ABSTRACT

Objective: To assess the short term clinical outcomes for a single-stent (SS) strategy versus a double-stent (DS) strategy in percutaneous coronary intervention (PCI) of distal unprotected left main coronary artery (ULMCA) lesions.

Study Design: Descriptive comparative study.

Place and Duration of Study: Armed Forces Institute of Cardiology, Rawalpindi, Pakistan from January 2017 to April 2018.

Methodology: SS treatment was defined as stenting of the main branch alone and DS treatment as stenting of both the main and side branches. Patients who underwent LMCA PCI were recruited in the study using consecutive sampling. Crossover technique, with or without kissing balloon (KB) dilatation, was employed in those getting PCI with a SS strategy; whereas, DK crush, mini-crush, culotte and T-stenting techniques were used in patients undergoing PCI with a DS strategy. The primary endpoints were a composite of major adverse cardiovascular event (MACE) i.e. myocardial infarction, stroke or death and target lesion revascularisation (TLR).

Results: A total of 103 patients were recruited in the study; out of which, 73 underwent LMCA PCI employing a SS technique; whereas, 30 of them were treated with a DS strategy. Mean age of the study participants was 63.2 ±10.6 years. The procedural success rate was 100% in both groups. There was a lower frequency of MACE with single-stent strategy (4.1%) versus the double-stent strategy (16.7%, p=0.031) during the 6-month follow-up period.

Conclusion: In comparison to the two-stent strategy of ULMCA bifurcation intervention, a single-stent approach seems to show favourable clinical outcomes and 6-month MACE-free survival. The choice of optimal revascularisation technique proves to be important for the prognosis; therefore, it requires pragmatic decision-making.

Key Words: PCI (percutaneous coronary intervention), ULMCA (unprotected left main coronary artery), SS (single-stent), DS (double-stent), MACE (major adverse cardiovascular events), CABG (cardiopulmonary bypass grafting), TLR (target lesion revascularisation).

How to cite this article: Nasir M, Shafique HM, Hussain S, Tuyyab F, Aziz S, Khadim R. Percutaneous Coronary Intervention for Left Main Coronary Artery Bifurcation Lesions: Two-stent versus one-stent Strategy for Comparison of 6-month MACE. J Coll Physicians Surg Pak 2020; 30(09):894-899.

INTRODUCTION

Bifurcation lesion PCI (percutaneous coronary intervention) is considered as the technically most difficult intervention when compared to non-bifurcation PCI, despite novel technological advances and introduction of innovative PCI strategies. It has a chance of plaque shift that can result in side branch occlusion. The risk increases manifold when distal unprotected left main coronary artery (ULMCA) is involved. Around 3-9% patients undergoing coronary angiography have significant left main disease, of which 60% have stenosis involving the distal left main stem.

Until late 1980’s, patients with distal ULMCA disease were rarely treated via percutaneous strategies and usually referred for CABG, which was considered the gold standard. There is an evolving interest in left main bifurcation PCI in current interventional scenario with promising results compared to the surgical treatment.

For non-left main bifurcation lesions, many studies were found comparing placement of a single-stent in the main vessel to placement of two-stent in the main vessel and side-branch, respectively, demonstrating no significant benefit of two-stent over a single-stent approach for the non-left main bifurcations. Similarly, some precursory studies depicted that double kissing (DK) crush and culotte techniques resulted in favourable outcomes in coronary artery bifurcation lesions. There were some contemporary studies also showing not so promising results where two-stent techniques were employed. For LMCA bifurcation lesions, there is little data suggestive of the optimal strategy for PCI and has always been largely dependent on operator’s preference.
In the current study, the aim was to evaluate and compare the 6-month clinical outcomes as MACE (major adverse cardiovascular events) in patients who underwent PCI for distal LMCA stenosis employing either a single-stent or a two-stent strategy.

**METHODOLOGY**

This descriptive comparative study was prospectively conducted at Department of Interventional Cardiology, AFIC/NIHD, Rawalpindi, Pakistan. Consecutive patients undergoing LMCA bifurcation stenting were recruited between January 2017 to April 2018. Diagnosis of distal ULMCA bifurcation lesion was based on coronary angiography. The inclusion criteria were patients with angiographic evidence of left main bifurcation disease; patients with favourable anatomy allowing percutaneous intervention, i.e. less than moderate calcification, optimal angulation, minimal disparity between the size of proximal vessel, main vessel and the side branch; patients who denied CABG despite effective counselling and patients with EuroScore >5%. The exclusion criteria were isolated ostial or shaft lesions and patients intolerant to dual antiplatelet therapy.

All the included patients underwent pre-procedural routine testing, including blood complete picture, testing cardiac enzymes and estimation of liver and renal function tests; an electrocardiogram, chest X-ray and transthoracic echocardiography. Risk factors for ischemic heart disease such as diabetes, hypertension, dyslipidaemias and smoking were assessed. Angiograms were reviewed and SYNTAX scores were applied. Patients with SYNTAX scores of >33 were recruited, if they were either unwilling for CABG or were declared unfit by the surgeons.

Radial or femoral access were acquired to perform the percutaneous intervention, mostly via a 7F sheath. Choice of using intravascular ultrasound (IVUS) guidance pre- and post-PCI was made upon interventionist’s preference, which was usually made use of in more complex procedures due to financial restraints, i.e. in patients with moderate to severe calcification and narrow angle between LAD and LCX (n=8 for SS and n=7 for DS). The choice between employment of a one-stent or a two-stent strategy was made by the operator, keeping in view the individual anatomical characteristics of the left main bifurcation lesion. Single-stent strategy was preferred when there was insignificant stenosis at LCX ostium, LCX diameter <2.5mm, angle of >60° between LAD and LCX; whereas, DS strategy was preferred when LCX diameter was >2.5mm or within 0.5mm of the LAD diameter, angle between LCX and LAD was <60° with concomitant diffuse disease in LCX. Medina classification system was applied to further help in deciding for the revascularisation strategy. True bifurcation lesions as described by Medina classification are 1,1,1; 1,1; 1,0,1; 0,1,1. A single-stent approach was described as a stent crossover technique usually from left anterior descending artery to LMCA with another wire parked in left circumflex (LCX) artery. It was followed by strut reopening and kissing balloon dilatation, if post-main vessel stenting the flow in LCX got compromised, i.e. TIMI II or less or in case the ostium of LCX showed a ≥75% narrowing by visual analysis (plaque shift or snow ploughing). The two-stent strategies employed in the current study were double kissing (DK) crush, mini-crush, culotte, T-stenting and other modified simpler techniques. Culotte was chosen when the LAD and LCX were of equal diameters and DK crush was preferred when the diameters; of LAD and LCX differed. Most deployments were achieved on higher than nominal atmospheric pressures. Post-deployment non-compliant balloons were made use of in all cases to ensure optimal stent apposition. Final serial kissing balloon inflations were religiously undertaken in all patients treated with a two-stent strategy; whereas, it was undertaken in single stent strategy where strut reopening was performed (n=10). As dilation of the side branch distorts, the geometry of the stent KBI becomes necessary to smoothen the orifice. Firstly, side branch balloon is inflated, then that of main branch, followed by simultaneous kissing balloon inflation. In addition, proximal optimization technique (POT) was used in all cases treated with DS strategy, which was followed by a REPOT for best post-procedural angiographic and clinical results. Proximal optimisation technique was undertaken in 36 patients dealt with SS strategy, followed by REPOT where KBI was done. It was not considered in cases where the left main was short, there was no carinal shift and the diameters of left main and proximal LAD were almost equivalent. Regular post-dilatation by NC balloon was done in all patients.

Intravenous infusion of glycoprotein IIa/IIIb inhibitors was continued for 12 hours post-PCI in patients who had antecedent acute coronary syndrome or enhanced thrombus activity during PCI. Post-PCI, 300 mg/day of aspirin was prescribed to all patients for one month, which was reduced to 75mg/day to be continued indefinitely thereafter. In addition, they received clopidogrel 300mg in divided doses for the first month, later reduced to 75mg/day for at least one year after the PCI. They were also prescribed adjunctive therapy, such as beta blockers and ACE inhibitors in absence of any contraindications.

Patients were followed up by telephone correspondence and clinic visits at one- and six-month post-PCI. Patients were observed during the entire study period for the development of major adverse cardiovascular events, such as myocardial infarction, stroke or death, acute stent thrombosis or target lesion revascularisation. They were also observed for the recurrence of anginal symptoms. The development of MACE was the primary endpoint. None of the patients were lost to follow-up.

The procedure was declared successful, if there was achievement of grade III thrombosis in myocardial infarction (TIMI) score at the completion of procedure with <20% residual disease, no on-table death, or no MI, need for emergency CABG or mortality during first two days post-PCI. All deaths were supposed to be cardiac in absence of identifiable non-cardiac cause. In-stent restenosis was described as >50% narrowing of the vessel diameter within 5 mm proximal or distal to the stent measured by quantitative coronary analysis. MI and in stent thrombosis were defined as per Academic Research Consortium (ARC) definition. TLR or TVR was defined as repeat revascularisation procedure done for in-stent restenosis as described above.
Table I: Baseline clinical characteristics of patients in both groups.

| Clinical characteristics | Single-stent group n=30 | Double-stent group n=30 | p-value |
|--------------------------|-------------------------|-------------------------|---------|
| Age (years)              | 64.0±10.8               | 61.5±10.0               | 0.281   |
| Gender, n (%) Male       | 53 (72.6%)              | 28 (93.3%)              | 0.019   |
| Female                   | 20 (27.4%)              | 2 (6.7%)                |         |
| Diabetes mellitus, n (%) | 32 (43.8%)              | 15 (50%)                | 0.568   |
| Diabetics                | 41 (56.2%)              | 15 (50%)                |         |
| Hypertension, n (%)      | 32 (43.8%)              | 8 (26.7%)               | 0.104   |
| Hypertensives            | 41 (56.2%)              | 22 (73.3%)              |         |
| Normotensives            |                         |                        |         |
| Smoking, n (%)           | 8 (11%)                 | 7 (23.3%)               | 0.158   |
| Active smokers           | 34 (46.6%)              | 15 (50%)                |         |
| Non-smokers              | 31 (42.5%)              | 8 (26.7%)               |         |
| Ex-smokers               |                         |                        |         |
| Chronic kidney disease, |                         |                        |         |
| n (%) CrCl <30           | 4 (5.5%)                | 3 (10%)                 | 0.704   |
| CrCl 30-50               | 17 (23.3%)              | 7 (23.3%)               |         |
| CrCl >50                 | 52 (71.2%)              | 20 (66.7%)              |         |
| LVEF, n (%)              |                         |                        |         |
| <35%                     | 2 (2.7%)                | 6 (20%)                 | 0.011   |
| 35-45%                   | 12 (16.4%)              | 7 (23.3%)               |         |
| 45-55%                   | 27 (37%)                | 10 (33.3%)              |         |
| >54%                     | 32 (43.8%)              | 7 (23.3%)               |         |
| Clinical presentation,   |                         |                        |         |
| n (%) Prior ACS          | 25 (34.2%)              | 14 (46.7%)              | 0.238   |
| Stable angina            | 48 (65.8%)              | 16 (53.3%)              |         |

LVEF: Left ventricular ejection fraction; ACS: Acute coronary syndrome; CrCl: Creatinine clearance.

Table II: Angiographic and procedural characteristics of two groups.

| Angiographic and procedural characteristics | Single-stent strategy (n=30) | Double-stent strategy (n=30) | p-value |
|--------------------------------------------|-----------------------------|-----------------------------|---------|
| Extent of CAD, n (%)                       | 13 (17.8%)                  | 0 (0%)                      | <0.001  |
| SVCAD                                      | 35 (47.9%)                  | 7 (23.3%)                   |         |
| 2VCAD                                      | 25 (34.2%)                  | 23 (76.7%)                  |         |
| SYNTAX Score                               |                             |                             | <0.001  |
| <22                                        | 16 (21.9%)                  | 0 (0%)                      |         |
| 22-33                                      | 56 (76.7%)                  | 21 (70%)                    |         |
| >33                                        | 1 (1.4%)                    | 9 (30%)                     |         |
| Medina classification, n (%)               |                             |                             | <0.001  |
| 1,1,1                                      | 33 (45.2%)                  | 30 (100%)                   |         |
| 1,1,0                                      | 35 (47.9%)                  | 0 (0%)                      |         |
| 0,1,1                                      | 5 (6.8%)                    | 0 (0%)                      |         |
| Intervention techniques, n (%)             |                             |                             |         |
| Cross-over                                 | 63 (86.3%)                  | 0 (0%)                      | <0.001  |
| Cross-over with KBI                        | 10 (13.7%)                  | 0 (0%)                      |         |
| DK crush                                   | 16 (53.3%)                  | 0 (0%)                      |         |
| Mini crush                                 | 4 (13.3%)                   | 0 (0%)                      |         |
| SKS                                        | 5 (16.7%)                   | 0 (0%)                      |         |
| Culotte                                    | 1 (3.3%)                    | 0 (0%)                      |         |
| T stenting                                 | 4 (13.3%)                   | 0 (0%)                      |         |
| Other two-stent modified techniques        | 0 (0%)                      | 0 (0%)                      |         |
| IVUS, n (%)                                | 8 (11.0%)                   | 7 (23.3%)                   | 0.106   |
| Rotablator, n (%)                          | 2 (2.7%)                    | 0 (0%)                      | >0.999  |
| POT, n (%)                                 | 36 (49.3%)                  | 29 (96.7%)                  | <0.001  |

SVCAD: Single vessel coronary artery disease; 2VCAD: Two vessel coronary artery disease; 3VCAD: Three vessel coronary artery disease; IVUS: Intravascular ultrasound; POT: Proximal optimisation technique.

Table III: Characteristics of patients who expired during the 6-month period.

| Clinical and angiographic characteristics of patients with MACE | Single-stent strategy mortality N=3 | Two-stent strategy mortality N=5 | p-value |
|---------------------------------------------------------------|-------------------------------------|----------------------------------|---------|
| SYNTAX score                                                 | 0 (0%)                              | 0 (0%)                           | 0.028   |
| <22                                                          | 3 (100%)                            | 1 (20.0%)                        |         |
| 22-33                                                        | 0 (0%)                              | 1 (20.0%)                        |         |
| >33                                                          | 1 (33.3%)                           | 1 (20.0%)                        |         |
| Age (years)                                                  | 0 (0%)                              | 0 (0%)                           | 0.659   |
| <40                                                          | 0 (0%)                              | 0 (0%)                           |         |
| 40-50                                                        | 2 (66.7%)                           | 2 (40.0%)                        |         |
| 51-60                                                        | 1 (33.3%)                           | 1 (20.0%)                        |         |
| 61-70                                                        | 1 (33.3%)                           | 1 (20.0%)                        |         |
| ≥71                                                          | 0 (0%)                              | 0 (0%)                           |         |
| Medina classification                                        | 1 (33.3%)                           | 5 (100%)                         | 0.035   |
| 1,1,1                                                        | 0 (0%)                              | 0 (0%)                           |         |
| 1,1,0                                                        | 2 (66.7%)                           | 0 (0%)                           |         |
| 1,0,1                                                        | 0 (0%)                              | 0 (0%)                           |         |
| Extent of IHD                                                | 0.0                                 | 0.0                              | 0.187   |
| 5VCAD                                                        | 2 (66.7%)                           | 1 (20.0%)                        |         |
| 2VCAD                                                        | 3 (60.0%)                           | 4 (80.0%)                        |         |
| 3VCAD                                                        | 1 (33.3%)                           | 0 (0%)                           |         |
| LVEF, n (%)                                                  | 0.0                                 | 0.0                              |         |
| <35%                                                         | 2 (66.7%)                           | 3 (60.0%)                        | 0.850   |
| 35-45%                                                       | 1 (33.3%)                           | 2 (40.0%)                        |         |
| 46-55%                                                       | 0 (0%)                              | 0 (0%)                           |         |
| >55%                                                         | 0 (0%)                              | 0 (0%)                           |         |
| DM                                                           | 2 (66.7%)                           | 3 (60.0%)                        | >0.999  |
| Diabetics                                                    | 1 (33.3%)                           | 2 (40.0%)                        |         |
| Non-diabetics                                                | 0 (0%)                              | 0 (0%)                           |         |
| HTN                                                          | 3 (100.0%)                          | 2 (40.0%)                        | 0.196   |
| Hypertensives                                                | 2 (66.7%)                           | 1 (20.0%)                        |         |
| Non-hypertensives                                           | 1 (33.3%)                           | 1 (20.0%)                        |         |
| Smoking                                                      | 2 (66.7%)                           | 3 (60.0%)                        | 0.688   |
| Active smoking                                               | 1 (33.3%)                           | 1 (20.0%)                        |         |
| Quitters                                                     | 0 (0%)                              | 0 (0%)                           |         |
| Non-smokers                                                  | 2 (66.7%)                           | 3 (60.0%)                        |         |
| CKD                                                          | 2 (66.7%)                           | 2 (40.0%)                        | 0.850   |
| CrCl <30                                                     | 3 (60.0%)                           | 2 (40.0%)                        |         |
| CrCl 30-50                                                   | 1 (33.3%)                           | 2 (40.0%)                        |         |
| CrCl >50                                                     | 0 (0%)                              | 0 (0%)                           |         |

Data analysis was done using SPSS 16.0 (SPSS Inc, Chicago, IL). Frequencies and percentages were used to depict qualitative variables. Chi-square/Fisher Exact test was employed to evaluate the relation between qualitative variables. Normally distributed continuous data were expressed as means ± standard deviation (SD) and the comparison was made using independent sample t tests. The p value <0.05 determined the statistical significance for the differences.

RESULTS

A total of 103 patients underwent percutaneous intervention for distal left main stem bifurcation lesions. Seventy-three (70.9%) of these patients underwent PCI with a single-stent technique; whereas, 30 (29.1%) patients were treated employing a two-stent strategy. The baseline clinical characteristics of patients in both groups are presented in Table I.

Table II shows the angiographic and procedural characteristics of the left main bifurcation stenting groups. Medina 1,1,1
lesions were demonstrated in 100% (n=30) of patients in whom two-stent strategy was employed as opposed to 45.2% (n=33) of patients in whom single-stent PCI was performed (p<0.001). Most patients with distal ULMCA disease had three vessel involvement 76.7% (n=23) in two-stent vs 34.2% (n=25) in one-stent group (p<0.001). Stent crossover technique was employed in the single-stent group with 13.7% (n=10) or without strut reopening 86.3% (n=63) with kissing balloon inflation. Patients in two-stent technique group were intervened using predominantly the crush techniques, i.e. DK crush (53.3%, n=16) and mini-crush (13.3%, n=4), followed by culotte (16.7%, n=5), T-stenting (3.3%, n=1) and other modified two-stent technique with final kissing balloon inflation (13.3%, n=4). IVUS guidance for PCI was utilised in 11% (n=8) patients in the single-stent group and 23.3% (n=7) patients in the two-stent group. POT was done in 36 (49.3%) patients treated with a single-stent strategy and all 30 (100%) patients in whom two-stent strategies were employed. No IABP was used pre- or post-procedure. Final kissing balloon inflations were done in all 30 (100%) patients dealt with a two-stent strategy and 10 (13.7%) patients of single-stent group.

The post-procedural clinical outcomes during the next 6-month follow-up demonstrated that total MACE seen in the single-stent group was 4.1% (n=3) as compared to 16.7% (n=5) in the two-stent group (p=0.031). One (1.4%) patient from the single-stent group experienced in-hospital mortality. She had concomitant history of recent myocardial infarction and reduced left ventricular ejection fraction. Two (6.7%) patients from the two-stent group died during hospital stay, both of whom had significant comorbidities predominantly CKD with reduced LVEF and higher SYNTAX scores. Recurrence of angina was observed in 4 (5.5%) patients treated with single-stent strategy versus 4 (13.3%) patients treated with two-stent strategy, incidence of which was more in patients with higher SYNTAX scores. TLR was observed in none of the patients. There was no acute stent thrombosis, fatal and non-fatal MI observed in both groups.

The angiographic and clinical characteristics of patients who suffered from MACE during the 6-month period are summarised in Table III.

**DISCUSSION**

Lesions involving LMS are found in 3-9% of patients undergoing coronary angiography; and such patients are more likely to develop adverse cardiovascular consequences, if not re-vascularised. ULMCA lesions differ from other angiographic lesions in characteristics such as larger luminal area, higher plaque burden with greater blood flow and reduced wall stress, more angulation of the bifurcation, parent and daughter vessel mismatch and adverse clinical outcomes in case the side branch gets compromised due to large myocardial territory supplied by it.

Previously, CABG was considered as a gold standard for LMCA lesions but recent data suggest equivalent results with statistically insignificant differences between either modality. The results of EXCEL, PRECOMBAT and SYNTAX trials depicted the non-inferiority of PCI for left main disease when compared to CABG. Bifurcation lesions, whether treated with CABG or PCI are still linked to adverse outcomes. Various strategies employing the use of two stents have been devised such as double kissing crush, simultaneous kissing stent techniques, mini-crush, culotte, T-stenting, V-stenting, most of them covering the side branch ostium satisfactorily. Current evidence from randomised trials for PCI in non-LMCA bifurcations favours single-stent strategy for routine intervention; and rather discourages the use of two-stent techniques. Unlike non-LMCA bifurcations, in distal LMCA lesions the side branch is of significant size and provides for a considerable myocardial territory, which makes it more substantial to protect its ostium.

Until recently, there has not been any substantial evidence to suggest as to what percutaneous technique for distal left main bifurcation lesions would be optimal and whatever little evidence is present, it is generated from various observational studies. In the current study, the authors sought to compare the short term clinical outcomes for double-stent versus the single-stent techniques for distal ULMCA bifurcation lesions. One-stent technique resulted into reduced MACE at 6-month (4.1%) versus the two-stent strategy, where MACE approaches (16.7%) at 6-month which turned out to be statistically significant (p=0.031). So, this study showed that SS strategy gives more promising results in LMCA bifurcations too, similar to non-LCMA bifurcations.

Various reasons could be postulated as to why DS (double-stent) approach is related to unfavourable outcomes when compared to SS (single-stent) strategy. The two-stent strategies are more complex and technically arduous, which lengthens procedure duration, contrast consumption and radiation exposure and there are higher chances of procedure linked injury to myocardium. If there is inadequate side branch coverage, the drug is not sufficiently released. Stent overlapping leads to layer upon layer of metal and its excessive concentration at lesion site. There are more chances of stent fracture that can eventually lead to adverse clinical outcomes. In contrast, not intervening upon the visually significant side branch stenosis is not linked to increased incidence of MACE, as it is postulated that most side branch lesions involving the ostia are pseudo lesions as also supported by a fractional flow reserve study by Koo et al. which demonstrates that side branch disease apparently perceived as >75% on visual or quantitative assessment, only 30% of it showed physiological significance.

In addition to the procedural factors, clinical predictors also play a significant role in determining outcomes in both SS and DS groups. In our study, we found that patients who experienced MACE at 6-month were over 50 years of age, had true bifurcation lesions i.e. Medina 1,1,1 (33.3% in SS and 100% in DS group). Most patients ending up with MACE at 6-month had extensive three vessel disease with significantly higher syntax scores (p=0.028) with evidence of moderate to severe LV dysfunction on transthoracic echo (all mortality observed in...
patients with EF <45% in both groups). The incidence of MACE was increased in diabetics, hypertensives, active smokers and in those with concomitant chronic kidney disease, in both groups.

In this study, the SYNTAX scores were calculated and lesions were classified according to Medina classification. The choice of stenting strategy was made by the operator according to the anatomical characteristics of the lesions and personal expertise. The DS approach mainly employed the crush technique (66.6%) followed by culotte (16.7%), so as to cover significant side branch lesion. POT followed by REPOT was done in 100% patients of DS group and in 49.3% of patients in the SS group. IVUS was utilised to gauge the post-procedural angiographic results in 11% patients of SS group and 23.3% patients of DS group. 4.1% of patients in the SS group suffered from MACE at 6-month in contrast to 16.7% of patients in the DS group. Apart from MACE, none of the patients had to undergo TLR. This indicated that both single stent strategies were quite safe and effective for treatment of distal LMCA bifurcation lesions with high procedural success rates. However, we inferred that single-stent strategies were not only found to be convenient and simpler technique wise; but also show more promising results, specially in patients with mild LCx ostial disease.

CONCLUSION

A single-stent strategy may be associated with reduced MACE at 6-month when compared to two-stent strategy for intervention of distal ULMCA bifurcation lesions. More data from randomised control trials would, however, be needed to support these outcomes.

ETHICAL APPROVAL:

Ethical approval was obtained from Institutional Ethics Review Board of Armed Forces Institute of Cardiology, Rawalpindi as per AFIC-IERB-SOP-15, prior to initiation of research work.

PATIENTS’ CONSENT:

Informed consents were taken from all patients and they had no objections whatsoever upon the publication of concerned data.

CONFLICT OF INTEREST:

Authors declared no conflict of interest.

AUTHORS’ CONTRIBUTION:

MN: Did the majority of manuscript writing, acquired all data by following up patients, and performed data analysis.

HMS: Contributed in manuscript writing.

SH: Did the critical review.

FT: Provided the work design and did the proofreading.

SA: Conception of work; and basic design of work was provided.

RK: Contributed in data analysis and interpretation.

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