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ORIGINAL ARTICLE

Prognostic Value of Left Ventricular Peak Global Longitudinal Strain in Mitral Valve Surgery for Primary Severe Mitral Regurgitation

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ABSTRACT

Background: Assessment of Preclinical systolic dysfunction using global longitudinal strain, a unique and sensitive technique for diagnosing left ventricular dysfuction. This study Aimed to clarify whether left ventricular global longitudinal strain is associated with a favourable clinical outcome after mitral valve (MV) surgery for primary severe mitral regurge (MR) Method: This prospective study included 30 patients with primary severe mitral regurgitation undergoing mitral valve surgery. The patients were classified into three groups according to their ejection fraction: Group I (10 patients with EF 30-45%), Group II (10 patients with EF 45-60%), and Group III (10 patients with EF >60%). LV-GLS was assessed preoperatively and postoperatively using speckle tracking echocardiography. Patients were monitored for three months after surgery. Results: Mitral valve replacement was done in 70% of Group I, 40% of Group II, and 50% of Group III. In contrast, MV repair was performed in 30%, 60%, and 50% of cases, with no significant difference seen (p=0.391). Echocardiographic results showed comparable patterns, with Group III maintaining superior GLS (-18±1.85), LVEF (67.1±3.6%), and reduced LVEDD and LVESD (p<0.05 for intergroup comparison). Only Group I (20%) experienced post-operative mortality, with no deaths reported in Groups II or III (p=0.12). ROC curve study revealed that GLS had the maximum sensitivity (100%) and specificity (78.26%) at -11.2, with an area under the curve of 0.839. **Conclusion**: left ventricular GLS is a significant predictor of clinical and echocardiographic outcomes after mitral valve surgery for primary severe mitral regurgitation in early detection of subclinical Left ventricular dysfunction.

Keywords: Mitral regurgitation, Global longitudinal strain, Echocardiography.

INTRODUCTION

ne of the most prevalent conditions affecting valves is mitral regurgitation (MR). Its presence is linked to a poor prognosis, and its frequency and severity rise with age. The most widely used classification divides MR into primary and secondary forms based on its mechanism [1]. A dynamic structure, the mitral valve (MV) apparatus interacts intricately with the surrounding anatomy. Mitral valve regurgitation may result from any disruption

of the surrounding anatomy or any part of the apparatus. Since the frequency of MR rises with age, there will be a significant increase in the number of MR patients who need hospitalization or intervention during the coming decades [2].

Because of the negative effects of chronic volume overload on the left ventricle, Poor outcomes are associated with untreated severe MR. When patients with suspected severe MR show up, early detection, etiology classification, and the timing of

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interventions is important because in primary MR, early intervention in patient with sub clinical LV dysfunction detected by GLS may produce excellent long-term results[3]. However patient showing Significant LV dysfunction detected by GLS carry poor prognosis after surgical correction.

Current guidelines emphasise the potential advantage of LV global longitudinal strain (GLS) over LVEF for risk categorisation in patients with severe primary MR [4]. As a more precise and sensitive indicator of LV function, GLS was introduced. The management of these patients is significantly influenced by the likelihood of MV repair [5]. Several authors showed the important role of LV global longitudinal strain (GLS) over LV EF as an index of subclinical cardiacdysfunction in MR. Its use might lead to an improvement in the detection of LV damage in these patients as well as a more accurate prognostic evaluation for surgical referral [6].

LV strain was able to identify subclinical LV dysfunction in asymptomatic patients with primary MR undergoing surgical (n = 30/71patients) or medical treatment and preserved LV EF undergoing exercise echocardiography, and to predict postoperative LV dysfunction [7]. According to **Hiemstra et al. [8],** LV-GLS may be useful in guiding surgical scheduling and is independently linked to cardiovascular events and mortality from all causes after MV surgery for primary MR.

Aim of the work:

The purpose of this study was to determine whether positive clinical outcomes after mitral valve surgery for severe mitral regurgitation are correlated with left ventricular global longitudinal strain.

Methods:

This prospective study was done at the Cardiology Department, Zagazig University Hospitals during the period between April 2023 to November 2024 included 30 patients with primary severe mitral regurgitation undergoing mitral valve surgery divided into 3 groups according to EF. Ten patients in

Group I had an ejection fraction (EF) of 30– 45%, ten patients in Group II had an EF of 45–60%, and ten patients in Group III had an EF > 60%. This grouping was done regardless social demographics (Age, Sex, etc...)

Ethical consideration:. An approval from the Research Ethics Committee of institutional review board, Faculty of Medicine, Zagazig University (IRB #10412/26-2-2023) and from patient's parents before the examination was obtained . An informed written consent from all patients before participation was obtained; it included data about aim of the work, study design, site, time, subject and methods, confidentiality. An official permission from the administrators of the defined Hospitals to conduct this study was obtained. All procedures were explained for each patient. The study was conducted in accordance with the World Medical Association's (Declaration of Helsinki) code of ethics for research involving human beings

Inclusion criteria were patients with primary severe MR who had either mitral valve (MV) replacement or repair surgery. Secondary mitral regurgitation, congenital heart disease, ischemic heart disease, primary cardiomyopathy, and severe Aortic valve dysfunction were the exclusion criteria.

Every patient underwent a clinical examination, with particular attention paid to blood pressure, pulse rate, rhythm, and any indications of heart failure. Commercially available devices were used to collect echocardiographic data. A certified sonographer performed standard 2dimensional, M-mode, and color Doppler ultrasonography on each participant in accordance with the American Society of Echocardiography's recommendations.

Mitral regurgige, pulmonary venous flow pattern, and valve morphology were measured using an integrated approach that involved computing Calculate the regurgitated volume and the proximal isovelocity surface area approach to determine the efficient regurgitant orifice[9].

Hussein, M., et al **2205** | Page Using methods for proximal isovelocity surface area, severe MR was established with a regurgitant volume of at least 60 ml and an effective regurgitant orifice area of at least 0.40 cm² [10].

Even though preoperative hemodynamic and non-strain echocardiographic parameters did not show significant differences in their absolute values to be clinically effective in predicting postoperative left ventricular dysfunction, End-diastolic and End-systolic dimensions and the thickness of the left ventricle wall were assessed through Mmode and two-dimensional imaging techniques. The left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) were calculated from the apical twochamber and four-chamber views, while the left ventricular ejection fraction (LVEF) was determined using the Simpson's biplane method. Right ventricular systolic pressure was measured using the peak velocity of tricuspid regurgitation.

Strain analysis:

Utilising frame-to-frame, tracking, and naturally occurring acoustic speckles, by the tissue's scattering of the ultrasonic beam, the speckle-tracking technology provides angleindependent assessment of myocardial deformation. This approach, which used magnetic resonance imaging to demonstrate correlation, was tried and tested. Twodimensional grayscale images were captured at the mid-papillary level using a parasternal short axis view, including apical 4-chamber, apical 3-chamber, and apical 2-chamber images. Strain measurements were performed with commonly used software. The digitally obtained images were uploaded for review after being extracted from the cardiac image archiving and communication system. In the end-systolic frame, the LV endocardial boundary was manually traced. The software automatically created a strain curve from the greyscale photos. Peak strain was defined as the strain curve's maximum negative value that occurred throughout the cardiac cycle. GLS was calculated by averaging the peak values of three apical images. Outcomes:

We monitored our patients for any problems, including heart failure, arrhythmia (ventricular tachycardia), and delayed weaning, while they were in the hospital following surgery. Three months following surgery, patients were monitored for clinical outcomes such as worsening heart failure (dyspnea, orthopnea), arrhythmia, and cardiac death.

Statistical analysis

All data were collected, tabulated, and statistically analyzed using IBM Corp. Released in 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. For quantitative data after testing normality using the Shapiro-Wilk test: normally distributed data presented with mean \pm SD and skewed data presented as median (IQR). Qualitative data were expressed as (percentage). The chi-square and Fisher's exact tests were used for qualitative data, while the independent sample t-test and Mann-Whitney U test were used for quantitative data between two distinct groups, and one way ANOVA was used for quantitative data between more than two distinct groups. The risk factors for developing complications were assessed by multivariable logistic regression analysis by a forward method using factors identified with a p-value < 0.05 in univariable analysis. The cut-off value of GLS was calculated using ROC, and cross tabs were created to find the sensitivity and specificity. All tests were two-sided. P-value ≤ 0.05 was considered statistically significant, p-value > 0.05 was considered statistically insignificant.

RESULTS

30 patients with primary severe mitral regurge suitable for mitral valve surgery were included in this study. They were divided into three groups based on EF: Ten patients in Group I had EF ranging from 30 to 45%. Their ages ranged from 38 to 71 years with a mean \pm SD of 59.5 \pm 11.52, 40% were females and 60% were males. Ten patients in group II had EF between 45 and 60%. Their ages ranged from 39 to 67 years with a mean \pm SD of 50.1 \pm 8.27, 50% were

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males and 50% were females. Ten individuals with EF > 60% in group III. Their ages ranged from 28 to 68 with a mean \pm SD of 50.4 \pm 12.24, 50% were males and 50% were females. The rest of the baseline characteristics are mentioned in (Table 1). The pathophysiology of mitral regurgitation varied significantly across the studied groups, as 80% of the patients in group I had degenerative MR, compared to 10% of patients in group II and 20% of patients in group III (P=0.003) (Table 1). On conducting ROC analysis (Receiver operation Curve) to determine the optimal cutoff value of GLS to detect complications, the analysis showed that GLS had the highest sensitivity (100%) and specificity (78.26%) at -11.2 with an area under the curve of (0.839) (Table 2 and Figure 1). Furthermore, there was a statistically significant association between preoperative and follow-up echocardiographic findings with GLS, as patients with GLS < -11.2 had a lower preoperative and follow-up GLS (P<0.001), a lower follow-up LA (P=0.01), a lower preoperative and follow-up LVEDD (P<0.001) and (P=0.002) respectively, a lower preoperative and follow-up LVESD

(P=0.002) and a higher preoperative and follow-up LVEF (P<0.001) when compared with patients with GLS > -11.2. Also, there was a statistically significant improvement of 8.5% in GLS (P=0.007), 5.5% in LA (P<0.001), and 4.2% in LVESD (P=0.002) among patients with GLS < -11.2 (P=0.007) (Table 3).

Also, there was a statistically significant association between GLS and outcomes, as most of the patients with GLS < -11.2 (66.7%) were NYHA class Ipostoperatively, while most of the patients with GLS > -11.2 (33.3%) were NYHA class III postoperatively (P<0.001). Also, (41.7%) of the patients with GLS > -11.2 had rehospitalization for HF, while none of the patients with GLS < -11.2 had rehospitalization for HF postoperatively (P=0.006). While there was no significant association between GLS and mortality (P>0.05) (Table 4).

After applying logistic regression analysis for predictors of complications, GLS and EF can be used as independent factors for predicting complications (Table 5).

Table 1: Baseline characteristics among the studied groups

Variables		Group I	Group I	Group II	P
		(n=10)	(n=10)	(n=10)	Value
Age	$Mean \pm SD$	59.5 ± 11.52	50.1 ± 8.27	50.4 ± 12.24	
(years)	Range	(38 - 71)	(39 - 67)	(28 - 68)	0.13^{1}
Sex (n. %)	Male	6 (60%)	5 (50%)	5 (50%)	
	Female	4 (40%)	5 (50%)	5 (50%)	0.88^{2}
Risk	Hypertension	6 (60%)	3 (30%)	6 (60%)	0.3^{2}
factors (n.	Diabetes mellitus	4 (40%)	4 (40%)	6 (60%)	0.59^2
%)	Smoking	5 (50%)	2 (20%)	4 (40%)	0.37^{2}
	Dyslipidemia	3 (30%)	1 (10%)	6 (60%)	0.08^{3}
Pathology	Degenerative	8 (80%)	1 (10%)	2 (20%)	
of MR	Rheumatic	2 (20%)	9 (90%)	8 (80%)	0.003^{3}
Type of	MV replacement	7 (70%)	4 (40%)	5 (50%)	
surgery	MV repair	3 (30%)	6 (60%)	5 (50%)	0.39^{2}

**IOne way ANOVA test, **2Chi-square test, **3Fisher exact test, Non-significant: P > 0.05, Significant: $P \le 0.05$

Table 2: ROC curve analysis of GLS in predicting complications

Variables	Cut point	Sensitivity	Specificity	PPV	NPP	AUC
		(%)	(%)	(%)	(%)	(%)
GLS	-11.2	100%	78.26%	58.33%	100%	0.839

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Table 3: Association between preoperative and follow-up echocardiographic findings with GLS among the studied patients

Variables		GLS < -11.2	GLS > -11.2	P
		(n=18)	(n=12)	Value
GLS (%)	Preoperative	-14.3 (3.17)	-10 (2.05)	<0.001 ²
Median (IQR)	Follow-up	-16 (5)	-9 (1.78)	< 0.001 ²
% of im	provement	↑8.5%	↓2.6%	
*P	value	0.0074	0.49^4	
LA (mm)	Preoperative	54.7 ± 5.11	58.4 ± 4.68	0.06^{1}
Mean ± SD	Follow-up	51.7 ± 5.49	57.5 ± 5.28	0.011
% of improvement		↑5.5%	↑1.5%	
*P value		<0.001 ³	0.37^{3}	
LVEDD (mm)	Preoperative	58.5 (4.25)	64.5 (3)	$<0.001^2$
Median (IQR)	Follow-up	56 (6.5)	65.5 (5.25)	0.002^{2}
% of im	provement	↑2.4%	↓1.6%	
*P	value	0.06^{3}	0.15^{3}	
LVESD (mm)	Preoperative	41 (2.75)	45.5 (5.5)	0.002^{2}
Median (IQR)	Follow-up	39 (4.5)	48 (5.75)	0.002^{2}
% of improvement		↑4.2%	↓2.9%	
*P value		0.02^{3}	0.051^3	
EF (%)	Preoperative	58.6 ± 9.95	44.4 ± 5.2	<0.001 ¹
Mean ± SD	Follow-up	59.9 ± 9.92	43.6 ± 9.85	<0.001 ¹
% of improvement		↑2.2%	↓1.8%	
*P	value	0.12^{3}	0.32^{3}	

^{*}¹Student T-test, ²Mann-Whitney U test, ³Paired sample T-test, ⁴Wilcoxon signed rank test, Non-significant: P >0.05, Significant: P ≤0.05

P value=Comparison between the two groups, *P value=Comparison within the same group **Table 4:** Association between outcomes and GLS among the studied patients

Variables		GLS < -11.2	GLS > -11.2	P
		(n=18)	(n=12)	Value
Post-operative	Class I	12 (66.7%)	1 (8.3%)	
NYHA	Class II	6 (33.3%)	2 (16.7%)	
	Class III	0 (0%)	4 (33.3%)	< 0.001
	Class IV	0 (0%)	3 (25%)	
	Died	0 (0%)	2 (16.7%)	
Mortality	Survived	18 (100%)	10 (83.3%)	
	Died	0 (0%)	2 (16.7%)	0.15
Rehospitalization	No	18 (100%)	7 (58.3%)	
for HF	Yes	0 (0%)	5 (41.7%)	0.006

^{*}Fisher exact test, Non-significant: P > 0.05, Significant: $P \le 0.05$

Table 5: Logistic regression analysis for predictors of complications

Variables	Univariate analysis		Multivariate analysis		
	P value	Odds (CI 95%)	P value	Odds (CI 95%)	
Age	0.45	1.03 (0.95 – 1.12)	-	-	
Gender	0.82	1.22(0.22-6.73)	-	•	
HTN	0.67	1.45 (0.26 - 8.01)	-	-	
DM	0.53	1.73 (0.31 – 9.57)	-	-	
Smoking	0.61	1.61 (0.26 – 10.13)	-	-	

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Dyslipidemia	0.54	1.71 (0.32 – 9.77)	-	-
Pathology of MR				
Degenerative				
Rheumatic	0.69	1.41 (0.25 - 7.9)	-	1
Type of surgery				
MV replacement				
MV repair	0.99	2.45(0.99 - 8.01)	-	-
GLS	< 0.001	1.97 (1.09 – 3.58)	0.03	1.51 (1.02 – 2.21)
LA diameter	0.34	1.09 (0.91 – 1.3)	-	-
LVEDD	0.008	2.14 (1.22 – 3.74)	0.06	2.53 (0.95 – 6.76)
LVESD	0.007	1.71 (1.16 – 2.53)	0.67	0.84(0.37-1.89)
LVEF	0.04	0.85(0.72-0.99)	0.05	0.85(0.72-0.99)
DD grade				
Grade I				
Grade II	0.41	2.13 (0.36 – 12.39)	-	-

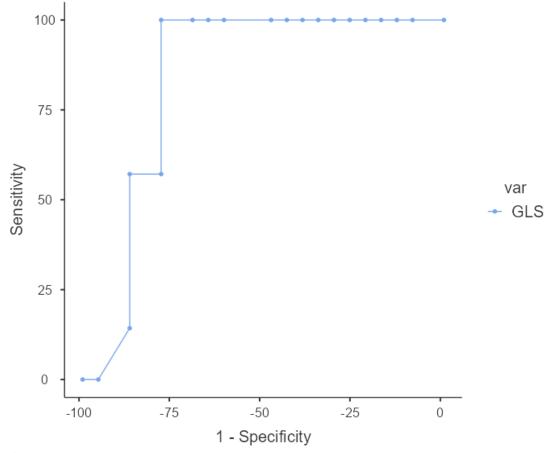


Figure 1: ROC curve analysis of GLS in predicting complications

DISCUSSION

The mean age of the groups in our current study was 59.5 ± 11.52 years for Group I, with no significant difference (p = 0.131), 50.1 ± 8.27 years for Group

II, and 50.4 ± 12.24 years for Group III. With 60% of the participants in Group I being men and 50% of the participants in Groups II and III being men, the gender distribution was comparatively

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balanced across the groups (p = 0.882). In terms of risk factors, 60% of Group I, 30% of Group II, and 60% of Group III had hypertension (p = 0.32). 40% of Group I, 40% of Group II, and 60% of Group III had diabetes mellitus (p = 0.592). Group I had a 50% smoking rate, Group II had a 20% smoking rate, and Group III had a 40% smoking rate (p = 0.372). Dyslipidemia affected thirty percent of Group I, ten percent of Group II, and sixty percent of Group III (p = 0.083). The findings of **Wu et al.** [11], who determined risk factors for surgery evaluated the frequency treatment of patients with severe MR at a tertiary medical centre support our findings. According to their research, the average age of the surgical patients was 62.6 ± 14.2 years, 30 (51%) of them were men, 32 (54%) had high blood pressure, and 16 (27%) had diabetes. The study by Daneshmand et al. [12] investigated the impact of patient age on risk-adjusted survival after mitral surgery. Their findings indicated that age was the most important multivariate predictor of late death [hazard ratio = 1.4 per 10-year increment, Wald χ 2 = 32.7, p < 0.0001]. Additionally, **Rahimi** et al. [13] shown that a higher risk of secondary primary and regurgitation is linked to prolonged exposure to high blood pressure in all of its manifestations. According to the most recent ACC/AHA study and ESC guidelines, pulmonary hypertension is one such poor prognostic indication. Patel et al. state that mitral valve surgery indicated class IIA as a recommendation for patients with asymptomatic MR [14].

The pathology of mitral regurgitation (MR) varied significantly between the groups, according to operational data. Degenerative MR was found in 80% of Group I, 10% of Group II, and 20% of Group III, whereas rheumatic MR was found in 20%, 90%, and 80% of the groups, respectively (p =

0.0032). In terms of surgical procedure, 70% of Group I, 40% of Group II, and 50% of Group III underwent mitral valve (MV) replacement. MV repair, on the other hand, was performed in 30%, 60%, and 50% of cases, respectively, with no discernible variation (p = 0.391).Our research contradicts **Delgado** et al. [15] discovered that 61% of MR cases requiring surgery are due to degenerative (organic) Furthermore, the previously unidentified etiology/ In one study, the community's mechanistic distributions of mitral regurgitation (MR), together with the associated clinical features were characterized outcomes, by Dziadzko et al. [16]. Lower ejection fractions (EF) are associated with degenerative MR, which is more common in Group I. It usually results in chronic volume overload. progressive LV dilatation. dysfunction (Edwards et al. [17], Vancheri et al. [18]). On the other despite severe regurgitation, rheumatic MR, which is more prevalent in Groups II and III, is linked to fibrosis and restricted leaflet motion, which results in less LV dilatation and comparatively preserved EF [19].

Significant improvements were observed in our examined groups' preoperative and follow-up echocardiographic results, especially in Group III. Group III experienced the greatest improvement in longitudinal strain (GLS) (9.9%, p = 0.044), while Groups I and II saw just slight changes. Group III experienced a 6.3% decrease in left atrial (LA) size (p = 0.0043), while Groups I and II saw less improvement. Ejection fraction (EF) and left ventricular dimensions (LVEDD and LVESD) varied somewhat between groups; Group III had a 1.2% rise in EF and a 4.8% improvement in LVESD, but these differences were not statistically significant.

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All things considered, Group III had the best postoperative results. Additionally, we discovered that individuals with a postoperative left ventricular ejection fraction of greater than 50% had improved preoperative global longitudinal strain and left ventricular ejection fraction. In their evaluation of patients having mitral valve replacement, Joung et al. [20] discovered that those with preoperative **LVEF** had larger immediate postoperative drop in LVEF. However, when preoperative LVEF was ≥50%, long-term LVEF plateaued at about 60%. This implies that individuals with greater preoperative LVEF would have improved ventricular function in the long run after surgery, which might be associated with fewer heart failure readmissions. Additionally, Kislitsina et al. [21] investigated if strain could enhance the left ventricular ejection fraction's (LVEF) capacity to forecast ventricular failure after mitral valve replacement for degenerative mitral regurgitation (DMR).Based on predischarge echocardiograms, they found that 449 patients (86.3%) had a postoperative LVEF of 50% or higher. Predischarge LVEF was less than 40% in 71 patients (13.7%), between 40% and 49% in 49 patients (9.4%), and 4.2% 22 people. Abnormal preoperative measurements of left atrial, right ventricular, and left ventricular strain were significantly associated with the development of postoperative LV dysfunction, although the preoperative Hemodynamic and non-strain echocardiographic parameters were not clinically useful as indicators postoperativeleft ventricular dysfunction because they did not exhibit appreciable variations in absolute values.

Due to less advanced myocardial remodeling and better-preserved myocardial function prior to surgery, Group III experienced greater postoperative improvements. As

demonstrated by improved GLS and decreased left atrial (LA) size, Group III demonstrated a substantial recovery with less chronic volume overload and less irreversible myocardial damage. However, because their hearts were less able to recover from the damage caused by prolonged MR, Groups I and II, who probably had more extensive LV dilatation and myocardial strain prior to surgery, only displayed minor changes. These results imply that improved postoperative outcomes result from early intervention prior to severe myocardial dysfunction [9, 22, 23]. Our findings are consistent with those of Stokke et al. [24], who found a significant decrease in GLS without a corresponding decrease in LVEF. This suggests that GLS may be able to identify subtle myocardial dysfunction that is not visible with LVEF measurement alone. This implies that the degree of cardiac damage may be underestimated if LVEF is the only measure used. Furthermore, Traunero et al. [25] showed that postoperative decline in left ventricular GLS can happen even when the ejection fraction is preserved, indicating that GLS may reveal myocardial dysfunction that is not picked up by more conventional metrics like LVEF. With the majority of patients in Class II (50% in Groups I and II, 40% in Group III) and a minority in Class III, the pre- and post-operative NYHA functional class comparison between the revealed significant groups no Significant improvement differences. was seen after surgery, especially in Group III, where 80% of patients achieved Class I, compared to 40% in Group II and 10% in Group I (p = 0.04). Group I experienced postoperative mortality (20%), whereas Groups II and III did not experience any recorded deaths. Consistent with our research, Pieri et al. [26] verified that patients undergoing heart surgery, MVR. including who had low preoperative LVEF are more likely to

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experience complications after the procedure. The results support your finding that patients with lower preoperative LVEF had higher postoperative mortality.

Our study's post-operative results revealed that Group I had a 20% mortality rate while Groups II and III experienced no deaths (p=0.12). 10% of Group I and 40% of Group II experienced rehospitalization for heart failure, whereas Group III experienced no cases, indicating a significant difference (p=0.04).

Modaragamage et al. [27] investigated global longitudinal strain in accordance with our investigation to ascertain the best time to do surgery for primary mitral regurgitation. According to their research, greater death rates were linked to impaired baseline GLS.

The ROC curve analysis of GLS in problem prediction showed that it had the highest sensitivity (100%) and specificity (78.26%) at -11.2 with an the under curve of (0.839).**Witkowski** al. et [28] discovered that, in agreement with our findings, a GLS cutoff of >-19.9% had a 90% sensitivity and a 79% specificity in predicting long-term LV dysfunction following mitral valve replacement. Additionally, Lee et al. [29] demonstrated that a hazard ratio of 1.229 (95% CI: 1.135 to 1.331; p < 0.001)indicates that a lower GLS is a significant predictor of cardiac events following mitral valve surgery.

Conclusion

After mitral valve surgery for initial severe mitral regurgitation, we conclude that left ventricular global longitudinal strain is a reliable indicator of clinical and echocardiographic results. Compared to patients with a preoperative GLS >-11.2, those with a preoperative GLS < -11.2 demonstrated significantly better postoperative recovery, including improved functional

status, lower rehospitalization rates, and improved echocardiographic parameters.

Conflict of Interest: None Financial Disclosures: None REFERENCES

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