Study of the effect of platelet rich plasma (PRP) on structural fat grafting for breast augmentation

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Abstract

Introduction: Lipoinjection can be used to give extra-volume during breast enhancement surgeries and to correct various types of depressed deformities of the breast, but certain problems remain as unpredictability and low graft survival which are a major concern in plastic surgery. Different trials and techniques were done for enhancement of the take and vascularity of the fat grafting. Platelet Rich plasma plays an important role in the process of vascularization of the grafted fat due to its high content of Platelet Derived Growth Factor(PDGF).

Methods: 20 patients who were candidates for breast augmentation were included in the study and were divided into two groups; one had fat injection with PRP in the breast and the other had breast lipoinjection with Coleman's technique. A questionnaire follow up was designed on Likert scale on grades 0-4 evaluating the volume and fat survival with statistical analysis.

Results: Group B (with PRP) showed overall better results. All patients with PRP showed moderate to good projection post-operatively. 50% of Group B showed no further requirement of secondary procedure. Whereas group A showed moderate to large requirement for secondary augmentation.

Conclusion: The increase in the level of PRP and in turn PDGF during the first days after lipoinjection will enhance the graft vascularity and overall survival, thus there is a need to further studies for standardizing the level of PDGF needed to have better take & results with lipoinjection.

Introduction:

The transfer of autologous fat as whole grafts has been performed since 1890s and as injectable grafts since 1920s. However, it is only within the past 20 years that the popularity of autologous fat transfer grafting has gained popularity in the field of soft tissue augmentation.[1]

Lipoinjection can be used to give extra volume during breast augmentation surgeries and also to correct various types of depressed deformities of the breast, but certain problems still remain, such unpredictability and low rate of graft survival due to partial necrosis of the transferred fat cells.[2]

Platelet derived growth factors acts as a very potent mitogen for mesenchymal cells which include fibroblasts and smooth muscle cells. It also acts as a chemo-attractant for neutrophils and it stimulates collagen and matrix formation.[3]

It was proved that PDGF signalling plays an important role in vascular mutation, maturation and remodelling. It has been also shown that expression of PDGF can increase pericyte density in a study done by Gehmert et al, 2011.[4] He proved that the capillary network formation expressed by the adipose progenitor cells is dependent on PDGF signalling pathway.

The main activating factor found in Plasma rich Plasma(PRP) is PDGF. Gehmert et al has proved that PDGF constitutes the main growth factors present in PRP's.[4] Previous studies showed that there is an increase in the percentage of fat graft survival and improvement of the skin quality after the use of PDGF during soft tissue fat grafting. Breast

enhancement and augmentation occurred with a minimal loss of injectable fat amount or volume of breast.[5]

The purpose of this study is to report our early experience with breast fat grafts enriched with platelet-rich plasma.

Material and methods:

20 patients underwent lipofilling either with or without PRP (Platelet Rich Plasma) Factor. The study was performed in Ain Shams University Hospitals, from the period of April 2009 to April 2011. The patients were divided into 2 groups:
- Group A: Patients underwent Lipofilling without PRP.
- Group B: Patients underwent Lipofilling with PRP.

All patients passed our inclusion criteria:
- Patient that had no aesthetic procedure to the breast ever, with an age ranging from 20 to 50 years old.
- Patient with chronic illness like DM, malignancies, and systemic immune diseases were in this study, also patients aiming to have huge or bigger sizes of breast were not included.
- Major asymmetry in breasts and patients with breast ptosis grade 3 where excluded.

Technique:

All patients underwent the procedure under general anathesia. Harvesting of fat from patient is the first step of the procedure after injection of tumescent fluid (1ml adrenaline+500cc ringer solution+10cc xylocaine 2%). The fluid injected to fat aspirated ratio was 1:1.

The harvesting procedure will be performed by the traditional methods of liposuction using Mercedes cannula of 3mm and 4mm diameter with suction pressure of 200-700mmHg that can be achieved with syringe system.[6]

The liposuction areas will be determined according to patient's excess fat and needs. Preparation of fat that will be injected into the breast was done according to Collman's technique[6] of fat injection.

Based on Kakudo's[7] technique;
- The preparation of PRP of 30cc whole blood of patient will be introduced to a centrifuge device (after using of 3cc citrate to prevent clotting).
- 15 minutes of centrifugation at 3000rpm for gravitational separation of whole blood into three fractions: erythrocytes, platelets poor plasma & platelet rich plasma (PRP).
- A total of 3-5cc of PRP will be activated by adding calcium chloride then injected to breast after fat injection through multiple puncture with a syringe 3mm diameter in fashion of 4-5 punctures to each quadrant after dividing breast into 4 quadrants.

Follow up documentation will be complete with pre and post-operative photographs standardized and taken in three views antero-posterior, lateral, and oblique with follow up of at least 6 months. All photographs were analysed and placed on a uniform coloured background.

A questionnaire was developed to evaluate the aesthetic outcome in all patients with their two groups, each page of questionnaire contained pre and postoperative photographs of one patient with the three views mentioned. Each page contained several questions to evaluate the aesthetic outcome and to determine the impression of the viewer and their opinion about the volume of breast , projection and requirement for further augmentation. The answers numerically marked based on 4 points likart scale [0 "worst" & 4 "best"]. This objective /subjective method of analysis was popularised by Moolenburg.[8] Page order was randomized, mixing the pre and post-operative pages throughout the survey and completion of the questionnaire was without time limit.

Data management and analysis:

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

I. Descriptive statistics:
1. Mean, standard deviation ($\pm$ SD) and range for parametric numerical data, while median for non parametric numerical data.
2. Frequency and percentage of non-numerical data.

II. Analytical statistics:

1. Fisher's exact test: was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.
2. Mann Whitney Test (U test) was used to assess the statistical significance of the difference of a non parametric variable between two study groups.
3. Wilcoxon signed rank test was used to assess the statistical significance of the difference of an ordinal variable (score) measured twice for the same study group.

   P- value: level of significance
   -P>0.05: Non significant (NS).
   -P< 0.05: Significant (S).
   -P<0.01: Highly significant (HS).

Results:

Personal patient satisfaction was omitted from this study as a form of efficacy analysis. Our analysis relied totally on the statistical analysis of the questionnaire. Follow up Photographs where performed at 6 months post-operatively.

One patient showed signs of minor infection at the injection site. He was prescribed an antibiotic from the quillon group which resolved the infection within5 days. Six of our patients showed signs of ecchymosis post operatively all of which resolved within 10 days without leaving any skin pigmentation .follow up of the patients was performed for an average of 6 months and proved to be uneventful in all cases.

Before (without PRP)

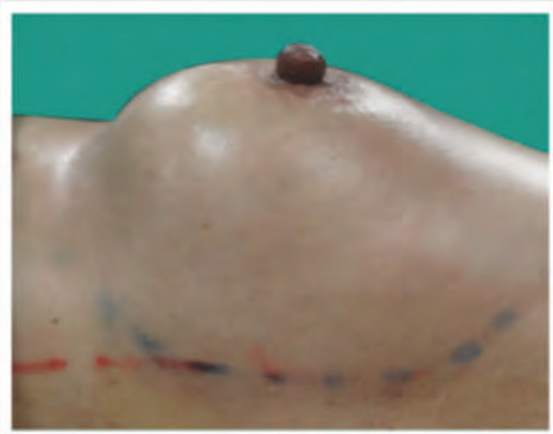*After (without PRP)*

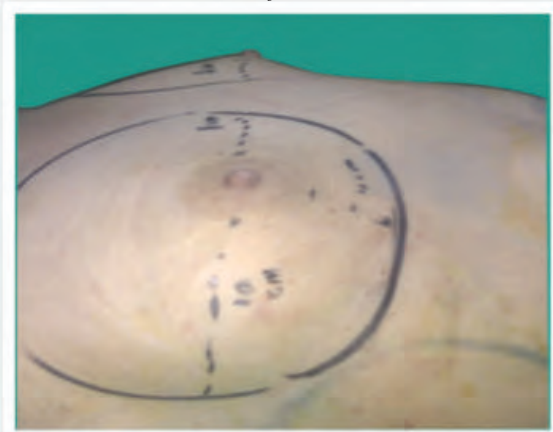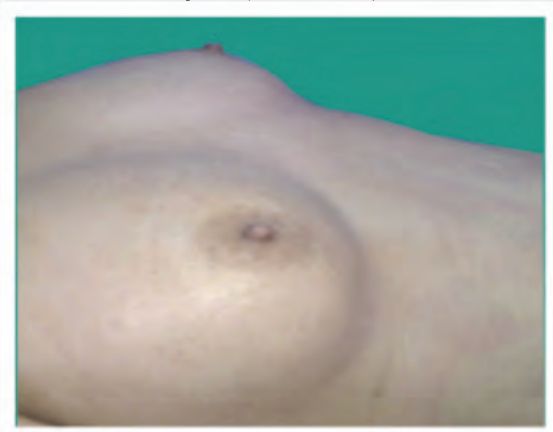*Before (with PRP)*

After (with PRP)

Before

After (with PRP)

Before (with PRP)

After (with PRP)

As for statistical analysis of the questionnaire, the results were all in favour of the patients that were injected with PRP enhanced fat.

Table (1): Description of comparison between post-operative cases among the two study groups as regard expert opinion.

		Group				P	Sig
		without PRPs		with PRPs			
		N	%	N	%		
Post-require augmentation	Major requirement	0	0.0%	0	0.0%	0.015	S
	Moderate requirement	4	40.0%	1	10.0%		
	Minimal requirement	6	60.0%	3	30.0%		
	No requirement	0	0.0%	6	60.0%		
Post symmetry	Symmetrical	2	20.0%	3	30.0%	1.00	NS
	Minor asymmetry	3	30.0%	3	30.0%		
	Moderate asymmetry	5	50.0%	4	40.0%		
	Major asymmetry	0	0.0%	0	0.0%		
Post-require further augmentation	Major further requirement	1	10.0%	0	0.0%	0.045	S
	Moderate further requirement	4	40.0%	0	0.0%		
	Minimal futher requirement	4	40.0%	5	50.0%		
	No further requirement	1	10.0%	5	50.0%		
Post-breast projection	Flat breast	0	0.0%	0	0.0%	0.071	NS
	Mininmal projection	5	50.0%	1	10.0%		
	Moderate projection	5	50.0%	6	60.0%		
	Good projection	0	0.0%	3	30.0%		

*Fisher exact test

There was a statistically significant difference between both groups as regards the need for further augmentation. The group with PRPs results showed that 60 % (p value 0.015) did not require further intervention while 40 % (p value 0.015) required further augmentation in the group with fat injection only.

When comparing breast symmetry, both groups showed similar results. Group A (without PRP) had a 50 % moderate asymmetry while Group B (with PRP) had 40 % moderate asymmetry. These values were not considered statistically significant difference.

Table (2): Description of comparison between post-operative cases among the two study groups as regard expert opinion (SCORES).

	without PRPs			with PRPs			P*	Sig
	Mean	±SD	Median	Mean	±SD	Median		
Post-require augmentation	1.60	0.52	2.00	2.50	0.71	3.00	0.007	HS
Post symmetry	1.30	0.82	1.50	1.10	0.88	1.00	0.598	NS
Post-require further augmentation	1.50	0.85	1.50	2.50	0.53	2.50	0.009	HS
Post-breast projection	2.50	0.53	2.50	3.20	0.63	3.00	0.021	S

Mann Whitney test
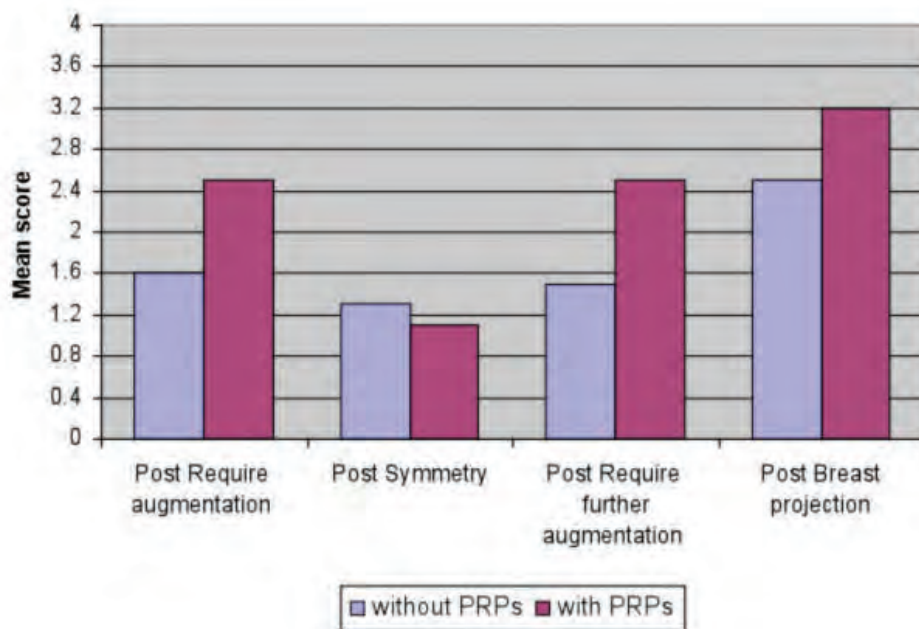
*Graph (1): Comparison between both groups as regards post-operative results.*

As for the projection of the breast; Group B (with PRP's) showed better projection results (3.20±.63 SD p value 0.021) which also proved to be statistically significant when compared with group A (without PRP's) which showed a mean of 2.50±053 SD.

When comparing each group separately Group A showed no significant difference in all questions between preoperative and post-operative values. This would translate into no or minimal improvement clinically at 6 months post-operative.

Table (3): Comparison between Pre and post-operative cases among study group A as regard expert opinion.

	Group A(without PRPs)						P*	Sig
	Pre			Post				
	Mean	±SD	Median	Mean	±SD	Median		
Require augmentation	1.30	0.82	1.00	1.60	0.52	2.00	0.317	NS
Symmetry	0.70	0.67	1.00	1.30	0.82	1.50	0.084	NS
Require further augmentation	0.90	0.88	1.00	1.50	0.85	1.50	0.167	NS
Breast projection	1.60	1.17	2.00	2.50	0.53	2.50	0.071	NS

*Wilcoxon signed rank test
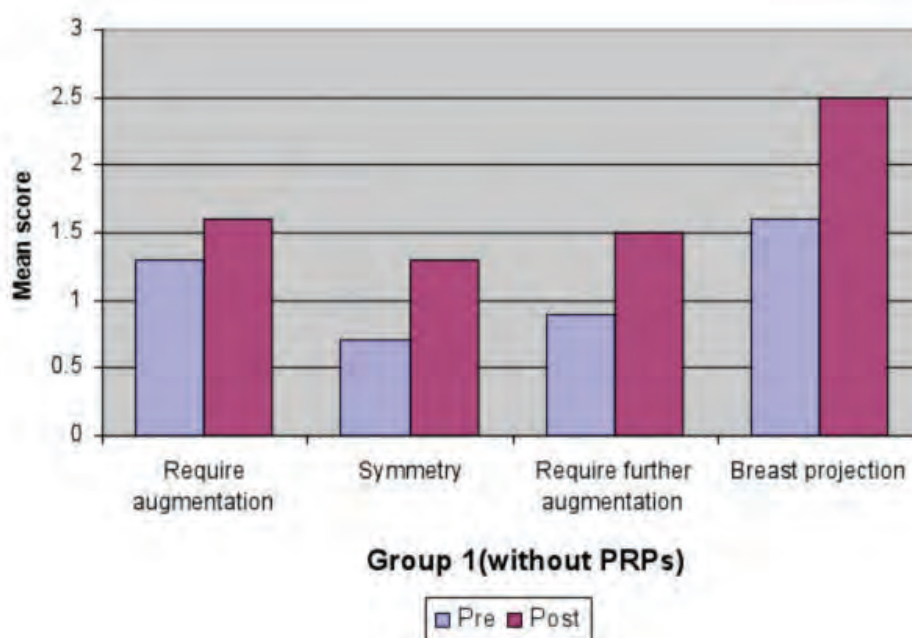
Graph (2):Comparison between pre and post-operative cases among study group A as regard expert opinion.

In the other group all scores showed a significant improvement in the post-operative values. Improvement was noted in projection, symmetry and the need for further augmentation.

Table (4): Comparison between Pre and post-operative cases among study group B as regard expert opinion (SCORES).

	Group A(without PRPs)						P*	Sig
	Pre			Post				
	Mean	±SD	Median	Mean	±SD	Median		
Require augmentation	1.10	0.57	1.00	2.50	0.71	3.00	0.009	HS
Symmetry	0.60	0.70	0.50	1.10	0.88	1.00	0.194	NS
Require further augmentation	0.80	0.79	1.00	2.50	0.53	2.50	0.004	HS
Breast projection	1.50	1.08	2.00	3.20	0.63	3.00	0.007	HS

*Wilcoxon signed rank test
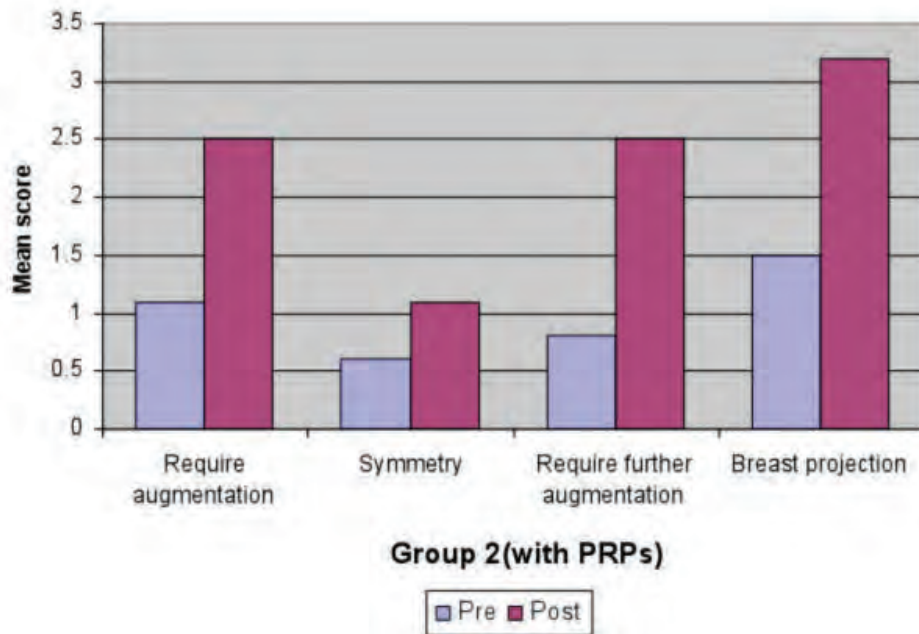
Graph (3): Comparison between pre and post-operative cases among study group B as regard expert opinion (SCORES).

Discussion:

PRPs are supposed to favour graft viability both by promoting cell proliferation and favouring new angiogenesis of the transplanted fat in the recipient site. This is mainly due to the high content of PDGFs.

In our hands we experienced a minor improvement in the group using fat enriched PRP at 10% ratio (PRP:Fat 1:9) when compared to grafting alone. Lipoinjection with PRP results showed that 90% of the cases showed moderate to good postoperative projection 6 months post-operative. 50 % of cases required minor augmentation while 50% proved to be good enough with the observers.

All results of the cases utilizing fat alone showed declined results when compared with the group with PRP. Not only that, but the questionnaire also showed only minor improvement between the pre and postoperative results in all cases when analysing observer results. Moreover, platelet rich plasma administration led to reduction of the need for secondary grafting.

Salgerllo et al, 2011[5] investigated the rate of fat necrosis using postoperative breast ultrasound, he concluded that unfortunately a higher rate of liponecrosis was observed in patients treated with PRP enhanced fat, this finding contradicts our results which are in correspondence with Nakamura et al, 2010[10] results, they found that PRP to fat ratio of 1:4 supports the fat grafting volume and viability by promoting capillary formation in the graft for at least 120 days when compared with fat grafting alone in rats.

Curvally et al, 2009[11] presented two series of patients treated for facial soft tissue defects using PRP to fat ratio1:2, they concluded that this technique improves adipose tissue maintenance and survival, when compared with fat grafting alone.

The unperidectibilty of fat graft survival has let surgeons worldwide to investigate an alternative technique in fat grafting in order to improve its take. Many approaches have been proposed in recent years, Coleman's techniques[6] remaining the most reliable.

In a study done by Michalevicz et al,[12] it was noted that vascular smooth muscles require mitogens such as PDGF in order to proliferate, not only that they proved that PDGF is one of the most potent mitogenic factors present in blood serum. PDGF activates fibroblasts and neutrophils, these functions are essential for the processes of inflammation and healing. In relation to self-proliferation, Kakudo et al, 2008[13] showed that activated PRP contains large amounts of PDGF-β and Transforming Growth Factor (TGF), and they promoted the

proliferation of human adipose derived stem cells and human dermal fibroblast in vitro. We believe that PDGF-α,β are the most essential growth factors present in PRP.

Adipose tissue angiogenesis is a complex process that involves many steps such as basement membrane breakdown, angiogenic remodelling, and vessel stability. Giusti et al, 2009[14] concluded that PDGF plays an integral role for promoting angiogenesis in human endothelial cells.

Hu et al[15] showed a negative effect on the proliferation of oral cells when using high concentrations of PRP, however Patiel et al[16] contradicted Hu's study and documented that collagen synthesis improvement was dose dependent. Thus in short saying a higher PRP to fat ratio would prove a better overall result.

We believe that a ratio 1:9 in fat injection with PRP would prove to sufficient in improving fat survival, not only sufficient, but also feasible to achieve in order to reach a ratio 1:2, we would require a minimal of 200cc of blood per breast. In our study we required an average of 225cc of fat per breast in order to reach a sufficient volume. Our requirements of PRP utilizing 1:9 ratio was 25cc of PRP per breast.

Our questionnaire was performed at 6 months postoperative interval, it showed that the group utilizing PRPs had better projection and overall result. This indicates that PRP's or its active ingredient PDGF improves fat survival and overall result. Salgarello et al, 2011[5] in a similar study showed no difference between two groups of fat transfer one of which utilizing PRP 10%.

We believe that primary take of fat results in increasing the longevity & results, therefore, in increasing factors aiding in neovascularization but should be administrated within the first week of the graft.

There are other factors that should be studied in order to reach a standardized protocol for fat injection in the breast in order to reach reliable outcome & results.

Conclusion:

In conclusion we believe that the increase of PDGF levels during the first 10 days of adipose tissue transfer enhances both take & fat survival. Also PDGF levels stabilization to a standard value should be studied further.

All clinical trials concerning adipose tissue transfer in order to correct human volume deficiencies aim to improvement of fat survival & standardization to reach reliable outcomes.

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