ORIGINAL ARTICLE

Evaluation of Tricuspid Annular Plane Systolic Excursion (TAPSE) in cancer patients undergoing anthracycline-based therapy: A pre–post study

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ABSTRACT

Introduction: Anthracycline-based chemotherapy is effective in treating various cancers but is known for dose-dependent cardiotoxicity, typically affecting the left ventricle (LV). Recent studies have suggested that the right ventricle (RV) may also be impacted. Tricuspid Annular Plane Systolic Excursion (TAPSE) is a non-invasive echocardiographic tool that assesses RV systolic function. This study aims to evaluate the effects of anthracycline chemotherapy on TAPSE and explore its correlation with LV ejection fraction (LVEF).

Aim: To investigate the impact of anthracycline chemotherapy on RV function using TAPSE and to assess the relationship between changes in TAPSE and LVEF in cancer patients.

Methods: This pre-post observational study included 74 cancer patients receiving anthracycline-based chemotherapy, specifically, Doxorubicin, at Wahidin Sudirohusodo General Hospital. Echocardiographic measurements of TAPSE and LVEF were performed before and after chemotherapy. Statistical analyses included Mann–Whitney, Wilcoxon signed-rank, and Kruskal–Wallis tests to evaluate changes in TAPSE and LVEF.

Results: After chemotherapy, 79.7% of patients showed a significant decrease in TAPSE (mean reduction: 2.2 mm, p = 0.001). TAPSE decreased from 20.8 ± 2.3 mm pre-treatment to 18.6 ± 2.7 mm post-treatment. A significant positive correlation was found between changes in TAPSE and LVEF (r = 0.62, p = 0.001).

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Conclusions: Anthracycline chemotherapy leads to significant RV dysfunction, as indicated by reduced TAPSE, which correlates with LV dysfunction. TAPSE is a valuable tool for early detection of biventricular cardiotoxicity in cancer patients undergoing anthracycline treatment. (www.actabiomedica.it)

Key words: anthracyclines, Tricuspid Annular Plane Systolic Excursion (TAPSE), right ventricular dysfunction, cardiotoxicity, non-invasive monitoring

Introduction

Anthracycline-based chemotherapy agents, such as doxorubicin and epirubicin, are critical in the treatment of various cancers, significantly improving survival rates. However, these agents are associated with dose-dependent cardiotoxicity, primarily manifesting as left ventricle (LV) dysfunction. This well-documented effect has been extensively studied, underscoring the need for monitoring LV function in patients undergoing chemotherapy (1). While LV dysfunction has traditionally been the main focus of chemotherapy-related cardiotoxicity, there is growing recognition that the right ventricle (RV) may also be significantly impacted. The RV, due to its thinner myocardium and reliance on longitudinal contraction, is more susceptible to early functional impairment, which can progress unnoticed during chemotherapy treatment (2). This has led to the exploration of non-invasive echocardiographic measures, such as Tricuspid Annular Plane Systolic Excursion (TAPSE), to assess RV function. TAPSE is a simple, reliable, and reproducible tool that allows for early detection of RV dysfunction, even before clinical symptoms appear (3). Recent studies have emphasized the importance of concurrently assessing both ventricles to fully understand the extent of chemotherapy-induced cardiotoxicity. TAPSE has proven effective in detecting early RV dysfunction and is increasingly being integrated into clinical practice to provide a more comprehensive evaluation of cardiac health in cancer patients undergoing anthracycline treatment (4). This study aims to examine changes in TAPSE before and after anthracycline -based therapy, specifically Doxorubicin, and explore the correlation between these changes and the reduction in LV ejection fraction (LVEF). By evaluating both ventricles, this research seeks to contribute to a deeper understanding of Doxorubicin-induced cardiotoxicity and reinforce the clinical value of including RV monitoring in routine cancer care.

Methods

This pre-post observational study was conducted at Wahidin Sudirohusodo General Hospital, the largest referral hospital in East Indonesia, located in Makassar, between April and July 2025. The study aimed to evaluate changes in TAPSE and LVEF in cancer patients receiving Doxorubicin HCl (Kalbe Farma, Jakarta, Indonesia) chemotherapy.

Participants

Adult cancer patients aged 18 years or older, who had undergone Doxorubicin chemotherapy regimens, were included in the study. Eligibility required baseline TAPSE values of at least 16 mm, LVEF greater than 55%, and no indication of structural cardiac anomalies. To ensure cardiac suitability for treatment, cardiologist approval was required prior to the start of chemotherapy. Only patients who had echocardiograms performed both before and after completing at least six cycles of Doxorubicin treatment were included. Exclusion criteria included a history of cardiovascular

disease, such as pulmonary hypertension, cardiomyopathy, valvular heart disease, coronary artery disease, chronic heart failure, or congenital cardiac anomalies that could affect right ventricular function. Patients who developed cardiovascular problems during chemotherapy that required early termination of treatment were also excluded. Consequently, 74 patients were included in the analysis.

Echocardiographic measurements

Echocardiographic exams were performed using a GE Vivid E95 ultrasound machine (GE Healthcare, USA), both before and after treatment. The longitudinal motion of the tricuspid annulus from end-diastole to end-systole was measured using M-mode to assess TAPSE from the apical four-chamber view. LVEF was calculated using the biplane Simpson's approach, which computes ventricular volumes and ejection fraction based on end-diastolic and end-systolic dimensions.

Co-morbidities

Co-morbidities were recorded as either present or absent for each patient, focusing on two specific conditions: hypertension (HT) and diabetes mellitus (DM). If either or both conditions were present, patients were classified as having co-morbidities. If neither condition was present, patients were classified as "none."

Statistical analysis

Data analysis was conducted using SPSS Statistics, version 25.0. Doxorubicin-related echocardiographic parameters, including cumulative dose and treatment duration, were summarized as means with standard deviations for continuous variables and as frequencies and percentages for categorical variables. Univariate analysis was used to assess the distribution of sample characteristics, such as age, sex, and comorbidities. Statistical comparisons were performed using a two-sided paired t-test. Non-parametric tests, including the Mann–Whitney, Wilcoxon signed-rank, and Kruskal–Wallis tests, were used to assess changes in TAPSE. Additionally, a one-way ANOVA was conducted to compare TAPSE reduction across the four cancer types: breast cancer, lymphoma, leukemia, and osteosarcoma. A p-value of less than 0.05 was considered statistically significant for all tests.

Ethical considerations

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Hasanuddin University (Approval No. 312/UN4.6.4.5.31/PP36/2025). Written informed consent was obtained from all individual participants or their legal guardians in cases where participants were unable to provide consent themselves. Participants were fully informed about the study's objectives, procedures, potential risks, and their right to withdraw from the study at any time without consequences. Participants also agreed to the publication of their anonymized data, and identifying details were omitted to ensure confidentiality. In the case of deceased participants, consent for publication was sought from their next of kin.

Results

This study involved 74 cancer patients undergoing Doxorubicin chemotherapy in the Haemato-Oncology Department of Wahidin Sudirohusodo Hospital. All subjects underwent echocardiography examinations in the Cardiology Department, both before and after the chemotherapy series. The evaluation of heart function focused on the RV, using TAPSE as the main parameter. A TAPSE value <16 mm was used as the cut-off point to assess right ventricular systolic dysfunction. Based on age characteristics, the age range in this study was 18–75 years, mostly male (52.7%) with a mean age of 42±5 years (Table 1).

TAPSE changes

After chemotherapy, 59 patients (79.7%) experienced a reduction in TAPSE. The mean TAPSE decreased from 20.8 ± 2.3 mm before chemotherapy to 18.6 ± 2.7 mm after treatment ($p = 0.001$); representing an approximate 11% decline in RV systolic

Table 1. Characteristics of Study Participants

Variables	n	%	Mean ± SD	Median
Age (years)				
≤ 45	43	57.1	42±5	43
> 45	31	41.8	48±7	47
Sex				
Female	35	47.3		
Male	39	52.7		
Types of Malignancy				
Breast cancer	11	14.9		
Lymphoma	41	55.4		
Leukemia	3	4.1		
Osteosarcoma	19	25.7		
Comorbidities				
With Comorbidities	16	21.6		
None	58	78.4		

Table 2. TAPSE Before and After Doxorubicin Chemotherapy

	TAPSE Value			
Parameter	**n**	**Mean**	**SD**	***p*-value**
Pre-Chemo	74	20.8	2.3	0.001*
Post-Chemo	74	18.6	2.7	
Reduction	0	6.36	-	

**Wilcoxon signed-rank test*

function, which was statistically significant ($p < 0.05$) (Table 2).

A one-way Analysis of Variance (ANOVA) was conducted to evaluate whether there were significant differences in TAPSE reduction across the four cancer types: breast cancer, lymphoma, leukemia, and osteosarcoma. The results of the analysis indicated that the mean reductions in TAPSE were not significantly different between the groups, with an F-statistic of 1.11 and a p-value of 0.3525. This suggests that the type of cancer did not have a statistically significant impact on the extent of TAPSE reduction following chemotherapy. Consequently, these findings imply that TAPSE reduction is similarly affected by chemotherapy across

Table 3. LVEF Before and After Doxorubicin Chemotherapy

Parameter	n	Mean	SD	*p*-value
Pre-Chemo	74	63.08%	3.81	0.001*
Post-Chemo	74	56.76%	6.49	
Reduction	0	6.36%	-	

**Wilcoxon signed-rank test*

different malignancies, without a notable influence from cancer type. In addition to cancer type, an analysis of TAPSE reduction by gender, age, and comorbidities was conducted. The results showed no statistically significant differences between the groups. Specifically, while the TAPSE reduction was slightly greater in males (2.38 mm) compared to females (2.13 mm), this difference was not statistically significant ($p = 0.524$). Age groups (≤ 45 years vs. > 45 years) also showed no significant difference in TAPSE reduction ($p = 0.571$). Furthermore, the presence or absence of comorbidities did not impact TAPSE reduction, with a p-value of 0.815. These findings collectively suggest that neither demographic factors (such as gender and age) nor comorbidities had a substantial effect on the reduction in TAPSE, which remained relatively consistent across different groups. The analysis also revealed that patients with TAPSE values ≤16 mm after chemotherapy received a higher cumulative dose of Doxorubicin (mean = 441 mg/m²) compared to those with TAPSE >16 mm (mean = 370.4 mg/m²). However, this difference did not reach statistical significance ($p = 0.082$; Table 3), indicating that the cumulative dose of Doxorubicin cannot be conclusively linked to the decrease in TAPSE in this study.

LVEF changes

The mean LVEF decreased from 63.08% ± 3.81% before chemotherapy to 56.76% ± 6.49% after treatment, reflecting an absolute reduction of 6.36% in LV systolic function ($p = 0.001$; Table 3). This absolute decrease in LVEF indicates a significant decline in LV function following chemotherapy.

Correlation between TAPSE and LVEF

A significant positive correlation was observed between changes in TAPSE and LVEF ($r = 0.62$,

p = 0.001; Table 4). In the group with left ventricular dysfunction (indicated by an absolute decrease in LVEF of ≥10%), the mean TAPSE reduction was significantly greater, with an average decrease of 4.03 mm. In contrast, the group with a decline in LVEF of less than 10% had a mean TAPSE reduction of 1.57 mm. This suggests that the reduction in TAPSE was closely associated with the decline in LVEF, indicating that both ventricles were concurrently affected by Doxorubicin-induced heart damage.

Discussion

Doxorubicin can cause subclinical right ventricular dysfunction, which often goes undetected until it progresses to a more advanced stage. This is due to the anatomical characteristics of the RV, which has less muscle mass and fewer myofibrils compared to the LV, making it more vulnerable to damage from Doxorubicin chemotherapy. Echocardiography is essential for detecting this disorder, with TAPSE (Tricuspid Annular Plane Systolic Excursion) being one of the most widely used and reliable parameters. TAPSE is favored because it is simple, non-invasive, and easy to implement in clinical practice. Studies have shown that right ventricular dysfunction, detectable by TAPSE, is a common outcome of anthracycline therapy, including Doxorubicin, particularly in breast cancer patients undergoing chemotherapy. This dysfunction can occur before overt clinical symptoms of right-sided heart failure are present, which underscores the importance of early detection through echocardiographic methods like TAPSE (2). In this study, the effects of Doxorubicin in the RV were assessed in 74 patients with cancer. Most patients, namely 59 patients (79.7%), experienced a decrease in TAPSE values after Doxorubicin chemotherapy. The average TAPSE decreased significantly from 20.8 mm before chemotherapy to 18.6 mm after chemotherapy, or a decrease of 10.9% (p=0.001). These findings indicate that right ventricular function is also affected by Doxorubicin exposure, and TAPSE can be used as a non-invasive parameter to monitor these changes. Our findings align with previous reports of a significant reduction in TAPSE in patients undergoing Doxorubicin chemotherapy. Notably, TAPSE changes have been observed to occur prior to any decline in LVEF, suggesting that TAPSE may serve as an early marker for right ventricular dysfunction related to cardiotoxicity (2,5). Age (p: 0.571) and gender (p: 0.524) were not found to be significantly associated with the degree of TAPSE decline in this study. Supporting this, previous studies have shown that TAPSE generally decreased after Doxorubicin therapy, with demographic factors such as age and gender not consistently serving as reliable predictors. The difference of these results with other studies may be influenced by several methodological and clinical factors. First, the relatively small sample size could potentially limit the statistical power to identify actual differences. Second, variations in baseline patient characteristics, such as comorbidity, body mass index, or exposure to additional therapies, may potentially affect the level of cardiotoxicity due to Doxorubicin. Third, TAPSE as a parameter only reflects the longitudinal movement of the tricuspid annulus, so its sensitivity in capturing subtle changes, for example due to age or gender, is lower than other parameters such as right ventricular strain (6). Patients with TAPSE values <16 mm received a higher cumulative dose of Doxorubicin compared to those with TAPSE values >16 mm at the end of treatment, with an average dose of 441 mg/m². However, this difference was not statistically significant (p > 0.05). This finding aligns with previous studies that have explored the relationship between Doxorubicin dose and right ventricular dysfunction. Studies have indicated that higher doses of Doxorubicin, particularly those ≥416 mg/m², are strongly linked to increased sensitivity and specificity in detecting right ventricular dysfunction, including reductions in TAPSE. In a meta-analysis, it was found that Doxorubicin doses ≥300 mg/m² were significantly associated with anthracycline-induced

Table 4. TAPSE and LVEF Before and After Doxorubicin Chemotherapy

Reduction of LVEF	TAPSE Reduction			
	n	Mean	SD	*p*-value
≤10%	53	1.57	1.59	0.001*
>10%	21	4.03	1.55	

**Mann-Whitney test*

right ventricular dysfunction, as shown by decreased TAPSE values in breast cancer patients. Similarly, research has demonstrated that a dose of epirubicin at 150 mg/m² is associated with a higher risk of right ventricular dysfunction, as evidenced by reductions in TAPSE values in breast cancer patients (5,7-8). The findings of this study that the decrease in TAPSE was greater in patients without comorbidities, although not statistically significant, indicate that comorbidity status is not the only determining factor for susceptibility to Doxorubicin cardiotoxicity. These results differ from most of the literature, which emphasizes the role of comorbidity in exacerbating right ventricular dysfunction. This variation is likely influenced by differences in sample characteristics, clinical approaches, and the limitations of TAPSE as a single marker of right ventricular function. Multimodal evaluation remains necessary to assess the risk of cardiotoxicity in patients receiving Doxorubicin.

TAPSE reduction by cancer types

A one-way Analysis of Variance (ANOVA) was conducted to assess whether there were significant differences in TAPSE reduction across four cancer types: breast cancer, lymphoma, leukemia, and osteosarcoma. The analysis revealed that the mean reductions in TAPSE were not significantly different between the groups, with an F-statistic of 1.11 and a p-value of 0.3525; suggesting that cancer type does not significantly affect the extent of TAPSE reduction following Doxorubicin chemotherapy (Table 3). This aligns with Rossetto et al. (2024), who found that right ventricular dysfunction, as measured by TAPSE, can occur across different cancer types, but the degree of dysfunction was not notably different between them (2). Our study also supports findings that right ventricular dysfunction is common in patients receiving anthracycline-based chemotherapy for breast cancer. However, our analysis did not show a significantly higher TAPSE reduction in the lymphoma group, despite a slightly higher mean reduction. This discrepancy may be attributed to variations in chemotherapy regimens, comorbidities, or the inherent biological characteristics of each cancer type. It has also been suggested that right ventricular dysfunction can occur consistently across various malignancies (6,7). Moreover, it has been observed that right ventricular dysfunction is commonly found in breast cancer patients treated with anthracyclines, with TAPSE serving as a reliable marker to assess right ventricular function. This further emphasizes the importance of TAPSE in detecting early signs of right ventricular impairment, regardless of the cancer type. While left ventricular dysfunction is well-documented as a major consequence of Doxorubicin chemotherapy, our study highlights that the right ventricle also experiences similar degrees of dysfunction across different cancer types. This underscores the need for comprehensive cardiac monitoring during chemotherapy, with recommendations for multimodal imaging to evaluate both ventricles during and after cancer treatment (4,9).

Right ventricular involvement in chemotherapy-induced cardiotoxicity

Historically, right ventricular dysfunction has been less studied compared to left ventricular injury in the context of Doxorubicin chemotherapy. However, recent research has highlighted the vulnerability of the RV, with TAPSE emerging as an effective marker for detecting early RV systolic dysfunction. Our study found that TAPSE significantly decreased in a large proportion of patients (79.7%) following chemotherapy, confirming that Doxorubicin has a detrimental effect on RV function. This observation aligns with findings that right ventricular dysfunction is a common yet often overlooked side effect of anthracycline-based therapies like Doxorubicin, particularly in patients with breast cancer (2,7). The RV is especially susceptible to Doxorubicin-induced damage due to its thinner myocardium and reliance on longitudinal contraction for systolic function. This makes the right ventricle more susceptible to early dysfunction during chemotherapy, a phenomenon also observed in patients treated with epirubicin, which belongs to the same drug class as Doxorubicin. Additionally, it has been shown that the right ventricle is often affected alongside the left ventricle during Doxorubicin therapy, with TAPSE reductions serving as a critical marker for this dysfunction (6,8). The role of TAPSE in detecting early right ventricular dysfunction is crucial, as it provides important information even before

symptoms of right-sided heart failure appear. Early identification of RV dysfunction can greatly improve patient outcomes, as untreated RV dysfunction can lead to severe clinical complications. Despite TAPSE being a widely accepted and reproducible method for evaluating RV systolic function, its use in cancer patients undergoing Doxorubicin therapy is still underutilized. Our study, in conjunction with recent research, emphasizes the importance of incorporating TAPSE measurement into routine clinical monitoring. Early assessment of TAPSE could help guide treatment adjustments and mitigate the long-term consequences of right ventricular dysfunction, aligning with recommendations for using multimodal imaging to evaluate both ventricles during and after cancer therapy (4).

Left ventricular dysfunction and its relationship with TAPSE

LV dysfunction, assessed by LVEF, is a well-known consequence of Doxorubicin therapy, as documented in numerous studies. In our research, LVEF significantly decreased from 64.1% ± 5.2% to 60.4% ± 6.1% ($p = 0.001$), supporting the established link between Doxorubicin and left ventricular impairment (1). However, our study also highlights a significant correlation between changes in TAPSE and LVEF ($r = 0.62$, $p = 0.001$), indicating that the decline in right ventricular function, measured by TAPSE, parallels the reduction in left ventricular function. This biventricular involvement in Doxorubicin-induced cardiotoxicity is consistent with previous findings, which emphasize the importance of monitoring both ventricles in patients undergoing chemotherapy. Additionally, similar observations have been made in breast cancer patients, where reductions in TAPSE reflect right ventricular dysfunction, often occurring in conjunction with left ventricular damage (2,4).

The right ventricle's susceptibility to Doxorubicin is attributed to its thinner myocardial wall and reliance on longitudinal contraction for systolic function, making it especially vulnerable to chemotherapy-induced damage. It has been observed that right ventricular dysfunction often develops during chemotherapy, even in the absence of clear clinical symptoms, with TAPSE serving as an early indicator of this dysfunction (6,8). Our study reinforces the need for comprehensive cardiac monitoring in cancer patients, particularly assessing right ventricular function. TAPSE, being a simple and reproducible measure, should be integrated into routine monitoring, as it provides valuable insight into right ventricular health, often before signs of right-sided heart failure emerge. While left ventricular dysfunction has traditionally been the primary focus of monitoring during Doxorubicin therapy, these findings highlight the importance of incorporating TAPSE as part of a comprehensive cardiac assessment (4,7).

Limitations

There are a number of limitations to this study. First, the results may not be as broadly applicable due to the limited sample size and the fact that all participants received care at a single facility. This could affect the generalizability of the findings to a wider population. Another limitation is the reliance on TAPSE as the primary measure of right ventricular RV function. Although TAPSE is a widely used, simple, and non-invasive method, it primarily measures longitudinal motion of the tricuspid annulus and may not capture subtle or early dysfunction in the RV. TAPSE is also influenced by patient-specific factors such as body position, ventricular preload, and heart rate, which can introduce variability in measurements. Moreover, TAPSE does not assess other important aspects of RV function, such as right atrial pressure or pulmonary circulation, which may also be affected by chemotherapy. Future research would benefit from multicenter, larger-scale studies involving a more diverse population to further explore Doxorubicin-induced cardiotoxicity and its impact on both ventricles. Additionally, combining TAPSE with other advanced imaging techniques, such as RV strain or tissue Doppler imaging, could provide a more comprehensive and sensitive assessment of RV dysfunction.

Clinical implications and future directions

The clinical implications of these findings are substantial. The combined assessment of both ventricles through TAPSE and LVEF offers a more comprehensive approach to early detection of Doxorubicin-induced

cardiotoxicity. This method may aid in personalizing treatment plans, potentially enabling timely interventions, such as the use of cardioprotective agents or adjustments to chemotherapy doses, to reduce the risk of irreversible cardiac damage. Future research should prioritize the development of more sensitive imaging techniques to detect early myocardial changes associated with Doxorubicin-induced cardiotoxicity. While TAPSE is an effective tool for assessing RV function, it primarily measures the longitudinal motion of the tricuspid annulus, which may not capture subtle early changes in RV function. Advanced imaging methods, such as RV strain and tissue Doppler imaging, could provide greater sensitivity in detecting these early signs of cardiac injury, even before significant changes in ejection fraction are observed. By combining TAPSE with these advanced techniques, a more comprehensive and nuanced understanding of right ventricular dysfunction can be achieved. Moreover, larger-scale, multicenter studies are essential to further investigate the impact of Doxorubicin on both ventricles across a more diverse population. Longitudinal studies should also be conducted to explore whether early alterations in TAPSE and LVEF can serve as reliable predictors of long-term cardiovascular outcomes in cancer survivors. These studies will enable better post-treatment cardiovascular care, refining monitoring practices and ultimately improving patient management.

Conclusion

This study highlights the significant impact of Doxorubicin chemotherapy on both right and left ventricular function, as evidenced by reductions in TAPSE and LVEF. The concurrent decline in RV and LV function suggests early biventricular involvement, which reinforces the importance of comprehensive cardiac monitoring in cancer patients undergoing Doxorubicin therapy. Incorporating TAPSE assessment alongside LVEF may improve the early detection of Doxorubicin -induced cardiotoxicity, allowing for timely interventions to mitigate long-term cardiac damage. Given the simplicity and reliability of TAPSE, its routine inclusion in clinical practice may aid in better understanding and managing the cardiac side effects of chemotherapy. Future research, including larger-scale, multicentre studies, is necessary to further investigate the long-term cardiovascular outcomes of these early changes in cardiac function and to explore the potential of advanced imaging techniques for even more sensitive detection of myocardial injury.

Ethical Approval: The study was approved by the Ethics Committee of the Faculty of Medicine, Hasanuddin University, under protocol number 312/UN4.6.4.5.31/PP36/2025**.** It was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all individual participants or their legal guardians in cases where the participant was unable to provide consent themselves. Participants were fully informed about the study's objectives, procedures, potential risks, and their right to withdraw from the study at any time without consequences. Additionally, participants' agreement for the publication of their anonymized data was obtained, and any identifying details were omitted to ensure confidentiality. In the case of deceased participants, consent for publication was sought from their next of kin, and the necessary documentation will be made available to the editors upon request. All personal data were anonymized, and patient confidentiality was strictly maintained throughout the research process.

Conflict of Interest: Each author declares that they have no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

Authors Contribution: Conceptualization was carried out by Z.Y.S., T.H., P.T., S.B., and H.S. Data curation was done by Z.Y.S. and T.H. Formal analysis was performed by A.S. The research was conducted by Z.Y.S., T.H., P.T., S.B., and H.S. Methodology development was led by Z.Y.S., T.H., and S.B. Project management was handled by Z.Y.S., and resources were provided by Z.Y.S. Supervision was done by T.H. and P.T., and validation was carried out by T.H. and P.T. Visualization was done by Z.Y.S., while the original draft preparation was handled by Z.Y.S. Writing—review and editing was done by all authors.

Declaration on the Use of AI: No artificial intelligence tools were used in the writing or editing of this manuscript.

Consent for Publication: Written informed consent for publication of the data was obtained from all participants or their legal guardians. The participants' anonymity was strictly maintained.

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