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Nipple–Areola Complex Ischemia or Necrosis in Freestyle Perforator Flap Reduction Mammoplasty Operations

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Abstract

Background Necrosis of the nipple–areolar complex (NAC) is one of the most devastating complications of reduction mammoplasty. A variable, asymmetrical pattern of blood supply from the main sources was found in the same individual. To solve this problem, we present a method to be sure that the blood supply to the NAC is contained within the designed pedicle during reduction mammoplasty. This is done using the colored duplex scan and handheld Doppler to perform freestyle perforator flap reduction mammoplasty.

Methods This is a prospective randomized study that has been performed on 105 patients in the period between January 2014 and 2019 at the Plastic and Reconstructive Surgery Department, Tanta University Hospitals. Freestyle perforator flaps were performed for all cases after perforator detection using handheld Doppler and confirmed by colored duplex scan.

Results The procedure was performed as freestyle on medial perforators in 35 (33.3%) cases, on lateral perforators in 20 (19%) cases, on combined medial and lateral perforators, as a bipedicle in 30 (28.7%) cases, on upper pole perforators in 12 (11.4%) cases, and on lower pole perforators in 8 (7.6%) cases.

Conclusions The use of handheld Doppler and the confirmation by duplex scan are mandatory to decrease the incidence of NAC necrosis or ischemia.

Level of Evidence IV This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine Ratings, please refer to Table of Contents or online Instructions to Authors www.springer.com/00266.

Keywords Breast reduction · Freestyle · Perforator flap · Nipple necrosis · Nipple ischemia

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Introduction

Necrosis of the nipple–areolar complex (NAC) is one of the most devastating complications of reduction mammoplasty. A variety of pedicles and skin patterns have been performed to avoid ischemic affection of the nipple–areola complex such as the inferior pedicle, the central pedicle, vertical bipedicle, transverse bipedicle, the superior, superolateral and superomedial techniques. In all these techniques, NAC vitality may be affected resulting in ischemia, with partial or total loss of the nipple–areola complex [1]. The reported rates of nipple necrosis vary with the use of different pedicles ranging from 0.8 to 2.3% with the inferior pedicle, 2.1% total nipple necrosis with the use of the superodermal pedicle and 2.3% with the superolateral pedicle technique [2].

With multiple variations, the breast is supplied with blood from three sources: branches of the internal thoracic artery, the axillary artery, and the intercostal arteries. In 18% of individuals, the breast may be supplied with blood

from the internal thoracic, the axillary, and the intercostal arteries. In 30%, the contribution from the axillary artery is negligible. In 50%, the intercostal arteries contribute little or no blood to the breast. In the remaining 2%, other variations may be found [3].

In another study, the source of the blood supply to the NAC was found to be via perforating branches of the internal thoracic artery in 48.8%, as well as from the anterior intercostal arteries in 24.4%, from the lateral thoracic artery in 23.2% and in 2.4% of the cases the NAC is supplied by a branch originating from the axillary artery which was described by Manchot as the superficial thoracic artery. The posterior intercostal arteries are of less importance, contributing only 1.2% of the blood to the NAC [4].

A variable, asymmetrical pattern of blood supply from the main sources was found in the same individual. In many cases, the right breast pattern of supply to the NAC differs from that of the left breast in the same cadaver. Anastomoses between the main sources of blood are abundant, and by examination of them, a basic pattern could be detected. Whereas various anastomoses were found, the most common were observed between the internal thoracic and lateral thoracic arteries, between the internal thoracic and anterior intercostal arteries, and between the anterior intercostal arteries and the lateral thoracic artery [5].

The internal mammary perforators enter the superior medial portion of the breast via the second through sixth intercostal spaces [6]. The second and third perforators are the predominant of these perforating vessels. Because of their larger caliber, the second or third perforators are the preferred recipient vessels for free tissue reconstruction using the internal mammary perforators [7].

The lateral thoracic or external mammary artery supplies the superolateral aspect of the breast. This vessel is a primary branch of the axillary artery and enters the breast after passing around the lateral border of the pectoralis major muscle at the inferior aspect of the axilla. It distributes its branches in the upper outer quadrant of the breast [8].

The lateral intercostal vessels represent an additional important blood supply of the breast. The lateral breast receives anterior intercostal arteries from the third through sixth interspaces. These vessels perforate the serratus anterior just lateral to the pectoral border. The lateral intercostal vessels enter the breast at the anterior margin of the latissimus dorsi to supply the lateral breast and overlying skin. The medial intercostal perforators are responsible for direct supply of the inferior central portion of the breast inferior to the nipple–areolar complex. These perforators pass from deep to superficial to supply the breast parenchyma as well as the nipple and nipple–areolar complex [9].

To keep the viability of the NAC at the designed pedicle, we present a method done using the colored duplex scan and handheld Doppler to perform freestyle perforator flap reduction mammoplasty.

Patients and Methods

The purpose of this study was to introduce a breast reduction technique designed to reduce the incidence of postoperative nipple–areola complex ischemia and necrosis, at the same time allowing all the other goals of breast reduction to be realized.

This is a prospective randomized study that has been performed on 105 patients in the period between January 2014 and 2019 at the Plastic and Reconstructive Surgery Department, Tanta University Hospitals.

Criteria for Patient Selection

Inclusion Criteria

Female sex, variable age-groups, variable marital or maternal status, and suffering from macromastia.

Exclusion Criteria

Patients having systemic diseases such as diabetes mellitus and/or bleeding tendency. Patients who are smokers were avoided unless they stopped smoking 4 months before surgery.

This study included female patients suffering from breast hypertrophy which mandated removal of 750 g of breast tissue or more. Variable age-groups, variable marital, and maternal status.

Signed consent was taken from each patient including the possibility of complications, agreement for clinical photography, and the possibility of using their data in medical publications.

Operative Techniques

All marking is done preoperatively with the patient seated, with the surgeon facing her, and with her arms at her sides. Midline was marked by connecting the suprasternal notch to the umbilicus (Fig. 1a). The inframammary fold was marked, and a point opposite to it was marked at the midline (Fig. 1b). The highest point of the new nipple–areola complex will be at the breast meridian along a line passing this point (Fig. 1c). Sometimes, the inframammary level was different on both sides, and in this case, the marking opposite to the higher side was chosen.
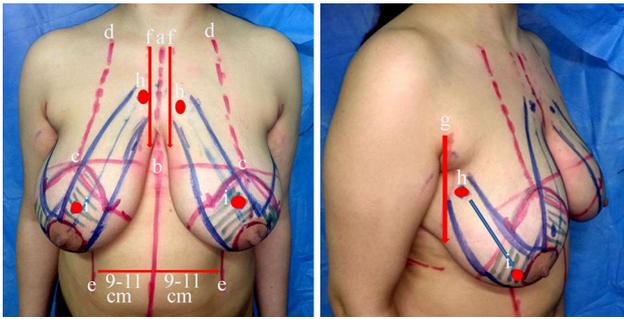
Fig. 1 Preoperative measurements and marking of breast with the patient in an upright position. (a) Midline was marked by connecting the suprasternal notch to the umbilicus (b). The inframammary fold was marked, and a point opposite to it was marked at the midline. (c) The highest point of the new nipple–areola complex will be at the breast meridian along a line passing this point. (d) Breast meridian was outlined by a line drawn from mid-clavicular point to the midpoint of the breast. (e) Another two vertical lines were drawn on the abdomen parallel to the midline and lateral to it by about 9–11 cm on each side to the level of inframammary fold. (f) Detection of the perforators supplying the NAC along a line parallel to and about one inch lateral to the sternal border for the internal mammary artery (IMA) perforators. (g) Same search was done along the anterior axillary line for the lateral thoracic artery (LTA), (h) along the upper pole of the breast for thoracoacromial artery perforators, and along the inferior pole of the breast for intercostal perforators, these markings represented the proximal perforators. (i) The probe was then placed at the breast around the areola. Strongly pulsed perforators around areola were marked; those represented the distal end of the perforator

The breast meridian was outlined by a line drawn from the mid-clavicular point to the midpoint of the breast (Fig. 1d). Another two vertical lines were drawn on the abdomen parallel to the midline and lateral to it by about 9–11 cm on each side to the level of inframammary fold (Fig. 1e). The areola was marked as a 42-mm-diameter circle in a sitting position. The breast vertical lines were drawn by rotating the breast laterally and cranially to first draw the medial vertical limb. Then, the breast was rotated medially and cranially to draw the lateral limb. The vertical lines will join each other at the bottom 2–4 cm above the inframammary fold (IMF), according to the size of the breast.

Preoperative Perforator Detection and Its Markings

While patients were in a supine position, a handheld Doppler, Bidop ES-100V3, with 10 MHz probe was used and detection of the perforators supplying the NAC was performed with the most audible signal. This was detected along a line parallel to and about one inch lateral to the sternal border for internal mammary artery (IMA) perforators (Fig. 1f). The same search was done along the anterior axillary line for the lateral thoracic artery (LTA) (Fig. 1g). Also detection of the perforator along the upper

pole of the breast for the thoracoacromial artery perforators was done, and along the inferior pole of the breast for the intercostal perforators. These markings represented the proximal end of the perforator (Fig. 1h). The probe was then placed at the breast around the areola; the probe was then traveling medially, laterally, caudally, and cranially. Strongly pulsed perforators around areola were marked; those represented the distal end of the perforators (Fig. 1i).

A complementary colored duplex ultrasonography, ACUSON X300 Ultrasound System, would be done to confirm these points of major perforators (Fig. 2). Vessels that had pulsations at the medial, lateral, upper, or lower poles of the breast and at the same time had pulsations around the NAC in a sectorial pattern were marked to be included in the pedicle as a freestyle flap. Then, the pedicle is designed to include the detected proximal perforator and the distal perforator at the areola.

Operative Steps

Operations were performed under general anesthesia, and the designed pedicle was deepithelialized starting from the outer margin of the nipple–areola complex (NAC) to the edge of the skin that will not be excised, as keeping this part of the dermis around the nipple will preserve the superficial veins (Fig. 3a). The dermis of the pedicle was divided just at the junction of the deepithelialized area with the remaining breast skin, and then subcutaneous dissection of the skin, using an intraoperative handheld Doppler, was performed keeping the skin thickness around 1–2 cm and stopping before the marked site of the previously marked perforators to preserve the arterial perforators of the pedicle.

With the aid of an intraoperative handheld Doppler, dissection of skin flaps medially was performed 1 cm lateral to the marked perforator if present (Fig. 3b), also laterally dissection was performed one centimeter medial to the marked perforator if present (Fig. 3c), superiorly up to the second intercostal space, and inferiorly up to the inframammary fold.

In the medially based freestyle perforator flap technique, skin flap dissection was stopped 1 cm lateral to the site of the pre- and intra-operatively perforators detected with Doppler to avoid its injury (Fig. 3b). Then, resections were performed above, below, and lateral to the pedicle as needed to achieve the required breast size.

In the laterally based freestyle perforator flap technique, skin flap dissection was stopped 1 cm medial to the site of the intraoperative Doppler-detected perforator (Fig. 3c), and then, resection was performed above, below, and medial to the pedicle as needed to achieve the required breast size.
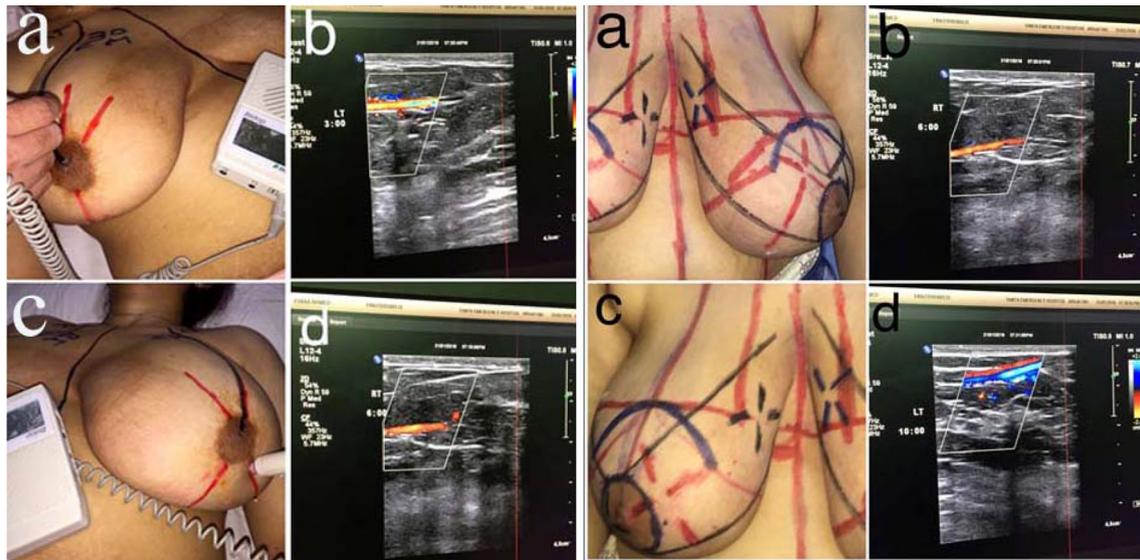
Fig. 2 a–d Using of a handheld Doppler in detection of the perforators of the pedicle and confirmation of the presence of the perforators using colored duplex ultrasonography
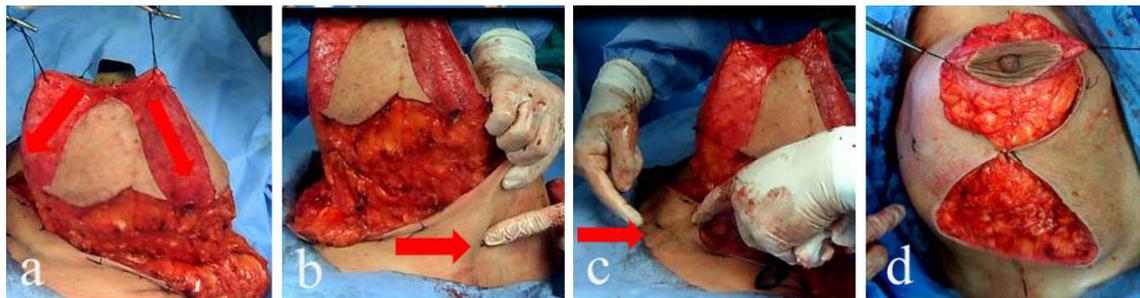
Fig. 3 a–d Deepithelialization of the pedicle and dissection of the skin to 1 cm before reaching the perforator site to avoid its injury and then breast shaping

In the inferiorly based freestyle perforator flap technique, dissection was stopped 1 cm above the site of the Doppler-detected perforators and then, resection was performed above, medial, and lateral to the pedicle as required to achieve the desired breast size.

In the superiorly based freestyle perforator flap technique, dissection was stopped 1 cm below the site of the Doppler-detected perforator. Then, resection was performed laterally, medially, and below the pedicle as required to achieve the desired breast size.

In the bipedicle, medially and laterally based freestyle perforator flap technique, dissection was stopped 1 cm medial and 1 cm lateral to the site of the intraoperative Doppler-detected perforators, and then, resection was performed above and below the pedicle as needed to achieve the required breast size.

The NAC was then transferred to the new site. The vertical and periareolar incisions were closed in two layers so that the vertical limb is 6–8 cm in length (Fig. 3d). A short horizontal limb is added as needed to deal with the dog ear formed at the lower end of the vertical incision. Gauze dressing and adhesive tape were applied.

The procedure was performed as freestyle on medial perforators in 35 cases, on lateral perforators in 20 cases, on combined medial and lateral perforators in 30 cases, on upper pole perforators in 12 cases, and on lower pole perforators in eight cases.

Patients were followed up postoperatively after discharge from the hospital after 1 week, 1 month, 3 months and 6 months or more. Preoperative and postoperative photographs (taken after 1, 3, and 6 months) were evaluated by the patient and three professors of plastic and reconstructive surgery not sharing in the work.

Results

This study was conducted on 105 patients complaining of large-sized breasts, admitted to the Plastic and Reconstructive Surgery Unit in Tanta University Hospitals in the period from January 2014 to 2019.

For all patients, preoperative detection of perforating vessels supplying the nipple–areola complex using a handheld Doppler and confirmed by colored ultrasonic Doppler was performed.

The excised tissue (in grams) was 750 ± 235.9 and the NAC elevation (in cm) was 14.4 ± 94. The procedure was performed as freestyle on medial perforators in 35 (33.3%) cases, on lateral perforators in 20 (19%) cases, on combined medial and lateral perforators, as a transverse bipedicle in 30 (28.7%) cases, on upper pole perforators in 12 (11.4%) cases, and on lower pole perforators in eight (7.6%) cases.

In the medial 35 cases, one perforator was found to come from the second intercostal space and reach the NAC in nine cases (25.7%), and also one perforator was found to come from the third intercostal space and reach the NAC in 11 (31.4%) cases. Two perforators came from the second and third intercostal spaces and reached the NAC in eight cases (22.9%), and two perforators came from the third and fourth intercostal spaces and reached the NAC in seven (20%) cases.

In the lateral 20 cases, a single perforator was found to come from the lateral thoracic and reach the NAC in ten cases and in the other ten cases (50%), two perforators were found to come from the lateral thoracic and reach the NAC in ten cases (50%).

At the upper pole of the breast, only a single perforator was found to come from thoracoacromial artery and reach the NAC in 12 cases. At the lower pole of the breast, a single perforator was found to come from the anterior intercostal arteries and reach the NAC in eight cases. In the 30 bipedicle cases, at the medial side, a single perforator from the second intercostal was present in 12 cases, a single perforator from the third in 14 cases and two perforators from the second and third in four cases.

At the lateral thoracic artery side of the bipedicle flap, a single perforator was found in 15 cases and two perforators in 15 cases (Table 1).

The NAC viability in the immediate postoperative course was good in 98 cases (93. 5%) and questionable in seven cases (6.5%). No case experienced NAC necrosis. All the seven ischemic cases were arterial ischemia. We have not been confronted with venous ischemia. Ischemic affection of the NAC occurred in three cases out of the 20 laterally based freestyle perforator flaps cases (15%), in two cases out of the eight cases of inferiorly based perforator flap (20%), and in two cases out of the 12 superiorly based perforator flap cases (16.7%). All seven cases healed under daily dressing using fusidic acid ointment and soaked saline dressing but showed areas of hypopigmentation. No affection of the NAC occurred in the medially based perforator flap cases.

The age of patients ranged between 24 and 54 years with a mean age 35.3. In medially based perforator flap patients (35), the suprasternal notch-to-nipple (SSN–N) distance ranged between 33 and 47 cm with a mean of 37.5 cm. In laterally based perforator flap patients (20), the SSN–N distance ranged between 34 and 43 cm, with a mean of 35.5 cm. In combined medially and laterally based perforator flap patients (30), the SSN–N distance ranged between 34 an 45 cm, with a mean of 36.5 cm. In superiorly based perforator flap patients (8), the SSN–N distance ranged between 33 and 41 cm, with a mean of 33.5 cm. In inferiorly based perforator flap patients (12), the SSN–N distance ranged between 33 and 46 cm with a mean of 37 cm (Figs. 4, 5, 6, 7).

Discussion

Breast hypertrophy has been and will be always a challenge to breast surgeons, who are always trying to address the problem and look for perfection. At the last few years, breast reduction techniques became numerous, but there is no specific technique that is suitable for all cases. NAC necrosis is one of the most annoying complications of reduction mammoplasty, and so, safety is mainly dependent on defining blood supply to the NAC [10].

In an anatomical study, Chiari found variations in the blood supply of the breast between the left and the right sides of the same cadaver in the form of partial or complete absence of branches to the NAC from the main sources. So, he concluded that it is difficult for a surgeon to suspect the blood supply of the breast that is to be operated upon [11]. This was also our observation during perforator detection where we found that in 21 cases the perforator supplying the NAC differed between the right and left breasts of the same patient.

NAC necrosis was reported to be 6% in the superior pedicle technique of reduction mammoplasty and 1.28% in the superolateral pedicle technique. [12]. In all our 105 cases, we had no one case of NAC necrosis, but seven cases (6.7%) suffered from NAC ischemia which healed under conservative treatment but resulted in patchy hypopigmentation.

To decrease the incidence of NAC necrosis, some authors tried a preoperative detection of the course of the arteries supplying the NAC and included them in their pedicles. Hall-Findlay [12] documented 126 breasts in 63

Table 1 Type of operation, number of perforators, NAC ischemia or gangrene, and SSN–nipple distance

Type of operation	Medial pedicle (35)	Lateral pedicle (20)	Lateral and medial pedicle (30)	Superior pedicle (12)	Inferior pedicle (8)
Perforator number	Single 20	Single 10	Single 35 (M& L)	Single 12	Single 8
	Double 15	Double 10	Double 25 (M&L)		
NAC ischemia	0	Three of single perforator cases	0	2	2
NAC gangrene	0	0	0	0	0
SSN–N distance	33–47 cm	34–43	24–45	33–41	33–46
Mean	37.5	35.5	36.5	33.5	37

Fig. 4 A case representing the transverse bipedicle flap technique in a 38-year-old patient with a SSN–N distance of a 38 cm, resection weight of 850 g from each breast, pre- and postoperative results showing good nipple vasculature and nice breast shape

patients with a predictable pattern of internal mammary artery perforators in true superomedial pedicled flaps.

Horta et al. reported the preoperative use of Doppler ultrasound for detection of perforators in 22 cases of breast hypertrophy. They reported excellent results with an average resection weight of 820 g per breast, and the incidence of NAC necrosis was nil [13].

In our study, we have used a predetermined perforator using a handheld Doppler and confirmed it by colored duplex scan in 105 cases. We have not encountered any nipple necrosis.

Seitz et al. [14] reported that there may be an absence of one or more of the branches of the internal mammary or lateral thoracic artery and the remaining branches constitute the dominant blood supply to NAC. This was also what we found that there is a great variability in the blood supply to NAC even in the same individual. This is what we have observed during our study, where we found that out of the medial pedicle cases (35), the perforator comes from the second intercostal space in nine cases, from the third intercostal space in 11 cases, from the second and third intercostal spaces in eight cases and from the third and fourth intercostal spaces in seven cases. As regards branches of the lateral thoracic artery, it reached the NAC, as a single perforator in ten cases (9.5%) and as two perforators in another ten cases (9.5%). So, we recommend the use of

Fig. 5 A case representing the medial pedicle technique in a 31-year-old patient with a SSN–N distance of 34 cm, resection weight of 750 g from each breast, pre- and postoperative results showing good nipple vasculature and nice breast shape

Fig. 6 A case representing the lateral pedicle flap technique in a 41-year-old patient with a SSN–N distance of 39 cm, resection weight of 950 g from each breast. Pre- and postoperative results show partial nipple ischemia of left breast that healed under daily dressings

Fig. 7 A case representing the superior pedicle flap technique in a 29-year-old patient with a SSN–N distance of 33 cm, resection weight of 750 g from each breast. **a–c** Before, early, late, right oblique view of the patient with sound healing of the right breast. **d–f** Before, early, late left lateral view of the same patient, showing partial NAC ischemia which healed under daily dressings leaving patchy hypopigmentation (white arrows)
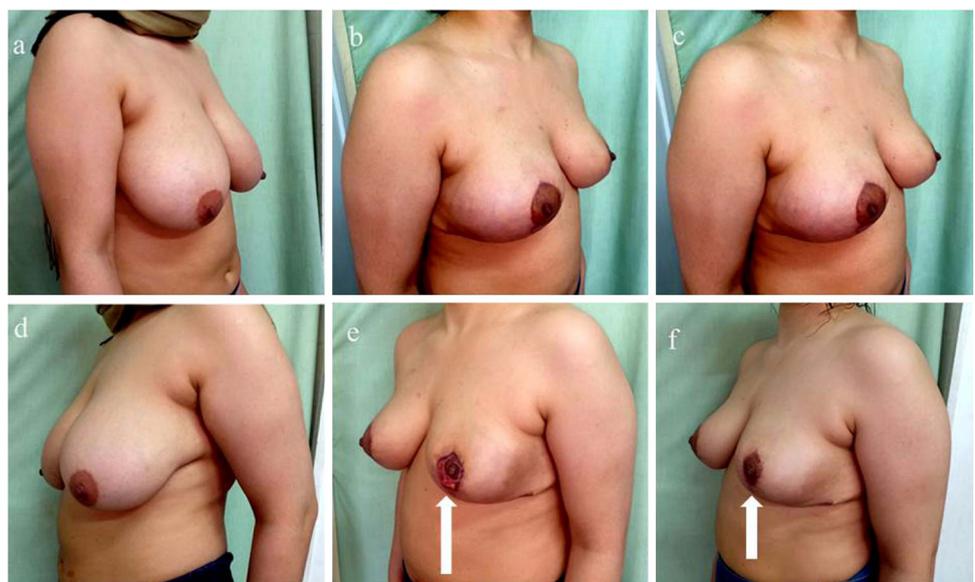
preoperative handheld Doppler to detect the sites of emergence of these dominant vessels with the strongest signal and detect that they are included in the pedicle used during the procedure.

The dependence on maintaining the medial and lateral pedicle attachments to the chest wall does not assure the safety of the pedicle used as one or both of these random flap attachments may not contain a blood supply to the NAC [15]. But in our 30 bipedicle cases, we were depending on the presence of perforators that have been detected by handheld Doppler and confirmed by colored duplex scan; that is why we have not encountered any cases of NAC necrosis. In our cases (105 patients), we found that the dominant perforators were found to come from the internal mammary artery in 35 cases, from the lateral thoracic artery only in 20 cases, from both arteries in 30 cases, from anterior intercostal arteries in eight cases, and from the thoracoacromial artery in 12 cases. Therefore, none of the cases of our study experienced NAC necrosis. But ischemia occurred in seven cases where the supplying perforators were small in size and with low power signals.

The classic medial pedicle without use of Doppler in marking may not be vascularized, and as in an anatomical study, the blood supply of superomedial pedicle came from the first and fourth internal mammary artery perforators with the absence of the second and third. The other breast in the same cadaver was supplied with the third perforator of the internal mammary artery with the first, second, and fourth perforators absent [16]. This was our observation that in our 35 medial cases, the perforator came from the second intercostal space in nine cases, from the third intercostal in 11 cases, from the second and third intercostal spaces in eight cases, and from the third and fourth intercostal spaces in seven cases.

This is a clear example that demonstrates the basis of our technique and the significant value of preoperative detection of perforators.

We found that the most common location of the vascular pedicle was medially from the internal mammary artery. It was present in 65 out of 105 cases (60.2%). The main perforator that supplied the nipple–areola complex was mainly from the third intercostal space in 25 cases, but it may come from the second in 21 cases or from both second and third in 12 cases, or from the third and fourth intercostal spaces in seven cases.

In our study, we found that the lateral thoracic artery perforators were the only supply to the NAC in 20 cases (19%) out of 105 cases. It reached the NAC as a single perforator in ten cases and as two perforators in another ten cases. The thoracoacromial was found to be the only supply to the NAC in eight cases. The anterior intercostal perforators were found to be the only blood supply in 12 cases.

In our study, we found also that internal mammary artery perforators (mainly second and third) run at distances of 10.3 and 4.2 mm from the skin surface, respectively. The accompanying veins run at 2.6 and 3 mm. So, the incisions we made at the dermal level of the base of the pedicle do not affect the perforators that run in the pedicle and at the same time, allow for shaping, maximum mobility, and rotation of the pedicle.

The venous drainage of the breast is composed of superficial and deep systems. The deep system includes the perforating branches of the internal mammary and posterior intercostal veins and tributaries of the axillary vein. The superficial system runs just deep to the superficial layer of the superficial fascia and includes transverse veins (91%), running medially to end in internal mammary vein perforators, and longitudinal veins (9%) running superiorly to empty in superficial veins of the lower neck [17].

Matthew et al. reported that although more common than arterial insufficiency, venous congestion of the NAC during breast reduction surgery is a relatively uncommon complication. They were confronted with a case of postreduction mammoplasty severe congestion, and they used leeches as treatment with successful results [18].

In our cases, we kept the dermis around the NAC and we also keep the dermis at the medial end of the pedicled flap. This helped to keep the superficial veins so we have no cases of venous congestion. Also in this work we performed all cases as freestyle perforator flaps so there is no kink for the deep veins of the flap. All this helped us to have no cases with venous congestion. Accordingly, medial, lateral, or the transverse bipedicle used in our cases includes most of the transverse veins that constitute most of superficial venous drainage of the NAC and this might help in the absence of venous gangrene in our cases.

Some surgeons attributed decreasing the incidence of NAC to the preservation of the horizontal breast septum that was described by Würinger et al. [19] and so improving the NAC vascularity. However, in our study, we found that the blood supply to the NAC was in sectorial manner and not through a septum.

O'Dey et al., in an anatomical microdissection study, stated that vascular variability and overlap may account for the remarkable safety of diverse nipple areola complexbearing pedicles, even though pedicle thickness influences vascular reliability. They observed that lateral and medial approaches, however, clearly show vascular advantages over that which can be observed in inferior and superior pedicles [20]. This was also our observation that out of 105 cases, only 12 cases were supplied by thoracoacromial artery and eight cases were supplied by anterior intercostal arteries.

Setala et al. [21] reported that with a body mass index (BMI) above 30, there is increased rate of complications,

especially nipple necrosis. Roehl et al. [22], in a retrospective study of 179 reduction mammoplasty patients, concluded that breast reduction is a safe operation for patients regardless of their BMI or size of reduction and with no increase in the rate of postoperative complications in the morbidly obese patients. In our study, the mean BMI in our patients was 28.4 ± 4.1. We found that the freestyle perforator-based reduction mammoplasty technique did not affect the rate of complications regardless of the weight status.

Conclusion

A variable, asymmetrical pattern of blood supply to the NAC may be found in the same individual. The use of handheld Doppler and the confirmation by duplex scan are recommended to decrease the incidence of NAC necrosis or ischemia. Freestyle perforator flap breast reduction operations were a safe one for patients regardless of their BMI.

Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest to disclose.

Ethical Approval All the procedures performed in this study that involved human participants were in accordance with the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or ethical standards. Accepted principles of ethical and professional conduct have been followed. This article was approved by Tanta University's Ethical Committee (2013/12/21).

Informed Consent Informed consent was obtained from all individual participants included in the study, and additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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