Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading doi: 10.1016/S2222-1808(14)60467-9

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

Utpal Kumar Biswas*[1], Arun Kumar[2]

[1]*Department of Biochemistry, NRS Medical College, Kolkata, India*

[2]*Department of Biochemistry, Manipal College of Medical Sciences, Pokhara, Nepal*

PEER REVIEW

Peer reviewer

Dr Kalyan Goswami, Professor, Mahatma Ghandhi Institute of Medical Sciences, Sebagram, Wardha, PIN-442102.
Tel: 9970030441
E-mail: Goswamikln@gmail.com

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

*Corresponding author: Dr. Utpal Kumar Biswas, Associate Professor & Head, Department of Biochemistry, NRS Medical College, Kolkata, India.
Tel: +919051642109
E-mail: drutpalkumarbiswas2010@gmail.com
Foundation Project: Supported by a grant from Confederation of Epidemiological Associations registered under Govt of Kerala (Grant No. 30-955/2012 CEA).

Article history:
Received 13 Nov 2013
Received in revised form 18 Nov, 2nd revised form 23 Nov, 3rd revised form 28 Nov 2013
Accepted 15 Dec 2013
Available online 28 Jan 2014

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-SO4 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
P value	ns	<0.05	<0.01	<0.05

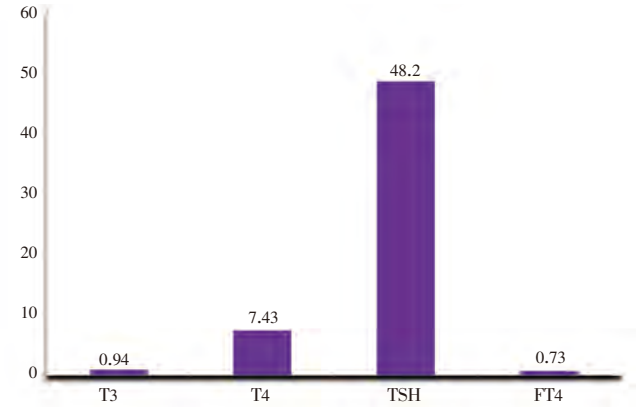

Figure 1. Serum levels of T3 (ng/dL), T4 (μg/L), TSH (μIU/mL) and FT4 (ng/dL) in patients with hypothyroidism.

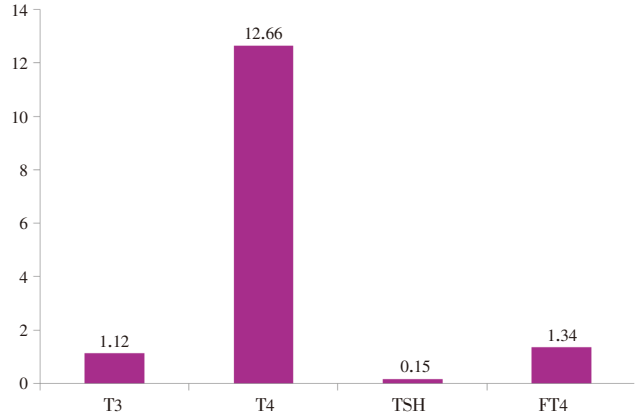

Figure 2. Serum levels of T3 (ng/dL), T4 (μg/L), TSH (μIU/mL) and FT4 (ng/dL) in patients with hyperthyroidism.

Table 2

Serum CA activity in patients with thyroid disorders.

Parameters	CA activity (U/mL)	*P* 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.

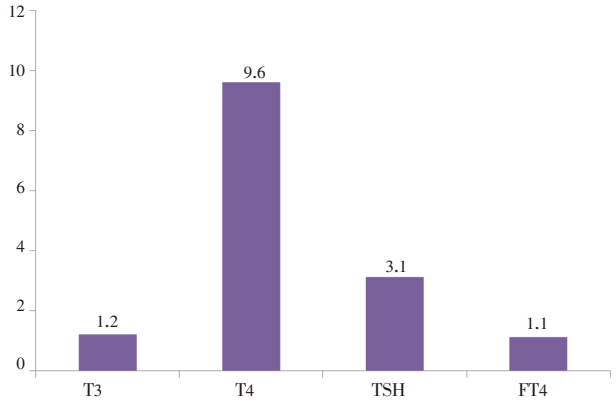

Figure 3. Serum levels of T3 (ng/dL), T4 (μg/l), TSH (μIU/mL) and FT4 (ng/dL) in euthyroid subjects.

Serum levels of CA activity in hypothyroid, hyperthyroid and euthyroid subjects are furnished in Table 2 and Figure 4.

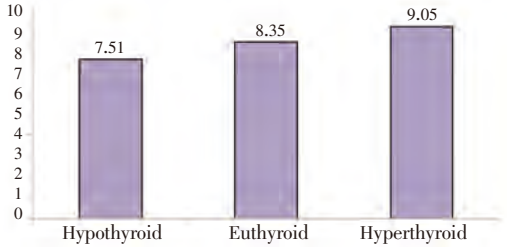

Figure 4. Serum CA activity in subjects with hypothyroidism, euthyroidism and hyperthyroidism.

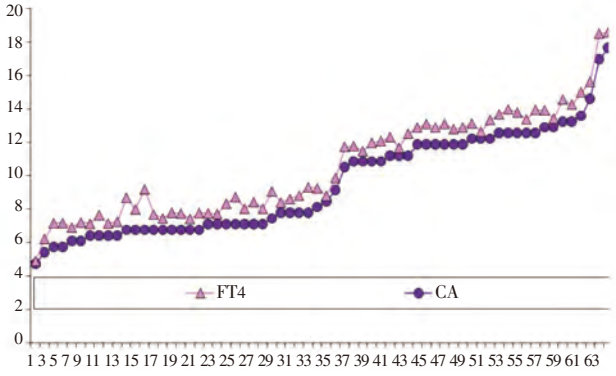

Figure 5. Serum CA activity vs FT4 levels.

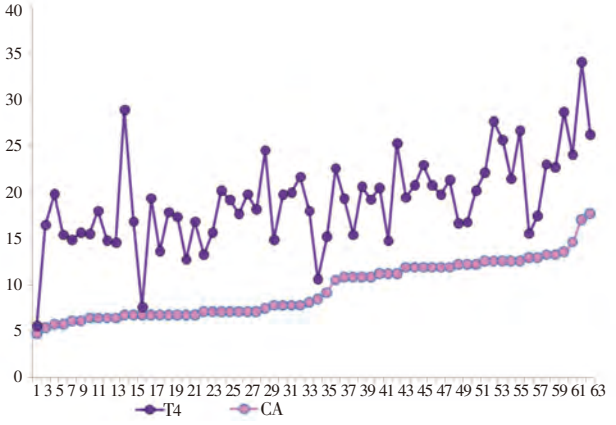

Figure 6. Serum CA activity vs total thyroxine (T4) levels.

4. Discussion

The aim of the current work was to observe the activity of CA in patients suffering from thyroid disorders. Though there is no significant difference which may be due to very small sample size, the subjects with hypothyroidism, euthyroid and hyperthyroidism had increasing levels of CA activity (Table 2). Previous study reported that T3 at a physiological free T3 concentration (0.54 nmol/L) significantly (32±7%, $P<0.01$) decreased CAI concentrations in YN-1 cells[8]. Similar study reported that T3 decreased the CAI concentration in BFU-E-derived cells in a dose-dependent manner[9]. Another study revealed that the decrease in CA activity of duodenal mucosa in hyperthyroidism was reversed in hypothyroidism, while both enzyme activities in the kidney were unrelated to thyroidal status[10]. Earlier study also showed that the zinc has some effects on TSH and thyroid hormone levels. Thyroid function influences zinc metabolism. In a study carried out in nephrectomized rats, it was observed that reduced thyroid function was strongly related to low serum zinc level[11]. It was reported that thyroid hormones modulated zinc transport activity in rat renal and intestinal brush border membrane[12]. Low zinc levels have a higher incidence of subclinical hypothyroidism and higher TSH levels that can be improved by zinc replacement[13]. Zinc effects on thyroid hormones are complex and include both synthesis and mode of action. A recent study observed that not only red blood cell (RBC) Zn but also the RBC CAI concentration in patients with Graves' disease reflected the patient's mean thyroid hormone level over the preceding several months. But in patients with sub acute thyroiditis, elevation of plasma thyroid hormone concentrations is transient and causes little change in the RBC CAI concentration[14]. Thyroid hormones are helpful in cell signaling and gene expression, so CA activity and expression may also be affected at the altered metabolism state[14]. Supplementation of zinc improves thyroid function in hypozincemic Down children[15]. In the current study, the serum CA activity is significantly associated with the T4 levels (Figure 6) and the FT4 levels (Figure 5). Further study with larger sample size is needed to establish a statistical significance of CA activity among different types of thyroid disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Supported by a grant from Confederation of

Epidemiological Associations registered under Govt of Kerala (Grant No. 30-955/2012 CEA).

Comments

Background

CA enzyme currently being studied in diseased state to diagnose the severity and progression as its activity is altered. Research reports have shown the link of CA activity in lung diseases, hemolytic anemia, diabetes mellitus, hypertension and lipid disorders. The CA activity is shown to alter in thyroid disorders, where hypothyroidism is associated with increased activity and hyperthyroidism with decreased activity. But still the mixed reports have been noticed. With keeping this view, the current study was designed to elucidate an association of CA activity with thyroid disorders.

Research frontiers

The CA activity is increased in hypothyroid and decreased in hyperthyroid disorders when compared to euthyroid patients. The levels of FT4 and T4 is directly proportional with the activity of CA, hence the assessment of thyroid disorder can be justified by assay of CA which is relatively cost-effective and gives valid diagnosis about the thyroid status.

Related reports

Earlier studies have not shown such valid results as some studies showed mixed results of CA activity with thyroid hormone and its status. Since this study have elaborately done on the various thyroid parameters with its correlation with CA activity hence, it is very informative and can be further conducted on large scale multi-centered study to substantiate the thyroid disorder by just doing CA assay.

Innovations & breakthroughs

The current study is innovative and gives us an idea to explore further and to re-think why and how CA activity is affecting the thyroid functions.

Applications

The CA activity can be used to analyze the thyroid disorder without doing thyroid parameters. This would help the poor patients who cannot afford to do hormonal analysis in a cost effective way.

Peer review

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

References

[1] Lionetto MG, Caricato R, Giordano ME, Erroi E, Schettino T. Carbonic anhydrase as pollution biomarker: an ancient enzyme with a new use. *Int J Environ Res Public Health* 2012; **9**(11): 3965-3977.

[2] Pirinççi N, Geçit I, Güneş M, Yksel MB, Kaba M, Tanik S, et al. Serum adenosine deaminase, catalase and carbonic anhydrase activities in patients with bladder cancer. *Clinics (Sao Paulo)* 2012; **67**(12): 1443-1446.

[3] Cobanoglu U, Demir H, Duran M, Şehitogullari A, Mergan D, Demir C. Erythrocyte catalase and carbonic anhydrase activities in lung cancer. *Asian Pac J Cancer Prev* 2010; **11**: 1377-1382.

[4] Biswas UK, Kumar A. Study on the changes of carbonic anhydrase activity in insulin resistance and the effect of methylglyoxal. *J Pak Med Assoc* 2012; **62**(5): 417-421.

[5] Das S, Biswas UK, Kumar A, Roy A. Study of serum carbonic anhydrase activity, uric acid, C-reactive protein levels and lipid parameters in patients with psoriasis. *Nepal J Dermatology Venereology Leprology* 2013; **11**(1): 20-27.

[6] Ertek S, Cicero AF, Caglar O, Erdogan G. Relationship between serum zinc levels, thyroid hormones and thyroid volume following successful iodine supplementation. *Hormones (Athens)* 2010; **9**(3): 263-268.

[7] Racker E. Glutathione reductase (liver and yeast). In: Colowick SP, Kaplan NO, editors. *Methods in enzymology*. New York: Academic Press; 1955: 722-725.

[8] Conrad AH, Zhang Y, Walker AR, Olberding LA, Hanzlick A, Zimmer AJ, et al. Thyroxine affects expression of KSPG-related genes, the carbonic anhydrase II gene, and KS sulfation in the embryonic chicken cornea. *Invest Ophthalmol Vis Sci* 2006; **47**(1): 120-132.

[9] Maragno A, Pironin M, Alcalde H, Cong X, Knobeloch KP, Tangy F, et al. ISG15 modulates development of the erythroid lineage. *PloS One* 2011; **6**(10): e26068.

[10] Carageorgiou H, Pantos C, Zarros A, Stolakis V, Mourouzis I, Cokkinos D, et al. Changes in acetylcholinesterase, Na_+,K_+-ATPase, and $Mg2_+$-ATPase activities in the forntalcortex and the hippocampus of hyper- and hypothyroid adult rats. *Metabolism* 2007; **56**(8): 1104-1110.

[11] Chen SM, Kuo CD, Ho LT, Liao JF. Effect of hypothyroidism on intestinal zinc absorption and renal zinc disposal in five-sixth nephrectomized rats. *Jpn J Physiol* 2002; **55**: 211-219.

[12] Prasad R, Kumar V, Kumar R, Singh KP. Thyroid hormones modulate zinc transport activity of rat intestinal and renal brush-border membrane. *Am J Physiol* 1999; **276**: E774-E782.

[13] Malik R, Hodgson H. The relationship between the thyroid gland and the liver. *QJM* 2002; **95**: 559-569.

[14] Halestrap AP, Wilson MC. The monocarboxylate transporter family—role and regulation. *IUBMB Life* 2012; **64**(2): 109-119.

[15] Bucci I, Napolitano G, Giuliani C, Lio S, Minnucci A, Di Giacomo F, et al. Zinc sulphate supplementation improves thyroid function in hypozincemic Down children. *Biol Trace Elem Res* 1999; **67**: 257-268.