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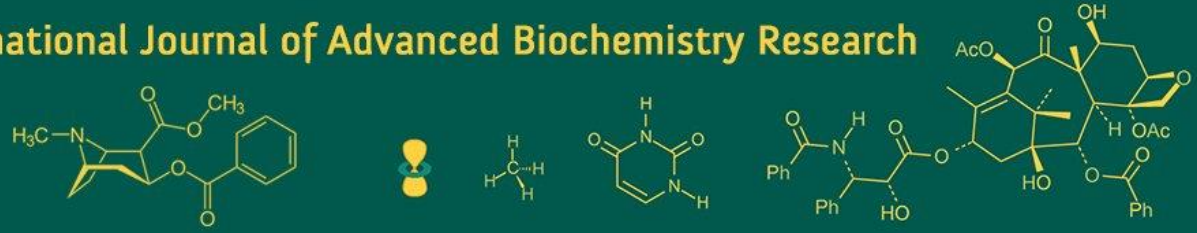

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Study of muscle biomarkers in hypothyroid patients

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Abstract
Background: Hypothyroidism is characterized by a decline in basal metabolic rate which can lead to glycogen build-up and decreased enzyme activity in energy synthesis and other regulatory systems. Thyroid hormones have a number of key targets including skeletal muscle. Thus, in hypothyroid patients altered glycolysis, glycogenolysis and mitochondrial metabolism have an impact on skeletal muscle function. Timely assessment of muscle biomarkers Creatine Kinase (CK) (NAC) [N-acetyl-cystein], CK-MM, Lactate dehydrogenase (LDH), Aspartate Transaminase (AST) and Aldolase in patients of hypothyroidism can estimate the usefulness of these enzymes as screening tests to detect muscle damage associated with hypothyroidism.
Objective: To estimate the serum CK(NAC), CK-MM, LDH, AST and Aldolase activity and compare with normal healthy controls.
Materials and Methods: The study was conducted in the Department of Biochemistry, Teerthanker Mahaveer Medical College & Research Centre Moradabad. CK (NAC), CK-MM, LDH, AST and Aldolase were measured in 60 patients with hypothyroidism under the age group of (20-60) years using automated method and their levels were compared with 60 healthy individuals as controls.
Results: Serum CK (NAC), CK-MM and Aldolase were significantly higher in cases than the controls (p<0.001) whereas LDH (p = 0.418) and AST (p=0.406) were slightly increased in hypothyroid patients as compared to the normal individuals but the difference was not statistically significant.
Conclusion: Muscle markers can be used to assess disease severity in hypothyroid patients. Early quantitation of the activity of these enzymes can help in the management of comorbidities associated with hypothyroidism as well as in improving the quality of life.

Keywords: Aldolase, AST, CK (NAC), CK-MM, hypothyroidism and LDH

Introduction
Thyroid hormones T_3 (triiodothyronine), T_4 (tetraiodothyronine) and Thyroid stimulating hormone (TSH) are produced by the thyroid gland and play a major role in the control of BMR, tissue differentiation and growth. T_3 & T_4 hormones are produced with the help of TSH and Thyrotropin releasing hormones (TRH) [1, 2]. Hypothyroidism (related to a malfunctioning thyroid gland) and hyperthyroidism (due to an overreactive thyroid gland) are the two types of thyroid disorders [3].
Primary hypothyroidism is a clinical illness caused by decreased T_3, T_4 and an increase in TSH hormones [4]. It is a widespread disorder that affects 4 to 15% of the world's population in mild to severe forms. In India, females are more often affected than males. Hypothyroidism affects approximately 11% of women and the risk of acquiring it increases as one reaches maturity [5]. Muscle's soreness, pale dry skin, a bloated face, mild fatigue, cold intolerance, reduced joint mobility, carpel tunnel syndrome, weight gain and some severe manifestation like cardiomyopathy and myxedematous coma are all signs of hypothyroidism [6].
Hypothyroidism is characterised by a decrease in baseline metabolism rate which can lead to glycogen build up and decreased enzyme activity in energy synthesis and other regulatory systems. Hypothyroidism lowers adenosine triphosphate (ATP) levels below a hazardous threshold by reducing glycolysis and oxidative phosphorylation [7].
Thyroid hormones have a number of targets, including skeletal muscle. Hypothyroidism has systemic effects due to metabolic disturbances or myxoedematous infiltration of connective tissue which includes the build-up of glycosaminoglycans in the tissues of many organs such as skeletal muscles, kidneys and other organs resulting in multiple organ dysfunction.

As a result, skeletal muscle performance in hypothyroid adults is affected by changes in glycogenolysis and mitochondrial metabolism [8, 9]. In hypothyroidism, clinical indicators of muscle damage include CK, Aldolase, AST and LDH [10].

The enzymes CK is found in skeletal muscles, cardiac muscles and the brain. In healthy people serum CK activity is affected by age, race, lean, body mass and physical activity [11, 12]. In people with primary hypothyroidism, the amount of CK in their blood can vary. In skeletal muscle, only a modest quantity of CK-MB isoenzyme is detected, ranging from 0.2 to 0.15 percent of total enzyme activity. In skeletal muscles and heart tissue, CK activity is highest. As a result, total CK activity may be one of the sensitive diagnostic markers for various muscle diseases [13].

Aldolase is an enzyme that catalyses a variety of reactions in distinct pathways. For instance, fructose 1-6 bisphosphate is converted to glyceraldehyde and dihydroxyacetone phosphate during glycolysis. Aldolase concentration has been demonstrated to be affected by myotonic muscle diseases including progressive muscular dystrophy. As a result, Aldolase could be used as a useful and specific biomarker for muscle injury [14, 15].

Lactate dehydrogenase is an enzyme which interconverts pyruvate and lactate as well as NADH and NAD+. Pyruvate, lactate, NADH and NAD+ are all converted to NADH and NAD+ by lactate dehydrogenase. LDH activity has been observed to be changed in hypothyroid conditions according to several previous investigations. In several disorders the LDH isoenzymes are increased in blood [16]. LDH elevation could indicate either increased or decreased hepatic clearance. It has been seen that patients with hypothyroidism produce more lactate during exercise than healthy people, a finding that may be associated with poor mitochondrial oxidative dysfunction [17].

Aspartate aminotransferase is a transaminase that catalyses the reaction between aspartate and amino acids. This process happens in the mitochondria and cytoplasm which gives energy to tissue [18]. AST has a substantially longer half-life than CK and is found to have a much wider distribution of tissues. It's a cytoplasmic and mitochondrial enzyme that catalyses aspartate deamination to produce oxaloacetate which can then enter the Kerb's cycle and so contribute to ATP synthesis [19, 20].

Previous research works on the activities of CK, Aldolase, LDH and AST in people with hypothyroidism have yielded mixed results. Some scientists have noticed that there is no change in the activity of these enzymes in hypothyroid patients while, others have found that the activity of these enzymes increases in hypothyroid patients.

Hence, this study was intended with the aim to estimate the changes in activity of serum enzymes CK (NAC), CK-MM, LDH, Aldolase and AST in patients with hypothyroidism and to estimate the usefulness of measurement of these enzymes as a screening method to detect muscle damage associated with hypothyroidism. This study was also intended to find whether timely estimation of muscle biomarkers CK (NAC), CK-MM, LDH, AST and Aldolase in hypothyroidism could help in better management of comorbidities associated with hypothyroidism and bring about an improvement in the quality of life of these patients.

Materials and Methods

The present study has been done in the Department of Biochemistry, Teerthanker Mahaveer College and Research Centre, General Medicine OPD in Moradabad, Uttar Pradesh from January 2021 to December 2021. Total 120 subjects were selected and divided into two groups. Group I included 60 diagnosed cases with hypothyroidism. They were recruited for the study after taking their due written consent. The subjects were in the age group of 20 to 60 years [3] including both males and females. Group II included 60 healthy control subjects who were age and sex matched with the cases.

Exclusion Criteria

Muscle ailments with causes other than hypothyroidism [3], Cardiac disorders [3], Patients on long term medications affecting muscle biomarkers [21], Hepatic disease [21] Crush injury (Rhabdomyolysis) [21].

After overnight fasting for 12 hrs, blood sample (8 ml) was obtained from all the subjects & dispensed into plain vials under proper aseptic conditions. After centrifugation 3000 RPM for 5 min, serum sample was used for analysis.

Various methods were used -

- Creatine Kinase (CK(NAC), CK-MM) by UV -Kinetic Method [22, 23]
- LDH by (UV Kinetic Method) [24]
- AST by (UV Kinetic Method) [25]
- Aldolase by ELISA (Enzyme linked immunosorbent assay) [26]
- T_3, T_4 & TSH by ELFA (Enzyme Linked Fluorescent Assay) [27]

Statistical analysis: SPSS version 28.0 for data analysis and Microsoft Word and Excel for graph generation. Mean and standard deviation was assessed & comparison was done using student's t-test & Pearson's coefficient correlation (*r*-value).

- *p*-value of less than 0.05 was considered as significant [S].
- *p*-value of less than 0.001 was considered as highly significant [HS].

Results

The results of the measured by different biochemical parameters in the research were listed below for hypothyroid patients and healthy subjects.

Table 1: Distribution of Hypothyroid Patients (According to age)

Age Group (years)	Number of patients (n=60)	Percentage (%)
20-30	14	23.3
30-40	12	20
40-50	9	15
50-60	15	25

Table 2: Distribution of Controls according to age

Age Group(years)	Controls(n=60)	Percentage (%)
20-30	17	28.3
30-40	18	30
40-50	8	13.3
50-60	11	18.3

We found that 25% of the patients in this study were between the ages of 50 and 60. This indicates that hypothyroidism is more common in this age range. Patients between the ages of 20 and 30 accounted for 23.33% of the total. Patients aged 30-40 years were seen 20% of the time, whereas patients aged 40-50 years were seen 15% of the time. This study showed that 30% controls were seen in the age group 30-40 years. 28.3% healthy controls were seen in the age group 20-30 years. 13.3% healthy subjects were found in the age group 40-50 years. 18.3% healthy controls were observed in the age group 50-60 years.

Table 3: Comparison of level of T_3, T_4 & TSH between Hypothyroid Control and Cases

Thyroid Profile	Hypothyroid Controls (Mean±SD)	Hypothyroid Cases (Mean±SD)	p Value	Significance Level
T_3 ng/dL (0.60-1.85 ng/dL)	1.12±0.77	0.36±0.30	<0.001	HS
T_4 µg/dL (4.80-11.80 µg/dL	7.53±4.13	1.88±0.88	<0.001	HS
TSH µIU / mL (0.390-6.160 µIU/mL)	2.53±1.23	13.91±7.00	<0.001	HS

The mean±SD of T_3 levels of patients with hypothyroidism 0.36±0.30 ng/dL is lower as compared to mean±SD of controls 1.12±0.77 ng/dL. The levels of mean±SD of T_4 levels of patients with hypothyroidism 1.88±0.88 µg/dL is lower as compared to mean±SD of controls 7.53±4.13 µg/dL and the Mean±SD of TSH levels of hypothyroid patients 13.91±7.00 µIU/L is significantly higher than that mean±SD of controls 2.53±1.23 µIU/L. (The above result shows that the cases and controls have been selected appropriately).

Table 4: Comparison of CK (NAC), CK-MM, LDH, Aldolase and AST between Control and Cases

Muscle Markers	Controls	Cases	p-value	Significance Level
CK(NAC) IU/L (39-190 IU/L)	150.4±30.65	282.70±60.21	<0.001	HS
CK-MM IU/L (5-25 IU/L)	19.95±5.52	45.49±16.78	<0.001	HS
LDH IU/L (140-250 IU/L)	244.95±16.31	249.68±42.01	0.418	NS
ALDOLASE IU/L (1.0- 7.5 IU/L)	4.59±2.69	16.86±9.10	<0.001	HS
AST IU/L (5-40 IU/L)	40.06±7.06	43.00±26.37	0.406	NS

The activity of CK (NAC) in patients with hypothyroidism was more in cases than in healthy controls. The mean value of CK-MM was raised in hypothyroid cases in comparison to normal subjects. The analysis showed that the mean value of LDH was slightly increased in cases as compared to healthy individuals but the difference is not statistically significant. The concentration of Aldolase was elevated in hypothyroid patients as compared to the healthy controls. There is no significant difference in the value AST between cases and controls.

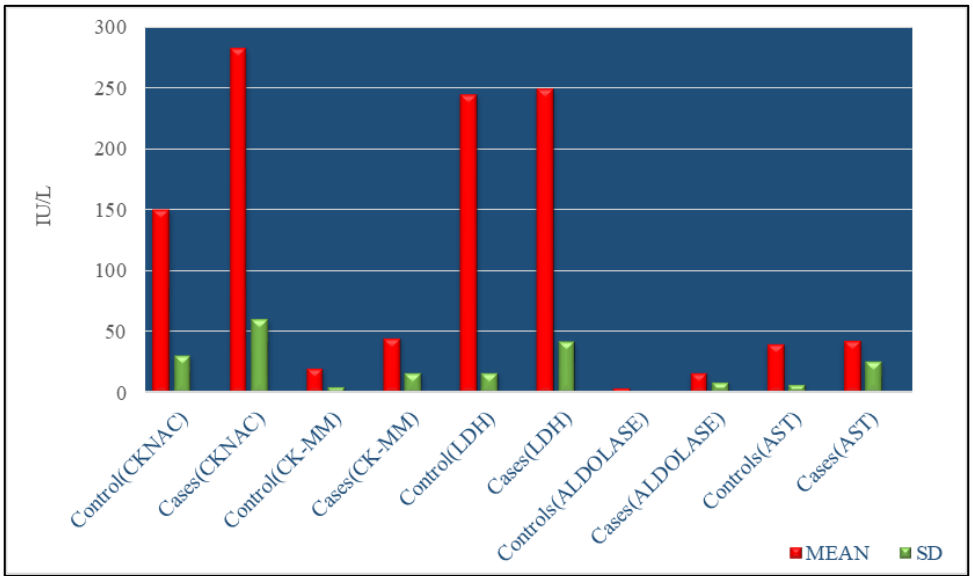

Fig 1: Muscle biomarkers in cases and controls

Discussion

The thyroid gland is a primary metabolic endocrine gland as T_3 and T_4 hormone secreted from thyroid gland are essential for the growth, development, and various other metabolic functions of the entire body organs. Hypothyroidism is a common endocrine condition caused by inadequate thyroid hormone production or decreased thyroid hormone activity [3].

Hypothyroidism can be caused by a variety of reasons including autoimmune disease, thyroiditis, iodine deficiency and hereditary conditions. Thyroid hormone production is impaired which impacts the functions of all other organs in the body [7]. These hormones have an impact on normal growth, metabolism and other processes as well as O_2 intake at cellular level. Weakness, fatigue, swollen face, cramps, weight gain, cold intolerance, exertional myalgias, muscle weakness and hair thinning are all symptoms of

hypothyroidism. The severity of the hormone deficiency determines the symptoms of hypothyroidism [5].

In overt hypothyroidism, the skeletal muscle damage is more common compared to subclinical hypothyroidism. Thyroid dysfunction results in a wide range of muscular aliments. It improves muscular sensitivity to circulating catecholamines and raises demand of micronutrients for muscles. Proximal muscular weakness, muscle pain and delayed tendon jerk relaxation are some of the heralding symptoms of muscle damage [13].

Therefore, this study was conducted to assess the effect of muscle markers such a CK (NAC), CK-MM, LDH, Aldolase and AST in patients with hypothyroidism.

In this cross-sectional study total of 120 patients were recruited of which 60 were healthy controls and 60 were patients suffering from hypothyroidism.

In table 1 the distribution of hypothyroid cases according to age has been shown. It was observed that 25% of the cases were in the age group of 50-60 years. 23.3% were in 20-30 years, 20% were in 30-40 years whereas 15% were in 40-50 years. Therefore, there is no significant effect of age distribution in hypothyroid patients.

In table 2 the distribution of hypothyroid controls according to the age was shown that 30% were in the age 30-40 years. 28.3% were in 20-30 years.18.3% were in 50-60 years and 13.3% were in 40-50 years.

In Table 3 the levels of T_3, T_4 and TSH in hypothyroid patients with that of controls has been compared. It was seen that the mean of T_3 levels in hypothyroid patients was 0.36±0.30 ng/dl whereas in healthy controls the mean value was 1.12±0.77 ng/dl.

We have found significant changes in the levels of T_3 in patients than healthy controls. The mean value of T_4 in cases of hypothyroid was 1.88±0.88 µg/dl and controls were 7.53±4.13 µg/dl. There was a significant decrease in the levels of T_4 in patients with hypothyroidism as compared to the healthy subjects. Our findings of mean value of TSH in cases of hypothyroid patients were 13.91±7.00µ IU/ml and 2.53±1.23 µIU/ml was in the healthy subjects. It shows that the levels of TSH in hypothyroid cases are high than the healthy controls (p<0.001).

In Table 4 the activity of CK (NAC), CK-MM, Aldolase, AST and LDH in hypothyroid patients was compared with that of controls. It has been assessed that the activity of CK (NAC) in patients with hypothyroidism was 282.70±60.21 IU/L and in the healthy subjects was 150.4±30.65 IU/L. There was a significant increase in the activity of CK (NAC) in hypothyroid cases as compared to the subjects.

These findings are similar with Mushtaq S, *et al*. 2014 [7] who found an increase in activity of CK in patients with hypothyroidism with mean value 108.79±34.92 IU/L as compared to controls 232.9±91.0 IU/L. There were 30 cases (25 females and 6 males) and 30 controls (25 females and 5 males). They concluded that T_3, T_4 and TSH along with muscles enzymes can be used to determine the severity as well as for screening and early diagnosis of hypothyroidism associated with myopathy.

In another study R. Shanti M, *et al*. 2017 [6] found elevation in CK activity in cases 224.94±76.7 IU/L compared to the healthy individuals 107±30.1 IU/L. There were 50 cases (36 females and 14 males) with 50 controls (31 females and 19 males). They observed a significant raised in serum CK and LDH activities in hypothyroid individuals.

Because CK facilitates the reversible transfer of high energy phosphate for storage of energy in the form of phosphocreatine by catalyses from ATP to creatine an increase in CK in hypothyroidism may be due to myofiber degeneration and a decreased rate of clearance of CK from circulation as a cause of raised CK in metabolic state of hypothyroidism that causes lowering in the site of glycolysis and oxidative phosphorylation resulting in reduce ATP production.

Another mechanism explaining the clinical outcome in hypothyroidism could be changes in sarcolemmal membranes which could lead to increased cell permeability and CK leakage from cells due to of the increased membrane permeability. Dystrophin deficiency makes the membrane more fragile, and contraction stress generates membrane rips, resulting in increased permeability.

In this study, it has been found that the mean value of CK-MM was 45.49±16.78 IU/L in hypothyroid cases and 19.95±5.52 IU/L in healthy controls. The activity of CK-MM in hypothyroid patients is slightly more as compared to controls.

Prakash *et al.* 2001 [28] also found the average rise in activity of serum CK 224.71±22.90 IU/L as compared to healthy individual 160.57±25.19 IU/L which is due to increased CK-MM (muscles isoform) which is similar to our findings.

LDH activity between the cases and the controls of hypothyroid was 249.68±42.01 IU/L and 244.95±16.31 IU/L. There is no significant rise in the activity of LDH in hypothyroid cases as compared to healthy controls.

This observation is different from A Vanangamudi, *et al.* 2018 [29] who had found increased activity of LDH in cases with hypothyroidism 428.70±146.39 IU/L than controls 343.64±93.75 IU/L. They studied 50 hypothyroid female patients and 50 healthy euthyroid females between the ages of 30 and 60. Hypothyroidism can produce a considerable rise in CK and LDH activity, according to their findings.

Similarly, Growder Mc DA, *et al*. 2011 [9] also found the high activity of LDH in subclinical 340.38±153.38 IU/L and in overt hypothyroidism patients 421.00±203.91 IU/L as compared healthy control 202.85±36.06 IU/L. In their study, they included 18 people with overt hypothyroidism 50 people with subclinical hypothyroidism and 99 healthy people as controls. They saw a considerable rise in LDH activity.

The LDH enzyme is found in all cells, although it is particularly abundant in the liver, muscle, and kidney. In the heart, LDH1 is the most important isoenzyme. LDH2 is found in both the heart and the muscles. The increased level of LDH could be attributed to a decrease in liver clearance. It can also rise as a result of myositis which is a skeletal muscle inflammatory disease seen in hypothyroidism. Similarly, LDH5 (M4) is elevated in muscular dystrophies. LDH1 (H4) levels are higher in myocardial infarction than LDH2.

In the present study the activity of Aldolase in hypothyroid cases was 16.86±9.10 IU/L and in control was 4.59±2.69 IU/L. There was a significant increase in the activity of Aldolase in hypothyroid cases compared with the healthy subjects.

Aldolase is a biomarker for muscle injury that is both important and specific. Myotonic muscle disorder, such as progressive muscular dystrophy causes a high concentration of Aldolase [10].

AST levels were 43.00±26.37 in patients of hypothyroidism and in controls 40.06±7.06 IU/L. There is no significant difference in the activity of AST between cases and controls. The findings of our study are different from Taywade O, *et al*. 2019 [5] who found elevation in the patients with hypothyroidism 57.97±19.33 IU/L as compared to controls 21.28±8.31 IU/L. They took 50 hypothyroid cases with 50 controls as study group. They also noticed an increase in blood enzymes, which can be used to screen for myopathy in hypothyroid patients and estimation of these enzymes can help to predict and monitor the outcome of hormone replacement therapy.

AST is found in all tissues except bone, with the liver, heart, kidneys, brain and skeletal muscles having the highest levels. Thyroid dysfunction is associated with an increase in AST levels. The half-life of AST is substantially longer than that of CK and is detected in a wider range of tissues than CK. Thus, the study included all the parameters CK-NAC, CK-MM, LDH, Aldolase and AST were increased in hypothyroid patients as compared with healthy subjects. The findings in the study are regarded as an important step to observe the relationship between muscle markers and hypothyroidism. They can be used as a diagnostic tool to prevent complications associated with increased activity in patients with hypothyroidism.

Conclusion

The activity of CK-NAC, CK-MM, LDH, Aldolase and AST was shown to be raised in individuals with hypothyroidism as compared to healthy controls in this study. Hypothyroidism is more common in the 50-60 age group according to this study. Males were not as affected as females. In hypothyroidism, skeletal muscles are affected which is reflected by an increase in these muscle enzymes. Myopathy in hypothyroidism is a significant cause of morbidity. Creatine kinase may be a better biochemical diagnostic for detecting muscle injury in hypothyroidism than other biochemical indicators. As a result, we conclude that biochemical muscle markers can be used to assess disease severity. Early detection and diagnosis of these enzymes can help with the management of comorbidities associated with hypothyroidism as well as to improve the quality of life in these patients.

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