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Evaluation of Right Ventricular Performance in Ischemic Patients with or without Significant Right Coronary Artery Disease

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ABSTRACT

Background: Optimal cardiac performance relies on integrity between left ventricle (LV) and right ventricle (RV) owing to variety of factors as shared coronary circulation. RV systolic dysfunction identified as a poor prognostic factor. **Objectives:** We aimed at assessing the impact of LV ischemia on RV performance in occurrence or absence of right coronary artery (RCA) disease. **Subjects and methods:** We enrolled 40 patients with proven LV ischemia. All patients were subjected to myocardial perfusion imaging and coronary angiography. LV and RV functional parameters were assessed in all individuals using conventional echocardiography, tissue Doppler imaging (TDI), two-dimensional speckle tracking echocardiography (2D-STE) and 4-dimensional echocardiography (4DE).

Results: We divided our patients based on presence or absence of angiographic significant RCA disease into two groups. Group I with RCA involvement (22 patients) and Group II without significant RCA involvement (18 patients). Group I was older, predominantly men and more hypertensive (HTN) compared to Group II, however it did not reach statistical significance. Our study demonstrated reduced RV performance in both groups however, it didn't reach the statistical significance.

Conclusion: RV performance was impaired in ischemic heart disease (IHD) but not significantly affected by the presence or absence of RCA affection, denoting that the right ventricular performance is directly interdependent on the left ventricular function in ischemic heart disease.

Keywords: Left ventricle, Right ventricle performance, Ischemia, Echocardiography, Myocardial perfusion imaging.

INTRODUCTION

For a very long time, the RV was viewed as the forgotten or neglected chamber of the heart since it was thought to be less important in cardiac disorders than its left counterpart. Therefore, the significance of the RV in the treatment and prognosis of numerous cardiac disorders is being acknowledged more and more **(1).**

Common coronary arterial blood supply among other factors closely linking the RV and LV performance. In extreme cases of myocardial infarction with LV injury, RV dysfunction develop, where LV infarction pattern affects the risk for RV insufficiency **(2).**

Extending LV ischemia may have an effect on RV function both directly by changing RV perfusions and indirectly by raising RV afterload. The RV and LV are very different from each other anatomically and physiologically. Once RCA occlusion happens, the RV becomes less susceptible to ischemia and less likely to sustain myocardial damage than when left coronary artery (LCA) obstruction happens due to the amount of LV dysfunction **(3).**

Because proximal RCA blockage is typically the responsible for acute RV infarction (RVI), inferior MI is frequently linked with RVI **(4)**.

The RV free wall, which is largely in charge of overall RV efficiency, is fueled by the RCA's RV branches. As some cases with proximal RCA occlusions show minor acute RV abnormalities, the location of RCA occlusions could be distal or proximal with different degrees of RV dysfunctions unrelated to the site of affection. RV dysfunctions may vary in severity from modest to severe RV performance depression **(5).**

The cost of echocardiography is lower than that of other methods used to assess RV function and it is readily accessible. It is a helpful instrument for a thorough assessment of RV dimensions and functions. Echocardiography evaluation of the RV is difficult because of its complicated structure, location, and anatomy. New echocardiography techniques, such 4DE and STE myocardial deformation imaging, offer prospective solutions to most of the problems with traditional echocardiography **(1).**

METHODOLOGY

Study population

Our study was a prospective observational study that enrolled 40 consecutive patients diagnosed as IHD who were candidate for myocardial perfusion imaging (MPI) and elective coronary angiography (CA) plus or minus intervention. The study was conducted through the period from November 2018 to November 2019.

Ethical consent: This study was ethically approved by Al-Azhar University's Research Ethics

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Committee (2022071408, 06/07/2022). Written informed consents from all the participants were obtained. The study protocol conformed to the Helsinki Declaration, the ethical norm of the World Medical Association for human testing.

Inclusion criteria: Our study included patients with chronic IHD and patients who had previous (old) myocardial infarction.

Exclusion Criteria:

1) Arrhythmia like atrial fibrillation and any other arrhythmias that interfere with gated Single Photon Emission Topography (SPECT) results, or valvular and congenital heart disease.
2) History of chronic obstructive airway disease, other pulmonary disease or systemic disease that may affect RV function.
3) Patients with poor echogenic windows.

Primary end point: Evaluation of the RV performance in ischemic patients with or without significant dominant RCA disease.

Secondary end point: Correlation of the right ventricular performance in the two groups evaluated by different imaging modalities as: end diastolic volume (EDV), ejection fraction (EF) and SWMA score by echo and MPI.

All cases were subjected to the following: Detailed history taking including risk factors of IHD (HTN, diabetes, smoking, dyslipidemia and FH of premature CAD), symptoms as chest pain and dyspnea. Thorough clinical examination was done including HR, BP, chest and cardiac examination in addition to resting twelve-lead surface ECG.

Echocardiographic study

An ultrasonography device called the Vivid E9 (GE Ultrasound, Herten, Norway) was used to do two-dimensional echocardiogram (2DE). Instances were studied using a multi frequency (2.5MHz) matrix probe (M3S).

At EchoPAC.GE version 201, all photos were electronically archived for future off-line processing. With ECG physio-signal presentation and looping recordings of 2-3 cycles, comprehensive TTE M-Mode, 2D, Doppler (pulsed and continual waves), colour flow mapping in the standard views across all available screens were acquired. Measurements of LV measurements and functionalities were made using all variables in accordance with American Society of Echocardiography norms [(6)].

RV inflow dimensions were calculated using the 4th chamber's apical perspective. Apical four chambers fractional areas changes (FAC), computed as [(end diastolic area) - (end-systolic area)/end-diastolic area)] x 100, and RV traditional myocardial performance index (RV-MPI) were used to produce right ventricular functioning measurements. Pulsed-wave Doppler measurements were made of the tricuspids flows velocities in the apical four-chamber viewpoint. The ensuing parameters were established: the ratio (E/A) TV between early diastolic maximum flow velocity (ETV) and later diastolic flow velocity (ATV) [(7)].

A sample size was put at the lateral segment of the tricuspid annulus to get the peak tricuspid annular systolic velocity (SRV), early diastolic velocities (ERV), and late diastolic velocities using TDI to measure RV myocardial velocity in the apical 4 chambered view (ARV). In order to evaluate RV-GLS, two dimensional RV strain scanning was carried out utilising three successive cardiac cycles of two dimensional images [(7)].

4D echocardiographic imaging of the right ventricle

Employing the same device and 4V transducers, six-beat full-volume 4D data sets (about 30 vol/s) were collected throughout breath-hold. Throughout collection, the 12-slice screen was applied to make sure that the RV was fully included in the data set [(8)]. The sets of data were then imported into the 4DAutoLVQ software program for evaluation.

The RV longitudinal axes in the standard end-diastolic frames were used to align the 4D data set. We defined the markers matching to the aorta annular diameters on the LV apical long-axis image, and the posterior and anterior connections of the RV free walls with the interventricular septum as well as the spacing between the septum and RV free wall on the RV short-axis view. A static RV shape modelling was adjusted to fit all the inputs by the software method, which also evaluates ultrasonic backscattering intensities. If necessary, the modeling was then manually modified. Using speckle-tracking technique, the RV contours were automatically followed throughout the full cardiac cycling, and automatic measures of the RV volumes, FAC, and EF were given.

Myocardial perfusion SPECT studies:

Stress protocol:

In accordance with normal Bruce procedure, treadmill exercising stress was administered to a total of 40 individuals in a row. Assessment was symptom-limited except when it was interrupted early for reasons advised in the revised exercise testing standards [(9)].

Myocardial Perfusion Imaging (MPI):

In accordance with a two-day procedure, a gated-SPECT MPI was performed for each patient. Using a dual-head (Philips) camera with low-energy,

high-resolution collimators, supine pictures were collected. The normal methods were followed in the processing of each radionuclides imaging and the related data. In all instances, 99mTc sestaMIBI was utilised (the routine tracer used in our laboratory). Based on the usual paradigm, myocardial perfusions were determined as the relative percent tracers' absorption in each of the 17 segments.

Experts in nuclear cardiology interpreted each MPI scan using both quantitative and qualitative analysis of the results. Every segment received a score from 0 to 4, with 0 representing ordinary uptake, 1 representing mild loss in uptake, 2 representing considerable reduction in uptake, and 4 representing absence of uptake. Additional high-risk perfusion scanning indicators, such as elevated lungs heart ratio (LHR), transient ischemic dilatation (TID), and aberrant regional and global wall movement anomalies, were described independently. Individuals with negative SPECT scans who also had TID and stress-induced stunning, two non-perfusion anomalies, were not included in the research.

Coronary angiography:

Depending on the American College of Cardiology/Society for Cardiac Angiography and Interventions clinically expert panel paper on cardiac catheterization laboratories guidelines, coronary angiography with or without interventions was carried out [(10)]**.** With the use of six French sheaths and diagnostics catheters, almost always Judkins catheters, all catheterizations were carried out beyond femoral artery accessibility. Participants were brought to the recovery room, in which the sheath was taken off and manual compression was used to achieve hemostasis. These participants spent 6 to 8 hours in bed rest. Interventional cardiologists with extensive training evaluated the angiograms. Over than 50% of the lesions were classified as substantial. Using the vessels that offer ascent to the posterior descending artery (PDA), one can determine the dominance of the coronary arteries.

Statistical analysis

Software SPSS for Windows (version 25; IBM SPSS Inc., Armonk, NY, USA) was used to assess the findings. Whereas categorical data were reported as percentages and frequencies for categorical variables, while continuously data were expressed as means and standard deviations (SD). The Shapiro testing was used to verify normality. Utilizing the unpaired Student's T-test and the Chi square for nominal data, a single-variable examination for group comparisons was carried out. The Pearson r correlation analysis was used to evaluate the relationships between the variables. $P \leq 0.001$ was regarded as very significant, while $P \leq 0.05$ was considered as statistically significant.

RESULTS

Demographic and clinical characteristics of the studied cases are shown in table (1). Patients were divided according to angiographic evidence of significant RCA involvement into Group I that included 22 patients with RCA involvement and 18 patients with no significant RCA atherosclerotic disease entitled Group II. Table (1) showed that group I patients where male gender was predominant, older age and higher incidence of HTN, diabetes and dyslipidemia as compared to group II, however it didn't reach statistical significance.

Table (1): Baseline anthropometric and clinical traits of the research population

Characteristics	Group I (n=22)	Group II (n=18)	P value
Age in years	54.73±7.45	50.50±7.91	0.09
Male gender	17 (77.27%)	11 (61.11%)	0.27
Hypertension	13 (59.01%)	8 (44.44%)	0.36
Diabetes	17 (77.27%)	13 (72.22%)	0.72
Dyslipidemia	17 (77.27%)	9 (50.00%)	0.08
Smoking	6 (27.27%)	6 (33.33%)	0.68
Family history of premature CAD	6 (27.27%)	4 (18.18%)	0.50
Chest pain	18 (81.82%)	15 (83.33%)	0.90
Dyspnea	5 (22.72%)	2 (11.11%)	0.34
HR (beat/min)	83.68±12.18	85.78±8.06	0.56
Systolic BP, mmHg	127.73±14.12	124.72±13.56	0.50
Diastolic BP, mmHg	82.27±8.13	81.67±7.86	0.81

Table (2) displayed the angiographic findings where 4 patients of group I had posterior descending artery (PDA) stenotic lesion (18.18%) and 3 patients had posterolateral (PL) stenotic lesion (13.64%). 15 patients had stenotic lesion proximal to RV branch (68.18%) while 9 patients had stenotic lesion distal to RV branch (40.91%). Group I patients had lower diagonal affection (p=0.03), higher left circumflex artery (LCX) affection (p=0.02) and higher SYNTAX score (p=0.01) compared to group II.

Table (2): Angiographic findings of the studied groups

	Group I (n=22)	Group II (n=18)	p-value
LM	6 (27.27%)	2 (11.11%)	0.20
LAD	14 (63.64%)	13 (72.22%)	0.57
D	3 (13.64%)	8 (44.44%)	0.03
LCX	13 (59.09%)	4 (22.22%)	0.02
OM	5 (22.73%)	3 (16.67%)	0.64
RCA	18	-	
PDA	4 (18.18%)	-	
PL	3 (13.64%)	-	
RCA lesion proximal to RV branch	15 (68.18%)	-	
RCA lesion distal to RV branch	9 (40.91%)	-	
SYNTAX score	18.48±10.54	9.86±8.16	0.01

Table (3) illustrated conventional echocardiographic parameters of RV dimensions and function. RV dysfunction was present in 22 patients (55%) of the whole studied population divided into 13 patients of group I (59.1%) and 9 patients of group II (50%). We didn`t find any significant difference between both groups apart from larger RV-ESA in Group I compared to Group II (p=0.03).

Table (3): shows conventional echocardiographic parameters of RV dimensions and function

Variable	Group I (n=22)	Group II (n=18)	P value
2D-LVEF, %	56.09±15.64	57.61±13.59	0.74
RV D1, mm	33.82±8.11	32.94±6.84	0.71
RV D2, mm	30.05±9.08	30.22±7.30	0.95
RV D3, mm	58.91±11.22	53.83±6.20	0.08
RV EDA, mm2	15.77±4.92	14.05±4.60	0.26
RV ESA, mm2	10.71±4.09	8.36±2.23	0.03
2D-RV FAC, %	32.31±11.43	33.41±11.55	0.76
2D-TAPSE, mm	19.55±3.28	19.87±5.68	0.83
2D-RV EDV, ml	33.59±16.47	30.33±18.04	0.56
2D-RV ESV, ml	19.27±12.53	14.44±6.91	0.13
2D-RVEF, %	44.86±13.83	45.89±13.34	0.81
Septal Sa, mm/s	5.18±1.4	5.83±1.28	0.12
Free Sa, mm/s	8.40±1.98	9.16±1.98	0.23
Avg RV Sa, mm/s	6.78±1.35	7.49±1.41	0.11
TE (cm/s)	0.53±0.14	0.49±0.14	0.43
TA (cm/s)	0.62±0.30	0.60±0.19	0.81
TE/A	0.96±0.31	0.91±0.42	0.72
PW-RV MPI	0.44±0.30	0.41±0.26	0.79
Septal Ea, mm/s	5.25±1.59	5.91±1.93	0.25
Free Ea, mm/s	7.05±2.08	7.50±2.46	0.53
Average Ea, mm/s	6.15±1.42	6.70±1.97	0.32
RV E/Ea	8.77±6.12	7.97±3. 23	0.60
Average Aa, mm/s	8.27±2.11	8.43±1.87	0.80

Abbreviations: EDA, end-diastolic area; ESA, end-systolic area; D1, basal diameter; D2, mid right ventricular diameter; D3, longitudinal diameter; E vel, early diastolic velocity; A vel, late diastolic or atrial velocity; TDI, tissue Doppler imaging; E/Ea, early diastolic velocity measured by pulsed Doppler/myocardial early diastolic excursion velocity measured by tissue Doppler

echocardiography; FAC, fractional area change; EDV, end-diastolic volume; ESV, end-systolic volume; TAPSE, tricuspid annular plane systolic excursion; Sa, myocardial systolic excursion velocity; MPI, myocardial performance index; TV, tricuspid valve; Aa, myocardial late diastolic or atrial excursion velocity; .* indicate that $p \leq 0.05$.

Regarding RV strain whether measured by TDI or STE, we didn`t find any significant difference between both groups. Table (4) presents the RV longitudinal strain parameters of the studied groups.

Table (4): RV longitudinal strain parameters of the studied groups

Variable	GI (n=22)	G II (n=18)	P value
TD-derived peak systolic strain			
Septal	-16.38±6.95	-16.91±6.73	0.81
Free wall	-25.02±10.04	-24.82±11.22	0.95
GLS	-20.70±7.19	-20.87±7.70	0.94
2D-longitudinal strain			
Septum, apical	-8.18±2.89	-11.17±6.37	0.08
Mid	-11.23±4.10	-12.22±5.79	0.54
Basal	-12.68±4.45	-13.89±6.04	0.49
Septal strain	-10.70±3.2	12.43±5.36	0.24
Free wall, apical	-10.32±5.2	-13.22±7.65	0.18
Mid	-14.45±8.73	-15.39±9.29	0.75
Basal	-15.91±8.71	-16.56±10.74	0.84
Free wall strain	-13.56±6.58	-15.06±8.11	0.53
2D-RVGLS	-11.84±5.28	-14.36±6.09	0.18
2D-LVGLS	-14.85±3.61	-14.57±3.48	0.82

4D-echocardiographic study

We didn`t find significant difference between both groups as summarized in table (5).

Table (5): 4D RV echocardiographic parameters

	G I (n=22)	G II (n=18)	P value
4D-EDV, ml	74.05±27.77	79.50±35.81	0.60
4D-ESV, ml	43.36±19.2	46.78±27.61	0.66
4D-SV, ml	30.68±15.62	32.67±13.05	0.66
4D-RVEF, %	38.59±10.32	41.04±11.75	0.49
4D-TAPSE, mm	11.95±3.85	12.28±3.34	0.78
4D-FAC, %	33.43±14.55	34.95±10.37	0.71

We found that 2D-RV septal strain was positively correlated with 2D-FAC, 2D-RVEF, average RV Sa, TD-RV GLS, 4D-RVEF, 4D-TAPSE, 4D-FAC and RV E/Ea while negatively correlated with PW-RV MPI as shown in table (6).

Table (6): Correlation between 2D-RV septal strain and different echocardiographic parameters of the right ventricle

Variable	2D-RV septal strain	P value
2D-FAC	0.647	<0.001
2D-RVEF	0.365	0.001
Average Sa	0.422	<0.001
TD-RV GLS	0.382	<0.001
4D-RVEF	0.361	0.001
4D-TAPSE	0.525	<0.001
4D-FAC	0.344	0.002
E/Ea	0.471	<0.001
PW-RV MPI	-0.361	0.001

Stress myocardial perfusion imaging study

No significant difference was found between G I and G II regarding ECG abnormalities during rest, METS, RV uptake, LHR or EID as shown in table (7).

Table (7): Stress myocardial perfusion imaging data

Variable	G I (n=22)	G II (n=18)	P value
ECG abnormalities at rest	15 (68.18%)	8 (44.44%)	0.54
METs	5.16±1.57	5.46±1.61	0.57
Inferior defect size, No	2 (9.09%)	4 (22.22%)	0.30
small	2 (9.09%)	1 (5.56%)	0.68
moderate	6 (27.27%)	2 (11.11%)	0.21
large	12 (54.55%)	11 (61.11%)	0.68
Reversibility			
Reversible	6 (27.27%)	2 (11.11%)	0.21
Partially reversible	12 (54.55%)	11 (61.11%)	0.68
Scar	2 (9.09%)	1 (5.56%)	0.68
Anterior, AL, AS, apical size			
No	14 (63.64%)	9 (50.00%)	0.39
Small	0	0	
Moderate	1 (4.55%)	1 (5.56%)	0.89
Large	7 (31.82%)	8 (44.44%)	0.42
Reversibility			
Reversible	6 (27.27%)	5 (27.78%)	0.97
Partially reversible	2 (9.09%)	4 (22.22%)	0.25
Scar	-	-	
Increased RV uptake	2 (9.09%)	1 (5.56%)	0.68
Lung Heart Ratio	0.39±0.07	0.39±0.06	0.83
Exercise-induced LV dilatation	8 (36.36%)	6 (33.33%)	0.84

We found a positive correlation between METs and 2D-RV GLS (r=0.310, p=0.05), while there was negative correlation between LHR and 4D-RVEF (r=-0.366, p=0.02). Also we found that impaired RV systolic function measured by 2D-strain was associated with presence of IHD risk factors as diabetes (r=240, p<0.001), HTN (r=77.42, p=0.03), dyslipidemia (r=80, p=0.02) and smoking (r=233.03, p<0.001). It was associated with large perfusion defect in MPI study (r=480, p<0.001), irreversibility of the perfusion defect (r=391, p<0.001), increased RV uptake (r=260, p=0.03) and EID (r=160, p=0.001). We found moderate positive correlation between 2D-LVGLS and TD-RVSS (p<0.001), 2D-

RVSS (p<0.001), 2D FRVS (p<0.001), 2D-RVGLS (p<0.001) and 4D-TAPSE (p<0.001), while there was weak positive correlation between 2D-LVGLS and TD-RVGLS (p=0.001), 4D-RVEF (p<0.001) and 4D-FAC (p=0.03) as shown in table (8)

Table (8): Correlation between 2D-LVGLS and different echocardiographic parameters of the right ventricle

Variable	2D-LVGLS	P value
TD-RVSS	0.622	<0.001
TD-RVS	0.363	0.001
2D-RV SS	0.646	<0.001
2D-RVFS	0.501	< 0.001
2D-RVGLS	0.626	<0.001
4D-RVEF	0.408	<0.001
4D-TAPSE	0.605	<0.001
4D-FAC	0.255	0.03

DISCUSSION

In our study, the mean age of our population was 52.83 ± 7.86 years which is much younger than the reported age for the development of IHD in other study conducted in England (64.5±12.7 years) [(11)]**.** Another study conducted in Italy evaluating RV dysfunction in RCA infarction reported the mean age of 65±12 years. This could be explained by the high-risk profile of Egyptian patients which promotes the development of coronary heart disease in earlier age [(12)]**.**

In group I we found that 68.18% of them had the lesion proximal to RV branch, in contrary to the Italian group who reported that most of the RCA involvement was distal (64% of all cases). We found that 55% of our patients had RV systolic dysfunction, however the location of RCA stenotic lesion didn`t affect the RV function in contrary **Santangelo *et al.*** [(14)] who reported that most of the patients with RV systolic dysfunction had a proximal significant lesion. It is worth to mention that we are reporting our data in non-acute setting and the Italian group data are in the setting of acute RCA infarction.

Kim *et al.* [(2)] studied the effect of RV insufficiency on physical functioning in individuals who are under exercise stresses, regardless of LV function. According to MPI and their findings, individuals with CAD risk factors had an increased incidence of RV insufficiency, which rose to 63% among those with established CAD**.**

Regarding MPI study results, we found no statistical significance between both groups in the inferior defect size or non-RCA related areas. In contrary, **Kim *et al.*** [(2)] reported that RV insufficiency was more common in individuals with inferior and lateral wall ischemia, indicating that regional LV perfusion had an effect on this condition's incidence, whereas corresponding anterior wall deficits were similar but they classified patients according to the degree of RV systolic function affection not RCA involvement as we did**.** Also, presence of inferior wall affection in group II may be explained by the presence of small CAD.

Strain is becoming a popular tool for assessment of LV function and is becoming increasingly utilized tool for assessment of RV function, we didn't find any significant difference between both groups regarding the RVGLS although RVGLS was lower in group I. We found that the 2D-RVGLS was positively correlated with 2D-FAC, 2D-RVEF, 4D-RVEF, 4D-TAPSE and 4DFAC, however most of the data in literature are comparing echo parameters with those derived from CMR and there are limited data directly comparing conventional echo methods against 4D and different strain techniques for the RV [(13)]**.**

We found a positive correlation between METs and 2D-RV GLS, which is also reported by **Kim *et al.*** [(2)] who identified a connection among RV dysfunction and less activity, corroborating the idea that RV dysfunctions alone can impair efforts tolerance, a physiological characteristic frequently used to stratify prognosis and previously demonstrated to anticipate cardiovascular and total mortality.

LIMITATIONS

Small number of the patients, which may have affected the validity of our results. Assessment of LV diastolic function was not included in our study. We need further studies to explain RV affection in IHD.

CONCLUSION

RV performance was not significantly affected by the presence or absence of RCA affection but right ventricular global longitudinal strain, which is a sensitive tool for detection of right ventricular systolic dysfunction in patients with IHD. It was affected in patients with right coronary artery disease and positively correlated with other 2D and 4D derived echocardiographic parameters for assessment of RV function. It appears that the right ventricular performance is directly interdependent on the left ventricular function in ischemic heart disease, so alleviation of left ventricular ischemia is expected to improve the global biventricular performance.

Abbreviations

CA	Coronary Angiography
EDV	End Diastolic Volume
IHD	Ischemic Heart Disease
HTN	Hypertension
LCA	Left Coronary Artery
LV	Left Ventricle
MPI	Myocardial Perfusion Imaging
RCA	Right Coronary Artery
RV	Right Ventricle
RVI	Right Ventricular Infarction
SPECT	Single Photon Emission Topography
STE	Speckle Tracking Echocardiography
SWMA	Segmental Wall Motion Abnormality
TDI	Tissue Doppler Imaging

DECLARATIONS

i. **Funding:** Not applicable.

ii. **Conflicts of interest:** The authors declared that they have no conflict of interest.

iii. **Ethics approval:** Ethics approval was done by the local University authorities.

iv. **Consent to participate:** A written consent was provided by all the study participants after explanation of the study steps.

v. **Consent for publication:** All the authors declared acceptance for publication.

vi. **Availability of data and material:** All data are available.

vii. **Code availability (software application or custom code):** Not applicable.

viii. **Authors' contributions:** All the authors have contributed equally to the current study.

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