Diabetes complications will not manifest until the second decade following hyperglycemia. This disease frequently has a prolonged period of asymptomatic hyperglycemia until diagnosed, people with T2DM may also have difficulties at the time of diagnosis. (Dennis et al., 2019)

COMPLICATION OF DIABETES
Microvascular/Neuropathic
Peripheral neuropathy (Sensory loss, Pain, Motor weakness) Foot disease (Ulceration, Arthropathy) Cataract, Retinopathy, (Impaired vision) Nephropathy (Renal failure) Autonomic Neuropathy (Gastrointestinal problems, Postural hypotension)
Macrovascular
Peripheral circulation (Claudication, Ischaemia) Coronary circulation (Myocardial infarction) Cerebral circulation (TIA, Stroke)

Table: 2. Complications of diabetes (Colledge et al.,2021)

DIAGNOSTIC CRITERIA OF DIABETES

FPG greater than or equal to 126mg/dL (7.0 mmol/L). Eight hours fasting without calorie intake
Or
2-h PG greater than or equal to 200 mg/dL (11.1 mmol/L) during the OGTT. 75 g of anhydrous glucose that is dissolved in water should be used to conduct the test as instructed by the WHO.
Or
HbA1c greater than or equal to 6.5% (48 mmol/mol) The test should be carried out with a glucose load that is equal to 75 g of anhydrous glucose dissolved in water as instructed by the WHO
Or
A random plasma glucose level of 200 mg/dL (11.1 mmol/L) was measured in a patient with classic hyperglycemia or hyperglycemic crisis.

Table: 3. Diagnostic criteria of diabetes (American Diabetes Association, 2022)

DCCT stands for “Diabetes Control and Complications Trial”;FPG stands for “Fasting Plasma Glucose”; OGTT stands for “Oral Glucose Tolerance Test”; WHO stands for “World Health Organization”, and (2hr- PG) stands for “2-hour plasma glucose”.

CREATINE KINASE

Creatine kinase is a compact enzyme having a molecular weight of 82-kDa enzyme present in the mitochondria and cytosol of tissues that required high energy. Creatine kinase is made of 2 polypeptide subunits each about 42 kDa present in the cytosol, which includes two distinguished forms of subunits found: M and B. And these subunits form 3 specific tissue isoenzymes: which include (CK-MM) skeletal muscle, (CK-MB) the cardiac muscle, and (CK-BB) the brain. This ratio of subunits differs according to muscle type: the skeletal muscle (98 % MM) and (2 % MB), the cardiac muscle (70-80 % MM) and (20-30% MB), and the brain has mostly BB. (Baird et al., 2012)

Creatine Kinase catalyzes the reversible phosphorylation of creatine to phosphocreatine and ADP (Adenosine tri-phosphate) to ATP (Adenosine diphosphate), and as such, can be essential for the regenerative process of cellular ATP.:

$$\text{Phosphocreatine (PCr)} + \text{MgADP}^- + \text{H}^+ \longrightarrow \text{MgATP}^{2-} + \text{creatine}$$

Phosphocreatine (PCr) circuit, which is an energy network, is composed primarily of CK. The cytosol isoenzymes in this circuit are strongly connected to glycolysis and the production of ATP for muscle activity. (Baird et al., 2012)

CK is a cytosolic compound that helps mitochondria to exchange phosphate through the cytoplasm as a source of high energy. It has a form of three isoenzymes CK-MM i.e., found in muscles and are considered as one of the muscle damage biomarkers that are released in the blood if muscle cells get damaged in diabetic patients, the dimension of creatine kinase will increase due to a rise in the isoenzyme and the source of this expansion is destruction in skeletal muscle caused by a decrease in vitality. Other isoenzymes, such as CK-MB (myocardial cell) and CK-BB

(cerebrum), are common. The main cause of elevated CK in diabetics is skeletal muscle damage, specifically CK-MM isoenzyme, with no cardiac problems. (Mohamed et al., 2019)

CREATINE KINASE AND TYPE 2 DIABETES MELLITUS

The skeletal muscle cells are affected by insulin deficiency or resistance, so a decline in glucose consumption reduces the amount of energy required by the muscle and causes muscle damage. (Mohamed et al., 2019) Insulin resistance (IR) in skeletal muscle is the major hallmark of T2DM, and it is thought to be largely explained by decreased non-oxidative glucose metabolism. Furthermore, insulin stimulation for glucose oxidation and suppression of lipid oxidation is significantly impaired in type 2 diabetes. (Awadalla et al.,2010)

In diabetes mellitus, the alteration in glucose, lipid, and protein metabolism, is especially evident in muscle cells (myocytes). Because of the disease, glucose utilization is reduced, phosphorylation of glucose is altered, glycogen synthesis is reduced, and glycolysis is suppressed. Because glycolysis has a lower capacity, oxaloacetate and pyruvate concentrations are reduced. (Jevrić-Causević et al.,2006) It may be due to many factors, including a defect in the Krebs cycle, which leads to an increase in proteolysis in diabetics. Defects of this cycle, as well as other metabolic pathways such as the respiratory chain, affect the morphology and function of mitochondria, resulting in a reduction in ATP production in skeletal muscle cells. (Mohamed et al., 2019)

The skeletal muscle of patients with Type II diabetes mellitus has smaller mitochondria than usual, limiting their capacity to produce energy. Low ATP can cause a decrease in creatine phosphate (CPK) synthesis, which may result in the activation of (AMP-activated protein kinase)

as a delay complication. A higher risk of muscle atrophy can be seen in diabetes patients. (Mohamed et al., 2019)

The previous study done by Adlija et al. found that cases with T1DM and T2DM had elevated in the activity of total CK activity. (Čaušević et al.,2006) It was also approved by Akram et al., who state that the level of CK activity is high in patients with type 2 diabetes cases. It was assumed that in this kind of diabetes, these alterations were represented in enzyme activity of creatine kinase, and an increase in activity was seen in type 2 diabetes cases (Awadalla et al.,2010)

LACTATE DEHYDROGENASE

Lactate Dehydrogenase is an enzyme having a molecular weight of 134 kDa and made up of 4 peptide chains of 2 forms: M (or A) and H (or B), which are genetically controlled separately. (Burtis et al.,2016) Lactate Dehydrogenase is present in almost body tissues and organ systems; however, serum LDH activity is rising in various diseases. (Klein et al., 2020) Lactate is produced by almost all tissues in the human body, with muscles producing the most. Lactate is eliminated by the liver and, to a smaller extent, whereas the kidneys under normal conditions. (Romero et al.2016)

Increased activity of LDH in body fluid and blood may help to demonstrate injury or disease. The rate of LDH activity in insulin-secreting cells is an essential regulator of physiological insulin production. LDH activity overexpression disrupts regular glucose metabolism and insulin production in the islet β-cell. It might be directly responsible for type 2 diabetes insulin secretion cause. (P Singh et al., 2022)

LACTATE DEHYDROGENASE AND TYPE 2 DIABETES MELLITUS

Lactate (2-hydroxypropanoic acid), previously thought to be a waste product of glycolysis, is now being recognized as an important factor in insulin resistance (IR), diabetes, cancer growth, development, and spread. Lactate is metabolized by various types of tissues and cells, including the hepatocytes, reproductive cells, and neurons, and converted to pyruvate via LDH and then to glycogen or carbon dioxide. Lactate is eliminated by the liver and kidneys under normal physiological conditions. Lactate, in general, can be transported to the liver and converted into glucose via the Cori cycle and used as a source of energy. (Wu et al., 2016)

The mechanisms leading to diabetes-related hyperlactatemia include significant modifications in the intracellular glucose metabolism in insulin-sensitive tissues, such as reduced glycogen synthesis, impaired glucose oxidative metabolism, and elevated whole-body nonoxidative glycolysis rates. Insulin resistance is essential for the pathophysiology of type II diabetes mellitus which serves as an early indicator of the condition. (Wu et al., 2016)

Elevated levels of insulin enhance glycolysis in insulin-resistant individuals by activating two rate-limiting enzymes, phosphofructokinase and pyruvate dehydrogenase. As a result, increased glycolysis activity can be seen in patients with insulin resistance/diabetes. Increased glycolysis leads to increased NADH and pyruvate formation and decreased NAD+ levels. In a redox reaction, LDH converts pyruvate to lactate while also producing NAD+ from NADH. This response may be exacerbated by insulin resistance because hyperinsulinemia causes increased glycolysis. (Wu et al., 2016)

In the previous research done by Johari et al., LDH activity did not change significantly in different groups as compared with control and diabetes subjects. (Johari et al,2018) In Huang's

study, significantly increased activity of LDH activity was seen in subjects with diabetes compared with healthy subjects. (Huang et al.,2006)

AIM AND OBJECTIVES

Aim:

The aim of the study is to evaluate levels of serum creatine kinase and serum lactate dehydrogenase in diagnosed cases of type 2 diabetes mellitus.

Objectives:

1. To estimate the serum creatine kinase level in patients with type 2 diabetes mellitus and apparently healthy controls.
2. To estimate the serum lactate dehydrogenase level in patients with type 2 diabetes mellitus and apparently healthy controls.
3. To compare serum creatine kinase and serum lactate dehydrogenase in type 2 diabetes Mellitus and apparently healthy controls.

MATERIALS AND METHODS

Research Question: Is there a change in the levels of serum creatine kinase and serum lactate dehydrogenase in newly diagnosed cases of type 2 diabetes mellitus as compared to controls?

Hypothesis

- *Null Hypothesis (H_0):* There is no significant change in the levels of serum creatine kinase and serum lactate dehydrogenase in newly diagnosed patients of type 2 diabetes mellitus as compared to controls.
- *Alternate Hypothesis (H_1):* There is a significant change in the levels of serum creatine kinase and serum lactate dehydrogenase in newly diagnosed patients of type 2 diabetes mellitus as compared to controls.

Type of study:

Case-Control study

Place of the study

Department of Biochemistry, Integral Institute of Medical Science & Research, Lucknow

Selection of subjects:

Controls

Inclusion criteria

1. Apparently healthy individuals
2. Subjects between the age of 18 to 70 years

Exclusion criteria

1. Conditions where phlebotomy is contraindicated
2. Subjects are suffering from chronic illness
3. Subjects are suffering from any acute illness

Cases

Inclusion criteria

1. Newly diagnosed cases of Type 2 Diabetes Mellitus (American Diabetes Association,2021)
2. Subjects between the age of 18 to 7o years

Exclusion criteria

1. Pregnant women
2. Subject suffering from thyroid disease
3. Subject suffering from Liver disease

Sample size

The formula for determining the sample-

$$n = \frac{(r+1)(SD^2)}{r} \frac{(Z\beta + Z\alpha/2)^2}{d^2}$$

Reference: Charan J Biswas, 2013

For quantitative variable

n= sample size in the case group

(r+1)/r= ratio of case to control

σ = standard deviation of the outcome variable (taken from previous studies)

Zβ= represents the desired power (typically 0.84 for 80% power)

Zα/2= represents the desired level of statistical significance (typically 1.96 for 95%)

d = effect size (the difference in means is taken from previous studies)

Then,

For 80% power, Zβ = (0.84)

For 0.05 significance level, Zα/2 = 1.96

r = 1 (equal no. of case and control)

σ= 10.0 (Mohamed et al., 2021)

d= 6.7

$$n = \frac{2(10)^2 (0.84+1.96)^2}{(6.7)^2}$$

n=34.9 ≈35

Therefore, the study includes **35** cases and **35** controls

Collection of samples

2.5 ml of venous blood was collected from the subjects under the aseptic condition in a plain vial. The blood samples were allowed to clot at room temperature for 15 minutes. The sample was then centrifuged at 1000xg for 10 minutes to separate the serum (Henry J. B., 1970).

500 microliter serum was used for the estimation of creatine kinase.

500 microliter serum was used for the estimation of lactate dehydrogenase.

Storage of the samples

The serum samples for the estimation of creatine kinase and lactate dehydrogenase were stored at -20°C until testing.

Laboratory Examination:

1. **Estimation of serum creatine kinase by IFCC method using Erba chem 7 semi autoanalyzer**

Principle:

CK catalyzes the reaction between CPK and ADP to form creatine and ATP. The ATP formed along with Glucose is catalyzed by Hexokinase to form G-6-Phosphate. The G-6- Phosphate reduces NADP to NADPH in the presence of G-6- Phosphate dehydrogenase. The rate of reduction of NADP to NADPH is measured as an increase in absorbance which is proportional to CK activity in the sample.

Creatine phosphate + ADP $\xrightarrow{CKBB/CKMB}$ Creatine + ATP

ATP + Glucose $\xrightarrow{HK}$ Glucose-6-phosphate + ADP

G6PDH + NAD $\xrightarrow{G6PDH}$ Gluconate-6-P+ NADH+H+

Procedure:

Type of reaction: Increasing

Wavelength/filter: 405 nm

Temperature: 37°C

Light path: 1 cm

Substrate Start Assay

Addition Sequence	**(T) 37°C**
Enzyme Reagent (L1)	0.8 ml
Sample	0.02 ml

Incubate at the assay temperature for 1 min. and add

Starter Reagent (L2)	0.2 ml

Mix well, and then read the initial absorbance A0 after 1 minute. Repeat the absorbance reading after 1, 2, and 3 minutes. Calculate the average absorbance change per minute (A/min).

Normal Reference Value:

Serum (Males): up to 24-195 U/L at 37°C

(Females): up to 24-170 U/L at 37°C

2. **Estimation of serum lactate dehydrogenase by DGKC method using Erba chem 7 semi autoanalyzer**

Principle:

Lactate Dehydrogenase catalyzes the conversion of pyruvate to NAD by reducing it with NADH. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance that is proportional to the sample's LDH activity.

Pyruvate + NADH $\Longrightarrow$ Lactate + NAD+ LDH

Procedure:

Type of reaction: Increasing

Wavelength/filter: 405 nm

Temperature: 37°C

Light path: 1 cm

Substrate Start Assay

Addition Sequence	**(T) 37°C**
Buffer Reagent (L1)	0.8 ml
Sample	0.02 ml

Incubate at the assay temperature for 1 min. and add

Starter Reagent (L2)	0.2 ml

Mix well, and then read the initial absorbance A0 after 1 minute. Repeat the absorbance reading after 1, 2, and 3 minutes. Calculate the average absorbance change per minute (A/min).

Normal Reference Value:

Serum: 230-460 U/L at 37°C

STATISTICAL ANALYSIS PLAN

Statistical analysis was performed using GraphPad and Microsoft Excel. All the data were expressed as mean ± standard deviation. An unpaired t-test was performed to compare the study parameters between cases and controls. Pearson correlation analysis was employed to determine the relationship between variables. p-values < 0.05 were considered statistically significant.

OBSERVATIONS AND RESULTS

CREATINE KINASE

The difference in the activity of serum creatinine kinase was not statistically significant (p= 0.3) between cases (35.72±16.82) as compared to controls (39.63±22.11) shown in Table: 3 & Fig: 1

Table: 3. Creatine Kinase in cases and controls

GROUP	N	MEAN	STANDARD DEVIATION	p-Value
CONTROLS	35	39.63	±22.11	p= 0.3
CASES	35	35.72	±16.82	

N- Number of Cases or Controls, p < 0.05 considered statistically significant

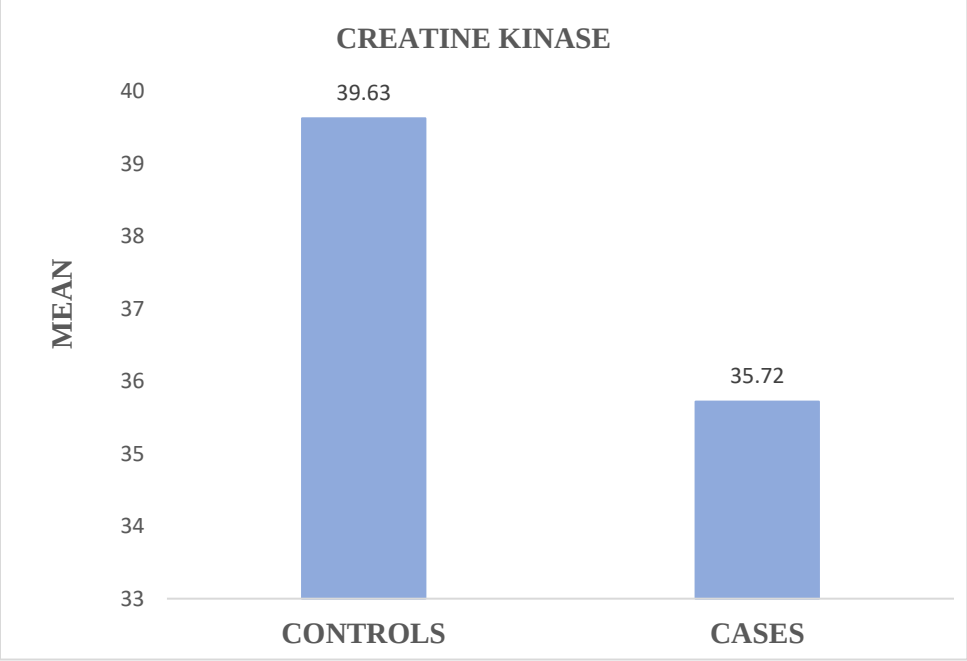


Fig: 1. Comparison of Creatine Kinase in controls and cases

LACTATE DEHYDROGENASE

The activity of serum lactate dehydrogenase was significantly low (p= 0.01) in cases (278.65±46.89) as compared to controls (316.48±71.18) shown in Table: 4 & Fig: 2

Table: 4. Lactate Dehydrogenase in cases and controls

GROUP	N	MEAN	STANDARD DEVIATION	p-Value
CONTROLS	35	316.48	±71.18	p= 0.01
CASES	35	278.65	±46.89	

N- Number of Cases or Controls, p < 0.05 considered statistically significant

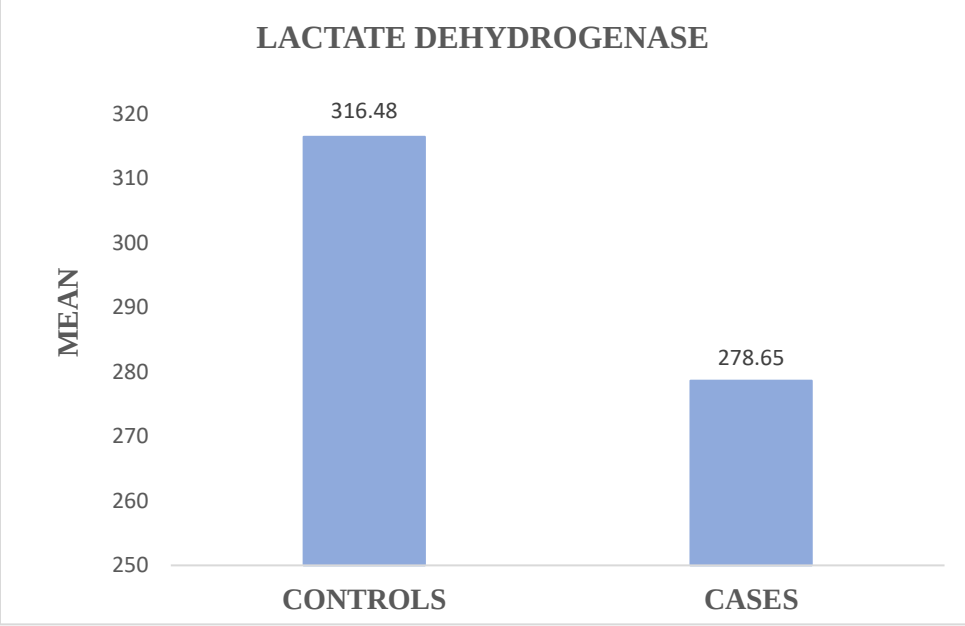


Fig: 2. Comparison of Lactate Dehydrogenase in controls and cases

BODY MASS INDEX

Body Mass Index did not show statistically significant difference (p= 0.7) between cases (22.96±2.39) and controls (22.69±3.87) shown in Table: 6 & Fig: 3

Table: 6. BMI distribution of cases and controls

GROUP	N	MEAN	STANDARD DEVIATION	p-Value
CONTROLS	35	22.69	±3.87	p= 0.7
CASES	35	22.96	±2.39	

N- Number of Cases or Controls, p < 0.05 considered statistically significant

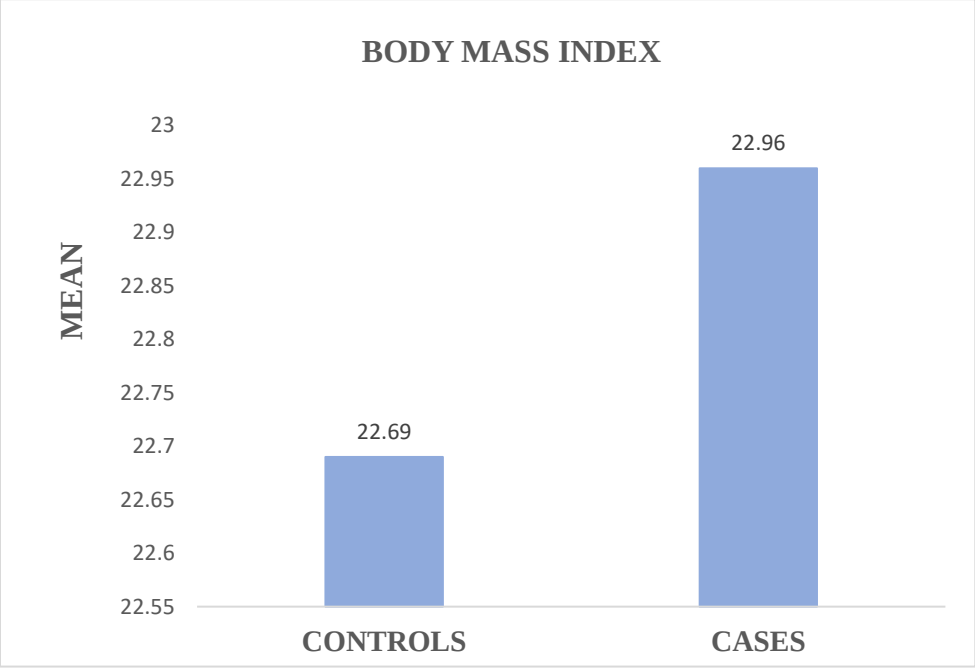


Fig: 3. Comparison of BMI in controls and cases

KARL PEARSON'S COEFFICIENT OF CORRELATION

A statistically significant positive correlation was observed between serum creatine kinase and serum lactate dehydrogenase (r= .405, p= .016)

		CK	LDH
CK	Pearson Correlation	1	.405*
	Sig. (2-tailed)		.016
	N	35	35
LDH	Pearson Correlation	.405*	1
	Sig. (2-tailed)	.016	
	N	35	35

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Table: 7. Pearson Correlation coefficient between Creatine Kinase and Lactate Dehydrogenase

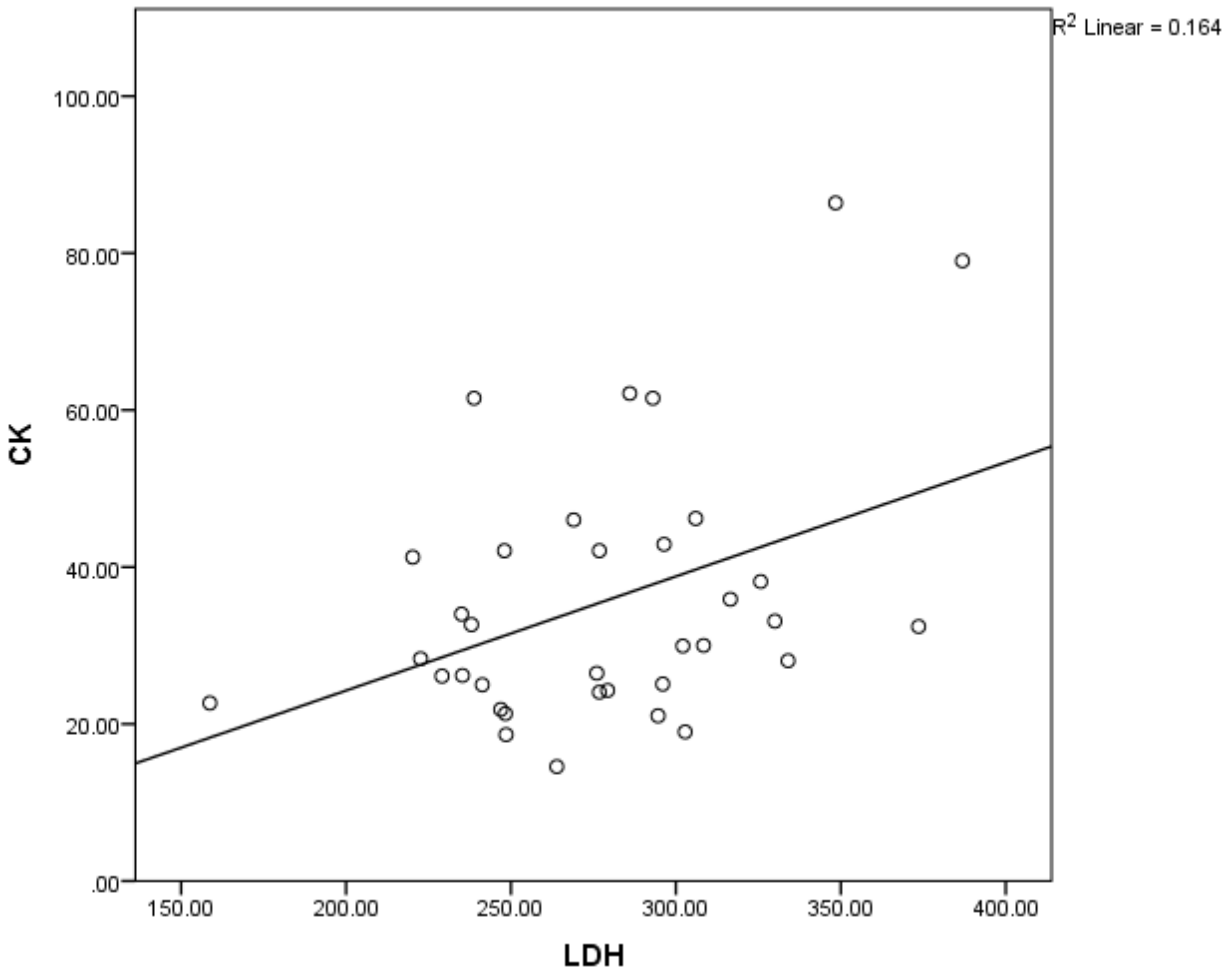


Fig: 4. Scatter diagram showing a correlation between Creatine Kinases and Lactate Dehydrogenase

DISCUSSION

Millions of people suffering from Type II diabetes mellitus continue to have poor health. One of the main pathogenic factors causing the condition is the body's resistance to the action of insulin in lowering blood sugar levels (insulin resistance). The principal issue for preserving glucose homeostasis is skeletal muscle, which does so by up taking glucose through both insulin-dependent and -independent processes. (Hulett et al., 2022)

In our study, we evaluated the status of serum creatine kinase activity in Type II diabetes mellitus patients. This study showed a decrease in serum activity of CK enzyme (35.72±16.82) in cases as compared with the controls group which was (39.63±22.11). This result shows the activity of serum creatine kinase was not significant between cases as compared to controls. There is no precise evidence, in the literature corresponding to the relationship between serum creatine kinase and Newly diagnosed Type II diabetes mellitus. However, limitation related to the number of examined patients points out the fact that further well-designed investigations are needed to give definite answers related to the possible mechanism of these changes.

Santos et al., suggested that the skeletal muscles type 2 diabetes patients have smaller mitochondria as compared to normal individuals, making energy production difficult. Low ATP can cause a decline in creatine phosphate, which can later activate the (AMP-activated protein kinase). Diabetics are more likely to experience muscular atrophy and alteration.

Several studies considered creatine kinase as a biomarker for the risk factor of cardiovascular problems in diabetes patients. Evliyaoğlu et al. reported that activity of the serum creatine kinase was significantly correlated with patients' glucose control who had type 2 diabetes, as compared with healthy controls. An elevated level of CK-MB/ CK activity was significantly high in diabetes patients who had uncontrolled glucose levels. The results support that both the

microvascular and macrovascular complications of diabetes may involve cardiovascular disease which may result in death and morbidity.

Mohamed et al., have reported that serum creatine kinase level was rise in diabetes mellitus patients either with cases of type 1 or type 2 in comparison to control groups. This finding is in support of the previous research done by Adlija et al., who found that T1DM and T2DM patients had elevated levels of serum creatine kinase. It was also proved by Akram et al., who concluded the level of serum CK activity was elevated in T2DM cases.

In our study, we observed that Type II Diabetes Mellitus patients had significantly decreased levels of serum lactate dehydrogenase activity as compared to normal individuals. Lactate dehydrogenase is an enzyme of the anaerobic metabolic pathway . (Johari et al, 2018)

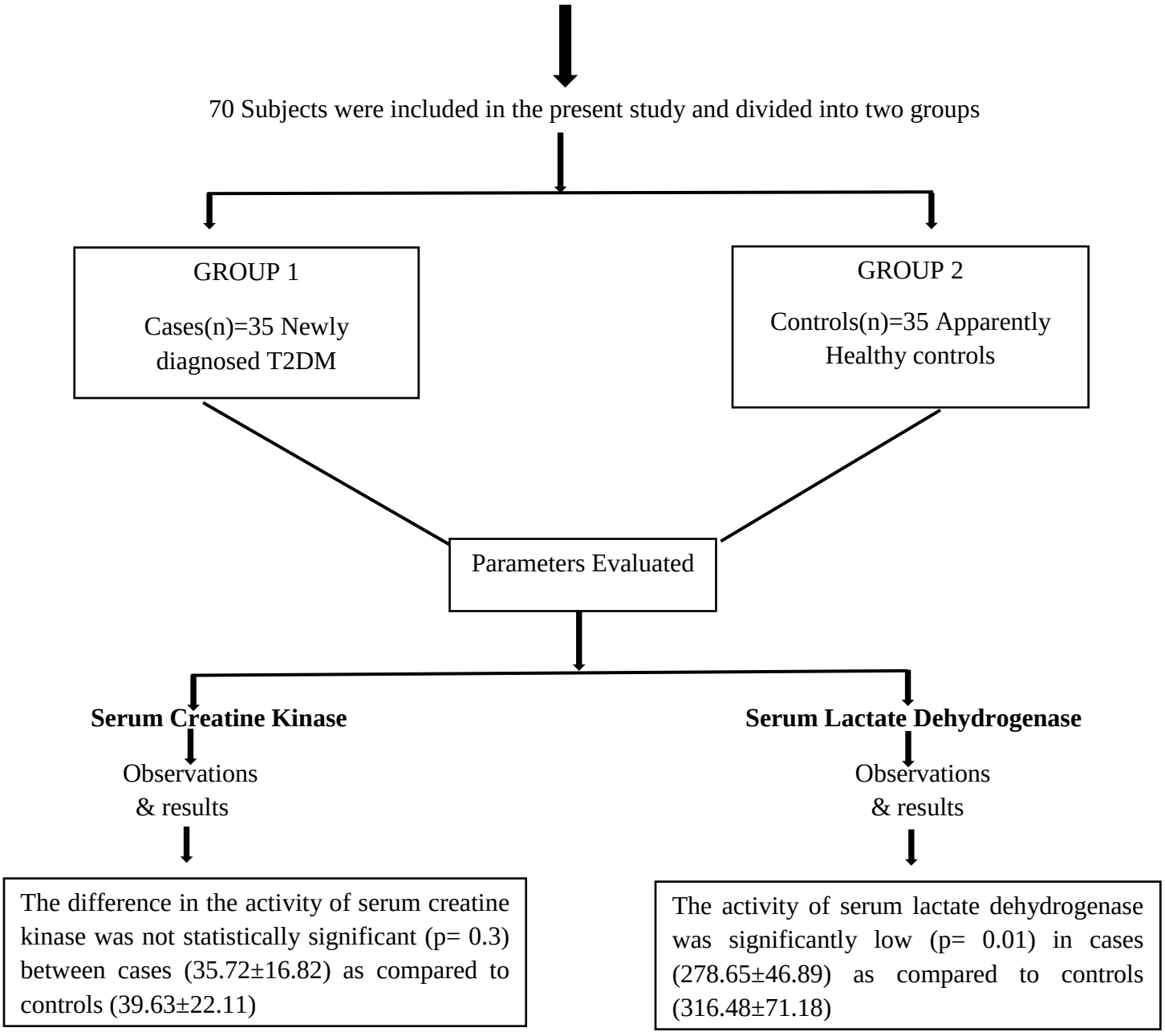
Oliver et al., did not observe any rise in the levels of serum LDH between diabetes mellitus and healthy controls. These findings are in agreement therewith Kamble et al., that mild diabetes mellitus does not affect LDH, but raised levels of LDH were highly significant in severe diabetes mellitus cases.

In Huang et al., study, LDH activity was increased among the cases of type 2 diabetes mellitus as compared to normal subjects. Sreenivasan et al. found elevated LDH activity in Type 2 diabetic Mellitus patients as compared to healthy subjects; the elevated enzyme activity seen in diabetes has also been linked to the influence of insulin on liver and muscle tissues. Because muscle and liver damage are frequently associated with diabetes, serum enzyme activities generated from muscle and liver may also contribute to elevated LDH activity in diabetics.

SUMMARY AND CONCLUSION

SUMMARY

The purpose of this study determines serum creatine kinase and serum lactate dehydrogenase in diagnosed cases of Type 2 Diabetes Mellitus & apparently healthy controls.



*****Serum Lactate Dehydrogenase activity was significantly low in cases as compared to controls.

*Serum Creatine Kinase and Serum Lactate Dehydrogenase are positively correlated and the correlation is statistically significant.

CONCLUSION

Diabetes is described as a group of disorders characterized by hyperglycemia. The present study found that the difference in the activity of serum creatine kinase between cases and controls was not statistically significant, whereas, serum lactate dehydrogenase activity was significantly decreased in cases with T2DM as a comparison to apparently healthy individuals.

Previous research has suggested that an increase in glucose level could damage liver cells and skeletal muscle which causes variation in the creatine kinase and lactate dehydrogenase activity in diabetes mellitus patients.

This study's findings may be helpful in understanding the role of serum lactate dehydrogenase activity in type 2 diabetes mellitus patients related to its pathogenesis. Further investigations are needed in order to confirm the current findings in larger a larger sample size.

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ANNEXURES

Unique Identification No:

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH LUCKNOW-226026

INCLUSION AND EXCLUSION CRITERIA- CASES

Inclusion criteria	YES	NO
1. Subject diagnosed with Type 2 Diabetes Mellitus (American Diabetes Association,2021)	☐	☐
2. Age group between 18- 70 years	☐	☐
3. Subject who has signed the informed consent form	☐	☐
Exclusion criteria		
1. Pregnant women	☐	☐
2. Subject suffering from Thyroid disorder	☐	☐
3. Subject suffering from Liver Disease	☐	☐

The subject is eligible for the study if all INCLUSION criteria are YES and all EXCLUSION criteria are NO

INVESTIGATOR'STATEMENT

I have verified the data entered in a case report form and have determined that it is complete, accurate, and compatible with the source documents.

Investigator's name Investigator's signature Date

Unique Identification No:

IDENTIFIERS- CASES

Registration No:

Contact No:

Name:

Father's Name /Husband's Name:

Address:

DEMOGRAPHICS- CASES

Age: ☐

Sex: Male ☐ Female ☐

Place of Residence: Urban ☐ Rural ☐

Social/Economical Status: a) Upper b) Upper Middle c) Lower Middle d) Upper Lower e) Lower

Educational: a) Illiterate b) Primary c) Middle d) High school e) Intermediates f) Graduate g) Postgraduate & above

ANTHROPOMETRIC PARAMETERS-CASES

Height(mts) ☐

Weight (kgs) ☐

Body Mass Index(kg/m^2) ☐

Unique Identification No:

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH
LUCKNOW-226026

INCLUSION AND EXCLUSION CRITERIA- CONTROLS

Inclusion criteria	YES	NO
1. Apparently healthy individuals	☐	☐
2. Subjects within the age of 18 to 70 years	☐	☐

Exclusion criteria

1. Conditions where phlebotomy is contraindicated	☐	☐
2. Subjects is suffering from chronic illness	☐	☐
3. Subjects is suffering from any acute illness	☐	☐

The subject is eligible for the study if all INCLUSION criteria are YES and all EXCLUSION criteria are NO

INVESTIGATOR'STATEMENT

I have verified the data entered in a control report form and have determined that it is complete, accurate, and compatible with the source documents.

_____	_____	_____
Investigator's name	Investigator's signature	Date

Unique Identification No:

IDENTIFIERS- CONTROLS

Registration No:

Contact No:

Name:

Father's Name /Husband's Name:

Address:

DEMOGRAPHICS- CONTROLS

Age: ☐

Sex: Male ☐ Female ☐

Place of Residence: Urban ☐ Rural ☐

Social/Economical Status: a) Upper b) Upper Middle c) Lower Middle d) Upper Lower e) Lower

Educational: a) Illiterate b) Primary c) Middle d) High school e) Intermediates f) Graduate g) Postgraduate & above

ANTHROPOMETRIC PARAMETERS-CONTROLS

Height(mts) ☐

Weight (kgs) ☐

Body Mass Index (kg/m²) ☐

INFORMED CONSENT FORM

Study title: "SERUM CREATINE KINASE AND SERUM LACTATE DEHYDROGENASE IN TYPE II DIABETES MELLITUS"

Subject's name…… Age…… Sex……

I confirm that I have read and understood/have been explained the information given by the researcher/moderator and I had an opportunity to ask questions. I understand that participation in the study is voluntary and I am free to withdraw at any time without giving any reason and without being my medical care and legal rights being affected. I understand that my identity will not be revealed to any third party or in publication. I understand that the researchers/ regulatory authorities/ ethics committee will not need my permission to access my health records if necessary for the current study. I agree not to restrict the use of any data or results that arise from this study provided such use is only for the scientific purpose(s). I agree to take part in the above study.

Signature of the subject……..… Date……..

Name of the Investigator(printed)……

Signature of the investigator…… Date……

Name and signature of the impartial witness with date if required

……

सूचित सहमति प्रपत्र

अध्ययन शीर्षक: सीरम क्रिएटिनकाइनेज ऐंड सीरम लैक्टेट डिहाइड्रोजनेज इन टाइप 2 डायबिटीज मिलिट्स

विषय का नाम …… उम्र …… लिंग ……

मैं पुष्टि करता हूं कि मैंने शोधकर्ता/मॉडरेटर द्वारा दी गई जानकारी को पढ़ और समझ लिया है/समझा गया है और मुझे प्रश्न पूछने का अवसर मिला है। मैं समझता हूं कि अध्ययन में भाग लेना स्वैच्छिक है और मैं बिना कोई कारण बताए और अपनी चिकित्सा देखभाल और कानूनी अधिकारों को प्रभावित किए बिना किसी भी समय वापस लेने के लिए स्वतंत्र हूं। मैं समझता हूं कि मेरी पहचान किसी तीसरे पक्ष या प्रकाशन में प्रकट नहीं की जाएगी। मैं समझता हूं कि वर्तमान अध्ययन के लिए यदि आवश्यक हो तो शोधकर्ताओं/नियामक प्राधिकरणों/नैतिकता समिति को मेरे स्वास्थ्य रिकॉर्ड तक पहुंचने के लिए मेरी अनुमति की आवश्यकता नहीं होगी। मैं इस अध्ययन से उत्पन्न होने वाले किसी भी डेटा या परिणामों के उपयोग को प्रतिबंधित नहीं करने के लिए सहमत हूं, बशर्ते ऐसा उपयोग केवल वैज्ञानिक उद्देश्यों के लिए हो। मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं।

विषय के हस्ताक्षर …… तारीख ……

अन्वेषक का नाम (मुद्रित)……

अन्वेषक के हस्ताक्षर …… तारीख ……

यदि आवश्यक हो तो तिथि के साथ निष्पक्ष गवाह का नाम और हस्ताक्षर

……

INSTITUTIONAL ETHICS COMMITTEE (IEC)

IIMS&R INTEGRAL UNIVERSITY, LUCKNOW

IEC/IIMS&R/2022/11

CERTIFICATE

This is to certify that research work entitled **"Serum creatine kinase and serum lactate dehydrogenase in Type 2 DM: A case control study"** submitted by **Fatma khatoon, Dr.Priyanka Thapa Manger** for ethical approval before the Institutional Ethics Committee IIMS&R.

The above mentioned research work has been approved by Institutional Ethics Committee, IIMS&R with consensus in the meeting held on **19 May 2022**.

Dr.Deepak Chopra
(Jt.Member Secretary)
IRC/IEC
IIMS &R

Dr.Q.S.Ahmed
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Diabetes is a metabolic disorder characterized by hyperglycemia either caused by insulin synthesis, insulin action, or both. The term "chronic hyperglycemia" of diabetes has been related to long-term effects, disorders, and failure of organs such as the heart, blood vessels, kidneys, nerves, and eyes. (American Diabetes Association, 2009).

Developed nations have been suffering from diabetes, and it has been the fourth leading cause of death. (Burtis et al., 2016) As stated by "The International Diabetes Federation," 537 million adult population of 20-79 age groups have diabetes mellitus. And the population is predicted to increase by 643 million by 2030 with a further increase to 783 million by 2045. Aside from this, it is predicted that nearly 541 million individuals will be affected by impaired glucose tolerance in 2021. About (6.7 million) people worldwide in the age group (20 to 79) are expected to die from diabetes-related diseases in 2021. (International Diabetes Federation,2021)

Diabetes is rapidly approaching epidemic levels in a country like India, with approx. of 62 million diagnosed cases. In the year 2000, India has the highest prevalence (31.7 million individuals) of diabetes cases globally, then came China with a number of 20.8 million and the United States with a number of 17.7 million. As stated by Wild et al, the worldwide diabetes prevalence can be anticipated to be increasing from (171 million) in 2000 to (366 million) by 2030, with India having greater growth. Diabetes mellitus is anticipated to impact 79.4 million Indian citizens in 2030, with significant increases in the number of people living in both the United States (42.1 million) and China (30.3 million). (Kaveeshwar et al., 2014)

Type I Diabetes Mellitus is triggered by an autoimmune response that occurs when the immune system targe β-cells of the pancreas which secret insulin. This type of diabetes can affect anyone at any age, although it most commonly affects children and young people. One of the most common chronic disorders in adolescents is Diabetes Mellitus Type I. The common symptoms can be considered as excessive thirst, frequent urination (polyuria), lack of energy or fatigue, blurred vision, and diabetic ketoacidosis. (International Diabetes Federation,2021)

T2DM is the most common widespread type of diabetes in the world, with 90 percent of cases. (Shlomo et al., 2016) Hyperglycemia is a complication of type II diabetes mellitus where the body's cells are unable to react and respond properly to insulin, a term known as insulin resistance (IR). Insulin resistance reduces hormone effectiveness, which leads to an increase in the production of insulin. (International Diabetes Federation,2021)

Insulin release and activity must be precise to fulfill the metabolic requirement; as a result, the molecular mechanisms involved in insulin secretion, release, & tissue insulin action must be closely controlled. The defects in the systems can cause metabolic imbalance, leading to T2DM pathogenesis. (Garcia et al., 2020) T2DM lowers life expectancy because it increases the chance of renal disease, peripheral neuropathy, heart failure, stroke, blindness, and amputation. (Smushkin et al.,2010) Both types of diabetes mellitus (DM) cause a decline in pancreatic β-cell function and mass, which is accompanied by a deterioration in

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glycemic control. Due to their significant overlap, it can be challenging to distinguish between T1DM and T2DM in daily practice. (Snehalatha et al., 2004)

Creatine kinase is an enzyme commonly known as Creatine phosphokinase (CPK) that is located inside cells in high concentrations in skeletal muscle, the brain, and the myocardium, with lower levels found in other visceral tissues. CK is a dimeric molecule made of the M and B subunits. The isoenzymes (CK-MM), (CK-MB), and (CK-BB) are mainly formed by combining these subunits. (Cabaniss et al.,1990)

All three isoenzyme species can be found in the cell's cytosol. However, a fourth variety does exist and that is distinct from others in the terms of electrophoretic mobility & immunology The isoenzyme (CK-Mt) is found between both the inner and outer membranes of mitochondria which accounts for 15 % of the total CK activity in the heart. The CK-Mt gene can be found on chromosome 15. (Burtis et al.,2016 7th edition)

An enzyme called creatine kinase catalyzes the (ATP-dependent phosphorylation) of creatine, which can be essential for energy buffering in tissues with different energy requirements, especially cardiac muscle, and skeletal muscle. The increase in the level of creatine kinase activity may indicate tissue injury and is seen in a variety of clinical diseases, also including myopathy. (Kristjansson et al.,2016)

A standard technique of determining the level of CK activity involves the rate of the preceding reaction using spectrophotometry. These results can differ greatly due to variations in analytical methodology, as well as differences in age, gender, race, and physical activity level. Electrophoresis, column chromatography, or radioimmunoassay are methods for separating CK into its isoenzymes. The majority of clinical laboratories employ cellulose acetate electrophoresis or agarose gel along with band quantification using fluorometric or spectrophotometric techniques. (Cabaniss et al.,1990)

The CKMB/CK level is a promising candidate which is used to determine the cardiovascular disorders associated with uncontrolled diabetes. These results supported the risk of cardiovascular disease-causing death and morbidity related to both macrovascular and microvascular diabetic complications. (Evliyaoğlu et al.,2010)

Lactate dehydrogenase (LDH) is a widely expressed enzyme. It is in charge of catalyzing the anaerobic, NADPH-dependent conversion of pyruvate to lactate, which can be required when muscle activity is elevated. (Kristjansson et al.,2016) The enzyme lactate dehydrogenase (LDH) is found in almost all cells of the body. It is worth noting that LDH is primarily found in the cytoplasm of the cell and becomes extracellular when the cell dies. It has been proposed that LDH concentrations vary depending on the energy requirements of different tissues. (Dmour H et al., 2020)

Lactate dehydrogenase is made up of 4 peptide chains of 2 types: Muscle(M) and Heart(H), which are genetically controlled separately. The structures (LD-M & LD-H) can be primarily determined by genes on human chromosomes no. 11 and 12, separately. (Burtis et al.,2016 7th edition)

Most tissues contain five LDH isoenzymes, which have multiple molecular forms while catalyzing the same reaction. Isoenzymes are made up of two separate types of subunits, H and M, that are randomly combined in a tetrameric structure. M4, M3H, M2H2, MH3, and H4 are the compositions of the five major isoenzymes, with M subunits predominating in skeletal muscle and the liver and H subunits predominating in the heart. (Nagler et al., 2001)

The activity of LDH can be altered with normal glucose metabolism and insulin release through the beta cells of the pancreas, and it may be responsible for insulin secretory abnormalities in type II diabetes mellitus. The above findings also agree with the concept of "that glucose-derived pyruvate metabolism in the mitochondrion is required for glucose-stimulated insulin secretion in the beta cell." (Ainscow et al., 2000)

The appearance of LDH extracellularly is used to detect cell damage or death. Increased levels have been seen in cardiac, hepatic, skeletal muscle, and renal diseases, as well as a variety of hematological and neoplastic conditions. (Klein et al., 2020)

As a result, determining serum creatine kinase and serum lactate dehydrogenase levels is critical when developing a diabetes treatment strategy.

REVIEW Of LITERATURE

Diabetes mellitus also referred to as diabetes, becomes a serious, long-term chronic disease in which blood glucose levels rise as a result of the body's inability to synthesize enough insulin or to adequately utilize the insulin that it does produce. (International Diabetes Federation,2021)

Diabetes, a disease characterized by "excessive urine emptying," can be dated back to 1500 B.C. in Egyptian writings. Indian doctors called it "honey urine" or "madhumeha" because of the fact that it attracted ants. In 400–500 A.D. An Ancient Indian physicians Sushruta and Charaka were the first to differentiate between the two kinds of diabetes, which were later referred to as Type I and Type II. (Lakhtakia, 2013) Aratus of Cappodocia (81-133AD) coined the term "Diabetes"(Greek; siphon). Thomas Willis of Britain coined the word "Mellitus" after re-discovering the sweetness of patients' urine and blood." (Latin; honey-sweet) in 1675. (Ahmed, 2002)

Diabetes is a serious lifestyle illness with a rising prevalence in the world. As the prevalence of diabetes rises in these nations, Asia accounts for more than 60% of the world's diabetic population. Similar to this, by the year 2030 (438 million) more persons aged 20 to 70 have type 2 diabetes than there were in 2010. (Indian Council of Medical Research, 2018) According to estimates, in 2021, 536.6 million people would have diabetes worldwide. And an increase of 12.2% will bring the total number up to (783.2 million) population in the year 2045. The prevalence of diabetes was similar among males and females and was highest among people age groups (75 to 79). In the year 2021, the prevalence was estimated to be greater in cities (12.1 percent) than in remote areas (8.3 percent) & in high-income countries (11.1 percent) rather than in low-income countries (5.5 percent). In comparison to high-income countries (12.2 percent) and low-income (11.9 percent) countries, it is predicted that middle-income nations will have the most rapid rise in diabetes cases between 2021 and 2045 (21.1%). In 2021, the total expenses of treating diabetes-related illnesses worldwide were expected to be 966 billion USD, with a rising to 1,054 billion USD by 2045. (Sun et al., 2021)

Diabetes is a potentially epidemic health problem that is quickly spreading throughout low-income and middle-income nations like India. According to projections, 69.9 million people are expected to be diagnosed with diabetes in India by 2025 , the great majority of which would go untreated. This is primarily caused by food changes and inadequate or no physical activity, which alters the physiological environment and causes overweight or obesity as well as diabetes. (Mathur et al., 2022)

PREVALENCE OF DIABETES WORLDWIDE

At Glance	2021	2030	2045
Total worldwide population	7.9 billion	8.6 billion	9.5 billion
Adult demographic age (20 -70yrs)	5.1 billion	5.7 billion	6.4 billion
Children population (0-14 years)	1.2 million	–	–
Diabetes (20-79 years)			
Global prevalence	10.5%	11.3%	12.2%
No. of individuals who have diabetes	536.6 million	642.7 million	783.2 million
No .of people dying due to diabetes	6.7 million	–	–

Table:1. Prevalence of Diabetes worldwide (International Diabetes Federation,2021)

CLASSIFICATION OF DIABETES (Colledge et al.,2021)

1. TYPE I DIABETES MELLITUS
2. TYPE II DIABETES MELLITUS
3. Other specific types
    - Genetic defects in the islet of β-cells function
    - Genetic defects of insulin action
    - Pancreatic disorders
    - Drug-induced
    - Infections with viruses
    - Uncommon types of immune-mediated diabetes
    - Associated with genetic disorders
4. Gestational diabetes mellitus

TYPE I DIABETES MELLITUS

Type I diabetes is an autoimmune disorder caused due the destruction of pancreatic beta cells that produce insulins. The anabolic hormone insulin is required for the regulation of glucose, lipid, protein, and mineral metabolism along with growth. Moreover, the insulin activates the liver to store the glucose as glycogen and synthesize fatty acids, which enables glucose to enter the muscles and adipose tissues inhibiting the breakdown of fat in adipose tissue and stimulating the uptake of potassium into cells. Insulin replacement therapy is essential for type 1 diabetes mellitus patients for the rest of their lives as part of the treatment. In absence of insulin diabetic ketoacidosis (DKA) can be developed which has a high risk of fatal. (Lucier J et al.,2022)

One of the most common chronic disorders among adolescents is T1DM, however, it can start at any age. The rate and prevalence of type 1 DM have been gradually rising and affecting a total of 5% to 10% of all diabetics. (Lucier J et al.,2022) This type of disease develops slowly, the triggering of the environmental event occurs years before the cell mass is sufficiently reduced to cause symptoms. Following diagnosis and early treatment, adolescents frequently experience a good recovery period. This kind of diabetes normally has a shorter course than any others. This youngster may have other autoimmune disorders such as thyroid disorder, hypoadrenalism, vitamin B12 deficiency anemia, alopecia areata, vitiligo, and other diseases of the thyroid-gastric complex. (Guthrie et al., 2004)

TYPE 2 DIABETES MELLITUS

90% of people have T2DM, a condition caused by inadequate insulin production by beta cells and tissue insulin resistance. (IR), and an insufficient compensatory insulin secretory response**.** Patients with this disease are typically overweight or have higher a percentage of body fat, which is mainly present in the abdomen regions. Under this situation, adipose tissue encourages insulin resistance through several inflammatory pathways, along with the increased synthesis of free fatty acids and deregulated adipokines. (Garcia et al.,2020)

The main causes of the T2DM epidemic include population aging, sedentary lifestyles, high-calorie meals, and global increases in obesity, which caused the increases in the rate and prevalence of T2DM to increase by four times. (Garcia et al.,2020) T2DM is most widespread among adults, however, it is also affecting children and adolescents (WHO, 2019)T2DM symptoms are comparable to Type 1 DM symptoms, despite the fact, that symptoms are much less severe and the disease may even have no symptoms at all. Furthermore, identifying the precise time of development of T2DM is usually difficult. (IDF, 2021)

Risk Factors of T2DM (Dennis et al., 2019)

- Obesity (termed as a BMI of 25 kg/m2 or appropriate criteria of overweight)
- Family history of diabetes
- Lack of physical exercise
- Racial or ethnicity
- Earlier diagnosed with Impaired Fasting Glucose, Impaired Glucose Tolerance, or an HbA1c of 5.7–6.4%
- History with GDM or a new-born weighing more than 4 kg (9 lb)
- Hypertension
- PCOD or acanthosis nigricans
- Cardiovascular disease history

PATHOGENESIS OF T2DM (Shlomo et al., 2016)

T2DM pathogenesis is complicated, involving the combination of hereditary and environmental factors. In particular, a sedentary lifestyle and high-calorie intake that results in obesity have been found to play a significant impact in the development of the condition. The clinical features vary widely in terms of onset of age with severity -related to hyperglycemia, and the degree of obese people. From a pathophysiological perspective, people having T2DM frequently exhibit these 3 primary defects:

- Resistance to insulin action in peripheral tissues, especially muscle and fat, and also in the liver
- Insufficient insulin production, particularly in response to a glucose stimulus

• Liver's rise in the ability to produce glucose

DIABETES MELLITUS COMPLICATION

Diabetes complications can affect numerous organ systems and this disease has a greater majority of morbidity and deaths. Diabetes complications will not manifest until the second decade following hyperglycemia. This disease frequently has a prolonged period of asymptomatic hyperglycemia until diagnosed, people with T2DM may also have difficulties at the time of diagnosis. (Dennis et al., 2019)

COMPLICATION OF DIABETES
Microvascular/Neuropathic
Peripheral neuropathy (Sensory loss, Pain, Motor weakness) Foot disease (Ulceration, Arthropathy) Cataract, Retinopathy, (Impaired vision) Nephropathy (Renal failure) Autonomic Neuropathy (Gastrointestinal problems, Postural hypotension)
Macrovascular
Peripheral circulation (Claudication, Ischaemia) Coronary circulation (Myocardial infarction) Cerebral circulation (TIA, Stroke)

Table: 2. Complications of diabetes (Colledge et al.,2021)

DIAGNOSTIC CRITERIA OF DIABETES

FPG greater than or equal to 126mg/dL (7.0 mmol/L). Eight hours fasting without calorie intake
Or
2-h PG greater than or equal to 200 mg/dL (11.1 mmol/L) during the OGTT. 75 g of anhydrous glucose that is dissolved in water should be used to conduct the test as instructed by the WHO.
Or
HbA1c greater than or equal to 6.5% (48 mmol/mol) The test should be carried out with a glucose load that is equal to 75 g of anhydrous glucose dissolved in water as instructed by the WHO
Or
A random plasma glucose level of 200 mg/dL (11.1 mmol/L) was measured in a patient with classic hyperglycemia or hyperglycemic crisis.

Table: 3. Diagnostic criteria of diabetes (American Diabetes Association, 2022)

DCCT stands for "Diabetes Control and Complications Trial"; FPG stands for "Fasting Plasma Glucose"; OGTT stands for "Oral Glucose Tolerance Test"; WHO stands for "World Health Organization", and (2hr- PG) stands for "2-hour plasma glucose".

CREATINE KINASE

Creatine kinase is a compact enzyme having a molecular weight of 82-kDa enzyme present in the mitochondria and cytosol of tissues that required high energy. Creatine kinase is made of 2 polypeptide subunits each about 42 kDa present in the cytosol, which includes two distinguished forms of subunits found: M and B. And these subunits form 3 specific tissue isoenzymes: which include (CK-MM) skeletal muscle, (CK-MB) the cardiac muscle, and (CK-BB) the brain. This ratio of subunits differs according to muscle type: the skeletal muscle (98 % MM) and (2 % MB), the cardiac muscle (70-80 % MM) and (20-30% MB), and the brain has mostly BB. (Baird et al., 2012)

Creatine Kinase catalyzes the reversible phosphorylation of creatine to phosphocreatine and ADP (Adenosine tri-phosphate) to ATP (Adenosine diphosphate), and as such, can be essential for the regenerative process of cellular ATP.:

$$\text{Phosphocreatine (PCr)} + MgADP^{-} + H^{+} \longrightarrow MgATP^{2-} + \text{creatine}$$

Phosphocreatine (PCr) circuit, which is an energy network, is composed primarily of CK. The cytosol isoenzymes in this circuit are strongly connected to glycolysis and the production of ATP for muscle activity. (Baird et al., 2012)

CK is a cytosolic compound that helps mitochondria to exchange phosphate through the cytoplasm as a source of high energy. It has a form of three isoenzymes CK-MM i.e., found in muscles and are considered as one of the muscle damage biomarkers that are released in the blood if muscle cells get damaged in diabetic patients, the dimension of creatine kinase will increase due to a rise in the isoenzyme and the source of this expansion is destruction in skeletal muscle caused by a decrease in vitality. Other isoenzymes, such as CK-MB (myocardial cell) and CK-BB (cerebrum), are common. The main cause of elevated CK in diabetics is skeletal muscle damage, specifically CK-MM isoenzyme, with no cardiac problems. (Mohamed et al., 2019)

CREATINE KINASE AND TYPE 2 DIABETES MELLITUS

The skeletal muscle cells are affected by insulin deficiency or resistance, so a decline in glucose consumption reduces the amount of energy required by the muscle and causes muscle damage. (Mohamed et al., 2019) Insulin resistance (IR) in skeletal muscle is the major hallmark of T2DM, and it is thought to be largely explained by decreased non-oxidative glucose metabolism. Furthermore, insulin stimulation for glucose oxidation and suppression of lipid oxidation is significantly impaired in type 2 diabetes. (Awadalla et al.,2010)

In diabetes mellitus, the alteration in glucose, lipid, and protein metabolism, is especially evident in muscle cells (myocytes). Because of the disease, glucose utilization is reduced, phosphorylation of glucose is altered, glycogen synthesis is reduced, and glycolysis is suppressed. Because glycolysis has a lower capacity, oxaloacetate and pyruvate concentrations are reduced. (Jevrić-Causević et al.,2006) It may be due to many factors, including a defect in the Krebs cycle, which leads to an increase in proteolysis in diabetics. Defects of this cycle, as well as other metabolic pathways such as the respiratory chain, affect the morphology and function of mitochondria, resulting in a reduction in ATP production in skeletal muscle cells. (Mohamed et al., 2019)

The skeletal muscle of patients with Type II diabetes mellitus has smaller mitochondria than usual, limiting their capacity to produce energy. Low ATP can cause a decrease in creatine phosphate (CPK) synthesis, which may result in the activation of (AMP-activated protein kinase) as a delay complication. A higher risk of muscle atrophy can be seen in diabetes patients. (Mohamed et al., 2019)

The previous study done by Adlija et al. found that cases with T1DM and T2DM had elevated in the activity of total CK activity. (Čaušević et al.,2006) It was also approved by Akram et al., who state that the level of CK activity is high in patients with

type 2 diabetes cases. It was assumed that in this kind of diabetes, these alterations were represented in enzyme activity of creatine kinase, and an increase in activity was seen in type 2 diabetes cases (Awadalla et al.,2010)

LACTATE DEHYDROGENASE

Lactate Dehydrogenase is an enzyme having a molecular weight of 134 kDa and made up of 4 peptide chains of 2 forms: M (or A) and H (or B), which are genetically controlled separately. (Burtis et al.,2016) Lactate Dehydrogenase is present in almost body tissues and organ systems; however, serum LDH activity is rising in various diseases. (Klein et al., 2020) Lactate is produced by almost all tissues in the human body, with muscles producing the most. Lactate is eliminated by the liver and, to a smaller extent, whereas the kidneys are under normal conditions. (Romero et al.2016)

Increased activity of LDH in body fluid and blood may help to demonstrate injury or disease. The rate of LDH activity in insulin-secreting cells is an essential regulator of physiological insulin production. LDH activity overexpression disrupts regular glucose metabolism and insulin production in the islet β-cell. It might be directly responsible for type 2 diabetes insulin secretion cause. (P Singh et al., 2022)

LACTATE DEHYDROGENASE AND TYPE 2 DIABETES MELLITUS

Lactate (2-hydroxypropanoic acid), previously thought to be a waste product of glycolysis, is now being recognized as an important factor in insulin resistance (IR), diabetes, cancer growth, development, and spread. Lactate is metabolized by various types of tissues and cells, including the hepatocytes, reproductive cells, and neurons, and converted to pyruvate via LDH and then to glycogen or carbon dioxide. Lactate is eliminated by the liver and kidneys under normal physiological conditions. Lactate, in general, can be transported to the liver and converted into glucose via the Cori cycle and used as a source of energy. (Wu et al., 2016)

The mechanisms leading to diabetes-related hyperlactatemia include significant modifications in the intracellular glucose metabolism in insulin-sensitive tissues, such as reduced glycogen synthesis, impaired glucose oxidative metabolism, and elevated whole-body nonoxidative glycolysis rates. Insulin resistance is essential for the pathophysiology of type II diabetes mellitus which serves as an early indicator of the condition. (Wu et al., 2016)

Elevated levels of insulin enhance glycolysis in insulin-resistant individuals by activating two rate-limiting enzymes, phosphofructokinase and pyruvate dehydrogenase. As a result, increased glycolysis activity can be seen in patients with insulin resistance/diabetes. Increased glycolysis leads to increased NADH and pyruvate formation and decreased NAD+ levels. In a redox

reaction, LDH converts pyruvate to lactate while also producing NAD+ from NADH. This response may be exacerbated by insulin resistance because hyperinsulinemia causes increased glycolysis. (Wu et al., 2016)

In the previous research done by Johari et al., LDH activity did not change significantly in different groups as compared with control and diabetes subjects. (Johari et al,2018) In Huang's study, significantly increased activity of LDH activity was seen in subjects with diabetes compared with healthy subjects. (Huang et al.,2006)

RESULTS

The difference in the activity of serum creatinine kinase was not statistically significant (p= 0.3) between cases (35.72±16.82) as compared to controls (39.63±22.11) shown in Table: 3 & Fig: 1

The activity of serum lactate dehydrogenase was significantly low (p= 0.01) in cases (278.65±46.89) as compared to controls (316.48±71.18) shown in Table: 4 & Fig: 2

Body Mass Index did not show statistically significant difference (p= 0.7) between cases (22.96±2.39) and controls (22.69±3.87) shown in Table: 6 & Fig: 3

DISCUSSION

Millions of people suffering from Type II diabetes mellitus continue to have poor health. One of the main pathogenic factors causing the condition is the body's resistance to the action of insulin in lowering blood sugar levels (insulin resistance). The principal issue for preserving glucose homeostasis is skeletal muscle, which does so by up taking glucose through both insulin-dependent and -independent processes. (Hulett et al., 2022)

In our study, we evaluated the status of serum creatine kinase activity in Type II diabetes mellitus patients. This study showed a decrease in serum activity of CK enzyme (35.72±16.82) in cases as compared with the control group which was (39.63±22.11). This result shows the activity of serum creatine kinase was not significant between cases as compared to controls. There is no precise evidence, in the literature corresponding to the relationship between serum creatine kinase and Newly diagnosed Type II diabetes mellitus. However, limitation related to the number of examined patients points out the fact that further well-designed investigations are needed to give definite answers related to the possible mechanism of these changes.

Santos et al. suggested that skeletal muscle type 2 diabetes patients have smaller mitochondria as compared to normal individuals, making energy production difficult. Low ATP can cause a decline in creatine phosphate, which can later activate the (AMP-activated protein kinase). Diabetics are more likely to experience muscular atrophy and alteration.

Several studies considered creatine kinase as a biomarker for the risk factor of cardiovascular problems in diabetes patients. Evliyaoğlu et al. reported that activity of the serum creatine kinase was significantly correlated with patients' glucose control who had type 2 diabetes, as compared with healthy controls. An elevated level of CK-MB/ CK activity was significantly high in diabetes patients who had uncontrolled glucose levels. The results support that both the microvascular and macrovascular complications of diabetes may involve cardiovascular disease which may result in death and morbidity.

Mohamed et al., have reported that serum creatine kinase level was a rise in diabetes mellitus patients either with cases of type 1 or type 2 in comparison to control groups. This finding is in support of the previous research done by Adlija et al., who found that T1DM and T2DM patients had elevated levels of serum creatine kinase. It was also proved by Akram et al., who concluded the level of serum CK activity was elevated in T2DM cases.

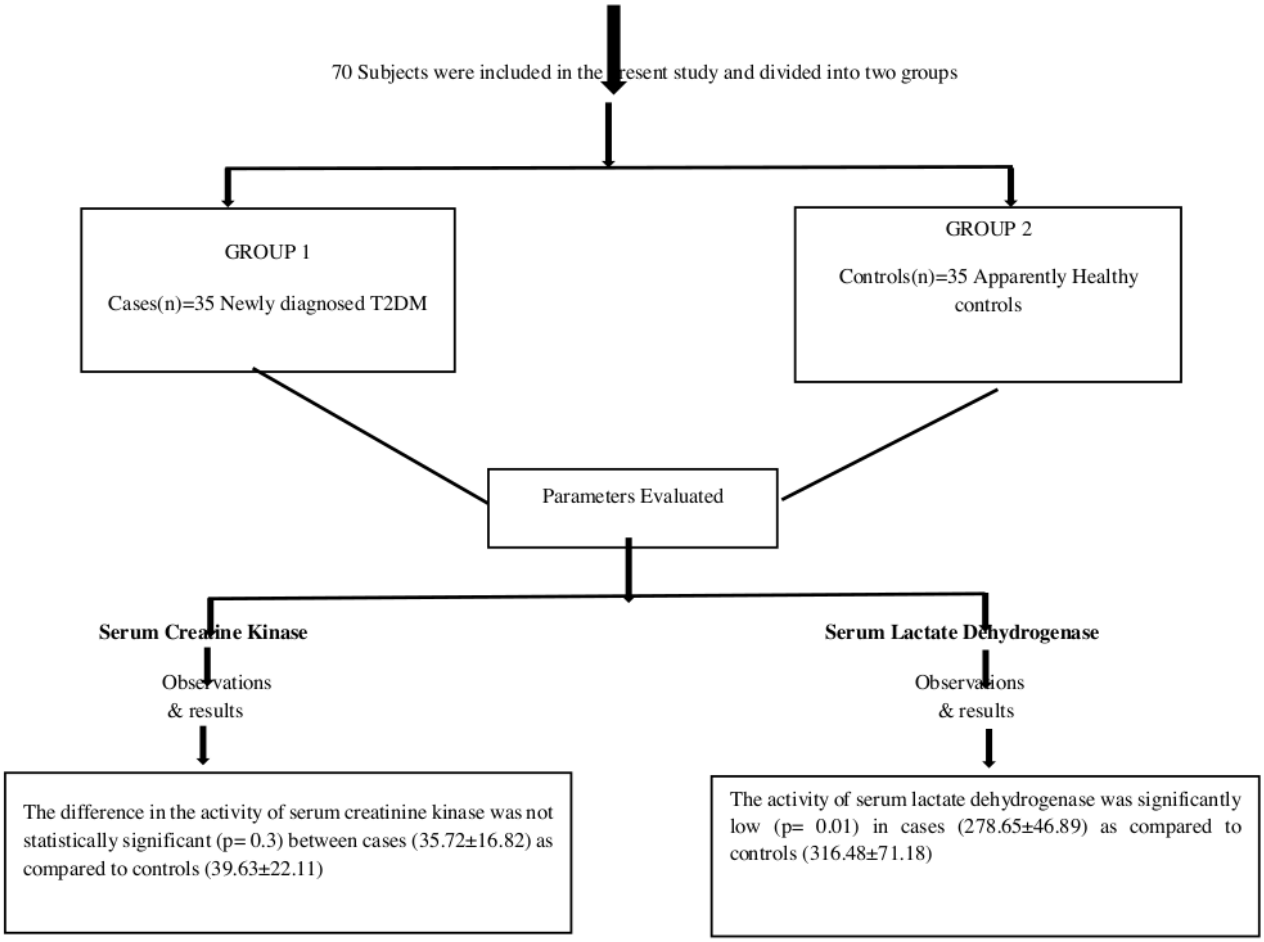
In our study, we observed that Type II Diabetes Mellitus patients had significantly decreased levels of serum lactate dehydrogenase activity as compared to normal individuals. Lactate dehydrogenase is an enzyme of the anaerobic metabolic pathway. (Johari et al, 2018)

Oliver et al., did not observe any rise in the levels of serum LDH between diabetes mellitus and healthy controls. These findings are in agreement therewith Kamble et al., that mild diabetes mellitus does not affect LDH, but raised levels of LDH were highly significant in severe diabetes mellitus cases.

In Huang et al., study, LDH activity was increased among the cases of type 2 diabetes mellitus as compared to normal subjects. Sreenivasan et al. found elevated LDH activity in Type 2 diabetic Mellitus patients as compared to healthy subjects; the elevated enzyme activity seen in diabetes has also been linked to the influence of insulin on liver and muscle tissues. Because muscle and liver damage are frequently associated with diabetes, serum enzyme activities generated from muscle and liver may also contribute to elevated LDH activity in diabetics.

SUMMARY

The purpose of this study determines serum creatine kinase and serum lactate dehydrogenase in diagnosed cases of Type 2 Diabetes Mellitus & apparently healthy controls.



*Serum Lactate Dehydrogenase activity was significantly low in cases as compared to controls.

*Serum Creatine Kinase and Serum Lactate Dehydrogenase are positively correlated and the correlation is statistically significant.

CONCLUSION

Diabetes is described as a group of disorders characterized by hyperglycemia. The present study found that the difference in the activity of serum creatine kinase between cases and controls was not statistically significant, whereas, serum lactate dehydrogenase activity was significantly decreased in cases with T2DM as a comparison to apparently healthy individuals.

Previous research has suggested that an increase in glucose level could damage liver cells and skeletal muscle which causes variation in the creatine kinase and lactate dehydrogenase activity in diabetes mellitus patients.

This study's findings may be helpful in understanding the role of serum lactate dehydrogenase activity in type 2 diabetes mellitus patients related to its pathogenesis. Further investigations are needed in order to confirm the current findings in larger a larger sample size.

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