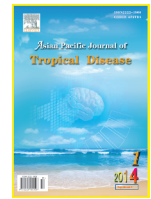




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# Does assessment of carbonic anhydrase activity support the diagnosis of various thyroid disorders

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## PEER REVIEW

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### Comments

Over all, the paper is very informative and gives very scientific information, which makes us rethink about the relationship of carbonic anhydrase activity and thyroid disorders.

Details on Page S341

## ABSTRACT

**Objective:** To evaluate the activity of carbonic anhydrase (CA) in various thyroid disorder patients and their results were compared with euthyroid acting as a control.

**Methods:** Two hundred and sixty-three participants were enrolled for the present study with ages ranging from 6 months to 74 years. Among them, hypothyroid, hyperthyroid and euthyroid subjects were chosen. Twelve hours' fasting blood samples were collected from all the patients reported to Medicine Out Patients Department and assessment of CA activity was done in all the three category patients. The data from the patients and controls were compared by using the student's *t*-test. The values were expressed as mean±standard deviation. Microsoft Excel for Windows 2003 was used for statistical analysis. *P*<0.05 were considered to indicate statistical significance.

**Results:** The serum levels of CA activities were observed to be higher in hyperthyroid patients compared to hypothyroid ones and the CA activity in euthyroid subjects were comparatively higher than hypothyroid subjects. It was also observed that the serum CA activity was significantly correlated with both T4 ( $r=0.997$ ,  $P<0.001$ ) and FT4 ( $r=0.590$ ,  $P=0.039$ ) values.

**Conclusions:** Though our study contains a very small sample size, as a pilot study we have demonstrated that the serum levels of CA activity is associated with the serum thyroxine levels.

## KEYWORDS

Hypothyroidism, Hyperthyroidism, Euthyroid, Carbonic anhydrase activity

## 1. Introduction

Carbonic anhydrase (CA) enzymes are virtually ubiquitous in all living systems having enormous functions, namely regulation of pH, fluid balance, carboxylation reactions and synthesis of bicarbonate[1]. Its serum levels have diagnostic implications in diseased states[2]. Reports from previous studies demonstrated increased CA activity in lung diseases associated with severe impairment of air passages, in certain liver diseases

and some hemolytic anaemias[3]. CA activities have been shown to alter in various pathological states, especially in diabetes mellitus, hypertension and lipid disorders[4,5]. The CA activity is shown to alter in thyroid disorders, where hypothyroidism is associated with increased activity and hyperthyroidism with decreased activity[6]. Since there is no concrete findings in association of CA activity with thyroid disorders, the current study was designed to establish the relation with CA activity to those with hypo and hyperthyroid patients and the findings

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were compared with euthyroid subjects.

## 2. Materials and methods

Two hundred and sixty–three participants were enrolled for the present study with ages ranging from 6 months to 74 years. Among them, hypothyroid 85 (16 men and 69 women, 13–74 years of age), hyperthyroid 65 (13 men and 52 women, 9–67 years of age) and euthyroid 113 (23 men and 90 women, 8–74 years of age) were recruited for the study. The study was pre–approved by the Ethical Committee of this Institution Review Board.

### 2.1. Inclusion criteria

Patients with thyroid disorder attending at Medicine Out Patients Department (OPD) confirmed by the laboratory tests were included in the study.

### 2.2. Exclusion criteria

Patients with hypertension, dyslipidemia and current smokers were excluded. Also patients on lipid lowering drugs and antioxidant vitamin supplements were also excluded.

### 2.3. Sample collection

Twelve hours' fasting blood samples were collected from all the patients reported to Medicine OPD with chief complaints of thyroid associated clinical symptoms. Also euthyroid patients were selected as controls. The patients selected for the study were registered in OPD of Nil Ratan Sarkar Medical College and Hospital, Kolkata. Five mL of blood samples was collected from the participants and was allowed to clot and then carefully centrifuged at 3000 r/min for 10 minutes. Clear serums were collected and kept in –4 °C until tests were performed. Serum samples obtained were used for analysis of biochemical parameters.

### 2.4. Assay of CA activity

The assay system consisted of 100 µL of sample (serum) containing 1.4 mL of 0.05 mol/L Tris–SO<sub>4</sub> buffer (pH 7.4) and 1.5 mL of 3 mol/L p–nitrophenyl acetate. The change in absorbance at 348 nm was measured over a period of 3 minutes, before and after adding the sample. One unit of enzyme activity was expressed as 1 mol/L of released p–nitrophenol per minute at room temperature[7].

### 2.5. Estimation of serum thyroid stimulating hormone (TSH), free thyroxine (FT4), triiodothyronine (T3) and total thyroxine (T4)

Serum TSH, T4, T3 and FT4 were estimated by enzyme–linked immuno sorbent assay (ELISA) methods using standard ELISA reagent kits (Lilac) and automated ELISA Reader with Washer

(Tecan) by the following assay principles.

Statistical analysis: The data from the patients and controls were compared by using the student's *t*–test. The values were expressed as mean±standard deviation. Microsoft Excel for Windows 2003 was used for statistical analysis. *P*<0.05 were considered to indicate statistical significance.

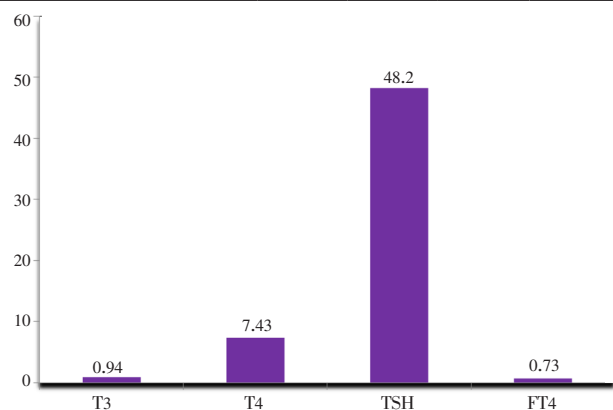
## 3. Results

Serum T3, T4, TSH and FT4 levels in subjects with hypothyroidism, hyperthyroidism and euthyroidism are depicted in Table 1 and Figures 1, 2 and 3 respectively.

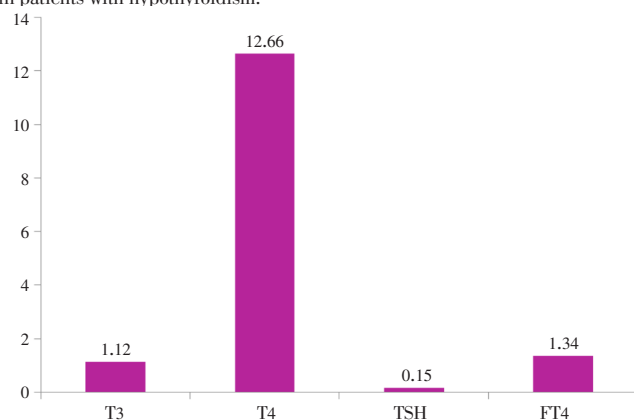
**Table 1**

Thyroid profile in study subjects.

Parameters	T3	T4	TSH	FT4
Hypothyroid patients (n=85)	0.94±0.48	7.43±3.1	48.2±86.1	0.73±0.31
Hyperthyroid patients (n=65)	1.12±0.48	12.66±5.8	0.15±0.14	1.34±0.41
Euthyroid subjects (n=113)	1.2±0.25	9.6±2.0	3.1±1.7	1.1±0.27
<i>P</i> value	ns	<0.05	<0.01	<0.05



**Figure 1.** Serum levels of T3 (ng/dL), T4 (µg/L), TSH (µIU/mL) and FT4 (ng/dL) in patients with hypothyroidism.



**Figure 2.** Serum levels of T3 (ng/dL), T4 (µg/L), TSH (µIU/mL) and FT4 (ng/dL) in patients with hyperthyroidism.

**Table 2**

Serum CA activity in patients with thyroid disorders.

Parameters	CA activity (U/mL)	<i>P</i> value
Hypothyroidism	7.51±1.68	ns
Euthyroidism	8.35±2.78	ns
Hyperthyroidism	9.05±3.22	ns

The serum levels of CA activity vs free thyroxine levels and total thyroxine levels among the study subjects (Figures 5 and 6). Pearson's correlation showed significant relationship of serum CA activity with both T4 (*r*=0.997, *P*<0.001) and FT4 (*r*=0.590, *P*=0.039) values.

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