A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

EVALUATION OF LDH ENZYME ACTIVITY AND SOME BIOCHEMICAL VARIABLES IN THE BLOOD OF PATIENTS WITH KIDNEY DISEASE IN KIRKUK

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

BY

SAMEER SALIH MOHAMMED MOHAMMED

ÇANKIRI

2022

EVALUATION OF LDH ENZYME ACTIVITY AND SOME BIOCHEMICAL VARIABLES IN THE BLOOD OF PATIENTS WITH KIDNEY DISEASE IN KIRKUK

By Sameer Salih Mohammed MOHAMMED

November 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor **:** Prof. Dr. Volkan EYÜPOĞLU

Examining Committee Members:

Chairman **:** Assoc. Prof. Dr. Şevki ADEM
Chemistry
Çankırı Karatekin University

Member **:** Asst. Prof. Dr. Ümit YIRTICI
Medical Laboratory
Kırıkkale University

Member **:** Prof. Dr. Volkan EYÜPOĞLU
Chemistry
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Sameer Salih Mohammed MOHAMMED

ABSTRACT

EVALUATION OF LDH ENZYME ACTIVITY AND SOME BIOCHEMICAL VARIABLES IN THE BLOOD OF PATIENTS WITH KIDNEY DISEASE IN KIRKUK

Sameer Salih Mohammed MOHAMMED

Master of Science in Chemistry

Advisor: Assoc. Prof. Dr. Volkan EYÜPOĞLU

November 2022

The aim of the thesis: Detection of LDH enzyme activity in the blood of patients with kidney disease compared to healthy people. Ninety samples of kidney patients were selected for the age group from 25 to 55 years and were divided into three groups. The first group consisted of 30 samples of patients ranging in age from 25 to 35 years, the second of 30 samples of the injured are for the age group from 35 to 55 years, and the third group: of 30 samples of healthy people. The results of the study indicated the effect of age directly on kidney patients, and that the success rate of kidney transplantation in the Middle Ages was greater than that of adults. Urea and creatinine are diagnostic chemical tests for kidney patients. Lactate dehydrogenase enzyme had an active role and its levels were significantly affected when compared to the control group, more studies are needed on the role of lactate dehydrogenase in the development of kidney disease. Total fat had important indicators with statistical significance. It could be a sign of fat accumulation and thus increase pressure on the kidneys in filtering rates and thus result in kidney failure. Liver enzymes and calcium did not have a clear effect, although there were some statistical differences. When conducting Pearson's test to find out the association between LDH enzyme with blood urea, creatinine, HDL, GOT, GPTK, and Cathe value of $r = 0.123$ at $P = 0.249$.

2022, 49 pages

Keywords: LDH, Kidney disease, Urea, Creatine, Heart attack

ÖZET

KERKÜK'TE BÖBREK HASTALIĞI OLAN HASTALARIN KANINDAKİ LDH ENZİM AKTİVİTESİ VE BAZI BİYOKİMYASAL DEĞİŞKENLERİN DEĞERLENDİRİLMESİ

Sameer Salih Mohammed MOHAMMED

Kimya, Yüksek Lisans

Tez Danışmanı: Prof. Dr. Volkan EYÜPOĞLU

Kasım 2022

Tezin amacı: Sağlıklı insanlara kıyasla böbrek hastalığı olan hastaların kanındaki LDH enzim aktivitesinin saptanması. 25 ila 55 yaş grubu için doksan böbrek hastası örneği seçildi ve üç gruba ayrılmıştır. Birinci grup, yaşları 25 ile 35 arasında değişen 30 hasta örneğinden, ikinci grup 35 ile 55 yaş arasındaki 30 yaralı örneğinden ve üçüncü grup: 30 sağlıklı insandan oluşuyormuştur. Çalışmanın sonuçları, yaşın doğrudan böbrek hastaları üzerindeki etkisini ve Orta Çağ'da böbrek nakli başarı oranının yetişkinlerden daha fazla olduğunu göstermiştir. Üre ve kreatinin, böbrek hastaları için tanısal kimyasal testlerdir. Laktat dehidrogenaz enziminin aktif rol oynadığı ve düzeylerinin kontrol grubuna göre anlamlı düzeyde etkilendiği, laktat dehidrogenazın böbrek hastalığının gelişimindeki rolüne ilişkin daha fazla çalışmaya ihtiyaç vardır. Toplam yağın istatistiksel anlamlılığı olan önemli göstergeleri varmıştır. Yağ birikiminin bir işareti olabilir ve bu nedenle filtreleme oranlarında böbrekler üzerindeki baskıyı artırabilir ve dolayısıyla böbrek yetmezliğine neden olabilmektedir. Karaciğer enzimleri ve kalsiyum bazı istatistiksel farklılıklar olmasına rağmen net bir etkiye sahip değildi. LDH enzimi ile kan üre, kreatinin, HDL, GOT, GPTK ve Cathe değeri arasındaki ilişkiyi bulmak için Pearson testi yapılırken, P = 0,249'da r = 0,123.

2022, 49 sayfa

Anahtar Kelimeler: LDH, Böbrek hastalığı, Üre, Kreatin, Kalp krizi

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Assoc. Prof. Dr. Volkan EYÜPOĞLU, for his patience, guidance and understanding.

Sameer Salih Mohammed MOHAMMED

Çankırı-2022

CONTENTS

LIST OF SYMBOLS

-	Minus
%	Percent
**	Significant
/	Divide
+	Plus
<	Greater than
=	Equal
>	Less than
±	Plus – minus
≤	Greater or equal to
≥	Less or equal to
Ca	Calcium
dL	Deciliter
g	Gram
gm	Gram
kg	Kilogram
L	Liter
m^2	Square meter
µg	Microgram
mg	Milligram
mIU	Milli-international units
min	Minute
mL	Milliliter
mmol	Millimole
mol	Mole
ng	Nanogram
nm	Nanometer
NS	Non-significant
rpm	Revolutions per minute
µL	Microliter

LIST OF ABBREVIATIONS

LDH	Lactic acid dehydrogenase
CKD	Chronic kidney disease
AKI	Acute kidney injury
CT	Computed tomography
ACR	Albumin-to-creatinine ratio

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

LDH is a cytoplasmic enzyme that may be discovered in almost every tissue; however, it is discovered in especially large concentrations in skeletal muscle, liver, and kidney. These enzymes can also be found in red blood cells, but in very lower concentrations. There are five distinct isomeric forms of LDH, and they may be found arranged in tetramers of either of the two types of subunits, which are referred to respectively as muscle or heart. These tetramers can be found in the body. Isoforms LDH-1 through LDH-5, which are also known as isozymes, have been given the designations LDH-1 through LDH-5. Each variant exhibits its gene in a distinct manner depending on whatever organ it is found in them (Zhang *et al.* 2017). It's possible that the H and M subunits that make up the LDH enzyme have a different composition in certain tissues than they do in others, but this only happens in certain tissues (this was covered in the "cellular" section above). This difference is due to the fact that the tissues have differing metabolic rates, energy needs, and roles, all of which are reflected in the ratio of LDHA to LDHB that they possess. LDHA is a long-chain omega-3 fatty acid, while LDHB is a short-chain omega-3 fatty acid The release of around forty percent of the lactate that is present in circulation is attributable to the skeletal muscle tissue (Miyamoto *et al.* 2018). This lactate is absorbed further, mostly by the liver and kidneys, where it then undergoes the process of oxidation in order to make glucose. Glucose is the end product of this process. In conditions of rest, about 10 percent of the lactate is oxidized in the brain to feed 8 percent of the cerebral energy needs, and the remaining lactate is released into circulation. This occurs when the brain is in a state of relative inactivity. When these circumstances are present, the brain's needs for energy are satisfied. On the other hand, hyperlactatemia and physical exercise may both lead to the intake of lactate. Although lactate is required for the upkeep of sixty percent of the metabolic activities that occur in the brain, cerebral lactate oxidation only contributes up to thirty-three percent of the total (Yang *et al.* 2017).

1.1 Aim of Study

The purpose of the study is to determine whether or not there is a difference in the levels of LDH enzyme activity found in the blood of healthy individuals as opposed to those who have renal illness. In this study, there will be a selection at random of 90 samples from patients with renal illness who are between the ages of 25 and 55, and then these samples will be divided into three groups.

2. LITERATURE REVIEW

Lactic acid dehydrogenase (LDH) is a cytoplasmic enzyme that may be found in practically every tissue, although it is found in particularly high quantities in skeletal muscle, liver, and kidney. These enzymes are also present in red blood cells, but at much lower numbers. There are five different isomeric forms of LDH, and they may be found organized in tetramers of either of the two kinds of subunits, which are designated as muscle or heart. LDH-1 through LDH-5 are the names given to the isoforms that are also termed isozymes. Each isoform has a unique expression pattern in the various organs (Mazzio *et al.* 2021).

The significance of LDH as a clinical diagnostic marker may be traced back to its ability to present itself in numerous ways. Isozyme LDH-1 is the primary isozyme that may be found in the tissue of the heart, and it consists of four heart subunits. Isozyme LDH-2 is the primary isozyme found in red blood cells and the reticuloendothelial system. It is composed of three heart subunits and one muscle subunit (3H1M). The LDH-3 isozyme is the primary isozyme found in the lungs. It is made up of two heart and two muscle subunits and has a molecular formula of 2H2M. Isozyme LDH-4 is the principal isozyme that may be found in the kidneys. It is composed of one heart subunit and three muscle subunits (1H3M). Although these five isoforms catalyze the same process in its entirety, they are distinguishable from one another due to differences in their affinity for the substrate and electrophoretic mobility. LDH zymography allows for the visualization of these five isoforms when they are in their active condition (Jung *et al.* 2019).

In different tissues, the H and M subunits that make up the LDH enzyme might have a different composition than in other tissues (this was covered in the "cellular" section above). This disparity is because the tissues have varying metabolic rates, energy requirements, and functions, all of which are reflected in the ratio of LDHA to LDHB that they contain. Skeletal muscle is responsible for the release of around 40 percent of the lactate that is found in circulation. This lactate is absorbed further, mostly by the liver and kidneys, where it then goes through the process of oxidation in order to produce glucose. During circumstances of rest, about 10 percent of the lactate is oxidized in the

brain to feed 8 percent of the cerebral energy demands, and the remaining lactate is discharged into circulation. During these conditions, the brain's energy requirements are met. On the other hand, hyperlactatemia and physical activity may both lead to the intake of lactate, which is necessary for the maintenance of sixty percent of the brain's metabolic processes, but cerebral lactate oxidation only contributes up to thirty-three percent (Vargas *et al.* 2016).

Quantification of LDH is of clinical importance because the tissue-specific pathological states that are reflected in a serum concentration of LDH (Figure 2.1) isozymes may be determined by LDH quantification. Because of the widespread prevalence of LDH and the isozyme form that it may take, it can be used as a marker for a wide variety of tissue damage. In the event that there is injury to the tissue, the cells will release LDH into the circulation. There is a possibility that the higher enzyme levels may continue to be present in the circulation for up to seven days, although this will depend on the sort of tissue damage. The substantial amount of cell death that happens as a consequence of organ damage causes a loss of cytoplasm, which leads to an increased level of LDH in the blood (Mazzoli *et al.* 2020).

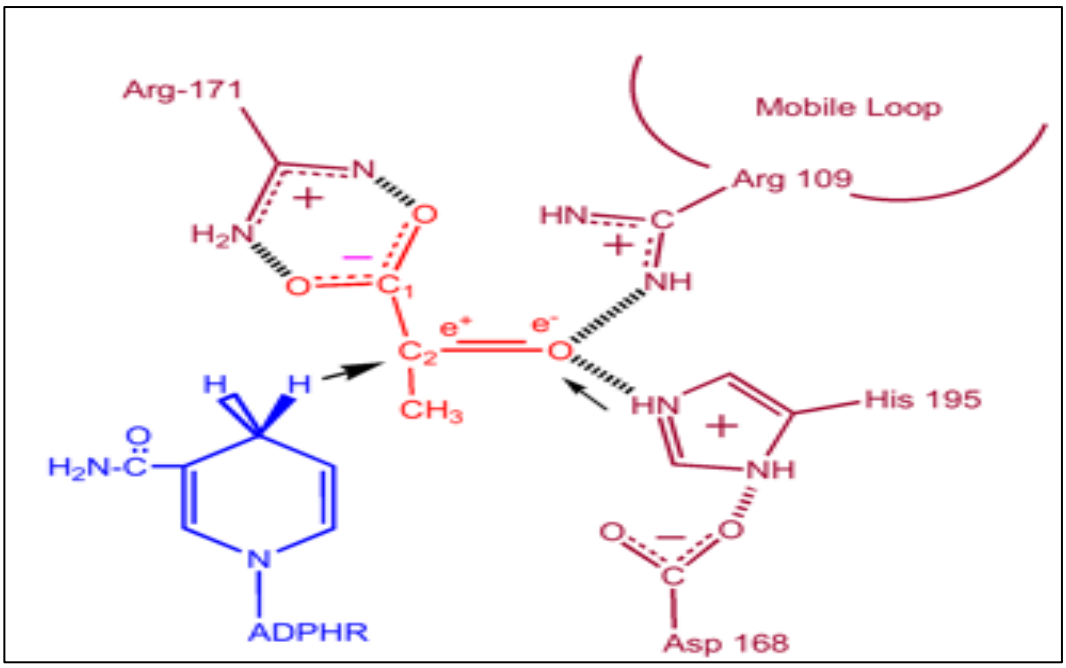

Figure 2.1 LDH structure (Juturu and Wu 2016)

Detection of LDH enzyme activity in the blood of patients with renal disease as compared to healthy persons is the objective of the research presented in this thesis. In which, there will be a random selection of 90 samples from persons with renal disease in the age range of 25 to 55 years old, and these samples will be split up into three groups. The first group included thirty samples from healthy individuals, the second group included thirty samples from infected individuals in the age range of 25 to 35 years old, and the third group included thirty samples from infected individuals in the age range of 35 to 55 years old (Ozaki *et al.* 2017).

The enzyme is found in a wide range of creatures, some of which are plants and others of which are mammals. It is found in every tissue and plays an essential role as a checkpoint in the processes of gluconeogenesis and DNA metabolism. An examination of LDH across all species reveals that its structure has been remarkably maintained, with just a few of differences occurring in the sequence of amino acids. Because of the structural similarities, which can be altered by changing just a few amino acids, it is possible to build functional compounds that can modify the catalytic potential and expression of the enzyme using this platform. The biochemical function, the testing methodologies, and the therapeutic significance of it will be the primary focuses of this part (Jia *et al.* 2018).

2.1 Lactic Acid Dehydrogenase

In general, LDH is an enzyme that is present in the majority of the tissues in the body. It is an essential component in the process known as cellular respiration, which is the transformation of glucose (sugar) derived from meals into energy. Even while LDH is present at high amounts in the cells of tissues, typical blood levels of the enzyme are very modest. On the other hand, increased levels of LDH are released into the circulation by tissues that have been injured by either an injury or a disease (Mazzoli *et al.* 2020).

Even though an LDH test is helpful in making a diagnosis of tissue damage, other tests are often required in order to locate the specific area where the damage has occurred. The LDH isoenzymes test is an example of this kind of examination. There are five distinct forms of the LDH enzyme, which are known as LDH isoenzymes. These isoenzymes are

located in various organs and tissues at varying quantities. By determining the quantities of these isoenzymes in the patient's blood, medical professionals are able to get a better understanding of the kind, location, and extent of the cellular damage. The liver, the heart, the pancreas, the kidneys, the brain, and the blood cells all contain LDH. Muscles, the pancreas, the liver, the kidneys, and the brain also contain LDH (Ozaki *et al.* 2017).

The LDH test's primary purpose is to assist in determining the extent of damaged tissue in the body as well as its location throughout the body. This test determines the amount of it, which is sometimes referred to as lactic acid dehydrogenase, that is present in the blood or, on occasion, in other bodily fluids. An enzyme is a kind of protein, and LDH is one of such enzymes. The production of energy in the body is significantly influenced by LDH. It is present in practically all of the tissues in the body, including those in the blood, kidneys, brain, and lungs, among others (Jia *et al.* 2018).

Damage to these tissues causes a release of the enzyme LDH into the circulation or other bodily fluids. If the amount of LDH in the blood or other fluids is high, it is possible that some tissues in the body have been destroyed as a result of illness or injury. This enzyme is released into the fluid component of the blood whenever there is a disruption or destruction of cellular structure. The medical community refers to this as "serum" or "plasma" (Gallo *et al.* 2015).

2.1.1 Cellular

There are five different isomeric forms of LDH, and they may be found organized in tetramers of either of the two kinds of subunits, which are designated as muscle (M) or heart (H). LDH-1 through LDH-5 are the names given to the isoforms that are also termed isozymes. Each isoform has a unique expression pattern in the various organs. The significance of LDH as a clinical diagnostic marker may be traced back to its ability to present itself in numerous ways. Isozyme LDH-1 is the primary isozyme that may be found in the tissue of the heart, and it consists of four heart subunits (4H). The LDH-3 isozyme is the primary isozyme found in the lungs. It is made up of two heart and two muscle subunits and has a molecular formula of 2H2M. Isozyme LDH-4 is the principal

isozyme that may be found in the kidneys. Although these five isoforms catalyze the same process in its entirety, they are distinguishable from one another due to differences in their affinity for the substrate, inhibitory concentration, isoelectric point, and electrophoretic mobility. LDH zymography allows for the visualization of these five isoforms when they are in their active condition (Vlachostergios *et al.* 2015).

Despite the fact that LDH is located mostly in the cytoplasm, evidence of its existence in mitochondria has been uncovered by a number of investigations. It has been shown that yeast, plants, and mammals all have some kind of mitochondrial L-lactate dehydrogenase (mL-LDH). Following that, mL-LDH acts as a catalyst in the mitochondrial matrix to speed up the process of converting L-lactate to pyruvate. In order to satisfy their increased need for energy, many cancer cells have reprogrammed the mitochondrial mechanisms. In cancer cells, glycolysis levels rise to abnormally high levels; as a result, mL-LDH may be a factor in the increased rate of oxidative phosphorylation (Sauer *et al.* 2017).

2.1.2 Molecular

The genes LDHA, LDHB, LDHC, and LDHD are all responsible for encoding LDH. The L-isomers of the enzyme are encoded by the LDHA, LDHB, and LDHC genes, whereas the D-isomer is encoded by the LDHD gene. The L-isomers are responsible for both the consumption of and production of L-lactate, which is the predominant enantiomeric form of lactate found in vertebrates. The LDHA version of the enzyme is encoded by a gene that can be found on chromosome 11p15.4. This gene is translated into a protein that has 332 amino acids. The LDHB gene, which can be found on chromosome 12p12.1, is responsible for the production of a protein that is 334 amino acids long. Both the LDHA gene and the LDHB gene are responsible for the production of the isozyme forms of lactate dehydrogenase enzymes, which range from LDH-1 through LDH-5. The lactate dehydrogenase enzyme is composed of three subunits: lactate dehydrogenase-A, lactate dehydrogenase-B, and lactate dehydrogenase-C. The two genes supply the instructions for creating these three subunits. There are five distinct types of LDH, and each one is composed of a total of four subunits (Sabel *et al.* 2017).

To complete the formation of an LDH tetramer in mammalian cells, an additional two subunits, designated LDHC and LDHBx, are required. While the LDHC gene is responsible for encoding the LDHC protein, which is unique to the testes, the LDHBx gene is responsible for encoding the LDHBx protein, which is unique to the peroxisome. The readthrough version of the LDHB gene is denoted by the symbol LDHBx. In order to produce LDHBx, the LDHB mRNA must first be translated, at which point the stop codon must be interpreted as encoding an amino acid. As a consequence of this, translation continues until it reaches the subsequent stop codon, which appends seven amino acid residues to the regular LDH-H protein. These residues encode the peroxisomal targeting signal, which enables LDHBx to be transported into the peroxisome (Liaud *et al.* 2015).

Both of the distinct subunits that make up LDH, known as the M subunit and the H subunit of LDH, have the same active site structure as well as the amino acids that are involved in the process. In the tertiary structure, the alanine that is found in the M-chain is switched out for glutamine that is found in the H-chain. These chemical reactions end up giving the two subunits distinct sets of biological characteristics. As a result, the H subunit is capable of binding at a higher rate, although having a catalytic activity that is five times lower than that of the M subunit. Pyruvate is converted into lactate, and NADH is converted into NAD+ by the action of the LDHA subunit, which bears a net charge of -6 and demonstrates a greater affinity for pyruvate (Elshaghabee *et al.* 2016).

When oxygen is scarce, LDH plays a crucial role in the process of keeping homeostasis stable. When doing intense activity, there is a rapid decrease in the amount of oxygen found in the muscular tissues. Because oxygen is often the last electron acceptor in the electron transport chain (ETC), the chain comes to a stop along with ATP synthase when oxygen is depleted (Lee *et al.* 2017). In spite of this, muscle cells are able to maintain their functionality by producing ATP through NAD+. Through a process of fermentation, LDH results in the production of lactic acid as a product. During the procedure, LDH will take electrons from NADH in order to produce NAD+. This NAD+ will then be channeled via the glycolysis route in order to produce ATP. In comparison to the ETC, this pathway results in a lower amount of ATP production; yet it enables the cell to continue carrying

out its physiological and biochemical processes even when oxygen is absent (Papadimitriou *et al.* 2016).

2.1.3 Function

One of the H transfer enzymes, or oxidoreductases, lactate dehydrogenase catalyzes the conversion of pyruvate to lactate in a direction that may be reversed. It does this by making use of NADH. In its most basic form, the enzyme plays a role in the anaerobic metabolism of glucose, which occurs when oxygen is either nonexistent or present in very low concentrations as shown in Equation (2.1) (Tsuge *et al.* 2019).

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \longrightarrow \text{Lactate} + \text{NAD}^+ \qquad (2.1)$$

When cells are subjected to anaerobic or hypoxic circumstances, the process of oxidative phosphorylation, which is responsible for the generation of ATP, gets disturbed. During this procedure, it is necessary for the cells to generate energy via a different metabolism. As a consequence of this, LDH is upregulated under these settings so that it can better meet the need for the creation of energy. On the other hand, the metabolic pathway leads nowhere for the lactate that is generated during the anaerobic conversion of glucose. It is only in the liver that any additional metabolic processes may be carried out on it. As a consequence of this, lactate is expelled into the bloodstream and carried to the liver. In the liver, LDH is responsible for carrying out the Cori cycle's opposite reaction, which converts lactate into pyruvate (Zhang *et al.* 2016).

During physical activity, the lactate dehydrogenase enzyme converts pyruvate into lactic acid by causing the muscles to use up all of the oxygen that is available to them. Pyruvate is not further digested in erythrocytes since there are no mitochondria present. Instead, pyruvate stays in the cytoplasm where it is eventually converted into lactate. During the course of this process, NADH is converted into NAD+. In order to successfully carry out the preliminary phase of glycolysis, it is important to have access to large concentrations of NAD inside the cell. When compared to oxidative phosphorylation, anaerobic

glycolysis produces just 2 molecules of ATP per molecule of glucose, while oxidative phosphorylation generates 36 molecules of ATP per molecule of glucose (Singhvi *et al.* 2017).

In different tissues, the H and M subunits that make up the LDH enzyme might have a different composition than in other tissues (this was covered in the "cellular" section above). This disparity is because the tissues have varying metabolic rates, energy requirements, and functions, all of which are reflected in the ratio of LDHA to LDHB that they contain. Skeletal muscle is responsible for the release of around 40 percent of the lactate that is found in circulation. This lactate is absorbed further, mostly by the liver and kidneys, where it then goes through the process of oxidation in order to produce glucose. During circumstances of rest, about 10 percent of the lactate is oxidized in the brain to feed 8 percent of the cerebral energy demands, and the remaining lactate is discharged into circulation. During these conditions, the brain's energy requirements are met. On the other hand, hyperlactatemia and physical activity may both lead to the intake of lactate, which is necessary for the maintenance of sixty percent of the brain's metabolic processes, but cerebral lactate oxidation only contributes up to thirty-three percent (Blaya *et al.* 2018).

When compared to normal cells, cancer cells have a function of LDH, especially LDHA, that is altered. Normal cells do not have this problem. Even when oxygen is present, cancer cells make use of the enzyme lactate dehydrogenase, or LDH, to speed up their aerobic metabolism and boost glycolysis, ATP synthesis, and lactate production. The name for this kind of process is the Warburg effect. By converting to an anaerobic metabolic phenotype, aberrant cancer cells are able to escape the production of oxidative stress caused by the ETC, which is beneficial to the cells (Zheng *et al.* 2015).

2.1.4 Mechanism

The pace of the process may be enhanced by a factor of types to LDH, which is responsible for catalyzing the synchronized inter-conversion of pyruvate to lactate and NADH to NAD+. If this is done, the rate of the process will be increased. As part of the ongoing chemical process, a hydride ion is being moved from NADH to pyruvate at its C2 carbon. This movement takes place at the same location in both molecules. The binding of NADH to the enzymes is needed to take place as the initial step of the molecular process. This stage is necessary for the process to continue. This binding requires participation from a large number of residues in the active site (Gallo *et al.* 2015). After NADH has been attached, it makes it easier for lactate to attach itself by facilitating a contact between the ring of NADH and the residues of LDH. Transfer of a hydride happens rapidly in both directions, ultimately leading to the formation of two tertiary complexes, which are denoted by the names LDH-NAD+-lactate and LDH-NADH-pyruvate, respectively. After that, pyruvate is first liberated from its connection to the enzyme, followed by NAD+ being freed. The rate at which NADH and NAD+ dissociate turns out to be the rate-limiting step (Pundir *et al.* 2016).

Regulation of enzymes: the transition from aerobic respiration to anaerobic respiration is required for LDH activity to take place. Allosteric modulation, regulation at the substrate level, and transcriptional regulation are the three modes of regulation that may influence LDH in varying degrees. LDH activity is controlled by both the relative availability of substrates and their concentration in the system (Mazzoli *et al.* 2020). During periods of intense muscle activity, when there is a concomitant increase in substrates, the enzyme's activity level rises. The buildup of ADP, AMP, and Pi is brought on by the imbalance between the demand for ATP and the supply of aerobic ATP. Because of this mechanism, the flow of pyruvate and NAD+ is directed via LDH, which then results in the production of lactate and NADH (Rodrigues *et al.* 2017).

High concentrations of ethanol result in the production of high concentrations of lactate and NADH, which in turn leads to the depletion of NAD+ in conditions where the ratio of NADH to NAD+ is elevated, as is typically the case in people who consume alcoholic

beverages. This is because drinking alcohol causes the NADH level to rise. Following the completion of this process, pyruvate is converted into lactate, which is then related to the regeneration of NAD+. Because of this, a high ratio of NADH to NAD+ causes the LDH equilibrium to move toward lactate (Mushegian 2016).

2.1.5 Testing

LDH tests are able to determine the quantity of LDH that is already present in the serum as a result of damaged tissues leaking LDH. As a foundation for the measurement of LDH activity, the catalytic feature of LDH that leads to the reversible oxidation of L-lactate to pyruvate, which is mediated by the hydrogen acceptor, NAD+, is used. In clinical diagnostic labs, the rate of NADH generation that alters the sample's optical density as determined spectrophotometrically at 340 nm is evaluated (Papadimitriou *et al.* 2016). Spectrophotometric analysis allows for the tracking of either the forward process of oxidation of L-lactate to pyruvate or the reverse reaction of pyruvate formation from pyruvate. For the purposes of research, the activity of LDH may be measured in a variety of materials, including plasma, serum, tissue, and cells, as well as in the culture medium. When working with serum and plasma samples, extreme caution is essential since hemolysis, which results in the release of enzymes from ruptured erythrocytes, has the potential to induce an artificial elevation in those levels (Kong *et al.* 2019).

LDH levels should fall somewhere in the range of 140 to 280 U/L to be considered normal. The clinical interpretation, on the other hand, is contingent upon the signs and symptoms shown by the patient. Because LDH is released during the clotting process, the serum will almost always have a greater amount of the enzyme than the plasma would. During intense physical activity, there is also an increase in LDH activity, which helps produce lactic acid under normal physiological settings. It is possible for a medicine or prescription to impact an LDH test, which might therefore prevent an accurate reading from being obtained. It is possible that a falsely low LDH result might be produced by the presence of high amounts of vitamin C (Jung *et al.* 2019). On the other hand, some anesthetics, aspirin, alcohols, opioids, and procainamide might lead to a falsely elevated

level of LDH. There is a possibility that the LDH test will only reveal an abnormally high concentration of one or more kinds of isozymes (Lee *et al.* 2017).

An increase in serum LDH levels may be the result of a number of different medical problems, including cancer, anemia, some infectious illnesses, pancreatitis, liver disease, renal disease, muscular damage, trauma, heart attack, and certain infectious infections. Infants and younger children, on average, have substantially higher normal levels of LDH levels compared to older children and adults. This is because the concentration of LDH fluctuates with age. The usual range for newborns is 135 to 750 U/L (units/L), the normal range for children up to the age of 12 months is 180 to 435 U/L, and the normal range for those older than 18 years old is 122 to 222 U/L (Jackson *et al.* 2016).

In addition to determining the amount of LDH present in samples, LDH isozyme testing provides insight on the kind, location, and extent of tissue injury. The tetrameric enzyme known as LDH is composed of H and M subunits. The assembly of the enzymes takes place in a defined ratio thanks to the tissue-specific synthesis of subunits, which is what provides tissue specificity. For example, the LDH that is specific to the heart (LDH-1) preferentially synthesizes all four H subunits, whereas the LDH that is specific to the liver (LDH-5) is exclusively made of all M-subunits. In a manner analogous, the subunits are synthesized by different tissues in a predetermined ratio (Eiteman and Ramalingam 2015).

The electrophoretic mobility shift is used in LDH isozyme testing to categorize the isozymes into one of five groups, numbered 1 through 5. Because of the unique composition of the subunits, there is a variation in the total charges, which results in a distinct movement when placed in an electric field. In a buffer with a pH of 8.6, an excellent separation pattern of LDH isozymes may be achieved (Yao *et al.* 2019).

2.2 Kidney Disease

The capacity of the body to clean the blood, filter excess water out of the blood, and assist maintain the blood pressure might be negatively impacted when you have a kidney (Figure 2.2) illness. Additionally, it may interfere with the creation of red blood cells and the metabolism of vitamin D, both of which are essential for maintaining bone health (Canaud *et al.* 2015). The kidneys are complex chemical factories that perform many vital functions, including, but not limited to, the following: I elimination of waste products from the body; (ii) elimination of drugs from the body; (iii) maintenance of the body's fluid balance; (iv) regulation of blood pressure; (v) regulation of the production of red blood cells; etc (Pruijm *et al.* 2014). Diseases of the kidneys develop when the organs themselves become damaged to the point that they are unable to function correctly. Risk factors, including diabetes, high blood pressure, and other chronic illnesses, may all contribute to the development of damage, which in turn can lead to health concerns (Fiorentino *et al.* 2018). It is possible for body to accumulate waste materials as well as fluid when the kidneys are impaired. Because of this, it may have swelling in the ankles, nausea, weakness, disturbed sleep, and shortness of breath. In the absence of therapy, the damage may get more severe, and you may ultimately lose the ability to use the kidneys. That is a major issue, and it may even pose a danger to one's life (Hosoya *et al.* 2015).

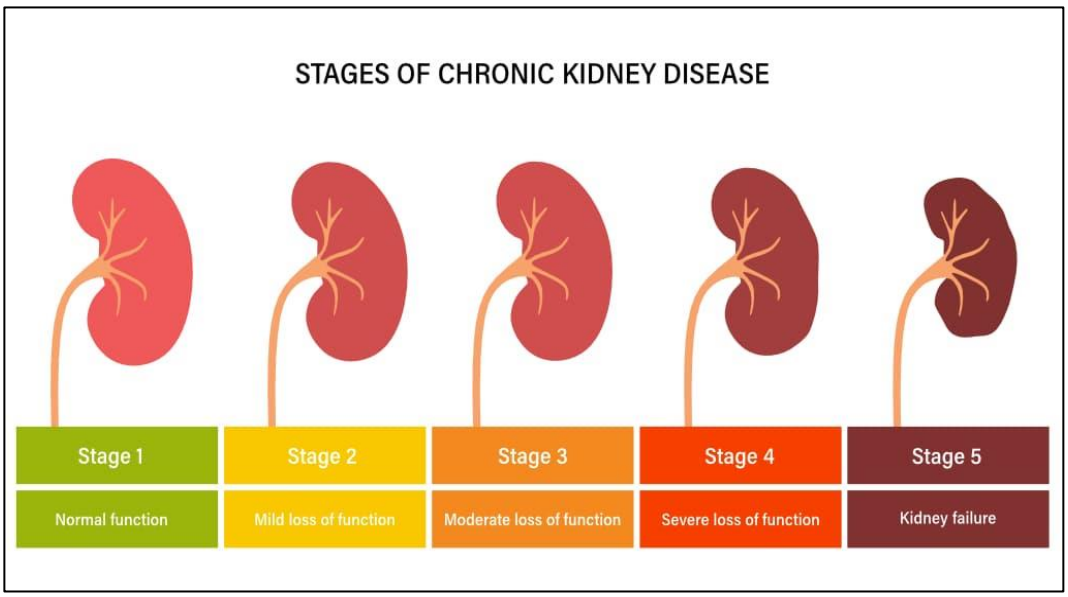

Figure 2.2 Stages of kidney disease (Ostermann *et al.* 2020)

2.2.1 Types

There are a number of disorders that may affect the kidneys, the most common of which are acute kidney injury (AKI), chronic kidney disease (CKD), and kidney failure. Chronic kidney disease (CKD) is a long-term condition in which the kidneys do not function as well as they should (Chawla *et al.* 2014). For instance, if you have AKI, waste products will begin to accumulate in your blood, and it will be difficult for your kidneys to maintain the correct fluid balance in your body as a result of this. The brain, the heart, and the lungs are among of the other organs that might be impacted by AKI. In most cases, the condition is brought on by a reduction in blood flow, direct injury to the kidneys, or obstruction of the urinary system (Hsu *et al.* 2016).

When the kidneys are unable to remove waste products from the blood effectively, a condition known as renal failure may develop. The most common causes of kidney failure include a reduction in the amount of blood that flows to the kidneys, issues with the elimination of urine, infections, glomerulonephritis, scleroderma, hemolytic uremic syndrome, drugs and alcohol, certain medications, chemotherapy medicines, and other causes (Stenvinkel *et al.* 2018).

2.2.2 Diagnosis and treatment

Evaluation of kidney function may be accomplished by a variety of tests; they are used in the diagnostic process for renal illnesses, to begin, the glomerular filtration rate, often known as the GFR, is widely regarded as the most accurate method for measuring kidney function (Rodríguez *et al.* 2018). This is determined by measuring the plasma clearance of a filtration marker in both the blood and the urine. Second, an ultrasound or computed tomography (CT) scan may be used to capture pictures of the kidney, which can then reveal any structural issues or malignancies that may be present in the kidney. In the third step of the diagnostic process, an anatomical change evaluation is performed using a kidney sample to get a conclusive diagnosis (Chacar *et al.* 2020).

When kidneys are injured or inflamed, chemicals like blood or protein may "leak" into the urine. This might be a sign of renal disease. The urine albumin-to-creatinine ratio (urine ACR) test is the most reliable method for determining the presence of protein in the urine. This test displays the total quantity of albumin, which is a form of protein, that is present in the urine sample (Bonventre 2014). If a person has diabetes or high blood pressure, an ACR test on their urine should be performed at least once per year, and if they have any of the additional risk factors listed for developing chronic kidney disease, the test should be performed every two years (Hickson *et al.* 2019). The glomerular filtration rate, often known as the GFR, is the most accurate indicator of kidney health. This rate may be calculated using the results of a blood test that measures creatinine levels (a waste product made by muscle tissue) (Saad *et al.* 2015).

GFR results that are more than 90 mL/min/1.73 m^2 are considered to be normal. A diagnosis of chronic kidney disease is established when a person's GFR is consistently lower than 60 mL/min/1.73 m^2 over a period of at least three months. It is possible to cure some manifestations of renal disease. These therapies are aimed at reducing the severity of the symptoms, preventing the illness from progressing further, and minimizing the risk of consequences. There is a possibility that some of your kidney function may be restored as a result of your therapy. There is currently no treatment available for chronic renal disease (Resztak *et al.* 2021).

What's causing your kidney illness will determine the treatment strategy that you and your physician choose to implement. Even when the underlying cause of your ailment is treated, there is always a possibility that your kidney disease may worsen. You will need therapy for end-stage renal disease once your kidneys reach the point where they are unable to process waste on their own. This may include the following. Dialysis. When your kidneys are no longer able to filter blood, waste and excess fluid are removed from your body via dialysis, there are two different kinds (Guan *et al.* 2014).

Hemodialysis is a process in which a machine cleans your blood by removing waste products and excess fluids. In order to do peritoneal dialysis, a small tube known as a catheter must first be inserted into the patient's belly. After that, a solution that absorbs

waste and fluids is inserted into your abdominal cavity and left there. After some time has passed, the solution will be expelled from your body. A healthy kidney from a donor will be transplanted into your body by a surgeon. This donor could be alive, or they might have already passed away (Seely 2017).

2.3 Urea

The breakdown of proteins into their constituent amino acids results in the production of urea as the primary nitrogenous waste product in the metabolism of all mammals and some fishes. In addition to being found in the urine of all animals, the substance may also be found in their blood, bile, milk, and sweat. Amino groups, represented by the molecule NH2, are stripped away from the amino acids that are a component of proteins during the process of their degradation (Mora-Bau *et al.* 2015). Ammonia (NH3), which is produced when these amino groups are broken down, is harmful to the body and must be eliminated by having the liver convert it to urea. After that, the urea travels to the kidneys, where it is processed further before being expelled in the urine. Urea (Figure 2.3), which is also known as carbamide, is an organic chemical that is risk-free, beneficial, and has a rich history. The breakdown of proteins results in the production of this naturally occurring chemical, which may be detected in high concentrations in the urine of mammals (Patel *et al.* 2017).

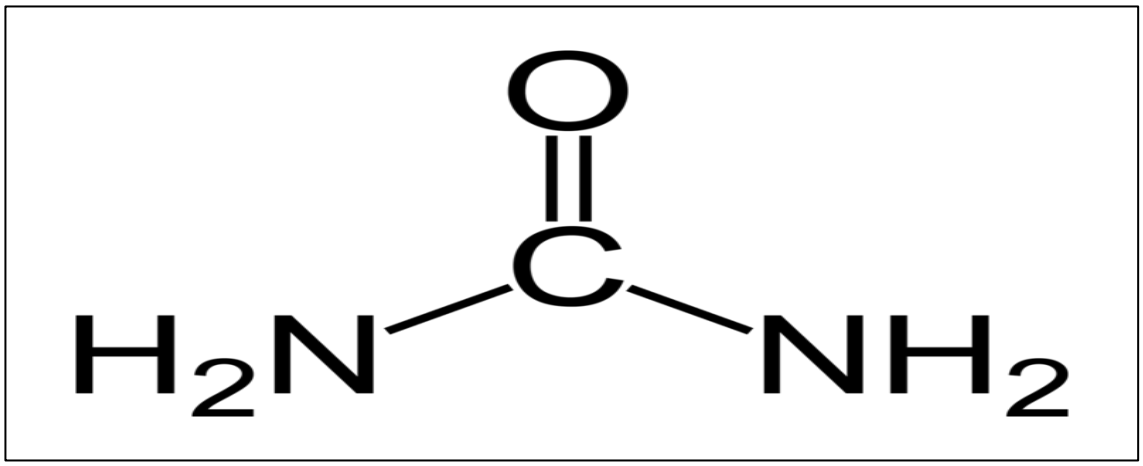

Figure 2.3 Urea structure (Tolulope and Deborah 2015)

The annual manufacturing capacity of urea across the world is around 220 million metric tons. There such a high demand for the urea that is produced, of all industrial chemicals, urea has the largest nitrogen concentration, and therefore, it is in high demand as a fertilizer. Ammonia has the second highest nitrogen content (Okojie and Omorokpe 2018). It breaks down into ammonia (technically known as ammonium ion) and carbon dioxide when it reaches the soil. Ammonium is oxidized by nitrogen-fixing bacteria to produce nitrate, which is then easily taken up by the roots of plants. Urea is particularly useful due to the fact that it can be applied as a solid, in the form of pellets; and its unusually high solubility in water allows it to be incorporated into solutions along with other plant nutrients (Stapleton 2017).

More than ninety percent of the urea that is produced is used in agricultural applications. The remaining twenty million metric tons that are produced each year are used for animal feed (cattle, among other animals, are able to convert it into protein), urea–formaldehyde resins, emollients for skin care, and the production of barbituric acid. In order to take advantage of urea's very low heat of solution in water, instant-cold packs are created (Keren *et al.* 2015). These packs are made of plastic pouches that hold urea and water in their own individual compartments. When the barrier between them is breached, the intermixing of the two generates a cooling effect that provides temporary relief for hurting muscles and joints (Hasan *et al.* 2021).

Even though agriculture makes extensive use of urea, the manufacture of urea in today's world is not in any way "green." The manufacturing of ammonia and urea together account for more than 2 percent of the world's total energy consumption and produce more carbon dioxide than any other industrial operation. At room temperature and pressure, Wang's team devised an electrochemical process that eliminates the need for ammonia production and instead transforms nitrogen gas, carbon dioxide, and water straight into urea. The synthetic path is difficult, and the procedure is neither currently effective nor adequately productive, but the goal is undeniably something that should be pursued since it is important (Gondim *et al.* 2018).

Urea may now be produced on a massive scale in commercial settings using liquid ammonia and liquid carbon dioxide as the starting materials. These two components are brought together in an environment characterized by high pressures and temperatures in order to produce ammonium carbamate, which is then broken down in an environment characterized by much lower pressures to produce urea and water (Saint *et al.* 2016).

It is one of the nitrogenous fertilizers that packs the biggest punch due to the significant amount of nitrogen it contains and the ease with which it may be broken down in the soil to produce ammonia. It is a relatively affordable component that is used in a variety of contexts, including incorporation in mixed fertilizers, application by itself to soil or foliage, and spraying. It produces methylene–urea fertilizers when combined with formaldehyde. These fertilizers release nitrogen gradually, continuously, and consistently, and they allow a whole year's supply to be administered at once. Even though urea nitrogen is not in the form of a protein, ruminant animals (such as cattle and sheep) are able to make use of it, and as a result, their needs for protein may be satisfied to a great extent using this method. Only its use as a fertilizer makes the use of urea in the production of urea-formaldehyde resin more significant than any other. In addition, substantial volumes of urea are required for the production of barbiturates (Sheerin 2015).

The reaction of urea with alcohols results in the formation of urethanes, while the reaction of urea with malonic esters results in barbituric acids. Urea may form crystalline inclusion compounds with certain straight-chain aliphatic hydrocarbons and their derivatives. These compounds are valuable for purifying the chemicals that are contained in the crystal structure (Vigil and Hickling 2016).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Studied groups

The aim of this study is to study the evaluation between lactate dehydrogenase (LDH) and some biochemical variables in patients' blood with kidney disease in Kirkuk city, north of Iraq. Experimental topics divided into three groups (Figure 3.1), group A consist of 30 healthy person (control group), group B consist of 30 patient with age between 25 and 35 years, group C consist of 30 patient with age between 35 to 55 years.

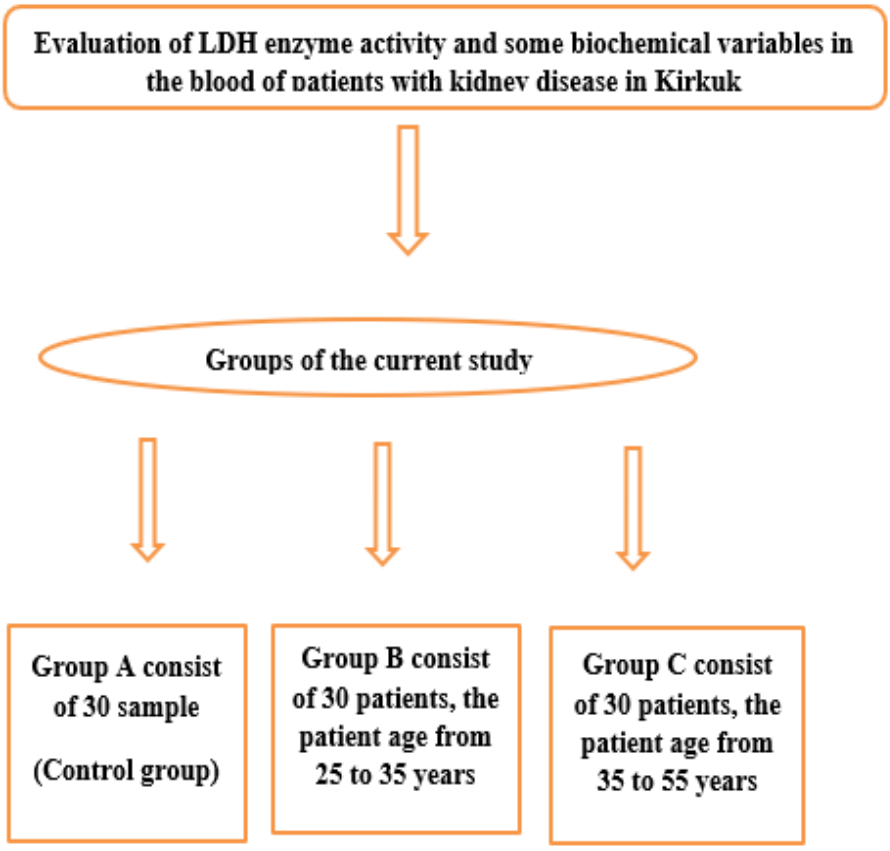

Figure 3.1 Groups of the study

3.1.2 Laboratory devices and other tools

In Table 3.1, it can be seen some of the important devices and other tools which is mainly used in vitro.

Table 3.1 Important materials, devices, and tools of the current study

Devices and tools		**Origins**
1.	Centrifuge	Korea
2.	UV-VIS Spectrophotometer	Apple, USA
3.	Water bath	Memmert , Germany
4.	Incubator	Thelco model 6, Uk
5.	Hot plate	Chemlab, UK
6.	Vortex	Uk
7.	pH meter	Korea
8.	Balance	Sartorius, Germany
9.	Oven	Gallen Hamp, UK
10.	Automatic micropipettes, to deliver 10 to 1000 L, and pipette tips	

3.1.3 Sampling of blood

Blood samples were collected and determine the biochemical measurements for kidney function and tests that include.

3.2 Methods

3.2.1 Assaying of serum GOT and serum GPT

Assaying of serum GOT and GPT were measured by a colorimetric method using commercially available kit (Giesse).

Principle

Aspartate aminotransferase, also known as GOT, is an enzyme that catalyzes the transfer of the amino group from aspartate to oxoglutarate, which results in the synthesis of glutatrate and oxaloacetate. This is shown rather clearly by Equation (3.1).

$$\text{L- aspartate +2-oxoglutarat} \xrightarrow[PH\ 7.4]{GOT} \text{L- Glutamate + Oxaloacetate} \quad (3.1)$$

Equation (3.2), Alanine aminotransferase, also known as GOT, is an enzyme that catalyzes the transfer of the amino group between alanine to oxoglutarate, resulting in the synthesis of glutarate and pyruvate.

$$\text{L- Alanine + 2-oxoglutarate} \xrightarrow[PH\ 7.4]{GOT} \text{L- Glutamate + Oxaloacetate} \quad (3.2)$$

The amount of oxaloacetate or pyruvate that is prepared by the transaminase activity after a predetermined amount of time can be determined through a reaction with 2,4-dinitropheylhydrazane (DNPH) and the measurement of the color that is produced in an alkaline solution. This activity is proportional to the amount of pyruvate that is formed.

-To prepare serum admix 100 µL of GOT with 100 µL of GPT, emplace it in water bath at 37 C for 60 minutes.
-To prepare DNPH admix 0.5 mL of blank with 0.5 mL of GOT and 0.5 mL of GPT. Stand for 20 minutes at 25°C.
-To prepare NaOH 0.4 N add 5 mL of blank to 5 mL of GOT and 5 mL of GPT.
-Mix for 5 minutes at room temperature.
-Read the absorbances (A) of the samples against a water blank.
-The color is stable for at least 60 minutes, read units of GOT or GPT from the curve.

3.2.2 Blood urea test

Procedure

1. Attend the reagents and samples to room temperature.
2. Put the solution into a cuvette.
3. Incubate the solution at 37 °C for 5 minutes.
4. Read the absorbance of the sample and standard at 600 nm against the blank.

The calculations can be done by applying the Equation (3.3)

(A sample /A standard) × C standard = mg/dL urea (3.3)

3.2.3 Creatinine test

1. Append 1000 µL of reagent to 100 µL of the sample and standard and incubate at 37°C.
2. Read the absorbance of the solution at 510 nm after 30 seconds (A1) and also after 90 seconds (A2). The calculations are easy when applying the Equation (3.4).

(A2-A1) sample/ (A2- A1) standard × C standard = mg / dL creatinine (3.4)

3.2.4 Calcium test

Bring in three test tubes—one each to serve as a blank, a standard, and a specimen—and add 0.5 milliliters of reagent no. 1 and 0.5 milliliters of reagent no. 2 to each of the test tubes. Add 25 microliters of distilled water to the blank tube, 25 microliters of standard solution to the standard tube, and 25 microliters of blood sample to the sample tube. Then, incubate the tubes for five minutes at 25 degrees Celsius, read the absorbance at 500 nanometers, and record the results. The calculation of calcium can be done using Equation (3.5).

Calcium (mg/ dL) = (A sample/ A standard) × Concentration (Standard) (3.5)

3.2.5 Measurement of lipid profile

Sample collection

After 10 to 12 hours of not eating or drinking anything, the samples for the lipid profile should be prepared. After adding 5 mL of blood serum to a tube, they should be

centrifuged for three minutes, and then the serum should be used for the test. Determine the total cholesterol, as well as the HDL and LDL levels. Two actions are required to get the desired outcome: LDL cholesterol is eliminated first, and then any remaining molecules are broken down by enzyme processes. In the second phase of the process, the HDL fraction's remaining cholesterol is measured using HDL-specific surfactants and well-defined enzymatic processes. These reactions take place in the presence of HDL.

Reaction principle

The reaction of the first step is done according to Equation (3.6), and Equation (3.7).

$$\text{LDL, VLDL, Chylomicrons} \xrightarrow{\text{CHE + CHO}} \text{Cholestenone} + H_2O_2 \quad (3.6)$$

$$2H_2O \xrightarrow{\text{Specific condition}} 2H_2O + O_2 \quad (3.7)$$

The reaction principle of 2nd step are done according to Equation (3.8) and Equation (3.9)

$$\text{HDL} \xrightarrow{\text{CHE + CHO}} \text{Cholestenone} + H_2O_2 \quad (3.8)$$

$$2H_2O + \text{chromogen} \xrightarrow{\text{Proxidase}} \text{Quinone pigment} \quad (3.9)$$

Reagent composition for Potassium test

R1: LDH 50 ku / L, NADH 10 mmol/ L , sodium azide 0.05 %; R2 : Pyruvate kinase 50 ku / L, Sodium azide 0.05 %; Cal.: Potssium standard, Potassium 6.0 mmol / L.

1. Add 1 mL of blank, sample and calibrator to R1 reagent tube, add 25 µL of sample-to-sample tube, also add 25 µL of calibrator to calibrator tube and mix well, incubate at 37 °C for five minutes.

2. Add 250 µL of blank, 250 µL of sample and 250 µL of calibrator to R2 reagent and mix, incubate for 1 minutes at 37°C then read the absorbance at 405 nm (A1), then read after 3 minutes (A2), The calculation can be done according to Equation (3.10).

Potassium (mmol/L) = (A1-A2)/ (A1-A2) Calibrator × C calibrator (3.10)

4. RESULTS AND DISCUSSION

4.1 Results

This study was conducted for the period from 2021 to 2022 in HAVIJE hospital, and the goal of the study was to detect the activity of LDH enzyme in the blood of kidney patients compared to healthy people. Ninety samples of kidney patients were selected for the age group from 25 to 55 years and were divided into three groups. The first group has 30 samples of patients ranging in age from 25 to 35 years, the second of 30 samples of the injured are for the age group from 35 to 55 years, and the third group: of 30 samples of healthy people.

4.1.1 Age

The relationship between LDH enzyme activity and age in the blood of patients with kidney disease (group AP = 30.566 ± 3.08146, group BP = 44.733 ± 5.65034), when compared to healthy people (CC= 39.300 ± 9.44366), indicates the presence of statistical significance with the control group at P = <0.05 as a shown in Table 4.1 and Figure 4.1. When conducting Pearson's test to find out the association between LDH enzyme with age, the value of r = 0.123 at P = 0.249, as shown in Table 4.11.

It is essential in the different phases of disease development for people who have renal disease. In terms of education and social standing, the vast majority of the adult patients had been rehabilitated, while just 14% were jobless. The findings suggest that age is also relevant throughout the period of kidney transplantation, which is a process by which children may achieve a high level of rehabilitation when they reach maturity. On the other hand, a kidney transplant has a finite lifespan, which indicates the need for more targeted immunosuppression with fewer adverse effects in order to achieve the aim of lifelong graft function (Offner *et al.*1999).

In guys older than 45 years, there is no longer a significant difference. Both women with one kidney and those with two kidneys saw the same level of function loss with advancing age. Loss of renal function may be more common in younger guys when compared with non-stone components of kidney stones. The progression of kidney function decline with age differs significantly between lithoformers and non-formers (Worcester *et al.* 2003).

Table 4.1 The mean of age for the kidney patients' group and for the control groups

Descriptive						
Age	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Group AP	30	30.566	3.08146	0.000	29.4160	31.717
Group BP	30	44.733	5.65034	0.000	42.6234	46.843
Group CC	30	39.300	9.44366	0.000	35.7736	42.826
Total	90	38.200	8.77458	0.000	36.3622	40.037

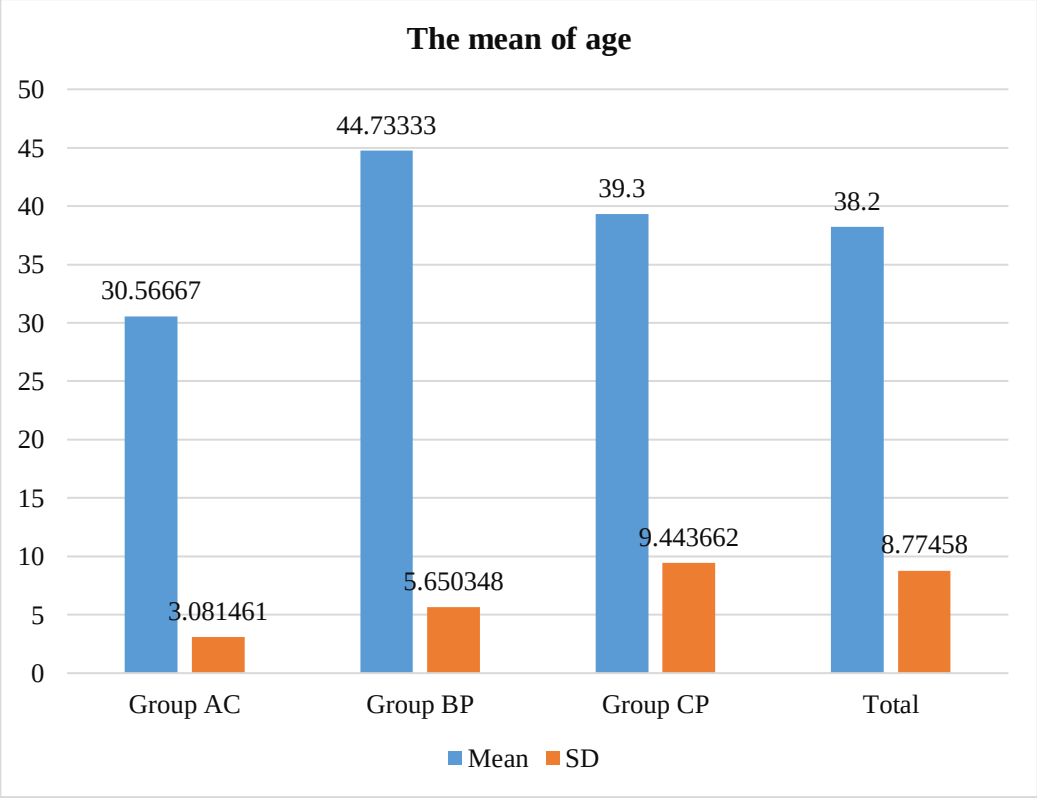

Figure 4.1 The mean of age for the kidney patients' group and for the control groups

4.1.2 Blood urea

The relationship between LDH enzyme activity and blood urea in the blood of patients with kidney disease (group AP = 152.200 ± 39.858, group BP = 157.500 ± 36.691), when compared to healthy people (CC= 31.500 ± 5.6431), indicates the presence of a high statistical effective when comparing the outcomes with the control group at P = <0.05 as a shown in Table 4.2 and Figure 4.2. When conducting Pearson's test to find out the association between LDH enzyme with blood urea, the value of $r = 0.557^{**}$ at P = 0.00, as shown in Table 4.11.

Urea, which is a marker of uraemic retention in chronic kidney disease (CKD) and of the adequacy of intradialytic solute clearance, has historically been thought of as being physiologically inactive. However, recent research has shown that urea may really play a role in kidney disease. On the other hand, a number of recent experimental findings demonstrate that urea is hazardous when present in quantities that are typical of CKD. To begin, there have been at least five studies that suggest that urea itself is responsible for inducing molecular changes that are linked to insulin resistance, the formation of free radicals, apoptosis, and the collapse of the protective intestinal barrier (Vanholder *et al.* 2018).

Table 4.2 The mean of blood urea kidney patients, and control groups

Descriptive						
Urea	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Group AP	30	152.200	39.858	0.000	137.316	167.083
Group BP	30	157.500	36.691	0.000	143.7992	171.200
Group CC	30	31.500	5.6431	0.000	29.39282	33.6071
Total	90	113.733	66.2612	0.000	99.85511	127.611

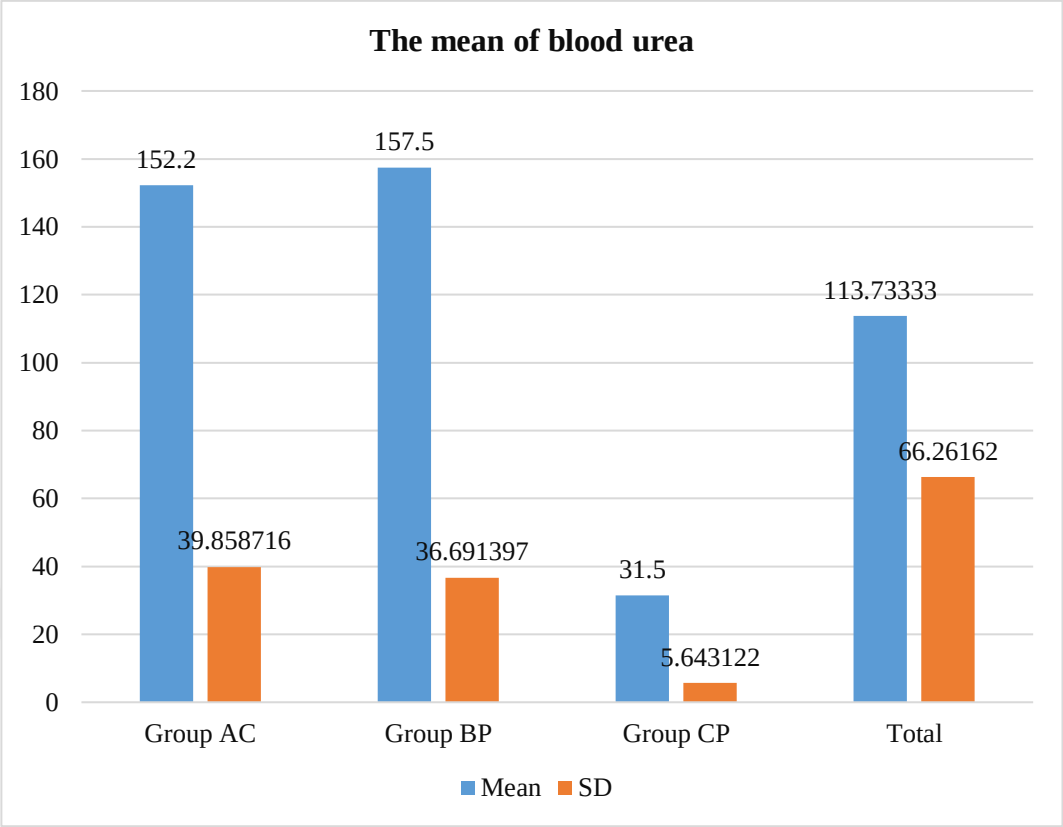

Figure 4.2 The mean of blood urea kidney patients, and control groups

4.1.3 Serum creatinine

The relationship between LDH enzyme activity and serum creatinine in the blood of patients with kidney disease (group AP = 6.21433 ± 2.340595, group BP = 7.32833 ± 2.4435), when compared to healthy people (CC= 0.7576 ± 0.10129), indicates the presence of a high statistical significance when comparing the results with the control group at P = <0.05 as a shown in Table 4.3 and Figure 4.3. When conducting Pearson's test to find out the association between LDH enzyme with serum creatinine, the value of $r = 0.503^{**}$ at P = 0.000, as shown in Table 4.11.

Patients with kidney loss made up 49.6 percent of the patient population, while normal gallstone patients made up 33.6 percent of the patient population. The three most prevalent reasons for kidney failure were an obstruction, a heavy stone load, and an infection. In individuals with a single kidney, the creatinine clearance was much lower. When all patients were taken into consideration, the rate of decline in renal function with

age was greater among guys who only had one kidney as opposed to those who had two kidneys (Worcester *et al.* 2003).

Table 4.3 The mean of creatinine kidney patients, and control groups

Descriptive						
Creatinine	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Group AP	30	6.21433	2.340595	0.000	5.34034	7.0883
Group BP	30	7.32833	2.443590	0.000	6.4158	8.2407
Group CC	30	0.75767	0.101291	0.000	.71984	.79549
Total	30	6.21433	2.340595	0.000	5.34034	7.0883

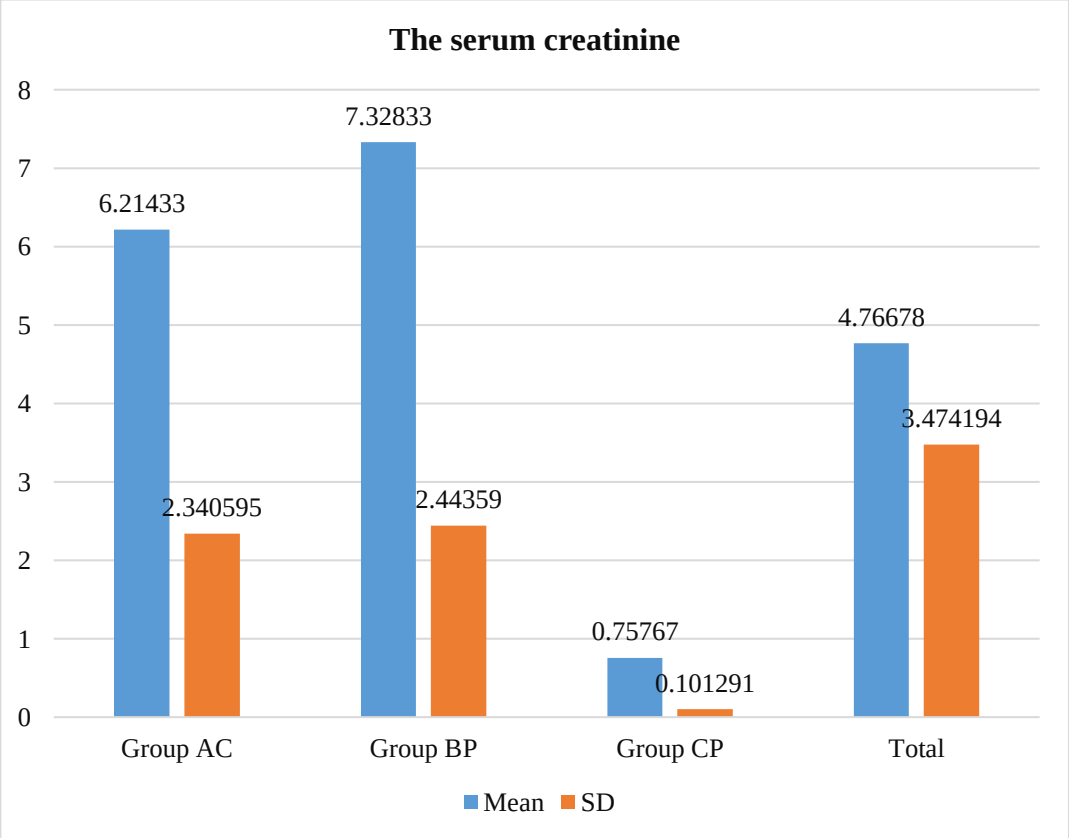

Figure 4.3 The mean of creatinine kidney patients, and control groups

4.1.4 Lactate dehydrogenase

The relationship between LDH enzyme activity in the blood of patients with kidney disease (group AP = 535.63 ± 125.7006, group BP = 542.100 ± 143.2748), when compared to healthy people (CC= 286.100 ± 34.6154), indicates the presence as compared with the findings of the control group, statistical significance was achieved at P = <0.05 as a shown in Table 4.4 and Figure 4.4.

Statistically significant correlations were seen between serum LDH-2 activity and serum total protein (r = –0.665, p 0.001), serum albumin (r = –0.615, p 0.001), proteinuria (r = 0.694, p 0.001), and serum total cholesterol (r = 0.723, p 0.001). According to the results of a linear regression study, serum total protein was shown to be an independent predictor of serum total LDH activity, while both serum total protein and proteinuria were found to be predictors of LDH-2. Conclusions: According to the results of Mohás et al. (2008), serum total LDH activity could be able to serve as a marker for the progression of the nephrotic syndrome.

Patients whose nucleic acid turned negative after 14 days had decreased LDH (P 0.05), the researchers discovered. ROC curve data suggested that lower LDH, predicted the nucleic acid of patients went negative within 14 days with statistical significance (P 0.05), AST, and LDH the statistical significance (P 0.05). After verification, the chance of nucleic acid becoming negative after 14 days was about four times greater in patients who had low LDH (256 U/L), low CRP (44,5 mg/L), and high ALB (>35.8 g/L) than in patients who had high LDH, high CRP, and low ALB (P 0.05) (Liu *et al.* 2021).

Table 4.4 The mean of LDH kidney patients, and control groups

Descriptive						
LDH	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	Upper Bound
Group AP	30	535.63	125.7006	0.000	488.69	582.57
Group BP	30	542.100	143.2748	0.000	488.60	595.59
Group CC	30	286.100	34.6154	0.000	273.17	299.02
Total	90	454.611	163.0715	0.000	420.45	488.76

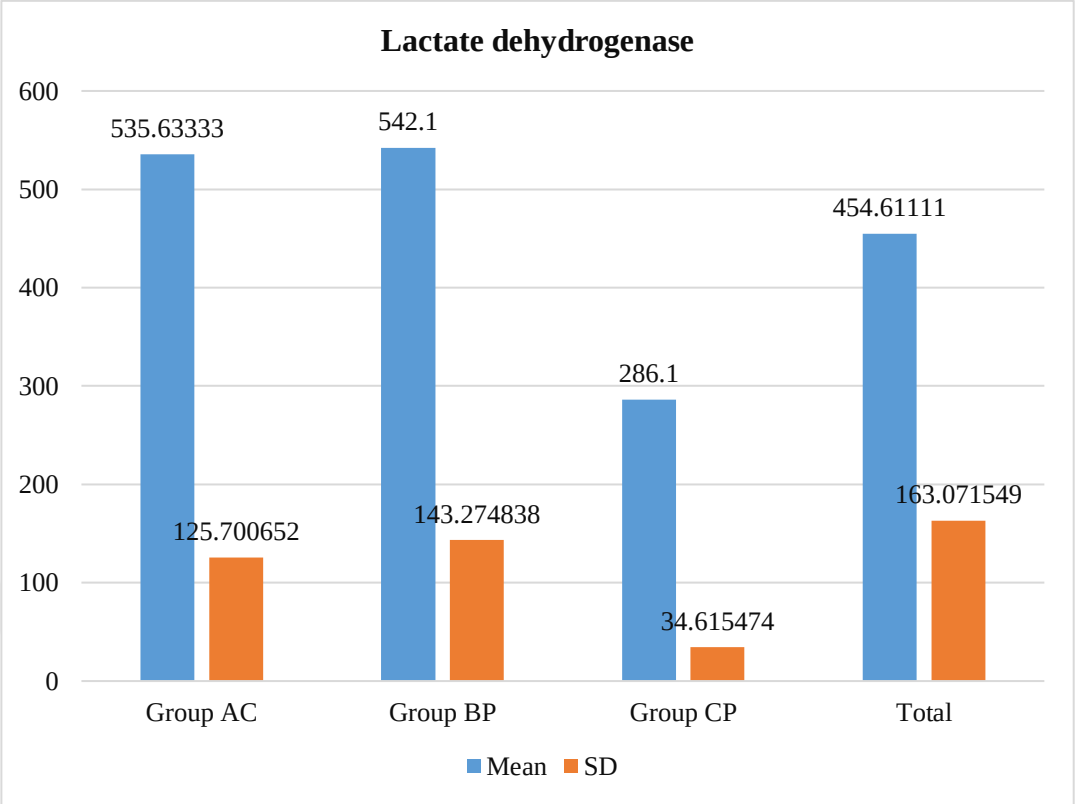

Figure 4.4 The mean of LDH kidney patients, and control groups

4.1.5 Total cholesterol

The relationship between LDH enzyme activity and total cholesterol in the blood of patients with kidney disease (group AP = 176.900 ± 24.2392, group BP = 161.33 ± 29.8297), when compared to healthy people (CC= 172.00 ± 18.8295), indicates the presence of a low as compared with the findings of the control group, statistical significance was achieved at P = <0.05 as a shown in Table 4.5 and Figure 4.5. When conducting Pearson's test to find out the association between LDH enzyme with total cholesterol, the value of r = 0.162 at P = 0.128, as shown in Table 4.11.

The link between cholesterol and the onset of renal dysfunction is not well understood, despite the vast information that exists about aberrant lipid patterns in patients who have reached the end stage of renal disease. Creatinine levels that were significantly raised and estimated creatinine clearance that was significantly lowered were the primary outcome measures. Cholesterol criteria were total cholesterol (200, 200 to 239, and 240 mg/dL),

HDL (40 or 40 mg/dL), total non-HDL cholesterol, and the ratio of total cholesterol to HDL. Total cholesterol was measured in milligrams per deciliter (mg/dL). The observed relationships between cholesterol parameters and lower creatinine clearance were analogous to one another but on a lesser scale. In particular, having a total cholesterol level that was elevated, having a non-HDL cholesterol level that was high, having a ratio of total cholesterol/HDL that was high, and having an HDL level that was low were significantly associated with an increased risk of developing renal dysfunction in men who had an initial creatinine level of less than 1.5 mg/dL. (Schaeffner *et al.* 2003).

Table 4.5 The mean of total cholesterol in kidney patients, and control groups

Descriptive						
Tc	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Group AP	30	176.9000	24.239253	0.049	167.848	185.95
Group BP	30	161.3333	29.829785	0.049	150.194	172.47
Group CC	30	172.0000	18.829544	0.049	164.968	179.03
Total	90	170.0777	25.290771	0.049	164.780	175.37

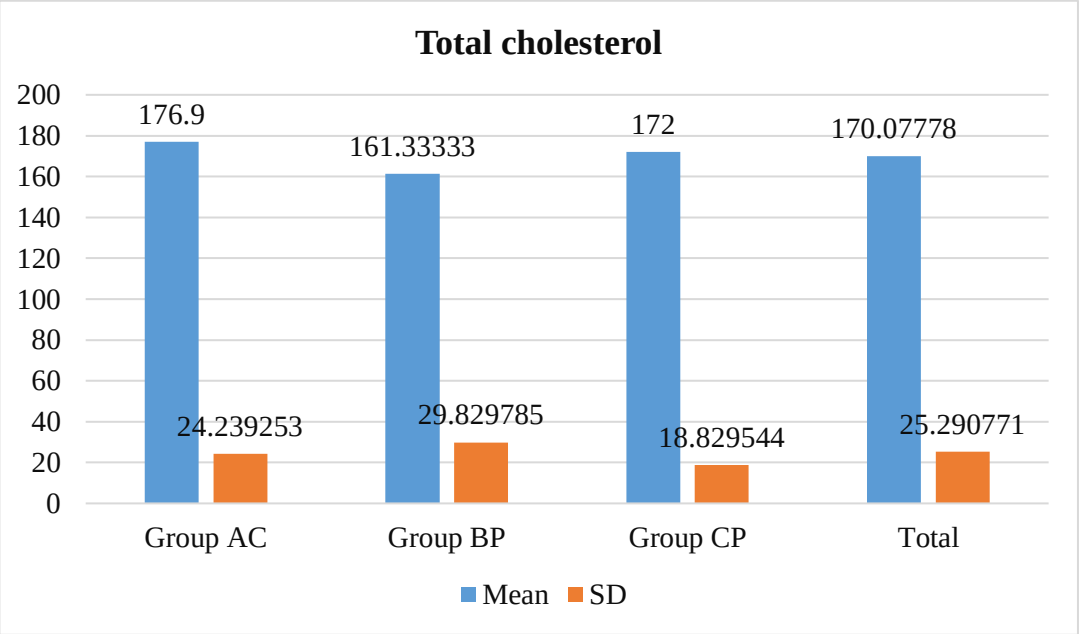

Figure 4.5 The mean of total cholesterol in kidney patients, and control groups

4.1.6 High-density lipoprotein

The relationship between LDH enzyme activity and HDL in the blood of patients with kidney disease (group AP = 36.700 ± 6.02380, group BP = 33.500 ± 5.17120), when compared to healthy people (CC= 47.866 ± 5.8471), indicates the presence of significant difference was noted as compared to the data obtained from the control group at P = <0.05 as a shown in Table 4.6 and Figure 4.6. When conducting Pearson's test to find out the association between LDH enzyme with HDL, the value of $r = 0.588^{**}$ at P = 0.000, as shown in Table 4.11.

Table 4.6 The mean of HDL kidney patients, and control groups

Descriptive						
HDL	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Group AP	30	36.7000	6.02380	0.000	34.4506	38.949
Group BP	30	33.5000	5.17120	0.000	31.5690	35.430
Group CC	30	47.8666	5.84709	0.000	45.6833	50.050
Total	90	39.3555	8.36836	0.000	37.6028	41.108

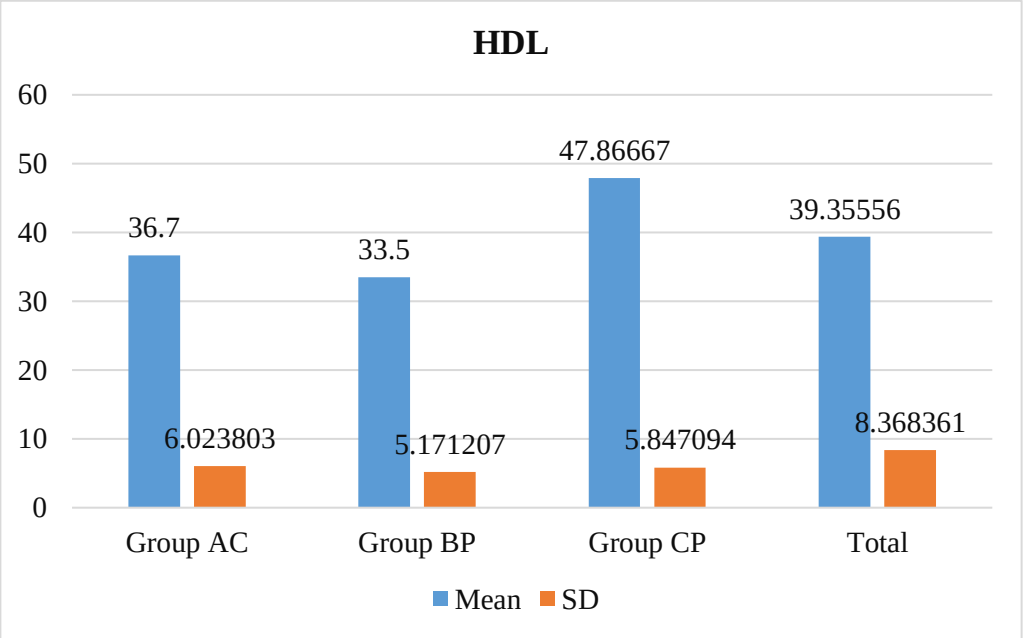

Figure 4.6 The mean of HDL kidney patients, and control groups

4.1.7 GOT

The relationship between LDH enzyme activity and GOT in the blood of patients with kidney disease (group AP = 23.733 ± 11.4859, group BP = 22.066 ± 8.5255), when compared to healthy people (CC= 15.566 ± 6.6316), indicates the presence of significant difference but non-statistical effective when comparing the findings with the control group at P = <0.05 as a shown in Table 4.7 and Figure 4.7. When conducting Pearson's test to find out the association between LDH enzyme with GOT, the value of $r = 0.482^{**}$ at P = 0.000, as shown in Table 4.11.

The current study was conducted to assess liver function and assess damage to some parts of the body, including the kidneys. GOT, GPT, ALP, and LDH homozygotes were estimated using the CUSIBIO protocol. The data revealed levels within normal levels of the liver enzyme GOT, GPT, and ALP in patients with kidney disease while increased in patients who had recovered from Covid-19. While the LDH analog showed an increase in the level of LDH2, LDH3, and LDH4 except for LDH1 showed a decrease in the level. We conclude that the dysregulation of hepatic function and the increased hepatic enzyme is due to the presence of ACE2 as receptors for β-cells in the liver cholangiocytes (Nazzal and Sabbar 2022).

Table 4.7 The mean of GOT kidney patients, and control groups

Descriptive						
GOT	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Group AP	30	23.7333	11.4859	0.002	19.444	28.022
Group BP	30	22.066	8.5255	0.002	18.883	25.250
Group CC	30	15.566	6.6316	0.002	13.090	18.042
Total	90	20.45556	9.672341	0.002	18.4297	22.481

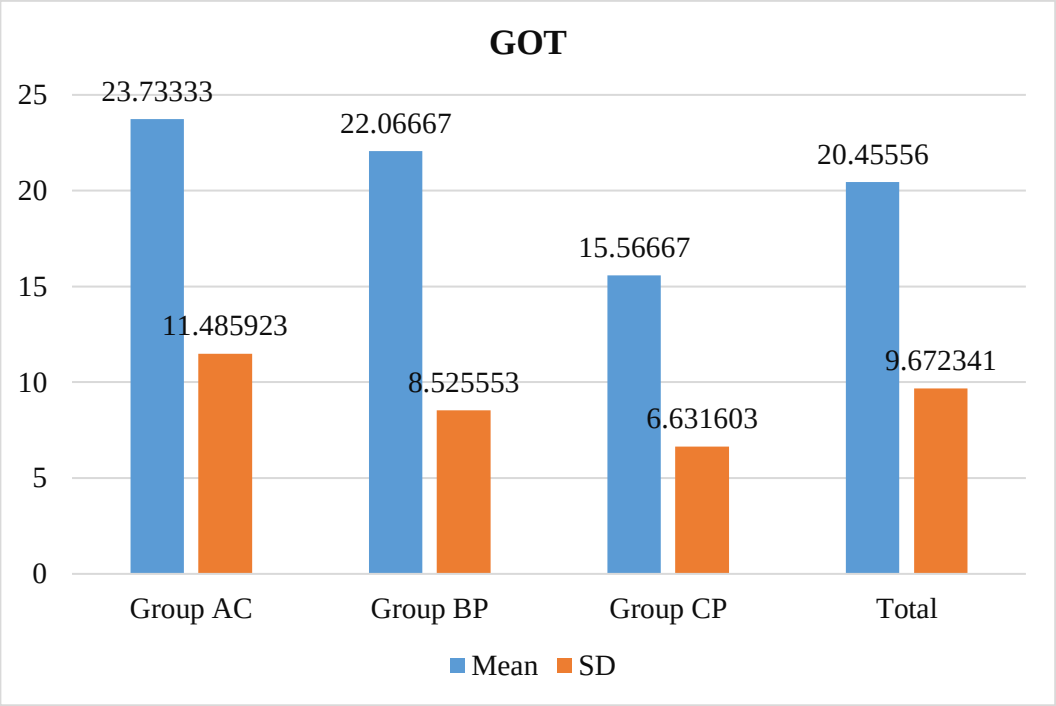

Figure 4.7 The mean of GOT kidney patients, and control groups

4.1.8 GPT

The relationship between LDH enzyme activity and GPT in the blood of patients with kidney disease (group AP = 16.8333 ± 11.70641, group BP = 16.334 ± 9.1588), when compared to healthy people (CC= 15.367 ± 5.9918), indicates the presence of significant difference but non-statistical significance statistical comparing the findings with the control group at P = <0.05 as a shown in Table 4.8 and Figure 4.8. When conducting Pearson's test to find out the association between LDH enzyme with GPT, the value of *r* = 0.243* at P = 0.021, as shown in Table 4.11.

Table 4.8 The mean of GPT kidney patients, and control groups

Descriptive						
GPT	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Group AP	30	16.8333	11.70641	0.823	12.462	21.204
Group BP	30	16.3333	9.15887	0.823	12.913	19.753
Group CC	30	15.3666	5.99127	0.823	13.129	17.603
Total	90	16.1777	9.16831	0.823	14.257	18.098

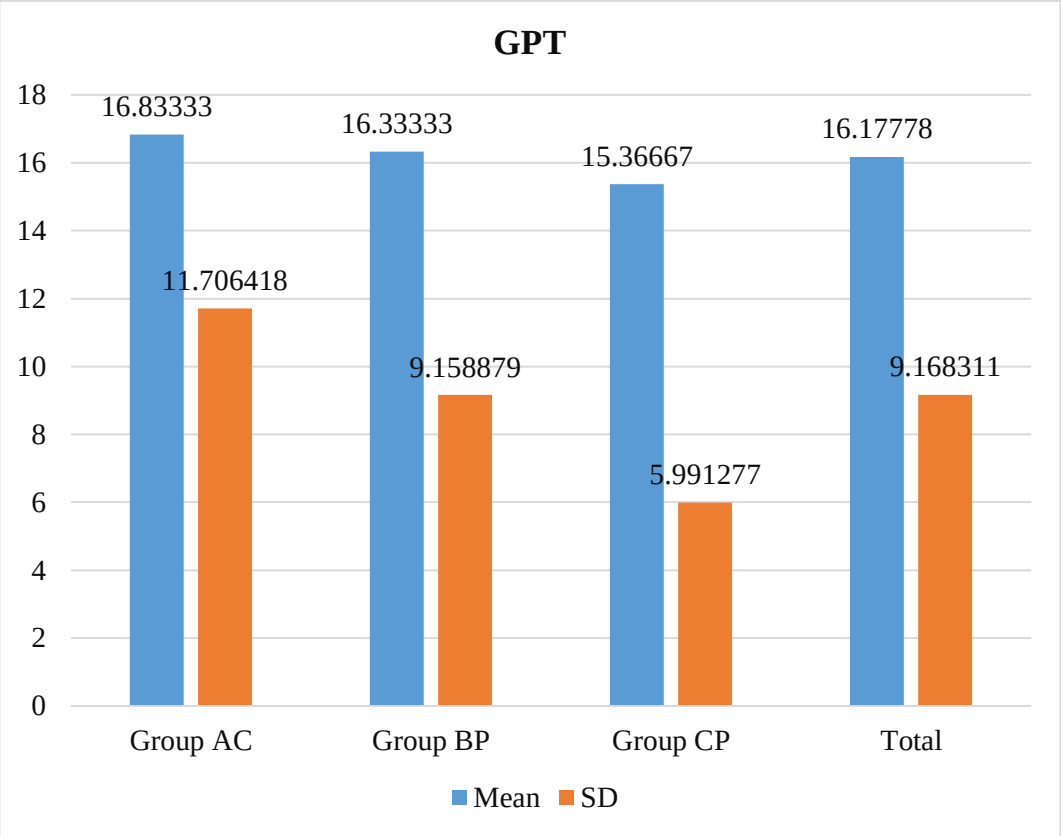

Figure 4.8 The mean of GPT kidney patients, and control groups

4.9 The potassium

The relationship between LDH enzyme activity and potassium (K) in the blood of patients with kidney disease (group AP = 5.665 ± 0.496475, group BP = 5.8406 ± 0.5808), when compared to healthy people (CC= 4.9190 ± 0.37648), indicates the presence of a non-significant difference was noted as compared to the data obtained from the control group at P = <0.05 as a shown in Table 4.9 and Figure 4.9. When conducting Pearson's test to find out the association between LDH enzyme with K, the value of $r = 0.433^{**}$ at P = 0.000, as shown in Table 4.11.

Table 4.9 The mean of K kidney patients, and control groups

Descriptive						
K	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Group AP	30	5.6653	0.496475	0.058	5.4799	5.8507
Group BP	30	5.8406	0.580879	0.058	5.6237	6.0575
Group CC	30	4.9190	0.376457	0.058	4.7784	5.0595
Total	90	5.4750	0.630814	0.058	5.3428	5.6071

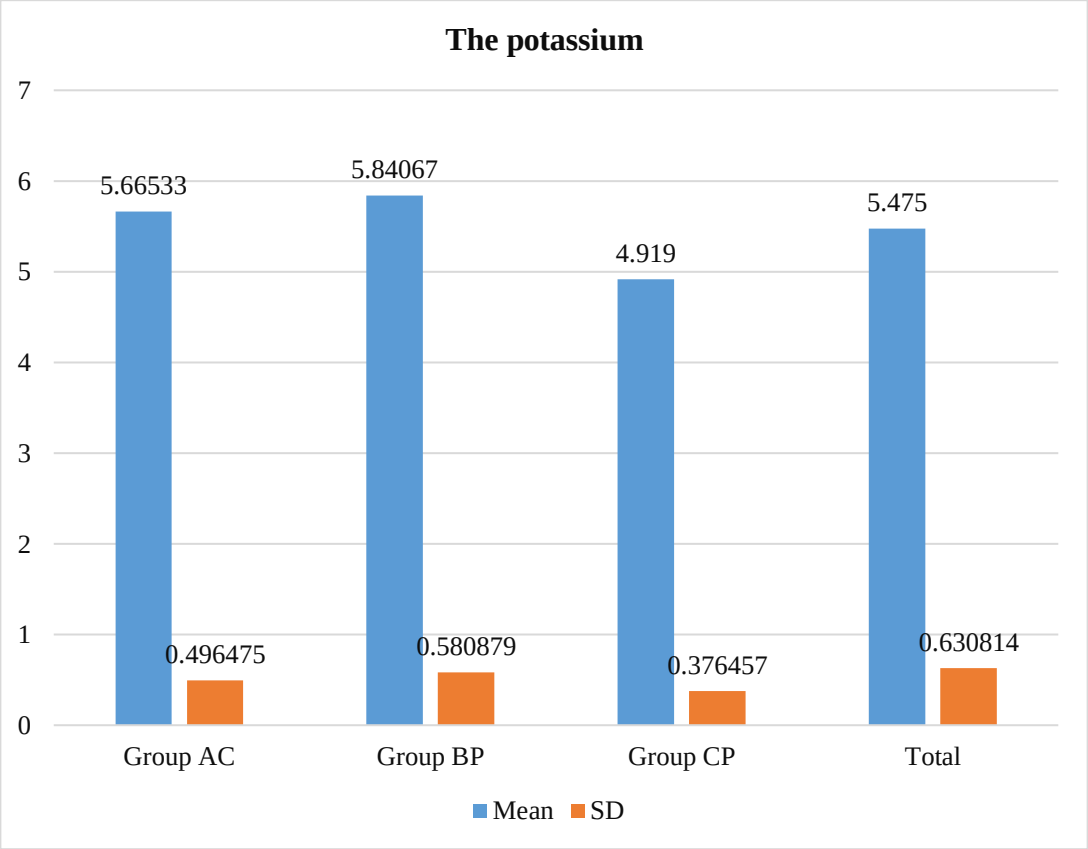

Figure 4.9 The mean of K kidney patients, and control groups

4.1.9 The calcium (Ca)

The relationship between LDH enzyme activity and the calcium in the blood of patients with kidney disease (group AP = 8.2783 ± 0.31461, group BP = 8.4293 ± 0.49516), when compared to healthy people (CC= 8.6866 ± 0.15024), indicates the presence of a non-significant difference was noted as compared to the data obtained from the control group

at P = <0.05 as a shown in Table 4.10 and Figure 4.10. When conducting Pearson's test to find out the association between LDH enzyme with calcium, the value of r = -0.265* at P = 0.011, as shown in Table 4.11.

Table 4.10 The mean of calcium kidney patients, and control groups

Descriptive						
Ca	**N**	**Mean**	**Std. Deviation**	**P-value**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Group AP	30	8.27833	0.31461	0.073	8.1608	8.3958
Group BP	30	8.42933	0.49516	0.073	8.2444	8.6142
Group CC	30	8.68667	0.15024	0.073	8.6305	8.7427
Total	90	8.46478	0.38501	0.073	8.3841	8.5454

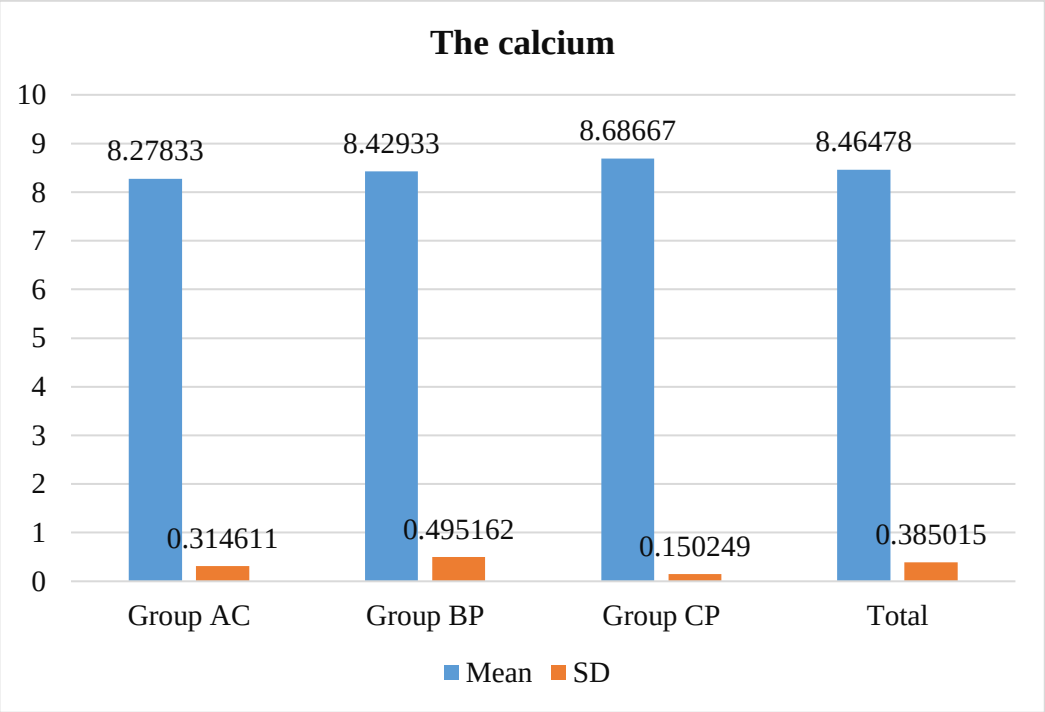

Figure 4.10 The mean of calcium kidney patients, and control groups

4.1.10 The correlation of biochemical markers

Table 4.11 shows the correlation between all biochemical markers used during the current study.

Table 4.11 The correlation between biochemical parameters with LDH in kidney patients

Variables		***r***
LDH - Age	Pearson Correlation	-0.123
	Sig. (2-tailed)	0.249
LDH – Blood urea	Pearson Correlation	0.557**
	Sig. (2-tailed)	0.000
LDH – serum creatinine	Pearson Correlation	0.503**
	Sig. (2-tailed)	0.000
LDH - Total cholesterol	Pearson Correlation	-0.162
	Sig. (2-tailed)	0.128
LDH - HDL	Pearson Correlation	-0.588**
	Sig. (2-tailed)	0.000
LDH - GOT	Pearson Correlation	0.482**
	Sig. (2-tailed)	0.000
LDH - GPT	Pearson Correlation	0.243*
	Sig. (2-tailed)	0.021
LDH - K	Pearson Correlation	0.433**
	Sig. (2-tailed)	0.000
LDH - Calcium	Pearson Correlation	-0.265*
	Sig. (2-tailed)	0.011

5. CONCLUSION AND RECOMMENDATION

The results of the study indicated the effect of age directly on kidney patients, and that the success rate of kidney transplantation in theMiddle Ages was greater than that of adults. Urea and creatinine are diagnostic chemical tests for kidney patients. Lactate dehydrogenase enzyme had an active role, and its amounts were substantially affected when compared to the control group, more studies are suggested on the role of lactate dehydrogenase in the development of kidney disease. Total fat had important indicators with statistical significance. It could be a sign of fat accumulation and thus increase pressure on the kidneys in filtering rates and thus result in kidney failure. Liver enzymes and calcium did not have a clear effect, although there were some statistical differences.

REFERENCES

Blaya, J., Barzideh, Z. and LaPointe, G. 2018. Symposium review: Interaction of starter cultures and nonstarter lactic acid bacteria in the cheese environment. Journal of Dairy Science, 101(4): 3611-3629.

Bonventre, J. V. 2014. Kidney injury molecule 1: a translational journey. Transactions of the American Clinical and Climatological Association, 125: 293.

Canaud, G. and Bonventre, J. V. 2015. Cell cycle arrest and the evolution of chronic kidney disease from acute kidney injury. Nephrology Dialysis Transplantation, (30): 575-583.

Chacar, F. C., Kogika, M. M., Zafalon, R. V. and Brunetto, M. A. 2020. Vitamin D Metabolism and Its Role in Mineral and Bone Disorders in Chronic Kidney Disease in Humans, Dogs and Cats. Metabolites, (10): 499.

Chawla, L. S., Eggers, P. W., Star, R. A. and Kimmel, P. L. 2014. Acute kidney injury and chronic kidney disease as interconnected syndromes. New England Journal of Medicine, (371): 58-66.

Eiteman, M. A. and Ramalingam, S. 2015. Microbial production of lactic acid. Biotechnology Letters, 37(5): 955-972.

Elshaghabee, F. M., Bockelmann, W., Meske, D., De Vrese, M., Walte, H. G., Schrezenmeir, J. and Heller, K. J. 2016. Ethanol production by selected intestinal microorganisms and lactic acid bacteria growing under different nutritional conditions. Frontiers in Microbiology, (7): 47.

Fiorentino, M., Grandaliano, G., Gesualdo, L. and Castellano, G. 2018. Acute kidney injury to chronic kidney disease transition. In Acute Kidney Injury-Basic Research and Clinical Practice, (193): 45-54.

Gallo, M., Sapio, L., Spina, A., Naviglio, D., Calogero, A. and Naviglio, S. (2015). Lactic dehydrogenase and cancer: an overview. Frontiers in Bioscience-Landmark, 20(8): 1234-1249.

Gondim, R., Azevedo, R., Braga, A. A. N. M., Veiga, M. L. and Barroso Jr, U. 2018. Risk factors for urinary tract infection in children with urinary urgency. International Braz. J. Urol., 44: 378-383.

Guan, Q., Alnasser, H. A., Nguan, C. Y. C. and Du, C. 2014. From humans to experimental models: The cytoprotective role of clusterin in the kidney. Med Surg Urol., 3(134): 2.

Hasan, K. A., Hussein, A. S. and Mohammed, T. K. 2021. Detection of Lasb and Plch Genes in Pseudomonas Aeruginosa Isolated from Urinary Tract Infections by PCR Technique. Annals of the Romanian Society for Cell Biology, 25(6): 123-134.

Hickson, L. J., Prata, L. G. L., Bobart, S. A., Evans, T. K., Giorgadze, N., Hashmi, S. K. and Kirkland, J. L. 2019. Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. E-BioMedicine., 47: 446-456.

Hosoya, K., Minakuchi, H., Wakino, S., Fujimura, K., Hasegawa, K., Komatsu, M. and Itoh, H. 2015. Insulin resistance in chronic kidney disease is ameliorated by spironolactone in rats and humans. Kidney International, 87(4): 749-760.

Hsu, R. K. and Hsu, C. Y. 2016. The role of acute kidney injury in chronic kidney disease. In Seminars in Nephrology, 36(4): 283-292.

Hudson, A., Romao, R. L. and MacLellan, D. 2017. Urinary tract infection in children. CMAJ., 189(16): 608-608.

Jackson, E., López-Gallego, F., Guisan, J. M. and Betancor, L. 2016. Enhanced stability of l-lactate dehydrogenase through immobilization engineering. Process Biochemistry, 51(9): 1248-1255.

Jia, B., Pu, Z. J., Tang, K., Jia, X., Kim, K. H., Liu, X. and Jeon, C. O. 2018. Catalytic, computational, and evolutionary analysis of the d-lactate dehydrogenases responsible for d-lactic acid production in lactic acid bacteria. Journal of Agricultural and Food Chemistry, 66(31): 8371-8381.

Jung, S., Hwang, H. and Lee, J. H. 2019. Effect of lactic acid bacteria on phenyllactic acid production in kimchi. Food Control, 106: 106701.

Juturu, V. and Wu, J. C. 2016. Microbial production of lactic acid: the latest development. Critical Reviews in Biotechnology, 36(6): 967-977.

Keren, R., Shaikh, N., Pohl, H., Gravens-Mueller, L., Ivanova, A., Zaoutis, L. and Hoberman, A. 2015. Risk factors for recurrent urinary tract infection and renal scarring. Pediatrics, 136(1): e13-e21.

Kong, X., Zhang, B., Hua, Y., Zhu, Y., Li, W., Wang, D. and Hong, J. 2019. Efficient L-lactic acid production from corncob residue using metabolically engineered thermo-tolerant yeast. Bioresource Technology, 273, 220-230.

Lee, J. W., In, J. H., Park, J. B., Shin, J., Park, J. H., Sung, B. H., Kweon, D. H. 2017. Co-expression of two heterologous lactate dehydrogenases genes in Kluyveromyces marxianus for l-lactic acid production. Journal of Biotechnology, 241: 81-86.

Liaud, N., Rosso, M. N., Fabre, N., Crapart, S., Herpoël-Gimbert, I., Sigoillot, J. C. and Levasseur, A. 2015. L-lactic acid production by Aspergillus brasiliensis overexpressing the heterologous ldha gene from Rhizopus oryzae. Microbial Cell Factories, 14(1): 1-9.

Liu, G., Zhang, B., Zhang, S., Hu, H. and Liu, T. 2021. LDH, CRP and ALB predict nucleic acid turn negative within 14 days in symptomatic patients with COVID-19. Scottish Medical Journal, 66(3): 108-114.

Mazzio, E., Mack, N., Badisa, R. B. and Soliman, K. F. 2021. Triple Isozyme Lactic Acid Dehydrogenase Inhibition in Fully Viable MDA-MB-231 Cells Induces Cytostatic Effects That Aren`t Reversed by Exogenous Lactic Acid. Biomolecules, 11(12): 1751.

Mazzoli, R., Olson, D. G. and Lynd, L. R. 2020. Construction of lactic acid overproducing Clostridium thermocellum through enhancement of lactate dehydrogenase expression. Enzyme and Microbial Technology, 141: 109645.

Mickiewicz, K. M., Kawai, Y., Drage, L., Gomes, M. C., Davison, F., Pickard, R. and Errington, J. 2019. Possible role of L-form switching in recurrent urinary tract infection. Nature Communications, 10(1): 1-9.

Miyamoto, T., Oguma, Y., Sato, Y., Kobayashi, T., Ito, E., Tani, M. and Nakamura, M. 2018. Elevated creatine kinase and lactic acid dehydrogenase and decreased osteocalcin and uncarboxylated osteocalcin are associated with bone stress injuries in young female athletes. Scientific Reports, 8(1): 1-10.

Mohás, M., Szigeti, N., Markó, L., Molnár, G. A., Cseh, J., Laczy, B. and Wittmann, I. 2008. Serum total LDH activity and LDH-2 isozyme in nephrotic syndrome. Kidney and Blood Pressure Research, 31(1): 47-54.

Mora-Bau, G., Platt, A. M., van Rooijen, N., Randolph, G. J., Albert, M. L. and Ingersoll, M. A. 2015. Macrophages subvert adaptive immunity to urinary tract infection. PLoS pathogens., 11(7): e1005044.

Mushegian, A. A. 2016. Bacteria stop fermentation with lactic acid. Science Signaling, 9457: ec288-ec288.

Nazzal, A. G. and Sabbar, A. G. 2022. Evaluation of liver function (GPT, GOT, ALP) and cardiac isoenzyme (LDH1, LDH2, LDH3, LDH4) in COVID19 patients after recovering. Al-Nisour Journal for Medical Sciences, 4(1): 1-13.

Offner, G., Latta, K., Hoyer, P. F., Baum, H. J., Ehrich, J. H., Pichlmayr, R. and Brodehl, J. 1999. Kidney transplanted children come of age. Kidney International, 55(4): 1509-1517.

Okojie, R. O. and Omorokpe, V. O. 2018. A survey on urinary tract infection associated with two most common uropathogenic bacteria. African Journal of Clinical and Experimental Microbiology, 19(3): 171-176.

Ostermann, M., Bellomo, R., Burdmann, E. A., Doi, K., Endre, Z. H., Goldstein, S. L. and Zarbock, A. 2020. Controversies in acute kidney injury: conclusions from a kidney disease: Improving Global Outcomes KDIGO Conference. Kidney International, 98(2): 294-309.

Ozaki, A., Konishi, R., Otomo, C., Kishida, M., Takayama, S., Matsumoto, T. and Kondo, A. 2017. Metabolic engineering of Schizosaccharomyces pombe via CRISPR-Cas9 genome editing for lactic acid production from glucose and cellobiose. Metabolic Engineering Communications, 5: 60-67.

Papadimitriou, K., Alegría, Á., Bron, P. A., De Angelis, M., Gobbetti, M., Kleerebezem, M. and Kok, J. 2016. Stress physiology of lactic acid bacteria. Microbiology and Molecular Biology Reviews, 80(3): 837-890.

Patel, R., Polage, C. R., Dien Bard, J., May, L., Lee, F. M., Fabre, V. and Hanson, K. E. 2022. Envisioning Future Urinary Tract Infection Diagnostics. Clinical Infectious Diseases, 74(7): 1284-1292.

Pruijm, M., Hofmann, L., Piskunowicz, M., Muller, M. E., Zweiacker, C., Bassi, I. and Burnier, M. 2014. Determinants of renal tissue oxygenation as measured with BOLD-MRI in chronic kidney disease and hypertension in humans. PloS One., 9(4): e95895.

Pundir, C. S., Narwal, V. and Batra, B. 2016. Determination of lactic acid with special emphasis on biosensing methods: A review. Biosensors and Bioelectronics, 86: 777-790.

Resztak, M., Sobiak, J. and Czyrski, A. 2021. Recent advances in therapeutic drug monitoring of voriconazole, mycophenolic acid, and vancomycin: a literature review of pediatric studies. Pharmaceutics, 13(12), 1991.

Rodrigues, C., Vandenberghe, L. P. S., Woiciechowski, A. L., de Oliveira, J., Letti, L. A. J. and Soccol, C. R. 2017. Production and application of lactic acid. In Current Developments in Biotechnology and Bioengineering, 543-556.

Rodríguez, E., Gimeno, J., Arias-Cabrales, C., Barrios, C., Redondo-Pachón, D., Soler, M. J. and Pascual, J. 2018. Membrane attack complex and factor H in humans with acute kidney injury. Kidney and Blood Pressure Research, 43(5): 1655-1665.

Saad, A., Herrmann, S. M. and Textor, S. C. 2015. Chronic renal ischemia in humans: can cell therapy repair the kidney in occlusive renovascular disease. Physiology, 30(3): 175-182.

Sabel, A., Bredefeld, S., Schlander, M. and Claus, H. 2017. Wine phenolic compounds: Antimicrobial properties against yeasts, lactic acid and acetic acid bacteria. Beverages, 3(3): 29.

Saint, S., Greene, M. T., Krein, S. L., Rogers, M. A., Ratz, D., Fowler, K. E. and Fakih, M. G. 2016. A program to prevent catheter-associated urinary tract infection in acute care. New England Journal of Medicine, 374(22): 2111-2119.

Sauer, M., Russmayer, H., Grabherr, R., Peterbauer, C. K. and Marx, H. 2017. The efficient clade: lactic acid bacteria for industrial chemical production. Trends in Biotechnology, 35(8): 756-769.

Schaeffner, E. S., Kurth, T., Curhan, G. C., Glynn, R. J., Rexrode, K. M., Baigent, C. and Gaziano, J. M. 2003. Cholesterol and the risk of renal dysfunction in apparently healthy men. Journal of the American Society of Nephrology, 14(8): 2084-2091.

Seely, J. C. 2017. A brief review of kidney development, maturation, developmental abnormalities, and drug toxicity: juvenile animal relevancy. Journal of Toxicologic Pathology, 30(2): 125-133.

Sheerin, N. S. 2015. Urinary tract infection. Medicine, 43(8): 435-439.

Singhvi, M., Zendo, T., Iida, H., Gokhale, D. and Sonomoto, K. 2017. Stimulation of d- and l-lactate dehydrogenases transcriptional levels in presence of diammonium hydrogen phosphate resulting to enhanced lactic acid production by Lactobacillus strain. Journal of Bioscience and Bioengineering, 124(6): 674-679.

Stapleton, A. E. 2017. The vaginal microbiota and urinary tract infection. Urinary Tract Infections: Molecular Pathogenesis and Clinical Management, 4(6): 79-86.

Stenvinkel, P., Painer, J., Kuro-o, M., Lanaspa, M., Arnold, W., Ruf, T. and Johnson, R. J. 2018. Novel treatment strategies for chronic kidney disease: insights from the animal kingdom. Nature Reviews Nephrology, 14: 265-284.

Sukkar Al-Farawn, M. A., Hussein, Z. M. and Abdullah, W. S. 2019. Effect of Laser Diode 820Nm on the Levels of Lactate Dehydrogenase, Creatin Phosphokinase, and Lactic Acid in Muscles of Students. Indian Journal of Public Health Research and Development, 10(6): p801-805.

Tolulope, A. and Deborah, O. 2015. Urinary tract infection amongst pregnant women in Amassoma, Southern Nigeria. African Journal of icrobiology Research, 9(6): 355-359.

Tsuge, Y., Kato, N., Yamamoto, S., Suda, M., Jojima, T. and Inui, M. 2019. Metabolic engineering of Corynebacterium glutamicum for hyperproduction of polymer-grade L-and D-lactic acid. Applied Microbiology and Biotechnology, 103(8): 3381-3391.

Vanholder, R., Gryp, T. and Glorieux, G. 2018. Urea and chronic kidney disease: the comeback of the century (in uraemia research). Nephrology Dialysis Transplantation, 33(1): 4-12.

Vargas, E., Ruiz, M. A., Campuzano, S., de Rivera, G. G., López-Colino, F., Reviejo, A. J. and Pingarrón, J. M. 2016. Implementation of a new integrated d-lactic acid biosensor in a semiautomatic FIA system for the simultaneous determination of lactic acid enantiomers. Application to the analysis of beer samples. Talanta, 152: 147-154.

Vigil, H. R. and Hickling, D. R. 2016. Urinary tract infection in the neurogenic bladder. Translational Andrology and Urology, 51: 72.

Vlachostergios, P. J., Oikonomou, K. G., Gibilaro, E. and Apergis, G. 2015. Elevated lactic acid is a negative prognostic factor in metastatic lung cancer. Cancer Biomarkers, 156: 725-734.

Worcester, E., Parks, J. H., Josephson, M. A., Thisted, R. A. and Coe, F. L. 2003. Causes and consequences of kidney loss in patients with nephrolithiasis. Kidney International, 64(6): 2204-2213.

Yang, Y., Zhang, J. and Yao, Y. 2017. Enantioselective effects of chiral pesticides on their primary targets and secondary targets. Current Protein and Peptide Science, 18(1): 22-32.

Yao, K., Zhou, Q. X., Liu, D. M., Chen, S. M. and Yuan, K. 2019. Comparative proteomics of the metabolic pathways involved in l-lactic acid production in Bacillus coagulans BCS13002 using different carbon sources. LWT., 116: 108445.

Zhang, C., Zhou, C., Assavasirijinda, N., Yu, B., Wang, L. and Ma, Y. 2017. Non-sterilized fermentation of high optically pure d-lactic acid by a genetically modified thermophilic Bacillus coagulans strain. Microbial Cell Factories, 161: 1-10.

Zhang, L., Li, X., Yong, Q., Yang, S. T., Ouyang, J. and Yu, S. 2016. Impacts of lignocellulose-derived inhibitors on l-lactic acid fermentation by Rhizopus oryzae. Bioresource Technology, 203: 173-180.

Zheng, Z., Zhao, M., Zang, Y., Zhou, Y. and Ouyang, J. 2015. Production of optically pure L-phenyllactic acid by using engineered Escherichia coli coexpressing L-lactate dehydrogenase and formate dehydrogenase. Journal of Biotechnology, 207: 47-51.

CURRICULUM VITAE

Personal Information

Name and Surname : Sameer Salih Mohammed MOHAMMED

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Chemistry	2020-2022
Undergraduate	Tikrit University College of Education for Pure sciences Department of Chemistry	2009-2013