

Alteration in Serum Creatine Phosphokinase in Hypothyroid Female Subjects of Reproductive Age Group in an Urban Population in Eastern India: A Cross Sectional Observational Study

Dr. Samarjit Koner¹, Dr. Arunima Chaudhuri²

¹Demonstrator, Department of Biochemistry, College of Medicine and Sagore Dutta Hospital (Affiliated to West Bengal University of Health Sciences), Kamarhati, West Bengal, India.

²Associate Professor, Department of Physiology, Rampurhat Government Medical College and Hospital (Affiliated to West Bengal University of Health Sciences), Rampurhat, West Bengal, India.

Corresponding Author: Dr. Arunima Chaudhuri

ABSTRACT

Background: Serum creatine phosphokinase (CPK) acts as important clinical parameter in muscle damage. Hypothyroid subjects may have elevated serum CPK level but correlation of the two is not well established till date.

Aims: To assess the significance of increased serum CPK level as biochemical tool in hypothyroidism in an urban young female population of reproductive age group in eastern India.

Materials & methods: This cross sectional study was conducted on 200 hypothyroid subjects in Burdwan Medical College in a period of 12 months after taking Institutional Ethical Clearance. 100 subjects were enrolled in the study as control. Serum TSH, FT4 and CPK levels were estimated.

The subjects were age matched. The computer software SPSS, version 16.0 was used for analyzing data.

Results: Among 200 hypothyroid subjects enrolled in the study, 50 hypothyroid patients had increased Serum CPK level (25%). Significant difference was observed between control and hypothyroid subjects for mean TSH ($P < 0.0001$), mean FT4 ($P < 0.0001$) and mean CPK ($P < 0.0001$). Serum CPK level was positively correlated with TSH levels ($r = 0.761$, $P < 0.00001$) and Serum CPK was negatively correlated with Sr. FT4 ($r = -0.328$; $P < 0.00001$).

Conclusions: It may be concluded from our study that Hypothyroid females in reproductive age group may have significant increase in serum CPK levels as compared to normal subjects and may be considered as important biochemical tool for the screening of muscle damage in hypothyroid individuals.

Keywords: Creatine phosphokinase (CPK), Hypothyroidism, Indian female population.

INTRODUCTION

Thyroid disorders are one of the common endocrine disorders among Indian population. [1,2,3] Thyroid hormones have a significant role in general body metabolism, development, growth and tissue differentiation. [4]

In 1959 serum CPK was first used as diagnostic tool in progressive muscular dystrophy and becomes an important clinical marker in muscle damage. The

serum CPK levels in healthy persons depend on age, race, lean body mass and physical activity. [1-7]

Hypothyroidism occurs as failure of thyroid gland to produce sufficient thyroid hormone or insufficient thyroid gland stimulation by hypothalamus or pituitary gland. [8] Thyroid diseases may give rise clinical features of neuromuscular dysfunction and has been found to be associated with proximal muscle weakness,

mononeuropathy and sensory motor polyneuropathy. [9-11] Muscular involvement is common with 30-80 % of hypothyroid patients with different symptoms. [4]

Elevated serum CPK levels have been found in hypothyroidism in different studies. [12-20] Different mechanism has been proposed for increased CPK activity in hypothyroidism with varying influence at various stages of diseases. They include the following: hypometabolic state in hypothyroidism causing reduction in glycolysis and oxidative phosphorylation leading to decrease ATP concentration; leakage of CPK from cells by alteration of sarcolemmal membrane; reduced turnover of CPK in hypothyroidism. [1,7,21] Decrease in enzyme clearance and subnormal temperature may also contribute to increase in serum CPK levels. [1]

CPK is found in both the cytosol and mitochondria and it is a compact enzyme. CPK plays an important role in muscle and brain and builds up a large pool of phosphocreatine for buffering of ATP levels. [17]

The present study was conducted to evaluate the occurrence of increased serum CPK level in hypothyroidism and to assess the significance of increased serum CPK level as biochemical tool in hypothyroidism in an urban young female population of reproductive age group in eastern India. Hypothyroidism is more common in females as compared to males, so they were taken as the study population. Thyroid disorders are common endocrine disorder in India but there is paucity of data on its prevalence in young women. So females of reproductive age group were chosen for our present study.

MATERIALS AND METHODS

This cross sectional study was conducted in Burdwan Medical College on 200 newly diagnosed female hypothyroid subjects in a period of 12 months after taking approval from institutional ethics committee and informed consent of the participating subjects. 100 controls (normal

thyroid status) were taken for the study. The formula we used for the calculation of the size of the required sample was $n=(z)^2p(1-p)/d^2$, n= sample size, z= z statistic for a level of confidence (95% level of confidence used, so z value =1.96), p= expected prevalence of proportion, d= desired precision taken as 6% and previous studies were taken into consideration.

Inclusion criteria:

Newly diagnosed 200 hypothyroid female subjects of reproductive age group attending in the Department of Biochemistry, Burdwan Medical College and 100 control subjects were enrolled for the study.

Exclusion criteria:

- Neuromuscular disorders
- Recent cardiovascular accident
- Recent cerebrovascular accident
- Drugs affecting serum CPK or thyroid hormone levels)
- Recent history of intramuscular injections
- Strenuous exercise
- Hypertension, diabetes mellitus

Parameters studied:

- Age
- TSH
- FT4
- Serum CPK

Methods:

Approval from the Institutional ethics committee was taken before conduction of the study and informed consent was taken from the participants. Subjects were recruited by random sampling using an online randomizer. Detailed history was taken from each subject as per case record format. Participants were also screened based on the inclusion and exclusion criteria.

Blood samples were drawn from subjects by sterile needle and syringes and sent to biochemical laboratory in sterile vials for analysis.

Estimation of Serum TSH was done by Quantitative determination of TSH concentration by Microplate immuno-enzymometric assay using Monobind Inc.

USA manufactured TSH AccuBind ELISA Kit (Normal value:0.39-6.16 micro IU/ml).

Estimation of Serum FT4 level was done by Quantitative determination of FT4 concentration by Microplate Enzyme Immuno assay using Monobind Inc. USA manufactured FreeT4 AccuBind ELISA Kit (Normal value:0.8-2.0 ng/dl)

Biochemical methods:

Estimation of TSH:

Measurement of serum TSH is generally regarded as the most sensitive indicator for the diagnosis of Hypothyroidism.

Test principle: (Method-Immunoenzymometric assay)

The immobilization occurs at the surface of microplate well between the interaction of streptavidin coated on the well and biotinylated monoclonal anti-TSH antibody. By mixing the monoclonal biotinylated antibody, the enzyme labelled antibody and a serum containing native antigen, reaction occurs between native antigen and the antibodies to form a soluble sandwich complex. Then the complex is deposited to the well. After equilibrium is achieved, the antibody bound fraction is separated from unbound antigen by aspiration or decantation. The enzymatic activity of the antibody bound fraction which is directly proportional to the native free antigen concentration is measured by adding substrate. By utilizing calibrators of known antigen concentrations, a dose response curve can be generated from which the antigen concentration of an unknown sample can be ascertained

Kit content:

1. Streptavidin Coated Microplates-96 wells coated with streptavidin and packaged in an aluminium bag with a drying agent.
2. TSH Enzyme Reagent- 13 ml/vial containing enzyme labelled polyclonal antibody, biotinylated monoclonal IgG in buffer, dye and preservative.
3. Thyrotropin Calibrators-seven vials(0.5ml/vial) of references for TSH Antigen at levels of 0,0.5,2.5,5.0,10,20 and 40 μ IU/ml

4. Substrate A(7ml/vial) -one bottle containing tetramethylbenzidine (TMB) in buffer.

5. Substrate B(7ml/vial)-one bottle containing hydrogen peroxide (H₂O₂) in buffer.

6. Stop Solution (8ml/vial)- one bottle containing a strong acid(1 N HCL)

7. Wash Solution Concentrate (20 ml)-one vial containing a surfactant in buffered saline. A preservative has been added.

Calculation of results:

- (1) Calculation of the mean absorbance value of calibrator and samples was done at 450 nm.

- (2) A point to point curve was plotted by plotting the absorbance of each calibrator on the vertical Y-axis against concentration of each calibrator on the horizontal or X-axis.

- (3) Using the absorbance value for each sample the corresponding concentration of TSH was determined in microIU/ml. from the standard curve.

Estimation of FT4: -

Thyroxine circulates in blood almost bound to carrier proteins. Thyroxine binding globulin (TBG) is the main carrier protein. Only the free (unbound) fraction of thyroxine is biologically active. Concentrations of the carrier proteins are altered in different clinical conditions. So the free thyroxine (FT4) concentration remains constant. The measurement of FT4 concentration correlates better than total thyroxine level.

Test principle: (Competitive Enzyme Immunoassay-EIA)

In competitive EIA, a competitive reaction results between the native free antigen and enzyme-antigen conjugate for limited number of insolubilized binding sites on antibody coated on the micro well. After the equilibrium is attained the antibody-bound fraction is separated from unbound antigen by aspiration or decantation. The enzymatic activity of the antibody bound fraction which is inversely proportional to the native free antigen concentration is measured by adding substrate. By utilizing calibrators of known antigen concentrations, a dose

response curve can be generated from which the antigen concentration of an unknown sample can be found out.

Kit contents: -

1. FT4 Antibody coated microplate (96 wells)-one 96 well microplate coated with anti-thyroxine serum.
2. Enzyme reagent (13ml/vial)-one vial of thyroxine-horseradish peroxidase (HRP) conjugate in a protein stabilized matrix.
3. FT4 calibrators (0.5 ml/vial)- six vials of human serum based reference calibrators for free thyroxine.
4. Substrate A (7 ml/vial)-one bottle containing tetramethylbenzidine (TMB) in acetate buffer.
5. Substrate B (7 ml/vial)-one bottle containing hydrogen peroxide (H₂O₂) in acetate buffer.
6. Wash solution concentrate (20 ml)-one vial containing a surfactant in buffered saline.
7. Stop solution (8 ml/vial)-one bottle containing a strong acid (1 N HCL).

Calculation: -

1. Calculation of absorbance value was done at 450 nm.
2. A point to point curve was plotted by plotting the absorbance of each calibrator on Y axis against concentration of each calibrator on X axis.
3. Using the absorbance value for each sample the corresponding concentration of FT4 was determined in ng/dl. from the standard curve.

Estimation of serum CPK:

Serum CPK activity was measured by modified International Federation of Chemical Chemistry (IFCC) method and was determined by a semi-automated analyzer (Erba Mannheim, Germany) using reagents supplied from Erba diagnostic Mannheim, Germany (normal value-24-145U/L in women)

Principle of CPK estimation:

Creatine kinase catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. ATP causes phosphorylation of glucose to Glucose 6 PO₄ in presence of Hexokinase. Glucose 6 PO₄ is oxidized to 6

phosphogluconate which reduces NADP to NADPH in presence of Glucose 6 PO₄ dehydrogenase. The rate of absorbance change at 340 nm is directly proportional to creatine kinase activity.

Hypothyroidism was defined as an elevated TSH (>6.16 micro IU/ml) with a decreased (<0.8ng/dl) or normal serum FT4 level (range:0.8-2.0 ng/dl) as per kit values. Increased CPK level was considered above 145U/L as per kit value.

Statistical data analysis:

The computer software “Statistical Package for the Social Sciences (SPSS) version 16 (SPSS Inc. Released 2007.SPSS for Windows, Version 16.0. Chicago, SPSS Inc.)” was used to analyze the data, P<0.05 was considered as significant and P<0.01 was considered as highly significant.

RESULTS

200 newly diagnosed hypothyroid female subjects and 100 controls were enrolled in our study. 50 subjects were having increased serum CPK level out of 200. There was no significant difference in age between the two groups (Age in years: 30.82±6.55 vs. 31.54 ±6.68; P value: 0.376). Prevalence of increased serum CPK was 25% in hypothyroid subjects.

Table 1: Shows Age, TSH, FT4 and serum CPK values of control and hypothyroid subjects

Parameter	Control (Mean±SD)	Hypothyroidism (Mean±SD)	P value
Age (years)	30.82 ± 6.55	31.54 ± 6.68	0.376
TSH(micro IU/ml)	1.90 ± 0.91	18.93± 9.50	<0.0001**
FT4(ng/dl)	1.32 ± 0.24	0.78±0.36	<0.0001**
Serum CPK(U/L)	43.58±11.86	112.92±40.40	<0.0001**

P<0.05*significant, <0.01** highly significant

Table 1 shows that the difference of TSH, FT4 and serum CPK were highly significant.

Significant difference was observed between control and hypothyroid subjects for mean TSH (P<0.0001), mean FT4 (P<0.0001) and mean serum CPK (P<0.0001). No significant difference was observed for mean age between control and hypothyroid subjects (Table 1, Figure 1).

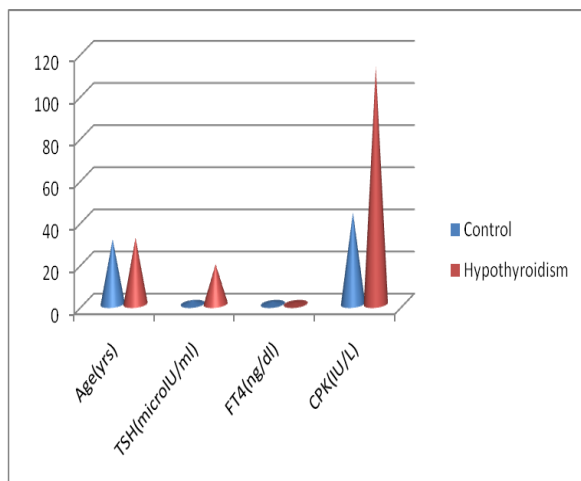


Figure 1: Shows the difference of Age, TSH, FT4 and serum CPK values between control and hypothyroid subjects

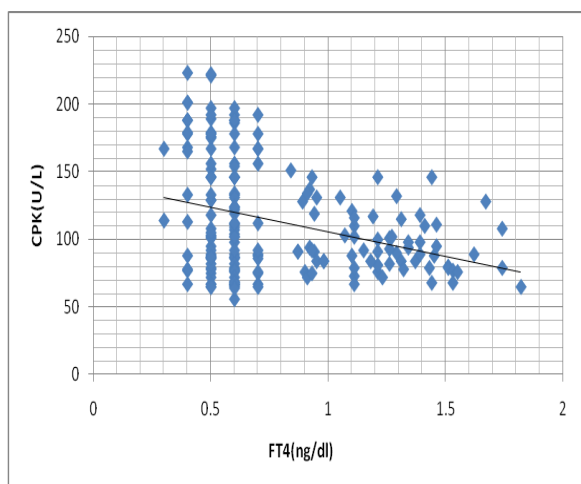


Figure2: Shows serum CPK level was negatively correlated with Sr.FT4 ($r = -0.328, P < 0.00001$)

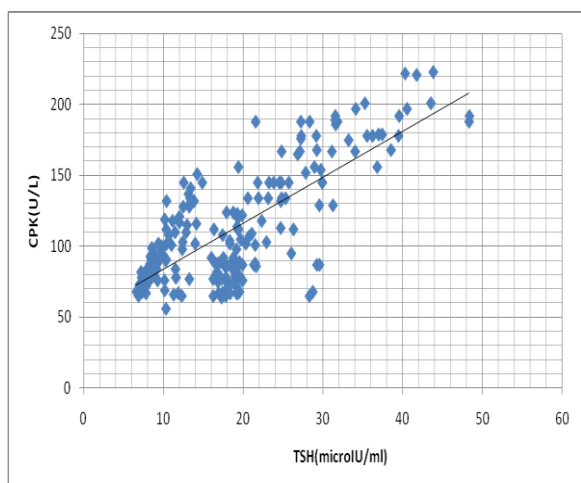


Figure 3: Shows serum CPK was positively correlated with TSH ($r = 0.761, P < 0.00001$).

Serum CPK level was negatively correlated with Sr. FT4 ($r = -0.328, P < 0.00001$) (Figure 2) and serum CPK was

positively correlated with serum TSH ($r = 0.761, P < 0.00001$) (Figure 3).

DISCUSSION

In India 42 million patients are suffering from thyroid disorders. [3] The reduced serum levels of triiodothyronine (T3) and thyroxine (T4) in hypothyroid patients is well documented but whether there is any correlation between creatine phosphokinase (CPK) and hypothyroidism is not well established. [12]

The present study was conducted to evaluate significance of increased serum CPK level in hypothyroid individuals in an urban female population of reproductive age group. We had taken 200 hypothyroid female subjects and 100 controls in our study. Significant difference was observed between control and hypothyroid subjects for mean TSH ($P < 0.0001$), mean FT4 ($P < 0.0001$) and mean serum CPK level ($P < 0.0001$). No significant difference of age was found between the two groups.

Alteration in cellular function and energy metabolism, abnormal glycogenolysis, mitochondrial oxidative metabolism and triglyceride turnover in muscle play the role in pathogenesis of muscle involvement in hypothyroidism. [22] Serum CPK measurement is important for evaluation of muscle weakness or myalgia and assessing myopathies or rhabdomyolysis. But increased CPK may be incidental finding without muscle symptoms. [23]

Severe hypothyroidism may be associated with highly elevated serum CPK and myopathy and adequate therapy leads to complete recovery. [24,25] Madhu et al. [26] in 2010 and Scott et al. [27] in 2002 in their studies found marked reduction of serum CPK level after giving thyroid replacement therapy along with resolution of clinical symptoms.

Increased serum CPK levels in hypothyroid individuals have been observed in many studies. [12-15,27-31] In the present study we observed 25% cases with increased

serum CPL level in hypothyroid subjects (50/200).

Musculoskeletal and neuromuscular findings may be noticed at duration of hypothyroidism disease process. [28] Muscle symptoms of hypothyroidism include muscle pain, cramp, stiffness, easy fatigability and weakness. [32-34] In some cases, myopathy may be manifested with increase in serum levels of CPK, Lactate dehydrogenase and aldolase level. [28]

A study by Lima et al [13] in 2012 observed a positive correlation between TSH and CPK ($r=0.065$) and negative correlation between CPK and FT4($r=-0.091$, $P<0.05$). Hekimsoy et al [15] in their study noticed skeletal muscle involvement by hypothyroidism more in overt hypothyroidism as compared to subclinical hypothyroidism. They found a positive correlation between CPK and TSH ($r=0.432$, $P=0.04$) and a negative correlation between FT3 and CPK ($r=-0.556$, $P=0.002$). Another study by Tejomani et al [28] in 2013 showed a significant positive correlation between CPK and TSH ($P<0.001$) in both overt and subclinical hypothyroid cases and a significant negative correlation between CPK and T3($P<0.001$) in overt hypothyroid cases.

In the present study we observed the prevalence of increased Serum CPK was 25%. Serum CPK was negatively correlated with serum FT4($r= -0.328$, $P<0.00001$) and serum CPK was positively correlated with serum TSH ($r=0.761$, $P<0.00001$).

CONCLUSION

It is evident from our study that hypothyroid females in reproductive age group may have significant increase in serum CPK levels as compared to normal subjects and may be considered as important biochemical tool for the screening of hypothyroidism and muscle damage.

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