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Original Article

Enhanced serum H_2S levels associated with TSH level In Hypothyroid Patients

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ABSTRACT

Background:
Hypothyroidism is one of the most common endocrine disorders in India and worldwide. Hydrogen sulfide (H_2S) is recently discovered as the third, gaso-transmitter, joining the ranks of the other two gaso-transmitters, namely nitric oxide (NO) and carbon monoxide (CO). H_2S has been implicated in regulating cardiovascular pathophysiology in experimental models [19, 20]. However, there is a paucity of information regarding the levels of H2S in thyroid disorders like hypo and hyperthyroidism.

Aims & Objective:
The current study aimed to estimate the serum H_2S levels in the patients with hypothyroidism and to find out its relationship with the serum Thyroid Stimulating Hormone (TSH) levels.

Methods:
Serum H_2S levels were measured in seventy recently diagnosed hypothyroid patients and compared with a similar number of healthy controls.

Results:
The serum H_2S level in patients is 38.84± 9.15micro mol/ml which is significantly (P< 0.001) higher than the value in controls which is 21.70 ± 8.70 micro mol/ml. We found there a significant positive correlation between serum TSH level and serum H_2S level. (Fig 26 showing r value=0.834 & p value=<0.001)

Conclusion:
The current study elucidated increased serum H_2S levels in patients with hypothyroidism. Serum H_2S level is positively correlated with the serum TSH (Thyroid Stimulating Hormone) level.

INTRODUCTION

Thyroid disorders are among the most common endocrine disorders worldwide[1, 2]. They affect almost 42 million people in India with a prevalence of 3.9% and 1.2% in different parts of the country[3-5]. As thyroid diseases can be definitively diagnosed using clinical examination and routine thyroid function tests, it is one of the most treatable and manageable endocrine disorders throughout the world provided it is diagnosed early. Failing an early diagnosis, thyroid disorders result in gross metabolic abnormalities encompassing almost all systems in the body and even may culminate in cancer of the thyroid gland.

Once the thyroid hormone-secreting cells, the thyrocytes are destroyed, it is difficult for the modern management system to help in their regeneration. Hydrogen sulfide (H_2S), one of the important gasotransmitters after nitric oxide and carbon monoxide[6, 7], has been reported to help at least in the partial regeneration of the thyrocytes[8]. The importance of this important gasotransmitter H_2S is reflected by the studies that have highlighted its important role in regulation of the arterial diameter, blood flow, and leukocyte adhesion, its anti-apoptotic action, anti-inflammatory function, and a strong nitric oxide like vasorelaxation function[6].

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The enzymes cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE) generate the H_2S in our body from L-cysteine Both of these enzymes are expressed in most of the tissues in the body. However, their expression is mostly in the central and peripheral nervous systems[9-12]. In these two enzymes, the CBS is mostly expressed in the brain whereas the CSE produces the H_2S in the thoracic aorta, portal vein, heart liver, and vascular and nonvascular smooth cells. On the other hand, enzymes like 3-mercapto pyruvate sulfur transferase are found to produce H_2S in both brain and vascular tissues[13, 14]. These two enzymes are regulated by several hormones and signaling factors and the direct inhibitory effects of other gaseous substances like nitric oxide (NO) and carbon monoxide (CO). The latter two gases NO and CO and stimulated also by the bacterial endotoxins[15, 16]. Enzymes like 3-mercapto pyruvate sulfur transferase (3MST), and cysteine aminotransferase (CAT), have been also found to produce this gaso-transmitter in the brain and vascular endothelium[13, 14].

The vascular relaxation effects of NO have been already well documented in both experimental animals and human subjects[17-20]. Its role in thyroid disorder has been highlighted recently where thyroid hormone levels were found to be inversely related to its blood concentration[21]. However, very few reports have been found that could highlight the relationship between thyroid function and H_2S. Keeping in mind the huge prevalence of thyroid disorder in our country we hypothesized that there is a role of this important gasotransmitter on thyroid function. Accordingly, the present study was designed to analyze the relationship between the blood levels of this gas and the thyroid function in hypothyroid patients.

MATERIALS AND METHODS:

This hospital-based case-control study was undertaken in the Department of Biochemistry in collaboration with the Department of Endocrinology in a tertiary care Medical College and Hospital in Kolkata, West Bengal, India.

A total number of 70 patients aged 20 to 60 years suffering from hypothyroidism were included in the study, from the outpatient department of Endocrinology of this Medical College and Hospital following pre-defined inclusion and exclusion criteria. Patients were selected using the method of convenience. This study was approved by the Institutional Ethics Committee. Patients having other endocrinal disorders like diabetes, pregnant women, polycystic ovarian disease, patients with malignant disease, and patients receiving antioxidant or H_2S inhibitors or donors were excluded from the study.

Sample collection:

The patients were selected using the method of convenience from the OPD of the Department of Endocrinology, after taking proper history. 6 ml of fasting blood samples were collected from both patients and controls aseptically in clotted vials. A collected blood sample was centrifuged at 2500 rpm for 5 minutes. The serum was separated and was kept in aliquots and stored in minus forty degrees Centigrade (-40˚C) in the deep freezer.

Serum Thyrotropin (TSH) levels and serum H_2S levels were measured in both patients and controls. Measurement of TSH done by standardized ELISA kit (Accubind).

Measurement of H_2S concentration in serum:

Principle:

Zn^{2+} was added to the serum sample to deposit H_2S, HS^- , and S^{2-}, as well as serum protein. ZnS deposition was re-dissolved by the addition of N, N-dimethyl-p-phenylenediamine, and the remnant protein was deposited by trichloroacetic acid. After centrifugation, ferric chloride was added to the supernatant fluid to generate methylene blue, which was analyzed by spectrophotometer at 670 nm.

Assay procedure:

425 microliters of PBS(Phosphate Buffer Saline) was taken in a glass tube and 75µl of serum was added along with 250µl of 10% tri-chloroacetic acid and the tube was capped. Next, the tube is centrifuged at 3000rpm for 15 mins the supernatant is decanted in another glass tube, and 250µl of 1% zinc acetate is added and capped again (with rubber cap or parafilm). Next 133µl of 20mM N, N-dimethyl- p- phenylene diamine sulfate, and 133µl of 30mM $FeCl_3$ were added and the tube recapped. 60µl of 10% NaOH was added and the resulting solution was incubated for 10 minutes at room temperature. The absorbance was taken in a spectrophotometer at 670 nanomater. All samples were assayed in triplicate and concentration in the solution was calculated against a calibration curve prepared using 25-250 micro mol/l concentrations of sodium sulfide (NaHS, Sigma-Aldrich,MO,USA) as shown in figure 1. Results of serum H_2S concentration were expressed in micromol/L.

Table-1 Intra and Inter-Assay Variations for Serum H_2S estimation:

Intra-assay variation				
Expt. No.	No. of replications	Mean	SD	CV (%)
1.	10	0.101	0.006	5.941
2.	10	0.066	0.005	7.576
3.	10	0.046	0.002	4.348

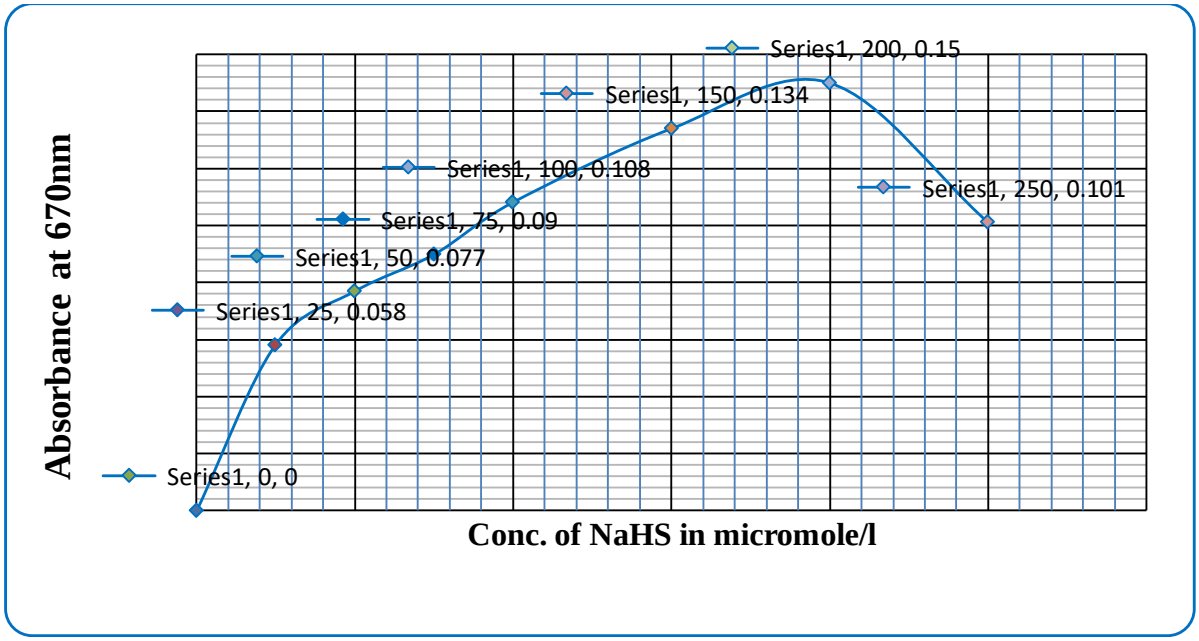
Inter-assay variation			
No. of replications.	Mean	SD	CV (%)
3	0.071	0.028	3.944

Intra and inter-assay coefficient of variation: The maximum intra-assay variation was 7.576 and inter-assay variation was 3.944. The linearity limit is from 25 to 200 µ mol/l of NaHS.

Statistical analysis:

Data were expressed as mean ± standard deviation(SD), Comparison of data was done using unpaired two-tailed student's t-test and Pearson's correlation, P<0.05 was considered as significant. The data were plotted in Microsoft Office Exel-2008 and statistical analysis were done using IBM SPSS version 2017. The data were analysed for normal distribution using Kolmogorov-Smirnov test.(It was found that the data were in normal distribution as P < 0.05).

Figure 1: Standard curve for assay of serum H_2S



RESULTS:

The clinical-biochemical parameters of the study subjects are depicted in Table 1. The serum TSH level in patients is 10.90±5.48 micro IU per ml which is significantly (P<0.001) higher than the value in controls which is 2.38 ± 0.936 micro IU per ml Figure 13). The serum fT4 level in patients is 0.96±0.29 nanogram/dL which is significantly (P<0.0019) lower than the value in controls which is 1.267 ± 0.14nano gm/dL (Fig 14). The serum fT3 level in patients is 1.73±0.55 picogram per ml which is significantly (P<0.001) lower than the value in controls which is 2.23 ± 0.175 picogram per ml(Fig 15).

The serum H_2S level in patients is 38.84± 9.15micro mol/ml which is significantly (P< 0.001) higher than the value in controls which is 21.70 ± 8.70 micro mol/ml (fig-18). The range of H_2S levels in patients varied from 21.66 to 68.33 micromol/ml and from 7.50 to 41micromol/ml in healthy controls. We found there a significant positive correlation between serum TSH level and serum H_2S level. (Fig 26 showing r value=0.834 & P value=<0.001)

Serum H_2S levels in the patients as well as in the control subjects in our study are comparable with earlier studies which is within the range, of 10 to 100 micromols per litre.

Table 1: The clinical and biochemical parameters of both patients and controls are shown

Variables	Patient mean (N=70)	Control mean (N=70)	P value
Age (years)	39.73 ± 9.66	40.07± 8.17	0.821
Height (m)	1.54± 0.05	1.545 ± 0.08	0.45
Weight (kg)	58.13 ± 10.97	60.9± 9.49	0.108
Body mass index (BMI)	24.67 ± 4.71	25.29 ± 2.85	0.342
TSH	10.90±5.48	2.38±0.94	<0.001
fT4	0.96±0.29	1.27±0.14	<0.001
fT3	1.73±0.55	2.23±0.18	<0.001
H_2S level (µ mol/l)	38.85 ± 9.15	21.7 ± 8.70	<0.001

Figure 1: Comparison of TSH level for patients and controls

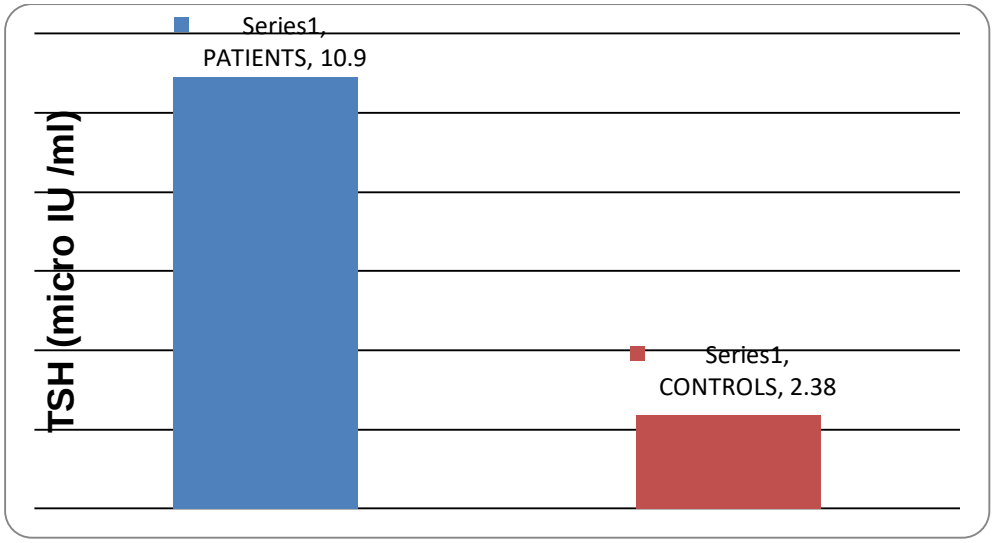


Figure 2: Comparison of H_2S level for patients and controls

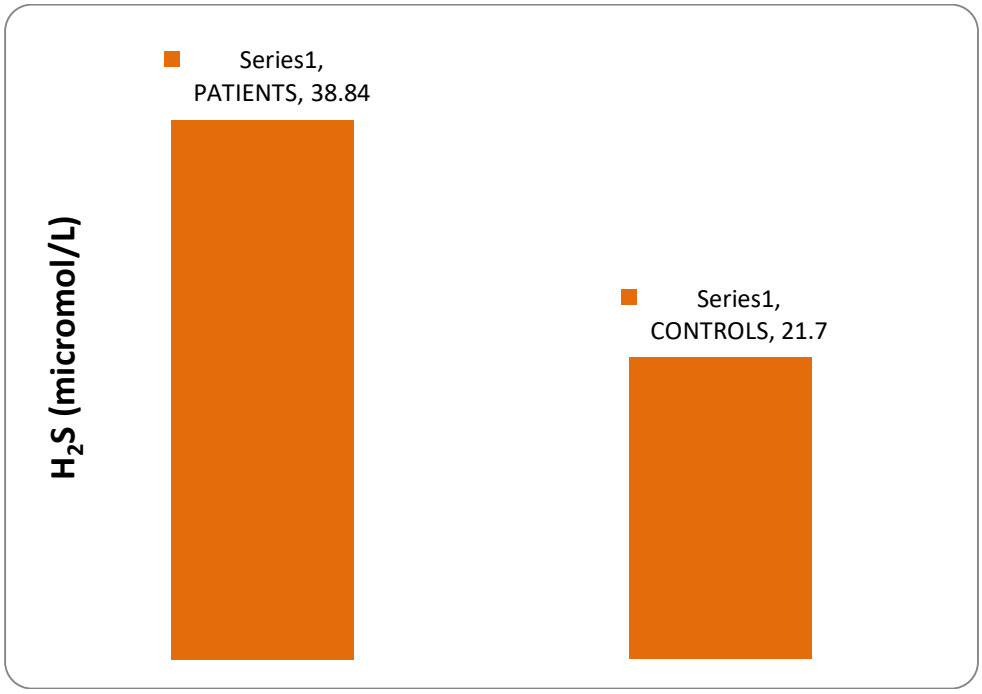
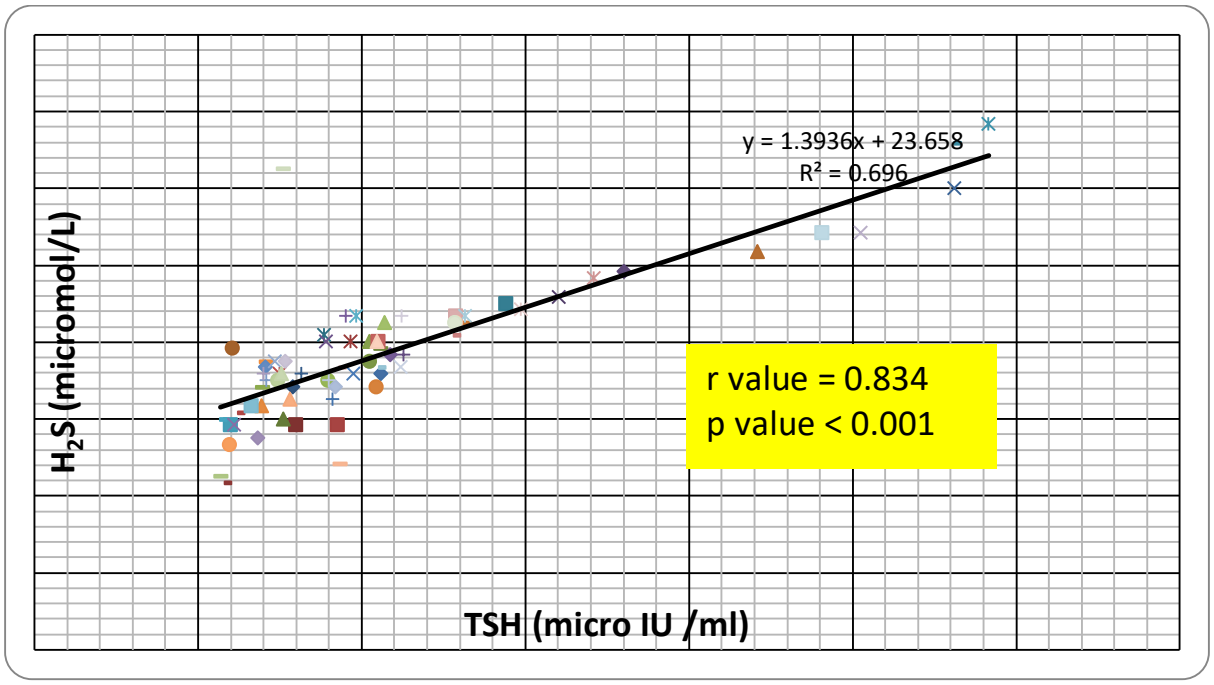


Figure 3: Scatter Diagram shows positive correlation between serum TSH levels with H_2S.



DISCUSSION:

Hypothyroidism is believed to be a common health issue in India as almost 42 million people are reported to suffer from thyroid disorders and 11% of the study population have been reported to suffer from hypothyroidism in some studies[22].

Previous studies have demonstrated the alteration of several vasoactive molecules, like Nitric Oxide (NO) in thyroid dysfunction and reported that endothelium-dependent vasodilation was blunted in hypothyroid rats (experimentally made by propylthiouracil) along with a reduction in their exercise tolerance [23]. Our study has indicated that the levels of hydrogen sulphide in the blood are significantly greater in the hypothyroid patients diagnosed as overt hypothyroid subjects with higher TSH and lower fT4 values ($p<.001$, Table 1 & 2, Figure 1). The data analysis revealed also that the degree of their increase is directly proportional to the raise in TSH values with a correlation coefficient of 0.834 and a P value <.001 (Figure 2 & 3). The effect of H_2S on the thyroid gland is supposed to be due to its effect on the blood vasculature and oxidative stress. Recent studies have indicated a role of endogenous H_2S in different pathophysiological condition including it's role in the vasculature[24].

Some recent reports suggest a cross talk between NO and H_2S in vascular reactivity and in other pathophysiological conditions that is explained by the vasodilator property of H_2S that is quite similar to the vasodilator property of the NO[24, 25]. Quite similar to the NO, H_2S can exhibit vasodilatory effects indirectly by delaying cGMP deactivation through PDE5 inhibition[25]. Several mechanisms like opening of the ATP-sensitive K+ (K_{ATP}) channels, induction of anti-oxidative molecules (e.g.-thioredoxin), reduction of lipid peroxide formation, up-regulation of the anti-apoptotic molecule Bcl-2, and activation of the anti-apoptotic signaling by Akt and MAP kinases have been suggested for the vasodilatory property of H_2S[26-28].

Our observation supports the fact that hydrogen sulfide plays a protective role in hypothyroidism by contributing to the antioxidant defense mechanisms as well as to the vasodilatory effect.

The potent antioxidant role of H_2S has been reported in many studies so far[29-31], particularly under more chronic conditions[29, 32]. The beneficiary effect of H_2S on thyroid hormone production is also demonstrated by its capability of inhibiting apoptosis of a number of cell types. This cytoprotective action may account for maintaining the normal lifespan of the thyroid cells, in turn, may improve thyroid function[33, 34]. H_2S has also been found to prevent ischemia-reperfusion injury in several organs like the heart, brain, liver, kidney, and lungs and to upregulate the superoxide dismutase activity in their mitochondria[35, 36].

Moreover, H_2S has been found to increase the cellular defence against the oxidative stress by enhancing the synthesis of glutathione in the mitochondria, increasing cellular uptake of cysteine, and regulating the activity of cytochrome oxidase of the respiratory chain[11]. In these ways, i.e by blunting the cellular respiration, decreasing oxidative stress and upregulating anti-oxidant defence, the H_2S promotes cell survival that most explicably improves the thyroid function. Through this ability to blunt cellular respiration, which in turn reduces mitochondrial ROS production and decreases mitochondrial uncoupling, H_2S can elicit cytoprotection. The present findings in our study indicates clearly that there was an increase in the H2S values in the patients which was directly proportional to the raise in their serum TSH levels. We propose this increase as a compensatory increase in the H_2S in response to the increase in TSH values and decrease in thyroxin levels (Table 1). This compensatory increase is most probably due to the body's effort for protecting its different organs and system against the hypothyroidism induced tissue damage. However, our study needs to be evaluated against the fact that it is a small scale horizontal study. For a more conclusive evidence vertically designed longitudinal studies and regression analysis are needed to provide definite proofs of this compensatory increase in the H2S gaso-transmitter hypothyroid disorders.

CONCLUSION:

The current study elucidated increased serum H_2S levels in the patients with hypothyroidism. Serum H_2S level is positively correlated with the serum TSH (Thyroid Stimulating Hormone) level. We propose this proportional increase as a compensatory increase of this cytoprotective gaso-transmitter to protect the cells against the adverse effects of hypothyroidism.

However, further larger scale longitudinal cohort studies are required in this direction to establish a potential role of H_2S and NO modulators towards the management of this non-communicable epidemic disorder.

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