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Assessment of Left Ventricular Mechanics in Patients with Severe Aortic Stenosis after Transcatheter Aortic Valve Implantation: 2-D Speckle Tracking Imaging Study

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Abstract

Background: Chronic pressure overload secondary to severe aortic stenosis causes impairment of left ventricular myocardial deformation and associated with adverse outcome. The present study aimed to assess the response of myocardial mechanics after transcatheter aortic valve implantation (TAVI).

Methods: Assessment of myocardial mechanics by quantification of LV longitudinal, circumferential strain and rotational deformation (apical, basal rotation and twist) by 2-D Speckle-tracking echocardiography at baseline and at midterm follow-up post-TAVI. The patients were divided into 2 groups based on baseline left ventricular ejection fraction. 46 patients had preserved LV EF $\geq 50\%$ preserved ejection fraction (PEF) and 34 patients had reduced left ventricular ejection (REF) $< 50\%$.

Results: 80 patients with severe AS and high surgical risk were evaluated. At a mean follow-up of 8 ± 3 months after TAVI, left ventricular longitudinal strain (LS) significantly improved in reduced ejection fraction (REF) group from $-9.88 \pm 3.93\%$ to $11.89 \pm 3.15\%$ ($P = 0.001$). In preserved ejection fraction (PEF) group, longitudinal strain improved from $-13.8 \pm 3.1\%$ to $-15.2 \pm 3.3\%$ ($P < 0.001$). Longitudinal strain rate (LSR) improved significantly in REF group, $-0.48 \pm 0.20 \text{sec}^{-1}$ to $-0.62 \pm 0.16 \text{sec}^{-1}$ ($P < 0.001$) and in PEF group, $-0.73 \pm 0.19 \text{sec}^{-1}$ to $-0.77 \pm 0.16 \text{sec}^{-1}$ ($P < 0.005$). In PEF group, LV twist angle was supra-physiological at baseline and decreased after TAVI towards normal values ($P = 0.006$). In REF group LV twist angle was reduced at baseline with significant increase towards normal value after transcatheter aortic valve implantation (TAVI), $P = 0.005$. That was attributed to severe LV dysfunction associated with reduction of left ventricular twist at baseline which improved in response to TAVI alongside with improvement of left ventricular systolic function. In reduced ejection fraction (REF) group circumferential strain and strain rate improved significantly after TAVI.

Conclusions: Myocardial mechanics of the left ventricle including strain, strain rate and twist are deformed in severe aortic stenosis. TAVI restores myocardial mechanics towards physiological values in patients with preserved and reduced ejection fraction.

1. Introduction

Impairment of global left ventricular (LV) longitudinal function in patients with severe aortic

valve stenosis was hypothesized three decades ago [1]. Using 2-D speckle-tracking technique, GLS value less than 15% was demonstrated to be associated with subtle LV dysfunction [2]. Global

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longitudinal strain was found to be more sensitive than left ventricular ejection fraction (LVEF) in patients with severe symptomatic aortic stenosis evaluated before AVR [2,3]. In an attempt to assess myocardial deformation immediately before AVR in patients with normal LVEF, GLS value was found low compared to rotation and twist which was found paradoxically high [3]. Limitations of LVEF, as a tool to assess left ventricular systolic function, were demonstrated in several studies in patients with pressure overload and hypertrophic remodeling of LV as a result of aortic stenosis [2–4]. Speckle-tracking echocardiography is a quantitative angle-independent method for assessment of myocardial deformation in multi directions. Strain and strain rate (SR) measurements by STE provides more sensitive predictors of subtle global and regional myocardial dysfunction [5]. Reduction in GLS carries a worsened prognosis in patients with AS [6]. In this study and by utilizing STE, We opted to study the impact of TAVI procedure on myocardial deformation mechanics in patients with AS undergoing TAVI procedure in Madinah Cardiac Center.

2. Patients and Methods

2.1. Study Population

The study was a retrospective study where symptomatic high-risk patients with severe AS who were deemed inoperable for conventional surgical AVR by a multidisciplinary team and subsequently had trans-catheter aortic valve implantation (TAVI) procedures in Madinah Cardiac Center (in Madinah city, KSA) were eligible for our study. Patients were included in this study if transthoracic echocardiograms obtained before and at mid-term follow-up after TAVI (between 6 and 12 months) were available for analysis. Exclusion criteria were (1) poor echocardiographic imaging for endocardial tracking in at least 2 adjacent myocardial segments and (2) Any rhythm other than sinus rhythm including atrial fibrillation during the echocardiographic study.

The study protocol was approved by the local Madina Cardiac Centre research ethics board.

The patients divided into two groups based on baseline LVEF. Forty six patients had preserved left ventricular ejection fraction (PEF); left ventricular ejection fraction (LVEF) $\geq 50\%$ and thirty four

Abbreviations

LV	left ventricle
AS	aortic stenosis
AVR	Aortic valve replacement
LVEF	left ventricular ejection fraction
LS	Longitudinal strain
GLS	Global longitudinal strain
LSR	Longitudinal strain rate
GLSR	Global longitudinal strain rate
STE	Speckle-tracking echocardiography
TAVI	Trans-catheter aortic valve implantation
PEF	Preserved left ventricular ejection fraction
REF	Reduced left ventricular ejection fraction
CS	Circumferential strain
GCS	Global circumferential strain
CSR	Circumferential strain rate
GCSR	Global circumferential strain rate

patients had reduced left ventricular ejection fraction (REF); left ventricular ejection fraction (LVEF) $< 50\%$.

2.2. Clinical Data

Demographic data, comorbidities, logistic European System for Cardiac Operative Risk Evaluation score [7] functional status, laboratory data and procedure outcomes were registered in our trans-catheter aortic valve implantation (TAVI) procedure database. Data pertinent to this study were analyzed.

2.3. Echocardiography

Transthoracic 2-D, Doppler and tissue Doppler imaging (TDI) echocardiographic measurements were carried out using Philips iE33 ultrasound system with a probe 3–5 MHz frequency before and after TAVI at Madinah cardiac center in the period of February 2013 to May 2017, according to American Society of Echocardiography guidelines [8]. Aortic valve area was calculated using the continuity equation. Severe aortic stenosis, was considered when aortic valve area $\leq 1.0 \text{ cm}^2$ and/or mean systolic gradient of the aortic valve $> 40 \text{ mm Hg}$ [8,9]. Speckle-tracking echocardiography (STE) was used to assess LV sub-endocardial mechanics, before and after TAVI using TOMTEC software. The software used the peak of QRS complex as a mark for end diastole. Apical views (apical 4,2&3 chambers) were used to obtain longitudinal strain and SR and averaged to 16 segments model [10]. Parasternal short-axis planes were used to obtain CS and SR at the level of the base, mid and apical LV, then averaging the 16 segments. Rotational mechanics

were obtained from rotational displacement of parasternal short axis at the basal and apical levels and maximal difference between values of the peak rotation at the apex and base levels were used to calculate the LV twist. Physiologic apical rotation is counterclockwise, so it was expressed as a positive angle. LV torsion was not obtained in 2D speckle tracking because it needs 3D to normalize the twist value to the distance between the respective image of basal and apical planes [11]. For standardization, the LV apical cross-section were obtained well beyond the papillary muscle, with either non or the smallest view of the right ventricle (RV) in the cross-section. The software used the peak of QRS complex as a mark for end diastole which is the time reference point, lagrangian strain and peak systolic strain were considered [11].

3. Results

All statistical analyses were conducted using SPSS version 20 (SPSS, Inc., Chicago, IL). Categorical variables are summarized as frequencies and percentages. Continuous variables are expressed as mean $\pm$ SD. Parameters of echocardiography, before and after TAVI were compared using McNemar's test for categorical variables and the paired t test for continuous variables.

3.1. Baseline Characteristics and Clinical Characteristics

Among the one hundred patients who underwent TAVI for severe AS from Feb 2013 to May2017, and who had available data of TTE, pre TAVI and at mid-term follow-up, 80 patients were included in this study. Five patients were excluded because of the presence of atrial fibrillation at the time of TTE and 15 patients were excluded because of poor endocardial tracking caused by insufficient endocardial definition during the cardiac cycle. Baseline characteristics are represented in Table 1. The mean age was 80 ± 11 years and 55 (68.8%) were males. The mean of Logistic European System for Cardiac Operative Risk Evaluation risk estimate was 14.8 ± 14 . There was no statistical significant differences between PEF and REF groups as regard to age, hypertension, diabetes, dyslipidemia and smoking. However, REF group patients had more severe functional limitation (NYHA IV in 41.2% vs 19.6%, $P = 0.035$), higher logistic European System for Cardiac Operative Risk Evaluation score (19.7 ± 13.8 vs 11.2 ± 13.1 , $P = 0.006$), and a greater prevalence of CAD (76.5% vs 39.1%, $P = 0.001$), compared to the PEF group.

Table 1. Clinical characteristics.

Clinical characteristics	All Patients (n = 80)	LVEF<50% Group A (n = 34)	LVEF>50 Group B (n = 46)	P
Age	80 ± 11	79 ± 12	80 ± 10	0.65
Gender				
Male	55(68.8%)	27(79.40%)	28(60.9%)	0.08
Female	25(31.30%)	7(20.60%)	18(39.10%)	
BSA	30.5 ± 7.1	30.3 ± 7.0	30.6 ± 7.2	0.8
BMI	1.8 ± 0.2	1.8 ± 0.2	1.80 ± 0.24	0.68
NYHA class III	20(25.0%)	7(20.6%)	13(28.3%)	0.4
NYHA class IV	23(28.7%)	14(41.2%)	9(19.6%)	0.035*
DM	46(57.5%)	23(67.6%)	23(50.0%)	0.11
HTN	56(70.0%)	22(64.7%)	34(73.9%)	0.37
Smoking	16(20.3%)	7(21.2%)	9(19.6%)	0.85
Dyslipidemia	18(22.5%)	7(20.60%)	11(23.9%)	0.72
Coronary artery disease	44(55.0%)	26(76.5%)	18(39.1%)	0.001*
Syncope	7(8.8%)	1(2.9%)	6(13.0%)	0.11
Life status (Expired)	14(17.5%)	6(17.6%)	8(17.4%)	0.97
CVA	4(5.0%)	3(8.8%)	1(2.2%)	0.17
Blood transfusion	26(32.5%)	13(38.2%)	13(28.3%)	0.3
Bleeding	76(95.0%)	33(97.1%)	43(93.5%)	0.46
Logistic EURO SCORE (%)	14.8 ± 14	19.7 ± 13.8	11.2 ± 13.1	0.006*
Admission duration	12.1 ± 8.6	12.9 ± 10.3	11.6 ± 7.2	0.5
Procedure duration	36.8 ± 12.1	35.6 ± 10.7	37.7 ± 13.2	0.47

BSA, Body Surface Area; BMI, body mass index; NYHA, New York Heart Association, DM, Diabetes mellitus, HTN: hypertension, CVA, cerebrovascular accident.

Data expressed as mean $\pm$ SD or as frequency (Number-percent).

SD: standard deviation P: Probability.

*:significance <0.05.

Table 2. Echocardiographic parameters before and after TAVI according to baseline LVEF.

Echocardiographic parameter	LVEF<50% Group A (n = 34)		P	LVEF≥50% Group B (n = 46)		P
	Pre-TAVI	Post-TAVI		Pre-TAVI	Post-TAVI	
AVMG	39.4 ± 15.5	9.80 ± 5.42	<0.001*	52.8 ± 20.0	11.09 ± 6.12	<0.001*
LVOT VTI	17.08 ± 3.98	19.13 ± 4.44	0.013*	23.85 ± 6.59	23.18 ± 5.85	0.484
AV VTI	83.38 ± 28.68	37.60 ± 10.39	<0.001*	105.86 ± 22.74	38.72 ± 7.81	<0.001*
SV	61.92 ± 19.50	68.95 ± 22.21	0.025*	81.09 ± 23.21	78.44 ± 20.18	0.417
SVI	34.88 ± 11.01	39.21 ± 14.01	0.020*	45.54 ± 13.54	44.48 ± 13.81	0.557
LVEDd	4.96 ± 0.74	4.86 ± 0.68	0.4	4.76 ± 0.74	5.51 ± 4.93	0.3
LVESd	3.94 ± 0.76	3.45 ± 0.72	<0.001*	3.15 ± 0.67	3.68 ± 3.86	0.35
IVSd	1.55 ± 1.86	1.22 ± 0.18	0.3	8.99 ± 6.77	1.60 ± 2.03	<0.001*
LVEF	34.7 ± 10%	49 ± 13%	<0.001*	63.8 ± 7.1%	64 ± 7%	0.85
AVA	0.719 ± 0.295	1.87 ± 0.44	<0.001*	0.728 ± 0.211	2.10 ± 0.76	<0.001*
Max PG	63.82 ± 23.33	18.67 ± 9.86	<0.001*	89.64 ± 32.47	24.22 ± 19.13	<0.001*
RV TAPSE	3.52 ± 4.93	13.50 ± 9.44	<0.001*	2.93 ± 4.51	15.71 ± 9.78	<0.001*
TDI tri S' velocity	10.48 ± 3.89	10.85 ± 3.84	0.664	12.66 ± 4.20	12.46 ± 2.57	0.77
PASP	55.03 ± 11.97	39.65 ± 11.46	<0.001*	46.13 ± 14.84	37.80 ± 11.83	<0.001*

AVMG: aortic valve mean gradient, LVOT VTI: left ventricular outflow tract velocity time integral, AV VTI: aortic valve velocity time integral, SV: stroke volume, SVI: stroke volume index, LVEDd: left ventricular end diastolic dimensions, LVESd: left ventricular end systolic dimensions, IVSd: interventricular septum in diastole, LVEF: left ventricular ejection fraction, AVA: aortic valve area, RV TAPSE: right ventricular tricuspid annular plane systolic excursion, TDI tri S': tissue Doppler imaging lateral tricuspid annulus S wave velocity; PASP: pulmonary artery systolic pressure.

Data expressed as mean ± SD. SD: standard deviation P: Probability *:significance <0.05.

3.2. Echocardiography Characteristics

3.2.1. 2D and Doppler Echocardiography

Transthoracic echocardiography was performed at mean of 8 ± 3 months of follow up after the TAVI procedures. The mean aortic valve area increased from 0.7 ± 0.2 cm² to 2.1 ± 0.8 cm², P < 0.001 in PEF group and from 0.72 ± 0.3 cm² to 1.9 ± 0.4 cm², P < 0.001 in REF group, with a significant decrease in the mean trans aortic valve pressure gradient from 52.8 ± 20.0 to 11.1 ± 6.1 mm Hg, P < 0.001 in PEF group and from 39.4 ± 15.5 to 9.8 ± 5.4 mmHg, P < 0.001 in REF group after TAVI. LVEF improved in both groups but only significant improvement was observed in REF group from 34.7 ± 10 % to 49 ± 13%,

P = <0.001. Table 2 shows echocardiographic parameters in both groups before and after TAVI.

3.2.2. Myocardial Mechanics

3.2.2.1. Longitudinal Deformation. According to baseline LVEF (Table 3), REF group had lower longitudinal strain and strain rate at baseline (before TAVI) compared to PEF group. At follow up, LV longitudinal strain significantly improved in both groups, in REF group improved from -9.88 ± 3.93% to -11.89 ± 3.15% (P = 0.001). In preserved ejection fraction (PEF) group, the improvement from -13.81 ± 3.08% to -15.22 ± 3.26% (P < 0.001) (Fig. 1). Also, LSR improved significantly in reduced ejection

Table 3. Myocardial mechanics before and after TAVI according to baseline LV function.

	Group I LVEF<50% (n = 34)		P	Group II LVEF≥50% (n = 46)		P
	Pre-TAVI	Post-TAVI		Pre-TAVI	Post-TAVI	
GLS (%)	-9.9 ± 3.9	-11.9 ± 3.2	0.001*	-13.8 ± 3.1	-15.2 ± 3.3	<0.001*
GLSR (sec ⁻¹)	-0.5 ± 0.2	-0.6 ± 0.2	<0.001*	-0.7 ± 0.2	-0.8 ± 0.2	0.005
Circumferential strain base	-15.4 ± 5.2	-17.5 ± 5.5	0.017*	-22.5 ± 8.1	-23.9 ± 11.3	0.433
Circumferential strain med	-14.8 ± 7.3	-19.6 ± 6.7	0.001*	-25.5 ± 7.7	-25.7 ± 7.7	0.769
Circumferential strain apex	-18.7 ± 11.0	-22.0 ± 12.2	0.102	-35.9 ± 11.5	-32.10 ± 9.20	0.024*
Global circumferential strain (%)	-16.30 ± 6.34	-19.71 ± 6.27	0.003*	-28.2 ± 7.0	-27.2 ± 6.3	0.210
Circumferential strain rate base	-0.82 ± 0.25	-1.06 ± 0.37	0.001*	-1.71 ± 2.95	-1.38 ± 0.49	0.461
Circumferential strain rate med	-0.82 ± 0.44	-1.20 ± 0.46	0.001*	-1.97 ± 3.92	-1.60 ± 0.65	0.503
Circumferential strain rate apex	-1.24 ± 0.84	-1.57 ± 0.78	0.089	-2.77 ± 3.07	-2.49 ± 1.75	0.572
Global circumferential strain rate (sec ⁻¹)	-0.96 ± 0.44	-1.28 ± 0.45	0.004*	-2.13 ± 3.25	-1.82 ± 0.77	0.508
Rotation (°) Base	-3.4 ± 4.1	-5.8 ± 5.4	0.005	-6.9 ± 3.7	-5.1 ± 3.1	0.007*
Rotation (°) Apex	5.7 ± 5.8	7.2 ± 4.1	0.006	13.5 ± 6.3	9.6 ± 7.6	0.009
Peak twist angle (°)	8.2 ± 7.0	12.98 ± 6.95	0.005	19.6 ± 8.8	14.2 ± 9.2	0.006

Data expressed as mean ± SD.

SD: standard deviation P: Probability.

*:significance <0.05.

fraction group (REF) group, $-0.48 \pm 0.20 \text{ s}^{-1}$ to $-0.62 \pm 0.16 \text{ s}^{-1}$ ($P < 0.001$) and in PEF group, $-0.73 \pm 0.19 \text{ sec}^{-1}$ to $-0.77 \pm 0.16 \text{ sec}^{-1}$ ($P < 0.005$) Circumferential Deformation.

At baseline preprocedural global circumferential strain (GCS) was higher in PEF group compared to REF group ($-28.19 \pm 6.95\%$ vs $-16.30 \pm 6.34\%$). After TAVI GCS showed no significant change in preserved ejection fraction PEF group ($-28.2 \pm 7.0\%$ vs $-27.2 \pm 6.23\%$, $P = 0.46$) while it significantly improved in reduced ejection fraction REF group ($-16.3 \pm 6.3\%$ vs $-19.7 \pm 6.3\%$, $P = 0.01$). Paradoxical significant improvement of apical circumferential strain (CS) was observed in PEF group (Fig. 2). In PEF group apical CS decreased from supra physiologic values $35.9 \pm 11.5\%$ towards normal values $32.1 \pm 9.2\%$ ($P = 0.024$). In REF group apical CS increased from depressed values $-18.73 \pm 11.04\%$

towards normal values $-22.0 \pm 12.2\%$ $P = 0.03$. GCSR improved significantly in REF group from $-0.96 \pm 0.44 \text{ sec}^{-1}$ to $-1.28 \pm 0.45 \text{ sec}^{-1}$ ($P = 0.004$) while no significant change observed in PEF group from $-2.1 \pm 3.3 \text{ sec}^{-1}$ to $-1.8 \pm 0.8 \text{ sec}^{-1}$ ($P = 0.508$). Table 3 shows myocardial mechanical parameters in both groups before and after TAVI. REF group patients had significant improvement in both CS and CSR; Fig. 2 shows an example of significant improvement in apical CS and CSR in one patient from REF group.

3.2.2.3. Rotation and LV Twist. In PEF group net LV twist angle before TAVI was supra physiological and after TAVI decreased toward normal values (from $19.56 \pm 8.79^\circ$ to $14.20 \pm 9.16^\circ$, $P = 0.006$).

In REF group, net LV twist before TAVI was low and after TAVI it increased towards normal values

Fig. 1. Pre versus post TAVI longitudinal strain in a representative patient with EF 58%. Segmental longitudinal strain curves (apical 3 chamber view) are illustrated. Longitudinal systolic strain is reduced at baseline (8%), with improvement after TAVI (12.5%).

Fig. 2. Pre versus post TAVI LV apical circumferential strain and circumferential strain rate in representative patient with EF 20% that improved to 56%. The apical CS increased from 6% to 31.8% and apical CSR increased from -0.4 sec^{-1} to -2.2 sec^{-1} .

Fig. 3. Pre versus post TAVI rotation and net twist angle in a representative patient with LVEF 20%. Basal clockwise and apical counterclockwise rotation illustrated. Twist angle increased from 8° to 16° with improved EF to 56%.

(from 8.15 ± 7.03 to 12.98 ± 28.95 $P = 0.005$), Fig. 3 shows an example of a patient with severe LV dysfunction EF 20% improved to 56% after TAVI and the net LV twist angle and apical counterclockwise rotation increased towards normal value.

4. Discussion

Our study demonstrated a significant beneficial impact of TAVI procedure on LV myocardial mechanics, including longitudinal, circumferential and rotational strain, in addition to the improvement of the conventional echocardiographic parameters in elderly patients with severe AS after TAVI procedure. This study included patients with a wide spectrum of baseline LVEF. Improvement in LV myocardial mechanics was evident in preserved and reduced LVEF groups. In severe AS, the long-standing pressure overload is responsible for the changes in the myocardial deformation; longitudinal strain is initially decreased as a result of sub endocardial ischemia [12]. In patients with severe AS and normal LVEF, some studies demonstrated impairment of myocardial deformation, both CS and radial strain [13–15]. Other studies described the increment of CS at the mid-level of LV and the increment of apical rotation and twist as an adaptive compensatory mechanism of reduced LV systolic function [3,15].

In consistent with previously reported studies, recovery of LV global longitudinal systolic strain post-TAVI was demonstrated in both groups of our patients regardless of the level of LVEF [11,16,17]. We demonstrated an improvement in both global GLS and LVEF post-TAVI which have been linked to favorable clinical outcome. Several researchers have reported a positive impact of the recovery of

the LV-GLS post-TAVI procedure on clinical outcome [18,19].

In consistent with previous studies, we observed in the preserved ejection fraction (PEF) group that there is an adaptive increase in the net of left ventricular (LV) twist and circumferential strain, which could participate in improvement of left ventricular systolic function. After TAVI the LV twist returned to the physiological levels which might indicate a relief of exhausted myocardial compensatory mechanism as a result of afterload reduction. The situation in REF group is the opposite with regard to circumferential, rotational, and torsional deformation after TAVI, there is reduced myocardial mechanics as regard circumferential strain and LV rotation and twist angle, that raised up towards normal values after TAVI. Pronounced improvement of myocardial mechanical deformation in REF group (the more risk group) give a hope to very sick patients with severe aortic stenosis (AS) to improve after TAVI [18,21–26]. Poulin et al. findings [20] are different if compared to this study. The GLS and GLSR in PEF patients are significantly improved in our patients but not in their patients. This could be explained by the cut point baseline EF between both groups which was 50% in our patients but 55% in the other study. This 5% difference may be reflected to the difference in strain and strain rate results. Also, in the rotation and twist angle results in REF group, our results showed significant improvement towards normal values but their results did not. This could be explained by the base line EF and its improvement after TAVI. In our patients the mean baseline EF was 34% that improved to 49% after TAVI. In their patients the mean ejection fraction (EF) was 45%

that improved to 51%. The other results are consistent with Poulin et al. findings [20].

The clinical implementation of this study may benefit patients with severe asymptomatic AS where significant reduction of left ventricular mechanics may warrant early intervention before symptoms appeared.

4.1. Limitations

Our study sample size is relatively small, furthermore exclusion of patients with poor images and/or arrhythmia reduced the intended study sample. Strain measurements were obtained by one researcher with no intraobserver variability assessment. There was no control group, the references values taken from the literature.

5. Conclusion

Improvement in left ventricular myocardial mechanics (left ventricular strain, strain rate and myocardial twist) after trans-catheter aortic valve implantation was observed in all patients regardless of the level of left ventricular ejection fraction. Patients with severe asymptomatic aortic stenosis where significant reduction of left ventricular mechanics may warrant early intervention before symptoms appeared. Further research are required to prove this statement.

Author Contribution

Conception and design of study: Hesham A. Naeim, Reda Abuelatta, Faisal O. Alatawi, Lamiaa Khedr. Acquisition of data: Hesham A. Naeim, Reda Abuelatta, Faisal O. Alatawi, Lamiaa Khedr. Analysis and interpretation of data: Hesham A. Naeim, Reda Abuelatta, Faisal O. Alatawi, Lamiaa Khedr. Drafting the manuscript: Hesham A. Naeim, Reda Abuelatta, Faisal O. Alatawi, Lamiaa Khedr. Revising the manuscript critically for important intellectual content: Hesham A. Naeim, Reda Abuelatta, Faisal O. Alatawi, Lamiaa Khedr. Approval of the version of the manuscript to be published: Hesham A. Naeim, Reda Abuelatta, Faisal O. Alatawi, Lamiaa Khedr.

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