Assessment of Right Ventricular Function by Speckle Tracking Echocardiography in Patients with COVID - 19

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ABSTRACT
Background: Coronavirus disease 2019 (COVID-19) is one of the most serious problems nowadays, it is a contagious illness triggered by SARS-CoV-2.
Aim of work: This study aimed to investigate the influence of active COVID-19 infection on right ventricular mechanics using speckle tracking echocardiography.
Subjects & methods: This was observational (cross-sectional) research that that was conducted in Agouza Specialized Hospital (which turned to isolation hospital after COVID – 19 pandemic) and Cardiology Department, Menoufia University Hospitals through the period from November 2021 to January 2023. The research was carried out on 50 admitted cases with confirmed COVID 19 infection.
Result: There was a significant affection of RV GLS in cases with severe covid-19 infection. A significant variance was observed among groups regarding CT severity index, the more severity of CT, the more affection of RV GLS. There was a significant relation between affection of RV GLS and presence of risk factors (DM and HTN).
Conclusion: Our results showed that there was subclinical impairment of RV function with 2D STE in cases infected with COVID-19 in relation to the degree of pneumonia.
Key words: Right ventricular function, Speckle tracking echocardiography, COVID - 19.

INTRODUCTION

COVID-19 is a respiratory disorder triggered via the SARS-CoV-2 virus that has been correlated with a wide range of cardiovascular complications, involving heart failure, myocarditis, and arrhythmias. Speckle tracking echocardiography is a non-invasive imaging technique utilized to evaluate cardiac function by measuring the deformation of myocardial tissue during the cardiac cycle. The year 2020 has been overshadowed by the global COVID-19 pandemic that has a profound influence on public health. The World Health Organization (WHO) stated over 762 million validated etiology of COVID-19, involving 6.8 million deaths, as of May 2023 [WHO Coronavirus (COVID-19) Dashboard with Vaccination Data, n.d.]. To combat the virus, more than 13 billion vaccine doses have been administered [(1)].

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), responsible for Coronavirus disease 2019, hasn't only affected the respiratory system but also showed implications for cardiovascular health. Increasing proof recommends that COVID-related cardiovascular illness have important role in illness progression and case results [(2)].

The objective of this work was to examine the effect of active COVID-19 infection on right ventricular mechanics applying speckle tracking echocardiography.

PATIENTS AND METHODS

This was an observational (cross-sectional) investigation that was conducted in Agouza Specialized Hospital (which turned to isolation hospital after COVID – 19 pandemic) and Cardiology Department, Menoufia University Hospitals through the period from November 2021 to January 2023. The research was carried out on 50 admitted patients with confirmed COVID 19 infection.

Inclusion criteria: Both females and males above the age of eighteen years old with confirmed positive PCR swap for COVID -19 infection and with moderate to severe chest CT affection as mentioned in C.T severity score in the study.

Exclusion criteria: Patients with primary pulmonary diseases (e.g.: IPF, COPD, bronchial asthma, etc.). History or any evidence of myocardial ischemia. Valvular affection more than mild form. Congenital heart diseases both cyanotic and acyanotic. History of cardiomyopathy of any type. Poor echogenic window. Any type of witnessed arrhythmia. Pericardial diseases.

Methods:
Tools: All patients have been subjected to the following:

A. **Complete history taking:** Including age & detailed history of cardiovascular risk factors (Hypertension, Diabetes mellitus, Hyperlipidemia and Smoking).

B. Full clinical examination:
1-General examination involving Height, Weight, Body mass index [The body mass index (BMI) evaluates body weight relative to body height (Body weight in Kilograms/Body height in square meters) expressed in Kg/m^2. underweight when below 18.5, overweight when above and above 30 is considered obese]. Heart rate obtained by manual palpatory method and EKG. Blood pressure both systolic and diastolic: Hypertension is described as office SBP values not less than 140 millimeters of

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mercury and/or diastolic BP (DBP) values not less than 90 millimeters of mercury. Oxygen saturation obtained by pulse oximeter on room air.

2-Cardiac examination including inspection, palpation of cardiac areas, auscultation (S1 + S2) and added heart sounds if present. Murmurs if present. Presence of pericardial rub or not. Air entry of both lungs for crepitations and wheezes.

C. Routine laboratory investigations for COVID 19 severity affection including inflammatory markers as following CBC with lymphopenia, serum D-dimer, CRP, serum IL-6 and serum ferritin.

D. Routine 12-lead surface ECG for all admitted patients.

E. Confirmed positive PCR swab for COVID-19 virus for all admitted patients, which was nasopharyngeal swap by RT-PCR technique obtained by trained health care personnel and tested in ministry of health central labs and patient is enrolled in such study if positive.

F. Chest C.T. assessment: All patients did C.T chest after arrival to hospital (Toshiba Aquilion 64 slice) and the result was analyzed by 2 scores for batter assessment:

Typical findings for Coronavirus disease 2019:

- Ground glass opacification 88%
- Bilateral involvement 88%
- Posterior distribution 80%
- Multilobular involvement 79%
- Peripheral distribution 76%
- Consolidations 32%

Conventional echocardiography:

1- Routine transthoracic echocardiograph (TTE) was done using 2D imaging, color Doppler, M-Mode, PW and CW for all patients.
2- TTE examination was done while patient was supine on left lateral position connected with single lead ECG cable.
3- Three successive cardiac cycles were acquired for apical four-chamber view for obtaining RV longitudinal view.

♦ **2D analysis:**
All required conventional echocardiographic data were taken using basic parasternal, apical and subcostal views for:
1- Chamber size and function quantification.
2- Valvular morphology and function assessment.
3- Great vessels measurements and assessment.

♦ **Conventional M-mode:** for measurement of LV dimensions, fraction shortening and ejection fraction, **TAPSE** (tricuspid annular plane systolic excursion) obtained in apical four by putting M-Mode curser on lateral part of tricuspid valve annulus and **MAPSE** (mitral annular plane systolic excursion obtained also in apical four chamber view by putting M-Mode curser on lateral part of mitral valve annulus. An example of MAPSE and TAPSE measured in a patient using M-mode in apical four chamber view [Figure 1]

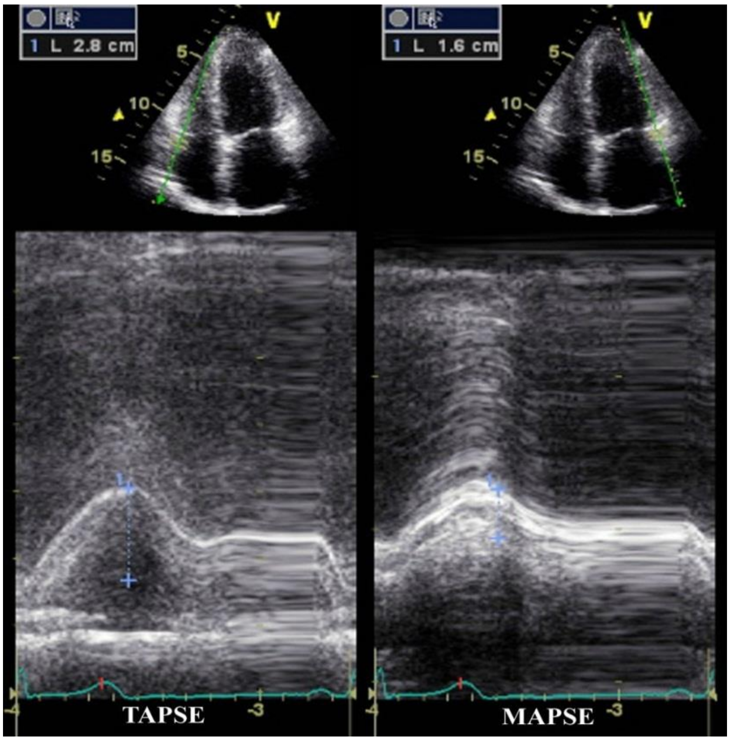

Figure (1): TAPSE and MAPSE.

- **Color CW and PW Doppler** PW Doppler for assessment of mitral valve flow and diastolic function also aortic valve flow and VTI for event timing and velocity, CW on tricuspid valve for assessment of PHT and presence of TR or PR. A sample of mitral inflow pattern in a patient with pseudonormal pattern (diastolic dysfunction grade II) with normal E/A ratio and reversed ratio with Valsalva maneuver [Figure 2].

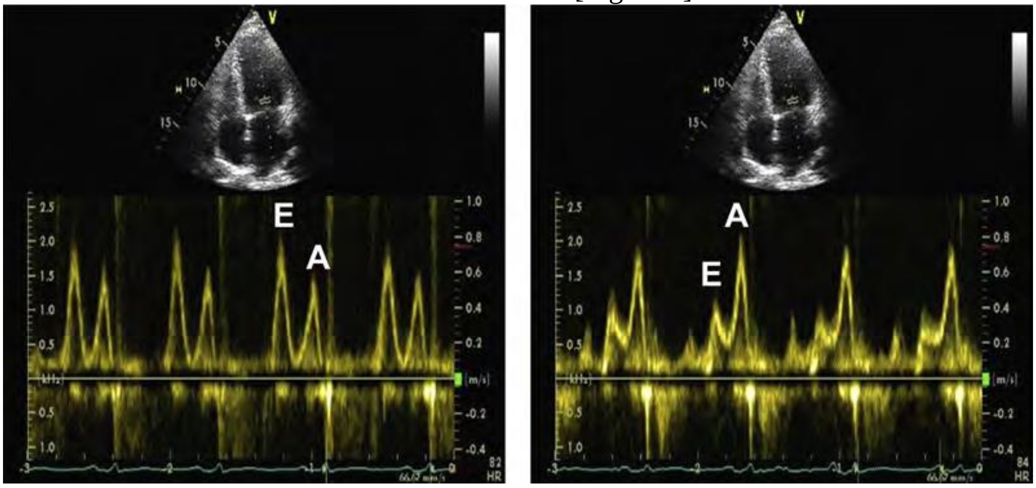

Figure (2): Color CW and PW Doppler.

- **Tissue Doppler imaging (TDI):** The procedure was carried out through activating the pulsed wave tissue Doppler function in the apical four-chamber view and locating a sample volume of five millimeters over the medial mitral annulus. 3 main myocardial velocities have been documented at the mitral annulus:
- The peak of major positive systolic velocity when the annulus moved towards the apex called **(S).**
- An early peak of major negative velocity throughout early diastolic filling when the annulus moved towards the base named **(e').**
- Another peak of negative velocity happening throughout late diastolic filling called (**a'**) velocity was measured.

A sample of TDI of a patient with normal diastolic function denoting S wave (peak of major positive systolic velocity), e' (early peak of major negative velocity) and a' (another peak of negative velocity) [Figure 3].

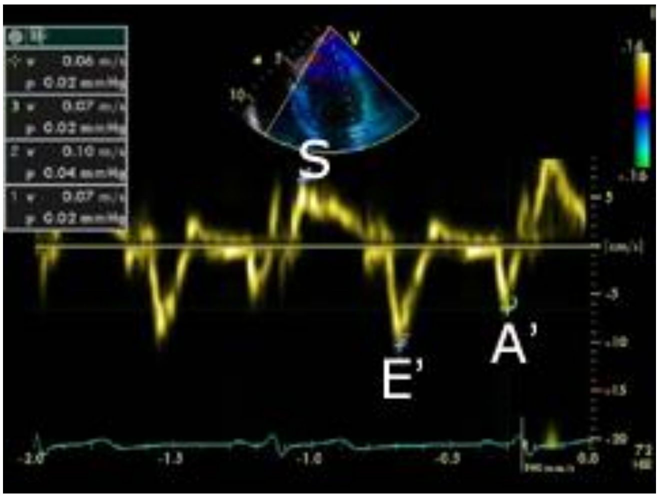

Figure (3): Tissue Doppler imaging.

G. Two dimensional speckle tracking analysis for right ventricle:

RV longitudinal strain: Longitudinal strain represents the shortening in systole (change in length) represented as a negative strain value of the myocardium along the long axis of the RV. The RV longitudinal strain was determined in apical four-chamber by estimating the longitudinal strain for IVS & right ventricular free wall [Figure 4].

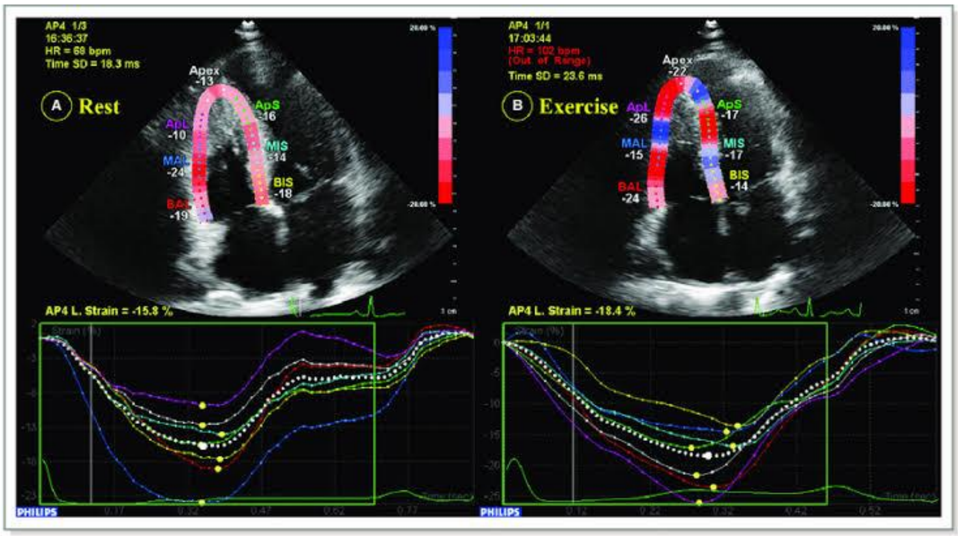

Figure (4): RV longitudinal strain determined in apical 4-chamber view.

Statistical analysis and data interpretation:
Information analysis has been carried out by SPSS software version 25 (SPSS Inc., PASW statistics for windows version 25, Chicago). Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) for non-normally distributed data and mean ± standard deviation for normally distributed data after testing normality using Kolmogorov-Smirnov test. Significance of the obtained results was judged at ≤ 0.05 level.

1- Fischer exact, Chi-Square test & Monte Carlo tests were utilized to compare qualitative information among groups as suitable
2- Mann Whitney U & Kruskal Wallis test were applied to compare among two examined groups and above two examined groups, correspondingly for non-normally distributed information.
3- Student t test was applied to compare two independent groups for normally distributed information.
4- One Way ANOVA test was utilized to compare over 2 independent groups with Post Hoc Tukey test to notice pair-wise comparison
5- The Spearman's rank-order association was applied to calculate the direction and strength of a linear correlation among 2 non-normally distributed continuous parameters.

Ethical Approval: The research was approved by The Ethics Committee of Cardiology Department, Faculty of Medicine, Menoufia University, Egypt. All participants gave written informed consents before enrolment. The research adhered to the Helsinki Declaration throughout its execution.

RESULTS

Table (1) demonstrated the comparison of conventional echocardiographic parameters between three chest CT score-groups. There were statistically significant variances in **FS** and **LVEF** between patients of group **A** (CT score 3) and patients of group **C** (CT score 5) with significantly lower **FS** and **LVEF** in group **C** and between patients of group **B** (CT score 4) and patients of group **C** (CT score 5) with significantly lower **FS** and **LVEF** in group **C**. Also, there was statistically significant difference in **TAPSE** between group **B** patients (CT score 4) and group **C** patients (CT score 5) with significantly lower **TAPSE** in group **C**. A statistically insignificant variances was observed among the groups regarding resting parameters.

Table (1): Conventional echocardiographic findings in CT score-based groups of the studied cases

	CT chest score			**Test of significance**	**within group significance**
	Group A (Score 3)	Group B (Score 4)	Group C (Score 5)		
	Mean				
LA	36.62±4.07	36.71±5.29	36.01±4.24	F=0.120 P=0.887	p1=0.960 p2=0.703 p3=0.655
Aorta	29.26±3.51	28.03±4.03	29.75±3.92	F=0.918 P=0.406	p1=0.370 p2=0.718 p3=0.192
LVEDD	51.31±4.77	48.85±4.59	48.83±6.56	F=1.08 P=0.347	p1=0.208 p2=0.198 p3=0.989
LVESD	32.77±4.08	29.98±4.11	32.77±6.61	F=1.65 P=0.203	p1=0.133 p2=0.997 p3=0.115
IVSD	9.5±1.78	9.6±1.83	9.4±1.91	KW=0.047 P=0.977	p1=0.835 p2=0.971 p3=0.868
LVPWD	9.5±1.78	9.6±1.83	9.4±1.91	KW=0.283 P=0.868	p1=1.0 p2=0.586 p3=0.715
FS	36.86±5.13	38.68±4.53	32.66±5.53	F=6.45 **P=0.003***	p1=0.317 **p2=0.02*** **p3=0.001***
LVEF	66.80±3.86	67.47±4.98	55.55±8.50	F=19.92 **P<0.001***	p1=0.763 **p2=0.001*** **p3=0.001***
E velocity	67.72±30.21	69.72±32.35	65.72±33.64	KW=3.01 P=0.222	p1=0.850 p2=0.068 p3=0.248
A velocity	61.87±15.41	61.59±16.12	65.81±19.62	F=0.325 P=0.724	p1=0.964 p2=0.517 p3=0.473
E/A	1.25±0.35	1.26±0.43	1.06±0.27	F=1.74 P=0.187	p1=0.941 p2=0.133 p3=0.103
e'	-8.72 (-13.37,-3.17)	-11.18 (-14.2,-3.17)	-7.63 (-13.24,-6.27)	KW=3.05 P=0.217	p1=0.344 p2=0.337 p3=0.102
E/e'	9 (5.07-19.61)	8.02 (4.52-17.03)	7.94 (4.5-15.88)	KW=0.671 P=0.715	p1=0.473 p2=0.480 p3=0.908
TR PG	23.6(13.98-31.85)	25.75(13.98-31.8	38.33(36.3-45.4)	KW=0.693 P=0.707	p1=0.820 p2=0.758 p3=0.345
Basal RVD	36.33±3.07	37.14±8.88	39.96±5.58	F=0.666 P=0.519	p1=0.296 p2=0.857 p3=0.362
TAPSE	1.89±0.38	1.99±0.37	1.66±0.29	F=4.13 **P=0.02***	p1=0.450 p2=0.06 **p3=0.008***

p1: variance among Group A & B, **p2:** variance among Group A & C, **p3:** variance among Group B & C, *Statistically significant, **F:** One Way ANOVA test, **MC:** Monte Carlo test, **KW:** Kruskal Wallis test

Table (2) demonstrated the comparison of RV Global Longitudinal Strain **(GLS)** parameters between three chest CT score groups. There was statistically significant difference in **right ventricular GLS** between group A patients (CT score 3) and group C patients (CT score 5) and between group B patients (CT score 4) and group C patients (CT score 5).

Table (2): RV strain echocardiographic findings in CT score-based groups of the studied cases

	CT chest score-based groups			Test of significance	within group significance
	Group A (Score 3) Number=15	Group B (Score 4) Number =17	Group C (Score 5) Number =18		
	Mean				
GLS	-16.47±2.17	-15.53±1.62	-12.82±2.63	F=17.08 **P<.001***	p1=0.233 **p2<0.001*** **p3<0.001***

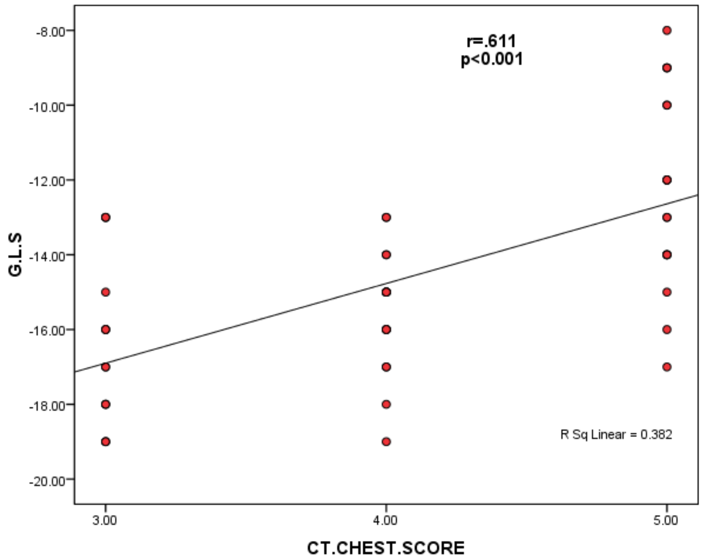

Figure (1): Scatter diagram illustrating association among CT chest score and GLS.

Table (3) compared patients with hypertension vs patients without hypertension and a statistically insignificant variance has been found among studied cases according to conventional risk factors.

Table (3): Relation between echocardiographic findings and presence of hypertension among studied cases

	No Hypertension N=18	**Hypertension N=28**	**Test of significance**
LA (mm)	35.20±4.19	37.25±4.58	t=1.59, P=0.117
Aorta (mm)	27.99±3.01	29.69±4.21	t=1.56, P=0.125
LVEDD (mm)	48.23±4.68	50.47±5.80	t=1.44, P=0.156
LVESD (mm)	30.19±4.10	32.91±5.65	t=1.84, P=0.072
IVSD (mm)	9.1(7.5-11)	9.7(1.1-86)	z=1.69, P=0.091
LVPWD (mm)	9.15(7.5-11)	9.8(1.1-90)	z=1.24, P=0.214
FS (%)	36.95±4.95	36.31±6.03	t=1.01, P=0.318
LVEF (%)	64.45±6.84	62±9.15	t=1.02, P=0.312
E velocity (cm/s)	72.14(40.64-124.37)	68.18(34.46-124.37)	z=0.376, P=0.707
A velocity (cm/s)	58.51±16.19	66.32±17.13	t=1.61, P=0.113
E/A	1.30±0.39	1.12±0.33	t=1.82, P=0.076
e' (cm/s)	-10.02(-14.2, -3.17)	-8.32(-13.89 , -3.17)	z=1.56, P=0.120
E/e'	7.66(4.57-17.03)	8.99(4.5-19.61)	z=1.27, P=0.205
TR PG (cm/s)	22.49(13.98-37)	32.99(23.98-45.4)	z=0.129, P=0.897
TAPSE (cm)	1.89±0.39	1.81±0.36	t=0.708, P=0.482
Basal RVD (cm)	35.49±3.66	35.44±8.95	t=0.029, P=0.977
Z: Mann Whitney U test, **t:** Student t test			

Regarding LV strain echocardiographic findings, there was statistically significant variance in both left ventricular GLS and Global Circumferential Strain (GCS) in cases that had hypertension against cases without hypertension with significantly less negative strain parameters in cases that had hypertension against cases without hypertension (Table 4).

Table (4): Relation among GLS & GCS findings and presence of hypertension among studied cases

	No hypertension N=18	**hypertensive N=28**	**Test of significance**
GLS (mean)	-15.35±2.70	-14.16±2.84	t=1.47 **P=0.148***
t: Student t test, **χ2:** Chi-Square test, ***:** Statistical significance			

In table (5), we compared COVID 19 cases with diabetes vs COVID 19 cases without diabetes and a statistically non-significant variance has been detected among studied cases according to conventional echocardiography parameters.

Table (5): Relation between echocardiographic findings and presence of diabetes among studied cases

	Non-diabetic N=11	**diabetic N=39**	**Test of significance**
LA (mm)	36.52±4.43	36.40±4.57	t=0.075 P=0.940
Aorta (mm)	29.01±4.13	29.02±3.80	t=0.009 P=0.993
LVEDD (mm)	30.56±4.52	32.18±5.40	t=0.109 P=0.914
LVESD (mm)	66.27±7.04	62.05±8.48	t=0.906 P=0.369
IVSD (mm)	9.5(1.1-12)	9.5(1.2-86)	z=0.365 P=0.715
LVPWD (mm)	9.5(1.1-12)	9.5.(1.2-90)	z=0.106 P=0.916
FS (%)	61.41±15.63	63.69±17.57	t=1.88 P=0.07
LVEF (%)	66.27±7.04	62.05±8.49	t=1.51 P=0.139
E velocity (cm/s)	74.6(53.99-107.7)	58.53(34.46-124.37)	z=1.32 P=0.186
A velocity (cm/s)	61.41±15.64	63.69±17.57	t=0.389 P=0.699
E/A	1.37±0.34	1.14±0.354	t=1.94 P=0.06
e' (cm/s)	-11.8(-14.2, -3.17)	-8.3(-13.89, -3.17)	z=1.64 P=0.101
E/e'	7.38(5.07-19.61)	8.95(4.5-17.03)	z=0.375 P=0.708
TR PG (cm/s)	24.98(13.98-37)	32.33(23.98-45.4)	z=0.352 P=0.725
Basal RVD (cm)	36.57±3.81	35.14±8.27	t=0.719 P=0.476
TAPSE (cm)	2.04±0.357	1.79±0.35	t=2.03 P=0.048*
Z: Mann Whitney U test			

Regarding LV strain echocardiographic findings, there was **statistically significant** variance in both left ventricular GLS and GCS in Coronavirus disease 2019 cases with diabetes vs Coronavirus disease 2019 patients without diabetes (Table 6).

Table (6): Relation between GLS & GCS findings in COVID 19 patients and presence of diabetes among studied cases

	Non-diabetic N=11	Diabetic N=39	Test of significance
GLS (mean)	-16.29±2.73	-13.78±2.49	t=3.26 **P=0.002***

t: Student t test, **χ2:** Chi-Square test, ***Statistically significant**

DISCUSSION

Significant differences were observed in fractional shortening (FS), RV GLS, and tricuspid annular plane systolic excursion (TAPSE) across different computed tomography (CT) severity stratified groups. Furthermore, there was an independent correlation between RV GLS and CT severity score, as well as between RV GCS and CT severity score, as shown in scatter diagrams.

Regarding RV GLS, we observed that 30% of patients exhibited deteriorated RV-GLS, with higher percentages found in more severe CT-score-based groups (13.3% in group A, 17.6% in group B, and 55.6% in group C) (P-value equal 0.004). Similar prevalence of abnormal RV GLS has been reported in previous studies. For instance, **Azeri *et al*.** [(3)] performed a study including 74 patients had active COVID-19 and found abnormal RV-GLS values (> -18) in 37.8% of patients [(3)]**.** These results emphasize the importance of evaluating right ventricular function in cases had COVID-19, as it can provide valuable insights into disease severity and prognosis. Additional investigation is required to fully understand the influence of COVID-19 on the right side of the heart and develop appropriate management strategies for patients with cardiovascular involvement. The WASE COVID-19 research, performed on 870 cases with active COVID-19 infection from various regions, including Asia, Europe, the United States and Latin America, shed light on the association between right-sided cardiac markers and disease severity [(4)]**.**

The study carried out central, blinded analyses of echocardiograms, including measurements of left ventricular ejection fraction (LVEF) and strain of the two ventricles. The results revealed that markers of both left-sided (LV GLS) and right-sided (RVFWS) function were independently associated with disease severity. Notably, the study found that LVEF didn't illustrate an independent correlation with illness degree, which differs from recently published findings by **Faridi *et al*.** [(5)]**.** Conversely, LV GLS demonstrated an independent association, emphasizing the importance of advanced echocardiographic assessment for a comprehensive risk evaluation in COVID-19 cases. Echocardiography plays a significant role in diagnosing and guiding clinicians towards specific diagnoses in COVID-19 patients, in addition to its association with disease severity and myocardial injury.

Other studies have also reported a strong association and a higher prevalence of abnormal echocardiographic parameters, particularly GLS, in COVID-19 patients. For instance, a study involved 218 COVID-19 patients and found a very high prevalence (80%) of abnormal RV GLS in the study population [(6)]**.** The wide range of association of strain parameters with COVID-19 infection may be attributed to different patient populations and varying severities of COVID-19 across studies. Nevertheless, these studies collectively suggest that abnormal RV GLS is indicator of increased cardiovascular risk and might be linked to worse clinical results in COVID-19 cases. The diminution in RV GLS found in COVID-19 patients could be influenced by several factors. Multiple mechanisms have been proposed to explain right ventricular involvement and myocardial injury in Coronavirus disease 2019 infection. These mechanisms include direct injury caused by myocarditis due to the binding of the SARS-CoV-2 spike protein to the ACE-2 receptor and indirect injury resulting from the cytokine inflammatory storm triggered via the immune response of the body [(7)]**.** Understanding the influence of COVID-19 on the right side of the heart is crucial for risk stratification and patient management. Advanced echocardiographic assessment including strain measurements, provides valuable insights into cardiac function and helps identify individuals at higher cardiovascular risk.

Our findings provided new light on, right ventricular free wall longitudinal strain (RVFWLS) alterations in cases had confirmed COVID-19 infection. The average RVFWLS and GLS of COVID-19 cases were found to be lower than that of a healthy control group, this was the case, indicating the existence of hidden myocardial damage. Even with maintained RVFWLS, imaging techniques for identification of cardiac damage in the acute environment. Even with this improvement, there was a tendency towards predicting in-hospital mortality or the requirement for mechanical ventilation when RVFWLS decline was moderate to severe. During admission, individuals who died had considerably greater levels of inflammatory markers including D-dimer than those who survived.

Our results showed a statistically significant association (p=0.008) between TAPSE and severity according to CT chest scores, which is generally consistent with those of current investigation in intensive care unit cases with COVID-19. The major breakthrough that abnormal RVFWLS was independently linked with severity risk and was discovered in this prospective investigation of RV STE in cases had COVID-19 needing IMV the association between right ventricular (RV) free wall longitudinal strain and severity among individuals with COVID-19.

Viral infiltration of the myocardium may result in cardiomyocyte inflammation and death, affecting both the left and right sides of the heart. This mechanism of direct myocardial injury is supported by multiple researches, involving a current study by **Churchill *et al.*** **[8]**, which found that more than half of the cases with elevated high-sensitivity troponin T (≥ 50 ng/mL) had evidence of left ventricular dysfunction**.** In addition to direct myocardial injury, myocardial involvement in COVID-19 may also be influenced by other factors such as hypoxia, proinflammatory cytokines and microvascular dysfunction **[9].** These mechanisms may result in secondary myocardial injury and affect both left and right ventricular function.

Regarding inflammatory markers, studies have shown a significant elevation in serum inflammatory cytokines and markers, including serum ferritin, IL-6, D-dimer and LDH in COVID-19 cases. These indicators are closely related to a diminution in right ventricular strain variables like RV GLS and RVFWS by **Bruhl *et al*.** **[10]**. Elevated inflammatory markers have been strongly associated with disease severity and may contribute to myocardial affection. A study by **Cameli *et al*.** **[11]** demonstrated a significant correlation among elevated inflammatory markers and disease severity in a study of 400 cases. Cardiovascular risk factors like hypertension and diabetes can also impact the right side of the heart. These conditions can affect the subendocardial fibers leading to left ventricular fibrotic processes and myocardial injury. Such myocardial injury is expected to have an impact on right ventricular strain parameters **[12].** Several studies have shown a significant reduction in right ventricular strain, including RV GLS, among patients with hypertension **[13].** Similarly, **Ghoreyshi-Hefzabad *et al.*** **[14]** demonstrated that patients with diabetes have been found to have impaired left ventricular strain parameters.

CONCLUSION

We have assessed the subclinical impairment of right ventricular function with 2D STE in cases infected with COVID-19 in correlation with the degree of pneumonia. Our study suggests that assessment of right ventricular function with 2D STE in COVID-19 infected patient as compared to conventional 2D echo variables especially TAPSE, was more reliable and earlier in detection of any dysfunction. Therefore, we propose the utilization of strain variables in addition to conventional variables in order to predict the prognosis and result of cases having a COVID-19 infection regarding RV function.

DECLARATIONS

Consent for publication: I certify that each author has granted permission for the work to be submitted.

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Availability of data & material: Available.

Conflicts of interest: None.

Competing interests: None.

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