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Autologous Biological Vitamin-C-added (ABC) Filler for Facial Volume Restoration

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Abstract

Purpose Face rejuvenation procedures with injectable agents continue to gain popularity. Nowadays, a variety of commercial products are available as filler material. Ideal fillers should be inexpensive, easily obtainable, nontoxic, and biocompatible. The aim of this study is to report a clinical perspective for an appropriate, feasible, and inexpensive protocol of a stable, autologous biological filler for facial volume restoring without any commercial kits.

Methods Eight patients were investigated who underwent facial injection with ABC filler. Eleven ml of whole blood was placed in standard tubes containing anticoagulant and for each patient, 8 tubes were prepared. After the centrifugation at 1630 xg for 5 minutes, the upper plasma was taken, calcium was added and cooled. After the addition of vitamin C, the syringes were incubated at 85 °C for 10 minutes. The autologous biological material obtained was used as filling material. For comparison, FACE-Q satisfaction questionnaires were used before and after the procedure.

Results All patients were followed up for a minimum of 4 months. No major complications were recorded. The patient-reported FACE-Q satisfaction and FACE-Q quality of life pre- and post-procedure results showed statistically significant improvement ($p < 0.05$). Overall satisfaction with the outcome was 89.12 ± 16.73 (range 55–100).

Conclusions ABC filler can be seen as a reliable, inexpensive, and easily obtainable material to restore facial volume with increased patient satisfaction and quality of life scores. We believe that our study will be encouraging to the application of autologous biological fillers for further clinical and scientific studies.

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 the Table of Contents or the online Instructions to Authors www.springer.com/00266.

Keywords Autologous · Biological · Filler · Plasma · Wrinkles

Introduction

Face rejuvenation procedures with injectable agents continue to gain popularity. Non-surgical facial rejuvenation procedures are defined as the most frequently applied aesthetic procedures in the United States of America (USA). According to the plastic surgery statistics report, 18.1 million cosmetic procedures were performed in the USA in 2019 [1]. Of these, 1.8 million were surgical procedures, and 16.3 million were reported as minimally invasive procedures [1]. Botulinum toxin ranks first with 7.7 million within those procedures, while soft tissue filling procedures rank second with a number of 2.7 million [1].

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Hyaluronic acid-based commercial fillers (such as Juvederm Ultra®, Restylane®, etc.) constitute 2.2 million of 2.7 million soft tissue filler procedures [1]. When the economic burden of such a frequent procedure is examined, it can be seen that the average fee of a hyaluronic acid filler procedure was $652 in 2019 and the total annual expenditure of the soft tissue filler procedure for the USA was reported to be approximately $1.4 billion [1]. Unfortunately, the large market share did not reduce the costs. For this reason, many studies have focused on autologous alternatives in recent years such as platelet-rich plasma, platelet-rich fibrin, fat grafting, skin dermal mixtures, etc., to improve skin quality and dermal remodeling [2–8].

Platelet-based biological materials have been used actively in the field of cosmetic surgery since the 2000s, and their importance and types have been understood more clearly day by day [9]. In a 1987 study, an increase in the high molecular weight (HMW) region was observed in the electrophoresis after the denaturation of the plasma at a temperature of 80 °C [10]. It may be hypothesized that this region is the accumulation area of the substances such as hyaluronic acid. Because hyaluronic acid is a type of HMW and in another study, it has been shown that a very small amount of hyaluronic acid is denatured at temperatures between 90 and 120 °C and in a period of 30 minutes. In June 2020, Gheno et al. reported that the resorption properties of heated albumin gel with liquid-platelet-rich fibrin (novel Alb-PRF) was significantly improved until 21 days compared to two standard preparations of PRF where they inserted platelet-poor plasma (PPP) into a device for the human plasma denaturation of proteins for 10 minutes at a temperature of 75 °C [11]. As noted by Gheno et al. heating and denaturing plasma proteins such as albumin is not a new procedure and has been known for more than 20 years [11, 12]. By the thermal denaturation process, a modification in the secondary structure transforms the plasma protein into a tridimensional structure whereby new hydrogen and disulfide ligations are created, with improved stability [11, 12].

In this regard, studies on heating plasma proteins after a centrifugation process are available in the literature for decades. In our study, we reported the patient satisfaction results with quality of life scales (using FACE-Q) of an applied autologous filler material that is biologically derived and stabilized in its solid form by adding calcium, cooling, and vitamin C. It is aimed to establish a clinical perspective for an appropriate, feasible, and inexpensive protocol (based on evidence-based medicine) without any commercial kits with highlighted tricks to prepare a stable, autologous biological filler for facial volume restoring and rejuvenation.

Materials and Methods

After approval by the Local Medical Ethics Committee, 8 patients who underwent facial biological filler procedures were enrolled in this study. This prospective study was approved by our Institutional Review Board (No: 0536/4470) and informed consent was taken from each patient. All protocols used in this study were conducted according to the recommended International Regulations and Declarations and complied with the Declaration of Helsinki.

Autologous Biological Vitamin-C-Added Filler (ABC Filler) Preparation

Eleven ml of whole blood was collected in sterile standard tubes containing anticoagulant citrate dextrose solution-formula A (ACD-A) with blood to anticoagulant ratio of 9:1. We obtained 8 tubes for each patient (Fig. 1). The 8 tubes were centrifuged for only 1 spin at a standard relative centrifugal force (RCF) of 1630 xg for 5 minutes using a multipurpose centrifuge device (NF 800®, NUVE Industrial Materials Manufacturing and Trading Co., Turkey) [13]. Blood components were separated into the plasma, buffy coat, and erythrocyte layers. The plasma layer formed after centrifugation is divided roughly into approximately 3 equal portions [9], and the uppermost one-third of plasma which can be called platelet-poor plasma (PPP) was gently aspirated and taken in sterile 2.5-mL syringes with Luer-Lock apertures. Then, a calcium-complex (Calciosel, Haver Pharma, Turkey, 100 mg/mL) with a proportion of 0.1 mL per 2.2 mL of PPP was placed in syringes for the activation of the platelets (Fig. 1). Immediately, the syringes were put at 0 °C for 10 minutes to enhance platelet activation [14]. After the activation with cold temperature and calcium, 0.3 mL vitamin C (L-ascorbic acid) (Selovita-C, Haver Pharma, Turkey, 100 mg/mL) was added to the syringes for stabilization of the biofiller. The syringes were incubated at a temperature of 85 °C for 10 minutes (Fig. 2a). Finally, the plasma in syringes was transformed into a viscous gel which is called autologous biological vitamin-C-added filler (ABC filler) (Fig. 2b–d, Video 1).

ABC Filler Injection

ABC filler was performed gently in the deep dermal planes and subdermally on each side of the face (Fig. 3). Injection areas are selected to correct the facial wrinkles, recreate the curves and the projection of the face. Under complete aseptic technique, ABC filler was injected slowly within deep dermal and subdermal planes using sterile disposable

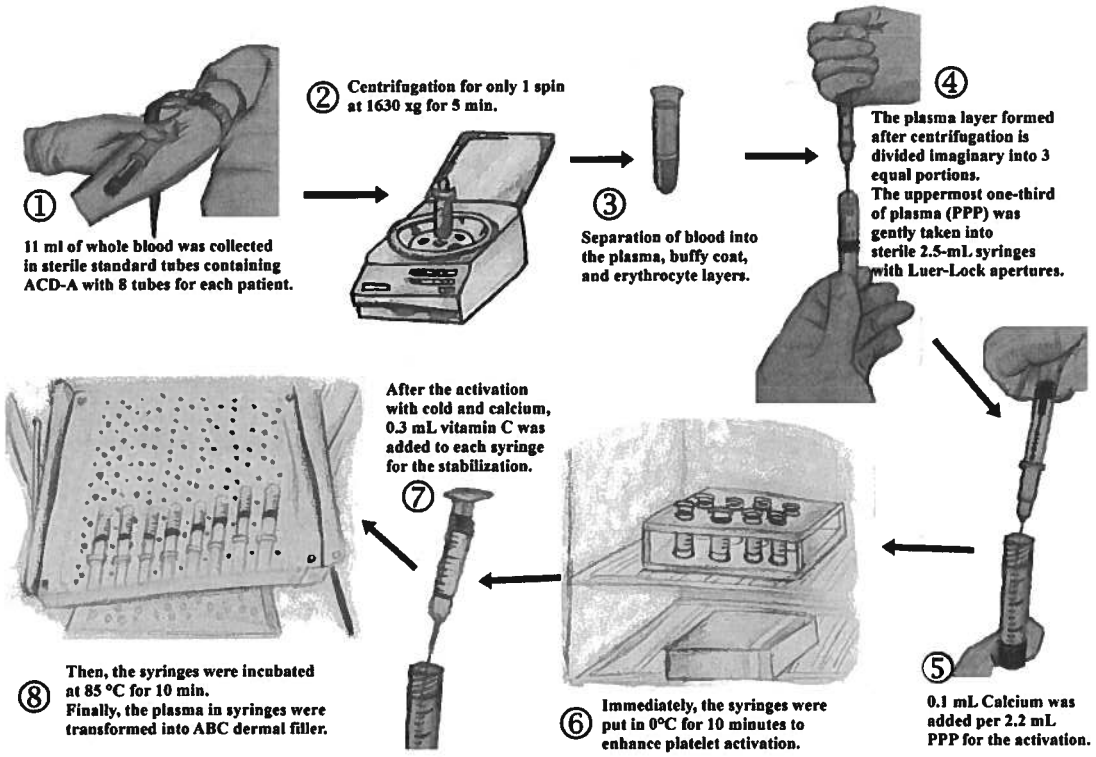

Fig. 1 An illustration of the study design

28 G needle. Care was taken to put in the hole upward from the face and away from any visible vessels. After injection, the sites were gently massaged to distribute the injected ABC filler to conform to the contour of the surrounding tissues. There was no touch-up session was planned or given.

Assessment Using FACE-Q Scales

The FACE-Q is a revolutionary patient-reported outcome instrument that evaluates specific outcomes in patients undergoing facial cosmetic procedures [15]. It utilizes several assessment scales to evaluate satisfaction with facial appearance, health-related quality of life, and satisfaction with the process of care. The FACE-Q satisfaction scales consist of questions relating to patient satisfaction concerning facial appearance, aging appearance appraisal, and other such parameters. Rating is performed based on the following scales: 1=very dissatisfied, 2=somewhat dissatisfied, 3=somewhat satisfied and, 4=very satisfied. Quality of life scales are rated as: "definitely disagree, somewhat disagree, somewhat agree, or definitely agree" [15].

A pre-procedure FACE-Q was administered including the following modules:

1. Satisfaction with facial appearance: This 10-item scale assesses the overall facial appearance using items including: "How your profile (side view) looks," "how fresh your face looks," and "how your face looks under bright lights" [16], etc.
2. Appraisal of overall lines: This 10-item scale assesses the entire facial lines how much bothering the patient by using items including: "How deep the lines," "how noticeable the lines," and "how old the lines on your face make you look" [15], etc.
3. Aging appearance appraisal: This 7-item scale includes statements about how the patient feels about the age his-her face looks. Respondents may agree/disagree with statements such as: "I look older than I want to look," "When I see my reflection, I am reminded of how old I look" [15], etc.
4. Psychological function: This 10-item scale includes a series of positively worded statements that respondents are invited to agree/disagree, for example, "I feel good about myself," "I feel confident," and "I feel attractive" [16], etc.
5. Social function: This 8-item scale includes a series of positively worded statements that measure social confidence. Respondents are invited to agree/disagree with statements such as: "I make a good first

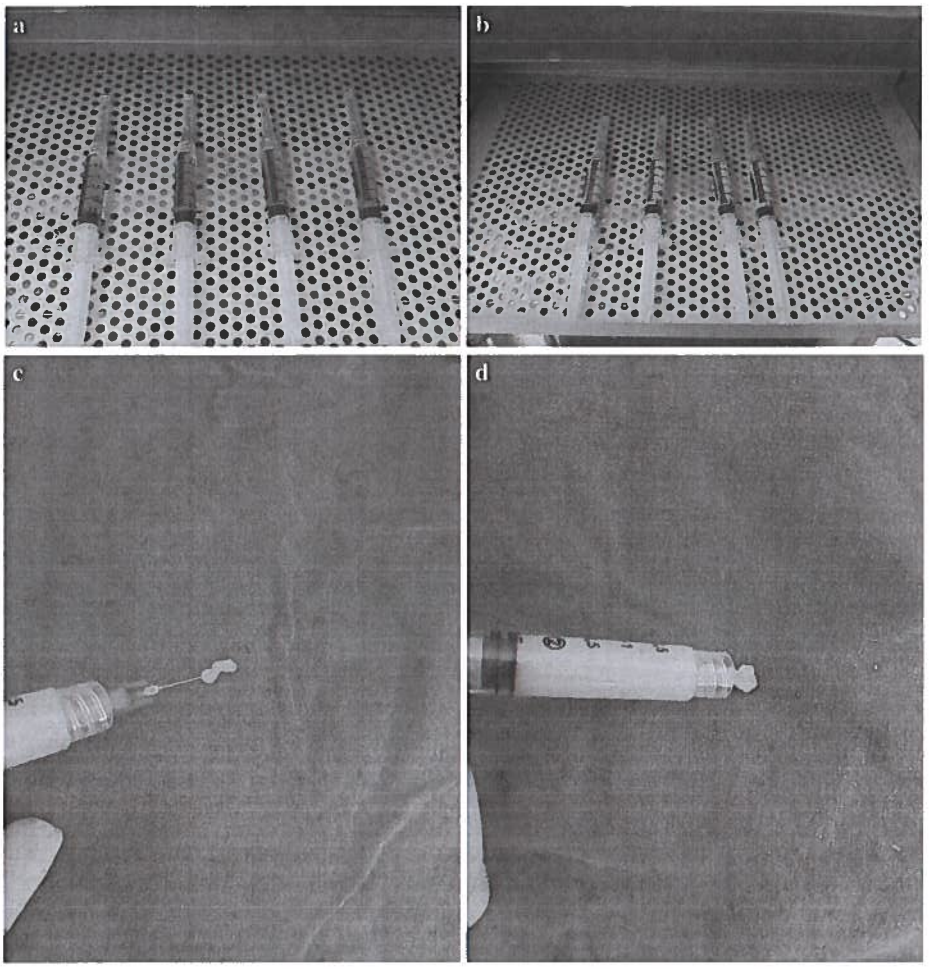

Fig. 2 The syringes before incubation at a temperature of 85 °C for 10 minutes can be seen (**a**). After the incubation period, the plasma in syringes was transformed into a viscous gel which is called ABC filler (**b**). ABC filler can easily pass from the needle (**c**). The stability of the ABC filler can be seen (**d**)

impression," "I am relaxed around people that I don't know well" [16], etc.

All patients included in this study completed a post-procedural FACE-Q at least after 4 months which was including the same modules as assessed during pre-procedural evaluation but only in addition to a 6-item satisfaction-with-outcome module. Each FACE-Q scale was scored using a lookup conversion table approach. Scores range from 0–100 with higher scores indicating better satisfaction/outcome and/or greater quality of life [15]. Scores were grouped to obtain the mean pre- and post-procedural results.

Statistical Analysis

All statistical analysis was performed using SPSS, version 15.0 software (SPSS Inc., IBM, Chicago, IL). The results were expressed as mean ± standard deviation and minimum–maximum values for the groups. Before statistical significance assessment, at first Kolmogorov–Smirnov Test for normality and Levene's Test for homogeneity were performed. Because of the small number of cases, non-parametric tests for related samples (Wilcoxon Signed Ranks Test) were used to determine the significance of difference to compare FACE-Q scores. "P-value" less than 0.05 was taken as significant.

Results

The mean age of 8 patients included in the study was 39.5 ± 7.9 years (range, 28–53 years). All patients were followed up over a minimum of 4 months. No major complications (infection, skin necrosis, nodulation, fibrosis, calcification, or vascular insults) were recorded, only temporary complications such as edema, erythema, and tenderness were observed. The patient-reported FACE-Q satisfaction (Fig. 4) and FACE-Q quality of life scores (Fig. 5) of pre- and post-procedure results showed a statistically significant improvement in all FACE-Q modules assessed. Satisfaction with overall facial appearance

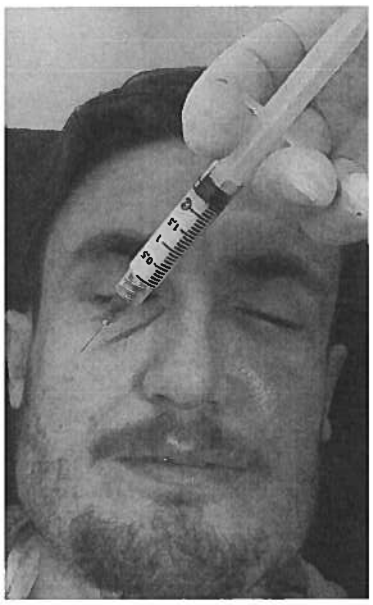

Fig. 3 Application of ABC filler to a 24-year-old man presented with malar atrophy. Transient erythema can be seen in the applied malar region

improved from scores of 46.63 ± 20.59 to 81.75 ± 20.85 ($p < 0.05$), whereas appraisal of overall lines and aging appearance appraisal from scores of 35.25 ± 13.30 to 75.00 ± 18.96 and from scores of 41.75 ± 6.30 to 82.00 ± 11.92, respectively (for both, $p < 0.05$) (Fig. 4). Quality of life scores (psychological and social functions) improved from scores of 60.75 ± 16.46 to 81.37 ± 14.45 and from scores of 58.62 ± 17.89 to 81.37 ± 12.48, respectively (for both, $p < 0.05$) (Fig. 5). The mean score of overall satisfaction with the outcome was 89.12 ± 16.73 (range 55–100). A few cases of ABC filler are illustrated in Figs. 6 and 7.

Discussion

Blood plasma differs from serum in that it is obtained by centrifugation in the presence of an anticoagulant to remove the cellular components and therefore contains approximately 10–20% more proteins than serum due to the presence of the clotting factors that are lost during serum collection [17]. Ninety percent of blood plasma is water and proteins constitute the majority of dissolved

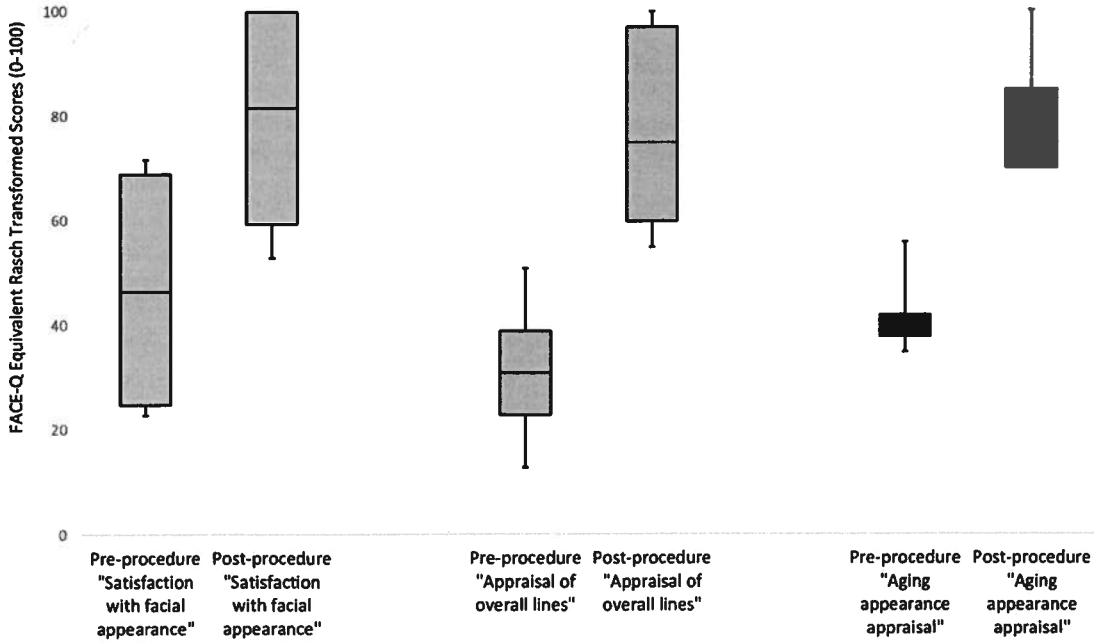

Fig. 4 Graphic analysis of FACE-Q scores comparing pre-procedure and post-procedure satisfaction with facial appearance, appraisal of overall lines, and aging appearance appraisal ($p < 0.05$). Data represent group means with minimum and maximum scores. Lower scores represent less satisfaction with the face, lines, and aging. An increase after the procedure can be seen

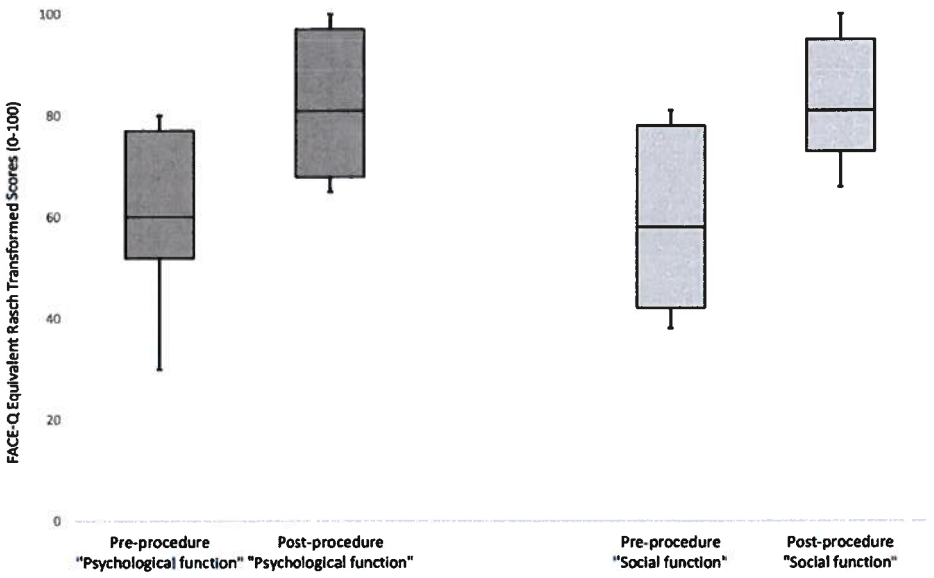

Fig. 5 The result of FACE-Q scores of quality of life comparing pre-procedure and post-procedure psychological function and social function ($p < 0.05$). Data represent group means with minimum and maximum scores. Lower scores represent less quality of life with facial appearance. Increased scores show the positive effects of the applied procedure on the social and psychological aspects of the patients' lives

substances in blood plasma. The total protein content of plasma in a healthy adult is on average 57–80 mg/mL, with albumin comprising the largest component (35–50 mg/mL) [17]. Therefore, Gheno et al. did not hesitate to use the term Alb-PRF for their novel membrane, which they heated and denatured platelet-poor plasma instead of albumin [11]. They heated a liquid platelet-poor plasma (PPP) layer and mixed the cell-rich buffy coat zone to improve the resorption properties of heated albumin gel with liquid-PRF (Alb-PRF) [11].

Garcia et al. used a commercial system named Endoret-Gel to treat atrophic scars [4]. Godfrey et al used the same commercial system to obtain plasma rich in growth factor gel (PRGF-gel) for facial volume restoration and skin rejuvenation [3]. In recent studies where biologically derived filling materials were studied, the authors and their colleagues used 9-mL commercial kits containing sodium citrate as anticoagulant and they collected 2 mL of platelet-rich plasma (PRP) just above the buffy coat after centrifugation at 580 xg for 8 min and then, incubated at 76 °C for 12 min to obtain the gel formula [2–7]. In our study, the biological filler preparation procedure differs from other studies at some points. Unlike them, there are several theoretical reasons why we prefer to use the uppermost plasma. As reported before, 6%, 14%, and 28% of the platelets present in whole blood are collected in the upper, middle, and lower thirds of the plasma, respectively, over the buffy coat layer after a centrifugation procedure and the smaller of these platelets accumulate in the upper third and the larger ones in the lower third of the plasma [9]. Small platelets could take up and store more plasma proteins relative to their size [18] and besides that, it is generally accepted that larger platelets contain more RNA content as compared to smaller platelets [19]. On the other hand, since all separations cannot be achieved at medium-low acceleration forces, the 2 ml portion –which was used in recent studies [2–7]– at the bottom makes about the lower half of the plasma, which may contain the transition stage to the buffy coat region and this region may be rich in leukocytes and larger platelets. It is obvious that the cytokines in white blood cells can be resistant to heat and have the ability to increase inflammation. The presence of neutrophils may be harmful, because they destroy surrounding tissue, even if the tissue is not injured [20]. After all, these neutrophils release nonselective and toxic reactive oxygen species that include hypochlorite, superoxide, and hydroxyl radicals at high levels [20]. Besides that, it is well known that DNA is highly stable to the high temperature in white blood cells and buffy coat lysates [21]. While considering that RNA is more stable than DNA [22], it would be more suitable to use the upper plasma which contains smaller platelets and plasma proteins rather than lower plasma including large platelets and leukocytes to produce a gel from plasma.

The platelets were irreversibly damaged with 10% calcium by balloon-shaped degeneration of the membrane and consequently influencing the release of proteins in the granules [23]. The activation of platelets leads to degranulation and release of contents. Egidi et al. in their study noted as low temperature appears to be a triggering factor for activation as well, thus yielding yet activated platelets [24]. In ABC filler, the reason for adding calcium and cooling is to activate smaller proteins that involving plasma proteins and glycocalyx. The glycocalyx is a carbohydrate-rich cell-surrounding agent that acts as a semipermeable barrier between plasma and interstitial fluid. The two biggest building blocks of glycocalyx are hyaluronic acid and

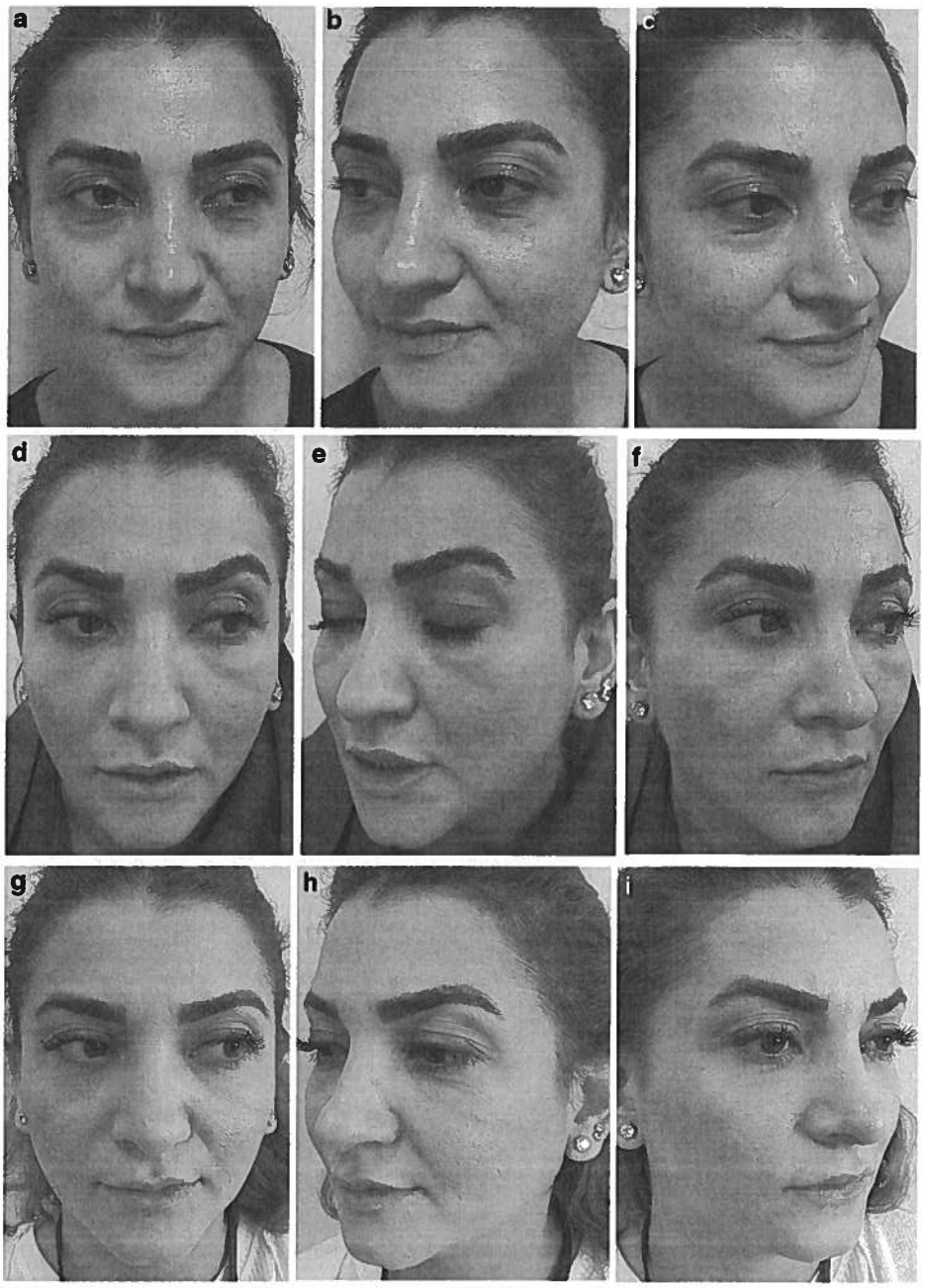

Fig. 6 Pre-procedure anterior (**a**) and oblique (**b**, **c**) views of a 38-year-old woman presented for facial recontouring to increase the youthful appearance. Malar atrophy, tear trough deformity and deep nasolabial folds can be seen. Results of 1-month views (**d**–**f**) and 4-month views (**g**–**f**) after one session application of ABC filler are shown. Decreased wrinkles with a well-rounded cheek and decreased tear trough deformity can be seen with an acceptable filling effect

heparan sulfate. Platelet glycocalyx has a thicker structure than other analogs in the blood when considering platelets that have become popular in recent years today. Researchers investigating Dengue fever in July 2017 found in their study that serum hyaluronic acid and heparan sulfate were significantly higher in the acute period in those with Dengue fever [25]. The researchers put forward the hypothesis of degradation of the glycocalyx present in the destroyed endothelium and cells, and in the same study, they attributed the same decreased platelet state to loss from the vascular space [25].

Self-crosslinking of protein can be achieved by heating relatively over 50 °C to produce a protein gel [26]. The thermal denaturation process transforms a modification in the secondary structure of the plasma proteins, glycocalyx of platelets, and proteins in the granules into a tridimensional structure with increased stability by adding vitamin C. The structure of thermally denatured proteins reflects the aggregation pattern of the protein molecules, which is mainly governed by the surface net charge and the hydrophobic area exposed by heating of the protein molecules [26]. Anitua et al. reported that their

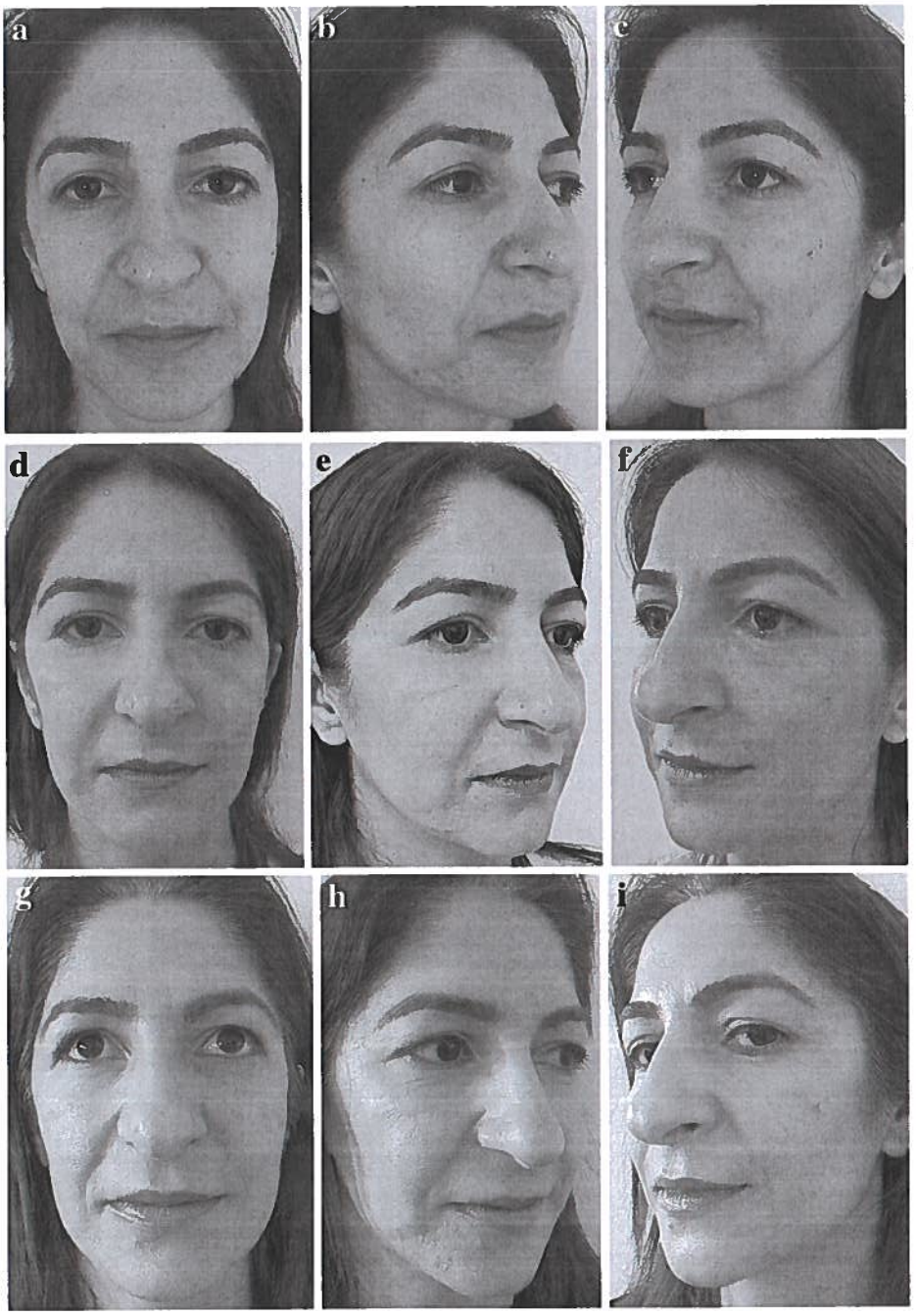

Fig. 7 Pre-procedure anterior (**a**) and oblique (**b**, **c**) views of a 42-year-old woman presented with malar and deep nasolabial folds can be seen. Results of 1-month views (**d**–**f**) and 4-month views (**g**–**l**) after one session application of ABC filler are shown. Primarily contouring of cheeks and decreased nasolabial folds could be seen with acceptable results

injectable formulation after thermal denaturation of plasma was able to sustain a saline binding behavior up to 72 h and a total of 15% swelling ratio [7]. They attributed this saline binding ability to both hydrophilicity and the three-dimensional maintenance of the biomaterial structure [7]. ABC filler may be seen as only a thermal degraded protein mixture in a form of a gel. The polymerized protein gel results in a volume effect in the applied skin [6].

In our study, vitamin C was also added before the thermal denaturation of PPP. Besides the effects on the skin such as supporting collagen biosynthesis and depigmentation, vitamin C protects tissues and cells against oxidative damage by free radicals in biological systems as an antioxidant [27]. Vitamin C readily undergoes two consecutive (yet reversible), one-electron oxidation processes to form the ascorbate radical, a relatively unreactive free radical, and is therefore considered an excellent reducing agent [27].

However, it is well known that the bioavailability of vitamin C in the skin is inadequate because of difficulty to deliver into the dermis in the optimum dosage when it is administered orally, and therefore, it is favored to use as topical in the practice of dermatology [28].

The FACE-Q module, which measures patient-reported outcomes of facial cosmetic treatments in a scientifically sound manner, confirmed marked post-treatment satisfaction with image perception in terms of its impact on social life and relationships [29]. We believe that a procedure performed on a patient should be combined with a specific questionnaire to assess the actual result on the perception of the patient's own to better understand the change in the quality of life [29]. In our study, with an autologous biological material prepared simply, the overall facial satisfaction of the patients improved dramatically and their psychological and social life scores became better. It could be discussed that particularly palpebromalar edema occurs in the first month. However, by ABC filler, we think that an acceptable result could be achieved which can be seen in the feedback of the results according to FACE-Q. On the other hand, ABC filler may be seen as a natural biological material that will increase the quality of the skin while providing a filling effect.

The biggest limitation of our study is the small number of patients. Our study can be seen as a preliminary study due to the significant decrease in the number of cosmetic patients due to the worldwide pandemic. Another limitation of the study could be seen as using the FACE-Q scales which can be argued as subjective assessments.

This biological filler material, which can be prepared as much as desired via a simple and inexpensive method, is perhaps a candidate to be made more suitable and longer-term by future studies. Future clinical trials including a higher number of patients and/or comparative long-term studies are needed to show the tissue augmentation potential of this autologous biological material.

Conclusion

This study shows that a biological material obtained from the patient's own plasma with a simple method without using a commercial kit enables facial volume restoration in the short-medium term. When the overall face perception of the patients is evaluated, it is observed that the improvement from the patients' own perspective creates an improvement in the social and psychological function scales. We think that our study will shed light on future studies as a pilot study and that an optimum filling can be obtained inexpensively with the contribution of the clinicians.

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Declarations

Conflict of interest No conflict of interest and all authors bear the responsibility of this letter.

Ethical Standards This prospective study was approved by our Institutional Review Board (No: 0536/4470) and informed consent was taken from each patient. All protocols used in this study were conducted according to the recommended International Regulations and Declarations and complied with the Declarations of Helsinki.

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