

## LATE LUMEN LOSS IN THE PROXIMAL AND DISTAL VESSEL WALLS ADJACENT TO THE STENT IS DUE TO INTIMAL HYPERPLASIA IN THE STENTED POPULATION WITH NON-RADIATION

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Edge-etkisinin intra koroner radyasyon uygulanmayan stent popülasyonunda da oluştuğu rapor edilmiştir. Bu edge-etkisinin mekanizması tam belli değildir. Bu çalışmada stent'e komşu damar segmentinde oluşan geç lümen kaybında intimal hiperplazinin rolü araştırıldı.

Çalışmaya 10 Yeni Zelanda türü erkek tavşan alındı. Tümüne Infra-Renal stent yerleştirildi. 3 ay sonra median laparotomi ile aort kesilip, abdominal ve iliak aort çıkarıldı. Proksimal için bu A<sub>1-5</sub>, distal için B<sub>1-5</sub> olarak numaralandırıldı.

Stente komşu damarda intimal hiperplasi belirtildi. Stentten uzaklaştıkça A<sub>4-5</sub> ve B<sub>4-5</sub> kısımda hiperplasi gözlenmedi. Proksimalde A<sub>1-A2</sub> (P=0,011), A<sub>1-A3</sub> (P=0,012) A<sub>1-A4</sub> (P=0,011) ve A<sub>1-A5</sub> (P=0,012) istatistiksel olarak fark izlendi. Bu

fark A<sub>4-A5</sub> arasında izlenmedi. Distal segment için B<sub>1-B2</sub> (P=0,012), B<sub>1 - B3</sub> (P=0,012), B<sub>1-B4</sub> (P=0,012) ve B<sub>1-B5</sub> (P=0,012) farklılık izlendi. B<sub>4-B5</sub> arasında bu fark izlenmedi (P=1). Proksimal ve distal uçlar karşılaştırıldığında A<sub>1-B1</sub> için p<0.001, A<sub>2- B2</sub> için p<0.001, A<sub>3-B3</sub> için p<0,001 farklılık izlendi. A<sub>4-B4</sub> ve A<sub>5-B5</sub> arasında farklılık izlenmedi (p=1 and p=1 sırasıyla).

Stente bitişik Proksimal ve distal damar duvarı stenten uzaklaştıkça geç lümen kaybına yol açan intimal hiperplazi derecesi azalıyordu

**Anahtar Kelimeler:** Edge etkisi, İntimal hiperplazi

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### INTRODUCTION

Stent implantation has been shown to reduce the rate of restenosis compared with balloon angioplasty<sup>1, 2</sup>, in-stent restenosis is a significant and a growing clinical problem. Intimal hyperplasia is the major mechanism of late lumen loss after stent implantation<sup>3-6</sup>. Although it is an effective therapy for in-stent restenosis, brachytherapy has an important limitation as it causes late lumen loss in the proximal and distal edges of the stent (Edge Effect)<sup>7-9</sup>. Edge effect has been reported recently in the stented population without intracoronary radiation<sup>10-12</sup>. Additionally, edge effect (in-segment restenosis) has been reported in drug-eluting stents which have been presented with great hopes<sup>13</sup>. The mechanism of this edge effect

is not clear yet. However, remodeling or intimal hyperplasia is proposed as the effective mechanism<sup>10-12</sup>.

In this study, we evaluated the role of intimal hyperplasia in the late lumen loss at the stent/vessel margin.

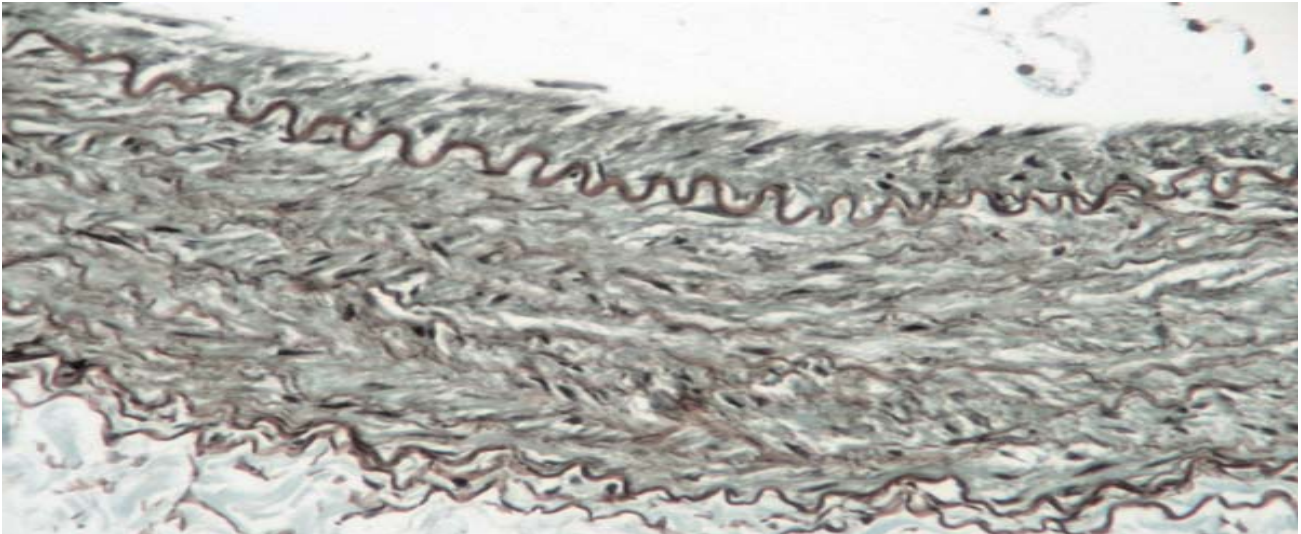
### MATERIAL and METHOD

The study was carried out on 10 New Zealand rabbits. The rabbits included in the study were chosen from among males to avoid the effect of estrogen. Administration of 17.5 mg of clopidogrel and 50 mg of acetylsalicylate was started three days before stent implantation. These drugs were given up to the one month of the procedure.

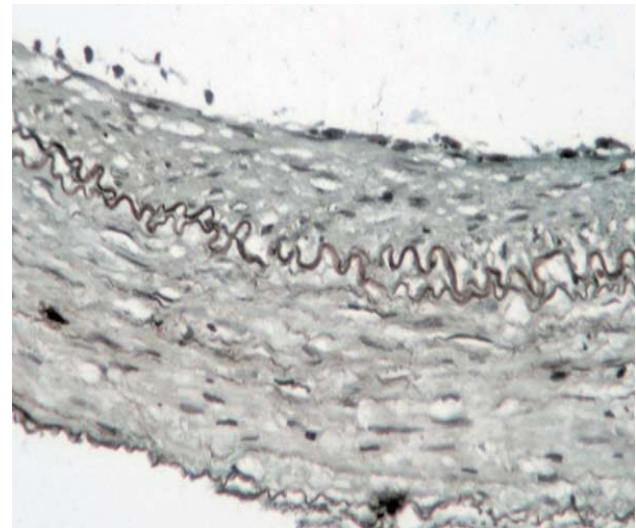
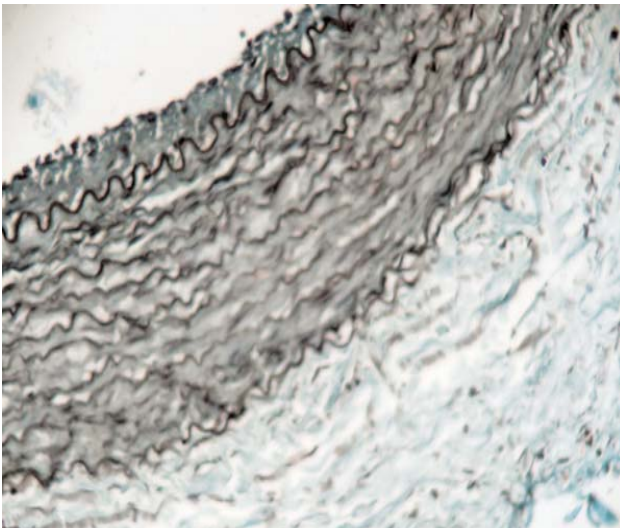
**Stenting:** The procedure was conducted by the aid of fluoroscopic guidance. Before the procedure, 50 mg/kg ketamine and 5 mg/kg xylazine were given intramuscularly for anesthesia. One thirds of this dose was repeated when necessary. 300 mg/kg heparin was injected intravenously. Right femoral artery was explored for angioplasty; after which a stent of 3x15 mm S7 (Medtronic AVE-USA) was implanted at 12

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**Figure 1:** Intimal hyperplasia characterized by the increase of smooth muscle cells and matrix at stent edge (arrow) (Verhoeff's elastic dye, x100)



**Figure 2a:** Intimal hyperplasia was becoming lesser when going far from the edge of the stent. (B3 section, Verhoeff's elastic dye, x100), **2b** Intimal hyperplasia at stent edge (arrow). (Verhoeff's elastic dye, x200):

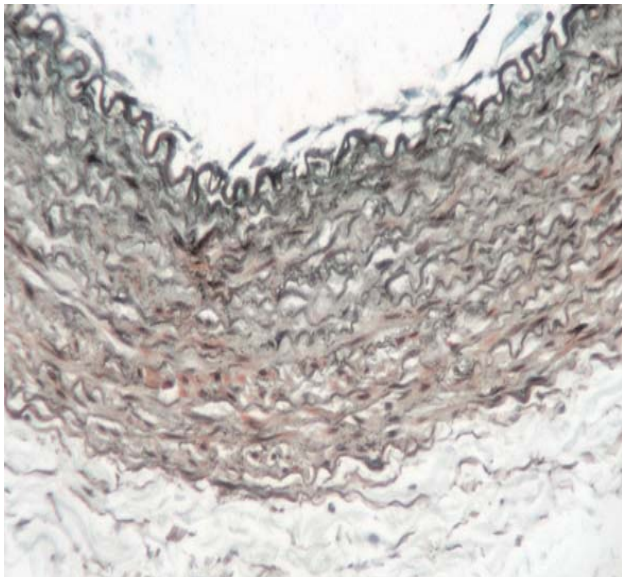


atm. pressure (stent-to-artery ratio of 1.1:1). After the procedure femoral artery was tied and the skin was sutured. Abdominal angiogram was made after stenting. Cases with angiographic dissection in the distal and proximal edges of the stent have been excluded from the study.

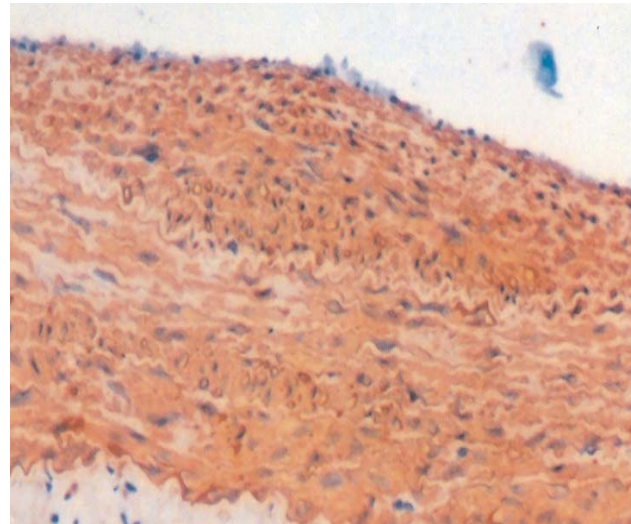
**Histopathological investigation:** On the 30<sup>th</sup> day after stent implantation, abdominal angiogram was made to the aorta under anesthesia. Then, euthanasia was performed by giving a high dose of pentobarbital. The abdomen was opened by median laparotomy incision, after which the site from the abdominal aorta to common iliac arteries was excised. Five sections each 1mm apart have been

obtained from vessels in the neighborhood of the stent. It was numbered as A<sub>1-5</sub> for proximal edge and as B<sub>1-5</sub> for distal edge. Neointimal area in the artery was measured for each millimeter. Tissue samples were fixated in 10% formaldehyde and buried in paraffin blocks. They were dyed with hematoxylin/eosin (H&E) for histological investigation. The cross sections were also dyed with Verhoeff's elastic dye and Masson Trichrom for detailed histopathological evaluation. Smooth-muscle actin immunohistochemical staining was performed for the activity of smooth muscle cells. The sections were examined by light microscope (Olympus BX50, Japan) and micrographs were obtained. The artery cross

**Figure 3:** Hyperplasia wasn't observed in A 4,5 and B 4,5 sections (Verhoeff's elastic dye, x200).



**Figure 4:** Smooth muscle cellular activity was observed in the smooth muscle actin staining (Smooth muscle actin immunohistochemical staining x200). Increased smooth muscle cell activity was observed pink color (arrow).



**Table 1:** Neointimal area values in the vessel walls adjacent to the stent and inter group comparisons.

	1.mm	2.mm	3.mm	4.mm	5.mm
A	0,068±0,005	0,031±0,002	0,023±0,002	0,00±0,00	0,00±0,00
B	0,195±0,007	0,062±0,005	0,038±0,001	0,00±0,00	0,00±0,00
P	<0,001	<0,001	<0,001	NS	NS

NS: Non significant

sections were also evaluated by computerized planimetry method that was described previously<sup>14</sup>. Isolated comparison were made for A<sub>1-5</sub> and B<sub>1-5</sub> separately. Additionally the degrees of hyperplasia in the distal and proximal edges were compared.

**Statistical analysis:** All values were expressed as mean ± SD. ANOVA was employed in the statistical analysis of histological data. P<0.05 was considered significant.

## RESULTS

Two rabbits in which dissection near the stent after procedure was observed were excluded from the study and the study was completed with eight rabbit. Intimal hyperplasia was evident in the vessel near the stent (A<sub>1</sub> ve B<sub>1</sub>, Figure 1) and hyperplasia was becoming lesser when going far from the edge of the stent (Figure 2a-b). Hyperplasia was not observed in A<sub>4,5</sub> and B<sub>4,5</sub> sections (Figure 3). Smooth muscle cellular activity was observed in the smooth muscle actin staining (Figure 4).

The measured values are seen in Table 1. Sta-

tistical significances as (0,068±0,005 mm<sup>2</sup>; 0,031±0,002 mm<sup>2</sup>) p:0,011 for A<sub>1</sub>-A<sub>2</sub>, (0,068±0,005 mm<sup>2</sup>; 0,023±0,002 mm<sup>2</sup>) p: 0,012 for A<sub>1</sub>-A<sub>3</sub>, (0,068±0,005 mm<sup>2</sup>; 0,00±0,00 mm<sup>2</sup>) p:0,011 for A<sub>1</sub>-A<sub>4</sub> and (0,068±0,005 mm<sup>2</sup>; 0,00±0,00 mm<sup>2</sup>) p:0,011 for A<sub>1</sub>-A<sub>5</sub> were observed when comparison was made for each section. However, there observed no significance for A<sub>4</sub>-A<sub>5</sub> (p=1; Table 2).

At the distal end, statistical significances as (0,195±0,007 mm<sup>2</sup>; 0,062±0,005 mm<sup>2</sup>) p:0,012 for B<sub>1</sub>-B<sub>2</sub>, (0,195±0,007 mm<sup>2</sup>; 0,038±0,001 mm<sup>2</sup>) p:0,012 for B<sub>1</sub>-B<sub>3</sub>, (0,195±0,007 mm<sup>2</sup>; 0,00±0,00 mm<sup>2</sup>) p:0,012 for B<sub>1</sub>-B<sub>4</sub> and (0,195±0,007 mm<sup>2</sup>; 0,00±0,00 mm<sup>2</sup>) p:0,012 for B<sub>1</sub>-B<sub>5</sub> were observed. No significance was observed for B<sub>4</sub>-B<sub>5</sub> (p=1; Table 2).

When comparison was made for the proximal and distal end, statistical significance was observed as (0,068±0,005 mm<sup>2</sup>; 0,195±0,007 mm<sup>2</sup>) p<0.001 for A<sub>1</sub>-B<sub>1</sub>, (0,031±0,002 mm<sup>2</sup>; 0,062±0,005 mm<sup>2</sup>) p<0.001 for A<sub>2</sub>-B<sub>2</sub>, (0,023±0,002 mm<sup>2</sup>; 0,038±0,001 mm<sup>2</sup>) p<0,001 for A<sub>3</sub>-B<sub>3</sub>. However, no

**Table 2:** The comparison of neointimal areas in the vessel walls adjacent to the stent

A Section	P <sub>A</sub>	B Section	P <sub>B</sub>
A <sub>1-2</sub>	0,011	B <sub>1-2</sub>	0,012
A <sub>1-3</sub>	0,012	B <sub>1-3</sub>	0,012
A <sub>1-4</sub>	0,011	B <sub>1-4</sub>	0,012
A <sub>1-5</sub>	0,011	B <sub>1-5</sub>	0,012
A <sub>2-3</sub>	0,012	B <sub>2-3</sub>	0,012
A <sub>2-4</sub>	0,012	B <sub>2-4</sub>	0,012
A <sub>2-5</sub>	0,012	B <sub>2-5</sub>	0,012
A <sub>3-4</sub>	0,012	B <sub>3-4</sub>	0,012
A <sub>3-5</sub>	0,012	B <sub>3-5</sub>	0,012
A <sub>4-5</sub>	NS	B <sub>4-5</sub>	NS

NS: Non significant

significances were observed between A<sub>4</sub>-B<sub>4</sub> and between A<sub>5</sub>-B<sub>5</sub> (p=1 and p=1 respectively; Table 1).

## DISCUSSION

We observed the development of intimal hyperplasia in the vessel walls adjacent to the stent and this hyperplasia was becoming lesser when going far from the edge of the stent. Additionally smooth muscle cellular activity was observed to increase when smooth muscle actin staining was performed.

The inevitable arterial injury due to balloon deployment of a stent coupled with the presence of a metallic foreign body causes inflammatory and proliferative responses<sup>15</sup>. Animal studies have shown that this results in neointimal hyperplasia not only within the stent but also at the edges, in the adjacent reference segments<sup>16</sup>. Stent caused the changes in neighbour tissues even in the absence of dissection after procedure. High pressure dilatation in the vessel walls adjacent to the stent may lead not only to acute dissections but also to severe barotrauma, triggering a cellular hyperplastic reaction<sup>4,10,11,17,18</sup>. There appeared to be a continuum of changes beginning at the stent/vessel margin and continuing out to the reference segment proximally and distally for at least 5 to 10mm<sup>10,11</sup>. Previous studies have shown lumen loss adjacent to the stent edge<sup>10-12</sup>. However, these studies differed on the mechanism of this edge effect.

Hoffmann et al<sup>10</sup> and Weissman et al<sup>11</sup> reported that the most important factor in the late lumen loss has been negative remodeling and it has become more evident when going far away from the stent edge. However, Mudra et al<sup>12</sup> suggested that it was related to an increase in plaque burden. They have

not been able to observe negative remodeling at the 1-3 mm of distal and proximal ends of the stent and has reported that intimal hyperplasia at this localization has been the main cause of late lumen loss. In the recent study, it has been reported that there has been late lumen loss at the proximal and distal ends of the stent and this edge effect has been caused by intimal hyperplasia. The results of our study support the results of the previous studies of Mudra et al and Weissmann et al. TAXUS II substudy<sup>19</sup> compared with BMS, there is instead a significant reduction in late lumen loss at the distal edge with TAXUS stents. ASian Paclitaxel-Eluting Stent Clinical Trial (ASPECT) reported serial IVUS edge analysis in small numbers of patients. In the ASPECT study<sup>20</sup> there were no significant changes at either edge, whereas in the study by Honda et al<sup>21</sup> there was significant lumen loss at the distal edge. SIRIUS trial<sup>13</sup>, which included patients with more complex lesions, edge stenoses, which were more frequently observed at the proximal than at the distal edges.

In our study, more evident hyperplasia was observed at the distal stent end when compared with the proximal one and it was interpreted that; it was the results of more barotrauma to the distal portion become minimal decrease in vascular diameter might occur when going from proximal to distal although the stent diameter did not change.

The SIRIUS study is a randomized multicenter, pivotal trial of 1058 patients with focal de novo native coronary lesions. In this trial, the binary in-stent restenosis rates were 3.2% versus a 35.4% restenosis rate in the control group<sup>13</sup>. However, there was moderate degree edge restenosis. Thus, the called in-segment restenosis rate was 8.9% for the sirolimus stent group versus 36.3% for the control group<sup>13</sup>. This result has caused a disappointment in the prevention of edge effect by drug eluting stents. Systemically used anti-proliferative drugs have been reported to prevent in-stent intimal hyperplasia<sup>22</sup>. However, the results are not sufficient yet.

As a result, late lumen loss in the proximal and distal vessel walls adjacent to the stent is due to intimal hyperplasia and the degree of the hyperplasia is reduced when going far away from the stent edge.

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