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Serum hydrogen sulphide levels in acute asthmatic children: a case control study

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Abstract

Background It's thought that respiratory epithelium-produced reduced hydrogen sulphide (H_2S) plays a role in the pathophysiology of acute asthma. In this case–control research, blood H_2S levels were examined between matched acutely asthmatic children and non-asthmatic controls. The grade of acute asthma, vital signs and absolute eosinophilic count in the asthmatic children were likewise associated with the blood H_2S level.

Methods Forty Egyptian asthmatic children had visited the emergency room and forty age- and sex-matched non-asthmatic controls had their blood H_2S levels measured using enzyme-linked immunosorbent assay (ELISA).

Results The serum H_2S in the two groups did not differ statistically significantly. Serum H_2S and respiratory rate showed a moderately significant inverse connection ($r = -0.325$, $p = 0.041$). However, serum H_2S and other clinical or laboratory variables exhibited no meaningful relationships. Patients' absolute and percentage eosinophil counts were considerably higher than healthy controls. Serum H_2S exhibited a sensitivity of 50% and a specificity of 32.5% for identifying children with acute asthma from non-asthmatic children.

Conclusion Children with asthma and those without asthma had similar serum H_2S levels. It has a lousy relationship with respiratory rate. It is indicated that it is an inadequate screening and diagnostic tool since it has low sensitivity (50%) and specificity (32.5%) in differentiating acute asthmatic children.

Keywords Acute asthma, Children, Hydrogen sulphide

Backgroud

Bronchial asthma (BA) is the most common chronic pediatric health problem with increasing morbidity and mortality [1]. This chronic inflammation is associated with exaggerated airway narrowing in response to specific triggers (such as viruses, allergens and/or exercise). Exaggerated airway narrowing causes recurrent episodes of wheezing, breathlessness, and chest tightness that can vary over time and in intensity. Symptom episodes are usually reversible either spontaneously or with appropriate asthma treatment such as fast-acting bronchodilators [2].

Different endogenous mediators have been suggested to be involved in regulation of airway inflammation/obstruction of BA [3]. One of these mediators is hydrogen sulphide (H_2S) [4]. H_2S is produced in the lung and airway tissues via cystathionine β-synthase (CBS) and/or cystathionine γ-lyase (CSE). The exact role of H_2S has not yet been fully defined, but it has been postulated that H_2S may exhibit "biphasic" effects; anti-inflammatory and pro-inflammatory. At low levels (micromole), H_2S has significant cell protection effects through anti-apoptosis, anti-necrosis and cell proliferation. However, at high levels (millimole), H_2S has cytotoxic effects via the production of free radicals and oxidants, glutathione depletion and initiation of proapoptotic gene expression, and stimulates apoptosis and

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cell death [5–7]. Accordingly; serum H_2S levels can vary depending on the underlying condition [8].

Published reports addressing the value of H_2S in human acute asthma are relatively few, especially in pediatric age groups [8, 9]. Hence, further studies are required to address this point. This study investigated serum H_2S level in a group of acute asthmatic children and compared it with that of a group of non-asthmatic matched controls. The relationship between serum H_2S level and acute asthma severity was also examined. It was hypothesized that serum H_2S level would be reduced in acute asthmatic children compared with controls. In addition, it was hypothesized that there would be negative correlation between serum H_2S level and acute asthma severity.

Methods

Study setting

This case control study was conducted on 80 Egyptian children who attended the outpatient clinic of Cairo University Children Hospital "Abu El Reesh", Faculty of Medicine, Cairo University from May 2020 to March 2021.

Study population

The children were divided into two groups; a study group "group A" and a control one "group B". Group "A" included 40 asthmatic children who consecutively attended the Emergency Room (ER), while group "B" included 40 age- and sex-matched non-asthmatic children who were recruited from the surgical clinic.

The inclusion criteria specified for the study group involved children with age ranging between 2–16 years, diagnosed with acute asthma (grade I [mild], grade II [moderate] and grade III [severe]) according to Global Initiative for Asthma (GINA) guidelines, with no intake of systemic corticosteroids or theophylline for two weeks before inclusion, and having no other associated diseases. Patients were excluded if they had grade IV acute asthma (eminent respiratory arrest), or refused to participate in the study.

Severity is assessed retrospectively from the level of treatment required to control symptoms and exacerbations, as follows: • Mild asthma: Well-controlled with as-needed reliever medication alone or with low-intensity controller treatment such as low-dose inhaled corticosteroids (ICSs), leukotriene receptor antagonists, or chromones. • Moderate asthma: Well-controlled with low-dose ICS/long-acting beta2-agonists (LABA) • Severe asthma: Requires high-dose ICS/LABA to prevent it from becoming uncontrolled, or asthma that remains uncontrolled despite this treatment [10].

Procedure

After taking informed consent, detailed child history was taken. This involved age, gender, residence, history of wheezes, cough and shortness of breath, seasonal variation of symptoms, nocturnal symptoms, previous history of similar attacks, history of other atopic manifestations and family history of atopy.

Patients were physically examined. This included both general examination (heart rate, respiratory rate and temperature) and local chest examination. The chest shape, respiratory movements, and trachea were inspected. The chest movements and trachea were palpated and chest tenderness was assessed. Percussion for dullness or hyperresonance and auscultation for air entry, type of breathing and rhonchi were carried out.

Laboratory investigations were carried out as follows. Two milliliters of venous blood on Etheylene-diamine-tetra-acetic (EDTA) were used for complete blood count (CBC) and absolute eosinophilic count (total leukocytic count multiplied by eosinophilic percentage) analysis. Assay of serum H_2S level was performed as follows. Three milliliters of venous blood were withdrawn from each participant. The blood sample was centrifuged at $1000 \times g$ and the serum was stored in plastic tubes at -20° C till assay was performed after all samples were collected. H_2S was analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer's specifications. ELISA test principle: The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human H_2S in samples. H_2S is added to monoclonal antibody enzyme which is pre-coated with human H_2S monoclonal antibody, incubation; then H_2S antibodies labeled with biotin is added to Streptavidin-HRP to form immune complex; then incubation is carried out and the resultant product is washed to remove the uncombined enzyme. Then Chromogen Solution A, B, is added, the color of the liquid changes to blue, and finally yellow by the effect of acid. The chroma of color and concentration of human H_2S substance of sample were positively correlated.

Study outcomes

The serum H_2S level was compared between acute asthmatic children and non-asthmatic controls. The serum H_2S level was also correlated with the grade of acute asthma, vital signs and absolute eosinophilic count in the study group.

Data management and statistical analysis

Data involving participant history, basic clinical examination, laboratory investigations and outcome measures were collected, coded and analyzed. Statistical Package

for the Social Sciences (SPSS version 20.0) software was used for analysis. Qualitative data were presented as frequencies and percentages, while quantitative data were presented as means ± standard deviation (SD). The alpha level of significance was set at $p<0.05$.

Descriptive statistics

Mean, Standard deviation (± SD) and range for parametric numerical data, while median and Inter-quartile range (IQR) for non-parametric numerical data. Frequency and percentage of non-numerical data. Mann–Whitney was used to assess the statistical significance of the difference of a non-parametric variable between both groups.

Results

The participants' demographic data were compared between both groups with no significant difference being revealed in-between. Clinical data involving the heart rate, respiratory rate, and body temperature were also compared between both groups. All clinical data were significantly higher in group "A" compared with group "B" ($p<0.05$). Table 1 shows the demographic and clinical data of both groups.

Regarding the clinical presentation in group "A", it was found that 36 (90%) of them presented with cough. Auscultatory wheezes was found in all of the 40 cases (100%). 17 (42.5%) cases had no respiratory distress, 17 (42.5%) had grade "I", and 6 (15%) had grade "II". The severity of asthma exacerbation was mild in 33 (82.5%) cases and moderate in 7 (17.5%) cases.

Regarding the hematological data, the eosinophilic percentage and absolute eosinophilic count were significantly higher in group "A" compared with group "B" ($p<0.05$) with no significant difference for the hemoglobin and white blood cells ($p>0.05$). As for the serum H_2S level, there was no significant difference in it between both groups ($p>0.05$). Table 2 shows both the hematological data and serum H_2S level in both groups.

Table 2 Hematological data and serum H_2S level in asthmatic and non-asthmatic children

	Cases (40) X ± SD	Controls (40) X ± SD	95% CI	*p*-value
Hb (g/dl)	11.76 ± 1.95	11.34 ± 1.66	-0.38—1.23	0.298
WBC (10^3/cmm)	9.73 ± 2.79	10.54 ± 8.57	-3.67—2.07	0.577
Eosinophils %	5.52 ± 4.62	2.09 ± 1.27	1.9—4.95	**< 0.001***
Absolute eosinophilic count (cells/cmm)	541 ± 495	213 ± 193	158—496	**< 0.001***
H_2S (pg/ml)	415.6 ± 396.1	360.8 ± 342.9	-0.110—219.8	0.510

X ± SD Mean ± standard deviation, *CI* Confidence interval

* Significant at $p<0.05$

The Pearson's correlation showed a significant negative correlation between the serum H_2S and respiratory rate ($r=-0.325$, $p=0.041$; Fig. 1). Table 3 shows the correlation findings among the tested variables.

Regarding the diagnostic accuracy of the serum H_2S, the receiver operating characteristic (ROC) curve showed that the serum H_2S has a sensitivity of 50% and a specificity of 32.5% in discriminating acute asthmatic children from non-asthmatic controls with a best cutoff-point of ≤ 299.5 pg/ml. The area under the curve (AUC) was 0.502. Figure 2 presents the ROC curve.

Discussion

Our results showed that there were no significant differences between both groups regarding the age and sex. This means they were age- and sex-matched. It was noted that the number of males was more than females in our study group. This is consistent with Chowdhury et al. [11] findings that young boys have more prevalent wheezes and asthma than young girls. This may be explained by the fact that young boys have smaller airway caliber and thus being more liable to airway narrowing compared with young girls [12].

Table 1 Demographic and clinical data of asthmatic children and non-asthmatic children

	Cases (40) X ± SD	Controls (40) X ± SD	95% CI	*p*-value
Age (years)	5.17 ± 2.92	4.83 ± 2.99	-0.96—1.66	0.598
Sex (male/female)	31/9	23/17	-	0.093
Residence (urban/rural)	36/4	33/7	-	0.517
Heart rate/min	102.5 ± 17.36	90.38 ± 13.08	5.28—18.97	**0.001***
Respiratory rate/min	37.38 ± 8.99	22.9 ± 3.99	11.36—17.56	**< 0.001***
Temperature °C	37.19 ± 0.48	36.94 ± 0.21	0.07—0.41	**0.005***

X ± SD Mean ± standard deviation, *CI* Confidence interval

* Significant at $p<0.05$

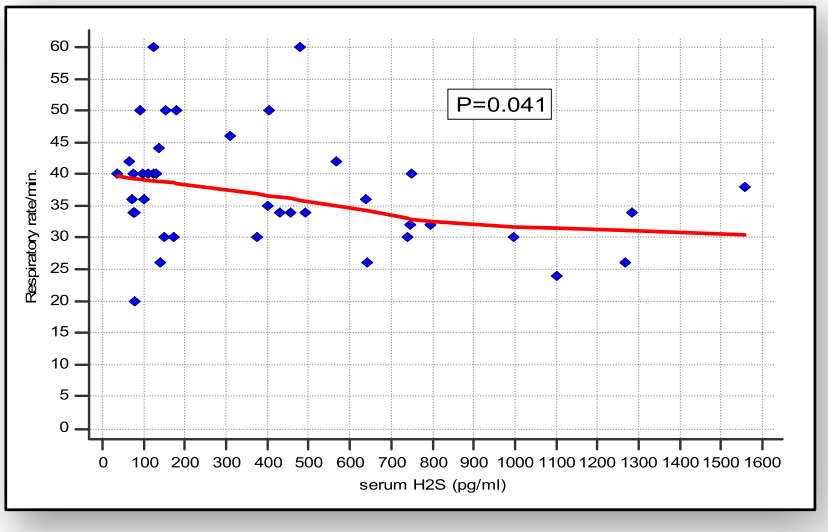

Fig. 1 Scatter plot between serum H_2S and respiratory rate

Table 3 Correlation between serum H_2S and other variables in asthmatic children

		Serum H_2S	Age	Cough	RD	HR	RR	Eosinophilic %
RD	**r- coefficient**	0.132	0.047	0.362*				
	***p*- value**	0.418	0.771	0.022				
HR	**r-coefficient**	0.093	-0.608*	-0.026	0.481**			
	***p*- value**	0.567	0	0.875	0.002			
RR	**r-coefficient**	-0.325*	-0.312*	0.127	0.412**	0.419**		
	***p*- value**	0.041	0.05	0.434	0.008	0.007		
Temp	**r-coefficient**	-0.115	-0.052	0.043	0.340*	0.302	0.058	
	***p*- value**	0.481	0.749	0.79	0.032	0.058	0.722	
Attack Severity	**r-coefficient**	-0.14	0.135	0.154	0.667**	0.29	0.152	
	***p*- value**	0.39	0.407	0.344	0	0.07	0.349	
Hb %	**r-coefficient**	-0.239	0.092	-0.065	-0.042	-0.21	0.319*	
	***p*- value**	0.138	0.572	0.69	0.796	0.193	0.045	
Absolute eosinophilic count	**r-coefficient**	-0.13	0.253	0.162	0.273	-0.031	0.077	0.889*
	***p*- value**	0.425	0.115	0.316	0.088	0.851	0.635	0

*$p < 0.05$

**$p < 0.01$

Our study showed that there was no statistical difference between both groups for the residence ratio (urban/rural). Acute asthmatics coming from urban areas were more than those coming from rural areas (ratio 4.7 to 1). This is consistent with the hygiene hypothesis that attempts to explain the observed trend of increased asthma prevalence in industrialized countries in urban areas compared with rural areas [13].

The mean temperature was significantly higher in group "A" compared with group "B". The main reason for the increased temperature is that they started with an acute respiratory infection (usually viral) that acted as the trigger of the acute asthma attack. For patients with asthma, viral respiratory tract infections, mainly those caused by human rhinoviruses, are associated with acute asthma exacerbations. There is also evidence that deficits in antiviral activity and integrity of the

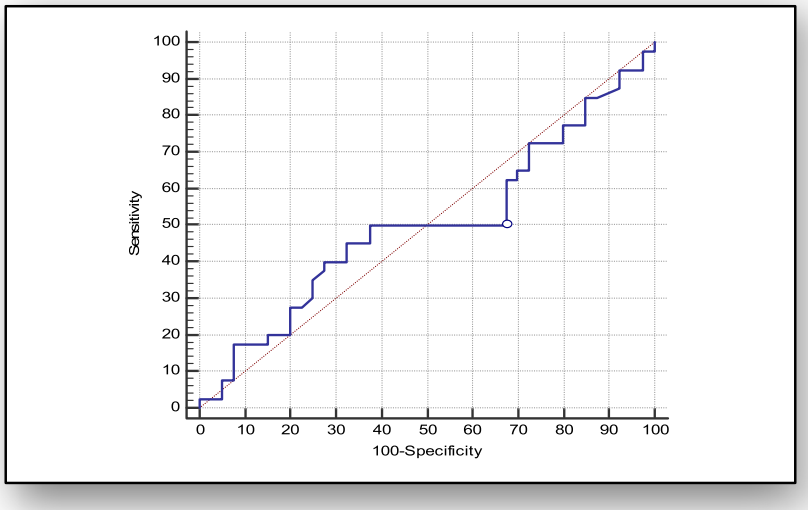

Fig. 2 Receiver operating characteristic (ROC) curve of serum H_2S in pediatric acute asthma

airway epithelial cells could make patients with asthma more prone to have severe viral respiratory infections of the lower airways, and thus increase the risk of asthma exacerbation [14].

The present study revealed that the mean respiratory and heart rates were significantly higher in group "A" compared with group "B". This significant increase in respiratory and heart rates might be explained by the fact that our cases presented with acute asthma exacerbation. In addition, the treatment received in the emergency room which is usually inhaled short acting beta-2 agonists (SABA) sometimes could result in tachycardia especially if nebulization was repeated several times [15].

A significant inverse correlation between the serum H_2S and respiratory rate was found in our study. It appears that as serum H_2S level decreases the severity of respiratory distress increases (reflected by increased respiratory rate). Our reported correlation is in concordance with Tian et al. [16] who found that the serum H_2S concentration was positively correlated with lung function indices. Thus, they suggested that reduced serum H_2S level indicates reduced lung functions. Since an inverse correlation was found in our study, our hypothesis is accepted.

Regarding the hematological data, no significant difference between both groups was found. Our findings are in contrast with those reported by Rhew et al. [17]. They found significant positive association between asthma and anemia with odds ratio of 1.71, and 2.64 respectively. This contradiction between our findings and these studies could be due to the difference in sample size and the fact that these studies were conducted on stable asthmatics. Further research is needed to elucidate any association between anemia and acute asthma in children.

In our study, there was a significant increase in the eosinophilic percentage and absolute eosinophilic count in group "A" compared with group "B". This is in agreement with Nakagome and Nagata [18]. The difference could be explained by the fact that eosinophils is involved in the pathogenesis of allergic disorders including BA.

In our study, no statistically significant difference in serum H_2S level was found between both groups. This is in contrast to Tian et al. [16] who reported the serum H_2S concentration was significantly decreased in children with acute asthma. The difference between our findings and those reported by Tian et al. might be explained by difference in the method of H_2S measurement. The ELISA method was used in our study versus the sensitive sulfur electrode [XS-270 ion meter, Leici®, China] that was used in their study. The severity of acute asthma was also different; in our study all our cases did not need hospitalization and the majority (82.5%) were classified according to GINA guidelines as mild attack. However, in Tian et al.'s study all cases needed hospitalization and treatment for 6 to 25 days, which suggests that their acute exacerbations were moderate or severe. Since our study revealed a non-significant difference in the serum H_2S level between both groups, our hypothesis is rejected.

To evaluate the diagnostic accuracy of serum H_2S in acute asthmatic children, the receiver operating

characteristic (ROC) curve of serum H_2S was plotted. It showed that the serum H_2S had sensitivity of 50% and specificity of 32.5% in discriminating acute asthmatic children from non-asthmatic. This suggests poor performance of serum H_2S as a screening tool in acute asthma. However, future studies with larger sample sizes might yield different results.

Our study had some points of strength; it is one of the few studies conducted on serum H_2S in acute asthmatic children, and it found a significant negative correlation between serum H_2S and respiratory rate. However, it had several limitations. The limitations involve the extremely short half-life of the H_2S (estimated to be between seconds and minutes), small sample size, measurement of H_2S in serum not sputum, not conducting pulmonary function tests, examining mild and moderate grades only of acute asthma, and not examining follow-ups with the study design being cross-sectional.

Few studies have been conducted on the biological effects of endogenous H_2S on airway and lung functions in respiratory diseases, and very few of them addressed the pediatric age group. Further investigation of this compound in the context of airway disease is required, possibly leading to improvement in the diagnosis and treatment of such diseases. Investigating sputum H_2S levels and sputum -to-serum H_2S ratio in acute asthmatic children is also required.

Conclusion

It was hypothesized that reduced H_2S production in the respiratory system could be used as an early detection biomarker, and consequently H_2S-based therapy would represent a new treatment strategy for asthma. Major challenges for establishing the diagnostic and therapeutic values of H_2S include the differential expression of CSE and CBS along the airway and their changes during asthma, the effects of H_2S on airway remodelling and bronchoconstriction, and the detection of the changes in H_2S levels in airway tissues and in exhaled air.

Unfortunately, in the current study there was no statistically significant difference in serum H_2S level between asthmatic children and healthy controls. There was a significant negative correlation between serum H_2S and respiratory rate. The serum H_2S has sensitivity of 50% and specificity of 32.5% in discriminating acute asthmatic children from healthy controls, so it is suggested to be a poor screening and diagnostic tool.

Abbreviations

H_2S	Hydrogen sulphide
BA	Bronchial asthma
CBS	Cystathionine β-synthase
CSE	Cystathionine γ-lyase
ER	Emergency Room
GINA	Global Initiative for Asthma
EDTA	Etheylene-diamine-tetra-acetic
CBC	Complete blood count
ELISA	Enzyme-linked immunosorbent assay
CI	Confidence intervals
SPSS	Statistical Package for the Social Sciences
ROC	Receiver operating characteristic
SABA	Short acting beta-2 agonists

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Authors' contributions

MAA drafted the work and revised it, being the major contributor in writing the manuscript. MAF made substantial contribution to the concept and design of the work and interpretation of data. ASA contributed to data acquisition. All authors read and approved the final manuscript. Each agreed both to be personally accountable for the author's own contributions and ensured that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Availability of data and materials

All data are available upon request.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Ethical Review Board, Faculty of Medicine, Cairo University, Approval number: MS 48 -2020.

Consent for publication

Not applicable (no individual details, images or videos).

Competing interests

The authors declare that they have no competing interests.

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