Research Article

Effect of Adding Platelet Rich Plasma and/or Nanofat on the Viability of Autologous Facial Fat Grafts: A Prospective Randomized Study using Volumetric CT Scan

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Abstract...

Volume retention remains a significant problem during the increased use of fat grafting in reconstructive and cosmetic surgery.

Aim of the work: To compare the effect of adding Nanofat and /or PRP to fat grafting to evaluate its effects on facial skin quality and on volume retention rate of the grafted fat using volumetric CT scan.

Design of the study: 60 patients seeking for a small volume ≤100 ml fat grafts (to enhance their facial appearance) were enrolled in this study between December 2019 and January 2022. Patients were randomly divided into three groups. Group A, B and C (where we added 20% of grafted fat as nanofat and/or PRP on the right side of the face. The left side of the face was taken as a control.

Patients and methods: Fat was harvested from the lower abdomen. The grafted fat was partially used for augmentation and partially emulsified to obtain a nanofat suspension. PRP was prepared by a double spin method. Volumetric scan was done before and 6 months after grafting to calculate resorption rate of grafted fat.

Results showed that no statistically significant effects of adding Nanofat and/or PRP on the resorption rate in all groups. Clinical results were excellent in 88% of cases without complications.

Conclusion: Adding Nanofat and/or PRP to the grafted fat had no statistically significant effect on reducing the resorption rate (P ≥ 0.05). Enhancement of the skin quality was reported on both sides of the face.

Abbreviations: PRP: Platelet rich plasma; ADSCs: Adipocyte Derived Stem Cells; AMFG: Autologous MicroFat Graft. SVF: Stromal vascular fraction; ASC: Adipocyte stem cell.

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Introduction

Volume retention remains a significant problem during the increased use of fat grafting in reconstructive and cosmetic surgery [1-4]. Controversy about infiltration, harvest, preparation, or placement techniques still present making a difficulty in reaching a consensus regarding the optimal technique [2]. To minimize these variables, we used the same technique and the same donor site (lower abdomen) and compared left and right side in the same patient in this prospective randomized clinical study. Adding PRP to fat injection may be a reliable way to bringing nutrients early and improves fat survival [3]. Nanofat grafting emulsifies fat and increases the progenitor cell, adipose-derived stem cells, endothelial progenitor cells, and soluble factors, this contributes to the regenerative effect of fat grafts [4,5]. In the current study, we compare the effect of adding 20% nanofat alone, 20% PRP alone and adding both PRP and nanofat 20% (10% each) to the grafted fat in the right side of the face to test their effect on the skin quality and on the resorption rate of the grafted fat using the volumetric CT scan. We used the left anatomical site as a control to rollout the effect of intrinsic factors that may affect the survival of fat grafts from one person to another. Due to the high number of SVF cells and elevated levels of MMP-9, IGF-1bFGF, and PDGF, Pallua et al suggested that abdominal wall would be the optimal site for at harvesting [6].

Patients and methods

Inclusion criteria were patients seeking for facial enhancement while exclusion criteria included patients with blood disease (hemophilia, coagulopathy), anemic patients (HGB<10 gm/dl), sever systemic disease and very thin patients with no donor site for liposuction.

All patients were subjected to:

Preoperative work up: Informed written consent for operation and for photography was obtained from all patients. Patients were informed that they might need another fat grafts after 6 months if they were not satisfied by the results. Local examination of the skin of the face (quality, texture, elasticity, tone, firmness, wide pores, and pigmentation). Investigations: including the routine laboratory work-up. Multislice CT with volumetric measurement before and 6 months after operation. (Figure 1).

Operative work up: PRP was prepared by manual double spin method. Thirty to sixty ml of blood was withdrawn and initially centrifuged at 800 rpm for 8 minutes. The separated plasma was collected and centrifuged again at 1,200 rpm for 12 minutes to obtain a small pellet of platelet concentrate. The lower one third of the plasma concentrate was used as PRP discarding the upper two third [6]. Fat preparation: Fat was collected using the Coleman method with some modifications [17]. Depending on each patient's condition, local or general anesthesia was used. Suitable amount of tumescent solution (0.9%saline 500 mL, lidocaine 15 mL, and half mL of epinephrine) was infiltrated into the donor site (lower abdomen). All patients had prophylactic antibiotic (Cefotaxime 1 gm.) with the induction of anesthesia and another dose 24 hours postoperatively. Using a 3 mm cannula with multiple sharp side holes attached to a 20 mL syringe, fat was harvested applying a gradual low negative pressure in the syringe. This was followed by low-speed centrifugation at (1000 rpm/min for 2 min). The harvested micro fat was partially used to increase volume. Another part was emulsified manually by at least thirty passes through a 10 mL syringes connected with a female Luer- Lock to get a nanofat which was left to decant for 60 minutes, the oily layer was discarded, and the yellow layer was used to be added alone or with PRP to the grafted fat. The patients were randomly divided into three groups. Group A (where we added 20 % of grafted fat as nanofat on the right side of the face), B (where we added 20 % of grafted fat as PRP on the right side of the face) and group C (Mixed where we added Nano 10% and PRP 10% of the grafted fat on the right side of the face). The left side of the face was taken as a control where we add normal saline (similar volume of PRP or Nanofat) to the grafted fat. Fat injection in the face was done on a similar mirror image sites on the left and right side of the face through a 2 mm port at Tragus crease (see Video, Supplemental Digital Content 1). The injecting cannula was 12 G in diameter and vary in length (5-9 cm) according to the volume and area to be grafted. Only a blunt tip injection cannula was selected to avoid accidental intravascular injection.

Follow up: Clinical evaluation was performed by three plastic surgeons from our department not involved in this work, through an overall score from 1 to 4 (poor, fair, good, and excellent). Overall satisfaction rate was reported by the patients according to the survey sheet answered by all of them after 6 months (Figure 2). The questions covered the improvements in skin quality (firmness, dilated pores, wrinkles, fine lines, skin tone, brightness/glow of the skin, rejuvenation of the face as a whole and the relative opinions as well). Each parameter had 1-10 score and the patient put the score of improvement. Distribution of injected fat on the face: The site of injections was on temple area, periorbital, malar, cheeks, and chin. The same sites on the face were injected as a mirror image.

Radiological evaluation

Volumetric CT was done twice one before operation and another one 6 months postoperative using a multislice 320 MDCT scanner (Toshiba Medical Systems, Japan). An axial volume acquisition using 0.5 mm slice thickness and 0 mm spacing was used, and dose reduction protocol was activated to avoid excessive exposure to radiation. The acquired images were transferred to a post processing workstation that had Slicer 3D opensource software. Fat was traced using a semi-automatic process in the consecutive axial images, allowing for high quality images for volumetric analysis and 3D views. Volumes were recorded in pre and postoperative scans for comparison and statistical analysis. Objective assessment of grafted fat was done by calculating the resorption rate of fat grafts before, after 6 months of grafting using the volumetric CT scan (Figure 1).

The resorption rate % was calculated as follow:

$$\frac{Volume\ of\ injected\ fat\ on\ each\ side - Volume\ after\ 6\ months}{Volume\ of\ injected\ fat\ on\ each\ side} \times 100 = Resorption\ rate\ \%$$

Retention volume % = 100 – Resorption rate

Statistical analysis: Record data were analyzed using the statistical package for social sciences SPSS 26ed. Normal distribution and homogeneity tests were performed on all data. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

* A one-way analysis of variance (ANOVA) when comparing between more than two means.

* Chi-square (X 2) test of significance was used to compare proportions between two qualitative parameters.

* The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the P-value was considered significant at ≤.05, highly significant at ≤0.01 and insignificant if P-value ≥0.05.

Results

Sixty Patients were enrolled in this study (54 female and six male). Mean age was 48.8 ± 4.03 (range 38.5 - 59 years). Mean BMI was 27.8 ± 1.4 (range 24.8 - 32.3). All patients were followed-up over a minimum 6 months. Clinical aesthetic results judged by plastic surgeons committee not sharing in this study were excellent in 53 cases (88%) without complications, good in 5 cases and fair in 2 cases without improvement in the scar after 6 months. Results of the questioner survey answered by the patients after 6 months showed improvement of the skin quality on both sides of the face (Figure 3):

* Highest improvement (score 87%) was in the brightness and glow of facial skin. Followed by improvement in skin firmness (84%), then rejuvenation of the face as a hole with score 81% (Figures 4,5,6).

* Improvement of circles around eyes in 55% of cases (Figure 6).

* Fine lines and wrinkles improvement was reported by 67-68% of patients (Figures 4,6).

* Improvement in the dilated pores of facial skin reported by 64% of patients (Figures 5,6,7).

* Relative opinions about the general improvement of the face were reported by 73% of patients.

* The lowest improvement was in the facial scars and reported by 52% of the patients (Figure 8).

* Resorption rate in male was highly significant compared to female patients (Figure 9).

Preoperative volumetric CT scan showed no significant difference between right and left side (P = 0.17) of the targeted areas on the face (Table 1). Mean volume of fat in the left side was 26.9 ml ± 4.94 SD (rang was 19-35 ml), Compared to 27.8 ml ± 3.84 SD on the right side (range was 21-35). The data of all sixty patients were split according to the type of additive on the fat grafts into three groups, A (Nano), B (PRP) and group C (mixed Nano and PRP) it showed the means of volumes of injected fat on each side, mean volume after 6 months and the resorption rate in each group (Table 2).

In group A (Nano fat group): Results showed that the resorption rate on the left side of the face was 31.7% (±2.66) compared to 28.9% (±4.02) which was statistically non-significant (P≥0.05).

In group B (PRP group): Results showed that the resorption rate on the left side of the face was 31.5% (±3.52) compared to 26.5% (±3.88) which was statistically non-significant. (P ≥ 0.05).

In group C (mixed Nano and PRP): Results showed that the resorption rate on the left side of the face was 33.4% (±4.01) compared to 26.4% (±4.2) which was also statistically non-significant (P≥0.05). This difference had no effect on final patient satisfaction scores, or on the aesthetic outcome judged by the surgical team or by the patients. For example, a five ml. difference in soft tissue volume between the two sides of the face (distributed all over the side) will represent a statistically significant difference on a sensitive volumetric tool as CT, nevertheless clinically speaking this is usually not noticeable and we had no patients complaining of asymmetry. It was noted that that preoperative CT revealed a degree of variability in soft tissue volume between both sides of the face (Table 1). A one-way analysis of variance (ANOVA) comparing between means according to the type of additive in the three groups showed that there was no significant reduction in the resorption rate between groups after 6 months from fat grafting P≥0.05 (Table 3). In each group, Paired Samples Test showed no statistical difference as regards the resorption rate between left and right side of the face P≥0.05 (Table 4). According to the sex, male showed high significant increase in the resorption rate compared to female patients (P = 0.005). No significant or persistent complications were observed in any patients during follow-up (including fat cysts, infection, foreign body reaction, permanent discoloration, or other side effects). Paresthesia occurred in seven patients only who received large volume of fat graft and resolved conservatively within 2 weeks.
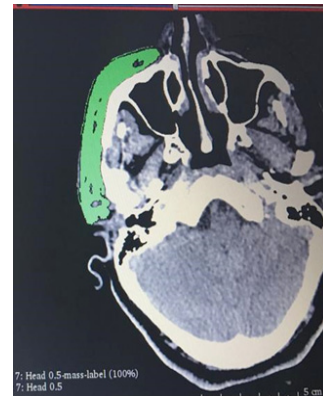
Figure 1: Axial CT scan of the cranium showing tagging of the subcutaneous fat in the right cheek as a step-in volume assessment.
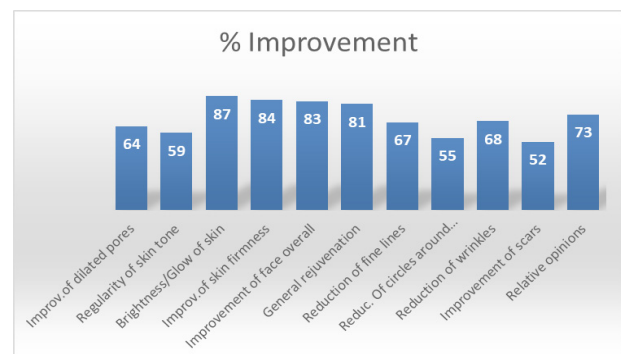
Figure 2: Results of improvements on facial skin parameters according to questioner survey.

Figure 3: Patient from group (A, nanofat): Pre (left) and 6 months post grafting (right).

Figure 4: Patient from group C (mixed nanofat and PRP): left side preoperative (left), 3 months postop. (middle), and 9 months postop. (right).

Figure 5: Patient from PRP group (B): Preoperative (left) and six months postop. (right).

Figure 6: Patient from group (A, nanofat): preoperative, (left) and 6 months postop. (right).

Figure 7: Patient from group B (PRP): Preoperative (left) and 6 months postop. (right).

Figure 8: Patient from group (A, nanofat): Pre (left) and six months postop. (right).

Figure 9: Patient from group A (nano): Pre (left) and six months postop. (right).

Table 1: Preoperative volumetric CT scan: Shows no significant statistical difference between the two sides of the face as regards to the volume of fat. (P = 0.17).

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Preop. CT. of fat on Lt. side Preop. CT. of fat on Rt. side	0.91667	5.13642	0.66311	-2.24354	0.41021	-1.382	59	0.172

Table 2: Descriptive Statistics according to additive type (Groups): Showing the mean of the volumes of injected fat on each side, volumes of fat remain on the left or right side and the resorption rate in each group.

Type of additive		N	Minimum	Maximum	Mean (ml)	Std. Deviation
(A) Nonofat	Vol.of in Lt or Rt side	20	18.00	38.00	**24.3**	5.60
	Vol after 6 months Lt. side	20	12.00	24.00	**16.5**	3.26
	Vol after 6 monthd Rt. side	20	13.00	24.00	**17.2**	3.28
	Resorp Vol % Lt side	20	27.30	36.80	**31.7**	2.66
	Resorp Vol % Rt side	20	22.70	36.80	**28.9**	4.02
(B) PRP	Vol.of in Lt or Rt side	20	19.00	40.00	**24.5**	5.68
	Vol after 6 months Lt. side	20	13.00	24.00	**16.6**	3.23
	Vol after 6 monthd Rt. side	20	14.00	26.00	**17.8**	3.41
	Resorp Vol % Lt side	20	27.30	40.00	**31.5**	3.52
	Resorp Vol % Rt side	20	21.70	36.00	**26.5**	3.88
	Vol after 6 months Lt. side	20	13.00	23.00	**16.7**	2.93
	Vol after 6 monthd Rt. side	20	15.00	25.00	**18.3**	3.03
	Resorp. Vol. % Lt side	20	27.30	41.70	**33.4**	4.01
	Resorp. Vol. % Rt side	20	20.80	33.30	**26.4**	4.20

Table 3: A one way analysis of variance (ANOVA) comparing between means according to the type of additive in the three group. Showing that there is no significant reduction in the resorption rate between groups (P ≥ 0.05).

		Sum of Squares	df	Mean Square	F	Sig.
Volume of inj. fat on Lt. or R.t side	Between Groups	11.200	2	5.600	0.189	0.828
	Within Groups	1689.400	57	29.639		
	Total	1700.600	59			
Volume after 6 months on Lt. side	Between Groups	0.633	2	0.317	0.032	0.969
	Within Groups	565.300	57	9.918		
	Total	565.933	59			
Volume after 6 months on Rt. side	Between Groups	13.233	2	6.617	0.628	0.538
	Within Groups	600.950	57	10.543		
	Total	614.183	59			

Table 4: Paired Samples Test showing insignificant improvement of resorption rate between left and right side of the face in each group. (P ≥ 0.05).

Type of additive			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
(A) Nonofat	Pair 1	Resorp. Vol. % Lt. side - Resorp Vol. % Rt. side	2.79500	2.19196	0.49014	1.76913	3.82087	5.702	19	0.000
(B) PRP	Pair 1	Resorp Vol % Lt side - Resorp Vol % Rt. side	5.01500	1.65697	0.37051	4.23951	5.79049	13.535	19	0.000
(C) Nano plus PRP	Pair 1	Resorp Vol % Lt. side - Resorp Vol % Rt. side	6.97500	2.25572	0.50440	5.91929	8.03071	13.828	19	0.000

Discussion

Volume retention remains a significant problem during the increased use of fat grafting in reconstructive and cosmetic surgery [1]. Because overall take rate of fat grafting by even experienced surgeons ranges from 50% to 90%, additional procedures are always necessary to achieve an optimal outcome [7-9]. We followed most authors recommendations on fat grafting, who stated that the cannula should attached to 10 ml syringe to provide slight negative pressure (the plug was withdrawn for 2 ml) [9-12]. It was proved that cytokines and growth factors are secreted by adipocyte derived stem cells (ADSCs) [10]. Our study had the advantage that we compared the effect on the same patient unlike other studies [11-13]. Assessing fat volume by CT is an objective sensitive method that can provide a better evaluation for the chosen fat graft techniques. "What is the percentage of volume loss (resorption) following fat grafting?" is a question we tried to answer in this study. Does the PRP or Nanofat enhance the sustainability of transplanted fat grafts? Does adding PRP and/ nanofat enhances the skin quality in the transplanted sites or this improvement was due to the grafted fat? Are questions that need also to be answered. CT volumetry was used to accurately quantify the degree of fat resorption. Our results showed that adding both Nanofat and/ PRP in a dose of 20% improves volume retention of the grafted fat but this improvement was not statistically significant in all groups (Tables 2,5). Nanofat grafting increases vascularization, neocollagenesis and tissue regeneration [13]. Cervelli et al observed that face fat grafting with PRP, showed 70% contour restoration after one year compared to only 31% in patients injected with fat only [14]. This was consistent with Gentile et al who observed contour restoration in 69% of patients treated with PRP and fat grafting after one year compared to 39% in patients treated without PRP [15]. Others concluded that the addition of PRP in a ratio 12:1 does not improve outcome [16]. One study showed a dose-dependent effect of the PRP on proliferation of ADSCs, the study used 4.5 mL of PRP on 25 mL lipograft (18%) [17]. Subcutaneous nanofat injection was used alone in another study for facial skin rejuvenation by modifying the pattern of the dermis [13]. All those previous authors did not compare the effect of adding Nanofat and/or PRP on the same patients to rollout the intrinsic or personal effects on fat resorption rate. To answer the second question whether adding PRP and/ nanofat enhances the skin quality in the transplanted sites or this improvement was due to the grafted fat? Our clinical aesthetic results and the results of questioner survey by the patients reported improvement in the facial skin on both sides of the face, so the local effect of adding PRP and/ nanofat can be excluded or can be explained by the effect of cytokines and growth factors derived from ADSC [10] or from progenitor cells and soluble regenerative factors present in lipoaspirate [4]. Facial rejuvenation and improving wrinkle depth can be obtained by microfat grafting so it is difficult to confine the definition of "skin rejuvenation" or "quality." in two studies lipografting gave a more homogenous skin color [19,20]. Improvement in skin elasticity was noticed in two studies [21,22], while others denied any improvement skin elasticity following fat grafting [25]. Other studies showed improvement in Skin texture [22-24]. Skin rejuvenation in the surrounding areas was reported by many after fat [25,26]. Recent scientific advances have shed more light on the mechanisms of adipose cell survival after autologous fat grafting. Both graft survival and graft replacement theories contribute to the outcome of fat graft retention [28]. Our results also showed that the resorption rate in males was significantly higher than in females (Table 5) probably because males are more active than females or due to different hormonal patterns in male and female.

Conclusion

Adding 20% Nanofat and/ PRP to fat grafts had no significant statistical effect on the improvement of fat survival rate in small volume autologous microfat graft (<100 ml). Improvement in the skin quality of the grafted areas on both sides of the face. Resorption rate in males was highly significant compared to female patients.

Recommendations: Application of this technique on a large number of patients in multicenter study is recommended.

Declarations

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