

# Factors affecting the occurrence and degree of luminal protrusion of carotid plaques after angioplasty stenting

## *An ex-vivo clinical study*

Serkan Guneyli, MD, Ismail Oran, MD, Celal Cinar, MD, Ibrahim Erdinc, MD, Cem Bilgen, MD.

### ABSTRACT

**الأهداف:** التحقق من إمكانية ظهور النتوءات من خلال دعامات الخلايا والعوامل التي تؤثر على النتوءات بعد دعامة جراحة الأوعية الدموية السببية والدعامة (CAS).

**الطريقة:** أجريت هذه الدراسة السريرية للمعالجة الجينية خارج الأحياء خلال الفترة من يوليو 2010م إلى أغسطس 2011م في قسم الأشعة، جامعة ايجه، مدرسة الطب، ازميز، تركيا. بعد الحصول على موافقة مجلس إدارة المستشفى، تم الحصول على 15 صفيحة سببية متتابعة وذلك بعد استئصال باطن الشريان وإدراجها ضمن الدراسة. تم وضع الصفيحات في طعم وعائي. كما أجريت زراعة للدعامة وجراحة الأوعية الدموية. بعد ذلك تم مسح النماذج ضوئياً مع الأشعة المقطعية والأسطح الداخلية من الدعامة عن طريق منظار الألياف البصرية. تم قياس البروز على الصور بالمنظار وفقاً لجدول افتراض عرض الخلية الدعامة كوحدة واحدة في نفس المستوى من كل نتوء للصفيحة اللويحية.

**النتائج:** لوحظ أن اللويحات أخف وزناً وأقل تكلساً. كان وزن الصفيحات مرتبطة عكسياً بأرقام البروز وأقطار شرائح الدعامة. على الرغم من أنها لم تصل إلى مستوى مهم إحصائي، كانت لوحات أرقام النتوء العالية أقل تكلساً وظهرت الأعراض لديها بشكل أكثر.

**خاتمة:** كانت نتوءات اللويحات التجويف واضحة لدى نموذجنا السابق. يبدو أن البروز هو العامل الرئيسي لأي زيادة تتعلق بظهور الأعراض قبل استئصال باطنة الشريان

**Objectives:** To investigate possible protrusions through stent cells, and the factors affecting protrusions after carotid angioplasty and stenting (CAS).

**Methods:** This ex-vivo clinical study was performed between July 2010 and August 2011 at the Department of Radiology, Ege University School of Medicine, Izmir, Turkey. After approval by the

Institutional Board, 15 successive carotid plaques, which were obtained intact after endarterectomies were included in the study. Plaques were placed into vascular grafts. Stent implantations and balloon angioplasties were performed. Afterwards, models were scanned with multislice CT and inner surfaces of stents were observed via fiberoptic endoscope. Protrusion measurement was carried out on endoscopic images according to a scale assuming the width of stent cell as one unit in the same level of each plaque protrusion.

**Results:** Symptomatic plaques were lighter, less calcified. Plaque weights were inversely correlated to protrusion numbers and diameters of the narrowest segments of stents. Although they did not reach to statistically significant level, plaques having high protrusion numbers were more symptomatic and less calcified.

**Conclusion:** Plaque protrusions into the lumen were apparent in our ex-vivo CAS model. The main factor increasing protrusions appeared to relate to the presence of symptoms before endarterectomy.

*Saudi Med J 2013; Vol. 34 (2): 166-170*

*From the Departments of Radiology (Guneyli, Oran, Cinar), Otolaryngology (Bilgen), Faculty of Medicine, Ege University, and the Department of Cardiovascular Surgery (Erdinc), Bozyaka Teaching and Research Hospital, Izmir, Turkey.*

*Received 5th November 2012. Accepted 16th December 2012.*

*Address correspondence and reprint request to: Dr. Serkan Guneyli, Department of Radiology, Faculty of Medicine, Ege University, Bornova 35040, Izmir, Turkey. Tel. +90 5336981477. Fax. +90 2367131507. E-mail: serkanguneyli@yahoo.com*

**Disclosure.** The authors have no conflict of interests, and the work was not supported or funded by any drug company.

Atherosclerosis of the carotid artery may trigger the pathophysiological mechanism underlying the stroke. Therefore, the therapeutic interventions were focused on the alleviation of the carotid artery stenosis. Since the medical treatment is usually insufficient to prevent the stroke, surgery has become the main and globally accepted therapeutic strategy. Initially, endovascular revascularization was mostly used in the patients for whom surgery was inapplicable. However, because this method is less invasive, recently it has become a good alternative to surgery for the carotid artery atherosclerotic disease. Acute or subacute thrombosis related complications were reported after angioplasty and stenting of the carotid artery stenosis. The main mechanism is still obscure, but the factors such as inappropriate anti-aggregant/anti-coagulant treatment, non-compliance to the treatment, resistance to the medications and protrusion of the atherosclerotic plaque material into the lumen may play role in the mechanism of the thromboembolic complications after carotid angioplasty and stenting (CAS).<sup>1</sup> In the literature, possible protrusions through stent cells after stenting and balloon angioplasty has been reported. However in our ex-vivo study, the factors, which affect protrusions after CAS were investigated. There were only a few similar studies regarding the endovascular treatment model (ex-vivo carotid artery stenting) on carotid plaques obtained by endarterectomy.<sup>1,2</sup> In Bicknell's study, plaques obtained by endarterectomy were put in 8 mm polytetrafluoroethylene (PTFE) graft and sutured with 6/0 polypropylene.<sup>1</sup> This graft was placed in a circular shaped apparatus having a pump and storage unit through which a fluid circulation occurred at 37°C. In this model, mimicking true blood circulation, percutaneous transluminal angioplasty (PTA) was performed on 25 plaques and formation of emboli was observed in all plaques. Numbers and maximum sizes of emboli are compared in 3 periods, both the number and size of the emboli gradually increased from the control period and guidewire advancement to the PTA period. However, the difference between these 3 periods was not statistically significant. The number and size of emboli seemed to be more evident in parallel to the degree of stenosis and inversely correlated with statin treatment longer than 4 weeks.<sup>1</sup> Another study was performed on 29 plaques by Bicknell et al<sup>2</sup> using the same ex-vivo model.<sup>1</sup> Levels of matrix metalloproteinases (MMP)-1, MMP-7, MMP-8, and MMP-13 were analyzed. Consequently, MMP-8 (neutrophil collagenase) was found to be correlated with the size of emboli. This study aimed to investigate possible protrusions through stent cells, and the factors affecting protrusions after CAS.

**Methods.** This ex-vivo study was performed between July 2010 and August 2011 at the Department of Radiology, Ege University School of Medicine, Izmir, Turkey. For this experimental study, the approval of the Institutional Board was obtained. Informed consents were obtained from all patients. Additionally, our study was performed according to principles of Helsinki Declaration. We designed this study similar to that of Bicknell et al.<sup>1</sup> In this study, 15 patients (12 male and 3 female), whose stenosis level of carotid artery lumen detected on Doppler Ultrasonography (US) (Siemens G40 Colour Doppler Ultrasound, Erlangen, Germany) were higher than 70% underwent to the carotid endarterectomy. Patients whose stenosis level of carotid artery was less than 70% were not included in the study. Demographics, the predisposing factors and the symptomaticities (8 asymptomatic, 7 symptomatic) of the patients were recorded. The mean age of the patients was 69. After the endarterectomies, their carotid plaques were obtained intact. For each plaque, these procedures were carried out. Dry weight of the carotid plaque was measured with a scale. After the measurement of the weight, the plaque was placed into an 8 mm PTFE vascular graft appropriately to its normal anatomic position. Stent implantation and balloon angioplasty was performed as usual. The graft's lumen, in which the plaque was located was dilated with a 7 mm steel carotid Wallstent (Boston Scientific, Boston, USA) implantation followed by a 5x20 mm angioplasty balloon. The obtained graft was placed into the saline containing 10% radiopaque material, and then its images were obtained from a 64 Slice CT at a section thickness of 0.5 mm (Toshiba Aquilion, Tokyo, Japan). The inner surface of the stent was observed with video-endoscopy using a fiberoptic endoscope (Karl Storz, Tuttlingen, Germany). For each plaque, all procedures were performed within 6 hours after the surgical removal of the plaque. The degree of protrusion was assessed. Demographic data was gathered not only by investigating the patients' files, but also by interviewing the patients and physicians. The calcification of the plaques and the narrowest segments of the stents were assessed with multislice CT scans. On endoscopic images, extension of the plaque material, which went through the stent cells into the lumen, was termed as protrusion and all of the protrusions were counted. The total number of protrusions was termed as protrusion number. The amount of any protrusion was calculated by multiplying the height of the protrusion with the length of the protrusion's base. The sum of the amounts of protrusions in the whole stent was termed as total amount of protrusions. The width of any neighbor cell

of stent on the same axial plane was accepted as one unit for the measurements of each protrusion. Average amount of protrusions was calculated by dividing the total amount of protrusions by the protrusion number. The presence of symptomatology, hyperlipidemia, hypertension, diabetes mellitus, coronary artery disease, smoking, and calcification in the plaque (on CT) were noted in all patients (7 non-parametric data). Additionally, the weight of the plaque, the protrusion number, the total and the average amount of protrusions, and the diameter of the narrowest segment of the stent on CT were recorded (5 parametric data).

Statistical analyses were performed using the Prism Version 5.01 (GraphPad Software Inc, CA, USA). Fisher exact test was used for the comparison of the non-parametric data. Mann-Whitney U test was used for the comparison of the parametric and non-parametric data. Single and multiple regression analyses were performed for the comparison of the parametric data. Statistical significant p value was accepted as  $p < 0.05$ .

**Results. Comparison of the parametric and non-parametric data.** There was a significant relation between the diameters of the narrowest segments of the stents and the symptomaticities of the patients ( $p < 0.05$ ,  $p = 0.002$ ). In other words, in symptomatic cases, the diameters of the narrowest segments of the stents were found to be larger. This was because asymptomatic plaques either had bigger size, or were more calcified and rigid (Figure 1 & Figure 2). The weights of the plaques were significantly related to the presences of calcification and the asymptomaticities of the patients. ( $p < 0.05$ ,  $p = 0.005$ ). As the plaques became heavier, they were less likely to be symptomatic. The weights of the calcified plaques were more than the weights of less calcified plaques. So with the combination of these 2 significant relations, we noted that, as the weights of the plaques increased, the plaques got more asymptomatic, and the diameters of the narrowest segments of the stents diminished. Although there was no (but very close to) statistical meaning, the higher the protrusion numbers, the more likely being symptomatic and the less likely having calcification ( $p > 0.05$ ,  $p = 0.07$ ). There was no more correlation between the other parametric and non-parametric parameters. ( $p > 0.05$ ).

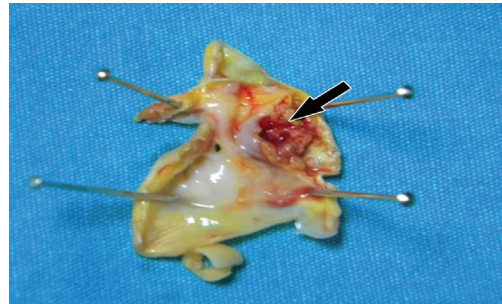
**Comparison of the parametric data with each other.**

There was a weak negative correlation between the protrusion numbers and the weights of the plaques. ( $p < 0.05$ ,  $p = 0.04$ ) (Figure 3 & Figure 4). There was a weak positive correlation between the diameters of the narrowest segments of the stents and the protrusion numbers ( $p < 0.05$ ,  $p = 0.02$ ). The protrusion numbers

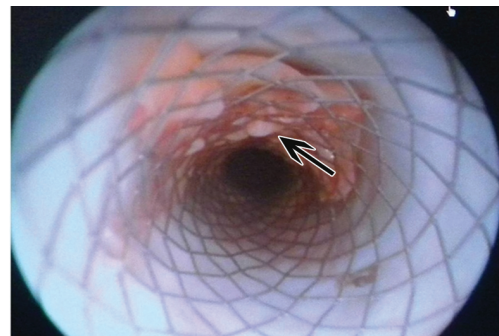
and the total amounts of protrusions were strongly positively correlated ( $p < 0.05$ ,  $p = 0.001$ ). The average amounts and the total amounts of protrusions were weakly correlated ( $p < 0.05$ ,  $p = 0.02$ ). The diameters of the narrowest segments of the stents and the weights of the plaques were found to be strongly inversely correlated ( $p < 0.05$ ,  $p = 0.002$ ). The diameters of the narrowest segments of the stents and the total amounts of protrusions were weakly correlated ( $p < 0.05$ ,  $p = 0.03$ ).

There was no statistical significant difference within the non-parametric data. (The presence of symptomatology, hyperlipidemia, hypertension, diabetes mellitus, coronary artery disease, smoking and calcification in the plaque on CT) ( $p > 0.05$ ). In our study results, the diameters of the narrowest segments of the stents, the weights of the plaques, the protrusion numbers, the total and the average amounts of protrusions, were demonstrated in Table 1.

**Discussion.** We found that protrusion number had significantly positive correlation with the

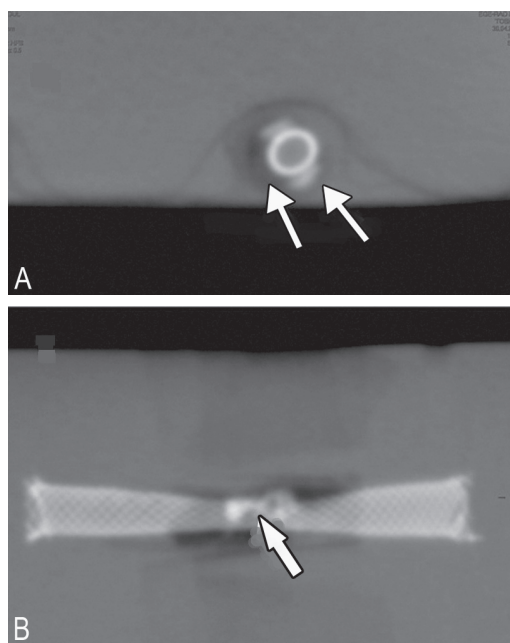


**Figure 1** - Macroscopic view of a non-calcified carotid plaque from a symptomatic patient. Arrow shows the plaque.

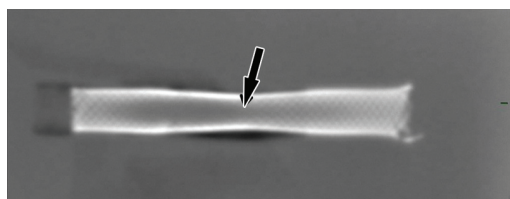


**Figure 2** - Video-endoscopic view of a non-calcified plaque protruding into the lumen. Arrow shows the protrusion of the plaque material.





**Figure 3** - A computed tomography (CT) images of the plaques. A) Axial CT scan demonstrating hypo- and hyper- dense plaque components around the stent. Arrows show hypo- and hyper-dense plaque components. B) Calcified plaque with smaller diameters of the narrowest segments of the stent on coronal CT scan. Arrow shows smaller diameter of the narrowest segments of the stent.



**Figure 4** - Non-calcified plaque with larger diameters of the narrowest segments of the stent on coronal CT scan. Arrow shows larger diameter of the narrowest segments of the stent.

diameter of the narrowest segment of stent ( $p=0.02$ ), and the protrusion number had significantly negative correlation with the weight of plaque ( $p=0.04$ ). The protrusion number had no direct significant relation with symptomaticity of the plaque and presence of calcification in plaque ( $p>0.05$ ,  $p=0.07$ ). However, the  $p$ -values were close to 0.05. Also, the protrusion number had direct significant positive correlation with the narrowest segment of stent (which correlated with symptomaticity), and less weight of plaque (which correlated with less presence of calcification in plaque). Therefore, when we combine these relations it was seen indirectly that the increased number of protrusions were found to be symptomatic and less calcified plaques. The calcified and larger plaques tend to be asymptomatic. In a larger plaque, the protrusion might be expected to occur more, but owing to its stable (asymptomatic) structure and the presence of calcification, the plaque itself might resist fragmentation, as well as protrusion. In a similar way, smaller and less calcified (symptomatic) plaques were less stable, and could readily fracture and protrude into the lumen.

In the comparison of the parametric data with each other, we noted that protrusion parameters were correlated within ( $p=0.001$ ). Like the relation between protrusion number and other parameters, the other parameters were correlated within. (For example; the diameters of the narrowest segments of the stents and the weights of the plaques were negatively correlated ( $p=0.002$ ). Every result in the study supported other one, there was no result, which was against the other one.

In the literature, only a limited number of articles have been published regarding the endovascular treatment model (ex-vivo carotid artery stenting) on carotid plaques obtained by endarterectomy. Clark et al<sup>3</sup> has performed stenting on 107 carotid artery

**Table 1** - The diameter of the narrowest segment of the stent (DSNS), protrusion number (PN), total amount of protrusions (TAP), average amount of protrusions (AAP), and the weight of the plaque with regard to the non-parametric data.

Parameter	Symptom		HL		HT		Smoking		CAD		DM		Calcification	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Mean DSNS	5.60*	4.53	4.87	5.35	5.11	4.5	4.93	5.1	4.87	5.13	4.95	5.08	4.56	5.20
Mean weight	0.70	0.96 <sup>†</sup>	0.82	0.86	0.83	0.91	0.81	0.87	0.91	0.79	0.73	0.91	0.99 <sup>‡</sup>	0.78
Mean PN	15.00	9.62	11.8	12.8	11.6	15.5	12.12	12.14	7.16	15.44	11.83	12.33	12.0	15.45
Mean TAP	3.04	2.05	2.50	2.53	2.36	3.51	2.63	2.37	1.19	3.39	1.94	2.89	2.7	2.44
Mean AAP	0.19	0.21	0.21	0.17	0.19	0.22	0.22	0.18	0.18	0.21	0.15	0.23	0.20	0.20

+ means presence of the non-parametric data, - means no presence of the non-parametric data. \* $p=0.002$ , <sup>†</sup> $p=0.005$ , <sup>‡</sup> $p=0.005$ , HL - hyperlipidemia, HT - hypertension, CAD - coronary artery disease, DM - diabetes mellitus

plaques, and intravascular ultrasonography (IVUS) had been applied on 87 of these cases before the stenting process. In patients with demonstrated plaque calcification on IVUS, stent expansion was less and the incidence of stroke was higher. This study has further supported to our results that the calcified plaques are more rigid. In another study, control of 23 patients with multislice CT after carotid artery stenting on later period postoperatively revealed that eccentric in-stent hypodense areas existed in 10 patients at the second week.<sup>4</sup> These areas were thought to be consistent with thrombosis, since these findings gradually subsided and disappeared at the third month. Formation of thrombosis was more common in patients in whom long stents extends to the carotid artery bifurcation.<sup>4</sup> Even though we could not demonstrate the protrusions to the graft lumen with CT, we measured the diameters of the narrowest parts of the stents and observed calcifications clearly. Video-endoscopy was used to determine the protrusions that were beyond the boundaries of multislice CT resolution. In the studies mentioned above, filling defects on CT that disappeared after stenting were termed as “thrombosis”. There are a few case reports, in which plaque protrusion into stent could be demonstrated. Wehman et al<sup>5</sup> performed carotid stenting with preservation of distal emboli using IVUS on a 68-year-old asymptomatic man. After stenting, an intraluminal lesion composed of ruptured plaque material was diagnosed in the distal part of the stent. This lesion could not be shown on angiography. Repetition of IVUS after 10 minutes revealed that the lesion was displaced and trapped in the filter, and distal embolization was evident. No symptom consistent with ischemia was observed on the patient.<sup>5</sup>

In Ferrero's study,<sup>6</sup> there were symptomatic embolism after CAS on 2 patients, and the plaque protrusions showed with Doppler US. In one case, it was 6 hours after CAS, and in the other one, 7 days after CAS. These 2 patients were treated with stent removal and carotid surgery. The patients had no neurological deficit after surgery. In Takigawa's study,<sup>7</sup> a 70-year-old man underwent CAS and after 10 days the patient underwent second time to CAS because of stent restenosis. After retreatment, the plaque protrusions showed in Doppler US. Therefore, the carotid surgery was performed and histological examination of the specimen revealed that, it was mainly plaque consisting of cholesterol crystals and macrophages.

There were some limitations for our study. The number of the patients was only 15. Additionally, there were a few performed studies in the literature.

In summary, we showed that plaque protrusion occurred through the stent pores during or after CAS. With the analysis of the parameters, we found that the main factor for plaque protrusion was symptomaticity of the plaque. After CAS, the symptomatic plaques are more likely to protrude into the lumen. The symptomatic plaques seem to be lighter, less calcified, and less rigid. The rupture of the protrusion began with the rupture of fibrous cap of the plaque material. Thrombosis and protrusion of the plaque are the causes of stent restenosis, which causes ischemic complications in the acute or subacute period. Stent restenosis is an emergency situation, which should be treated quickly. In the future studies, stent design will be improved and stent stenosis problem will be less with those stents.

## References

1. Bicknell CD, Cowling MG, Clark MW, Delis KT, Jenkins MP, Hughes AD, et al. Carotid angioplasty in a pulsatile flow model: Factors affecting embolic potential. *Eur J Vasc Endovasc Surg* 2003; 26: 22-31.
2. Bicknell CD, Peck D, Lau NM, Alkhamisi NA, Cowling MG, Clark MW, et al. The relationship between plasma MMP-1,-7,-8 ve -13 levels and embolic potential during carotid endoluminal intervention. *Eur J Vasc Endovasc Surg* 2004; 28: 500-507.
3. Clark DJ, Lessio S, O'Donoghue M, Schainfeld R, Rosenfield K. Safety and utility of intravascular ultrasound-guided carotid artery stenting. *Catheter Cardiovasc Intervent* 2004; 63: 355-362.
4. Watarai H, Kaku Y, Yamada M, Kokuzawa J, Tanaka T, Andoh T, et al. Follow-up study on in-stent thrombosis after carotid stenting using multidetector CT angiography. *Neuroradiology* 2009; 51: 243-251.
5. Wehman JC, Holmes DR, Ecker RD, Sauvageau E, Fahrback J, Hanel RA, et al. Intravascular ultrasound identification of intraluminal embolic plaque material during carotid angioplasty with stenting. *Catheter Cardiovasc Intervent* 2006; 68: 853-857.
6. Ferrero E, Ferri M, Viazzo A, Nessi F. Carotid stent removal of symptomatic plaque protrusion after carotid angioplasty stenting. *Interact Cardiovasc Thorac Surg* 2010; 11: 254-256.
7. Takigawa T, Matsumaru Y, Kubo T, Fukuhara N, Hayakawa M, Usui M. Recurrent subacute in-stent restenosis after carotid artery stenting due to plaque protrusion. *Neurol Med Chir* 2009; 49: 413-417.