

# Anatomical Effectiveness of Endoscopic Dilatation in Plummer–Vinson Syndrome: A Prospective Observational Study from a Tertiary Care Hospital in Pakistan

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**Abstract: Background:** Plummer–Vinson syndrome (PVS) is a rare triad of dysphagia, iron deficiency anemia, and esophageal webs. Endoscopic dilatation is the primary mechanical intervention for relieving web-related obstruction. Evaluating its anatomical effectiveness, alongside hematological changes attributable to combined treatment, can help guide patient management.

**Objective:** To evaluate the effectiveness of endoscopic dilatation in patients with Plummer-Vinson Syndrome PVS treated at a tertiary care hospital.

**Materials and Methods:** This Prospective observational study, was conducted at Fauji Foundation Hospital, Rawalpindi, from 14<sup>th</sup> March 2025 to 30<sup>th</sup> December 2025. Ethical approval was granted by the Ethical Review Committee of Fauji Foundation Hospital (Ref# 935/RC/FFH/RWP; approval date: 13<sup>th</sup> March 2025). All consecutive patients aged 30–70 years with PVS were enrolled. All patients received oral ferrous sulphate 200 mg daily and underwent endoscopic dilatation with Savary–Gilliard bougie dilators to achieve a luminal diameter of 15 mm under general anesthesia. Dysphagia was assessed using a standardized 5-point ordinal scale at baseline and follow-up. Hemoglobin, serum ferritin, and esophageal web status were assessed at three months. Anatomical resolution of the esophageal web, along with hematological changes and improvement in dysphagia, were noted as outcome.

**Result:** Of 27 patients, complete anatomical resolution of esophageal webs was observed in all patients (100.0%; 95% CI:87.2%–100.0%). Dysphagia improvement was reported in 25 patients (92.6%; 95% CI:75.7%–99.1%). Median hemoglobin increased from 8.7g/dL (IQR:8.1–9.3) to 12.8g/dL (IQR:9.2–14.0), and median serum ferritin from 9.0ng/mL (IQR:7.8–10.7) to 19.8ng/mL (IQR:10.6–26.9) ( $p < 0.001$  for both). Iron deficiency anemia persisted in 17 patients (63.0%; 95% CI:44.2%–78.5%).

**Conclusion:** Endoscopic dilatation achieved complete anatomical resolution of esophageal webs and dysphagia in most PVS patients.

**Keywords:** Plummer-Vinson syndrome, Esophageal web, Endoscopic dilatation, Iron-deficiency anemia, Dysphagia.

## INTRODUCTION

Plummer-Vinson syndrome (PVS) is defined by the classical triad of dysphagia, iron-deficiency anemia, and upper esophageal webs [1]. Patient presents with a history of progressive dysphagia for solids, which worsens over the years, and symptoms of iron deficiency anemia. In addition, the patient may have brittle, easily broken nails, glossitis, koilonychia, fissuring or cracks at the corners of the mouth, and atrophic oral mucosa [2].

Middle-aged women are more likely to have PVS and are also at a higher risk of getting squamous cell carcinoma of the proximal esophagus and pharynx, which

is considered a precancerous disease [3]. The frequency of post-cricoid carcinoma in patients with PVS can vary from 4–16% in recent studies [1]. Only anecdotal accounts from areas in which PVS was first described have been available in recent years due to improvements in nutritional status and a decline in the frequency of iron deficiency. However, PVS still affects people of all sexes, ages, and ethnicities in developing nations [4].

The etiology of PVS remains unknown. Autoimmunity, genetic predisposition, and dietary and iron depletion are some of the hypothesized etiopathogenic processes. Diagnosis of PVS typically involves a complete blood count showing hypochromic microcytic anemia, along with a barium esophagogram or upper gastrointestinal endoscopy to detect esophageal webs. A biopsy is

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essential to rule out malignancy [5]. Treatment typically involves iron supplementation and endoscopic dilatation. Recent studies suggest that a combination of endoscopic dilatation and iron supplementation effectively alleviates dysphagia in most cases [6-9].

In Pakistan, limited data exist on the management of PVS, underscoring the need for further investigation into the anatomical and clinical outcomes of endoscopic dilatation. This study aims to contribute data from a tertiary care centre on web resolution rates, dysphagia outcomes, and hematological response to combined treatment, and to explore factors potentially associated with persistent iron deficiency.

## MATERIALS AND METHODS

This prospective observational study was conducted at Fauji Foundation Hospital, Rawalpindi, Pakistan, from 14<sup>th</sup> March, 2025 to 30<sup>th</sup> December, 2025. Ethical approval was granted by the Ethical Review Committee of Fauji Foundation Hospital (Ref# 935/RC/FFH/RWP; approval date: 13<sup>th</sup> March 2025), prior to study initiation. Written informed consent was obtained from all participants before enrollment.

As PVS is a rare condition, a formal sample size calculation was not performed. All patients who met the eligibility criteria and presented to the hospital during the study period were enrolled using consecutive non-probability sampling. The inclusion criteria were patients diagnosed with PVS, aged 30 to 70 years, of either gender. Exclusion criteria included patients unfit for general anesthesia, those with a history of head and neck malignancies, individuals who had undergone prior upper gastrointestinal surgeries, and pregnant or lactating females.

The simultaneous presence of dysphagia, iron deficiency anemia, and esophageal webbing defined PVS. Dysphagia was defined by one or more of the following: coughing, choking, or throat clearing during or within one minute of swallowing food or liquid; voice changes such as a wet or gurgly voice following swallowing; difficulty initiating a swallow or prolonged mealtime; and the reported sensation of food or liquid sticking in the throat or chest [1-6].

Dysphagia severity was quantified at baseline and at each follow-up visit using a standardized 5-point ordinal scale: 1 = able to eat normal diet; 2 = able to eat some solid foods; 3 = semi-solid foods only; 4 = liquids only; 5 = complete dysphagia. Clinically meaningful improvement

was defined as a reduction of  $\geq 1$  point at three-month follow-up relative to baseline. Although this scale was not independently validated in this population, it is consistent with grading systems widely used in esophageal disease research and was applied consistently by the same clinical team throughout the study.

All patients underwent endoscopic dilatation using Savary–Gilliard polyvinyl bougie dilators (Cook Medical, USA) under general anesthesia, performed by endoscopists with a minimum of two years of procedural experience. Dilatation was initiated with the smallest bougie encountering minimal resistance and advanced progressively by approximately 1–2 mm (up to two sequential sizes) per session, according to patient tolerance and procedural safety. The target esophageal luminal diameter was 15 mm. Inter-session intervals were standardized at four weeks. Patients with persistent dysphagia at two-week clinical review underwent additional sessions; a maximum of three sessions were performed. Mucosal biopsies from the post-cricoid region and web site were obtained at initial endoscopy for histopathological assessment (malignancy defined as squamous cell carcinoma or high-grade dysplasia).

All patients received oral ferrous sulphate 200 mg (elemental iron ~65 mg) once daily (Fefan<sup>®</sup>) for three months. Adherence was assessed at each monthly visit by tablet counting and patient self-report. All 27 patients reported complete adherence.

The primary outcome was anatomical resolution of the esophageal web on barium swallow or endoscopy at three months. Secondary outcomes were: (1) improvement in dysphagia severity score at three months; and (2) change in hemoglobin and serum ferritin levels at three months, interpreted as reflecting the combined effect of endoscopic dilatation and concurrent iron supplementation, as both were administered to all patients. Correction of iron deficiency anemia was defined as hemoglobin >12 g/dL (females) or >13.5 g/dL (males) with ferritin >30 ng/mL. Clinical review was at two weeks and then monthly.

Immediate procedural complications (bleeding, perforation, aspiration, cardiorespiratory events) were assessed during and within 24 hours of each dilatation. Delayed complications (stricture formation, web recurrence) were assessed at monthly follow-up visits.

## STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS Statistics version

27. Continuous variables are reported as medians and interquartile ranges (IQRs); categorical variables as frequencies and percentages with 95% confidence intervals (CIs) for key proportions (Wilson method). The Wilcoxon Signed-Rank test compared hemoglobin and serum ferritin before and after treatment. The Mann–Whitney U test compared continuous variables between outcome groups. Fisher's Exact test was applied throughout for categorical comparisons given the small sample size and expected cell counts below 5; Chi-square was used only where all expected cell counts were  $\geq 5$ . A p-value  $< 0.05$  was considered statistically significant. All subgroup analyses are explicitly exploratory and hypothesis-generating due to the small sample size; results should not be interpreted as confirmatory. Confidence intervals are provided alongside p-values for key outcomes.

## RESULT

A total of 27 patients with PVS were consecutively enrolled and completed three-month follow-up. Complete anatomical resolution of esophageal webs was achieved in all patients (100.0%; 95% CI: 87.2%–100.0%). Clinically meaningful dysphagia improvement ( $\geq 1$ -point reduction on the 5-point ordinal scale) was documented in 25/27 patients (92.6%; 95% CI: 75.7%–99.1%). Iron deficiency anemia persisted in 17 patients (63.0%; 95% CI: 44.2%–78.5%); 10 patients (37.0%; 95% CI: 21.5%–55.8%) achieved anemia correction following combined treatment.

In exploratory subgroup analyses, patients with persistent post-treatment iron deficiency were significantly younger (median: 51.0 [IQR: 35.5–56.0] vs 63.0 [IQR: 44.0–66.5] years;  $p = 0.020$ ). Females constituted 85.2%

of the cohort; sex was not significantly associated with persistent iron deficiency ( $p = 0.088$ ). Median dysphagia duration did not differ between groups (6 [3–7] vs 4.5 [1.8–6.8] months;  $p = 0.604$ ). Type 2 diabetes mellitus (present in 55.6%) was significantly associated with persistent iron deficiency (Fisher's Exact test:  $p = 0.007$ ). Pre-existing malignancy (present in 44.4%) was similarly associated (Fisher's Exact test:  $p = 0.006$ ). These associations are exploratory (Table 1).

All dilatations used Savary–Gilliard bougie dilators targeting 15 mm luminal diameter, under general anaesthesia, by operators with  $\geq 2$  years of experience, with four-week inter-session intervals. Number of sessions required was significantly associated with outcome: 90.9% of patients requiring three sessions had persistent iron deficiency versus 9.1% who achieved correction (Fisher's Exact test:  $p = 0.030$ ). The median maximum dilator diameter achieved was significantly higher in patients who achieved anemia correction (15.0 mm [IQR: 14.7–17.2]) versus those with persistent iron deficiency (14.1 mm [IQR: 12.5–15.7]; Mann–Whitney U,  $p = 0.046$ ). One immediate complication was recorded: minor mucosal bleeding in one patient (3.7%), managed endoscopically without sequelae. No perforations occurred (Table 2).

Significant hematological improvement was observed following combined treatment. Median hemoglobin increased from 8.7 g/dL (IQR: 8.1–9.3) to 12.8 g/dL (IQR: 9.2–14.0) (Wilcoxon Signed-Rank test,  $p < 0.001$ ). Median serum ferritin increased from 9.0 ng/mL (IQR: 7.8–10.7) to 19.8 ng/mL (IQR: 10.6–26.9) ( $p < 0.001$ ). These changes reflect the combined effect of endoscopic dilatation and concurrent oral iron supplementation, and cannot be attributed to dilatation alone (Table 3).

**Table 1.** Comparison of Post-treatment Iron Deficiency with Baseline Characteristics of the Patients.

Characteristics	Total (n=27)	Post-treatment IDA		p-value
		Yes (n=17)	No (n=10)	
Age, years, [median (IQR)]	53 (41-64)	51.0 (35.5-56.0)	63.0 (44.0-66.5)	0.020a*
<b>Gender, n (%)</b>				
Male	4 (14.8)	1 (5.9)	3 (30.0)	0.088b
Female	23 (85.2)	16 (94.1)	7 (70.0)	
Duration of dysphagia, months, [median (IQR)]	5 (3-7)	6 (3-7)	4.5 (1.8-6.8)	0.604a
Dysphagia severity score† [median (IQR)]	3 (3–4)	3 (3–4)	3 (2–4)	0.541a
<b>Comorbidities, n (%)</b>				
Type II diabetes mellitus	15 (55.6)	13 (86.7)	2 (13.3)	0.007b*
Hypertension	11 (40.7)	8 (47.1)	3 (30.0)	0.384b

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Residential Status, n (%)				
Rural	14 (51.9)	10 (58.8)	4 (40.0)	0.345b
Urban	13 (48.1)	7 (41.2)	6 (60.0)	
Educational Status, n (%)				
Illiterate	5 (18.5)	3 (17.6)	2 (20.0)	0.843b
Primary/Secondary	12 (44.4)	7 (41.2)	5 (50.0)	
Matric and above	10 (37.0)	7 (41.2)	3 (30.0)	
Pre-existing Malignancy, n (%)	12 (44.4)	11 (91.7)	1 (8.3)	0.006b*

†Dysphagia severity: 1=normal diet; 2=some solid food difficulty; 3=semi-solid only; 4=liquids only; 5=complete dysphagia. IDA=Iron deficiency anemia. a: Mann-Whitney U test; b: Fisher's Exact test (expected cell count <5) or Chi-square. \*p<0.05.

**Table 2.** Procedural Details and Comparison by Post-treatment Iron Deficiency Status (n=27).

Characteristics	Total (n=27)	Post-treatment IDA		p-value
		Yes (n=17)	No (n=10)	
Dilator type	Savary–Gilliard bougie (all cases)	—	—	—
Target luminal diameter (mm)	15	—	—	—
Dilation protocol	Progressive; +1–2 mm per session	—	—	—
Anesthesia	General (all cases)	—	—	—
Operator experience	≥2 years (all cases)	—	—	—
Inter-session interval	4 weeks	—	—	—
Iron supplementation	Ferrous sulphate 200 mg/day × 3 months (all patients)	—	—	—
Adherence to iron therapy, n (%)	27 (100.0)	—	—	—
Sessions of dilatation, n (%)				
1 session	11 (40.7)	4 (36.4)	7 (63.6)	0.030b*
2 sessions	5 (18.5)	3 (60.0)	2 (40.0)	
3 sessions	11 (40.7)	10 (90.9)	1 (9.1)	
Max dilator diameter achieved, mm [median (IQR)]	14.9 (12.7–15.8)	14.1 (12.5–15.7)	15.0 (14.7–17.2)	0.046a*
Immediate complications, n (%)				
Mucosal bleeding	1 (3.7)	1 (100)	0 (0)	—
Perforation	0 (0)	—	—	—
Histopathology, n (%)				
Squamous mucosa inflammation	17 (63.0)	10 (58.8)	7 (70.0)	0.682b
Dysplasia	1 (3.7)	1 (5.9)	0 (0)	
Inconclusive	9 (33.3)	6 (35.3)	3 (30.0)	

IDA=Iron deficiency anemia. a: Mann-Whitney U test; b: Fisher's Exact test (expected cell count <5) or Chi-square. \*p<0.05. All subgroup analyses are exploratory.

**Table 3.** Median Difference of Hemoglobin and Serum Ferritin Level at Baseline and at 3 Months of the Treatment (n=27).

Variables	Median (IQR)	p-value
Hemoglobin at baseline (g/dL)	8.7 (8.1-9.3)	<0.001
Hemoglobin at 3 months (g/dL)	12.8 (9.2-14.0)	
Serum ferritin at baseline (ng/mL)	9.0 (7.8-10.7)	<0.001
Serum ferritin at 3 months (ng/mL)	19.8 (10.6-26.9)	

Wilcoxon Signed-Rank test applied. p<0.05 considered significant. Hematological changes reflect the combined effect of endoscopic dilatation and concurrent oral iron supplementation and cannot be attributed to dilatation alone.

## DISCUSSION

The present study demonstrates that endoscopic dilatation with Savary–Gilliard bougie dilators is anatomically effective for PVS, achieving complete resolution of esophageal webs in all 27 patients at three months. Dysphagia improvement was documented in 25/27 patients (92.6%), supporting the clinical relevance of the anatomical outcome. Significant hematological improvement was also observed; however, this must be interpreted in the context that all patients received concurrent oral iron supplementation. The hematological changes, therefore, reflect the combined effect of both interventions, not endoscopic dilatation alone.

Despite complete anatomical resolution of esophageal webs in all patients, iron deficiency anemia persisted in 63.0% at three months. This underscores an important clinical message: anatomical correction of the esophageal web does not guarantee complete hematological recovery. Structural repair removes the mechanical barrier to adequate oral intake but does not address the underlying causes of iron deficiency, including inadequate iron stores, occult blood loss, chronic inflammation associated with malignancy, diabetes-related metabolic disturbances, impaired gastrointestinal absorption, or insufficient duration of replacement therapy. The term 'anatomically effective' is therefore a more accurate characterization of the primary outcome than 'clinically effective', and the conclusions have been revised accordingly.

The demographic profile of the present cohort, predominantly female (85.2%) with a middle to older age distribution, is consistent with classical descriptions of PVS. Novacek *et al.* [10] described PVS as a disorder predominantly affecting women in the fourth to seventh decades of life, a pattern observed across many case series and reviews. Similarly, Alzamzamy *et al.* [11], in a large multicenter study involving 56 patients across Egypt, India, and Iraq, reported a female predominance of 80.5% and a mean age of  $41 \pm 17$  years.

Our cohort showed a female predominance (85.2%) and a middle-to-older age distribution, in keeping with prior case series and reviews that describe PVS as rare, predominantly affecting women in the fourth to seventh decades [12]. In large contemporary series, median ages around the early 40s and female proportions of ~80% have been reported, e.g., a multicenter study of 56 patients [11]. Similar distributions were noted in another study where female predominance and upper esophageal membranes were typical [13].

Our findings of significant improvement in hemoglobin and ferritin mirror the general observation that iron repletion improves hematologic parameters and may alleviate dysphagia, particularly when webs are not severely obstructive. However, the high proportion of persistent iron deficiency (63%) in our cohort contrasts with reports where clinical response after a single dilatation session is frequent. For instance, the multicenter study reported single-session sufficiency in 76.8% of patients and no major post-dilatation complications [11]. Likewise, conference data suggest better early clinical results with balloon dilatation and frequent sufficiency with one session (82%), though this is an abstract and may reflect selection and technique differences [14]. Although endoscopic dilatation effectively resolves the anatomical obstruction caused by the esophageal web, it does not directly address the underlying causes of iron deficiency. Persistent iron deficiency may reflect inadequate iron stores at baseline, ongoing occult blood loss, chronic inflammatory states, malignancy, impaired gastrointestinal absorption, diabetes-related metabolic disturbances, or insufficient duration of iron replacement therapy. Therefore, successful anatomical correction should not be expected to result in immediate normalization of hematological parameters in all patients.

The divergence may be explained by patient factors (younger age, diabetes), disease severity (tight webs), and procedural intensity (maximal dilator diameter). Prior guidance notes that advanced webs rarely respond to iron alone and usually require mechanical disruption/dilatation, with single large bougie passage often adequate [15]. Case reports also document rapid dysphagia relief with iron therapy alone in selected patients, highlighting heterogeneity in web biology and obstruction [16].

The current study data shows that requiring three sessions was associated with persistent iron deficiency, and achieving a larger maximal diameter correlated with correction. This is consistent with the principle that greater luminal expansion yields better symptom relief in benign esophageal narrowing. Although the bougie vs balloon debate persists for benign strictures, meta-analysis suggests similar efficacy and safety (with less post-procedure pain for balloons), whereas some contemporary series in other etiologies report higher technical success with bougies, differences that may be context-specific [17].

The observed association between diabetes and the persistence of iron deficiency plausibly reflects multifactorial impairment of iron metabolism and nutrition, though diabetes is not a classical hallmark of PVS epidemiology.

Autoimmune associations (e.g., celiac disease, autoimmune thyroiditis) are more often highlighted in previous studies [18-20]; our cohort did not systematically assess autoimmunity, which may partly explain differences.

Taken together, the data suggest that endoscopic dilatation with adequate luminal expansion plus iron repletion is effective but not uniformly sufficient for hematologic normalization in all patients. Attention to modifiable procedural factors (e.g., achieving larger diameters) and patient factors (screening for autoimmune disease and occult malignancy) may optimize outcomes. Given the premalignant risk, structured surveillance should be incorporated into follow-up protocols [21-23]. Moreover, a study also emphasized the importance of oral health and PVS while managing the disease as well [24].

In short, endoscopic dilatation is considered the main therapeutic intervention for dysphagia in PVS caused by esophageal webs. Studies have shown that a single session of endoscopic dilatation can relieve dysphagia in the majority of patients. A prospective study by Goel *et al.* reported complete symptomatic relief in 94% of patients after the first dilatation session, with minimal complications and low recurrence rates [24]. A notable finding in the present study was the high prevalence of pre-existing malignancy at baseline (44.4%). Plummer–Vinson syndrome is recognized as a premalignant condition, with previous studies reporting an estimated risk of upper aerodigestive tract squamous cell carcinoma ranging from 4% to 16% [24]. The substantially higher prevalence observed in our cohort should be interpreted with caution. Several factors may explain this finding, including referral bias associated with a tertiary care center, where patients with more complex clinical presentations and suspected malignancies are more likely to be evaluated. In addition, the relatively small sample size may have contributed to an overestimation of the observed prevalence.

The association between chronic iron deficiency and malignant transformation has been attributed to mucosal atrophy, depletion of iron-dependent oxidative enzymes, impaired epithelial repair, and increased susceptibility to DNA damage [25]. Although our findings support the importance of careful malignancy assessment in patients with PVS, the observed prevalence in this study should not be interpreted as representative of the general PVS population. Nevertheless, thorough endoscopic evaluation and biopsy of suspicious lesions remain important during the initial assessment, and appropriate long-term

surveillance should be considered given the recognized premalignant nature of the syndrome [2, 24].

## LIMITATIONS

Several important limitations should be acknowledged. First, this was a single-centre prospective observational study with a small sample (n=27); all subgroup analyses are exploratory and hypothesis-generating, not confirmatory. Second, all patients received both iron supplementation and endoscopic dilatation concurrently, precluding attribution of hematological improvement to either intervention in isolation a limitation inherent to the real-world management of this condition. Third, the dysphagia scale used, while standardized and consistently applied, was not independently validated in this population; future studies should employ validated instruments such as the Sydney Swallowing Questionnaire or the Watson Dysphagia Scale. Fourth, follow-up was limited to three months, precluding assessment of long-term web recurrence or malignant transformation. Fifth, adherence to iron therapy was assessed by self-report and tablet counting, which may overestimate true adherence. Future multi-centre prospective studies with larger samples, validated dysphagia instruments, systematic autoimmune assessment, and longer follow-up are needed to establish the durability of endoscopic dilatation and long-term outcomes in PVS.

## CONCLUSION

Endoscopic dilatation using Savary–Gilliard bougie dilators achieved complete anatomical resolution of esophageal webs in all patients with PVS, with dysphagia improvement documented in 92.6% of cases. Hematological improvement was observed following combined endoscopic dilatation and oral iron supplementation; however, iron deficiency anemia persisted in 63.0% of patients, confirming that anatomical correction alone is insufficient for complete hematological recovery. The procedure should therefore be characterized as anatomically effective rather than comprehensively clinically effective. Continued iron replacement therapy, evaluation and management of underlying causes of persistent iron deficiency including metabolic comorbidities and malignancy and structured surveillance for post-cricoid malignancy remain essential components of PVS management. All subgroup analyses in this study are exploratory given the small sample size. Prospective multi-centre studies with validated outcome instruments, systematic comorbidity assessment, and longer follow-up are required to confirm these findings.

## ABBREVIATIONS

**CBC:** Complete Blood Count.

**Hb:** Hemoglobin.

**IQR:** Interquartile Range.

**PVS:** Plummer–Vinson Syndrome.

## AUTHORS' CONTRIBUTION

**Mahnoor Iftikhar:** Conceptualization, Study Design, Methodology, Data analysis and interpretation, Writing Draft, Critical review and revision of the manuscript, and Final approval, final proof to be published.

**Sayyad Nusrat Raza:** Conceptualization, Critical review and revision of the manuscript, and Final approval, final proof to be published.

**Sadia Faisal:** Methodology, Data analysis and interpretation and Writing Draft.

**Kiran Bukhari:** Writing Draft.

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Declared none.

## ETHICAL DECLARATIONS

### Data Availability Statement

Data will be available from the corresponding author upon a reasonable request.

### Ethical Approval

This study was approved by the Ethical Review Committee of Fauji Foundation Hospital (Ref# 935/RC/FFH/RWP; approval date: 13<sup>th</sup> March 2025).

### Consent to Participate

Signed informed consent was obtained from all eligible study participants.

### Consent for Publication

All authors have given the consent for publication.

### Conflict of Interest

Declared none.

### Competing Interest/ Funding

Declared none.

## Use of AI-Assisted Technologies

The manuscript underwent language editing with Quill-Bot AI tools to improve clarity. All scientific content was reviewed and verified by the authors.

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