Pulmonary Circulation

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RESEARCH ARTICLE OPEN ACCESS

Assessment of the Relationship Between the Six-Minute Walk Test (6MWT) With Serum Chloride Level and Mean Pulmonary Arterial Pressure in Patients With Pulmonary Arterial Hypertension

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Received: 31 January 2025 | **Revised:** 14 March 2025 | **Accepted:** 21 March 2025

Funding: The Mashhad University of Medical Sciences funded this study. The data in this article were taken from the thesis of Maryam Masinaei at the Mashhad University of Medical Sciences. (Reg. No. 4001798).

Keywords: mean pulmonary arterial pressure | pulmonary arterial hypertension | serum chloride levels | 6-min walk test

ABSTRACT

Pulmonary artery hypertension (PAH) is a fatal disease associated with high mortality, especially in countries with limited health resources in terms of lack of access to diagnostic and therapeutic evaluations. Therefore, it is necessary to discover inexpensive and available serum biomarkers for examining patients. This study investigates the relationship between PAH patients' six-minute walk (6MWT) distance, serum chloride levels, and mean pulmonary arterial pressure (mPAP). In this cross-sectional study, patients with PAH referring to the pulmonary hypertension clinic of our tertiary hospital were included. Then, the patient's demographic information and clinical findings were recorded, and the serum level of chloride and the 6MWT were examined in the patients. In the present study, 70 PAH patients were evaluated. All patients were female, and the mean age of the patients was 39.44 ± 8.33 years old. Hypochloremia was considered as serum chloride < 97 mmol/L in our study. The mPAP of patients with hypochloremia was significantly higher than non-hypochloremia patients ($p < 0.001$). The serum chloride levels had a significant positive correlation with the 6MWT distance ($r = 0.634$, $p < 0.001$). According to the linear regression analysis results, serum chloride level was a significant predictor of 6MWT distance even after adjustment for age and creatinine ($\beta = 0.48$; $p = 0.002$). Serum chloride level can be used as an inexpensive method for the evaluation of disease severity in PAH patients, especially in patients with higher time since the diagnosis of PAH.

1 | Introduction

Pulmonary arterial hypertension (PAH) is a subgroup of pulmonary hypertension (PH) characterized by the presence of vascular remodeling and vasoconstriction of precapillary arterioles [1, 2]. The PAH can cause right-side heart failure and even death, especially in untreated patients [3]. Despite recent progressions in the treatment of PAH patients, their mortality is

Article type: Original Research Article

high, and their 10-year survival estimates are about 65% [4]. The 6-min walk test (6MWT) is a valuable instrument for objective assessment of pulmonary hypertension severity and provides essential information about the functional status and exercise capacity of patients with pulmonary hypertension (PH) [5].

Recent studies show serum chloride can be involved in the pathophysiology of pulmonary hypertension and heart failure and may act as a predictor of severity and mortality in patients with PAH [1, 6–8]. Several proposed mechanisms exist for the relationship between serum chloride and cardiovascular diseases. Serum chloride influences body fluid balance, volume status, and diuretic resistance [9]. Besides, serum chloride can affect the With No Lysine (WNK) pathway and have an essential role in embryonic cardiac development, angiogenesis, and controlling blood pressure [7, 10].

Elevated mean pulmonary arterial pressure (mPAP) is the hallmark of PH and contributes to these patients' physical activity limitations [11]. Understanding the relationship between 6MWD, serum chloride levels, and mPAP could provide critical insights into the mechanisms underlying exercise intolerance in PAH patients and potentially identify biomarkers for disease severity. Finding a new, inexpensive biomarker for assessing the severity of PAH is more important in resource-limited countries [12].

This study aimed to assess the relationship between 6MWD, serum chloride levels, and mPAP in patients diagnosed with PAH. By clarifying these relationships, we hope to enhance the understanding of PAH pathophysiology, find new biomarkers for disease severity, and improve clinical management strategies, leading to better patient outcomes.

2 | Methods

2.1 | Study Design and Setting

This cross-sectional study included patients with pulmonary hypertension referred to the pulmonary hypertension clinic of Ghaem Hospital in Mashhad from 22 June 2022 to 21 August 2024.

2.2 | Participants

Patients between 18 and 75 years old diagnosed with PAH who had undergone Right heart catheterization (RHC) and echocardiography while ruling out other causes of PH were included in the study. Patients underwent RHC only once at the time of diagnosis, and that measurement was recorded. The "Time since PAH diagnosis (years)" variable represents the duration since the catheterization. The inclusion criteria for this study were as follows:

- Consent to participate in the study.
- Definitive diagnosis of PAH based on Right heart catheterization:
  - Mean pulmonary artery pressure (mPAP) $\geq$ 20 mmHg
  - Pulmonary artery wedge pressure (PAWP) $\leq$ 15 mmHg
  - Pulmonary vascular resistance (PVR) > 3 Wood units (WU)

  In 2022, the European Society of Cardiology (ESC) and the European Respiratory Society (ERS) proposed new diagnostic criteria for pulmonary hypertension (PH). The threshold for PVR has been adjusted from $\geq$ 3 Wood units (WU) to > 2 WU. These changes are reported to capture approximately 55% more patients with PH [13, 14]. However, our study adhered to the previous standard of 3 WU, which was the criterion at the time of patient recruitment and aligns with many prior PAH studies.
- A pulmonary function test, echocardiography, lung computed tomography (CT) scan, and pulmonary ventilation/perfusion scan sorted out other possible etiologies of pulmonary hypertension.

Patients who did not consent to participate in the study and those who could not perform 6MWT due to joint problems (like knee osteoarthritis) were excluded.

2.3 | Measurement

Based on a designed checklist, patient information, including gender, age, etiology of PAH (idiopathic or non-idiopathic), and time since PAH diagnosis, was recorded.

NYHA functional class of patients is determined according to their physical activity compliance [15].

The level of right ventricular dysfunction was determined according to the patients' echocardiography findings. The right atrial pressure, mean pulmonary artery pressure (mPAP), pulmonary artery wedge pressure (PAWP), cardiac index, and pulmonary vascular resistance (PVR) were recorded for all of the patients according to the findings of right heart catheterizations (RHC) performed previously.

All patients underwent the 6MWT using a standard method, and the 6MWT distance was recorded in a checklist.

After the patients consented to participate in the study, five cc of venous blood were taken from them after fasting for at least 6 h for laboratory evaluations.

The serum levels of sodium, potassium, urea, creatinine, and chloride of the patients were checked in the laboratory of our tertiary hospital (Ghaem Hospital, Mashhad, Iran), and the results were recorded in the checklist.

2.4 | Ethical Consideration

Ethical consent was obtained from all patients by the Declaration of Helsinki. This study was approved by the Ethics Committee of Mashhad University of Medical Sciences with code IR.MUMS.MEDICAL.REC.1401.667.

2.5 | Sample Size Estimation

So far, no published study has examined the correlation between 6MWD and serum chlorine levels. Therefore, the

sample size was calculated using the correlation formula, based on the assumption of a correlation coefficient of 0.5 between 6MWD and serum chlorine levels, and considering alpha of 0.05 and beta of 0.2, the sample size was estimated at 29 cases:

$\alpha(\text{two-tailed}) = 0.05$

$\beta = 0.2$

$r = 0.33$

The standard normal deviate for $\alpha = Z\alpha = 1.9600$

The standard normal deviate for $\beta = Z\beta = 0.8416$

$C = 0.5 * \ln[(1+r)/(1-r)] = 0.5493$

Total sample size $= [(Z\alpha + Z\beta)/C]2 + 3 = 29$

The sample size was also calculated based on the other primary outcome (correlation coefficient between the 6-min walk distance test and mean pulmonary artery pressure). According to a study published by Wernhart et al. in 2020 [16], the correlation coefficient (r) between the 6-min walk distance test and mean pulmonary artery pressure in patients with pulmonary hypertension was reported to be −0.33. The required sample size was calculated as 70 cases using the correlation formula and considering alpha of 0.05 and beta of 0.2:

$\alpha(\text{two-tailed}) = 0.05$

$\beta = 0.2$

$r = 0.33$

The standard normal deviate for $\alpha = Z\alpha = 1.9600$

The standard normal deviate for $\beta = Z\beta = 0.8416$

$C = 0.5 * \ln[(1+r)/(1-r)] = 0.3428$

Total sample size $= N = [(Z\alpha + Z\beta)/C]2 + 3 = 70$

Therefore, based on the larger sample size calculated, 70 patients were included in this study.

2.6 | Statistical Analyses

The collected data was entered into SPSS version 21. Qualitative data were reported using numbers and percentages. The distribution of quantitative data was examined using the Kolmogorov-Smirnov statistical test. Quantitative data with normal distribution were reported as mean ± standard deviation (SD) and qualitative data as median (interquartile range (IQR)). The chi-squared test was used to compare qualitative variables between the two groups. An independent samples *t*-test was used to compare quantitative variables between two groups in normal distribution, and a Mann-Whitney U test was used for non-normal distribution. Pearson correlation coefficient test was used to examine the correlation between quantitative variables in case of normal distribution of both variables, and Spearman's rank correlation coefficient test was used otherwise. Linear regression was used to examine serum chloride level in predicting 6MWT distance after adjustment for age and creatinine. In all calculations, $p < 0.05$ was considered a significant level.

3 | Results

In the present cross-sectional study, 70 PAH patients were evaluated. All patients were female, and the mean age of the patients was 39.44 ± 8.33 years old. Based on the median serum chloride and 6MWT of the patients, they were divided into two groups (serum chloride equal to or more than 97 mmol/L and lower than 97 mmol/L; 6MWT equal to or more than 230 m and lower than 230 m). The results of the variables compared between these groups are shown in Table 1.

Hypochloremia was considered as serum chloride < 97 mmol/L in our study. The mean age of patients with and without hypochloremia was insignificant ($p = 0.214$). The etiology of PAH in 26 (74.3%) hypochloremia and 28 (80.0%) patients without hypochloremia was idiopathic ($p = 0.569$). The median time since PAH diagnosis in patients with and without hypochloremia was 5 and 4 years ($p = 0.055$). The median NYHA functional class of patients with hypochloremia was significantly higher than non-hypochloremia patients ($p < 0.001$). Right ventricular dysfunction in 9 (30.0%) of hypochloremia patients and 2 (6.3%) of non-hypochloremia patients was severe ($p = 0.001$). The mPAP of patients with hypochloremia was significantly higher than non-hypochloremia patients (75.17 ± 11.51 vs 62.03 ± 13.84 mmHg respectively; $p < 0.001$). Right atrial pressure ($p = 0.922$), PAWP ($p = 0.108$), Cardiac index ($p = 0.132$), and PVR ($p = 0.530$) did not show any significant difference between hypochloremia and non-hypochloremia patients. Among laboratory findings, serum sodium level was significantly higher in hypochloremia patients than in non-hypochloremia patients (144.67 ± 3.21 vs. 136. 17 ± 2.32 MEq/L; $p = 0.002$), and serum Urea, Creatinine, and Potassium were similar between the two groups.

In our study, the mean age of patients with 6MWT distance < 230 m was significantly higher than 6MWT distance ≥ 230 m (42.34 ± 8.91 vs 36.54 ± 6.65; $p = 0.003$). The etiology of PAH in 29 (82.9%) patients with 6MWT distance < 230 m and 25 (71.4%) patients with 6MWT distance ≥ 230 m was idiopathic ($p = 0.255$). The median time since PAH diagnosis in patients with 6MWT distance < 230 m was significantly higher than the other group (median (IQR) of 5.0 (3.0–7.0) vs 3.0 (2.0–5.0) respectively; $p = 0.001$). The Median NYHA functional class of patients with 6MWT distance < 230 m was significantly higher than the patients with 6MWT distance ≥ 230 m ($p < 0.001$). Severe right ventricular dysfunction was observed in 10 (34.5%) patients with a 6MWT distance < 230 m and 1 (3.0%) of patients with a 6MWT distance ≥ 230 m ($p < 0.001$). The mPAP ($p = 0.001$) and PAWP ($p = 0.043$) were significantly higher in patients with 6MWT distance < 230 m. The cardiac index ($p = 0.118$) and PVR ($p = 0.133$) did not show any significant difference between the PAH patients with 6MWT distance < 230 and ≥ 230 m. The difference between the mean serum levels of urea ($p = 0.325$), creatinine ($p = 0.054$), sodium ($p = 0.589$), and potassium ($p = 0.736$) between PAH patients

TABLE 1 | Comparison of findings between the PAH patients with different serum chloride levels and 6-min walking test distances.

		Serum chloride			6MWT distance		
	Total $N = 70$	**< 97 mmol/L** $N = 35$	**≥ 97 mmol/L** $N = 35$	***p*-value**	**< 230 m** $N = 35$	**≥ 230 m** $N = 35$	***p*-value**
Age (years)	39.44 ± 8.33	40.69 ± 9.12	38.20 ± 7.38	0.214[a]	42.34 ± 8.91	36.54 ± 6.65	0.003[a]
Female, *n* (%)	70 (100)	35 (100)	35 (100)		70 (100)	35 (100)	
Etiology of PAH, *n* (%)							
Idiopathic	54 (77.1)	26 (74.3)	28 (80.0)	0.569[b]	29 (82.9)	25 (71.4)	0.255[b]
Non-idiopathic	16 (22.9)	9 (25.7)	7 (20.0)		6 (17.1)	10 (28.6)	
Time since PAH diagnosis (years)	5.0 (3.0–6.0)	5.0 (3.0–7.0)	4.0 (3.0–5.0)	0.055[c]	5.0 (3.0–7.0)	3.0 (2.0–5.0)	0.001[c]
NYHA functional class (median (IQR))	2.0 (2.0–3.0)	3.0 (2.0–3.0)	2.0 (1.0–2.0)	< 0.001[c]	3.0 (2.0–3.0)	2.0 (1.0–2.0)	< 0.001[c]
I	13 (18.6)	1 (2.9)	12 (34.3)	< 0.001[b]	1 (2.9)	12 (34.3)	< 0.001[b]
II	33 (47.1)	15 (42.9)	18 (51.4)		15 (42.9)	18 (51.4)	
III	20 (28.6)	16 (45.7)	4 (11.4)		15 (42.9)	5 (14.3)	
IV	4 (5.7)	3 (8.6)	1 (2.9)		4 (11.4)	0 (0.0)	
Right ventricular dysfunction							
Mild	19 (30.6)	3 (10.0)	16 (50.0)	0.001[b]	3 (10.3)	16 (48.5)	< 0.001[b]
Moderate	32 (51.6)	18 (60.0)	14 (43.8)		16 (55.2)	16 (48.5)	
Severe	11 (17.7)	9 (30.0)	2 (6.3)		10 (34.5)	1 (3.0)	
Right atrial pressure (mm Hg)	16.76 ± 4.46	16.85 ± 4.43	16.67 ± 4.68	0.922[a]	18.08 ± 4.61	15.33 ± 3.98	0.126[a]
mPAP (mmHg)	68.79 ± 14.23	75.17 ± 11.51	62.03 ± 13.84	< 0.001[a]	74.14 ± 12.78	63.12 ± 13.64	0.001[a]
PAWP (mmHg)	12.0 (10.0–14.0)	13.0 (10.5–14.0)	12.0 (10.0–12.75)	0.108[c]	13.0 (11.5–14.0)	11.5 (10.0–12.7)	0.043[c]
Cardiac index (L/min/m^2)	2.0 (1.8–2.6)	1.9 (1.7–2.0)	2.3 (1.8–2.8)	0.132[c]	1.9 (1.6–2.0)	2.0 (1.8–2.8)	0.118[c]
PVR (Woods unit)	12.0 (9.2–19.5)	12.0 (9.7–19.5)	10.2 (8.2–19.7)	0.530[c]	18.0 (11.0–20.0)	10.0 (8.6–17.2)	0.133[c]
Serum chloride (mmol/L)	96.5 (92.0–99.0)	92.0 (90.0–95.0)	99.0 (98.0–101.0)	< 0.001[c]	94.0 (91.0–97.0)	99.0 (95.0–101.0)	< 0.001[c]
6MWT distance (m)	227.90 ± 98.14	174.37 ± 78.79	281.43 ± 86.26	< 0.001[a]	146.77 ± 58.40	309.03 ± 50.85	< 0.001[a]
Urea (mg/dL)	26.76 ± 9.29	29.50 ± 11.04	24.74 ± 7.43	0.148[a]	28.86 ± 12.46	25.21 ± 5.94	0.325[a]
Creatinine (mg/dL)	0.87 ± 0.36	0.96 ± 0.44	0.81 ± 0.29	0.261[a]	1.01 ± 0.46	0.77 ± 0.23	0.054[a]
Sodium (MEq/L)	139.0 ± 4.90	144.67 ± 3.21	136. 17 ± 2.32	0.002[a]	140.25 ± 7.27	138. 0 ± 2.35	0.589[a]
Potassium (MEq/L)	4.04 ± 0.32	3.90 ± 0.36	4.12 ± 0.31	0.374[a]	4.0 ± 0.41	4.0 ± 0.28	0.736[a]

Note: Data are *N* (%), mean ± SD, or Median (IQR).

Abbreviations: CHD, congenital heart disease; CTD, connective tissue disorders; mPAP, mean pulmonary artery pressure; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; 6MWT, 6-min walk test.

[a]Independent samples *t*-test.

[b]Chi-squared test.

[c]Mann-Whitney U test.

with 6MWT distance of < 230 m and ≥ 230 m was not significant.

The results of the correlation analysis between quantitive variables and serum chloride level and 6MWT distance are shown in Table 2. The serum chloride levels had a significant positive correlation with cardiac index ($r = 0.541$, $p = 0.005$) and 6MWT distance ($r = 0.634$, $p < 0.001$). The correlation between serum chloride level and 6MWT distance is shown in Figure 1. The serum chloride level had significant negative correlation

TABLE 2 | Analysis of the correlation between quantitative variables with the chloride level and 6MWT distance.

	Chloride level		6MWT distance	
Variable	***r***	***p*-value**	***r***	***p*-value**
Age (years)	−0.250	0.037[b]	−0.467	< 0.001[a]
Time since PAH diagnosis (years)	−0.324	0.006[b]	−0.433	< 0.001[b]
NYHA functional class	−0.587	< 0.001[b]	−0.661	< 0.001[b]
Right atrial pressure (mm Hg)	−0.148	0.481[b]	−0.274	0.185[a]
mPAP (mmHg)	−0.585	0.001[b]	−0.503	< 0.001[a]
PAWP (mmHg)	−0.386	0.056[b]	−0.481	0.015[a]
Cardiac index (L/min/m2)	0.541	0.005[b]	0.342	0.095
PVR (Woods unit)	−0.274	0.185[b]	−0.311	0.130[b]
6MWT distance (m)	0.634	< 0.001[b]	1.0	—
Serum chloride (mmol/L)	1.0	—	0.634	< 0.001[b]
Urea (mg/dL)	−0.383	0.028[b]	−0.274	0.122[b]
Creatinine (mg/dL)	−0.346	0.049[b]	−0.429	0.013[a]
Sodium (MEq/L)	−0.494	0.177[b]	0.022	0.956[a]
Potassium (MEq/L)	−0.103	0.792[b]	−0.030	0.940[a]

Abbreviations: mPAP, mean pulmonary artery pressure; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; 6MWT, 6-min walk test.
[a]Pearson correlation coefficient.
[b]Spearman's rank correlation coefficient.

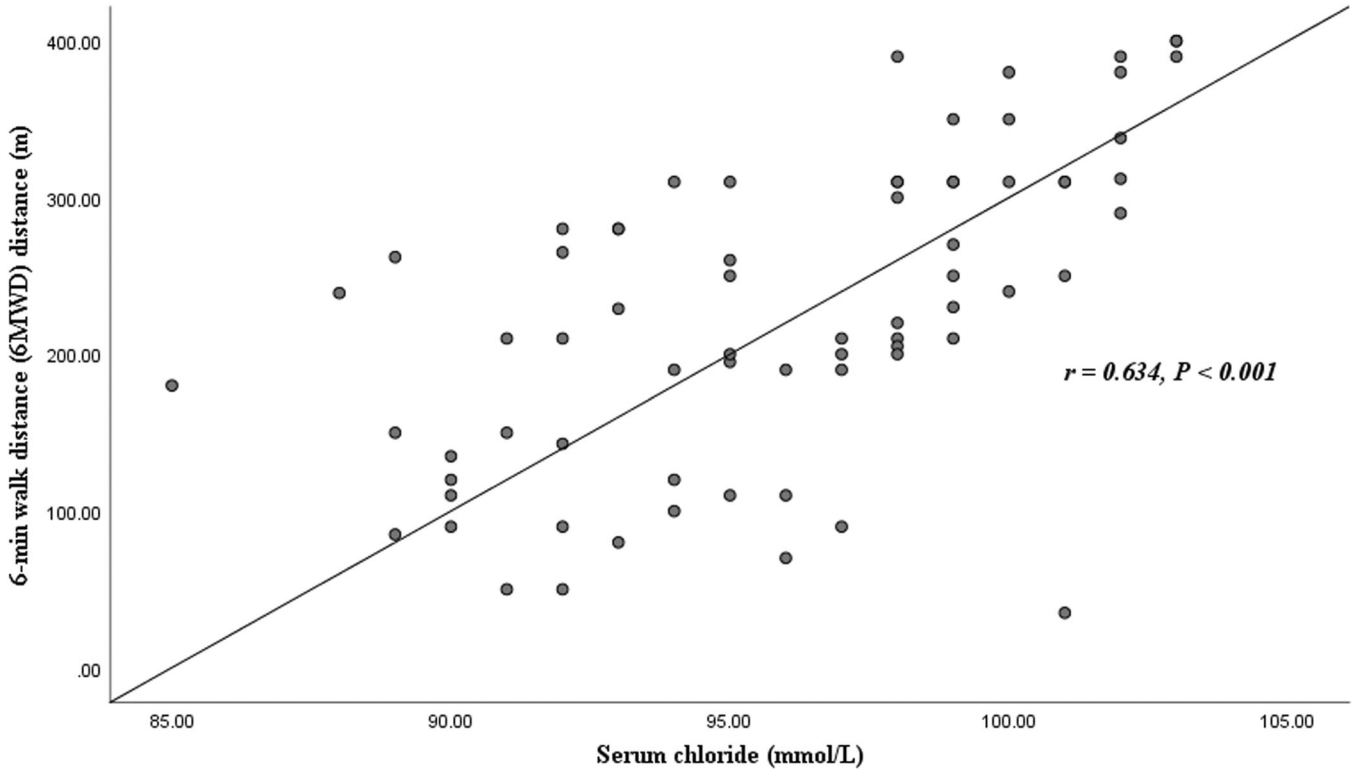

FIGURE 1 | The correlation between serum chloride level and 6MWT distance.

TABLE 3 | The results of the linear regression analysis to check the serum chloride level and hemodynamic parameters in the prediction of 6MWT distance after adjustment for age and creatinine.

Variable	β	95% CI	*p*-value
Serum chloride (mmol/L)	0.48	3.931, 16.602	0.002
Right atrial pressure (mm Hg)	−0.18	−16.076, 8.440	0.515
mPAP (mmHg)	−0.37	−4.850, −0.196	0.035
PAWP (mmHg)	−0.46	−49.106, 2.351	0.072
Cardiac index (L/min/m2)	0.205	−68.551, 147.180	0.447
PVR (Woods unit)	−0.224	−13.252, 6.144	0.445

Abbreviations: mPAP, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance.

with age ($r = -0.250$, $p = 0.037$), time since PAH diagnosis ($r = -0.324$, $p = 0.006$), NYHA functional class ($r = -0.587$, $p < 0.001$), mPAP ($r = -0.585$, $p = 0.001$), urea ($r = -0.383$, $p = 0.028$), and creatinine ($r = -0.346$, $p = 0.049$).

The 6MWT distance had a significant negative correlation with age ($r = -0.467$, $p < 0.001$), time since PAH diagnosis ($r = -0.433$, $p < 0.001$), NYHA functional class ($r = -0.661$, $p < 0.001$), mPAP ($r = -0.503$, $p < 0.001$), PAWP ($r = -0.481$, $p = 0.015$), and creatinine ($r = -0.429$, $p = 0.013$).

Linear regression analysis was used to check the serum chloride level's ability to predict the 6MWT distance after adjustment for age and creatinine (Table 3). According to the linear regression analysis results, serum chloride level was a significant predictor of 6MWT distance even after adjustment for age and creatinine ($\beta = 0.48$, 95% CI: 3.931, 16.602; $p = 0.002$). The mPAP was also a significant negative predictor of 6MWT distance after adjustment for age and creatinine ($\beta = -0.37$, 95% CI: −4.850, −0.196; $p = 0.035$). Other hemodynamic predictors were not significant predictors of 6MWT distance after adjustment for age and creatinine.

4 | Discussion

According to the results of our study, hypochloremia, even after adjustment for age and creatinine, was associated with lower 6MWT distance and more severe pulmonary hypertension. Naal et al. reported that serum chloride is an independent predictor of mortality in PAH patients [6]. Prins et al. reported the association of hypochloremia with right ventricular dysfunction and higher mortality [1].

The mechanism of the relationship between serum chloride level and pulmonary hypertension is still unknown, but there are some possible mechanisms. Intracellular chloride acts as a signaling effector to regulate physiological functions [17, 18]. For example, WNK kinases are a family that senses intracellular chloride and is involved in hypochloremic signal transduction [1, 19]. Hypochloremia can activate WNKs through a phosphorylation mechanism [1, 18].

WNKs have an essential role in embryonic cardiac development, angiogenesis, and controlling blood pressure, as reported in previous studies [7, 10]. WNK1 upregulates Glut1 and Glut4, which are both glucose transporters. Their upregulation has been reported in animal models with right ventricle overload [1].

As reported in previous studies, inflammation has an important role in the pathophysiology of PAH [20]. In PAH patients, macrophage accumulation and increased levels of inflammatory cytokines have been reported [21]. As reported in the study of Yin et al., macrophages with lower levels of intracellular chloride secrete more inflammatory cytokines, including TNF-α, IL-1β, and IL-6 [18].

Hypochloremia was associated with diuretic resistance in previous studies, and it can worsen right-side heart failure by this mechanism [22, 23].

In contrast to our study, in the study of Prins et al., the serum chloride had no relationship with mPAP [1]. In the study of Naal et al., only about 6% of patients at the time of PAH diagnosis and 6 months after diagnosis had serum chloride levels of ≤ 96 mmol/L and only serum chloride level after 6 months was a predictor of mortality [6], but in our study half of patients had hypochloremia (serum chloride < 97 mmol/L. One of the causes of higher prevalence of hypochloremia in our study could be higher time since PAH diagnosis in our study (minimum one, and maximum 8 years). The significant negative correlation of serum chloride levels with time since PAH diagnosis can confirm this hypothesis in our study. Another proposed mechanism could be variation in volume status following diuretic and other therapies and variation in the neurohormonal axis [6]. Differences in age etiology of PAH between our study and these studies are another possible reason for these discrepancies in results. Our patients were younger than the patients in the study of Naal et al. and Prins et al. [1, 6]

In our study, the 6MWT distance had a significant negative correlation with NYHA functional class, mPAP, and PAWP. In accordance with our study, Gupta et al. found a significant negative correlation between 6MWT distance and mPAP in Sarcoidosis-associated PH patients. However, in the study of Gupta et al., the correlation between 6MWT distance and PAWP was insignificant [24]. Although previously reported that the severity of patients' daily activity limitation did not necessarily correlate with the hemodynamic severity, we found a significant correlation between 6MWT distance and some hemodynamic parameters [25]. So, more studies on the

relationship between hemodynamic parameters and the severity of physical activity limitations of PAH patients are required.

Considering that the number of our patients was almost low, we could not divide and compare the patients according to the serum chloride tertile, so we divided the patients according to the median serum chloride level and 6MWT distance. The time since the diagnosis of PAH in our patients was not similar or in a narrow range, so future studies in patients with a distict long time since diagnosis can help to understand the role of serum chloride on predicting PAH severity. We acknowledge that diuretic use may have influenced serum chloride levels. However, the effect of the routine treatments of PAH on the serum chloride level and their effect on the severity of the disease can be evaluated in future studies. Long-term follow-up of the serum chloride level of PAH patients can help discover the ability of serum chloride levels to predict these patients' mortality.

We do not have direct data on whether correcting hypochloremia improves outcomes such as 6MWT or mPAP. However, further studies are required to examine the efficacy of supplemental chloride therapy on 6MWT distance and other prognostic factors of hypochloremic PAH patients. In addition, the role of serum chloride in predicting the severity of other types of pulmonary hypertension can be investigated.

5 | Conclusion

According to the results of our study, serum chloride levels can be used as an inexpensive method for evaluating disease severity in PAH patients, especially in patients with a longer time since the diagnosis of PAH.

Author Contributions

S.H, M.M, M.E, D.A. and A.B designed the study and collected data, A.B, S.B, S. A. and M.J.N analyzed data, wrote the first draft of the paper and submitted the manuscript, D.A, A.B, S.A. and M.J.N contributed to writing and revision of the manuscript. All authors contributed to finalizing the manuscript.

Acknowledgments

The researchers thank all individuals who participated in the execution of the project in any way.

Ethics Statement

Ethical consent was obtained from all patients by the Declaration of Helsinki. This study was approved by the Ethics Committee of Mashhad University of Medical Sciences with code IR. MUMS. MEDICAL. REC.1401.667.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

References

1. K. W. Prins, R. Kalra, L. Rose, et al., "Hypochloremia is a Non-invasive Predictor of Mortality in Pulmonary Arterial Hypertension," *Journal of the American Heart Association* 9, no. 5 (2020): e015221.

2. N. F. Ruopp and B. A. Cockrill, "Diagnosis and Treatment of Pulmonary Arterial Hypertension: A Review," *Journal of the American Medical Association* 327, no. 14 (2022): 1379–1391.

3. S. Rosenkranz, L. S. Howard, M. Gomberg-Maitland, and M. M. Hoeper, "Systemic Consequences of Pulmonary Hypertension and Right-Sided Heart Failure," *Circulation* 141, no. 8 (2020): 678–693.

4. A. Boucly, L. Savale, X. Jaïs, et al., "Association Between Initial Treatment Strategy and Long-Term Survival in Pulmonary Arterial Hypertension," *American Journal of Respiratory and Critical Care Medicine* 204, no. 7 (2021): 842–854.

5. P. Agarwala and S. H. Salzman, "Six-Minute Walk Test," *Chest* 157, no. 3 (2020): 603–611.

6. T. Naal, B. Abuhalimeh, G. Khirfan, R. A. Dweik, W. H. W. Tang, and A. R. Tonelli, "Serum Chloride Levels Track With Survival in Patients With Pulmonary Arterial Hypertension," *Chest* 154, no. 3 (2018): 541–549.

7. J. Xie, T. Wu, K. Xu, I. K. Huang, O. Cleaver, and C. L. Huang, "Endothelial-Specific Expression of WNK1 Kinase Is Essential for Angiogenesis and Heart Development in Mice," *The American Journal of Pathology* 175, no. 3 (2009): 1315–1327.

8. X. He, C. Liu, Y. Chen, J. He, and Y. Dong, "Risk of Cardiovascular Mortality Associated With Serum Sodium and Chloride in the General Population," *Canadian Journal of Cardiology* 34, no. 8 (2018): 999–1003.

9. F. B. Rivera, P. Alfonso, J. M. Golbin, et al., "The Role of Serum Chloride in Acute and Chronic Heart Failure: A Narrative Review," *Cardiorenal Medicine* 11, no. 2 (2021): 87–98.

10. M. Murthy, T. Kurz, and K. M. O'Shaughnessy, "WNK Signalling Pathways in Blood Pressure Regulation," *Cellular and Molecular Life Sciences* 74, no. 7 (2017): 1261–1280.

11. P. Douschan, G. Kovacs, A. Avian, et al., "Mild Elevation of Pulmonary Arterial Pressure as a Predictor of Mortality," *American Journal of Respiratory and Critical Care Medicine* 197, no. 4 (2018): 509–516.

12. S. Gidwani and A. Nair, "The Burden of Pulmonary Hypertension in Resource-Limited Settings," *Global Heart* 9, no. 3 (2014): 297–310.

13. K. Zeder, E. Brittain, G. Kovacs, and B. A. Maron, "The Management of Mild Pulmonary Hypertension in Clinical Practice," *Annals of the American Thoracic Society* 21, no. 8 (2024): 1115–1123.

14. K. Kusaka, K. Takeda, M. Kawashima, and Y. Morio, "New Diagnostic Criteria and Current Issues for Pulmonary Hypertension," *Respiratory Investigation* 62, no. 6 (2024): 1034–1036.

15. C. W. Yancy, M. Jessup, B. Bozkurt, et al., "2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines," *Circulation* 128, no. 16 (2013): e240–e327.

16. S. Wernhart and J. Hedderich, "Prediction of Pulmonary Hypertension in Older Adults Based on Vital Capacity and Systolic Pulmonary Artery Pressure," *JRSM Cardiovascular Disease* 9 (2020): 2048004020973834.

17. Á. G. Valdivieso and T. A. Santa-Coloma, "The Chloride Anion as a Signalling Effector," *Biological Reviews* 94, no. 5 (2019): 1839–1856.

18. T. Yin, L. He, Y. Du, et al., "Macrophage WNK1 Senses Intracellular Hypo-Chlorine to Regulate Vulnerability to Sepsis Attack During Hypochloremia," *International Immunopharmacology* 139 (2024): 112721.

19. M. Shekarabi, J. Zhang, A. R. Khanna, D. H. Ellison, E. Delpire, and K. T. Kahle, "WNK Kinase Signaling in Ion Homeostasis and Human Disease," *Cell Metabolism* 25, no. 2 (2017): 285–299.

20. T. Klouda and K. Yuan, "Inflammation in Pulmonary Arterial Hypertension," *Advances in Experimental Medicine and Biology* 1303 (2021): 351–372.

21. L. C. Price, S. J. Wort, F. Perros, et al., “Inflammation in Pulmonary Arterial Hypertension,” *Chest* 141, no. 1 (2012): 210–221.

22. C. Masella, D. Viggiano, I. Molfino, et al., “Diuretic Resistance in Cardio-Nephrology: Role of Pharmacokinetics, Hypochloremia, and Kidney Remodeling,” *Kidney & Blood Pressure Research* 44, no. 5 (2019): 915–927.

23. J. S. Hanberg, V. Rao, J. M. Ter Maaten, et al., “Hypochloremia and Diuretic Resistance in Heart Failure: Mechanistic Insights,” *Circulation: Heart Failure* 9, no. 8 (2016): e003180.

24. R. Gupta, R. P. Baughman, S. D. Nathan, et al., “The Six-Minute Walk Test in Sarcoidosis Associated Pulmonary Hypertension: Results From an International Registry,” *Respiratory Medicine* 196 (2022): 106801.

25. S. Saxer, M. Lichtblau, C. Berlier, E. D. Hasler, E. I. Schwarz, and S. Ulrich, “Physical Activity in Incident Patients With Pulmonary Arterial and Chronic Thromboembolic Hypertension,” *Lung* 197, no. 5 (2019): 617–625.