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SHORT REPORT

Plasma hydrogen sulphide does not predict severity of acute pancreatitis in humans

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Abstract The primary aim of this study was to assess the usefulness of plasma hydrogen sulphide (H_2S) level at admission as a predictor of severity of acute pancreatitis. The secondary aims were to examine whether the level of H_2S after 48 h correlated with severity and whether level of H_2S correlated with pulmonary, renal or infectious complications. Plasma hydrogen sulphide was measured within 24 h of admission and 48 h later, in patients with acute pancreatitis. Patients were classified as having mild or severe pancreatitis, and H_2S levels in the two groups were compared. A total of 55 patients had H_2S estimation carried out within 24 h of admission. H_2S levels were similar in patients with mild (mean 31.8 ± 18.8, range 7.1 to 81.4 μmol/L) and severe pancreatitis (mean 28.2 ± 21.6, range 6.1 to 74.4 μmol/L; $p = 0.339$). There was no difference found between the groups after 48 h (mild $n = 28$, mean 26.8 ± 19.4 μmol/L, and severe $n = 20$, mean 34.6 ± 21.0 μmol/L; $p = 0.127$). There was also no difference in the levels between patients with or without lung injury, kidney injury or sepsis. Performing H2S estimation to predict severity in acute pancreatitis is not beneficial.

Keywords Acute pancreatitis · Hydrogen sulphide · Prognosis · Severity

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Introduction

Acute pancreatitis can vary in intensity from mild self-limited inflammation to severe necrotising pancreatitis with myriad complications, significant mortality and high medical cost [1]. About 80 % of patients admitted with acute pancreatitis have mild disease and have good outcomes [2]. Acute pancreatitis has been traditionally classified as mild and severe, based on the absence or presence of local complications and distant organ failure, which may occur anytime during the course of illness. Severe pancreatitis should be identified as early as possible to provide more intensive monitoring, appropriate therapy and accurate prognosis. Thus, every clinician who treats pancreatitis will appreciate a tool that is easy to use, that can be used early in the course of illness (ideally at admission) and that accurately identifies those who have or will develop severe acute pancreatitis.

Recent studies have established hydrogen sulphide (H_2S) to be an important 'gasotransmitter' in the body. Data from animal studies suggest that there is good correlation between H_2S levels and the severity of pancreatitis, and the presence of acute lung injury [3]. To our knowledge, barring one conference abstract [4], no detailed studies have been carried out in humans to examine the role of H_2S as a marker of severity in acute pancreatitis.

Hence, the primary aim of this study was to assess the usefulness of H_2S level at admission as a predictor of severity of acute pancreatitis, by comparing the levels in patients with mild and severe acute pancreatitis. The secondary aims were to examine whether the level of H_2S after 48 h correlated with the severity of pancreatitis and to investigate whether the level of H_2S correlated with pulmonary, renal or infectious complications. Sepsis was suspected if there was clinical deterioration along with fever and leucocytosis after the first week or if a pathogenic organism was grown in culture.

Methods

Patients

This was a prospective single-centre study conducted at the Christian Medical College Hospital, Vellore, Tamil Nadu, India, from July 2012 to September 2013. The study protocol was approved by the Institutional Review Board. Patients of either sex between the ages of 18 and 80 admitted with the diagnosis of acute pancreatitis were enrolled after informed consent. The diagnosis was made if the patient fulfilled two out of the following three criteria [1].

1. Pancreatic type abdominal pain
2. Serum amylase or lipase more than three times upper limit of normal
3. Imaging study (USG or CT) suggestive of acute pancreatitis

Pregnant women and patients with a diagnosis of chronic pancreatitis, pseudocyst or pancreatic tumors were excluded. Initial samples for H_2S measurement were collected within 24 h of admission, followed by a second sample 48 h later.

Hydrogen sulphide measurement

H_2S levels in plasma were measured immediately after sample collection, spectrophotometrically as described [5]. Briefly, 100 μL of aliquots of the samples was mixed with 100 μL of distilled water in micro-centrifuge tubes containing 300 μL of trapping solution (mixture of 0.5 % zinc acetate, 0.5 % EDTA, 0.8 % NaOH, *w*/*v*) to trap H_2S. The reaction was terminated after 10 min by adding 200 μL of *N*, *N*-2dimethyl-*p*-phenylenediamine sulphate (20 mM in 7.2 M HCl), immediately followed by addition of 200 μL of FeCl3 (30 mM in 1.2 M HCl). The mixture was then kept in the dark for 30 min, following which; 200 μL of trichloroacetic acid (10 % *w*/*v*) was added to precipitate protein. The mixture was then centrifuged at 9300*g* for 10 min, following which, the absorbance of the resulting supernatant was measured at 670 nm. H_2S concentration in the plasma was then calculated against a calibration curve of NaHS standard solution.

Statistical analysis

Results are expressed as mean and median with appropriate measures of dispersion. If the data did not satisfy the assumption of normality, a non-parametric approach was used. To calculate paired differences, Wilcoxon signed-rank test was used. Mann-Whitney *U* test was used to compare groups.

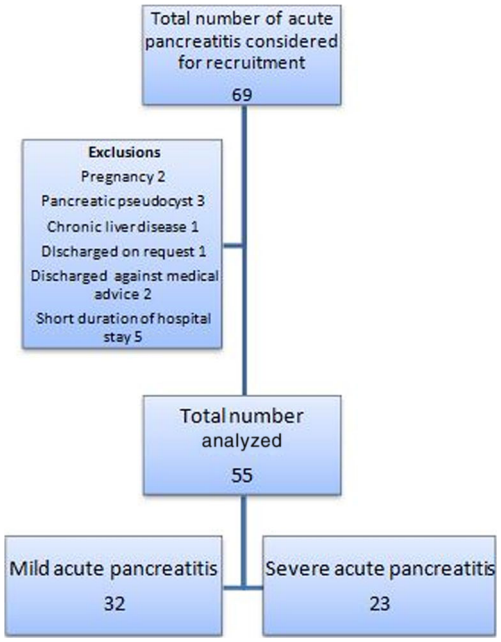

Fig. 1 Flow of patient recruitment

Results

A total of 55 patients had H_2S estimation carried out within 24 h of admission (Fig. 1). There were 43 males and 12 females. Thirty-six patients presented within 72 h of onset of pain. Alcohol (45.5 %) and gallstones (27.3 %) were the most common etiologies. No cause could be ascertained in 23.6 %.There were 32 cases of mild pancreatitis and 23 cases of severe pancreatitis. A more detailed break-up of the types of complications is given in Table 1.

H_2S levels at admission in patients with mild pancreatitis (mean 31.8 ± 18.8, range 7.1 to 81.4 μmol/L) were similar to those with severe pancreatitis (mean 28.2 ± 21.6, range 6.1 to 74.4 μmol/L, $p = 0.339$, Fig. 2). H_2S levels were measured in 48 patients after 48 h. There was no difference in H_2S levels at 48 h (mild $n = 28$, mean 26.8 ± 19.4 μmol/L and severe $n = 20$, mean 34.6 ± 21.0 μmol/L; $p = 0.127$) or the temporal trend of H_2S (rising or falling trend in relation to the admission H_2S value), between the mild and severe cases. Among the mild cases, 12/28 showed an increase in H_2S, while among the severe cases 11/20 showed an increase in H_2S when the levels

Table 1 Multivariate logistic regression analysis of factors significantly associated with maternal mortality on univariate analysis

Parameter	Odds ratio (95 % CI)	*p*-value
Hemoglobin	0.457 (0.223, 0.935)	0.032
Total leukocyte count (cells per μL)	1.004 (1.000, 1.001)	0.003
INR	92.9 (1.497, 5.76)	0.031

INR international normalized ratio

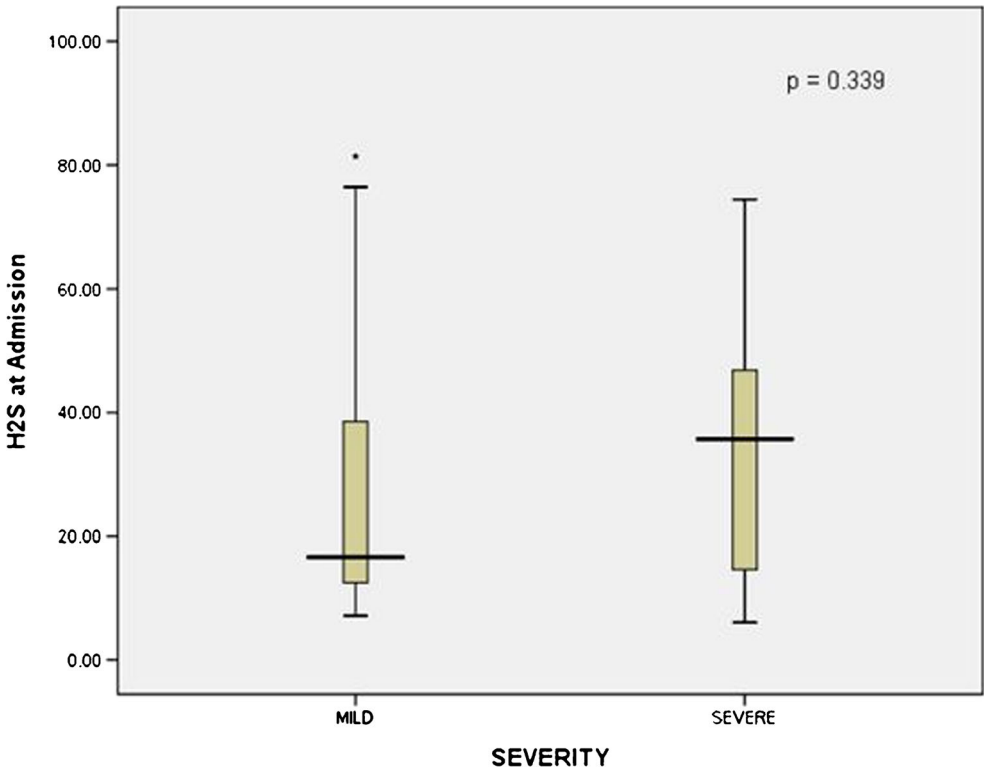

Fig. 2 Comparison of hydrogen sulphide level at admission in mild and severe acute pancreatitis

measured at 48 h was compared with those at admission ($p = 0.538$).

H_2S levels of 36 patients, who came to the hospital within 72 h of onset of pain, were not significantly higher than those who presented later (early presenters, mean 30.6 ± 17.9 μmol/L vs. late presenters, mean 27.8 ± 24.9 μmol/L; $p = 0.176$). Among the early presenters, there was no significant difference between the mild and severe groups (mild pancreatitis $n = 21$, mean 29.5 ± 19.1 μmol/L and severe $n = 15$, mean 32.2 ± 16.7 μmol/L; $p = 0.470$, Fig. 3).

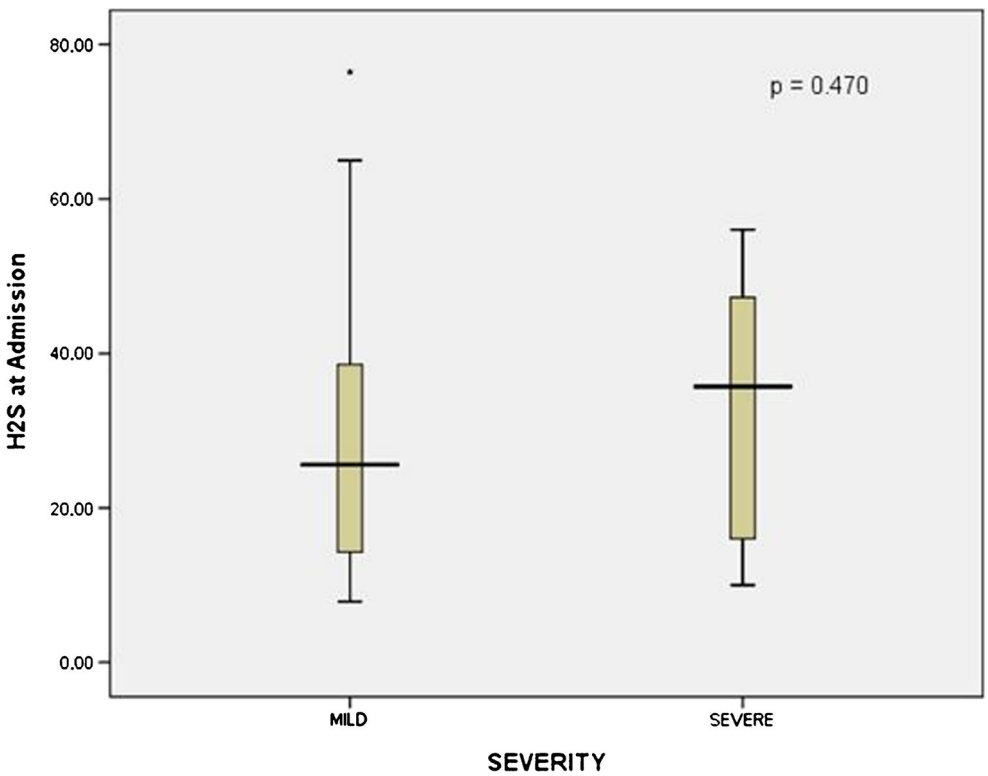

Fig. 3 Comparison of hydrogen sulphide level at admission in mild and severe acute pancreatitis, among patients who present to the hospital within 72 h from the onset of pain

H_2S levels at admission also did not predict the development of acute kidney injury, acute lung injury or infectious complications during hospital stay. H_2S levels in two patients who died (10.0 and 47.3 μmol/L) were also not dissimilar to the rest of the group.

CRP was done at 48 h from onset of pain or at admission if the patient came later. Forty-three patients had C-reactive protein (CRP) measurements, 25 mild and 18 severe cases. Mean CRP values were significantly higher in severe cases as compared to mild cases (155.1 ± 66.1 vs. 66.2 ± 57.1 mg/L, p value < 0.001) [6]. We did not find a correlation between CRP and admission H_2S values ($p = 0.658$).

Discussion

Animal studies have shown a correlation between severity of pancreatitis and H_2S levels [3, 7, 8]. Data in humans, though scanty, also suggest that H_2S levels may correlate with severity especially when patients present early in the course of illness (with 72 h) [4]. The current study did not support these findings, when either the entire cohort was compared or a subgroup of early presenters were separately analyzed.

Animal studies have also shown that hydrogen sulphide levels correlate with severity of acute lung injury and that attenuation of the H_2S production led to less severe lung injury [3, 8–11]. We could not establish any correlation between H_2S and acute lung injury, in this study.

Many markers of severity that have performed well in the controlled environment of animal studies are often found to perform poorly in real life situations. The differences in aetiology of pancreatitis, severity of insult, duration of illness, development of complications and genetic variations in inflammatory response may be responsible for the wide variation in plasma H_2S that has rendered this test unhelpful.

A possible limitation of our study was that the samples for H_2S were analysed on the first day of admission and not necessarily on first day of acute pancreatitis. Studies using animal models of disease have shown that hydrogen sulphide levels are increased in the early stages of inflammation, and it is possible that in some patients who were admitted long after symptom onset, the peak would have been missed. However in real life scenario, only a subset of patients will present on the first day of onset of pancreatitis to the hospital.

In conclusion, our data shows that there was no difference in hydrogen sulphide levels measured at admission or at 48 h in patients with mild and severe acute pancreatitis. Levels of H_2S did not correlate with systemic complications (acute lung injury, acute kidney injury and sepsis) or mortality. Temporal trends of H_2S in the initial days of admission were also not found to be helpful. Thus, the present study suggests that performing H_2S estimation as a predictor of severity is acute pancreatitis is not beneficial.

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Compliance with ethical standards

Conflict of interest INQ, DD, KRT, RTK, SDC, AG, AKD, EGS, AR, KAB, and AJJ declare that they have no conflict of interests.

Ethics statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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