Original article:

Biochemical evaluation of myopathy in Patients of hypothyroidism.

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Abstract:

Background: 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. The aim of the present study was to confirm the involvement of muscles in hypothyroidism using biochemical markers of muscle damage such as serum Creatine Kinase (CK), its isoenzyme CKMB, serum Lactate Dehydrogenase (LDH) and serum Aspartate Aminotransferase (AST/SGOT) and also to correlate the activity of these muscle enzymes with T3, T4, and TSH levels.

Methods: In this study thyroid function tests (T3, T4 &TSH), serum CK, CKMB, LDH and AST/SGOT were measured in 30 patients with newly detected overt hypothyroidism and the results were compared with that of 30 healthy adults who were taken as control.

Results: The results show that mean CK, LDH and SGOT/AST were significantly increased in patients with hypothyroidism while the rise in CKMB was statistically insignificant as compared to control subjects. A positive correlation was found between CK, CKMB, LDH & SGOT/AST levels with TSH levels and negative correlation was found with T3 and T4 levels.

Conclusion: The significant elevation of serum CK, LDH and SGOT activities indicate muscle involvement in hypothyroidism and that these enzymes can be used as parameters for screening of hypothyroid patients.

Keywords: Hypothyroidism, Creatine Kinase, Lactate Dehydrogenase

Introduction:

Overt abnormalities in thyroid function are common endocrine disorders affecting 5-10% of individuals over a lifespan[1]. Clinical symptoms and signs are often nonspecific and the diagnosis and monitoring of therapy depends crucially on measurement of thyroid hormones (tri-iodothyronine-T3 and thyroxine-T4) and thyroid stimulating hormone (TSH) in blood[2]. Hypothyroidism can cause several symptoms, ranging from mild (e.g. fatigue, weight gain, cold intolerance, mental slowing, muscle cramping) to severe (e.g. heart enlargement, myxodema coma). Without regards to the cause of hypothyroidism, neuromuscular and musculoskeletal manifestations can be observed in many patients with the condition [3]. The patients with hypothyroidism do have myopathy rather than functional muscle disease. Nonspecific muscle stiffness related to myalgia may be associated with serum muscle enzyme elevations. Serum creatine kinase (CK) was first used as a diagnostic aid in progressive muscular dystrophy in 1959 by Ebashi et al [4]. It has since become an important clinical marker for muscle damage. The serum CK level in healthy individuals depends on

age, race, lean body mass and physical activity [5]. Serum creatine kinase (CK) elevation can be observed in 57%–90% of patients with hypo-thyroidism[6]. CK MB- one of the iso forms of CK, is a marker for diagnosis of myocardial infarction. It is also increased in various inflammatory conditions of skeletal muscles and muscle damage and along with CK and troponin levels has also been reported to increase above reference values in hypothyroid patients without apparent myocardial damage[7]. Skeletal muscle is affected more profoundly in cases of overt hypothyroidism and less so when subclinical hypothyroidism is present[8] .

A majority of patients with hypothyroidism have been shown to have an increased serum CK. Furthermore only a few studies have investigated serum lactate dehydrogenase (LDH) and aspartate transaminase (AST) activity in patients with thyroid dysfunction. The present study was carried out to study muscle enzymes (creatine kinase, creatine kinase-MB, lactate dehydrogenase and aspartate aminotransferase) in cases of overt hypothyroidism and to correlate their levels with tri-iodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) levels.

Materials and Methods:

The current prospective case-control study was conducted in the Department of Biochemistry at Padmashree Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune-18. The proposal of the study was put forth in the meeting of ethical committee of the institute and necessary permissions and clearance was obtained. An informed consent was also obtained from each participant of the study population which consisted of sixty subjects aged between 20-50 years. Thyroid function tests (T3, T4 & TSH), serum CK, CKMB, LDH and AST/SGOT were measured in 30 patients with newly detected overt hypothyroidism *(0vert* hypothyroidism was defined as a TSH level of 20μIU/ml or more or a TSH level of more than 4.0μIU/ml with T3 or T4 concentration below normal)[9] and the results were compared with that of 30 healthy euthyroid adults who were taken as control. Exclusion criteria were taken to rule out other diseases which can alter the results of study like neuromuscular disorder or any recent cardiovascular or cerebrovascular event. Patients were also screened for any drug history, especially which can affect CK or thyroid hormone levels and recent history of intramuscular injections or strenuous exercise was ruled out.

Under all aseptic precautions about 5ml of fasting morning blood samples were collected from antecubital vein in a plain vial without applying tourniquet. The blood was allowed to clot and was centrifuged at 1500 RPM for 10 minutes and serum was separated, and analyzed immediately on the same day for thyroid function tests (T3, T4 & TSH), serum CK, CK-MB, LDH and AST/SGOT levels. In case the sample analysis was delayed, it was refrigerated at 2-8°C for a maximum period of 3 days.

Serum T3, T4 and TSH were measured by microplate enzyme immuno assay (ELISA method). The kit used to analyze thyroid hormones was Accubind Elisa Microwells and measured on Lisa Plus Microplate reader (RT – 2100C). Serum CK, CKMB, LDH and AST/SGOT were measured on semi auto analyzer.

Data Analysis:

The data obtained was compiled and analysed statistically using students *t*-test. *p* value of less than 0.05 was considered to be statistically significant. Pearson's correlation coefficient was used to find out the correlation.

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Results:

The study population consisting of the control group and study group having 30 subjects each were comparable with regard to age (mean age of 33.3 ± 7.89 and 34.6 ± 5.6 years respectively) and gender (female/male 24/6 and 25/5 respectively) [Table 1]. Females constituted majority of study population probably because of higher incidence of hypothyroidism in them.

The mean values of T3, T4 and TSH were found to be 0.4 ± 0.2 ng/ml, 3.7 ± 1.5 µg/dl and 33.0 ± 12.5 µIU/ml in the study group (overt hypothyroid subjects) as compared to 1.13 ± 0.37, 7.63 ± 1.61 & 2.78 ± 1.25 respectively in the control group [Table 2]. Elevation of CK activity was found in 16 patients (53.33%, 16/30) with overt hypothyroidism as against only 2 patients (6.67%, 2/30) in the control group and the mean CK activity was found to be 232.9 ± 91 IU/L in study group as compared to 108.79 ± 34.92 IU/L in the control group. The result was statistically highly significant ($p < 0.001$). The isoenzyme of creatine kinase (CK-MB) was found elevated in only two patients with overt hypothyroidism and the result was statistically insignificant ($p=0.21$)[Table 3,Fig 1].

Elevation of LDH activity was found in 12 patients (40%, 12/30) with overt hypothyroidism as against only 1 patient (3.33%, 1/30) in the control group. The mean LDH activity in the study group was 242.5 ± 71.91 IU/L as compared with 157.79 ± 29.52 IU/L in the control group. The result was statistically highly significant ($p<0.001$). Similarly the SGOT levels were elevated in 11 patients (36.67%, 11/30) in the study group as against only 1 patient (3.33%, 1/30) in the control group. Also the mean SGOT levels were higher in the study group (45.0 ± 25.9 IU/L) as compared to the control group (20.04 ± 7.60 IU/L). The result was again statistically highly significant ($p<0.001$) [Table 3,Fig 1].

This study also indicates that serum enzyme activity of these muscle enzymes correlates with the degree of hypothyroidism. A positive correlation was found between CK, CK-MB, LDH and SGOT/AST levels with TSH levels (r = +0.82, +0.72, +0.75, +0.65 respectively) and a negative correlation was found between CK, CK-MB, LDH & SGOT/AST levels with T3 (r = -0.5, -0.44, -0.40, -0.39 respectively) and T4 levels (r = -0.76, -0.67, -0.65, -0.65 respectively)[Table 4,Fig 2,3,4].

TABLE No. 1: AGE AND GENDER DISTRIBUTION OF STUDY POPULATION

Group	No.(F/M)	Range	Age (years) Mean ± SD	p value
Control	30(24/6)	20-50	33.3 ± 7.89	0.46^{NS}
Study	30(25/5)	26-47	34.6 ± 5.6	

[NS: $p > 0.05$; Not Significant.]

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TABLE No. 2: LEVELS OF SERUM T3, T4 & TSH IN STUDY POPULATION.

VARIABLES (Normal Range)	Control (Mean ± SD)	Study (Mean ± SD)	p value
Total T3 (0.5-1.85ng/ml)	1.13 ± 0.37	0.4 ± 0.2	0.0001 **
Total T4 (4.8-11.6µg/dl)	7.63 ± 1.61	3.7 ± 1.5	0.0001 **
TSH (0.5-6.05µIU/ml)	2.78 ± 1.25	33.0 ± 12.5	0.0001 **

[NS: $p > 0.05$: Not Significant; * $p < 0.05$: Significant; ** $p < 0.001$: Highly Significant.]

TABLE No. 3 : COMPARISON OF SERUM CK, CKMB, LDH & AST/SGOT LEVELS BETWEEN CONTROL AND STUDY GROUPS.

VARIABLES (Normal Range)	Control (Mean ± SD)	Study (Mean ± SD)	p value
CK (24-170IU/L)	108.79 ± 34.92	232.9 ± 91.0	0.0001**
CK-MB (< 25IU/L)	10.27 ± 3.35	11.8 ± 5.6	0.21^{NS}
LDH (103-227IU/L)	157.79 ± 29.52	242.5 ± 71.91	0.0001 **
AST/SGOT (5-34IU/L)	20.04 ± 7.60	45.0 ± 25.9	0.0001**

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TABLE No. 4: CORELLATION.

PARAMETERS CORELLATED	r value	p value
T3 with		
CK	-0.5	0.009*
CK-MB	-0.44	0.014*
LDH	-0.40	0.027*
SGOT/AST	-0.39	0.020*
T4 with		
CK	-0.76	0.0001**
CK-MB	-0.67	0.0001**
LDH	-0.65	0.0001**
SGOT/AST	-0.65	0.0001**
TSH with		
CK	+0.82	0.0001**
CK-MB	+0.72	0.0001**
LDH	+0.75	0.0001**
SGOT/AST	+0.65	0.0001**

NS, $p > 0.05$: Not Significant; * $p < 0.05$: Significant; ** $p < 0.001$: Highly Significant.

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Fig 1: Comparison of serum CK, CKMB, LDH and AST/SGOT levels between Control group and Study group.

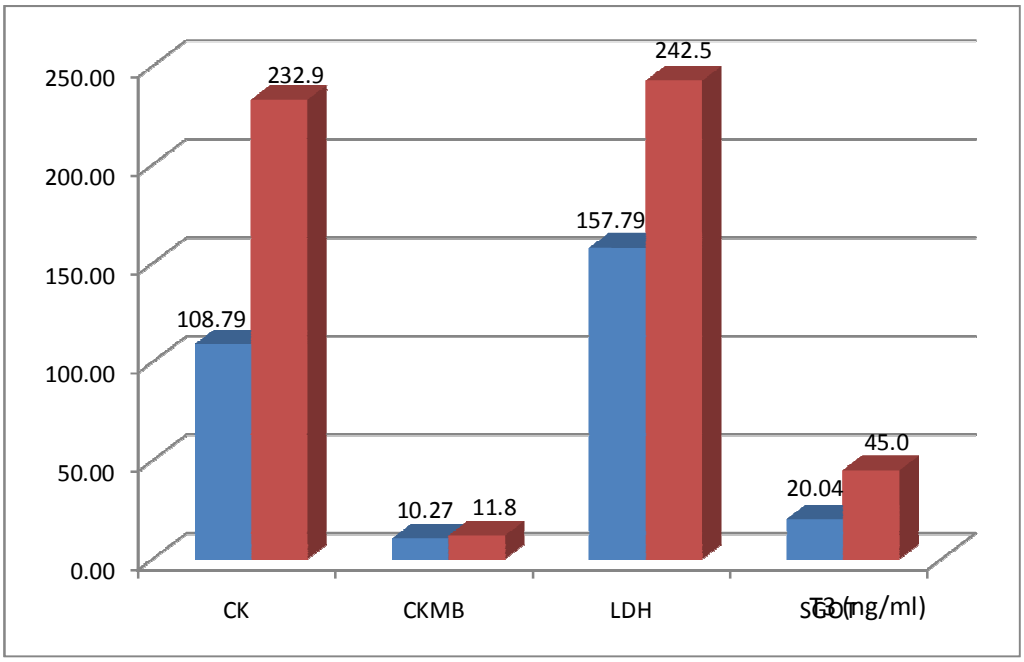

Fig 2 : Correlation of CK, CKMB, LDH & SGOT with T3 levels.

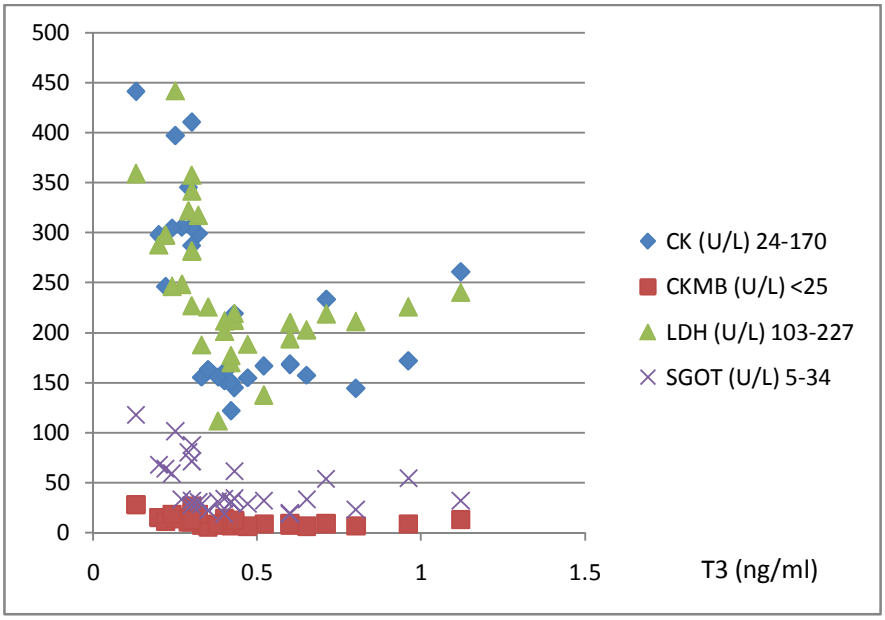

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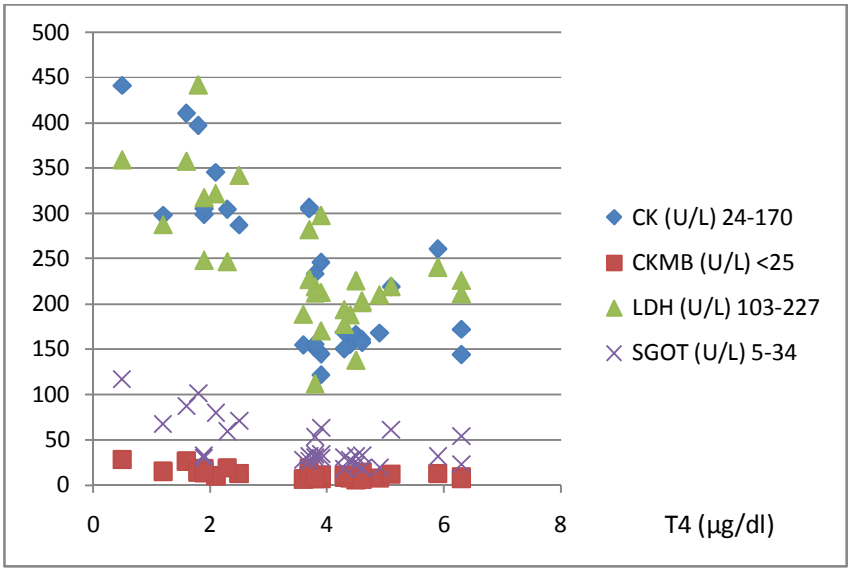
Fig 3 : Correlation of CK, CKMB, LDH & SGOT with T4 levels.



Discussion:

The findings of this study confirm that serum CK activity is frequently increased in patients with overt hypothyroidism. These findings are in accordance with those of other studies, which report a 43% to 97% elevation of serum CK activity in hypothyroidism[9,10,11]. However this is in contrast to the findings of Hartl et al**,** who found an elevation of CK activity in only 2 of 69 patients[12] . Prakash A et al in her study also reported average elevation of serum CK in 60% of hypothyroid subjects that is mostly due to increased CK-MM (muscle isoform)[13]. However, some studies reported CK-MB (heart isoform) levels to also increase above reference values in hypothyroid patients[7,14].The study also found increase in LDH and AST/SGOT activity in patients with overt hypothyroidism. Fleisher GA et al also reported 37% of hypothyroid patients to have elevated LDH levels[15] . In another study elevation of LDH activity was found in 33% of patients with overt hypothyroidism and in 74% of patients with subclinical hypothyroidism[2] and in a latter study 27 of 45 hypothyroid patients had elevated total LDH levels[16]. The levels of AST/SGOT were reported to be elevated in 60% of patients with hypothyroidism by Griffith PD[17]. **S**enzyme activity of these muscle enzymes correlated with the degree of hypothyroidism which is in accordance with other studies[2,9,13,18,19,20] .Involvement of skeletal muscle is among the most prevalent clinical consequences of hypothyroidism. Histologically the muscle fibres show enlargement, focal myofibrillar degeneration, increase in central nuclei, glycogen accumulation and mitochondrial aggregations and type II fibre atrophy[23]. Slowed muscle contraction and relaxation, known as hypothyroid myopathy may be caused by a shift in the distribution of muscle fibre types from fast-twitch fibres to slow-twitch fibres. A reduction in muscle mitochondrial oxidative capacity and beta-adrenergic receptors, as well as the induction of an insulin-resistant state, may result in these changes. Evidence from a study by Sinclair and colleagues suggests that a decrease in muscle carnitine in patients with either hypothyroidism or hyperthyroidism may contribute to thyroid myopathy[24].

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The hypo-metabolic state of hypothyroidism can cause a reduction in glycolysis and oxidative phosphorylations and thus reducing adenosine triphosphate (ATP) concentrations beyond a critical limit. The alteration in sarcolemmal membranes can cause increased cell permeability and the leakage of enzymes from cells[25]. Another possibility is reduced turnover of enzymes because of hypothyroidism, allowing serum enzyme activities to rise generating a marked release of muscle enzymes through the altered sarcolemmal membranes[22].

The significant elevation of serum activities of these enzymes indicate muscle involvement in hypothyroidism which may present as clinical or subclinical myopathy and needs high index of suspicion. These enzymes also correlate with disease severity and show a positive correlation with TSH levels and a negative correlation with T3 and T4 levels.

Conclusion: We conclude that these muscle enzymes along with T3, T4 and TSH can be used to determine the severity as well as for screening and early diagnosis of hypothyroidism and any associated underlying myopathy.

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