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Comparative Assesment of Lactate Dehydrogenase and Creatine Phosphokinase Level in Hypothyroid and Euthyroid Patients

Biochemistry

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ABSTRACT

Thyroid hormones regulate metabolism. According to different studies worldwide it has been seen that in hypothyroid subjects, LDH and CPK levels were markedly elevated due to involvement of hepatocytes. The aims of this study were to measure the level of serum creatine kinase (CK) and lactate deydrogenase (LDH) in hypothyroid patients, and to compare the level of CK & LDH levels in hypothyroid & euthyroid individuals.92 patients coming to Dept. of Biochemistry & Dept. of Endocrinology in NRS Medical college were tested for CPK,LDH,FT4 & TSH. In hypothyroid individuals mean CPK level was 185.4012+-61.73301IU/L & mean LDH level was 270.8472+-44.05418 IU/L,both the values were found to be statistically significantly higher than the euthyroid group.(p value < 0.001). Limitation of this study was small sample size etc.

INTRODUCTION

Thyroxine(T4) and tri-iodothyronine(T3) are essential for normal organ growth, development and function. These hormones regulate the basal metabolic rate of all cells, including hepatocytes, and thereby modulate hepatic function; the liver in turn metabolizes the thyroid hormones and regulates their systemic endocrine effects. Thyroid dysfunction may perturb liver function. Hyperthyroidism (caused by an overactive thyroid gland) and hypothyroidism (due to a poorly functioning thyroid gland),depending on whether serum thyroid hormone levels (T4 and T3) are increased or decreased respectively. The symptoms of hypothyroidism are weight gain,increased sensitivity to cold,muscle weakness,joint or muscle pain,depression,fatigue,pale dry skin,a puffy face,a hoarse voice,etc.

Most affected organs in thyroid hormone alteration include liver and heart. So, it alters the liver enzymes like ALP, AST, ALT, GGT and cardiac enzymes like CPK, LDH and AST.[1][2][3][4]

Lactate dehydrogenase is a cytoplasmic enzyme mainly found in organs like heart, liver, kidney and skeletal muscles. Elevated LDH levels in serum are observed in several hemolytic, neoplastic, cardiac, skeletal muscle and renal diseases.These may also be found in destructive renal disease, progressive muscular dystrophy, megaloblastic anaemia, liver cirrhosis, hepatitis, hepatic metastasis, hepatoma and pulmonary embolism. Lactate dehydrogenase catalyzes the oxidation of lactate to pyruvate accompanied by the simultaneous reduction of NAD to NADH.[5]

CPK is mainly found in all muscle and brain tissue. It plays an important role in energy storing mechanism of the tissues.CPK catalyses the reaction between Creatine Phosphate and ADP to form creatine and ATP. The ATP formed along with Glucose is catalysed by Hexokinase to form Glucose 6 phosphate.The Glucose 6 phosphate reduces NADP to NADPH.[6]

The main aim of study was to determine the relationship between thyroid hormone alteration (i.e.hypothyroidism) and serum enzymes level(CPK & LDH). The study included 92 subjects visiting Endocrinology Department & Department of Biochemistry, NRS Medical College for thyroid function test, after which they were classified into hypothyroid & euthyroid groups & were tested for CPK & LDH levels.

AIMS & OBJECTIVES

To compare the LDH & CPK level in hypothyroid and euthyroid patients and to find whether there is a difference in the LDH and CPK levels in hypothyroids and euthyroids.

MATERIALS AND METHOD:

This study was conducted in the Dept.of Biochemistry and Dept. of Endocrine of NRS Medical College and Hospital between 26th of July 2016 to 26th of August 2016 (one month) . It was a tertiary hospital based non-interventional, observational, comparative study. This prospective case-control study was conducted in the department of Biochemistry at NRS Medical College and Hospital.Thyroid function tests (TSH,fT4)were done & serum LDH & CPK were measured in 45 euthyroid control and 47 patients who were detected with hypothyroidism. Hypothyroidism was defined as having TSH level greater than 6.16 microIU/ml.

Inclusion criteria :

The individuals with 18-65 age groups and without any chronic condition other than thyroid or any of the conditions mentioned in the exclusion criteria were included in this study.

Exclusion criteria :

Exclusion criteria were taken to rule out other diseases which can alter the results like neuromuscular disorder or any recent cardiovascular events or any severe life threatening disease. Individuals with an active infection or a recent infection including liver disease or any other liver disorder, malignancy, bone and muscle disease or disorder related with myopathy, muscular dystrophy or injury, cardiac, pancreatic or hepatobillary system. Patients suffering from diabetes, hypertension, malignancy, pregnancy, alcoholics, drug abusers and patients taking statins ,fibrates, ARV, Angiotension-II receptor antagonists or oral contraceptive pills(OCP) were excluded as they are well known to have side effects over hepatocytes causing abnormal liver enzyme levels.

Case & controls were selected randomly after going through the inclusion and exclusion criteria.

Laboratory Investigation :

LDH - LDH is measured using a UV-Kinetic spectrophotometer.LDH catalyzes the oxidation of lactate to pyruvate accompanied by the simultaneous reduction of NAD to NADH.LDH serum activity is proportional to the increase in absorbance due to the reduction of NAD.

LDH REFERENCE RANGE:

114-240 IU/L at 37 degree celsius (Adults).[5]

CPK - CPK is measured using a UV-Kinetic spectrophotometer.Creatine phosphokinase catalyses the reaction between creatine phosphate and ADP to form creatine and ATP.The ATP formed along with Glucose is catalysed by Hexokinase to form Glucose 6 phosphate which reduces NADP to NADPH.The rate of reduction of NADP to NADPH is measured as an increase in absorbance which is proportional to CPK activity in the sample.

CPK REFERENCE RANGE:
Serum (Male) 24-195 U/L at 37 degree celsius
(female) 24-170 U/L at 37 degree Celsius[6]

TSH - TSH measurement is actually the determination of it's concentration in the serum by a Microplate Immunoenzymometric assay.

REFERENCE RANGE:
0.39-6.16 micro IU/ml (Adults).[7]

fT4 - fT4 or free Thyroxine concentration determination in serum is performed using a Microplate Enzyme Immunoassay.

REFERENCE RANGE:
0.8-2.0 ng/dl (Adults).[8]

Patient Consent - Patient consent was obtained before starting the study.

Resources -
A. Human resources - All faculty members ,technicians and other staff members of the Department,all laboratory personnelswho helped in the collection and recording of the data.

B. Material resources - Reagents,instruments and other materials needed was supplied by the Department.

C. Financial - Financial resources were not needed,all reagents were procured from laboratory store.

REVIEW OF LITERATURE:
Hypothyroidism is one of the most commonly occurring thyroid disorders worldwide. Muscle involvement in hypothyroidism is common with 30-80% of hypothyroid patients presenting with muscular symptoms varying from myalgia to true myopathy.

Lactate dehydrogenase catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+. The molecular weight of 134,000 Da and is composed of four peptide chains of two types: M(or A) and H(or B).The subunit compositions of the five isoenzymes are LD-1 (HHHH;H4); LD-2 (HHHM;H3M); LD-3 (HHMM;H2M2); LD-4 (HMMM;HM3); and LD-5 (MMMM;M4).A different,sixth LDH isoenzyme is LD-X, present in postpubertal human testes.It has been noted that various conditions can raise the LDH levels including hypothyroidism which is a highly prevalent disorder. [5]

Serum CK is increased in nearly all patients with injury,inflammation,or necrosis of skeletal and heart muscles occurs.The CK isoenzymes are numbered as CK-1,CK-2 and CK-3. All the three of isozymes are found in the cytosol of the cell or are associated with myofibrillar structures[6] .As thyroid hormones are essential for normal organ growth, development function and regulate the basal metabolic rate of all cells, its alteration can affects the entire metabolism and can alter the activity of serum enzymes. Most affected organs include liver and heart. So,according to some studies done worldwide alteration of thyroid hormone levels can also alter the liver enzymes like ALP, AST, ALT, GGT and cardiac enzymes like CPK, LDH and AST.[3][4]

Recognition of a pattern of elevations in commonly measured serum enzymes [creatine phosphokinase (CPK), lactic dehydrogenase (LDH), and glutamate oxalacetate transaminase (SGOT)] can facilitate the diagnosis of hypothyroidism, especially when muscle weakness is a symptom. Special attention should be given to the serum enzyme values which initially were elevated and fell to normal levels during thyroid replacement therapy. Isoenzyme fractionation of LDH and CPK indicated skeletal muscle as the source of the elevated enzyme activity.[9]

Study by Ayodeji F. Ajayi et al reveals that although hyperthyroid state is not associated with altered liver function, hypothyroidism caused hepatic dysfunction. It was therefore suggested that liver function indices should be monitored in altered thyroid states, especially in hypothyroidism.[10]

There is negotiation regarding the increased levels of LDH and CPK in thyroid disorders with some studies stating that LDH and CPK can be used as a parameter for screening the thyroid disorder (McGrowder, 2011).[11]

RESULTS & STATISTICAL ANALYSIS
Patients visiting Endocrinology Department & Department of Biochemistry, NRS Medical College,Kolkata with the request of TFT were recruited for the study after going through inclusion exclusion criteria. Two variables were measured for hypothyroidism and controls(euthyroidism), i.e., serum enzymes level CPK and LDH. Thyroid profile FT4 and TSH were measured to categorize hypothyroidism and euthyroidism. The data were analyzed to compare the mean values between hypothyroidism and controls and to find out correlation between thyroid profile and serum enzymes in the hypothyroidism and controls. Overall the cases and controls were in the age range of 20–60 years. About 92 patients were included. All the statistical analysis were performed using SPSS and Microsoft excel. The significant differences between two groups was compared using Student's T-test. Data were presented as mean ± SD. Probability values were calculated throughout, and $p<0.05$ was considered statistically significant.

Table 1: Thyroid Profile of the Hypothyroidism and Control.

Study group	Normal range	Control (n=45)	Hypothyroid (n=47)
FT4 (ng /dl) Mean ± SD range	0.8-2.0 ng/dl	1.46±0.29 (0.84-1.91)	0.52±0.36 (0.14-1.55)
TSH (μIU/ml) Mean ± SD range	0.39-6.16 micro IU/ml	2.92±1.14 (0.74-5.01)	29.92±12.15 (14.02–42.20)

Healthy Control- (Euthyroid)(Sample Number = 45)

	VALID NO.	MEAN (IN IU/L)	STD. DEV. (IN IU/L)	MINIMUM (IN IU/L)	MAXIMUM (IN IU/L)	95% CONFIDENCE INTERVAL LOWER BOUND (IN IU/L)	95% CONFIDENCE INTERVAL UPPER BOUND (IN IU/L)
LDH	45	147.82	14.12454	126.1	179.7	119.58	176.06
CPK	45	64.5088	28.90786	29.43	111	6.7	122.31

Table – 3 - Descriptive Statistics Of Numerical Variables - Group Hypothyroid- (Sample Number = 47)

	VALID NO.	MEAN (IN IU/L)	STD. DEV. (IN IU/L)	MINIMUM (IN IU/L)	MAXIMUM (IN IU/L)	95% CONFIDENCE INTERVAL LOWER BOUND (IN IU/L)	95% CONFIDENCE INTERVAL UPPER BOUND (IN IU/L)
LDH	47	270.8472	44.05418	204	375.7	182.75	358.95
CPK	47	185.4012	61.73301	129.95	372.7	61.94	308.86

Taking CPK reference range as stated below :
Serum (Male) 24-195 U/L at 37 degree celsius
(female) 24-170 U/L at 37 degree Celsius
& taking LDH reference range as stated below:
114-240 IU/L at 37 degree celsius (Adults).

We can see that the level of LDH is more in hypothyroid patients than euthyroids .Same is the case for CPK levels,as is evident from the mean values.

DISCUSSION AND CONCLUSION
Thyroid hormones regulate the rate of metabolic activity and their alterations can perturb the activity of serum enzyme.

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The aim of the present study was comparative assessment of LDH & CPK levels in hypothyroid and euthyroid patients .CK activity may also be found in macromolecular form-the so-called macro-CK.It exists in two forms: types 1 and 2.Type 1 is a complex of CK,typically CK-BB,and an immunoglobulin,often IgG, but other complexes have been described such as CK-MM with IgA.Macro-CK type 2 is oligomeric CK-Mt.The apperance of this isoenzyme in serum is usually associated with a poor prognosis.Macro-CK can interfere with the assay of CK-MB by some immunoinhibition method.Hypothyroidism is the main cause of myopathy.About 60% of hypothyroid subjects show an average elevation of CK activity fivefold greater than the URL.The major isoenzyme present is the CK-MM,suggesting muscular involvement.[14][15]

LDH activity is present in many cells of the body and is invariably found in the cytoplasm of the cell.Therefore leakage of the enzyme from even a small mass of damaged tissue increases the observed serum activity of LDH to a significant extent.In heart,liver,kidney and erythrocytes LD-1 and LD-2 predominate ,whereas in liver and skeletal muscle,LD-4 and LD-5 isoenzymes predominate.Other isoenzyms are present in various sources like spleen, lungs,lymph nodes,leukocytes,platelets).Elevations of LDH activity are observed in liver diseases and since in hypothyroidism the hepatocytes are damaged so LDH level in the serum increases.

Thyroid hormones exert their effect on all tissue and modulate the rate of metabolic activity.Alterations in thyroid function can affect the various organ system of body and perturb measures like AST, ALT, GGT, ALP, CPK, LDH.In this we are mainly concerned with CPK and LDH.[1][3][4]

In this study, in majority of the patients, it was found that the increase in the levels of CPK and LDH in hypothyroid patients is several times more than in euthyroid patients.45 euthyroid controls and 47 hypothyroid patients were included in this study and their LDH and CPK levels were tested, it was found that the mean for LDH levels in hypothyroids is several folds higher than the mean for the euthyroids. Similarly, for CPK,it's mean was also found to be about 2.83 times in hypothyroids than that for the euthyroids.

There is the recognition of a pattern of elevations of serum enzymes in hyperthyroid and hypothyroid patients. Most probably the reason behind this increase is LDH and CPK are cytosolic and in hypothyroidism the membrane permeability increases so these hormones leak into the blood streams.

The findings of this study is supported by similar finding of Raju Pandey et al[12]. Saha B et al [13]also found similar increase & showed that replacement therapy with thyroxine resulted in remarkable lowering of creatine kinase not only from high level to normal as early as 3 weeks even before normalization of thyroid stimulating hormone, but also from high normal to low normal level. Gaede JT et al [9]showed serum enzyme values which initially were elevated and fell to normal levels during thyroid replacement therapy. His study showed isoenzyme fractionation of LDH and CPK indicated skeletal muscle as the source of the elevated enzyme activity.

LIMITATION

More individuals should have been added into this present study.The increase in the sample size to 1000 or more would have given a better conclusion with less chances of error. More liver enzyme parameters should have been included & with the help of polyacrylamide gel electrophoresis it should have been identified whether the raised fraction of LDH & CPK was of hepatic origin or of muscular origin.

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