Abstract: Identifying children with chronic kidney disease (CKD) at high risk of cardiovascular disease (CVD) and ensuring they receive appropriate treatment can prevent CVD events and mortality later in life. Hydrogen sulfide (H\textsubscript{2}S) is a gaseous signaling molecule participating in CVD and CKD. Thiosulfate is not only an oxidation product of H\textsubscript{2}S but is also a H\textsubscript{2}S donor. We examined whether H\textsubscript{2}S, thiosulfate, and their combined ratio have differential associations with CVD risk markers in 56 children and adolescents aged 6–18 years with CKD stages G1–G4. Up to two-thirds of CKD children showed higher BP load on 24 h ambulatory blood pressure monitoring (ABPM), even in the early stage. CKD children with ABPM abnormalities had a higher H\textsubscript{2}S-to-thiosulfate ratio, while H\textsubscript{2}S-related parameters were not affected by the severity of CKD. The H\textsubscript{2}S-to-thiosulfate ratio was positively correlated with 24 h systolic BP (SBP), nighttime SBP, and carotid artery intima-media thickness (cIMT). After adjusting for confounders, H\textsubscript{2}S was negatively associated with LV mass, thiosulfate was positively associated with 24-DBP, and the H\textsubscript{2}S-to-thiosulfate ratio was positively correlated with nighttime SBP and cIMT. Our data demonstrate differential associations in circulating H\textsubscript{2}S, thiosulfate, and their combined ratio with CVD risk in childhood CKD. Further studies are required to determine whether targeting the H\textsubscript{2}S signaling pathway can develop novel therapeutic strategies against CVD in this high-risk population.

Keywords: cardiovascular disease; ambulatory blood pressure monitoring; chronic kidney disease; children; hydrogen sulfide; nitric oxide; hypertension; thiosulfate

1. Introduction

Hydrogen sulfide (H\textsubscript{2}S) is a gaseous signaling molecule of biological impact in illness involving cardiovascular disease (CVD) [1,2]. Despite being a poisonous gas in excess, H\textsubscript{2}S executes important biological functions at the physiological level [3]. Nitric oxide (NO) has long been known as a vasodilator. Increasing experimental evidence indicates that NO deficiency is involved in hypertension and chronic kidney disease (CKD) [4]. Similar to NO, H\textsubscript{2}S has a key role in the regulation of blood pressure (BP) and renal physiology [1,5]. The production of H\textsubscript{2}S can occur through enzymatic or non-enzymatic reactions [1]. H\textsubscript{2}S can be non-enzymatically generated from organic thiol. Thiosulfate is one product formed during oxidative H\textsubscript{2}S metabolism. Alternatively, thiosulfate can be reduced and regenerate H\textsubscript{2}S [6].
Accordingly, thiosulfate is a metabolite of H₂S and an index of the sulfide pool [7]. In adult patients who received hemodialysis, the H₂S-generating pathway was down-regulated [8].

Hypertension in children and adults with CKD is an important clinical concern resulting in high risk of CVD morbidity and mortality as well as CKD progression. Although overt cardiovascular (CV) events rarely happen in children, atherosclerosis and hypertension can begin in early childhood [9]. We and others have shown that hypertension is extraordinarily prevalent in CKD children, even in the early stages [10,11]. Accordingly, early identification of children at risk for CVD may facilitate timely intervention and reduce the burden of CKD. To date, several noninvasive functional and structural assessments have been reported to identify high-risk CKD children for CVD [12]. These surrogate markers for CVD include 24 h ambulatory blood pressure monitoring (ABPM), carotid artery intima-media thickness (cIMT), left ventricular (LV) mass, and arterial stiffness indices. [12–14]. Our previous study revealed that several NO-related parameters are correlated with certain CVD risk surrogate markers in the early stages of pediatric CKD [10]. However, little information currently exists with regard to H₂S and thiosulfate levels in CKD children [15]. Importantly, early identification of subphenotypes has led to insights into their pathogenesis and the development of personalized approaches to CKD care.

Given this background, we hypothesized that the H₂S signaling pathway may be a crucial mechanism contributing to the development of hypertension and CVD in childhood CKD. We therefore evaluated the associations between H₂S, thiosulfate, and their combined ratio with CVD risk markers in children with early-stage CKD.

2. Materials and Methods

2.1. Patients and Study Design

We used data from a prospective cohort study of children and adolescents aged 6–18 years with CKD who were recruited between November 2018 and April 2022 from the Pediatric Nephrology Outpatient Clinic at the Kaohsiung Chang Gung Memorial Hospital, a tertiary medical center in Taiwan. This project was approved by the ethics committee of Chang Gung Medical Foundation, Taoyuan, Taiwan (201701735A3C501 and 202001973A3C601), and written informed consent was obtained from each participant and their parents. The CKD was defined and staged by the KDIGO 2012 clinical practice guidelines [16]. Based on body height and blood creatinine (Cr) level, the estimated glomerular filtration rate (eGFR) was calculated with the bedside CKiD equation formula [17]. Staging of CKD was classified according to eGFR (mL/min/1.73 m²) as G5 < 15, G4 15–29, G3 30–59, G2 60–89, and G1 ≥ 90. The etiologies of CKD were divided into two groups: congenital anomalies of kidney and urinary tract (CAKUT) and non-CAKUT. The following exclusion criteria were applied: if a participant was pregnant, had congenital heart disease, had CKD stage G5, had received dialysis or a kidney transplant, or was unable to cooperate with the CV assessment, then they were excluded from the selection.

This ancillary study was conducted on a subgroup of 56 participants who received the measurement of H₂S and thiosulfate, 24 h ABPM, as well as CV assessments. We collected fasting blood samples and spot urine samples. These aliquots were refrigerated immediately before being moved to −80 °C storage. Blood urea nitrogen, Cr, hemoglobin, total cholesterol, triglyceride, low-density lipoprotein (LDL), glucose, sodium, potassium, calcium, phosphate, uric acid, and total protein-to-creatinine ratio were analyzed by the hospital central laboratory as stated before [11].

2.2. Analysis of Plasma H₂S and Thiosulfate Levels

We measured concentrations of H₂S and thiosulfate in the plasma according to our validated protocol by an Agilent Technologies 1290 high-performance liquid chromatography (HPLC) system connected to an Agilent 6470 Triple Quadrupole LC/MS Spectrometry (MS) (Agilent Technologies, Wilmington, NC, USA) [18]. Phenyl 4-hydroxybenzoate (PHB) was added to samples as an internal standard. The column used for chromatographic separation was a Supelco C18 column (5 cm × 2.1 mm, 3 μm; Sigma–Aldrich, Bellefonte,
PA, USA) protected by an Ascentis C18 column (2 cm × 2.1 mm, 3 µm; Merck KGaA, Darmstadt, Germany). The solvent system was composed of 0.1% formic acid in water and acetonitrile. The flow rate was 300 µL/min. The LC/MS was equipped with an electrospray ionization (ESI) source. We detected thiosulfate derivative pentafluorobenzyl (PFB)-S$_2$O$_3$H and H$_2$S derivative sulfide dibimane (SDB). Selected reaction monitoring mode was applied to detect target compounds with a target of $m/z$ 415–223, $m/z$ 292.99–81, and $m/z$ 212.99–93, for SDB, PFB-S$_2$O$_3$H, and PHB, respectively. The intra-assay coefficient of variation was 4% and 6% for H$_2$S and thiosulfate, respectively.

2.3. Blood Pressure Measurement and Cardiovascular Assessment

Children were instructed to measure office BP at the clinic visit. After an initial 5 min of rest, seated BP was measured in triplicate at 1 min intervals. Hypertension was diagnosed according to the 2017 American Academy of Pediatrics (AAP) guidelines [19]. Data from 24 h ABPM were collected from subjects using the Oscar II monitoring device (SunTech Medical, Morrisville, NY, USA). BP and heart rate (HR) were recorded by 24 h ABPM every 20–30 min for 24 h. Participants and their parents were asked to keep a personal record documenting activities and sleeping times. We used the following definition to examine abnormalities on ABPM profile: (1) daytime or nighttime systolic or diastolic BPs greater than the 95th percentile in reference to gender and height; (2) daytime or nighttime systolic or diastolic BP load greater than 25%; and (3) nighttime BP load dipping less than 10% based on comparison with ABPM reference data [20].

Echocardiograms were performed on Philips IE33 system (Philips, Bothell, WA, USA) by experienced pediatric cardiologists. For each patient, measurement of LV mass was performed in the parasternal long-axis view using two different modalities (M-mode imaging and 2D imaging). The LV mass index (LVMI) was calculated by indexing LV mass to height$^{2.7}$ [21]. Images of the common carotid artery were obtained using Doppler ultrasound (ProSound α7, Aloka Co., Tokyo, Japan) with a 5–12 MHz linear array transducer. The cIMT was measured during end diastole as determined at the R-wave on the electrocardiography. Indices of arterial stiffness, augmentation index (AI), and pulse wave velocity (PWV) were analyzed by echo-tracking technique (e-TRACKING system; Aloka Co., Tokyo, Japan).

2.4. Statistical Analysis

Descriptive characteristics were expressed as median (25–75th percentile), mean ± standard deviation, or number (%). Mann–Whitney U-test, t-test, or Chi-squared test was employed to evaluate differences in variables between the two groups. Spearman’s rank correlation coefficient analysis was performed to measure the degree of association between two variables. Multivariable linear regression models were used to examine the associations among H$_2$S-related parameters with CVD risk markers. The level of statistical significance was set at $p < 0.05$. All data were analyzed using the Statistical Package for the Social Sciences (SPSS) software 14.0 (Chicago, IL, USA).

3. Results

3.1. Patient Characteristics

As Table 1 shows, the study participants comprised 56 children and adolescents with CKD. This study group had a median age of 11.4 years, was 57.1% male, and consisted of 51.8% with CAKUT. Among the 56 subjects with CKD stages G1–G4, 35 (62.5%), 17 (30%), 3 (5.4%), and 1 (1.8%) had CKD stages G1, G2, G3, and G4, respectively. CKD children and adolescents were stratified into two groups based on eGFR: the G1 group (eGFR ≥ 90 mL/min/1.73 m$^2$) and the G2–G4 group (eGFR < 90 mL/min/1.73 m$^2$). The G2–G4 group was higher in age, systolic BP, blood urea nitrogen, Cr, and uric acid, but lower in eGFR than those in the G1 group (Table 1). A total of 12 CKD children (21.4%) were diagnosed as having hypertension according to office BP measurement; however, CKD children with this characteristic did not differ between the two groups. Regarding
antihypertensive therapy, five of the G1 group (14.3%) and six of the G2–G4 group (28.6%) received angiotensin II receptor blocker (ARB) alone or with calcium channel blocker.

Table 1. Demographics and biochemical data.

| CKD Stage | G1 | G2–G4 |
|-----------|----|-------|
| Case numbers | 35 | 21 |
| Age (years) | 10.7 (8.7–13.9) | 14.4 (10.5–16.1) * |
| Male gender (%) | 17 (42.9%) | 15 (71.4%) |
| Body height (percentile) | 50 (25–85) | 50 (25–85) |
| Body weight (percentile) | 75 (25–85) | 75 (25–85) |
| Body mass index (kg/m²) | 17.9 (15.1–20.9) | 18.6 (15.6–23.1) |
| Systolic blood pressure (mmHg) | 106 (100–112) | 120 (112–125) * |
| Diastolic blood pressure (mmHg) | 68 (63–77) | 70 (64–80) |
| CAKUT (%) | 15 (42.9%) | 14 (66.7%) |
| Hypertension (% by office BP) | 6 (20.7%) | 6 (40%) |
| Blood urea nitrogen (mg/dL) | 11 (10–13) | 16 (12.5–18.5) * |
| Creatinine (mg/dL) | 0.5 (0.45–0.55) | 0.9 (0.75–0.96) * |
| eGFR (mL/min/1.73 m²) | 120 (112–126) | 80 (70–85) * |
| Hemoglobin (g/dL) | 13.5 (13–14.1) | 14.3 (12.9–15.5) |
| Total cholesterol (mg/dL) | 158 (141–181) | 155 (133–178) |
| Low-density lipoprotein (mg/dL) | 80 (65–102) | 75 (62.5–96.5) |
| Triglyceride (mg/dL) | 61 (49–101) | 65 (50.5–103) |
| Uric acid (mg/dL) | 5.1 (4–6) | 6.4 (5.7–7.7) * |
| Glucose (mg/dL) | 87 (83–89.5) | 86 (83–91.5) |
| Sodium (mEq/L) | 141 (140–142) | 141 (140–142) |
| Potassium (mEq/L) | 4.3 (4.1–4.5) | 4.5 (4.2–4.6) |
| Calcium (mg/dL) | 9.5 (9.2–9.9) | 9.8 (9.3–10.1) |
| Phosphate (mg/dL) | 4.8 (4.5–5.2) | 4.8 (4.2–5.1) |
| Urine total protein-to-creatinine ratio (mg/g) | 52.5 (37.3–327.9) | 43.3 (30.5–225.4) |
| On antihypertensive therapy | 5 (4.3%) | 6 (28.6%) |

Values are presented as median (25th, 75th percentile) or n (%). * p < 0.05 by the Mann–Whitney U-test. eGFR = estimated glomerular filtration rate; CAKUT = congenital anomalies of kidney and urinary tract; CKD = Chronic Kidney Disease.

3.2. Plasma H₂S and Thiosulfate Levels

We analyzed the plasma levels of H₂S and thiosulfate in children with CKD and determined the ratio of H₂S to thiosulfate (Table S1, Supplementary Materials). As presented in Figure 1, our data revealed there was no difference in plasma levels of H₂S (Figure 1A), thiosulfate (Figure 1B), nor in the ratio of H₂S to thiosulfate (Figure 1C) between the two groups. Analyses that evaluated the existence of associations between levels of H₂S-related parameters with renal function and office BP indicated that there was no correlation between plasma Cr level with H₂S (p = 0.565), thiosulfate (p = 0.408), nor the ratio of H₂S to thiosulfate (p = 0.448). Additionally, systolic BP was positively correlated with plasma H₂S level (r = 0.264, p = 0.049).

3.3. Cardiovascular Assessment

From the study of 56 pediatric patients with CKD who concurrently had laboratory tests and a comprehensive CV assessment performed, 38 (67.9%) had at least one BP load abnormality on ABPM. This included 11 subjects (19.6%) with 24 h BP greater than the 95th percentile, 6 subjects (10.7%) with daytime BP greater than the 95th percentile, 18 subjects (32.1%) with nighttime BP greater than the 95th percentile, 25 subjects (44.6%) with BP load greater than the 25th percentile, and 33 patients (58.9%) with a nocturnal decrease in BP of less than 10% (Table 2). There was a greater proportion of CKD children with 24 h BP greater than the 95th percentile in the G2–G4 group vs. the G1 group. We observed that the LV mass was higher in children with CKD stages G2–G4 compared to G1, while LVMI did not differ between the two groups. Likewise, the cIMT in the G1 and G2–G4 group did
not differ. Using the AI and PWV to evaluate arterial stiffness, we observed neither was different between the two groups.

Figure 1. Plasma (A) H\textsubscript{2}S and (B) thiosulfate levels and (C) H\textsubscript{2}S-to-thiosulfate ratio (HTR) in CKD children.

Table 2. Cardiovascular assessments.

| CKD Stage          | G1          | G2–G4       |
|--------------------|-------------|-------------|
| 24 h ABPM          | 35          | 21          |
| Abnormal ABPM profile (with any of the following abnormalities) | 22 (62.9%) | 16 (76.2%) |
| Average 24 h BP > 95th percentile | 4 (11.4%) | 7 (33.3%) * |
| Average daytime BP > 95th percentile | 2 (5.7%) | 4 (19%) |
| Average nighttime BP > 95th percentile | 9 (25.7%) | 9 (42.9%) |
| BP load ≥ 25%      | 15 (42.9%)  | 10 (47.6%)  |
| Nocturnal decrease in BP of <10% | 21 (60%) | 12 (57.1%) * |
| Left ventricular mass (g) | 86.4 (65.6–99.3) | 118 (72.2–171) * |
| Left ventricular mass index (g/m\textsuperscript{2.7}) | 30.3 (25.9–36.1) | 34.2 (28.4–42.2) |
| Carotid artery intima-media thickness (mm) | 0.3 (0.3–0.4) | 0.3 (0.3–0.4) |
| Augmentation index (%) | −2.8 (−10.2−0.8) | −6 (−16.8−4.1) |
| Pulse wave velocity (m/s) | 3.8 (3.4–4.1) | 4 (3.6–4.7) |

Values are presented as median (25th, 75th percentile) or n (%). *p < 0.05 by the Chi-squared test or the Mann–Whitney U-test. ABPM = 24 h ambulatory blood pressure monitoring. BP = blood pressure.

3.4. Association between H\textsubscript{2}S and Cardiovascular Risk Markers

Table 3 illustrates correlations between H\textsubscript{2}S, thiosulfate, and H\textsubscript{2}S-to-thiosulfate ratio with CV risk markers across all CKD patients. Spearman’s rank correlation analysis revealed H\textsubscript{2}S was positively correlated with nighttime SBP (r = 0.275, p = 0.04). Conversely, thiosulfate negatively correlated with nighttime SBP (r = −0.27, p = 0.044). The H\textsubscript{2}S-to-thiosulfate ratio exhibited positive correlations with 24 h SBP (r = 0.306, p = 0.002), nighttime SBP (r = 0.336, p = 0.011), and cIMT (r = 0.267, p = 0.047).
Table 3. Correlation between plasma H$_2$S and thiosulfate levels with cardiovascular markers.

| Cardiovascular Markers                           | H$_2$S  | Thiosulfate | H$_2$S-to-Thiosulfate Ratio |
|-------------------------------------------------|---------|-------------|----------------------------|
|                                                 | $r$     | $p$         | $r$           | $p$       | $r$     | $p$       |
| 24 h systolic blood pressure                     | 0.239   | 0.076       | $-0.263$      | 0.05      | 0.306   | 0.022 $^*$|
| Daytime systolic blood pressure                  | 0.234   | 0.082       | $-0.218$      | 0.106     | 0.238   | 0.077     |
| Nighttime systolic blood pressure                | 0.275   | 0.04 $^*$   | $-0.27$       | 0.044 $^*$| 0.336   | 0.011 $^*$|
| 24 h diastolic blood pressure                    | 0.241   | 0.074       | $-0.124$      | 0.362     | 0.18    | 0.184     |
| Daytime diastolic blood pressure                 | 0.139   | 0.307       | $-0.117$      | 0.39      | 0.103   | 0.449     |
| Nighttime diastolic blood pressure               | 0.226   | 0.094       | $-0.084$      | 0.54      | 0.159   | 0.242     |
| Left ventricular mass                            | $-0.034$| 0.804       | $-0.093$      | 0.495     | 0.095   | 0.484     |
| Left ventricular mass index                      | $-0.201$| 0.137       | $-0.129$      | 0.343     | 0.045   | 0.742     |
| Carotid artery intima-media thickness           | 0.244   | 0.07        | $-0.173$      | 0.201     | 0.267   | 0.047 $^*$|
| Augmentation index                               | $-0.127$| 0.351       | $-0.115$      | 0.397     | 0.076   | 0.579     |
| Pulse wave velocity                              | 0.004   | 0.979       | $-0.039$      | 0.777     | 0.054   | 0.694     |

* $p < 0.05$ by Spearman’s correlation coefficient.

We next analyzed plasma H$_2$S and thiosulfate levels, and their combined ratio, stratified according to the ABPM profile. Table 4 shows the H$_2$S-to-thiosulfate ratio was significantly higher in CKD children with 24 h hypertension, nighttime hypertension, high BP load, non-night dipping, and abnormal ABPM profile. Analysis of individual H$_2$S or thiosulfate did not show any significant difference between CKD children with normal and abnormal ABPM profile.

Table 4. Plasma H$_2$S and thiosulfate levels vs. ABPM profile.

| ABPM Profile       | n   | H$_2$S          | Thiosulfate | H$_2$S-to-Thiosulfate Ratio |
|--------------------|-----|-----------------|-------------|-----------------------------|
|                    |     | $\mu$mol/L      | $\mu$mol/L | $\mu$mol/$\mu$mol          |
| 24 h BP            |     |                 |             |                             |
| Normal             | 45  | $15.5 \pm 9.6$  | $1.07 \pm 1.44$ | $39.2 \pm 46.9$            |
| Abnormal           | 11  | $18.1 \pm 8.8$  | $1.02 \pm 1.47$ | $82.3 \pm 86.7 ^*$         |
| Daytime BP         |     |                 |             |                             |
| Normal             | 50  | $15.9 \pm 9.8$  | $1.05 \pm 1.42$ | $45.6 \pm 59.1$            |
| Abnormal           | 6   | $17.1 \pm 5.6$  | $1.18 \pm 1.65$ | $64.7 \pm 55.9$            |
| Nighttime BP       |     |                 |             |                             |
| Normal             | 38  | $14.3 \pm 6.6$  | $1.17 \pm 1.54$ | $30.3 \pm 29.6$            |
| Abnormal           | 18  | $19.5 \pm 13.1$ | $0.84 \pm 1.18$ | $84.5 \pm 84.1 ^*$         |
| BP load            |     |                 |             |                             |
| Normal             | 31  | $12.9 \pm 5.3$  | $1.1 \pm 1.52$ | $28.4 \pm 28.8$            |
| Abnormal           | 25  | $19.8 \pm 11.6 ^*$ | $1.02 \pm 1.35$ | $71.7 \pm 75.7 ^*$         |
| Night dipping      |     |                 |             |                             |
| Normal             | 23  | $13.9 \pm 5.4$  | $0.94 \pm 1.05$ | $30 \pm 24.9$              |
| Abnormal           | 33  | $17.5 \pm 11.3$ | $1.15 \pm 1.66$ | $60 \pm 71.3 ^*$           |
| ABPM profile       |     |                 |             |                             |
| Normal             | 18  | $13.1 \pm 5.6$  | $0.94 \pm 1.11$ | $24.7 \pm 15$              |
| Abnormal           | 38  | $17.4 \pm 10.6$ | $1.12 \pm 1.57$ | $58.6 \pm 67.9 ^*$         |

* $p < 0.05$ by the $t$-test.

Table 5 illustrates the associations between H$_2$S, thiosulfate, and their ratio with cardiovascular risk markers using multivariate linear regression analyses adjusted for age, sex, eGFR, uric acid, and H$_2$S metabolites. In applications of regression analysis for prediction models ($r = 0.488$, $p = 0.002$), night SBP was associated with the H$_2$S-to-thiosulfate ratio ($p = 0.009$). Additionally, thiosulfate was associated with 24 h DBP ($p = 0.004$), daytime DBP ($p = 0.012$), and nighttime DBP ($p = 0.012$), controlling for age and eGFR. We also noted that LV mass was negatively associated with H$_2$S ($p = 0.004$) in the adjusted model controlling for age ($r = 0.765$, $p < 0.001$). Moreover, a positive association was found between cIMT and the H$_2$S-to-thiosulfate ratio ($p = 0.021$) in the adjusted model controlling for sex.
(\(r = 0.458, p = 0.004\)). Our data suggest a significant influence of the \(H_2S\) pathway on the CVD risk.

Table 5. Adjusted regression model estimates of the association of plasma \(H_2S\), thiosulfate, and their ratio with cardiovascular risk markers.

| Dependent Variable | Explanatory Variable | Adjusted \(^a\) Model |   |
|--------------------|----------------------|------------------------|---|
|                    |                      | Beta   | \(p\) Value | \(r\) | \(p\) Value |
| Nighttime SBP      | \(H_2S\)-to-thiosulfate ratio | 0.356  | 0.009       | 0.488 | 0.002       |
| 24 h DBP           | Thiosulfate          | 0.354  | 0.004       | 0.628 | <0.001      |
| Daytime DBP        | Thiosulfate          | 0.317  | 0.012       | 0.589 | <0.001      |
| Nighttime DBP      | Thiosulfate          | 0.32   | 0.012       | 0.574 | <0.001      |
| Left ventricular mass | \(H_2S\)          | –0.291 | 0.004       | 0.765 | <0.001      |
| cIMT               | \(H_2S\)-to-thiosulfate ratio | 0.315  | 0.021       | 0.458 | 0.004       |

\(^a\) Adjusted for age, gender, eGFR, and uric acid. BP = blood pressure. SBP = systolic blood pressure. DBP = diastolic blood pressure. cIMT = carotid artery intima-media thickness.

4. Discussion

This is the first study describing the different predictive abilities of plasma \(H_2S\), thiosulfate, and \(H_2S\)-to-thiosulfate ratio as biomarkers for subclinical CVD in CKD children. The key findings are (1) \(H_2S\)-related parameters were not affected by the severity of CKD; (2) more than two-thirds of CKD children exhibited BP load abnormalities on ABPM, even in the early stage; (3) the \(H_2S\)-to-thiosulfate ratio was positively correlated with 24 h SBP, nighttime SBP, and cIMT; (4) CKD children with abnormalities on ABPM had a higher \(H_2S\)-to-thiosulfate ratio compared to those with a normal profile; and (5) linear regression models indicated \(H_2S\) was inversely associated with LV mass; thiosulfate was positively associated with 24-DBP and daytime and nighttime DBP; and the \(H_2S\)-to-thiosulfate ratio was positively correlated with nighttime SBP and cIMT.

Plasma \(H_2S\) level has been reported to be reduced in CKD patients and animal models [5,8,18], possibly due to inhibition of \(H_2S\)-generating enzyme expression by CKD. Conversely, sodium thiosulfate therapy aids in preventing CKD-induced hypertension accompanied by increases in plasma levels of \(H_2S\) and thiosulfate [18]. Notably, thiosulfate can serve as a sulfide donor to increase \(H_2S\). In contrast, a significant quantity of \(H_2S\) is oxidized to thiosulfate. Accordingly, we determined not only \(H_2S\) and thiosulfate levels but also calculated their combined ratio to investigate their bidirectional relationship. The ratio of \(H_2S\)-to-thiosulfate may reflect the recycling of \(H_2S\) [22].

Plasma \(H_2S\) and its metabolites have served as biomarkers for many diseases involving CVD [23]. So far, a plethora of different analytical methods has evolved for \(H_2S\) detection. Given the diverse chemistries of \(H_2S\) detection methods, orders of magnitude differences in the physiological sulfide levels have been reported with a relatively high concentration [23]. Our data analyzed by the HPLC-MS/MS method tie well with recently developed methods revealing that the free \(H_2S\) level in plasma is relatively low, as reported at low \(\mu\)mol/L level [24,25]. Importantly, in addition to its free forms, within the biological matrix, \(H_2S\) also presents in other bound forms, which are involved in releasing free \(H_2S\) in physiological response to stimulus [23,24].

We observed that there was no difference of plasma levels of \(H_2S\), thiosulfate, and \(H_2S\)-to-thiosulfate ratio in CKD children between the G1 and the G2–G4 groups. These results contradict previous observations in adult patients with CKD, in whom plasma \(H_2S\) levels gradually decreased with CKD stage climbing [25]. We believe that the population comprising this study differs somewhat from those formerly reported since a significant fraction of children were in the early stages of CKD.

Our study shows that a high prevalence of CKD children had BP load abnormalities on ABPM irrespective of their CKD stages. Our data indicated that high BP load on ABPM in children with early stages of CKD is often disguised by office BP measurements, consistent with prior studies [10,11]. In line with prior findings in pediatric CKD [21,26], left
Ventricular hypertrophy represented as increased LV mass tends to develop in advanced CKD. However, several CVD risk markers, such as cIMT, AI, and PWV, were not different between the two groups.

Although there were few associations between abnormal ABPM profile with individual H\textsubscript{2}S or thiosulfate level, the H\textsubscript{2}S-to-thiosulfate ratio was significantly higher in CKD children with BP load abnormalities on ABPM, including 24 h hypertension, nighttime hypertension, high BP load, and non-night dipping. Additionally, in our study, a positive association between the H\textsubscript{2}S-to-thiosulfate ratio and nighttime SBP was found, even after controlling for confounding. We observed that there were strong positive associations between thiosulfate with 24 h DBP, daytime DBP, and nighttime DBP. These findings are in remarkable contrast to recent animal studies showing thiosulfate had therapeutic potential in hypertension and kidney disease [18,22,27]. Considering that H\textsubscript{2}S and thiosulfate can be converted to each other, a high H\textsubscript{2}S-to-thiosulfate ratio may reflect increased release of free H\textsubscript{2}S or reduced sulfide pool capacity to augment the thiosulfate-to-H\textsubscript{2}S conversion. This mechanistic relationship supports the notion that the higher H\textsubscript{2}S-to-thiosulfate ratio does not represent a secondary response but rather a compensatory effect to high BP load in the early stage of CKD children.

For further investigations of the impact of H\textsubscript{2}S against subclinical CVD in CKD children, we examined the correlation of H\textsubscript{2}S and thiosulfate with CVD risk markers, including LV mass, cIMT, and arterial stiffness parameters. Increases in LV mass and cIMT have been observed in adults and children with advanced CKD [15]. A lower plasma H\textsubscript{2}S level was reported as an independent predictor of increased LV mass in adult CKD [28]. This notion was supported by our data, which show a negative association between H\textsubscript{2}S and LV mass. We also found cIMT was positively associated with the H\textsubscript{2}S-to-thiosulfate ratio. In view of the fact that a high H\textsubscript{2}S-to-thiosulfate ratio represents increased conversion of thiosulfate to H\textsubscript{2}S, our data suggest an increase in this ratio might be a compensatory response against increased cIMT in children with early stages of CKD. Nevertheless, in the current study, we did not observe abnormal arterial stiffness parameters in children with early stages of CKD and their associations with H\textsubscript{2}S-related parameters. Given that validation studies of arterial stiffness have not been reproduced in childhood CKD [29], further studies are necessary to clarify the prognostic abilities of AI and PWV for cardiovascular outcomes in larger pediatric CKD cohorts.

Overall, the current study has some limitations. First, it is probable that a small number of CKD children from one hospital do not represent an entire population and provide sufficient power to determine slight differences in the CVD risk markers. Further multicenter studies of larger numbers may be warranted. Second, we did not recruit healthy children in this cohort since children with CKD stage G1 served as the control to compare the differences of BP load and CV risk markers between two different levels of eGFR (i.e., ≥90 vs. <90 mL/min/1.73 m\textsuperscript{2}). Third, reference values for ABPM were based on studies conducted on participants of white ethnicity [20]. Ethnic differences must be considered, and our findings await further validation in other populations.

5. Conclusions

There is a paucity of data on the role of H\textsubscript{2}S on childhood CKD, especially in the early stage. Our study casts a new light on the different predictive abilities of plasma H\textsubscript{2}S, thiosulfate, and the H\textsubscript{2}S-to-thiosulfate ratio as biomarkers for subclinical CVD in children with CKD. A better understanding of the role of biomarkers will aid in risk stratification and personalized approaches for CKD care. Exploiting the possibilities that the study of H\textsubscript{2}S holds in this regard can have potential implications for therapeutic strategies against CVD in pediatric CKD.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jpm12081241/s1, Table S1: Plasma H\textsubscript{2}S, thiosulfate, and H\textsubscript{2}S-to-thiosulfate ratio in CKD children.
Author Contributions: Conceptualization, C.-N.H. and Y.-L.T.; data curation, W.-T.L., W.-L.C.,
and Y.-L.T.; funding acquisition, Y.-L.T.; project administration, C.-N.H. and Y.-L.T.; methodology,
G.-P.C.-C., S.L., and Y.-L.T.; writing—original draft, C.-N.H., W.-T.L., W.-L.C., and Y.-L.T.; writing—
review and editing, C.-N.H., W.-T.L., W.-L.C., G.-P.C.-C., S.L., and Y.-L.T. All authors have read and
agreed to the published version of the manuscript.

Funding: This work was supported by the Chang Gung Memorial Hospital, Kaohsiung, Taiwan,
grant CORPG8M0151, and the Ministry of Science and Technology, Taiwan, grants MOST 110-2314-B-
182-020-MY3 and MOST 110-2314-B-182A-029.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration
of Helsinki, and approved by the Institutional Review Board and Ethics Committee of Chang Gung
Medical Foundation, Taoyuan, Taiwan (201701735A3C501 and 202001973A3C601).

Informed Consent Statement: Informed consent was obtained from all subjects and parents involved in
the study.

Data Availability Statement: Data are contained within the article.

Acknowledgments: We would like to thank the Institute of Environmental Toxin and Emerging-
Contaminant, the Super Micro Mass Research and Technology Center and the Center for Environmental
Toxin and Emerging Contaminant Research, Cheng Shiu University, Kaohsiung, for technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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