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Significance of Various Biomarkers in Pre-Clinical and Clinical Studies

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ABSTRACT
The biomarkers are the biomolecule that indicate pathological process or pharmacological response to the drug treatment. The clinical uses of biomarkers is easy to identify disease condition and less expensive and also biomarkers are usually measured over a shorter time span. The proteomics techniques are used to separation of serum samples and identifies disease names. Some biomarkers are mainly used to recognize the disease names like cancer of prostate, breast, ovary, lung and heart disease, arthritis, asthma, cystic fibrosis. Whereas the advancement of technology few biomarkers have been approved by the Food and Drug Administration for disease diagnosis.
Objectives: -The objective of study was to measure the selected biomarkers to diagnose the diseases.
Result:-There was neither increase nor decrease in the serum biomarkers, all the biomarkers were shown within the normal range.
Conclusion:-The estimation of all the biomarker are very much essential to measure the metabolic and physiological function of the body.
Keywords: Serum biomarkers, disease diagnosis and prognosis, biochemical indicators,

I. INTRODUCTION

The biomarkers are the biomolecule that act as an indicator of a pathological process or pharmacological response to the drug treatment." Biomarkers are the measures used to perform a clinical examination such as blood pressure or cholesterol level and are used to monitor and predict health states in individuals or across populations.

Variety of biomarkers

Nowadays we are usingmany biomarkers. Every biological system (for example the cardiovascular system, metabolic system or immune system) has its own specific biomarkers. Many of these biomarkers are relatively easy to measure and also used for medical examinations[2].

For example, a general health check may include assessment of

- ✓ blood pressure,
- ✓ heart rate,
- ✓ cholesterol,
- ✓ triglycerides and
- ✓ fasting glucose levels.
- ✓ body measurements such as weight,
- ✓ body mass index (BMI) and
- ✓ Waist-to-hip ratio are routinely used for assessing conditions such as obesity and metabolic disorders.

➢ Characteristics of an ideal biomarker[2]

The ideal biomarker has certaincharacteristics that make it appropriate for checking a particular disease condition. Ideally, an ideal marker should have the following features:

- ➢ Safe and easy to measure
- ➢ Cost efficient to follow up
- ➢ Modifiable with treatment
- ➢ Consistent across gender and ethnic groups
- ➢ Biomarkers for Diabetes[3]:

01. Estimation of amount of glucose in serum
02. Estimation of amount of various lipids such as
- ✓ Total cholesterol
- ✓ Triglycerides
- ✓ LDL
- ✓ VLDL

03. Estimation of amount of protein in serum
04. Histopathology of pancreas in preclinical studies

- ➢ Biomarkers for Cardiovascular disease[4]:

01. ECG
02. Troponin test
03. Estimation of various enzymes such as
- ✓ LDH
- ✓ CPK/CK
- ✓ SGOT
- ✓ SGPT

04. Estimation of amount of various lipids such as
- ✓ Total cholesterol
- ✓ Triglycerides

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- ✓ LDL
- ✓ VLDL

05. Histopathology of heart in preclinical studies

- ➢ Biomarkers for Hepatic dysfunction[5]:

01. Estimation of various enzymes and biochemical constituents such as

- ✓ LDH
- ✓ CPK/CK
- ✓ SGOT
- ✓ SGPT
- ✓ Total Bilirubin
- ✓ Direct Bilirubin

2. Estimation of amount of various lipids such as

- ✓ Total cholesterol
- ✓ Triglycerides
- ✓ LDL
- ✓ VLDL

03. Histopathology of Liver in preclinical studies

- ➢ Biomarkers for Renal dysfunction[6]:

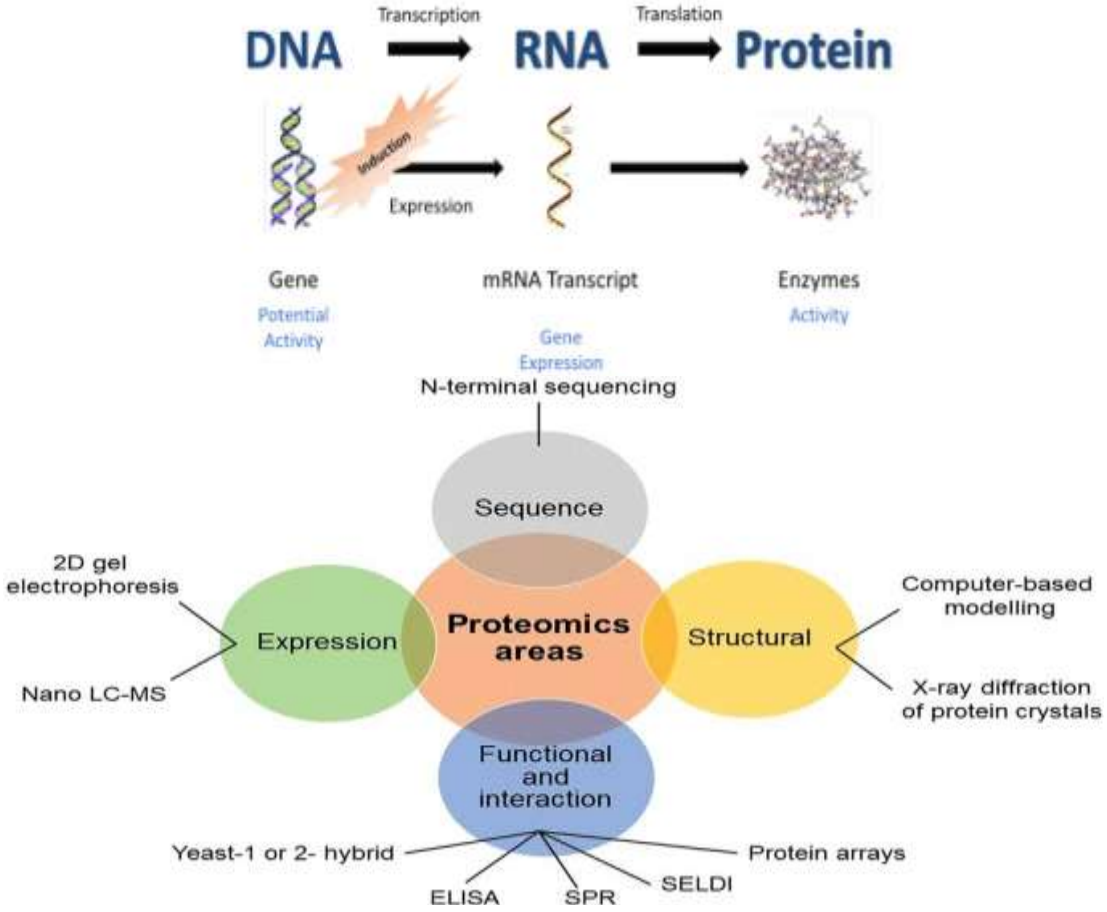
01. Estimation of amount of biochemical parameters such as

- ✓ Serum Creatinine
- ✓ Blood Urea Nitrogen

02. Histopathology of Kidney in preclinical studies

Proteomics

Proteomics is the science and technology of separating and identifying proteins from crude biological samples.The main aim of clinical proteomics is to determine which protein or group of proteins are responsible for specific function of phenotype.



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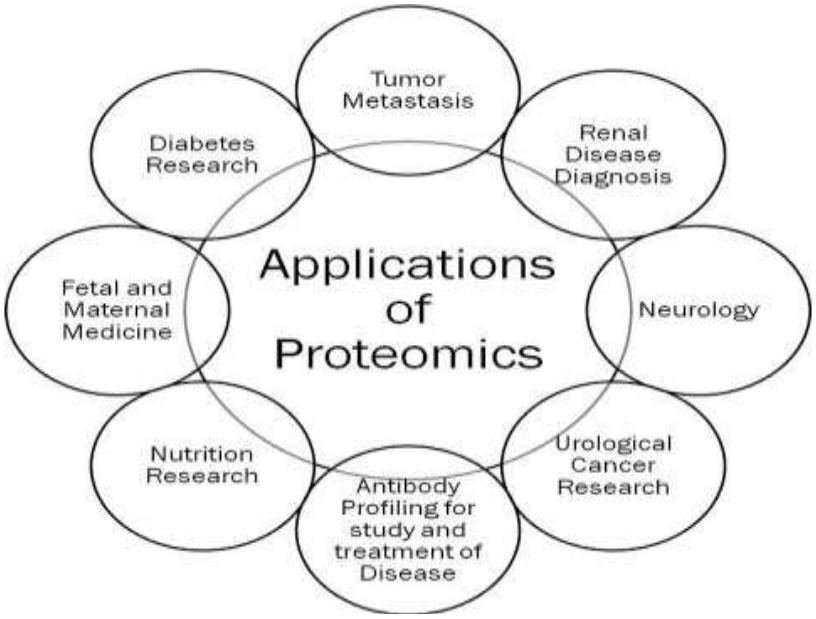

The biomarkers are the biomolecule that act as an indicator of pathological process or pharmacological response to the drug treatment. The clinical uses of biomarkers is easy to identify disease condition and less expensive and also biomarkers are usually measured over a shorter time span." Biomarkers are the measures used to perform a clinical examination such as diabetic research, neurology, fetal and maternal medicine, urological cancer research, nutrition research, tumor metastasis, renal disease[7].

II. METHODOLOGY

➢ Estimation of fasting blood glucose[8, 9].

Method: Endpoint Calorimetric

Principle: Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalyzed reaction, the oxygen liberated is accepted by the chromogen system to give a red colouredquinoneimine compound. The red colour so developed is measured at 505 nm and is directly proportional to glucose concentration.

Procedure:

Table 01: Estimation of fasting blood glucose

Pipette into tubes marked	Blank	Standard	Test
Pipette into tubes marked	--	--	20 µL
Serum or Plasma	--	20 µL	--
Working Glucose Reagent	1.5 ml	1.5 ml	1.5 ml
Mix well. Incubate at 37°C for 10 minutes or at R.T. for 30 min.			
Purified Water	1.5 ml	1.5 ml	1.5 ml

Mix well. Read absorbance at 490-550 nm against a reagent blank.

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

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➢ **Estimation of Serum Total Cholesterol[10,11].**

Principle: Cholesterol reacts with hot solution of ferric perchlorate, ethyl acetate and sulphuric acid (cholesterol reagent) and gives a lavender colored complex which is measured at 560nm.

Clinical Significance: Measurement of serum cholesterol levels are useful in evaluation of the risk of the coronary arterial occlusion, atherosclerosis, myocardial infarction, liver function, biliary function, intestinal absorption, thyroid function and adrenal disease.

Increases: Increased levels are found most characteristically in primary myxodema, hyperlipoproteinemia's, in nephrotic syndrome, obstructive jaundice and in diabetes mellitus.

Decreases: Low values are frequently obtained in anaemias, in haemolytic jaundice, in malabsorption syndrome, severe malnutrition, acute infections and in terminal state. Very low values are occurring in betalipoproteinaemia and to a lesser degree in familial hypobetalipoproteinaemias.

Procedure:

Table 02: Estimation of Serum Total Cholesterol

Pipette into tubes marked	Blank (B)	Standard (S)	Test (T)
Reagent 1 : Cholesterol Reagent	3.0 ml	3.0 ml	3.0 ml
Reagent 2 : Working Cholesterol Standard, (200 mg %)	--	0.015ml	--
Serum / Plasma	--	--	0.015ml

Mix well and keep the tubes immediately in the boiling water bath exactly for 90 seconds (1½ minutes). Cool them immediately to R.T. under running tap water. Measure the O.D. of Standard (S) and Test (T) against Blank (B) on a colorimeter with a yellow green filter or on a spectrophotometer at 560 nm.

Calculation:

$$\text{Serum / Plasma Cholesterol (mg/dl)} = \frac{\text{O.D Test}}{\text{O.D Standard}} \times 200$$

➢ Estimation of serum urea[12,13]:

Method: DAM method

Principle: Urea reacts with hot acidic Diacetylmonoxime in presence of Thiosemicarbazide and produces a rose–purple colored complex, which is measured colorimetrically.

Clinical Significance: Urea is the major end product of protein metabolism in humans. It constitutes the largest fraction of the non-protein nitrogen component of the blood. Urea is produced in the liver and excreted through the kidneys in the urine. Consequently, the circulating levels of urea depend upon protein intake, protein catabolism and kidney function.

Increases: Elevated serum urea concentrations are observed in impaired kidney function, liver diseases, congestive cardiac failure, diabetes, infections and diseases which impair the kidney function.

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Procedure:

Table 03: Estimation of serum urea.

Pipette into tubes marked	**Blank (B)**	**Test (T)**	**Standard (S)**
Solution I	2.5 ml.	2.5 ml.	2.5 ml.
Sample	--	0.01 ml	--
Reagent 3 : Working Urea Standard, 30mg%	--	--	0.01 ml
Reagent 2 : Diacetylmonoxime (DAM)	Mix well		
	0.25 ml	0.25 ml	0.25 ml

Mix well and keep the tubes in the boiling water exactly for 10 minutes. Cool immediately under running water for 5 minutes, mix by inversion and measure the color intensity within 10 minutes using a green filter against blank.

Calculation:

$$\textbf{Serum / Plasma: Urea in mg/100 mg} = \frac{\textbf{O.D Test}}{\textbf{O.D Standard}} \times 30$$

➢ **Estimation of serum creatinine[14,15]:**

Method: Alkaline Picrate Method.

Principle: Creatinine in a protein free solution reacts with alkaline picrate and produces a red colored complex, which is measured colorimetrically.

Clinical significance: Creatinine is a waste product formed in muscles from the high energy storage compound creatine phosphate. The amount of creatinine produced is fairly constant and is primarily a function of muscle mass. It is removed from plasma by glomerular filtration and then excreted in urine without any appreciable reabsorption by the tubules. Creatinine is a useful indicator of renal function.

Increases: Elevated creatinine level in serum is usually associated with various renal diseases. In the earlier stage of renal disease creatinine clearance test is a sensitive index of impaired renal function.

Procedure: Step A. Deproteinization of test sample:

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Table 04: Estimation of serum creatinine procedure step -1

Serum / Plasma / Dilute urine	:	0.5 ml
Purified water	:	0.5 ml
Reagent 1 : Picric Acid	:	3.0 ml

Mix well; keep in a boiling water bath exactly for one minute. Cool immediately under running tap water and centrifuge or filter.

Step 2. Colour Development:

Table 05: Estimation of serum creatinine procedure step -2

Pipette into tubes marked	**Blank (B)**	**Standard (S)**	**Test (T)**
Filtrate/Supernatant (From Step A)	--	--	2.0 ml
Working Standard	--	0.5 ml	--
Purified water	0.5 ml	--	--
Reagent 1 : Picric Acid	1.5 ml	1.5 ml	--
Reagent 2 : Sodium Hydroxide, 0.75 N	0.5 ml	0.5 ml	0.5 ml

Mix well and allow standing at R.T. exactly for 20 minutes and measure immediately the optical density of Blank (B), Standard (S) and Test (T) against Purified water on a colorimeter with a green filter.

Calculation:

$$\text{Serum Creatinine in mg/100 ml} = \frac{\text{O.D. test-O.D. Blank}}{\text{O.D. std-O.D. Blank}} \times 3.0$$

➢ **Total serum protein[16]:**

Principle: Cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a colored complex.

The protein contents of brain homogenates were determined by Lowry's method. This assay is based on the principle that proteins react with folin's reagent to give a coloured complex. The blue colour so formed was due to the reaction of the alkaline copper tartarate with the protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of

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blue colour formed was measured at 660 nm using bovine serum albumin as standard.

➢ **Estimation of serum triglycerides[17]:**

Principle: The enzyme, lipoprotein lipase catalyzes hydrolysis of TGs to glycerol and FAs. Glycerol then is phosphorylated in an ATP - requiring reaction catalyzed by glycerophosphate. The formed glycerophosphate is oxidized to dihydroxyacetone and H2O2 in a glycerophosphate oxidase catalyzed reaction. H2O2 then reacts with 4 -AAP and 4 -chlorophenol under the catalytic influence of peroxidase to form colouredquinoneimine complex, the intensity of which was measured at 505nm.

Lipase Triglyceride + 3 H2O Glycerol + 3 fatty acids

Glycerokinase Glycerol + ATP Glycero –3-phosphate + ADP

Glycerophosphate oxidase Glycero –3-Phosphate + O2DHAP + H2O2

Peroxidase 2H2O2+ 4-AAP + 4 - chloro phenol quinoneimine + HCl + 4H2O Procedure: Fresh clear and unhaemolysed serum was used for the estimation. The reaction mixtures were mixed well and incubated for 10 min at 370C. The absorbance of sample and standard were measured against reagent blank at 505 nm. The absorbance was measured by using a Shimadzu spectrophotometer. This procedure is followed for the estimations of following enzymes.

➢ **Estimation of serum high density lipoprotein cholesterol (HDL-C)[18]:**

Erba diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase / peroxidase (CHOD-POD) method.

Principle:

HDL-C is measured in the supernatant after the precipitation of the lipoproteins including chylomicrons, very low density lipoproteins and low density lipoproteins intermediate density lipoproteins directly from serum polyanions likephosphotungstic acid and along with MgCl2 are added to an aliquot of serum an immediate heavy precipitation is formed. The precipitate then is sedimented by centrifugation and HDL cholesterol is measured in the clear supernatant, which is estimated by enzymatic method as described earlier in estimation serum of TC.

Standard of assay details:

1. 0.5ml of serum was taken into test tube and 0.5ml of precipitating reagent was added, mixed well and kept at room temperature for 15min.
2. Centrifuged for15 min at 4000rpm.
3. The clear supernatant was separated & immediately used to determine the cholesterol.

➢ **Estimation of serum low-density lipoprotein cholesterol (LDL-C)[19]:**

Using the data obtained including total cholesterol, HDL cholesterol and triglycerides, the LDL cholesterol levels were calculated using the empirical equation of **Friedewald.** Serum LDL cholesterol=(Triglycerides/5)+HDL cholesterol-Total cholesterol.

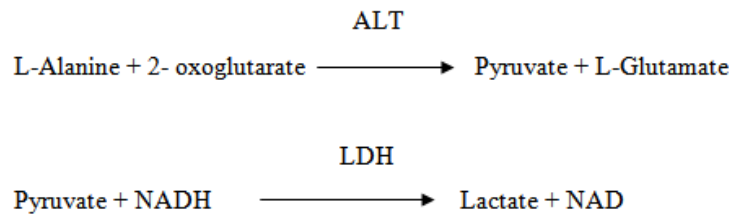
➢ **Estimation of Alanine aminotransferase (ALT)[20] :**

ALT (formerly called Serum Glutamate Pyruvate Transaminase; SGPT), is mainly present in cytosol of the liver cell, but also in smaller amounts in kidneys, heart, muscles and pancreas.

Low levels of ALT are normally found in the blood. However, when the liver is damaged or diseased, it releases ALT into the blood stream (due to increased permeability of cell membrane), which makes ALT level group; hence evaluating ALT is an index of the extent of liver damage.

Alanine amino transferase catalyses the transfer of amino group from Alanine to 2-oxoglutarate with the formation of glutamate and pyruvate. The liberated pyruvate is reduced to lactate dehydrogenase (LDH) in the same reaction an equivalent amount NADH is oxidized to NAD.

The reaction thus can be written as:



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Clinical significance: ALT is present in high concentrations in the liver and to a lesser extent in kidney, heart, skeletal muscle, pancreases, spleen and lungs.

Increases: Increased levels are generally result of primary liver diseases such as cirrhosis's carcinoma, viral or obstructive jaundice, viral hepatitis, lead poisoning, drug reactions, exposure to carbon tetrachloride, decay of large tumor (necrosis) and shock.

Decreases: Decreased levels may be observed in renal dialysis patients and that of vitamin B6 deficiency.[91]

➢ **Estimation of Aspartate aminotransferase (AST)[21]:**

Aspartate aminotransferase (formerly called Serum Glutamate Oxaloacetate Transaminase) is located in the cytosol of liver. In addition, it is also found in the mitochondria and in many tissues of heart, liver, skeletal muscle and kidney. The hepatic cell damage leads to increased levels of AST in blood serum.

Serum Glutamate Oxaloacetate Transaminase (SGOT) is a tissue enzyme that catalyses the exchange of amino and keto group between alpha amino acids and alpha keto acids.

AST is widely distributed in tissue principally cardiac, hepatic muscle and kidney; injury to these tissues results in increase in the AST (SGOT) enzyme level into general circulation. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma and viral hepatitis will also increase serum AST levels.

Clinical Significance: AST occurs in all human tissues and is present in large amounts in the liver, renal, cardiac and skeletal muscle tissue.

Increases: Increased levels are associated with liver disease or damage, myocardial infarction, heart attack, alcohol abuse, with high doses of vitamin A, mononucleosis, cancer and myositis.

Principle: L-Aspartate + 2-Oxoglutarate ⟶ Oxaloactate + L-Glutamate

$$\text{Oxaloacetate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}$$

$$\text{Sample pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}$$

➢ **Estimation of Lactate Dehydrogenase (LDH)[22]:**

Clinical significance: The enzyme LDH is concentrated in heart, kidney, liver muscle and other body tissues.

Increases: LDH levels increases in case of myocardial infarction, renal damage, hepatitis, anemia, malignancies and other muscular disease or injury.

Principle:

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}$$

➢ **Estimation of Creatine Kinase (CK)[23]:**

Clinical significance:

Creatine kinase is found in cardiac, skeletal and cerebral muscle tissues.

Increases: levels increase in myocardial infarction, acute cerebro-vascular disease, muscular dystrophy or injury. Following a myocardial infarction, CK

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activity begins to rise within 4-6 hours, peaks between 18-30 hours and to normal by third day.

Principle:

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{Creatine kinase}} \text{Creatine} + \text{ATP}$$

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{ADP} + \text{Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + \text{NADP}^{+} \xrightarrow{\text{G6PDH}} \text{6-Phospho gluconate} + \text{NADPH} + \text{H}^{+}$$

G6PDH: Glucose-6-phosphate dehydrogenase

III. RESULTS

Table 06: Estimation of various serum biomarkers

Serum Creatinine Mg/dl	BUN Mg/dl	LDH IU/l	CPK IU/l	AST IU/l	ALT IU/l	TG Mg/dl	TC Mg/dl	HDL Mg/dl	LDL Mg/dl	VLDL Mg/dl	Glucose Mg/dl	Protein Mg/dl
0.3558± 0.012	66.76± 0.115	90.82± 0.210	168.2± 0.052	37.06 ± 0.116	38.81± 0.209	182.30± 0.012	143.9 ± 0.516	71.40± 0.212	94.50± 0.543	63.72± 0.652	88.52± 0.426	0.653± 0.351
Normal Range												
0.4-0.8 Mg/dl	15-22 Mg/dl	146-184 IU/l	10-120 IU/l	50-150 IU/l	10-40 IU/l	76.13 ±2.38 Mg/dl	113.99±2.18 Mg/dl	49.14± 1.05 Mg/dl	49.64± 1.8 Mg/dl	15.22±0.48 Mg/dl	50-135 Mg/dl	6.0-8.3 Mg/dl

Table 06

There was neither increase nor decrease in the serum biomarkers, all the biomarkers were shown within the normal range.
The results are summarized in table 07 and graphically depicted in fig.1

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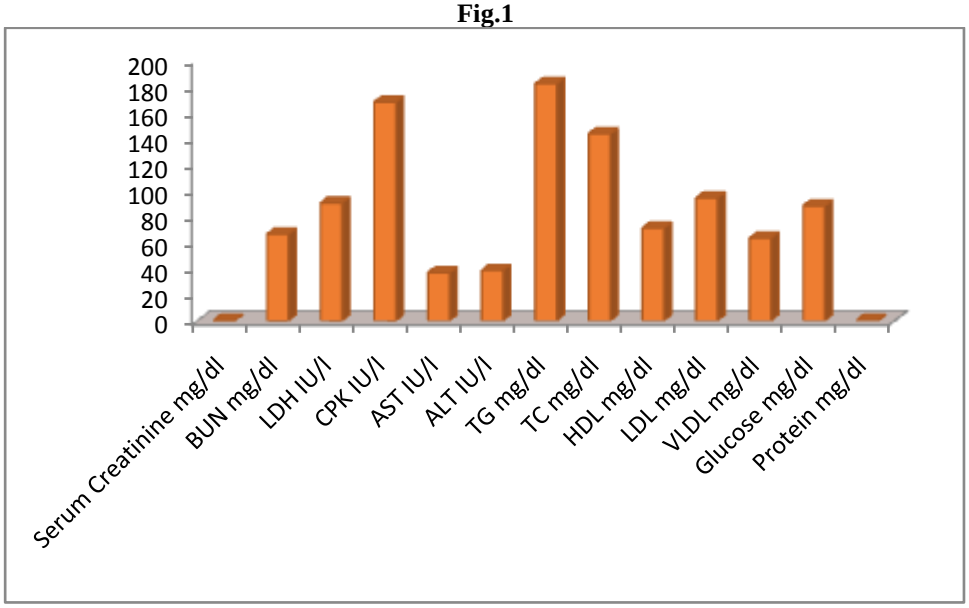
Serum biomarker level

Fig.1



IV. DISCUSSION

The biomarkers are the biomolecule that act as an indicator of a pathological process or pharmacological response to the drug treatment." Biomarkers are used to perform a clinical examination such as blood pressure or cholesterol level and are used to monitor and predict health states in individuals or across populations.A tumor marker is synthesized and released into circulation system or cell surface in large quantity and also these markers used to identifying the metastasis because changing the concentration of malignant tumor.Repeating test of serum biomarker allows following treatment and assessing response to treatment, monitoring tumor progression and metastasis[24].

The biomarkers are also used to identification of creatinine concentration in urine samples to clarify the glomerular filtration rate (GFR). Creatinine is formed within in the body by glomerulus filtration. And creatinine markers also used to clearance the creatinine. Once the GFR rate is decreased it will causes renal damage and increases in plasma creatinine concentration. Creatinine markers and BUN can also used as a an indicators of renal function. But BUN is not favorite marker for the clearance because it influenced by factors like high protein diet, varies in protein synthesis, hydration status[25].

Glucose is the most abundant monosaccharide. And its made by plants and diet. the total carbohydrates are present in the form of polysaccharides and simpler sugars. The sucrose, which is a disaccharide of glucose and fructose.Gluconeogenesis and glycogenolysis maintain glucose concentrations. The micro glucose oxidase technologymeasure blood glucose concentration and estimation of blood glucose in the blood chemistry.

V. CONCLUSION

The estimation of all the biomarker are very much essential to measure the metabolic and physiological function of the body.

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