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Left Ventricular Longitudinal Strain Assessment by Speckle Tracking Echocardiography in Patients with Chronic Aortic Regurgitation

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Abstract

Background: Chronic aortic regurgitation (AR) is associated with subtle myocardial changes that will lead eventually lead to ventricular dysfunction. To establish a proper treatment plan, speckle tracking echocardiography (STE) has emerged as a sensitive tool to detect such changes compared to the limited conventional echocardiography.

Aim of Study: This study was conducted to evaluate left ventricular performance in patients with chronic AR using 2-D STE.

Patients and Methods: This prospective case control study included 30 cases diagnosed with moderate to severe AR along with 30 age and gender matched healthy controls. All patients were clinically assessed. Additionally, all subjected were assessed with conventional and 2-D STE. Global longitudinal strain was evaluated and recorded.

Results: Both ejection fraction and fractional shortening showed a significant decrease in cases versus controls. On the other hand, aortic root diameter, left atrial dimensions, interventricular septal thickness in diastole, left ventricular internal dimensions during systole and diastole, together with interventricular septal thickness in systole showed a significant increase in cases compared to controls. Global longitudinal strain had mean values of –18.33±7.932 and –21.70±3.631 in cases and controls respectively, with a significant decrease in cases versus controls (*p*-value 0.030).

Conclusion: Subtle or substantial reduction of LV systolic function was present in AR group as evidenced by a significant reduction of left ventricular global longitudinal strain. Hence, strain may act as a sensitive indicator for subclinical dysfunction in such cases.

Key Words: *Aortic regurgitation – Speckle tracking echocardiography – Global longitudinal strain.*

Introduction

THE overall prevalence of chronic aortic regurgitation (AR) is about 13% and 8.5% in men and women respectively [1,2]. Deterioration of cardiac function is a known and dreadful complication of chronic aortic regurgitation (AR). It generates left ventricular (LV) volume overload leading to its dilatation. Although ejection fraction (EF) is maintained at early stages, LV systolic dysfunction will eventually develop, and it is manifested by a drop in EF [3,4].

The problem is that disease progression is not associated with clinical manifestations in most AR cases [5]. Of note, about 25% of these cases with asymptomatic LV dysfunction develop heart failure every year, and the mortality in this special group is about 10% [6,7].

Traditional echocardiography may delay surgical intervention as it has limitations in the detection of subclinical myocardial dysfunction [8,9]. Therefore, more accurate diagnostic procedures are needed for early prediction of irreversible myocardial dysfunction. This is of crucial importance, as complete recovery after surgical outcome would be questionable [5].

The term "myocardial strain" is defined as the fractional change in a myocardial segment length relative to its baseline value. It is expressed as percentage, Myocardial strain echocardiographic imaging has been widely used as a clinical index of both global [10,11] and regional left ventricular dysfunction [12,13].

Previous studies reported the ability of global longitudinal strain to detect subtle changes in myocardial function [14].

Therefore, this study was conducted to evaluate LV performance in patients with chronic AR using two-dimensional strain speckle tracking echocardiography (2-D STE).

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Patients and Methods

The current prospective case control study was conducted at the Cardiology Department, Specialized Medicine Hospital, Mansoura University, Egypt over the period of one year, starting from March 2017 till March 2018. After gaining an informed written consent and obtaining the approval from the Institutional Review Board (IRB), a total of 60 subjects were included in the study. They were divided into two equal groups; Group 1 included 30 cases diagnosed with AR, and Group 2 included 30 healthy controls.

For the cases group, we included any adult cases diagnosed with moderate to severe AR whatever the cause. Contrarily, cases with associated aortic stenosis, other valvular lesions, acute AR, ischemic heart disease, cardiomyopathy, diastolic hypertension, diabetes mellitus or chronic kidney disease were excluded from the current study.

All of the included cases were subjected to detailed history taking (age, gender, and duration of the disease), clinical examination (blood pressure (BP), pulse, neck veins, chest and cardiac auscultation), electrocardiogram along with routine laboratory investigations (complete blood count, serum creatinine and random blood sugar).

Furthermore, all of the included subjects underwent conventional transthoracic echocardiography and 2-D STE using Philips Affiniti C50 machine. Examination was performed when the patient was lying in the left lateral decubitus position. Echocardiographic examination was done based on the recommendations of American society of echocardiography (ASE) as we started the evaluation by assessment of the anatomy of aortic valve and aortic root to determine the etiology and mechanism of regurgitation. This was followed by assessment of LV size, geometry, and function.

We obtained parasternal long axis view, short axis view and apical four, three and two chambers views. Under guidance of the parasternal long axis view, the following m-mode parameters were obtained (Fig. 1A,B); end-diastolic and end-systolic diameters of the LV, interventricular and posterior left ventricular wall thickness in systole and diastole. Both aortic root and left atrial diameters were measured in the same view.

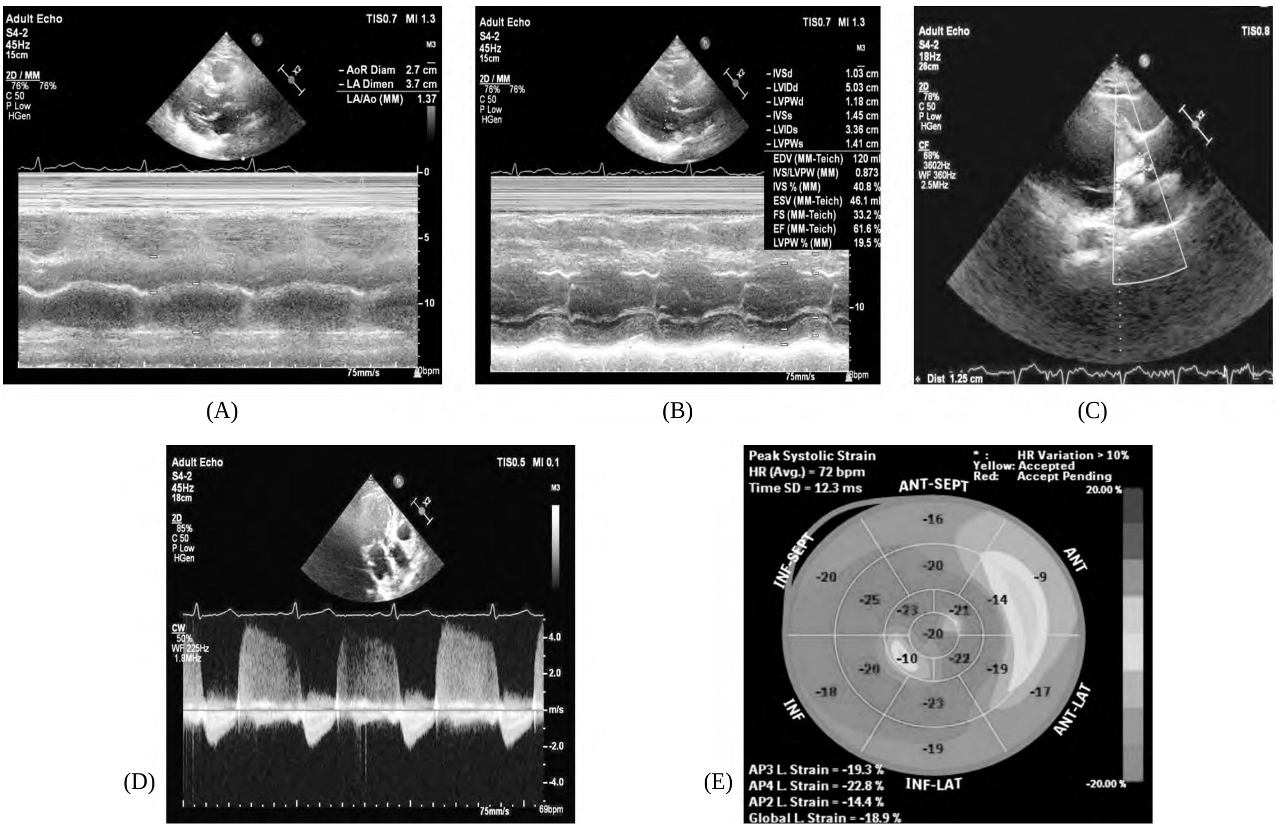

Fig. (1): Forty-seven-year-old female with history of rheumatic heart disease. (A) M mode at AO and left atrium. (B) M mode at mid ventricular level to evaluate EF. (C) Color Doppler represents about >2/3 LVOT width. (D) CWD at AV. (E) Regional and global longitudinal strain.

Conventional Doppler echocardiography:

We used 2-D color Doppler interrogation of apical 5-chamber view to guide cursor placement in the most turbulent area of transaortic flow. Continuous Doppler was recorded; peak gradient and pressure half time were measured. Color Doppler was used to evaluate 3 components (flow convergence, Vena Contracta, jet size and direction in LV outflow) (Fig. 1C,D).

Speckle tracking echocardiography:

Speckle tracking was done by Automated Function Imaging (AFI) which is a software tool that systematizes 2D speckle tracking after obtaining real time apical views including apical four, three and two chambers views to measure in real-time regional as well as global longitudinal strain of the myocardial wall (Fig. 1E).

Statistical analysis:

Collection, and analysis of data entered were conducted by the Statistical Package for the Social Sciences (SPSS 26, IBM/SPSS Inc., Chicago, IL) software for analysis. Categorical data were expressed as number and percent within groups while the quantitative data were expressed as mean and standard deviations (SD). To compare the collected data, Chi-Square test (or Fisher's exact test) was used to compare qualitative data groups while quantitative data groups were compared via either independent-Samples *t*-test or Mann-Whitney U test for parametric and non-parametric quantitative data respectively. *p*-values <0.05 are considered statistically significant.

Results

Starting with demographic characteristics, cases and controls had mean ages of 43.27 and 41.43 years respectively. Males represented 67% and 53% of cases in the same groups respectively. Both age and gender were not significantly different between the two study groups ($p>0.05$).

When it comes to the clinical data, systolic blood pressure had significantly higher values in cases versus controls (124.67 vs. 118.33mmHg respectively - $p=0.001$). However, diastolic blood pressure showed no significant difference between the two groups ($p=0.53$). Also, heart rate was not significantly different between cases and controls ($p=0.348$). All the previous data are summarized in Table (1).

As shown in Table (2), most conventional echocardiographic parameters showed a statistically significant difference between cases and controls ($p<0.05$) apart from left atrial diameter ($p=0.209$) and posterior wall thickness during systole (0.387). Both ejection fraction and fractional shortening showed a significant decrease in cases versus controls. On the other hand, aortic root diameter, left atrial dimensions, interventricular septal thickness in diastole, left ventricular internal dimensions during systole and diastole, together with interventricular septal thickness in systole showed a significant increase in cases compared to controls.

Table (1): Demographic and clinical criteria of the included groups.

	Group 1 (30 cases)	Group 2 (30 controls)	*p*-value
Age (year)	43.27±5.076	41.43±4.944	0.148
Gender:			
- Male	20 (67%)	16 (53%)	0.3
- Female	10 (33%)	14 (47%)	
SBP	124.67±5.561	118.33±7.915	0.001 *
DBP	77.33±5.040	78.17±5.167	0.530
HR	80.50±7.528	82.43±8.291	0.348

DBP : Diastolic blood pressure.
HR : Heart rate.
SBP : Systolic blood pressure.

Table (2): Conventional echocardiographic parameters of the included groups.

	Group 1 (30 cases)	Group 2 (30 controls)	*p*-value
ARD (cm)	3.23±0.728	2.67±0.479	0.001 *
LAD (cm)	3.40±0.724	3.17±0.699	0.209
IVSD (cm)	1.17±0.379	0.97±0.183	0.012*
LVIDd (cm)	5.60±1.102	4.33±0.661	0.000**
IVSS	1.67±0.479	1.37±0.490	0.020*
LVIDs	3.53±0.973	2.43±0.504	0.000**
PWTs (cm)	1.63±0.490	1.77±0.679	0.387
EF %	63.30±9.777	71.83±6.859	0.000**
FS %	36.40±7.356	41.40±8.505	0.019*

ARD : Aortic root diameter.
EF : Ejection fraction.
FS : Fractional shortening.
IVSD : Interventricular septal thickness in diastole.
IVSS : Interventricular septal thickness in systole.
LAD : Left atrial dimension.
LVIDD : Left ventricular internal dimensions during diastole.
LVIDS : Left ventricular internal dimensions during systole.
PWTs : Posterior wall thickness during systole.

Table (3) shows that there was a significant difference between the two groups regarding the mean value of cumulative longitudinal peak systolic strain of basal anterior, mid anterior, basal inferolateral and apical inferior walls ($p<0.05$). Nevertheless, other measurements showed no significant differences between the two groups ($p>0.05$).

Table (3): Left ventricular peak systolic strain in the study groups.

	Group 1 (30 cases)	Group 2 (30 controls)	*p*-value
Basal Anterosep	–17.10±5.006	–17.60±5.367	0.710
Basal Anterior	–15.97±4.263	–19.67±4.205	0.001 *
Basal AnteroLat	–16.33±5.142	–18.43±3.588	0.072
Basal InferoLat	–15.70±4.801	–18.27±5.071	0.049*
Basal Inferior	–16.13±4.531	–17.03±4.030	0.420
Basal Inferosep	–16.57±5.157	–17.90±3.367	0.241
Mid Anterosep	–18.20±4.397	–21.30±5.011	0.14
Mid Anterior	–19.23±4.240	–22.80±4.429	0.002*
Mid Anterolat	–18.43±5.643	–20.63±3.709	0.080
Mid InferoLat	–19.33±6.784	–19.97±4.263	0.667
Mid Inferior	–17.47±5.829	–19.60±4.288	0.112
Mid Inferosep	–17.97±5.269	–19.23±4.861	0.337
Apical Anterior	–22.17±4.488	–22.60±4.651	0.715
Apical Lateral	–22.47±5.355	–23.47±6.740	0.527
Apical Inferior	–23.40±5.805	–27.73±6.432	0.008 *
Apical Septum	–24.80±7.058	–25.87±5.296	0.511
Apical Apex	–23.07±4.525	–24.07±4.741	0.407

As shown in Table (4), comparing the two groups as regard time to peak showed no significant difference between cases and controls (p>0.05), apart from the basal inferior wall that showed a significant increase in that parameter in cases versus controls (316.97 vs. 296.63 respectively - p=0.04).

The net result of peak systolic longitudinal strain showed significant reduction of apical 2 longitudinal strain and global strain in cases versus controls. The former had mean values of 19.57 and –22.07, while the latter had mean values of –18.33 and –21.7 in cases and controls respectively. Conversely, apical and 4 longitudinal strains showed no significant difference between the study groups. Table (5) illustrates these data.

Table (4): Time to peak in the included groups.

	Group 1 (30 cases)	Group 2 (30 controls)	*p*-value
Basal Anterosep	308.20±29.741	302.93±36.068	0.540
Basal Anterior	314.43±33.374	305.27±38.172	0.326
Basal AnteroLat	323.23±42.796	324.23±49.475	0.934
Basal InferoLat	318.77±41.773	301.00±36.497	0.85
Basal Inferior	316.97±37.910	296.63±36.898	0.040*
Basal Inferosep	330.90±54.790	321.00±45.351	0.449
Mid Anterosep	307.60±28.662	303.27±35.493	0.605
Mid Anterior	311.60±31.900	300.40±37.731	0.219
Mid Anterolat	316.97±40.685	311.63±43.617	0.626
Mid InferoLat	305.97±37.701	296.47±37.923	0.335
Mid Inferior	312.67±39.050	294.57±36.392	0.068
Mid Inferosep	320.67±48.389	309.83±45.559	0.376
Apical Anterior	311.40±27.014	298.23±34.237	0.104
Apical Lateral	308.47±29.860	304.97±27.723	0.640
Apical Inferior	310.00±31.044	297.47±37.816	0.166
Apical Septum	315.17±40.356	313.90±39.438	0.903
Apical Apex	311.23±26.264	301.73±27.870	0.179

Table (5): The net result of peak systolic longitudinal strain in the study groups.

	Group 1 (30 cases)	Group 2 (30 controls)	*p*-value
AP2 L strain	-19.57±3.645	-22.07±3.982	0.014*
AP4 L strain	-20.03±4.694	-20.97±3.113	0.368
AP3 L strain	-18.30±7.557	-19.30±9.308	0.649
Global strain	-18.33±7.932	-21.70±3.631	0.039*

Table (6): Roc curve for prediction of cases by Net result.

	AUC	95% CI	Cut off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
AP2L strain	0.689	0.554-0.823	>-21.6	83.3%	53.3%	64.1 %	76.2%	68.3%
AP3L strain	0.595	0.449-0.741	>-20.85	70.0%	53.3%	60%	64%	61.7%
AP4L strain	0.576	0.430-0.722	>-20.65	56.7%	53.3%	54.8%	55.2%	55%
Global strain	0.678	0.540-0.815	>-21.75	73.3%	53.3%	61.1%	66.7%	63.6%

AUC: Area under the curve. CI: Confidence interval. PPV: Positive predictive value. NPV: Negative predictive value.

The area under receiver operating characteristic curve for AP2 L strain in the prediction of cases was 0.689 (95% confidence interval): 0.554-0.826. By using Roc curve Sensitivity, Specificity. PPV, NPV and accuracy at cutoff >-21.6 were (83.3%, 53.3%, 64.1%, 76.2% and 68.3% respectively).

Also, AUC for AP3 L strain in the prediction of cases was 0.595 (95% confidence interval): 0.449-0.741. By using Roc curve Sensitivity, Specificity. PPV, NPV and accuracy at cutoff >-20.85 were (70%, 53.3%, 60%, 64% and 61.7% respectively).

In addition, AUC for AP4 L strain in the prediction of cases was 0.576 (95% confidence interval): 0.430-0.722. By using Roc curve Sensitivity, Specificity. PPV, NPV and accuracy at cutoff >–20.65 were (56.7%, 53.3%, 54.8%, 55.2% and 55% respectively). So, AP2 L strain was better in prediction of cases.

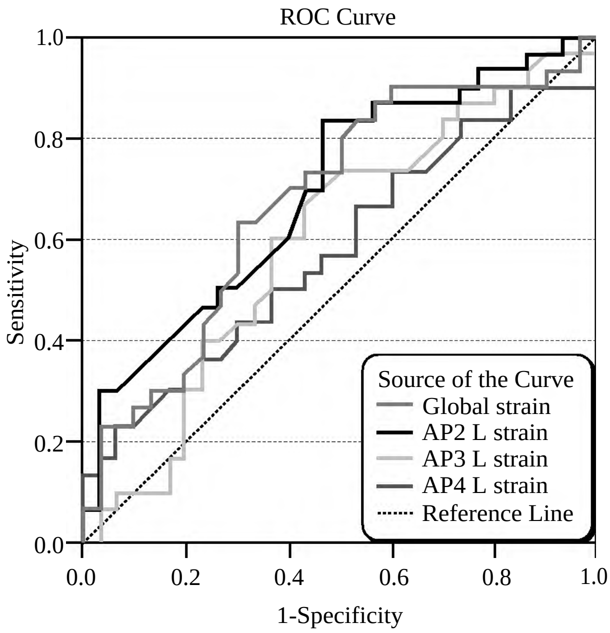

Fig. (2): Roc curve for prediction of cases by Net result.

Discussion

LV dilatation is a basic component in the pathophysiology of chronic AR, and it is frequently encountered in patients with chronic AR. It occurs secondary to volume overload. Additionally, LV hypertrophy also occurs due to the need for increased contractility. These measures help to preserve LVEF. Nevertheless, when it fails, irreversible LV dysfunction occurs **[15]**.

As most patients with LV dilatation and hypertrophy secondary to that disease are asymptomatic, it is essential to detect subclinical LV functional changes. The present study aimed to evaluate the early detection of left ventricular dysfunction in patients with chronic aortic regurgitation using strain imaging. To achieve this target, we recruited 30 patients with isolated chronic moderate to severe AR in addition to 30 age and sex matched healthy controls.

The general demographic data did not show any significant difference between cases and controls, and that should nullify any bias that may have skewed the results in favor of one group rather than the other one.

Our findings showed that systolic blood pressure had significantly higher values in cases versus controls (124.67 vs. 118.33mmHg respectively - p=0.001). However, diastolic blood pressure showed no significant difference between the two groups.

Another study reported that systolic blood pressure was significantly elevated in AR cases compared to controls (135 vs. 116mmHg). Also, diastolic blood pressure showed no significant between the two groups (58 and 66mmHg respectively - p=0.54) **[16]**. This study agreed with us regarding both parameters.

In contrast with our findings, Smedsrud et al., negated any significant difference between cases and controls regarding systolic blood pressure, which had mean values of 142 and 135mmHg in cases and controls respectively (p=0.26). The same authors reported a significant decrease of diastolic blood pressure with AR cases (66 vs. 77mmHg in controls - p<0.01) **[17]**. The heterogenicity between different studies could be explained by different disease stage, patient criteria, sample size, and statistical tests performed.

In the current study, no significant difference was detected between cases and controls regarding heart rate (p=0.348), which had mean values of 80.5 and 82.43bpm in the two groups respectively.

Similarly, another study also negated any significant difference between cases and controls regarding heart rate (p=0.41), which had mean values of 68 and 71 bpm in the two groups respectively **[17]**. A recent study also confirmed the previous findings **[5]**.

In our study, intraventricular septal thickness showed a statistically significant increase in AR cases compared to controls, either in systole or diastole (p<0.05). Left atrial dimensions had higher values in cases compared to controls (3.4 vs. 3.17cm respectively) despite being non-significant.

In line with the previous findings, a recent study also reported an increase in left atrial dimensions and interventricular septum in AR cases versus controls (p<0.001). The former had mean values of 3.59 and 3.81 cm in moderate and severe AR cases compared to 3.24cm in controls. The latter had mean values of 0.96 and 1.08cm in the cases groups respectively compared to 0.88cm in controls **[5]**.

Marciniak et al., also reported a significant increase of IVS in cases with severe AR compared to controls (1 and 0.8cm respectively - p<0.001) **[15]**.

Our findings showed that aortic root diameter showed a significant increase in cases versus controls (3.23 vs. 2.67cm respectively - p=0.001), and this was in accordance with Abd Alaziz et al., who reported that aortic root diameter had mean values of 3.66 and 2.69cm in the same groups respectively, with a significant increase in cases versus controls (p<0.001) [18].

Our results showed no significant difference between the two groups regarding posterior wall thickness (p=0.387). Likewise, another study reported no significant differences between cases and controls regarding PWT, as it had mean values of 1 and 0.8cm respectively. However, the same study reported a significant increase in the same parameter in the subgroup with severe disease (1.1cm) [15].

In the current study, cases with AR showed a significant reduction in LVEF compared to controls (71.83 vs. 63.3% respectively - p<0.001). Zeng et al., confirmed our findings as there was a significant decrease in LVEF in AR cases compared to controls (p=0.024). EF had mean values of 63.58% and 5.17% in cases with moderate and severe AR respectively, whereas controls had a mean value of 64.49% [5].

On the other hand, another study showed no significant difference between cases and controls regarding left ventricular ejection fraction (p=0.59). It had a mean value of 59% in both cases and controls [17].

In the current study, the cases had significantly lower fractional shortening when compared to controls (p=0.019). it had mean values of 36.4 and 41.4% in the two groups respectively.

Likewise, another study reported a significant decrease in fractional shortening (p=0.008) in cases with AR compared to controls (35.8 and 39.3% respectively) [18].

When it comes to STE findings, both apical 2 longitudinal and global longitudinal strain showed a significant decrease in cases against controls (p=0.014 and 0.039 respectively). The former had mean values of –19.57 and –22.07, while the latter had mean values of –18.33 and 21.7 in cases and controls respectively.

It was previously reported that longitudinal strain undergoes more evident changes in AR compared to the circumferential one. The longitudinal strain was even reduced without normalization of the preload. Also, changes implying subclinical left ventricular dysfunction associated with AR starts in the subendocardium, where longitudinal fibers predominate [17]. In cases with AR, coronary blood flow is decreased despite increased ventricular O_2 demand. In turn, subendocardial ischemia occurs leading to decreased longitudinal strain [19].

In agreement with our findings, Smedsrud and his coworkers also showed a significant decrease in longitudinal strain measured by 2-D speckle tracking echocardiography (p<0.01). It had mean values of –17.5 and –22.1% in cases and controls respectively. The same authors reported that global systolic longitudinal strain is more superior to EF in the detection of myocardial dysfunction in chronic AR cases [17].

Zeng and his associates also reported a significant decrease in GLS in cases compared to controls (p<0.001). While controls had mean a mean value of 22.08%, the same parameter had mean values of –18.88% and –16.06% in cases with moderate and severe disease respectively [5].

Furthermore, Marciniak et al., reported that cases with severe AR had significant impairment of LV longitudinal strain compared to healthy controls [15].

In the same context, Di Salvo et al., conducted a study handling the same perspectives but in patients aged less than 16 years. Authors reported a significant decrease in LV average longitudinal strain in cases with progressive AR compared to cases with stable disease (–17.8 vs. –22.7% respectively - p=0.001). Using a cut off value of –19.5%, LS had sensitivity and specificity of 77.8% and 94.1 % respectively to detect cases with progressive disease [9].

In our opinion, we recommend to use that diagnostic modality in the assessment of patients with AR. This will not only help in the detection of subtle dysfunctional changes in the LV, but also it will help cardiologists to optimize the treatment strategy for each patient as surgical intervention may be needed earlier. Moreover, GLS has been used to predict post-operative outcomes as published in a recent systematic review [20].

Our study has some limitations. First of all, it is a single center study that included a small sample size. Additionally, we should have followed the patients to detect cases who develop more deterioration of LV systolic function.

Conclusion:

According to the previous findings, subtle or substantial reduction of LV systolic function was present in AR group as evidenced by significant reduction of left ventricular global longitudinal strain. Hence, strain may act as a sensitive indicator for subclinical dysfunction in such cases.

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تقييم وظيفة البطين الأيسر لدى مرضى الارتجاع الأبهرى المزمن بواسطة دوبلر القلب النسيجى والتتبع الرقطى

أن معدل انتشار الارتجاع الابهرى المزمن حوالى ١٣٪ عند الرجال و ٨٪ عند السيدات، وبوجه عام فإن الارتجاع الابهرى المزمن من الأمراض التى يمكن تحملها وعادة ما تكون بدون أعراض حتى مرحلة الفشل الشديد لعضلة القلب، وقد وجد أن الوظيفة الانقباضية للبطين الأيسر عامل مهم قبل الجراحة لهؤلاء المرضى قبل أن يقل قيمة طرد البطين عن ٥٠٪ والبعد الانقباضى لأكثر من ٥٠مم.

ومع ذلك فإن استخدام المعايير المعتمدة على الحجم مثل طرد البطين لتقييم وظيفة عضلة القلب قد تنطوى على قيود فى حالة تغيير حمولة القلب كما أن التغيير لحجم الارتجاع أو زيادة الضغط الانقباضى والانبساطى فى البطين الايسر يؤديان إلى إخفاء التطورات المرضية فى عضلة القب، بالإضافة إلى أن الايكو التقليدى يقيم الكلية للبطين الايسر فى حين أنه فى حالة الارتجاع الابهرى من المهم تقييم وظيفة البطين الاقليمية، حيث أنه إذا تم تأجيل الجراحة حتى يعانى المريض، قد يكون هناك خلل فى وظيفة القلب غير قابل لعلاج أو لا رجعة فيه.

وعلى الرغم من وجود الدراسات الإكلينيكية المتعددة التى أوضحت الخلل فى وظيفة عضلة القلب لدى مرضى الارتجاع الابهرى، فإنه لا يوجد واحدة منها تستطيع أن توضح الخلل الذى يصيب وظيفة عضلة القلب قبل ظهور الأعراض الإكلينيكية للمرض.

وتعتبر معدل الشد الانقباضى الطولى طريقة مثلى لقياس وظيفة عضلة القلب الكلية والاقليمية حيث أنه وجد بالرغم من قيمة طرد البطين فى المعدل الطبيعى إلا أن قيمة الشد فى البطين الايسر قد تقل لدى مرضى الارتجاع الابهرى وقد استخدمت القيم الادنى كمؤشر لاجراء الجراحة فى وقت مبكر لدى هؤلاء المرضى.

الهدف من البحث: إيجاد وسيلة دقيقة لتقييم وظيفة البطين الايسر عن طريق أشعة الايكو دوبلر واستخدام التتبع الرقطى لدى المرضى الذين يعانون من الارتجاع فى الصمام الابهرى ولا يعانون من أعراض.

المرضى وطرق الدراسة: الدراسة تشمل جميع المرضى الذين لديهما ارتجاع فى الصمام الابهرى، لإسباب مختلفة.

المعايير المستبعدة: أمراض الصمامات الأخرى المصاحبة للأرتجاع الأبهرى.

- أمراض قصور الشريان التاجى
- هبوط عضلة القلب
- مرضى الفشل الكلوى المزمن
- مرضى البول السكرى
- مرضى ارتفاع الضغط الأنبساطى.

وجميع هؤلاء المرضى سيخضعون للآتى:

- التاريخ المرضى الكامل.
- الفحص البدنى الكامل.
- تخطيط القلب الكهربائى.
- موجات صوتية على القلب.
- دوبلر القلب النسيجى والتتبع الرقطى.
- فحوصات معملية.