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ORIGINAL ARTICLE

Comparison of Histopathological Findings and Flap Survival Following Four Different Dermal Filler Material-Induced Vascular Occlusion

Dört Farklı Dermal Dolgu Maddesinin Neden Olduğu Damar Tıkanıklığı Sonrasında Histopatolojik Bulgular ve Flep Sağ Kalımının Karşılaştırılması

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ABSTRACT

Aim: To evaluate comparatively the damage, histopathological findings, and flap necrosis rates due to vascular occlusion caused by the intraarterial injection of dermal fillers calcium hydroxylapatite, polycaprolactone, agarose gel, hyaluronic acid, and serum physiologique.
Material and Methods: The study involved 100 rats, divided into five groups, and 40 arteries. Dermal fillers and serum physiologic were injected into the inferior epigastric arteries, with samples taken at various intervals. The study evaluated degeneration-necrosis, inflammation, fibroblast and collagen densities, thrombus presence, edema, polymorphonuclear leukocyte (PMNL), lymphocyte, mast cell, macrophage, and eosinophil percentages, and calcification. Additionally, 20 rats with 40 flaps created by the inferior epigastric arteries were divided into five injection groups to evaluate flap necrosis rates a week after injection.
Results: There was a statistically significant difference in the percentages of flap necrosis values between the groups. Post-HOC showed that the difference was due to the hyaluronic acid (HA) group with a higher necrosis percentage. Thrombus filling the whole lumen was observed to be higher in the HA group compared to the other groups at all hours and in the first week. Given intima and media degenerations, there was a statistically significant difference at all times and in all groups and a significant difference at certain times when examining the distribution of PMNL, lymphocyte, mast, and macrophage. When eosinophil measurement, inflammation, fibroblast and collagen densities, edema, and calcification findings were evaluated, no significant difference was found between the groups.
Conclusion: It was concluded that besides statistically significant histopathological differences between each filler within the artery, in particular, the HA group caused thrombus, completely occluding the lumen, significantly more than the other groups; accordingly, the flap necrosis rate was significantly higher than the non-HA filler groups. This result highlighted the importance of antithrombotic and thrombolytic therapy, especially in HA fillers.

Keywords: Agarose gel, calcium hydroxylapatite, hyaluronic acid, polycaprolactone, vascular occlusion

ÖZ

Amaç: Bu deneysel çalışma, intraarteriyel dermal dolgu maddeleri kalsiyum hidroksilapatit, polikaprolakton, agaroz jel, hyaluronik asit ve serum fizyolojik enjeksiyonu ile oluşan vasküler oklüzyona bağlı hasar, histopatolojik bulgular ve flep nekroz oranlarını karşılaştırmalı olarak değerlendirmek için tasarlanmıştır.
Gereç ve Yöntemler: 100 sıçan, beş gruba ve 40 atardamara bölünmüştür. Dermal dolgu maddeleri ve serum, alt epigastrik arterlere enjekte edilmiş ve çeşitli aralıklarla örnekler alınmıştır. Çalışmada dejenerasyon-nekroz, inflamasyon, fibroblast ve kollajen yoğunlukları, trombüs varlığı, ödem, PMNL, lenfosit, mast hücresi, makrofaj ve eozinofil yüzdeleri ve kalsifikasyon değerlendirilmiştir. Ek olarak, alt epigastrik arterler tarafından oluşturulan 40 flebe sahip 20 sıçan, enjeksiyondan bir hafta sonra flep nekroz oranlarını değerlendirmek için beş enjeksiyon grubuna ayrılmıştır.
Bulgular: Gruplar arasında flep nekroz yüzdesi değerinde istatistiksel olarak anlamlı bir fark vardı. Post-HOC, farkın daha yüksek nekroz yüzdesine sahip HA grubundan kaynaklandığını gösterdi. Tüm lümeni dolduran trombüsün HA grubunda diğer gruplara göre tüm saatlerde ve ilk haftada daha yüksek olduğu görüldü. İntima ve medya dejenerasyonları incelendiğinde tüm zamanlarda ve tüm gruplarda istatistiksel olarak anlamlı bir fark ve PMNL, lenfosit, mast ve makrofaj dağılımı incelendiğinde belirli zamanlarda anlamlı bir fark vardı. Eozinofil ölçümü, inflamasyon, fibroblast yoğunlukları ve kollajen yoğunlukları, ödem ve kalsifikasyon bulguları değerlendirildiğinde gruplar arasında anlamlı bir fark bulunmadı
Sonuçlar: Her bir dolgu maddesi arasında arter içinde istatistiksel olarak anlamlı histopatolojik farklılıkların yanı sıra, özellikle hyaluronik asit grubunun lümeni tamamen tıkayan trombüse diğer gruplardan anlamlı olarak daha fazla neden olduğu ve buna bağlı olarak flep nekroz oranının HA dolgusu olmayan gruplara göre anlamlı olarak daha yüksek olduğu sonucuna varıldı. Bu sonuç, özellikle hyaluronik asit dolgularında antitrombotik ve trombolitik tedavinin önemini vurguladı.

Anahtar Kelimeler: Agaroz jel, kalsiyum hidroksiapatit, hyaluronik asit, polikaprolakton, vasküler oklüzyon

Introduction

Dermal fillers have become increasingly popular in the field of aesthetic medicine, offering a non-surgical solution for addressing various cosmetic concerns, such as wrinkles, fine lines, and volume loss. The growing demand for these procedures can be attributed to the perceived benefits of a more youthful and rejuvenated appearance, as well as the relatively quick and minimally invasive nature of the treatments. However, the widespread use of dermal fillers has also been accompanied by a growing frequency of

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complications, some of which can be quite serious (1). It is of paramount importance for healthcare providers and patients to have a comprehensive understanding of the risks and considerations associated with these treatments.

Vascular complications, such as arterial occlusion and tissue necrosis, are considered the most serious and potentially devastating adverse events associated with dermal filler injections (1,2). The incidence of vascular complications is relatively low, but the consequences can be severe, ranging from temporary discoloration to permanent disfigurement, necrosis, or even blindness (1). Numerous studies have reported cases of vascular complications, highlighting the importance of understanding the underlying mechanisms and appropriate management strategies (3,4).

The pathophysiology of filler-induced vascular complications is complex, involving a cascade of inflammatory and thrombotic processes ultimately resulting in tissue ischemia and damage. Upon intravascular injection, the filler material can trigger an immediate inflammatory response, leading to the activation of platelets and the coagulation cascade (5,6). This can result in the formation of thrombi occluding the affected vessels, cutting off blood supply to the surrounding tissues. The severity of the inflammatory response is largely dependent on the specific properties of the dermal filler material, such as its viscosity, particle size, and biocompatibility (5).

The specific mechanisms by which different filler materials interact with the vascular system and trigger this cascade of events are still not fully elucidated, a better understanding of these processes is crucial for improving both prevention and management of these complications.

This experimental study was designed to comparatively evaluate the inflammatory and thrombotic processes and the damage caused by intraarterial injection of these four different structures of dermal fillers.

Material And Methods

This study was conducted using 120 male Wistar-Albino rats in 2023, following ethical approval from the Konudam Local Ethics Committee for Animal Experiments of Necmettin Erbakan University with the protocol number, 2002-047. Of 120 rats, 100 were used to observe the histopathological effects of fillers on the vessels. For this purpose, the animals were divided into five different groups, numbered one to five, to receive different filler injections. Group 1: Calcium hydroxylapatite (CaHa) Radiesse, Group 2: Polycaprolactone (PCL) Ellanse S, Group 3: Agarose gel (AG) Algeness 1.5%, Group 4: Hyaluronic acid (HA) Yvoire Classic 20mg/mL, Group 5: Serum Physiologique (SP)

Approximately 0.02 mL of dermal fillers were injected into both sides of inferior epigastric arteries following a 2-cm oblique incision under anesthesia. In this way, 40 arteries were used in each group, 200 in total. For each group, samples for histopathological examination were taken from eight vessels at hour 0, eight vessels at one hour, eight vessels at three hours, eight vessels at 24 hours, and eight vessels at the first week. Following the collection of biopsy samples, the relevant animals were sacrificed under the Care and Use of Laboratory Animals guidelines.

The biopsy samples obtained were evaluated by a single pathologist. Degeneration-necrosis, inflammation, fibroblast and collagen densities in the intima and media layers of the vessels, the presence of thrombus in the vessels, the presence of edema, polymorphonuclear leukocyte (PMNL), lymphocyte, mast cell, macrophage, and eosinophil percentages and the presence of calcification were evaluated (Figure 1).

The remaining 20 of the 120 experimental rats were also divided into five different injection groups and used for the evaluation of flap survival after inferior epigastric artery occlusion. Rats were anesthetized, and 40 flaps of 2 × 2 cm on both sides of the lower abdomen solely nourished by the inferior epigastric arteries were designed and elevated, The purpose of this flap design is to avoid feeding from surrounding tissue and collaterals. Then, approximately 0.02 mL of dermal fillers were injected into both sides of inferior epigastric arteries in each injection group (Figure 2). After the injections, the flaps were sutured back into place, and postoperative follow-up was started. Photographs of flaps in each group were taken at the end of the first week after injection. The percentage of the surviving area of each flap fig was measured with Digimizer Image Analysis Software (Ostend, Belgium) (Figure 2). Differences in flap necrosis occurrence rate and mean percentage of surviving flap area of each group were compared.

Statistical analysis

Data analysis was performed using the Statistics Package for Social Science (SPSS, Version 29.0,

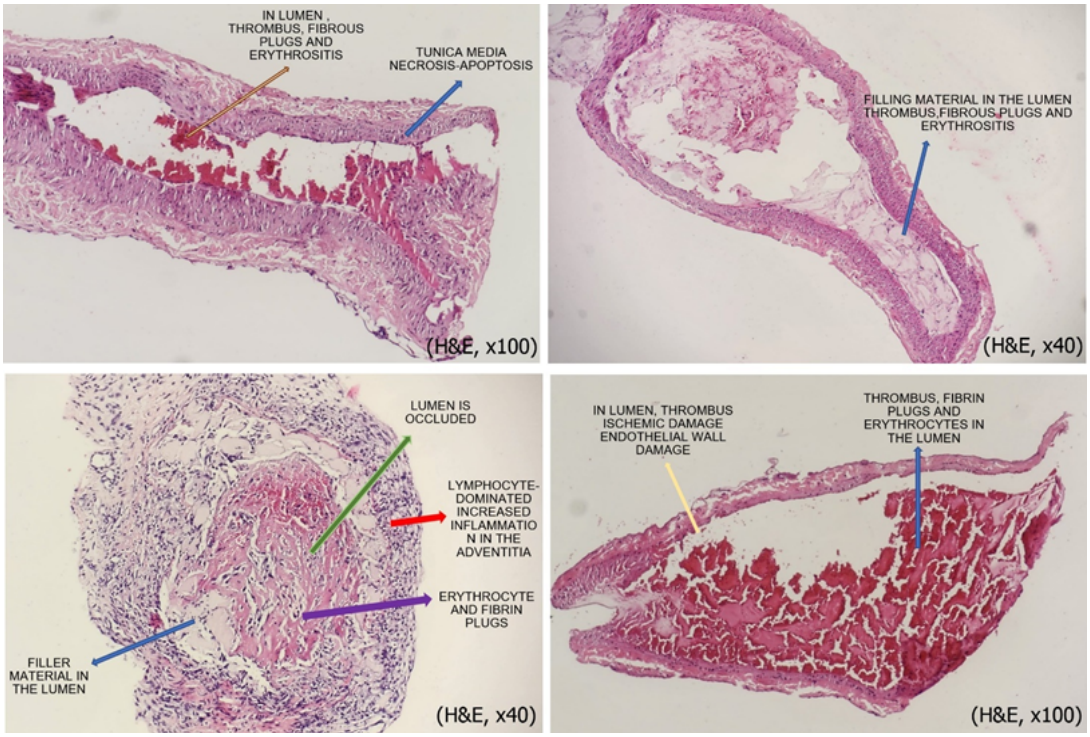

Figure 1. Degeneration-necrosis, inflammation, fibroblast and collagen densities in the intima and media layers of the vessels, the presence of thrombus in the vessels, the presence of edema, PMNL, lymphocyte, mast cell, macrophage and eosinophil percentages and the presence of calcification were evaluated histopathologically.

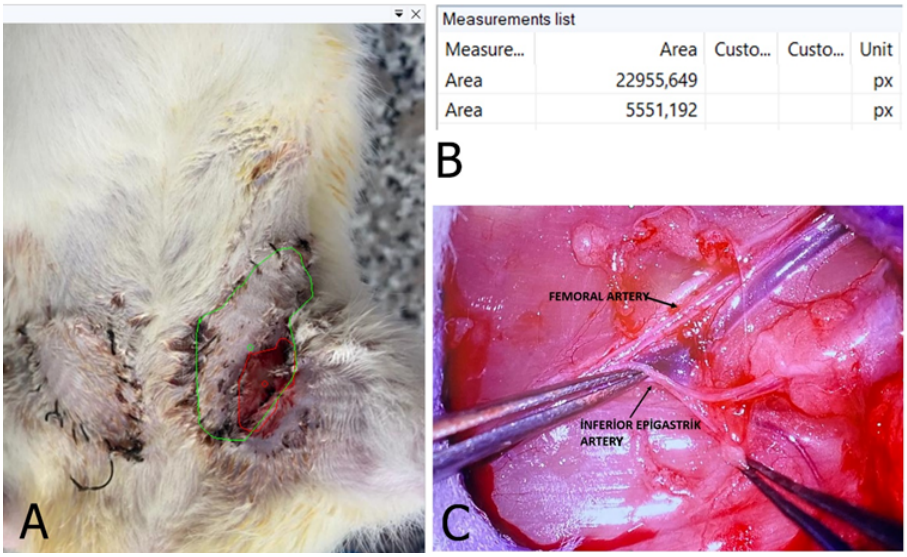

Figure 2. (A,B) The percentage of surviving area of each flap was measured with Digimizer Image Analysis Software, (C) inferior epigastric artery.

IBM Corp., NY, USA). Characteristics of patients, as n (percent) or median (minimum-maximum) for categorical and continuous variables, respectively, were reported. The Kruskal Wallis H test was used to compare the median of independent groups. Nominal variables were compared using a two-tailed chi-square or Fisher test. In pairwise comparisons between dependent measures, multiple tests were evaluated with the Bonferroni correction. A p-value was set at <0.05 for statistical significance.

Results

A total of 40 flaps in 20 rats were divided into five groups with eight flaps in each group. When the percentage of flap necrosis measured in the first week was examined, the mean values were 0.6% (min 0.3-max 13.7) in the CaHa Group 1, 1.7% (min 0.2-max 22.7) in the PLLA Group 2, 0.7% (min 0.2-max 12.6), in the AG Group 3, 25.4% (min 1.2-max 99.4)) in the HA Group 4 and 1.5% (min 0.5-max 5.9) in the SP Group 5 (p=0.036) (Table 1). It was determined that there

Table 1. Percentage of flap necrosis between the groups measured at the first week

Groups (n=40)	CaHa[1] (n=8)	PCL[2] (n=8)	AG[3] (n=8)	HA[4] (n=8)	SP[5] (n=8)	p	Post-HOC
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)		
Flap necrosis (%) (1st Week)	0.6 (0.3-13.7)	1.7 (0.2-22.7)	0.7 (0.2-12.6)	25.4 (1.2-99.4)	1.5 (0.5-5.9)	0.036	1-4;2-4;3-4

AG: Agarose gel, CaHa: Calcium hydroxylapatite, HA: Hhyaluronic acid, PCL: Polycaprolactone

was a statistically significant difference in flap necrosis value between the groups ($p<0.05$). Post-HOC test was performed to determine which groups caused the difference and determined that the differences were due to the HA group (group 4) in which the highest percentage of necrosis was detected.

In the histopathological examination of biopsy samples, the intima and media degenerations occurring in Groups 1-5 according to hours are summarized in Table 2. When the data were examined, it was seen that there was a statistically significant difference between the groups at all times and in all groups ($p<0.05$). At 24 hours, the highest intima damage in the vessels was in the CaHA group (group 1), while the highest media damage was in the HA group (group 4). At week 1, the highest intima and media damage was in the HA group (group 4).

whole lumen was seen at all times in the HA group, but it was seen in only one subject at the 24th hour in the PCL group and was not seen in the other groups.

The distribution of PMNL, lymphocyte, mast cell, macrophage, and eosinophil measurements by hours in Groups 1-5 is given in Table 4. When the table is examined, it was determined that there was a statistically significant relationship between the groups in PMNL measurements at the 1st and 24th hours and the 1st week, in all measurements except the initial measurement in lymphocytes, in mast measurements at the 24th hour and the 1st week, and in macrophage measurements only at the 1st week ($p<0.05$). No statistically significant relationship was observed between the groups in eosinophil measurements.

When inflammation, fibroblast and collagen densities, edema, and calcification findings were evaluated, no

Table 2. Intima and media degenerations that occurred in Groups 1-5 according to hours and distribution of degeneration-necrosis measurements among groups

Degeneration-Necrosis (%) (N=40)	CaHa[1] (n=8)	PCL[2] (n=8)	AG[3] (n=8)	HA[4] (n=8)	SP[5] (n=8)	p	Post-HOC
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)		
Intima							
Hour 0	10 (5-20)	0.5 (0-3)	2 (1-2)	0 (0-0)	0 (0-0)	<0.001	1-2;1-4;1-5;3-4;3-5
1st hour	35 (10-40)	12.5 (10-40)	60 (10-80)	7.5 (1-40)	0 (0-5)	<0.001	1-4;1-5;2-3;2-5;3-4;4-5
3rd hour	35 (20-60)	20 (10-40)	45 (20-60)	20 (15-40)	15 (0-30)	0.002	1-2;1-5;2-3;3-4;3-5
24th hour	55 (40-60)	40 (20-60)	45 (20-60)	35 (20-60)	2 (0-5)	<0.001	1-5;2-5;3-5;4-5
1st Week	30 (10-50)	20 (10-40)	22.5 (10-40)	40 (20-50)	5 (2-10)	<0.001	1-5;2-4;2-5;3-5;4-5
Media							
Hour 0	10 (3-20)	0.5 (0-3)	1 (1-2)	1 (0-2)	0 (0-0)	<0.001	1-2;1-3;1-4;1-5;3-5;4-5
1st hour	35 (30-40)	12.5 (10-20)	50 (30-70)	15 (2-50)	0 (0-2)	<0.001	1-2;1-5;2-3;3-4;3-5;4-5
3rd hour	40 (30-60)	30 (20-40)	50 (30-60)	35 (20-50)	25 (0-40)	0.002	1-2;1-5;2-3;3-5
24th hour	60 (30-70)	45 (10-70)	50 (20-70)	60 (30-70)	5 (2-10)	<0.001	1-5;2-5;3-5;4-5
1st Week	40 (30-60)	30 (20-40)	30 (20-80)	45 (20-60)	7.5 (0-10)	<0.001	1-5;2-5;3-5;4-5

AG: Agarose gel, CaHa: Calcium hydroxylapatite, HA: Hhyaluronic acid, PCL: Polycaprolactone

Thrombus formation within the artery after filler injection according to hours data in Groups 1-5 are summarized in Table 3. When the data were examined, it was seen that there was a statistically significant difference between the groups at all times and in all groups. ($p<0.05$). It was observed that thrombus filling the

significant difference was found between the groups.

Discussion

Dermal fillers have become increasingly prevalent in the field of cosmetic procedures, with a surge in demand over the past decade. The ease of use,

Table 3. Distribution of Thrombus Measurements Among Groups

Thrombus (n=40)	CaHa [1] (n=8)	PCL [2] (n=8)	AG [3] (n=8)	HA [4] (n=8)	SP (n=8)	p
	n (%)	n (%)	n (%)	n (%)	n (%)	
Thrombus (Hour 0)						0.002
None	3 (37.5)	8 (100)	8 (100)	6 (75)	8 (100)	
Lightly layered fibrin thrombus adhesive to the endothelium (+)	5 (62.5)	0 (0)	0 (0)	2 (25)	0 (0)	
Thrombus filling 50% of the lumen (++)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Thrombus filling the whole lumen (+++)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Thrombus (1st hour)						<0.001
None	0 (0)	5 (62.5)	3 (37.5)	0 (0)	8 (100)	
Lightly layered fibrin thrombus adhesive to the endothelium (+)	6 (75)	3 (37.5)	2 (25)	1 (12.5)	0 (0)	
Thrombus filling 50% of the lumen (++)	2 (25)	0 (0)	3 (37.5)	2 (25)	0 (0)	
Thrombus filling the whole lumen (+++)	0 (0)	0 (0)	0 (0)	5 (62.5)	0 (0)	
Thrombus (3rd hour)						**<0.001**
None	5 (62.5)	8 (100)	2 (25)	0 (0)	5 (62.5)	
Lightly layered fibrin thrombus adhesive to the endothelium (+)	3 (37.5)	0 (0)	4 (50)	1 (12.5)	1 (12.5)	
Thrombus filling 50% of the lumen (++)	0 (0)	0 (0)	2 (25)	3 (37.5)	0 (0)	
Thrombus filling the whole lumen (+++)	0 (0)	0 (0)	0 (0)	4 (50)	2 (25)	
Thrombus (24th hour)						**<0.001**
None	0 (0)	3 (37.5)	2 (25)	0 (0)	8 (100)	
Lightly layered fibrin thrombus adhesive to the endothelium (+)	4 (50)	2 (25)	2 (25)	1 (12.5)	0 (0)	
Thrombus filling 50% of the lumen (++)	4 (50)	2 (25)	4 (50)	4 (50)	0 (0)	
Thrombus filling the whole lumen (+++)	0 (0)	1 (12.5)	0 (0)	3 (37.5)	0 (0)	
Thrombus (1st Week)						**<0.001**
None	0 (0)	8 (100)	2 (25)	1 (12.5)	8 (100)	
Lightly layered fibrin thrombus adhesive to the endothelium (+)	5 (62.5)	0 (0)	5 (62.5)	5 (62.5)	0 (0)	
Thrombus filling 50% of the lumen (++)	3 (37.5)	0 (0)	1 (12.5)	1 (12.5)	0 (0)	
Thrombus filling the whole lumen (+++)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	

efficacy, and perceived safety of these treatments have contributed significantly to their popularity. However, despite their impressive safety profile, the growing prevalence of dermal filler injections has also been accompanied by a concerning rise in adverse events and complications, although proper patient selection, appropriate product selection, and meticulous injection techniques are crucial in minimizing the risk of adverse events (2).

Complications associated with dermal fillers can be broadly classified into early, occurring up to several days post-treatment such as injection site reactions, infection, hypersensitivity, lumps, asymmetries, vascular complications, and late complications occurring from weeks to years post-treatment such as atypical infection, biofilm, foreign body granuloma, migration (5). Although the probability of vascular injury caused by dermal fillers is reported to be less than 0.05%, when dermal fillers are accidentally injected into the blood vessels, they can lead to a range of serious complications, including persistent skin necrosis, ophthalmoplegia, permanent unilateral or bilateral vision loss, and stroke (7,8). It was reported that the most common vascular occlusion complication reported in patients undergoing facial filler was vision loss, and most cases resulting in blindness did not show complete recovery despite treatment due to the vulnerability of the retina to ischemia (7). In a previous review conducted by Beleznay et al., autologous fat was found to be responsible for 47.9% of cases of unilateral permanent blindness, followed by HA (23.5%), collagen (8.2%), poly-L-lactic acid (3.1%), and calcium hydroxylapatite (2%) (7).

Therefore, it is very important to have detailed knowledge about vascular complications that are more serious and require urgent intervention compared with other complications. To our knowledge, there is no study in the literature comparing the effects of different filler materials following intravascular injection. This experimental animal study was planned to investigate the rate of flap necrosis and histopathological changes developing within the artery following the intravascular injection of four different structural fillers.

In vascular complications, which can also be encountered as embolia cutis medicamentosa (ECM) in the literature, there may be an increase in vascular

Table 4. Distribution of PMNL, Lymphocytes, MAST Cells, Macrophage and Eosinophil Measurements among Groups

Variables (N=40) (%)	CaHa[1] (n=8)	PCL[2] (n=8)	AG[3] (n=8)	HA[4] (n=8)	SP[5] (n=8)	p	Post-HOC
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)		
PMNL							
Hour 0	0 (0-0)	0 (0-100)	0 (0-0)	0 (0-0)	0 (0-0)	0.406	
1st hour	0 (0-0)	0 (0-100)	0 (0-100)	100 (50-100)	100 (100-100)	<0.001	1-4;1-5;2-4;2-5;3-4;3-5
3rd hour	0 (0-98)	0 (0-100)	0 (0-100)	0 (0-50)	92.5 (0-100)	0.163	
24th hour	0 (0-50)	100 (99-100)	100 (95-100)	20 (20-40)	91.5 (80-96)	<0.001	1-2;1-3;1-5;2-4;2-5;3-4
1st Week	0 (0-3)	7.5 (0-100)	0 (0-2)	2 (0-10)	0 (0-0)	0.002	1-2;2-3;2-5;3-4;4-5
Lenfocytes							
Hour 0	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
1st hour	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-50)	0 (0-0)	0.013	1-4;2-4;3-4;4-5
3rd hour	0 (0-100)	0 (0-100)	0 (0-0)	100 (50-100)	0 (0-10)	<0.001	1-4;2-4;3-4;4-5
24th hour	25 (0-100)	0 (0-0)	0 (0-5)	75 (20-80)	5 (2-10)	<0.001	1-2;1-4;2-4;2-5;3-4
1st Week	69 (50-95)	80 (0-87)	77.5 (0-86)	73.5 (58-85)	100 (100-100)	<0.001	1-5;2-5;3-5;4-5
Mast							
Hour 0	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
1st hour	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
3rd hour	0 (0-2)	0 (0-2)	0 (0-0)	0 (0-0)	0 (0-0)	0.545	
24th hour	0 (0-0)	0 (0-1)	0 (0-0)	0 (0-0)	1 (0-10)	0.007	1-5;2-5;3-5;4-5
1st Week	0 (0-0)	1 (0-3)	2 (0-4)	0 (0-15)	0 (0-0)	<0.001	1-2;1-3;2-5;3-4;3-5
Macrophage							
Hour 0	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
1st hour	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
3rd hour	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
24th hour	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
1st Week	27 (0-40)	4 (0-10)	15 (10-20)	24 (0-40)	0 (0-0)	<0.001	1-2;1-5;2-3;2-4;3-5;4-5
Eosinophil							
Hour 0	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
1st hour	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
3rd hour	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	
24th hour	0 (0-2)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.406	
1st Week	1.5 (0-10)	0 (0-0)	0 (0-5)	0 (0-2)	0 (0-0)	0.058	

AG: Agarose gel, CaHa: Calcium hydroxylapatite, HA: Hhyaluronic acid, PCL: Polycaprolactone

problems depending on the amount of drug, and similarly, different fillers such as collagen (9), or fat (10).

While it is not clear why some fillers of the same amount cause a greater degree of vascular compromise, it may be due to the tendency of dermal fillers to activate the inflammatory process and/or the coagulation process resulting in the progression to irreversible necrosis of the involved tissues (1). For these reasons, it is important to know the structure, properties, tissue behavior of filling materials, and pathophysiology of vascular occlusions induced by dermal fillers.

Calcium Hydroxylapatite (CaHA)

CaHA consists of 30% calcium hydroxylapatite microspheres and 70% sodium carboxy-methylcellulose (CMC), after injection, the CMC gel is rapidly broken down while the CaHA microspheres act as a sort of platform for newly synthesized collagen (10). It is used in the face for mid-deep to deep wrinkles and folds and to correct volume loss and contours. Microscopically, CaHA is easily recognizable as 25- to 45- µm blue-gray, round-oval particles, surrounded by fibrin (11).

The onset of nodules is the most commonly reported adverse event, and its incidence was found to be 3% in a cohort study (12). In vitro analysis has shown limited reversibility of vascular compromise with calcium hydroxyapatite (13).

Polycaprolactone (PCL)

The PCL-based filler is composed of PCL microspheres (30%) suspended in an aqueous carboxymethyl cellulose (CMC) gel carrier (70%) (14). CMC gel is absorbed within the first 6-8 weeks, and the volume loss caused by the absorption of the gel is replaced by neocollagenesis caused by PCL (15).

Polycaprolactone (PCL) based fillers can rarely

cause complications such as nodules or granulomas. Nodule formation is a minor complication frequently associated with fillers, including HAs. In the periorbital region and very superficial applications, PCL-based fillers may very rarely cause xanthelasma-like products (16). It is reported in a study that, in 10 cases where PCL fillings caused vascular embolism complications, heparin and nitroglycerin treatment resulted in significant improvement in blood flow and reduction in ischemia (17).

Agarose Gel (AG)

Agarose is a saccharide polymer (D-galactose and 3,6-anhydro-L-galactopyranose sugars) extracted from red algae (Rhodiphyta) (18). AG is viscous-elastic at temperatures below 45C°. It is slowly degraded by macrophages, and intracellular metabolism through the pentose cycle (19). It is hydrocolloid and nonhydrophilic and causes no mid-term or long-term edema in the surrounding tissues (18). It contains no chemical cross-linking materials such as BDDE, or polyethylene glycol (PEG), which are found in crosslinked HA fillers.

Although there is no reported case of vascular occlusion in the literature, in an experimental study conducted on rats, it was determined that the mean flap survival rate was 92% after the urokinase treatment of arterial occlusion created with agarose gel (20).

Hyaluronic Acid (HA)

The use of cross-linked HA fillers has become the most commonly used filling material in aesthetic practices. Cross-linking is a process that modifies the molecular structure of HA, making it more resistant to degradation and increasing its durability within the skin. Among the various crosslinking agents, 1,4-Butanediol diglycidyl ether (BDDE) has emerged as a widely used option due to its versatility and effectiveness. Different brands have different crosslinking technologies, the degree of modification ranging from 1 to 10%, the molecular weight of 100-600 kDa, and HA concentrations (HAC) ranging from 15 to 24 mg/mL with different shear elastic moduli (G') (21,22). Cohesivity or the capacity to resist fragmentation and dispersal, MoD, and the ability of a gel to take up water (swelling factor) may be important in the intravascular behavior of dermal fillers and their response to reversal agents (22,23).

The vaso-occlusive process induced by intravascular injection of HA gel may further increase the size of the plug, creating a prothrombotic state (24). HA platelet interaction appears to trigger hemostasis and platelet aggregation. Experimental studies on murine have shown that the platelet-rich white thrombus within the gel plug subsequently forms a fibrin-rich red thrombus (25). This rapid process suggests an inflammatory process initiated by the HA bolus rather than anoxic endothelial damage, and the nature of the early white thrombus points to a direct role in platelet interaction with intravascular HA (26). The pro-inflammatory, platelet-activating effect of HA, which begins with platelet-derived hyaluronidase 2 and leukocyte binding, has been described in many studies (27).

In a comparative study showing that HA induces a thrombotic response, thrombus formation was evident in rabbit ears after Restylane (HA) injection and showed a higher rate of total, irreversible vessel occlusion compared with injections of lower viscosity, small-particle polymethylmethacrylate (PMMA) filler whereas this effect was not seen (28).

Besides the thrombogenicity of vasoinoculated HA gels, it is considered an advantage in terms of safety that HA fillers are reversible with hyaluronidase and can be applied around the affected blood vessels. In animal studies a significant flap survival benefit has been shown in animals treated with dual therapy—featuring thrombolytic agents (urokinase or alteplase) combined with hyaluronidase when compared with hyaluronidase alone (25). Cavallini et al showed in animal models that immediate intervention within the first 4 hours after a vascular event significantly reduced ear skin necrosis (29).

In our study, it was observed that the thrombus filling the entire lumen was significantly higher in the HA group than in the non-HA groups, starting from the first hour at all times. It was also observed that this finding was reflected in the flap necrosis rates that there was a significant difference in favor of the HA group compared to the non-HA filler groups, and that this was consistent with the thrombus formation potential of HA.

To our knowledge, there is no similar study in the literature. It is noteworthy that the rate of flap necrosis and thrombus formation completely occluding the vessel lumen was statistically significantly higher in the HA group (Group 4) than in the other groups. The low percentages of flap necrosis in Groups 1, 2, and 3, despite no treatment, are worth evaluating in terms of questioning the flap model or the amount of filler injected. The main limitations of our study are the use

of a limited number of rats for the measurement of flap necrosis percentages and the exclusion of the effects of hyaluronidase and antithrombotic and thrombolytic therapies.

Conclusion

It was concluded that besides statistically significant histopathological differences between each filler within the artery, in particular, the HA group caused thrombus, occluding completely the lumen, significantly more than the other groups, and accordingly, the flap necrosis rate was significantly higher than the non-HA filler groups. This result highlighted the importance of antithrombotic and thrombolytic therapy, especially in HA fillers.

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