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Speckle Tracking Echocardiographic Assessment of Left Ventricular Function by Myocardial Strain Before and After Aortic Valve Replacement

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Abstract

Background & objectives: In patients of aortic stenosis and regurgitation, pressure and volume effects on left ventricular function are occult and missed by routine echocardiography markers like ejection fraction (EF). Speckle tracking analysis by measuring global longitudinal strain and global circumferential strain seems to ascertain this occult LV function parameters at an early phase in a more comprehensive manner. Limited studies have examined these parameters pre/post aortic valve replacement (AVR).

Methods: 94 consecutive patients with symptomatic severe aortic stenosis (AS) or aortic regurgitation (AR), planned for AVR were included (as per set inclusion criteria) along with 15 normal controls-15 months prospective study. Routine echocardiography and speckle tracking imaging was done at baseline (pre AVR) and post AVR at 1st week, 1st month and 3rd month of follow up.

Results: 90 patients completed study (70 in AS and 20 in AR group). In AS group mean values (± 2 standard deviations) of global longitudinal strain (GLS) improved from a baseline -10.9% (± 3.9) to -19.4% (± 3.8) at 3rd month (p value < 0.0001). Mean values of global circumferential strain (GCS) too improved from -17.3% (± 4.5) to -21.4% (± 3.6) respectively (p value < 0.0001). In AR group too mean values of global longitudinal strain progressed from a baseline -12.6% (± 3.9) to -19.4% (± 3.4) at three months of follow (p value < 0.0001) and mean values of global circumferential strain also progressed from -15.3% (± 3.4) at baseline to -21.7% (± 3.1) respectively (p value < 0.0001).

Conclusion: Magnitude of recovery of GLS and GCS after AVR was more as compared to recovery in EF. Poor GLS/GCS values at baseline were associated with lesser recovery pressing need for an earlier intervention.

Keywords: Aortic valve disease, Global longitudinal strain, Global circumferential strain

1. Introduction

Patients with severe aortic stenosis (AS) develop LV hypertrophy and dysfunction due to long-standing pressure overload of the left ventricle [1]. On the other hand, severe aortic incompetence mainly induces the dilatation of left ventricular (LV) volume. Studies have reported that more than one-third of patients with severe aortic stenosis and more than one-fourth with severe aortic regurgitation (AR) become symptomatic (angina, dyspnoea

and syncope in aortic stenosis/fatigue, palpitations, dyspnoea on exertion as most common symptoms in aortic regurgitation) [2–4]. Both severe AS and severe AR patients may deteriorate into irreversible myocardial dysfunction such as left ventricular heart failure which may increase the risk of sudden death. Aortic valve replacement (AVR) is an effective therapy which may reduce the potential risk of sudden death and improve left ventricular heart function as well [5,6]. The predicting factors for the timing of surgery are still being discussed. Besides,

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the mechanism of the improvement on the left ventricular function remains unclear. LV dysfunction may be underestimated by the standard methods such as cardiac catheterization and routine echocardiography especially when such patients have preserved ejection fraction. This occult LV dysfunction in AS/AR has important implications for morbidity and mortality [7]. Since most of the patients of aortic stenosis have preserved ejection fraction the speckle tracking echocardiography is a useful tool to detect subtle LV dysfunction in patients with severe AS especially with preserved ejection fraction [8–12]. Many recent studies have documented the prognostic value of Global myocardial strain. By documenting this subtle LV dysfunction, this study may help in better estimation of prognosis of such patients. LV strain function worsens with time in untreated patients and detecting subtle myocardial dysfunction at an early stage may have therapeutic implications. Our current study explores the extent of the reversibility of LV strain function following AVR and whether the pre AVR values of myocardial strain have a bearing on the extent of its recovery. Data generated may be valuable in determining the optimum timing of AVR. The application of these indices to AS/AR and its clinical significance are still in early stages and have not been fully established yet [13–17]. There is scarcity of data touching this important aspect of valvular heart disease with very few studies with a limited number of patients [18–22,30].

2. Materials and methods

The study was conducted at Department of Cardiology and Cardiothoracic and vascular surgery in a tertiary care hospital after taking informed consent from every enrolled patient. Due clearance taken from the ethics committee of the institute (S.M.S Medical College and Attached hospitals, Jaipur) which is in accordance with the ethical standards of the Helsinki Declaration (1964, amended most recently in 2008) of the World Medical Association. A hospital based prospective, observational analysis.

2.1. Inclusion criteria

Consecutive patients planned to undergo AVR (As per latest Guidelines) for symptomatic - severe valvular aortic stenosis and severe aortic valve regurgitation in the hospital from July 2019 to September 2020 were screened and 94 patients who fulfilled the inclusion criteria of severe AS/AR were enrolled.

- Severe AS defined as (Aortic V max $\geq$ 4 m/s or mean pressure gradient $\geq$ 40 mm Hg, AVA $\leq$ 1.0cm²)
- And severe aortic valve incompetence was defined as Jet width $\geq$ 65% of LVOT, Vena contracta $>$ 0.6 cm, with holo diastolic flow reversal in proximal abdominal aorta, regurgitation volume $\geq$ 60ml/beat, RF $\geq$ 50%, ERO $\geq$ 0.3 cm²
- Apart from these 15 normal patients were taken as controls
- Symptoms included (angina, dyspnoea and syncope in aortic stenosis/fatigue, palpitations, dyspnoea on exertion as most common symptoms in aortic regurgitation)

2.2. Exclusion criteria

The patients were excluded if they had a history or presented with:

- (1) One or more coronary artery disease needing myocardial revascularisation;
- (2) Previous myocardial infarction
- (3) Any previous cardiac surgery and also Concomitant coronary artery bypass graft surgery (CABG)
- (4) More than mild additional valve diseases.
- (5) Patients with poor echo window and unsatisfactory tracking quality on strain imaging were excluded
- (6) Patients later found to have prosthesis dysfunction
- (7) Patients with prior history of hypertension

Detailed History, examination, NYHA functional class and all routine investigations were recorded. Detailed Echo with speckle tracking was recorded (As per Echo protocol) pre AVR, then at 1 week, 1 month and 3 months post AVR. (LVEDV, LVESV, EF, Global longitudinal peak Strain, Global circumferential peak strain were calculated and statistically analyzed).

2.3. Echocardiography protocol

The commercially available echocardiographic systems (Philips Epiq 7, Philips Medical Systems Corporation, MA, USA) were applied, equipped with S5-1 (1–5 Mhz). All echocardiographic examinations were recorded and 20% examinations were verified by the second observer. The patients received conventional echocardiography, two-dimensional and speckle tracking echocardiography before the surgery as well as at 1 week, 1 month and

3 months after AVR. All the images were analysed using QLAB (cardiac motion quantification, aCMQ) from Philips Medical Systems etc. For data acquisition, three complete cardiac cycles (3 consecutive heart beats only) were stored digitally, with patients held in a breath holding state in left lateral supine position. LV Ejection Fraction (by biplane Simpson's method), left ventricular end diastolic volume (LVEDV), left ventricular end systolic volume (LVESV) were derived. Examination and calculations as per the American Society of Echocardiography 2018 guidelines [31].

Aortic valve area will be calculated using the continuity equation.

$$(AVA = CSALVOT * VTILVOT / VTIAV)$$

where CSALVOT is cross sectional area of LVOT, VTI LVOT is velocity time integral across LVOT, VTI AV is velocity time integral across aortic valve.

For STE analysis, standard parasternal short-axis views at the level of base, papillary muscles and apex along with apical four-, three-, and two-chamber views will be acquired with frame rates between 50 and 80 per second. This technique is based on a tracking algorithm able to identify natural acoustic markers ('features') within the myocardium, to track their motion from frame, and to calculate from that velocity and deformation of the myocardium. For data evaluation, the left ventricle was divided into six walls (septal, lateral,

anterior, inferior, anteroseptal, posterior) and each wall into three segments (basal, mid) and 5 segments at apical level (anterior, septal, inferior, lateral and apex). Longitudinal strain was calculated for each of the 17 segments. Circumferential strain (CS) will then be measured in six segments of the parasternal short-axis view at basal, mid level and 4 segments at apical level. The software automatically calculates the average of segmental peak strain (Global longitudinal strain and Global circumferential strain) (see Figs. 7 and 8).

2.4. Statistical analysis

All results were illustrated as mean $\pm$ SD. The independent Student's t test was applied for comparing values of AS/AR group. The paired Student's t test was applied for comparing changes within AS/AR groups. Results were considered statistically significant for $p < 0.05$. Data was analysed using SPSS 26.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

Total 90 patients completed study- 70 patients with severe aortic stenosis (AS), 20 patients with severe aortic regurgitation (AR). Among 70 patients of severe aortic stenosis (54 patients had degenerative calcific tricuspid valvular aortic stenosis, 12 patients had bicuspid aortic valve with fibrocalcific disease and 4 patients had rheumatic etiology).

Scheme of Study

Fig. 1. Results in patients of symptomatic severe aortic stenosis (AS) who underwent aortic valve replacement (AVR) ($n = 70$), Left ventricular end diastolic volume (LVEDV)/left ventricular end systolic volume (LVESV) in ml. Data expressed as mean values.

Among 20 patients of severe aortic valve regurgitation – 14 were calcific degenerative aortic valves (of which 2 were bicuspid), 4 were myxomatous degenerations, 2 had Marfan's syndrome. All patients underwent surgical aortic valve replacement and received mechanical prosthesis, with size of prosthesis varying from 19 mm to 23 mm. All patients included in the study underwent a coronary angiogram before AVR and had normal or insignificant coronary artery disease. All patients followed a set institutional protocol for pre-operative assessment/routine and were prepared accordingly, transesophageal echocardiography was used intra-operatively/peri operatively for guidance as well. Along with both groups, 15 normal controls were also studied and there was no significant inter observer, intra observer bias on consecutive follow up echocardiography seen (Fig. 9). The group of patients with severe aortic stenosis had 52.9% males

and 47.1% females with a mean age of 61.6 years, and group with severe aortic regurgitation had 65% males and 35% females with a mean age of 59.4 yrs. 66% patients in the AS group were either in NYHA functional class II or III and 85% patients in AR group were in either NYHA class II or III at time of enrolment. AS group had 22.8% diabetics and 28.6% smokers, whereas similar numbers for AR group were 15% and 20% respectively. (See Table 1. Baseline characteristics of patients). 61% patients in group of aortic stenosis had preserved ejection fraction and 39% had LV dysfunction (which had very poor LV function) – over all mean ejection fraction came out to be 44.2% for all values. Among patients in group of aortic regurgitation too most patients (65% had preserved ejection and 35% had LV dysfunction-however very poor LV function values in these 35% brought the mean value of ejection fraction down to 43.2% in this group.

Fig. 2. Results in patients of symptomatic severe aortic stenosis (AS) who underwent aortic valve replacement (AVR) ($n = 70$), Left ventricular ejection fraction (%). Data expressed as mean values.

Fig. 3. Results in patients of symptomatic severe aortic stenosis (AS) who underwent aortic valve replacement (AVR) ($n = 70$), global longitudinal strain (GLS), global circumferential strain (GCS) in negative %. Data expressed as mean values.

Fig. 4. Results in patients of symptomatic severe aortic regurgitation (AR) who underwent aortic valve replacement (AVR) ($n = 20$), Left ventricular end diastolic volume (LVEDV)/left ventricular end systolic volume (LVESV) in ml. Data expressed as mean values.

Fig. 5. Results in patients of symptomatic severe aortic regurgitation (AR) who underwent aortic valve replacement (AVR) ($n = 20$), Left ventricular ejection fraction (%). Data expressed as mean values.

Table 1. Baseline characteristics of patients.

	Aortic stenosis patients (n = 70)	Aortic regurgitation patients (n = 20)	Controls (15)
Men/female (n,%)	37/33 (52.9%/47.1%)	13/7 (65%/35%)	9/6 (60%/40%)
Age (years)	61.6 ± 2.3yrs	59.4 ± 3.4yrs	60.1 ± 2.2yrs
Diabetes (n/%)	16 (22.8%)	3 (15%)	—
Smoker (n/%)	20 (28.6%)	4 (20%)	—
Systolic Blood Pressure (Mean in mm Hg)	125.6 ± 5.6	149.2 ± 10.7	119.5 ± 4.5
Diastolic Blood Pressure (Mean in mm Hg)	84.3 ± 5.6	56.1 ± 4.5	79.3 ± 6.4
Body Surface Area (m ²)	1.6 ± 0.3	1.7 ± 0.4	1.65 ± 0.5
Hemoglobin (g/dl)	11.1 ± 1.6	10.8 ± 1.5	12.9 ± 1.8
Serum Creatinine (Mean-mg/dl)	1.1 ± 0.2	0.84 ± 0.1	0.72 ± 0.2
Hypercholesterolemia (n,%)	4 (19%)	1 (16.6%)	—
NYHA Functional Class			
NYHA I	0	0	—
NYHA II	13	7	
NYHA III	47	10	
NYHA IV	10	3	

*Values expressed as mean ± Standard deviations

Mean values of Left ventricular end diastolic volume (LVEDV in ml) in the AS group improved from 101.6 ml (2SD ± 14.3) at baseline to 93.5 ml (± 11.4) at third month of follow up and mean values of left ventricular end systolic volume (LVESV in ml) improved from 55.7 ml (± 10.3) at baseline to 39.8 ml (± 9.8) at third month of follow up (p value 0.0003 and 0.0001 respectively). There was an improvement in mean values of ejection fraction from 44.2%

(± 14.8) to 48.6% (± 13.4) but results were not statistically significant (p value 0.067). Mean values of Global longitudinal strain (GLS in minus %) too improved from a baseline −10.9% (± 3.9) to −19.4% (± 3.8) (at 3rd month) with a significant p value < 0.0001 after a small dip at 1st week of follow up (initial myocardial depression after AVR has been reported in previous studies too) [29]. Mean values of Global circumferential strain too followed

Table 2. (Results) Echocardiographic findings of patients with severe aortic stenosis, severe aortic regurgitation (pre and post aortic valve replacement-AVR) and normal controls.

Patients with severe aortic stenosis											
	Before AVR		1 week After AVR			1 month After AVR			3 months After AVR		
	Mean	SD	Mean	SD	P-value	Mean	SD	P-value	Mean	SD	P-value
LVEDV	101.6	14.3	94.8	13.8	0.005	92.1	12.9	0.0001	93.5	11.4	0.0003
LVESV	55.7	10.3	53.2	9.9	0.145	49.4	10.1	0.0001	39.8	9.8	0.0001
LVEF	44.2	14.8	42.9	15.1	0.607	45.6	13.1	0.554	48.6	13.4	0.067
GLS	−10.9	3.9	−11.3	3.8	0.539	−14.2	4	0.0001	−19.4	3.8	<0.0001
GCS	−17.3	4.5	−15.6	4.2	0.0223	−18.1	4.4	0.289	−21.4	3.6	<0.0001
Patients with severe aortic regurgitation											
	Before AVR		1 week After AVR			1 month After AVR			3 month After AVR		
	Mean	SD	Mean	SD	P-value	Mean	SD	P-value	Mean	SD	P-value
LVEDV	167.5	16.5	124.2	14.9	0.0001	113.6	13.9	0.0001	109.4	13.8	0.0001
LVESV	93.4	11.2	84.5	10.8	0.014	66.9	10.4	0.0001	54.1	9.9	0.0001
LVEF	42.3	18.2	43.2	17.3	0.874	45.9	15.9	0.51	49.8	14.2	0.155
GLS	−12.6	3.9	−10.2	3.1	0.0377	−11.6	3.5	0.398	−19.4	3.4	<0.0001
GCS	−15.3	3.4	−12.7	3.8	0.0283	−14.6	4	0.554	−21.7	3.1	<0.0001
Normal Controls											
	Baseline		Repeat after 1 week			Repeat after 1month			Repeat after 3 months		
LVEDV (ml)	75.6		75.1			74.9			75.5		
LVESV(ml)	31.8		32.1			32.3			31.9		
LVEF (%)	55		55			55			55		
GLS (%)	−21.3		−21.9			−21.8			−21.7		
GCS(%)	−23.3		−23.9			−23.1			−23.5		

Abbreviations: LVEDV (left ventricular end diastolic volume), LVESV (left ventricular end stroke volume, LVEF (left ventricular ejection fraction), GLS (global longitudinal strain), GCS (global circumferential strain).

the pattern of GLS with values changing from -17.3% (± 4.5) to -21.4% (± 3.6) with p value < 0.0001 (Table 1, Table 2, Also see Figs. 1–3).

Mean values of Left ventricular end diastolic volume (LVEDV in ml) in AR group also improved from 167.5 ml (± 16.5) at baseline to 109.4 ml (± 13.8) at third month of follow up and mean values of left ventricular end systolic volume (LVESV in ml) reduced from 93.4 ml (± 11.2) at baseline to 54.1 ml (± 9.9) at third month of follow up. LV ejection fraction mean value at baseline was 42.3% (± 18.2) and 49.8% (± 14.2) at three months of follow up (p value 0.155). Mean values of Global longitudinal strain (GLS in minus %) too improved from a baseline -12.6% (± 3.9) to 19.4% (± 3.4) (significant p value < 0.0001) at three months follow up examination after a minor dip at 1st week follow up. Mean values of Global circumferential strain also progressed from -15.3% (± 3.4) at baseline to -21.7% (± 3.1) at three months follow up speckle tracking analysis (significant p value < 0.0001) (Table 2, Figs. 4–6). Mean values of GLS in 15 controls were -21.3% (± 2.6) and GCS was -23.3% (± 3.1) (significant p values on comparison with respective values in both AS and AR group) (Table 2).

4. Discussion

A total of 90 patients (70 in AS group and 20 in AR group) completed the three months of follow up apart from 15 other normal patients which served as controls. Even though many patients in both AS and AR group had preserved ejection fraction, the GLS and GCS on speckle tracking analysis established occult LV dysfunction in that subset of patients in form of reduced GLS and GCS values at baseline. The magnitude of improvement in GLS/GCS was much larger compared to a minor insignificant

recovery of ejection fraction over 3 months post AVR. The diagnostic ability multiplies when both GLS/GCS and routine 2D echocardiography markers (LVDEV/LVESV/LVEF) are applied together. Both Global longitudinal strain and global circumferential strain improved after aortic valve replacement with significant improvements at three months follow up.

Also the magnitude of LV function recovery as measured on GLS and GCS on consecutive follow up intervals was larger when their respective baseline values were healthier. This clearly shows that more poor LV function (GLS/GCS) at baseline is associated with a lesser recovery of LV - signalling a need for early detection of LV dysfunction by global longitudinal and global circumferential strain to decide on timing for aortic valve replacement. Speckle tracking analysis identifies the subtle LV function changes at a much earlier stage in contrast to LV ejection fraction, left ventricular end diastolic volume or left ventricular end systolic volume. Our study shows gives impetus to the thought of intervening early in aortic valve disease by incorporating strain analysis in evaluation of patients for aortic valve replacement. Similar results were observed in many other studies [5,23–26]. Al-Rashid et al. found that the global longitudinal strain (GLS) 1 week following TAVR was comparable to that at baseline (-15.9 ± 4.3 vs -16.8 ± 4.1 ; $p = \text{NS}$) but significantly improved at 3 months following TAVR ($-15.9 \pm 4.3\%$ vs. $-19.5 \pm 3.5\%$; $p < 0.001$). GLS improves at 3 months after TAVR, while LV ejection fraction does not show a substantial change, signalling an early recovery of LV longitudinal function after the intervention [23]. Lozano Granero VC et al. found that between baseline and discharge, only a modest but statistically significant improvement in GLS (global longitudinal strain) could be seen (GLS%

Fig. 6. Results in patients of symptomatic severe aortic regurgitation (AR) who underwent aortic valve replacement (AVR) ($n = 20$), global longitudinal strain (GLS), global circumferential strain (GCS) in negative %. Data expressed as mean values.

Fig. 7. Echocardiography images acquired for a patient with severe AS, 4 chamber view (top left), 2 chamber view (bottom left), 3 chamber view (top right), and short axis view at basal level with delineation of endocardial and epicardial borders (bottom right). AFI was later applied.

-14.6 ± 5.0 at baseline; -15.7 ± 5.1 at discharge following AVR, $p = 0.0116$) [25]. Iwahashi et al. demonstrated that although LV mass index and LV systolic function did not change significantly after 2 weeks (LV mass index, 137 ± 54 vs 125 ± 36 g/m²; LV ejection fraction, 60 vs 58%, both $P =$ not significant), peak strain and strain rates increased ($P < 0.001$) and time to peak strain and strain rate shortened after AVR ($P < 0.001$) [34]. Kafa R et al. documented interesting findings in their study of patients of severe aortic stenosis pre and post AVR

where all patients included had preserved ejection fraction. Although a longer follow up but GLS values showed a significant improvement from pre AVR -14.8% to post AVR -17.2% . In patients with severe aortic stenosis, approximately 20% of patients who survived more than 1 year after aortic valve replacement had an abnormal LV-GLS value on postoperative echocardiography, despite a preserved postoperative LVEF and demonstrable left ventricular mass regression which again highlights the need for early intervention [32]. In our study we

Fig. 8. Bull's Eye plot diagram showing (extreme left image) global longitudinal strain values for each of the 17 segments in a patient with severe AS before aortic valve replacement with average GLS value at baseline of -8.3% (LVEF = 48%, GCS was -14.6%). After undergoing aortic valve replacement (Mid image) average GLS improved to -18.1% and GCS in same patient improved to -22.1% at 3 months follow up, LVEF = 52%).

Fig. 9. Bland Altman Plots for inter observer and intra observer variability. [Dotted lines represent 1.96 standard deviations of difference of values of mean of GLS (Global longitudinal strain)/GCS (Global circumferential strain) expressed in %, no significant difference found].

observed an improvement (although not significant) in the ejection fraction post AVR but in studies by Iwahashi N et al., Bauer et al. and Strotman JM et al. contradictory data can be found about the extent of improvement of LVEF [34–36]. The EARLY TAVR study (ClinicalTrials.gov Identifier: NCT03 042104) is an ongoing trial which will evaluate the clinical outcomes of an earlier intervention in

patients with asymptomatic severe aortic AS undergoing TAVR. Recently concluded AVATAR trial concluded that even among patients with asymptomatic severe aortic stenosis, early surgery was beneficial. Early surgery compared with conservative therapy was associated with a significantly lower incidence of composite adverse events over a median follow-up of 32 months. 30 day mortality:

1.4% in the early surgery group vs. 4% in the conservative therapy group ($p = \text{not significant [NS]}$) and heart failure hospitalization: 9.5% in the early surgery group vs. 20.1% in the conservative therapy group ($p = \text{NS}$) [33].

5. Conclusion

Current guidelines for aortic valve replacement rely only on 2D echo and Doppler analysis which might be hindering the quantum of benefit of aortic valve replacement for these patients [27,28]. We as authors believe that there is a bright scope for strain analysis in evaluation of aortic valve disease patients as even asymptomatic patients with normal ejection fraction and moderate aortic valve disease but significantly reduced global longitudinal and global circumferential strain values may be appropriate candidates to receive aortic valve replacement (AVR).

6. Limitations

Sample size of the study was limited due to low number of patients undergoing AVR amid covid pandemic. Further, none of the patients underwent TAVR (transcatheter aortic valve replacement) which is rapidly becoming a favoured option of intervention. Applying same results on TAVR patients may not be justified.

Author contribution

Conception and design of Study: Gajinder Pal Singh Kaler, Rakesh Mahla. Literature review: Gajinder Pal Singh Kaler, Rakesh Mahla, Himanshu Mahla, Sarita Choudhary. Acquisition of data: Gajinder Pal Singh Kaler, Rakesh Mahla, Himanshu Mahla, Sarita Choudhary, Gurdarshan Singh, Raghuveer Prasad Patel. Analysis and interpretation of data: Gajinder Pal Singh Kaler, Rakesh Mahla, Himanshu Mahla, Sarita Choudhary, Gurdarshan Singh, Raghuveer Prasad Patel, Navjot Kaur Kaler. Research investigation and analysis: Gajinder Pal Singh Kaler, Rakesh Mahla, Himanshu Mahla, Sarita Choudhary, Gurdarshan Singh, Raghuveer Prasad Patel, Navjot Kaur Kaler. Data collection: Gajinder Pal Singh Kaler, Rakesh Mahla, Himanshu Mahla, Sarita Choudhary, Gurdarshan Singh, Raghuveer Prasad Patel, Navjot Kaur Kaler. Drafting of manuscript: Gajinder Pal Singh Kaler, Rakesh Mahla, Himanshu Mahla, Sarita Choudhary. Revising and editing the manuscript critically for important intellectual contents: Gajinder Pal Singh Kaler, Rakesh Mahla, Himanshu Mahla, Sarita Choudhary, Navjot Kaur Kaler. Data

preparation and presentation: Gajinder Pal Singh Kaler, Rakesh Mahla, Himanshu Mahla, Sarita Choudhary, Navjot Kaur Kaler. Supervision of the research: Gajinder Pal Singh Kaler, Rakesh Mahla. Research coordination and management: Gajinder Pal Singh Kaler, Rakesh Mahla.

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Conflicts of interest

There are no conflicts of interest.

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