Panacea Journal of Medical Sciences 2023;13(3):824–828

Content available at: https://www.ipinnovative.com/open-access-journals

Panacea Journal of Medical Sciences

Journal homepage: http://www.pjms.in/

Original Research Article

An observational study to determine relationship of serum hydrogen sulphide level in hypothyroid patients in a medical college in Kolkata

Ankita Bhattacharya[1], Sadajit Banerjee[2], Ipsita Bhattacharyya[1], Pinaki Saha[1]*, Debmalya Sanyal[3], Indira Bhaskar Biswas[1]

[1]*Dept. of Biochemistry, KPC Medical College and Hospital, Kolkata, West Bengal, India*
[2]*Dept. of General Medicine, Techno India DAMA Healthcare & Medical Centre, Kolkata, West Bengal, India*
[3]*Dept. of Medicine, KPC Medical College and Hospital, Kolkata, West Bengal, India*

ARTICLE INFO

Article history:
Received 13-01-2023
Accepted 21-05-2023
Available online 07-12-2023

Keywords:
Hypothyroidism
Thyroid

ABSTRACT

Hypothyroidism is an endocrinopathy eventuate due to destruction of thyroid cells incapacitating synthesis of thyroid hormones. H_2S which is a gas signaling molecule that might protect synthesis and secretion of thyroid hormone by upregulating the expression levels of thyroid hormone synthesis-related proteins and promotingting Thyroid peroxidase (TPO) activity through S-sulphydration of sirtuin-1 (SIRT-1). Depending on this fact we measured the H_2S level in a group of hypothyroid patient. The plasma H_2 S level in case in our study was 33.95 ± 4.14 micromol/l with the range from 27.82 to 43.94 micromol/l. This was significantly (P< 0.001) lower than age/sex matched healthy controls which was 64.67±4.25 micromol/l, with a range from 49.81 to 80.02 micromol/l. this study also elucidated that plasma H_2S levels were significantly correlated with level of diagnostic parameters of hypothyroidism.

1. Introduction

Dysfunction of thyroid gland broadly classified as hypothyroidism and hyperthyroidism. Thyroid hormone plays indispensable role in regulating various physiological functions. Depleted Thyroid hormone production and secretion causes hypothyroidism. In contrast, unconfined and excess free thyroid hormones leads to hyperthyroidism. Thyroid peroxidase (TPO) Pendrin, sodium/iodide symporter (NIS) and monocarboxylate transporter 8 (MCT8) are some of the major bio molecules which are prerequisite for synthesis and secretion of thyroid hormone in thyrocytes.[1]

After carbon monoxide (CO) and nitric oxide (NO), Hydrogen Sulphide is most commonly produced endogenously through enzymatic processes such as cystathionine β-synthase (CBS),[2] cystathionine γ-lyase (CSE)[3] and 3-mercaptosulphurtransferase (3-MPST).[4]

H_2S takes part in antiatherogenesis by S-sulphydration of sirtuin-1 (SIRT1), thereby diminishes apoptosis and cellular senescence in alveolar epithelial cells by modulating SIRT1. Auxiliary information suggest elevated glucose level has an antagonistic impact on thyroid cell line with consequential thyroid hormone deficiency through SIRT1 inactivation**.** Based on these derivations, contribution of H_2S in synchronising SIRT1 for thyroid function can be presumed.[1]

This probe is stationed to analyse the change in H_2S levels in hypothyroid patients. Also the regulatory effect of H_2S on thyroid function and the detailed mechanism were further explored.

* *Corresponding author*.
E-mail address: spinaki1973@gmail.com (P. Saha).

https://doi.org/10.18231/j.pjms.2023.150

2. Background

Hypothyroidism is an endocrinopathy eventuate due to destruction of thyroid cells incapacitating synthesis of thyroid hormones.[5] Xue Zhao et al postulated that H_2S, a gas signalling molecule perhaps foster and endorse the expression of thyroid hormone synthesis regulatory proteins and stimulating activity of TPO by way of SIRT1. In mammalian tissue, H_2S is a by-product created by action of two enzymes, cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS) pivoted by pyridoxal-5'-phosphate on amino acid termed L-cysteine.[6] Contemporary studies concluded a third enzyme coined 3-mercaptopyruvate sulfur transferase (3MST), along with cysteine aminotransferase (CAT), which synthesise H2 S in the brain and vascular endothelium.[7,8] CSE manifests principally in the thoracic aorta, ileum, heart, portal vein, vascular smooth muscle, kidney and liver whereas CBS is pronounced in the peripheral and central nervous systems.[9–13] Bulk of H2S is metabolized to sulphate and thiosulphate in mitochondria via oxidative metabolism. Low level H_2S transform to less toxic compounds through a non enzymatic course exclaimed as cytosolic detoxification pathway. Thiosulphate further remoulds to sulphate and/or sulphite catalyzed by thiosulphate cyanide sulphur-transferase (TST).[14] Thereafter, above-mentioned metabolised substances are excreted across the intestinal tract, kidney and lungs in less than 24 hours span to retain the normal magnitude of plasma H2S as it is non-toxic and dissipate.

3. Materials and Methods

We conducted a case control study in the department of Biochemistry and Medicine, KPC Medical College, Jadavpur, Kolkata, India & department of medicine, Techno India DAMA Healthcare & Medical Centre. Overall, 45 confirmed Hypothyroid patients were chosen of which 21 were male and 24 were female aged above 18 years as case with age matched healthy volunteers as controls consisting of 22 male and 23 females. Institutional Ethics Committee has pre-approved this piece for evaluation. After acquiring consent from all voluntary participants, blood samples to be collected aseptically in clot activator containing vials. Serum to be separated by centrifugation will be deep-frozen at -20°C for further analysis.

3.1. Inclusion criteria

3.1.1. Case

45 patients of age18 and above, both males and females, suffering from hypothyroidism (clinically evaluated and serum TSH>5.0 mIU/ml, fT4 value < 0.89 ng/dl) those who are willing to participate in the study procedure.

3.1.2. Control

45 healthy volunteers (euthyroid) of the same age group both males and females proposed to be included as controls in the study. Healthy controls are selected from staff, faculty and relatives of patients who are clinically healthy and euthyroid after laboratory evaluation.

Since it is a pilot study, few number of study subjects and controls have been recruited initially, later planned to workup evaluate in a larger sample size for both study subjects and controls.

3.2. Exclusion criteria

Patients are proposed to be excluded from the study who are suffering from:

1. Diabetes mellitus or other endocrinal disorders
2. Receiving antithyroid drugs
3. Anti TPO level high
4. Pregnancy
5. Autoimmune diseases

3.3. Measurement of H_2S concentration in plasma

Serum H_2S levels were assessed following modified versions of the methods reported earlier.[15–17] These procedures were calibrated in our setup.[18]

3.4. Principle

On addition of Zn^2+ to plasma, deposits of H_2S, $H^2–$ and $S^2–$, along with serum protein were obtained and these serum protein were again solvated using NaOH. ZnS deposits dissolved with addition of N, N -dimethyl-p-phenylenediamine, and trichloroacetic acid sediments residual protein in exist. Supernatant obtained following centrifugation was mixed with ferric chloride that produced Methylene blue. Reading was evaluated in spectrophotometer at 670nm.

3.5. Assay procedure

425 microlitres of PBS were mixed with 75 microlitres of serum and further diluted with 250 microlitres 10% trichloroacetic acid and heterogeneously stored in a well capped test tube. Subsequently, tube was centrifuged at 3000 rpm for 30 minutes. Thereafter, supernatant obtained after centrifugation was transferred to a fresh test tube. 250 microlitres of 1% zinc acetate was added to the tube containing supernatant and corked tightly. Additionally, 133 microliters of 20 mmol N, N-dimethyl-p- phenylenediamine sulphate and 133 microliters of 30 milimol of FeCl3 were put in the tube and recapped. The resulting solution was prepared by further addition of 60 microlitres of 10% NaOH and the tube was incubated for 10 minutes at room temperature. Each and every samples

were triplicates and concentration of H_2S in solution was measured against sodium sulphide (NaHS diluted in deionised water) calibration curve under micromol/L denomination.

3.6. Standardization of serum H_2S level assay

When NaHS is dissolved in water, HS- is released to forms H_2 S, with H+ ions in water. This is one of the mainstay for using Sodium Sulphide as standard for devising calibration curve plotted on a graph by dilution of stock solution under same conditions and technique for estimation of H_2S (Figure 1). The linearity limit is 25-250 micromol/l for NaHS. Hereunder, calibration curve is extrapolated with plasma concentration of H_2S in NaHS standards of manifold dilution.[6]

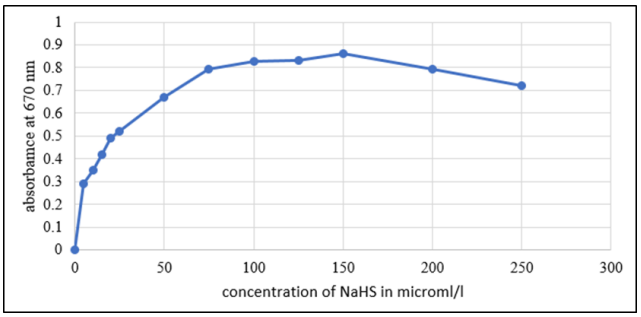

Figure 1: Standard curve of serum H2S assay

4. Results

The clinico-biochemical parameters of the study subjects are presented in Table 1. The serum H_2 S level in case in our study was 33.95 ± 4.14 micromol/l with the range from 27.82 to 43.94 micromol/l. This was significantly (P< 0.001) lower than age/sex matched healthy controls which is 64.67±4.25 micromol/l, with a range from 49.81 to 80.02 micromol/l (Table 1 and Figures 3 and 4). The level of TSH and FT4 in case was 61.02±21.78 and 0.78±0.067 respectively. Where as in age sex matched healthy control group, the TSH and fT4 levels are 3.85±2.23 and 1.31± 0.23 consecutively.

Table 1: The clinical and biochemical parameters of the study subjects

Variables	Mean ± SD		P value
	Case	Control	
Age (Years)	52.03±5.29	53.13±4.86	NS
Sex (M/F)	21/24	22/23	
TSH (mIU/ml)	61.02±21.78	3.85±2.23	<0.001
FT4 (ng/dl)	0.78±0.067	1.31± 0.23	<0.001
H2S (μ mol/L)	33.95±4.14	64.67±4.25	<0.001

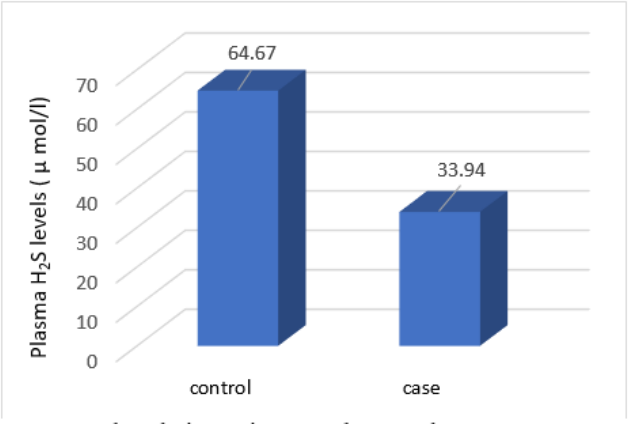

Figure 2: Comparison of serum H_2S levels in patients and controls

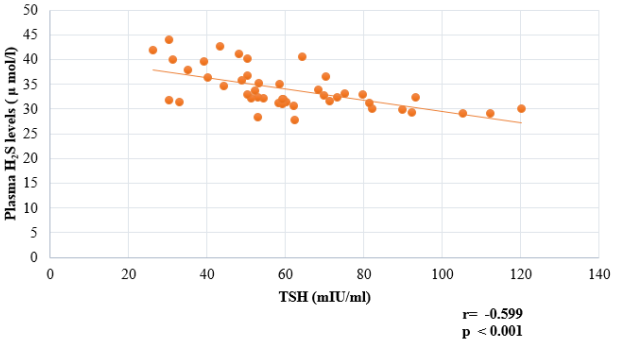

Figure 3: Scatterdiagram showing correlation between serum H_2S and TSH values (r= -0.599, P=<0.001)

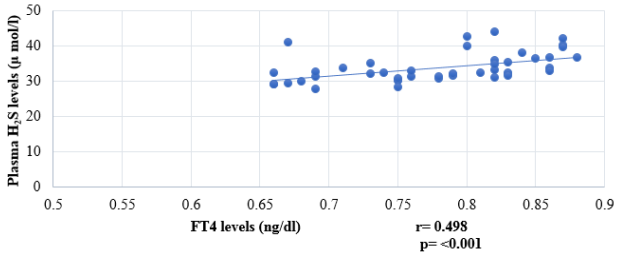

Figure 4: Scatter diagram showing correlation between serum H_2S and FT4 values (r= 0.498, P=<0.001)

5. Discussion

Numerous proof endorse and substantiate the role and involvement of H_2S in multiple physiological conditions such as angiogenesis,[19,20] energy production,[21] vascular relaxation,[22] neuronal activity,[23] and glucose regulation.[24] Notwithstanding that, atherosclerosis,[25] diabetes,[26] asthma,[27] hypertension, and neurodegenerative diseases[28] are some of the pathological conditions linked to unusual & atypical H_2S metabolism. Hypothyroid patients were reportedly manifested with depleted concentration of

plasma H_2S in an article done by Xeu Zhao et al.[1] The above statement was clarified by demonstrating ascending modulation of SIRT1which was enriched via H_2S surge enhancing synthesis & secretion of thyroid hormones. In another investigation, Dongdong Wu et al illustrated the controlling potential of exogenous H_2S on proliferation, viability, migration and invasion of thyroid carcinoma cells. Role of external H_2S is crucial and of paramount importance in progression of human thyroid carcinoma cells via ROS/PI3K/AKT/mTOR signalling pathway. Moreover, H_2S level regulates RAS/RAF/MEK/ERK signaling pathway and correspondingly has escalating effect on thyrocytes.[29] On the basis of volatility, propensity to undergo oxidation, binding capability to organic molecules and surface adsorption to glass and rubber, it is inconvenient to accurately estimate the concentration of Sulphide in biotic (Richardson et al, 2000).[27] Sulphide concentrations are distinctively measured In biological tissues and fluids by numerous analytical procedures. Unionized sulphide can be further calculated from concentration of dissolved sulphide.[30] Analytes like blood or plasma are estimated performing specifically gas chromatography either coupled with flame photometric detection (GC/FPD) or flame ionizing detection (GC/FID), iodometric titration, potentiometry and spectrophotometry, with ion selective electrodes (ISE), and high performance liquid chromatography (HPLC). Our technique of choice for this article is modified methylene blue method originally developed by Siegel L M in 1965, and later modified by Stipanuk M H et al in 1982.[18] This colorimetric method is cost effective and easy to set-up at any simple laboratory. This method has disadvantages and limitations over higher end equipment based procedures due to interferences in the likes of viscosity, turbidity and low detection limit.

6. Conclusion

The current study elucidated decreased levels of serum H_2S in hypothyroid patients. Thereby, this study suggest serum H_2S levels are significantly correlated with level of diagnostic parameters of hypothyroidism. Further study is needed in this direction to focus on the role of H_2S modulators towards the management of this disorder.

7. Limitation

The current study was a pilot study. The sample size taken for study was very small. Further studies will be required to validate the results by involving larger pool of samples.

8. Source of Funding

None.

9. Interest of Conflicts

None.

References

1. Zhao X, Cao Y, Zhao E, Li T, Cong T, Gao Y, et al. The Expression Levels of SARS-CoV-2 Infection-Mediating Molecules Promoted by Interferon-γ and Tumor Necrosis Factor-α Are Downregulated by Hydrogen Sulfide. *Int J Mol Sci*. 2022;23(21):13624. doi:10.3390/ijms232113624.
2. Braunstein AE, Goryachenkova EV, Lac ND. Reactions Catalysed by Serine Sulfhydrase from Chicken Liver. *Biochim Biophys Acta*. 1969;171(2):366–8.
3. Chiku T, Padovani D, Zhu W, Singh S, Vitvitsky V, Banerjee R, et al. H2S Biogenesis by Human Cystathionine Gamma-Lyase Leads to the Novel Sulfur Metabolites Lanthionine and Homolanthionine and Is Responsive to the Grade of Hyperhomocysteinemia. *J Biol Chem*. 2009;284(17):11601–12.
4. Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, et al. 3-Mercaptopyruvate Sulfurtransferase Produces Hydrogen Sulfide and Bound Sulfane Sulfur in the Brain. *Antioxid Redox Signal*. 2009;11(4):703–13.
5. Gillam MP, Kopp P. Genetic Defects in Thyroid Hormone Synthesis. *Curr Opin Pediatr*. 2001;13(4):364–72.
6. Saha P, Banerjee P, Pal P, Auddya L, Sen S, Sau TJ, et al. Enhanced plasma H2 S levels associated with fasting blood glucose in Type-2 diabetes mellitus. *Asian J Med Sci*. 2015;6(6):11–5.
7. Shibuya N, Tanaka M, Yoshida M, Ogaswara Y, Togawa T, Ishii K, et al. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid Redox Signal*. 2009;11(4):703–14.
8. Shibuya N, Mikami Y, Kimura Y, Nagahara N, Kimura H. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J Biochem*. 2009;146(5):623–6.
9. Jain SK, Bull R, Rains JL, Bass PF, Levine SN, Reddy S, et al. Low Levels of Hydrogen Sulfide in the Blood of Diabetes Patients and Streptozotocin-Treated Rats Causes Vascular Inflammation? *Antioxid Redox Signal*. 2010;12(11):1333–7.
10. Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci*. 1996;16(3):1066–71.
11. Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun*. 1997;237(3):527–31. doi:10.1006/bbrc.1997.6878.
12. Jha S, Calvert JW, Duranski MR, Ramachandran A, Lefer DJ. Hydrogen sulfide attenuates hepatic ischemia reperfusion injury: Role of antioxidant and antiapoptotic signaling. *Am J Physiol Heart Circ Physiol*. 2008;295(2):801–6.
13. Lefer DJ, Granger DN. Monocyte rolling in early atherogenesis:genesis:vital role in lesion development. *Circ Res*. 1999;84(11):1353–5.
14. Caliendo G, Cirino G, Santagada V, Wallace JL. Synthesis and Biological Effects of Hydrogen Sulfide (H2 S): Development of H2 S-Releasing Drugs as Pharmaceuticals. *J Med Chem*. 2010;53(17):6275–86.
15. Ang AD, Konigstorfer A, Giles GI, Bhatia M. Measuring free tissue sulfide. *Adv Biol Chem*. 2012;2:360–5. doi:10.4236/abc.2012.24044.
16. Yusuf M, Kwong HBT, Hsu A, Whiteman M, Bhatia M, Moore PK, et al. Streptozotocin-induced diabetes in the rat is associated with enhanced tissue hydrogen sulfide biosynthesis. *Biochem Biophys Res Commun*. 2005;333(4):1146–52.
17. Ali MY, Whiteman M, Low CM, Moore PK. Hydrogen sulphide reduces insulin secretion from HIT-T15 cells by a K-ATP channel dependent pathway. *J Endocrinol*. 2007;195(1):105–12.
18. Yang Z, Feng L, Junbao DU, Chaoshu T, Guoheng XU, Bin G, et al. Modified methylene blue method for measurement of hydrogen sulfide level in plasma. *Acta Physiologica Sinica*. 2012;64(6):681–6.
19. Li Z, Organ CL, Kang J, Polhemus DJ, Trivedi RK, Sharp TE, et al. Hydrogen Sulfide Attenuates Renin Angiotensin and Aldosterone Pathological Signaling to Preserve Kidney Function and Improve

Exercise Tolerance in Heart Failure. *JACC Basic Transl Sci*. 2018;3(6):796–809.
20. Cai W, Wang M, Moore P, Jin H, Yao T, Zhu Y, et al. The novel proangiogenic effect of hydrogen sulfide is dependent on Akt phosphorylation. *Cardiovascular Res*. 2007;76(1):29–40.
21. Fu M, Zhang W, Wu L, Yang G, Li H, Wang R, et al. Hydrogen sulfide (H2S) metabolism in mitochondria and its regulatory role in energy production. *Proc National Acad Sci*. 2012;109(8):2943–8.
22. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, et al. H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science*. 2008;322(5901):587–90.
23. Ma DK, Vozdek R, Bhatla N, Horvitz HR. CYSL-1 interacts with the O2-sensing hydroxylase EGL-9 to promote H2S-modulated hypoxia-induced behavioral plasticity in C. elegans. *Neuron*. 2012;73(5):925–40.
24. Pichette J, Gagnon J. Implications of hydrogen sulfide in glucose regulation: how H2S can alter glucose homeostasis through metabolic hormones. *Oxid Med Cell Longev*. 2016;doi:10.1155/2016/3285074.
25. Mani S, Li H, Untereiner A, Wu L, Yang G, Austin RC. Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. *Circulation*. 2013;127(25):2523–34.
26. Cheng Z, Garikipati VNS, Nickoloff E, Nickoloff E, Wang C, Polhemus DJ, et al. Restoration of Hydrogen Sulfide Production in Diabetic Mice Improves Reparative Function of Bone Marrow Cells. *Circulation*. 2016;134(19):1467–83.
27. Wang P, Wu L, Ju Y. Age-dependent allergic asthma development and cystathionine gamma-lyase deficiency. *Antioxid Redox Signal*. 2017;27(13):931–44.
28. Paul BD, Sbodio JI, Xu R. Cystathionine γ-lyase deficiency mediates neurodegeneration in Huntington's disease. *Nature*. 2014;509(7498):96–100.
29. Wu D, Li J, Zhang Q, Tian W, Zhong P, Liu Z, et al. Exogenous Hydrogen Sulfide Regulates the Growth of Human Thyroid Carcinoma Cells. *Oxid Med Cell Longev*. 2019;p. 18. doi:10.1155/2019/6927298.
30. Kolluru GK, Shen X, Bir SC, Kevil CG. Hydrogen sulfide chemical biology: pathophysiological roles and detection. *Nitric Oxide*. 2013;35:6–20. doi:10.1016/j.niox.2013.07.002.

Author biography

Ankita Bhattacharya, Assistant Professor

Sadajit Banerjee, Consultant

Ipsita Bhattacharyya, Assistant Professor

Pinaki Saha, Associate Professor https://orcid.org/0000-0002-7591-1568

Debmalya Sanyal, Professor

Indira Bhaskar Biswas, Professor and HOD

Cite this article: Bhattacharya A, Banerjee S, Bhattacharyya I, Saha P, Sanyal D, Biswas IB. An observational study to determine relationship of serum hydrogen sulphide level in hypothyroid patients in a medical college in Kolkata. *Panacea J Med Sci* 2023;13(3):824-828.