ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Enhanced plasma H_2S levels associated with fasting blood glucose in Type-2 diabetes mellitus

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Submitted: 05-05-2015 Revised: 30-06-2015 Published: 01-07-2015

ABSTRACT

Introduction: A number of recent literatures suggest a potential role of H_2S and H_2S modifying agents in the etiology and management of type-2 diabetes mellitus. **Objective:** The current study was aimed to evaluate the plasma levels of H_2S in the patients with type 2 Diabetes mellitus and to find out if there is any relationship of H_2S concentrations with the fasting blood glucose levels. **Methods:** Plasma H_2S levels were measured in sixty two recently diagnosed type 2 diabetic patients and compared with similar number of healthy volunteers as controls. **Results:** The plasma H_2S level in the patients (81.17 ± 16.40 micromol/l) is significantly higher ($P < 0.001$) than the healthy controls (50.69 ± 8.69 micromol/l) and the H_2S levels in plasma have significant positive correlation ($r = 0.359$, $P = 0.004$) with fasting blood glucose levels. **Conclusion:** The present study has elucidated that the patients with type-2 diabetes mellitus are associated with elevated plasma H_2S levels which are well correlated with glucose levels. This reveals a potential role of H_2S modulators towards the management of this non-communicable epidemic disorder.

Key words: Type 2 diabetes mellitus, Plasma H_2S levels, FBG

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v6i6.12532

BACKGROUND

The prevalence of diabetes mellitus type-2 have drastically increased to several folds in the last two decades.[1] Though several etiological background of this non-communicable epidemic disease has been well explained and being efficiently treated, yet, a number of recent literatures suggested a potential role of H_2S and H_2S modifying agents in the etiology and management of this metabolic disorder.[2]

Hydrogen sulfide is endogenously produced in the mammalian tissues from the amino acid L-cysteine by the action of two enzymes, cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE). Both of these enzymes are dependent on pyridoxal-5'-phosphate. Recent studies have shown a third H_2S-producing enzyme, 3-mercaptopyruvate sulfur transferase (3MST), along with cysteine aminotransferase (CAT), which produces H_2S in the brain as well as in the vascular endothelium.[3,4] Expression of CSE or CBS is tissue specific. CSE is expressed mainly in the thoracic aorta, portal vein, ileum, heart, liver, kidney, and vascular smooth muscle, whereas CBS is highly expressed in the central and peripheral nervous systems.[5-9] The majority of H_2S is metabolized to sulfate and thiosulfate via oxidative metabolism in mitochondria, while only low levels of H_2S can be converted into less toxic compounds by the cytosolic detoxification pathway. This oxidation is not enzymatically driven, while thiosulfate conversion to sulfate and/or sulfite is catalyzed by thiosulfate cyanide sulfur-transferase (TST).[10] These metabolic products are then expelled within 24 hours via the kidneys, intestinal tract and lungs to maintain the normal levels of H_2S in plasma. Under normal circumstances, H_2S does not accumulate, which means that under physiological conditions, endogenous H_2S is not toxic to cells.

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Presently it has emerged as a mediator of important physiologic functions in humans.[11] Recent reports suggests that the changes in the balance of hydrogen sulfide (H_2S) play an important role in the pathogenesis of β-cell dysfunction that occurs in type 1 and type-2 diabetes. In addition, changes in H_2S homeostasis also play a role in the pathogenesis of endothelial injury, which develop on the basis of chronically or intermittently elevated circulating glucose levels in diabetes. Experimental evidences have been published implicating H_2S overproduction as a causative factor in the pathogenesis of β-cell death in diabetes.[12,13] Some other experiments have suggested H_2S deficiency due to increased H_2S consumption by hyperglycemic cells, in the pathogenesis of diabetic endothelial dysfunction, diabetic nephropathy, and cardiomyopathy.[12] There is possibility that the modulation of H_2S production may be a potential therapeutic strategy for diabetes mellitus.[13] This has led researchers to investigate H_2S related substances for treatment of diabetes.[14,15]

However, very few literatures have reported the plasma levels of H_2S in human. Most of the reports are in the animal model and the H_2S concentrations are reported mainly at the tissue level.[16,17] Previous studies have also given controversial findings. Some reported that the levels of H_2S in streptozotocin induced diabetic rats is reduced.[13] Others have found an elevated levels of H_2S in plasma in the similar animal models.[17]

Since there is paucity of data in this aspect and no previous report in our population, the aim of our study is to evaluate the plasma levels of H_2S in the patients with type 2 Diabetes mellitus and to find out if there is any relationship of H_2S concentrations with the fasting blood glucose levels.

MATERIALS AND METHODS

This case control study was conducted in the department of Biochemistry and Medicine, NRS Medical College, Kolkata, India. Sixty two recently diagnosed type 2 diabetic patients within 20 to 50 years of age, consisting 30 males and 32 females, were enrolled for the study along with similar number of age matched healthy volunteers as controls (37 males and 25 females). The study was pre approved by the Institutional Ethics Committee. Pregnant mothers, Patients with Type-1 diabetes mellitus and other endocrine disorders, polycystic ovarian disease, renal failure, malignant disease, receiving antioxidant and H_2S modifying agents were excluded from the study.

Sample collection

Fasting blood samples were collected aseptically in heparin containing vials from the patients and healthy controls after obtaining informed consent. Whole blood was used for estimation of HbA_{1c}. The plasma was separated by centrifugation, immediately used for estimation of H_2S and FBG. Rest is kept stored at 4°C for estimation of other biochemical parameters, done within 48 hours. Buffers and reagents were kept inside appropriate temperature zones in refrigerators.

Measurement of H_2S concentration in plasma

Plasma H_2S levels were estimated following methods described earlier[13,18,19] with further modification and standardization in our laboratory. This spectrophotometric method involves the reaction of sulfide with N, N-dimethyl-p-phenylenediamine sulfate in the presence of the oxidising agent Fe^{3+} in hydrochloric acid to form methylene blue which is read at 670nm.

Assay procedure

Seventy-five ml of plasma was added to 425 microliter of PBS and 250 microliter of 10% tri-chloroacetic acid in a capped glass tube. Then it was centrifuged at 3000 rpm for 30 minutes. The supernatant was taken in another glass tube and 250 microliter of 1% zinc acetate, 133 microliter of 20 milimolar N, N-dimethyl- p- phenylene diamine sulphate in 7.2 mM HCl, 133 microliter of 30 milimolar $FeCl_3$ in 1.2 mM of HCl and 60 microliter of 10% NaOH was added, capped and incubated for 10 minutes at room temperature. All samples were assayed in triplicates and the plasma of H_2S levels calculated against a calibration curve prepared using 25-250 micromol/l concentrations of sodium sulfide (NaHS, Sigma-Aldrich, MO, USA) as shown in Figure 1. The intra-assay and inter-assay variation of this method was 7.576 and 3.944 respectively and the maximum sensitivity was up to 25 micromol/l.

Estimation of fasting blood glucose and other biochemical parameters were done using standardized reagent kit.

Statistical analysis

Data were expressed as mean ± standard deviation (SD), comparison of data was done using unpaired two-tailed Students' t-test and Pearson's correlation, $P<0.05$ was considered as significant. Statistical analysis was done using Microsoft Office Excel-2007 and SPSS Statistics version 2020.

RESULTS AND DISCUSSION

The clinico-biochemical parameters of the study subjects are depicted in Table 1. The plasma H_2S level in the patients in our study is 81.17 ± 16.40 micromol/l with the range from 52.50 to 110.83 micromol/l. This was significantly ($P< 0.001$) higher than age/sex matched healthy controls which is 50.69 ± 8.69 micromol/l, with a range from 41.67 to 71.67 micromol/l (Table 1 and Figure 2). Plasma H_2S

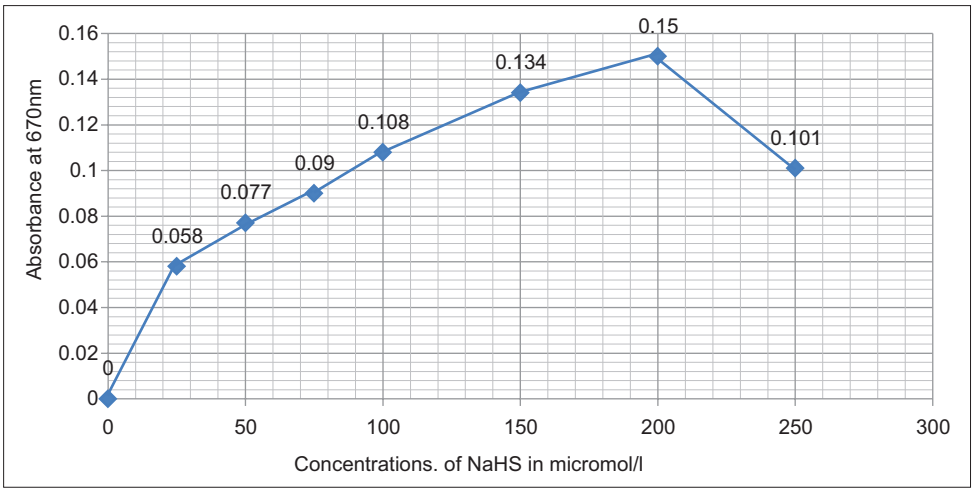

Figure 1: Standard curve of plasma H_2S assay

levels in the patients as well as control subjects in our study are comparable with earlier studies which is within the range of 10 to100 micromol/l in human subjects.[5]

Elevated hydrogen sulfide levels in plasma were also reported in patients with proliferative diabetic retinopathy[20] as well as patients with cardiovascular disease[21] cerebral ischaemic damage[22] and septic shock[23] M. Yusuf *et al.* has earlier reported that the streptozotocin-induced diabetes in rat is associated with enhanced tissue hydrogen sulfide biosynthesis.[17] The activities of H_2S producing enzymes and the tissue H_2S contents are known to increase under diabetic conditions.[24]

The other studies have reported reduced levels of H_2S in the plasma as well as in the tissues in diabetes mellitus. Jain *et al.* demonstrated lower circulatory levels of H_2S in type-2 diabetic patients,[5] but their observations were associated with increased levels of pro-inflammatory cytokines which may have affected the outcome. In another study[13] Brancaleone *et al.* demonstrated impaired H_2S in the non-obese diabetic (NOD) mice.[25] Whiteman *et al.* also reported decreased plasma H_2S levels in overweight participants and patients with type-2 diabetes mellitus. They have suggested that adiposity is a major determinant of plasma H_2S levels.[26] However, in the current study the subjects were mostly non-obese and non-overweight. Mean body mass index of our patients was within the reference range. So the findings of the current study could not demonstrate whether adiposity plays a role in determination of plasma levels of H_2S in patients suffering from type-2 diabetes.

Measurement of sulfide concentrations in biological materials is difficult due to its volatility, tendency to undergo oxidation, adsorption to glass and rubber and binding to organic molecules (Richardson et al, 2000).[27,28] In biological tissues and fluids, the sulfide concentrations

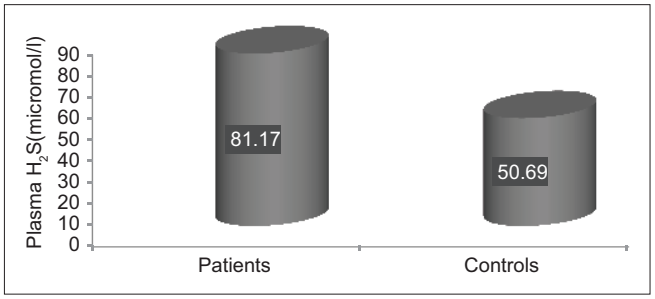

Figure 2: Comparison of plasma H_2S levels in patients and controls

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

Variables	Mean±SD		P value
	Patient	Control	
Age (years)	44.03±5.27	43.63±5.56	
Sex (M/F)	30/32	37/25	
Body mass index (BMI)	24.15±4.09	24.81±2.63	NS
Fasting blood Glucose (mg/dl)	119.14±26.88	79.06±12.64	<0.001*
Post prandial blood Glucose (mg/dl)	176.08±46.26	108.76±11.16	<0.001*
Glycosylated haemoglobin HbA_{1C}(%)	6.85±1.14	3.8±0.34	<0.001*
Plasma H_2S level (micromol/l)	81.17±16.40	50.69±8.69	<0.001*

Student's t-test was done, *- significant (P<0.05), with 95% confidence level: NS=Not significant

are typically determined and the concentrations of unionized sulfide can be further calculated from the concentration of dissolved sulfide.[28,29] A number of analytical techniques have been used for measuring hydrogen sulfide in biological tissues and fluids like blood or plasma including gas chromatography coupled with flame ionizing detection (GC/FID), gas chromatography coupled with flame photometric detection (GC/FPD), iodometric titration, potentiometry with ion selective

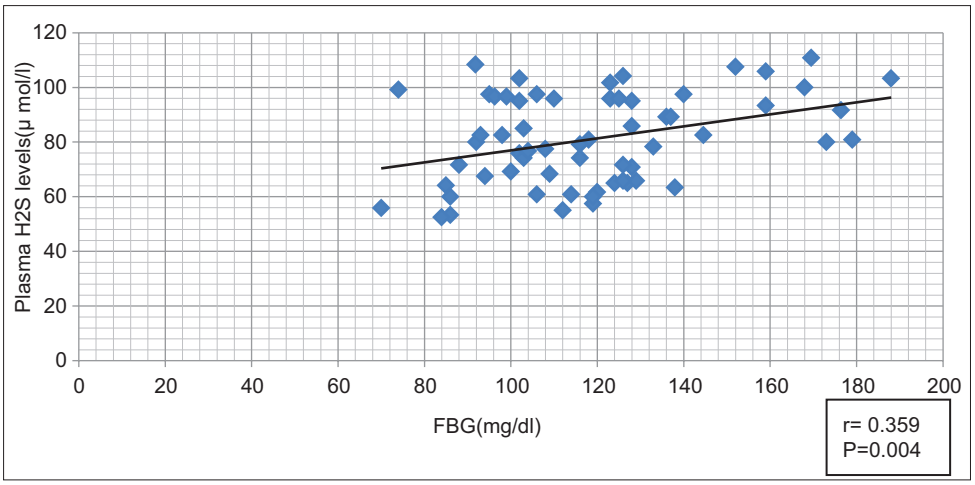

Figure 3: Scatter plot showing correlation between plasma H_2S and FBG values in patients (Or) (Or) Plasma H_2S levels show significant positive correlation (r= 0.359, P=0.004) with fasting blood glucose levels

electrodes (ISE), spectrophotometry and high performance liquid chromatography (HPLC). However, we have used here the methylene blue method with further modifications for estimation of plasma H_2S levels, originally developed by Siegel L M,1965, and later modified by Stipanuk M H and Beck P W,1982, Richardson *et al*, Zheng Y. *et al*.[19,27,31-33] This colorimetric method has limitations because of viscosity and turbidity and a detection limit of 0.25 micromol/l, but this simple colorimetric method used in our study is cost-effective and can be performed in a simple laboratory even at the rural set-up where other sophisticated methods are not feasible.

CONCLUSION

The current study elucidated increased levels of H_2S in type-2 diabetes mellitus and the plasma H_2S levels are significantly correlated with glucose levels. Further study is needed in this direction to establish the role of H_2S modulators towards the management of this non-communicable epidemic disorder.

Limitations of the study

The sample size for the current study was small as it was a pilot study. Further studies are required to validate the results of the current study with large sample size.

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Authors Contribution:
PS − Designed the study, Data Acquisition, Data Analysis and Drafting of Manuscript. **UKB** − Data Analysis, Drafting of Manuscript, Review of Manuscript.
AK − Manuscript Preparation, Data Analysis, Review of Manuscript, Final Approval.

Source of Support: Nil, **Conflict of Interest:** None declared.