ISSN: 2639-0353

Madridge Journal of Dermatology & Research

Research Article

Open Access

Comparative Study of the Efficacy and Safety of Calcium Hydroxyapatite Fillers

Ya. A. Yutskovskaya[1]*, E.A. Kogan[2], A. Yu. Koroleva[3], A.D. Lokastova[3]

[1]*Department of Dermatovenereology and Cosmetology, Pacific State Medical University, Ministry of Health of the Russian Federation, Moscow, Russian Federation*

[2]*Department of Pathological Anatomy, Sechenov University, Moscow, Russian Federation*

[3]*Center for the treatment of complications of Professor Yutskovskaya's Clinic, Russian Federation*

Article Info

***Corresponding author:**
Ya. A. Yutskovskaya
Department of Dermatovenereology and Cosmetology
Pacific State Medical University
Ministry of Health of the Russian Federation, Moscow
Russian Federation
Email: yutsk@mail.ru

Received: June 28, 2025
Accepted: July 30 05, 2025
Published: August 08, 2025

Citation: Yutskovskaya YA, Kogan EA, Koroleva AY, Lokastova AD. Comparative Study of the Efficacy and Safety of Calcium Hydroxyapatite Fillers. *Madridge J Dermatol Res*. 2025; 8(1): 146-157. doi: 10.18689/mjdr-1000136

Published by Madridge Publishers

Abstract

Background: Endogenous calcium hydroxyapatite (CaHA) is the main mineral substance of human teeth and skeleton. Bioceramic material CaHA has exceptionally high biocompatibility, lack of antigenicity, when injected stimulates regeneration of collagen types I and III, elastin and proteoglycans, as well as formation of de novo vascular network.CaHA-based fillers are widely used due to their safety and efficacy; currently, there are limited data comparing their biostimulatory effects, which is critical to understanding their clinical behavior and biocompatibility.

Methods: The article presents the results of a pilot, randomized, prospective, split-face trial that evaluated the efficacy and safety of Radiesse, Crystalys, and Facetem in healthy female volunteers aged 30-45 years. The study included 19 patients with nasolabial fold (NLF) severity at rest of grade II or higher on the 5-point Merz scale who met the study selection criteria.The study participants were observed for 18 months. Clinical efficacy was based on the results of MRI of the maxillofacial area, assessment of histomorphological and immunohistochemical changes in tissues after injections, 3D photography, questionnaires on the GAIS scale and clinical examination of patients during the entire study period - before the procedure, as well as 1, 4, 9, 12, 18 months after the procedure.

Results: Radiesse, Crystalys and Facetem demonstrated clinical efficacy, high safety profile and good tolerability of the contour plastic procedure. According to MRI datagreater efficiency of Radiessewas noted compared to Crystalys and Faceteam. Histomorphological and immunohistochemical changes after injections of CaHA-based fillers revealed a remodeling effect on the skin. The features of the studied CaHA-based fillers include earlier and more pronounced stimulation of elastin production after using Radiesse with positive dynamics maintained for up to 12 months; earlier stimulation of type 3 collagen synthesis after using Radiesse and Facetem; higher rates of accumulation of types 1 and 3 collagens with dynamic restructuring of their content and new formation of microvessels in the dermis after using Radiesse.

Clinical evaluation of efficacy and safety showed a high level of satisfaction with the results of the procedures performed by both the doctor and the patient.

Keywords: Dermal fillers, CaHA, Radiesse, Crystalys, Facetem, immunohistochemistry, histological features, collagen type I, collagen type III, CD34, Ki-67.

Abbreviations

CaHA — Calcium Hydroxyapatite;
ICH-GCP —International Conference on Harmonisation of Good clinical practice;

IHC — Immunohistochemical study;
GAIS — Global Aesthetic Improvement Scale;
NLF — Nasolabial folds;
PMMA — Polymethyl Methacrylate.

Highlights: This article describes the immunohistochemical and histological changes of tissues after the use of CaHA-based dermal fillers for skin remodeling.

1. Introduction

Endogenous calcium hydroxyapatite (CaHA) is the main mineral substance of human teeth and skeleton. Bioceramic material CaHA has exceptionally high biocompatibility, lack of antigenicity, when injected stimulates regeneration of collagen types I and III, elastin and proteoglycans, as well as formation of de novo vascular network. Clinically, restoration of these structures is accompanied by increased density, elasticity, radiance and hydration of the skin, reduction of wrinkles and general rejuvenation of the skin. The results of histological and immunohistochemical studies have shown that, unlike a number of other products, such as PLLA, CaHA provides regeneration of soft tissues with minimal participation of immunocompetent cells of adaptive immunity and without development of chronic inflammation. According to Lemperle G et al (2003), no foreign body reactions were recorded with the use of CaHA [1]. The low frequency of granuloma formation and low potential for foreign body reactions were subsequently confirmed by other researchers [2].

CaHA-based fillers are widely used due to their safety and efficacy; currently, there are limited data comparing their biostimulatory effects, which is critical to understanding their clinical behavior and biocompatibility. Kunzler C et al (2023) showed that the size, shape uniformity, and surface character of CaHA microspheres can vary between products and influence the patient's physiological and immune response [3].

Radiesse (Merz North America, USA) is a bioceramic material based on CaHA, which is almost identical in molecular composition to endogenous CaHA, and has been used for biomedical purposes for many years. Synthetic CaHA in Radiesse is produced in the form of homogeneous microspheres (20-45 μm in diameter) distributed in a carboxymethylcellulose gel, which makes up 70% w/v of the filler. The use of the dermal filler CaHA Radiesse (CaHA-R) is characterized by high efficiency and safety. Correction with CaHA-R provides an immediate aesthetic result and promotes long-term improvement of the skin by stimulating the production of components of the extracellular matrix of the dermis. Patient satisfaction is usually high, which indicates that the treatment results meet expectations. [4-8].

Crystalys (Panaxia LTD, Germany) is a biodegradable product consisting of CaHA particles (55.7%), ranging in size from 25 to 45 microns, uniformly distributed in a gel that mainly consists of phosphate buffer and glycerol. The gel structure is formed by adding a small amount of carboxymethylcellulose. In a study on the anisomorphism of CaHA particles in some fillers for the Crystalys product, the presence of crater-like defects on the surface of the particles and the presence of particle fragments (which distinguishes them from Radiesse fillers) were determined. This circumstance leads to a decrease in surface tension on their surface, which contributes to the development of a macrophage reaction [9-11].

Facetem (CGBio, South Korea) is a biodegradable product consisting of CaHA particles (30%) ranging in size from 24 to 45 microns, uniformly distributed in a carboxymethylcellulose gel (70%). CaHA microparticles have a cellular microspherical structure created using the patented Lattice-pore technology. The spatial structure of Faceteam microspheres is organized according to the "cabbage fork" principle and promotes slow progressive biodegradation, which provides a prolonged augmentation effect compared to other CaHA-based fillers. Under conditions of its prolonged presence in the dermis, it provides an increase in the volume of the extracellular matrix of the dermis by these cells due to the synthesis of collagen by fibroblasts. The mechanism of the dermis's response is played out according to the type of inflammatory reaction. The identified phase is a reaction to a foreign body with the deposition of plasma proteins on the surface of the implant (provisional matrix), caused by modulation of the activity of macrophages with their subsequent proliferation and activation of fibroblasts with the transition of acute inflammation to chronic with the subsequent effect of remodeling of the extracellular matrix. [12, 13].

The aim of this study was to compare the biostimulating properties of Radiesse, Crystalys and Facetem fillers by means of histomorphological and immunohistochemical evaluation, as well as to study the tomographic behavior of the compared fillers and their clinical efficacy and safety indicators.

2. Material and Methods

Study design

The study was designed as a pilot, randomized, prospective, split-face trial to evaluate the efficacy and safety of Radiesse, Crystalys, and Facetem in healthy female volunteers aged 30-45 years for histomorphological and immunohistochemical reasons. Participants were recruited at a single research center (Center for the Treatment of Complications, Professor Yutskovskaya Clinic, Moscow) from January 2024 to December 2024. All patients signed an informed consent form to participate in the study. The study was approved by the ethics committee. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and ICH-GCP.

Participants of the Study

The study included 19 patients with nasolabial fold (NLF) severity at rest of grade II or higher on the 5-point Merz scale who met the study selection criteria. Patients were excluded if they were pregnant or breastfeeding, had used any absorbable fillers, botulinum toxin type A injections in the treatment area within 24 months before the study, had persistent drugs in the facial area, including polylactic acid, polymethyl methacrylate (PMMA), silicone, fat grafts (regardless of the time since implantation), had

any cosmetic procedures in the facial area and biopsy area within the last 6 months before the study, and patients with a history of severe or uncontrolled systemic diseases.

Intervention under study

The study included six visits. All patients were randomly assigned to two groups: 7 people in the Radiesse/Crystals group and 12 people in the Radiesse/Facetem group. The procedure was performed during the 1st visit (D01). At 1, 4, 9, 12, 18 months after the treatment, the patients visited the study center for evaluation visits.

In the Radiesse/Crystalys group, at visit 1, participants received Radiesse and Crystalys injections into different halves of the face in the midface, subdermally into the nasolabial folds, and into the peri-auricular area. Facial markings were performed according to Hinderer by drawing a straight line from the lateral canthus of the eye to the oral commissure, perpendicular to the line from the lateral canthus of the eye to the lower jaw, and a line from the ala of the nose to the mid-tragal point. At the intersection of these lines, the McGregor and Hinderer apex points were injected, and the midpoint between them was also injected. (*Figure 1*). Injections were performed periosteally with a 27G x 19mm needle. 0.2 ml of filler was injected into the medial point, 0.3 ml into the middle point, and 0.3 ml into the lateral point. In the NLF area, injections were performed using a 22G x 50mm cannula and 1 ml of filler was injected. In the periauricular area, injections were performed using a 27G x 38mm cannula and 1 ml of filler was injected. (*Figure 1*).

In the Radiesse/Facetem group, at visit 1, participants received injections of Radiesse and Facetem into different halves of the face in the midface, subdermally into the nasolabial folds, and into the periauricular area. Face markings were performed according to Hinderer. Injections were performed at the McGregor, Hinderer, and midpoint between them (*Figure 1*). Injections were performed periosteally with a 27G x 19mm needle. 0.2 ml of filler was injected into the medial point, 0.3 ml into the middle point, and 0.3 ml into the lateral point. In the NLF area, injections were performed using a 22G x 50mm cannula and 1 ml of filler was injected. In the periauricular area, injections were performed using a 27G x 38mm cannula and 1 ml of filler was injected. (*Figure 1*).

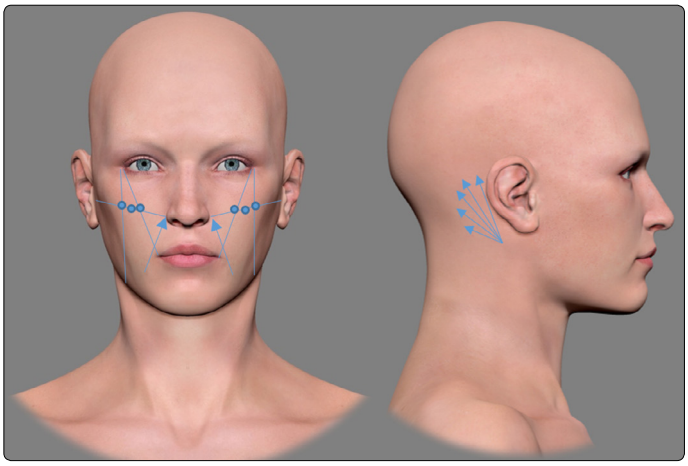


Figure 1. Scheme of drug administration

Study endpoints

Efficiency: The study participants were observed for 18 months, at stage D01, as well as 1, 4, 9, 12, 18 months after the procedure, photo documentation and 3D scanning were performed using a LifeViz camera (France) for clinical evaluation of the treatment effectiveness. An assessment of the volume deficit of the midface, the severity of NLF, and an assessment of the clinical picture by the doctor and the patient were performed.

Comparative histomorphological analysis of skin biopsies was performed before the procedure, as well as 1, 4, 9, 12, 18 months after the procedure. (*Figure 2*). Punch biopsies (219 skin punch biopsies from 19 patients) were fixed in 10% neutral formalin and embedded in paraffin according to a standard protocol. Serial paraffin sections (4 pm) were prepared and then stained with hematoxylin and eosin (H&E). Tissue samples obtained by punch biopsy were subjected to qualitative and quantitative analysis to determine collagen types I and III and elastin expression. In addition, the presence of drug globules, the degree of lymphohistiocytic infiltration and the severity of angiogenesis were qualitatively and quantitatively analyzed. For immunohistochemical analysis, sections were stained after antigen retrieval in a retrieval solution according to a standard protocol. Monoclonal antibodies to collagen I (mouse monoclonal antibodies manufactured by Santa Cruz (sc-293182), clone 3G3, dilution 1:100), collagen III (mouse monoclonal antibodies manufactured by Santa Cruz (sc-166316), clone B-4, dilution 1:100), CD34 (Huabio, Polyclonal Rabbit Antibody, dilution 1:2000, dilution 1:100), Ki-67 (rabbit monoclonal antibodies, clone SP6, GeneTex, dilution 1:500) were used. A semiquantitative method was used to analyze immunohistochemical parameters, studying 10 fields of view at high magnification (x 400) in two sections, according to a standard scale. A semiquantitative method was used to assess the staining intensity of histological and immunohistochemical preparations (scoring system). In case of weak staining, the marker expression was estimated at 2 points, in case of moderate staining – 4, in case of high staining – 6.

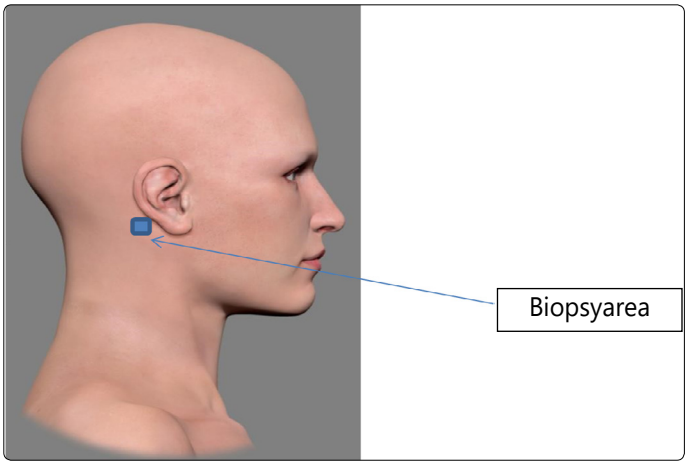


Figure 2. Schematic representation of punch biopsy zones

Magnetic resonance imaging of the maxillofacial region was performed before the procedure, immediately after (2–5

days), after 1 and 12 months. (figure 3). MRI of the maxillofacial region was performed on a high-field magnetic resonance tomograph Siemens MagnetomEspree with a magnetic field strength of 1.5 Tesla. Scanning was performed according to the following protocol: use of a special Head coil; patient position - strictly on the back, eyes closed; slice orientation: strictly axial, coronal, sagittal; visualization level: axial projection - from the upper walls of the frontal sinuses to the upper parts of the neck, coronal projection - from the tip of the nose to the external auditory canals; sagittal projection - visualization of the entire volume of the head; slice thickness - 1-5 mm; used sequences - 3D T1, 3D T2, 3D T2 fat sat, T2 STIR, T1 fat sat. Based on the obtained MRI results, the following were performed: assessment of the degree of correction of the volume and shape of the middle third of the face, assessment of the immediate "depot" zones of the CaHA-based drug, assessment of the soft tissues of the middle third of the face on the right and left at the level of the zygomatic bones in the projection of the periosteal administration of drugs, assessment of the regional lymph nodes in the scanning zone.

Figure 3. Schematic representation of the magnetic resonance imaging area of the maxillofacial region

Safety: Safety and tolerability of treatment were assessed throughout the study. Safety information was collected based on questionnaires given to participants and personal interviews with patients before treatment. At each visit, patients were interviewed about adverse events (AEs) and serious AEs; data on concomitant medications were recorded.

Statistical analysis: The study was planned as a pilot. The sample size was not determined. Descriptive data are provided for each studied parameter. The results were assessed using the statistical method for small sample criteria - Mann Whitney and Student's criterion. When comparing more than two dependent samples, the Friedman criterion was used.

3. Results

Study population

All study participants met the inclusion criteria for the study. During the study, each participant received a single injection correction procedure using a CaHA-based filler in a split-face design. Patients were followed for 18 months. All participants completed the study program.

Efficiency parameters

Clinical evaluation of effectiveness: According to the results of statistical analysis using the Friedman criterion with an additional grouping factor in the form of the study medicinal product, it was shown that in the case of both generic drugs in participants of both the 1st group (Radiesse/Crystalys) and the 2nd group (Radiesse/Facetem), when assessing the clinical efficacy of injections according to the parameter of midface volume deficit, assessed according to the validated Merz scale, no statistically significant differences were found between the generic and the original medicinal product at any time point (pholm>0.05) (*Figure4*).

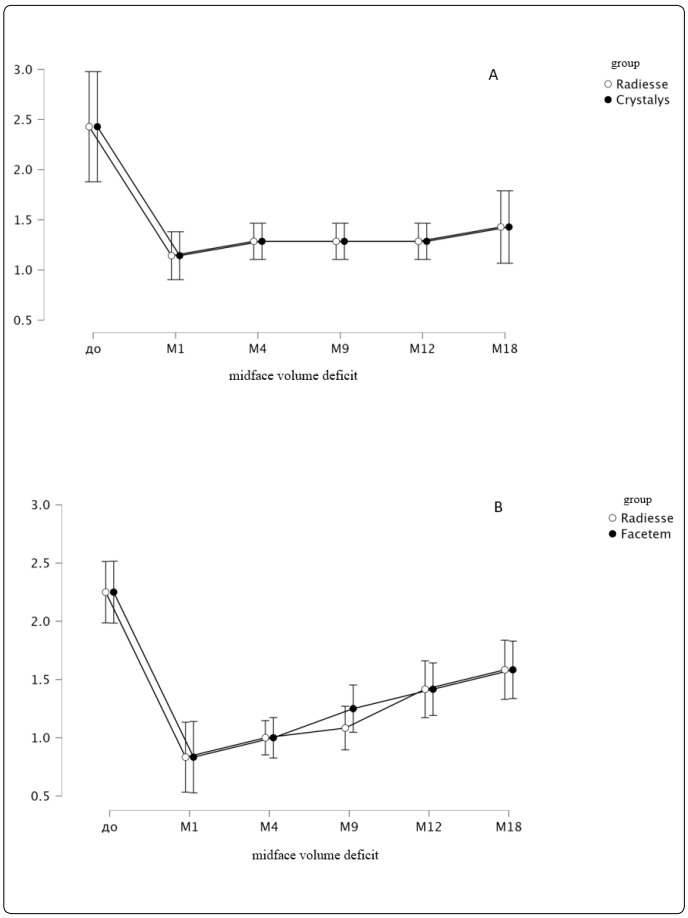


Figure 4. Parameters of midface volume deficit in dynamics: (A) in group 1 (Radiesse/Crystalys); (B) in group 2 (Radiesse/Facetem)

It is noteworthy that with subdermal administration in the area of NLF, 12 months after the administration of the medicinal products, a more pronounced clinical result was observed after the use of Radiesse in the 1st group (Radiesse/Crystalys), which may be associated with a more pronounced collagen stimulating effect. (*Figure 5*). A large sample is required to obtain statistically significant results.

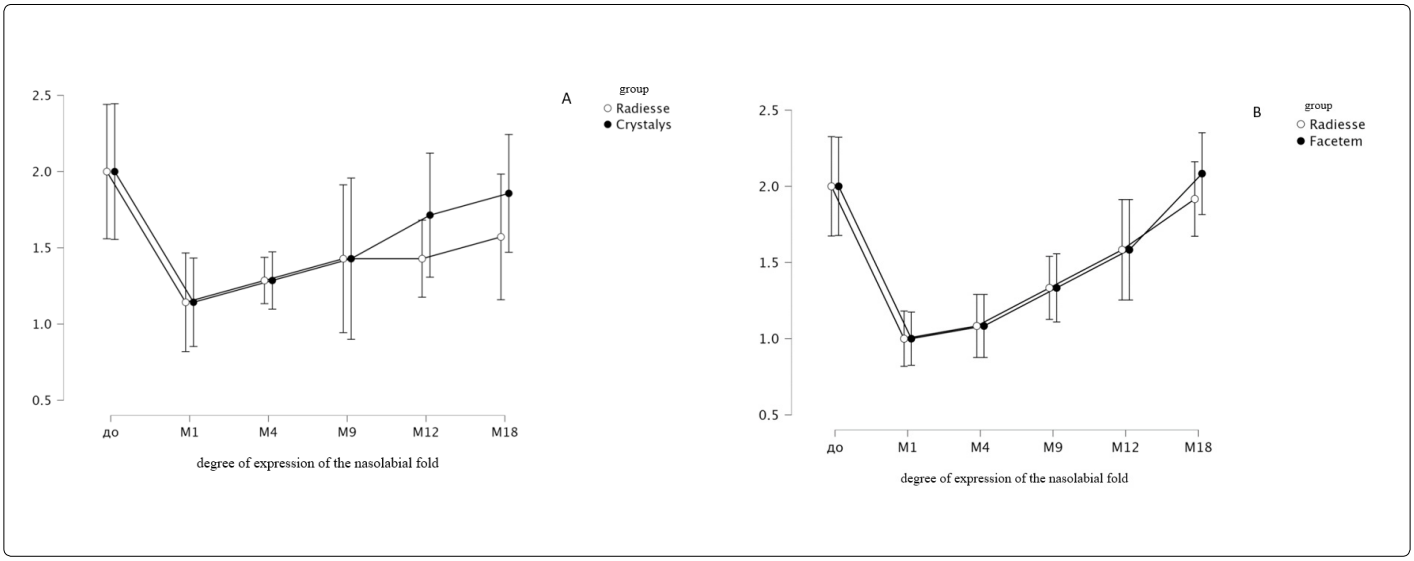


Figure 5. Parameters of NLF expression in dynamics: (A) in group 1 (Radiesse/Crystalys); (B) in group 2 (Radiesse/Facetem)

The results of statistical analysis using the Friedman criterion with an additional grouping factor in the form of an assessor (doctor/patient) showed that at no time point were statistically significant differences found between the doctor's and patient's assessments on the GAIS scale (pholm>0.05). In the case of both generic medicinal products (Radiesse/Facetem), at no time point were statistically significant differences found between the generic and the original medicinal product (Figure 3).

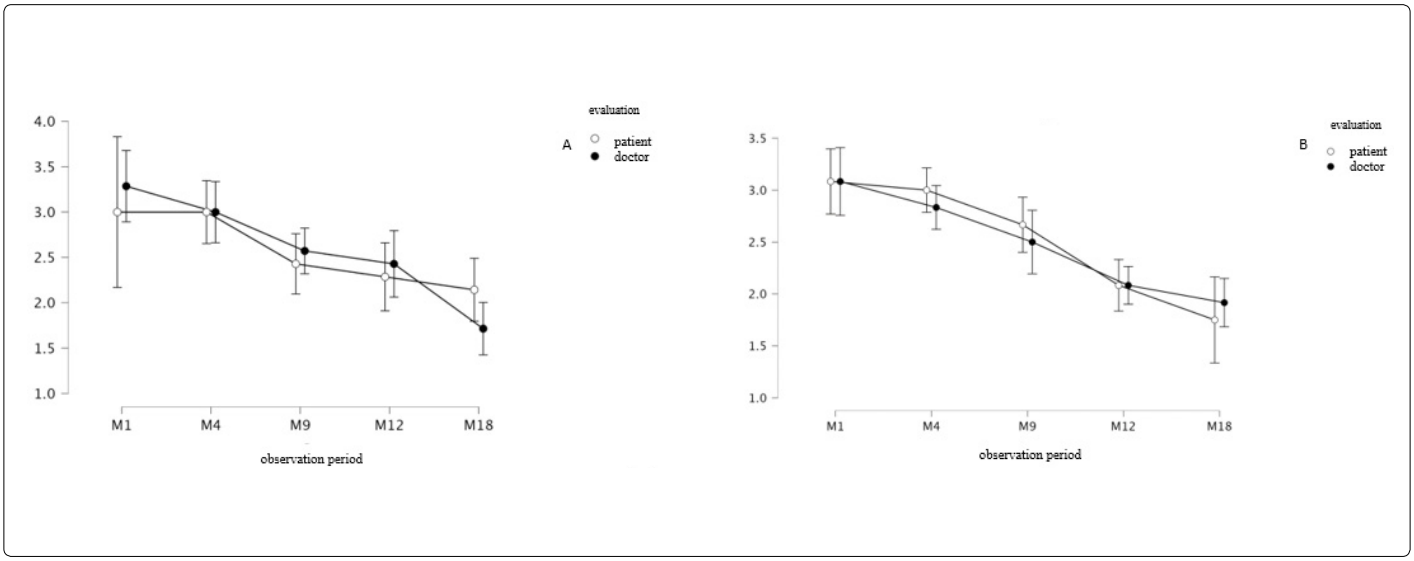


Figure 6. GAIS score over time: (A) in group 1 (Radiesse/Crystalys); (B) in group 2 (Radiesse/Facetem)

Evaluation of effectiveness based on MRI results

All patients underwent maxillofacial MRI at control time points. The CaHA-based preparations used in this study: Radiesse, Crystalys, Facetem in standard form were available for visualization, qualitative and quantitative assessment by MRI in both the early and late post-injection periods. Within a week after the injection of the studied preparations, all patients had more pronounced reactive edema of the facial soft tissues along the periphery of the Radiesse depot injected into the right half of the face compared to reactive edema of the facial soft tissues along the periphery of the Crystalys and Facetem depots. (*Figure 7A*).However, already 1 month after the injection, signs of reactive edema of the perifocal soft tissues were not determined (*Figure 7B*). During the entire observation period, all patients showed symmetrical distribution of the injected drugs and a stable increase in the thickness/volume of the soft tissues of the middle third of the face compared to the MRI data performed before the administration of the study medicinal products. After 12 months of the study, all patients on the side where the correction was performed with Radiesse showed greater efficiency in maintaining the correction of the volume and shape of the soft tissues of the face compared to the correction performed with Crystalys and Facetem. After 12 months of the study, all patients showed subtotal or total biodegradation of the gel carrier of the CaHA-based drugs: Radiesse, Crystalys, Facetem (*Figure7C*).

Preservation of the correction of the volume and shape of the soft tissues of the middle third of the face after biodegradation of the gel carrier of CaHA-based drugs was more pronounced on the half of the face where the correction was performed with Radiesse.

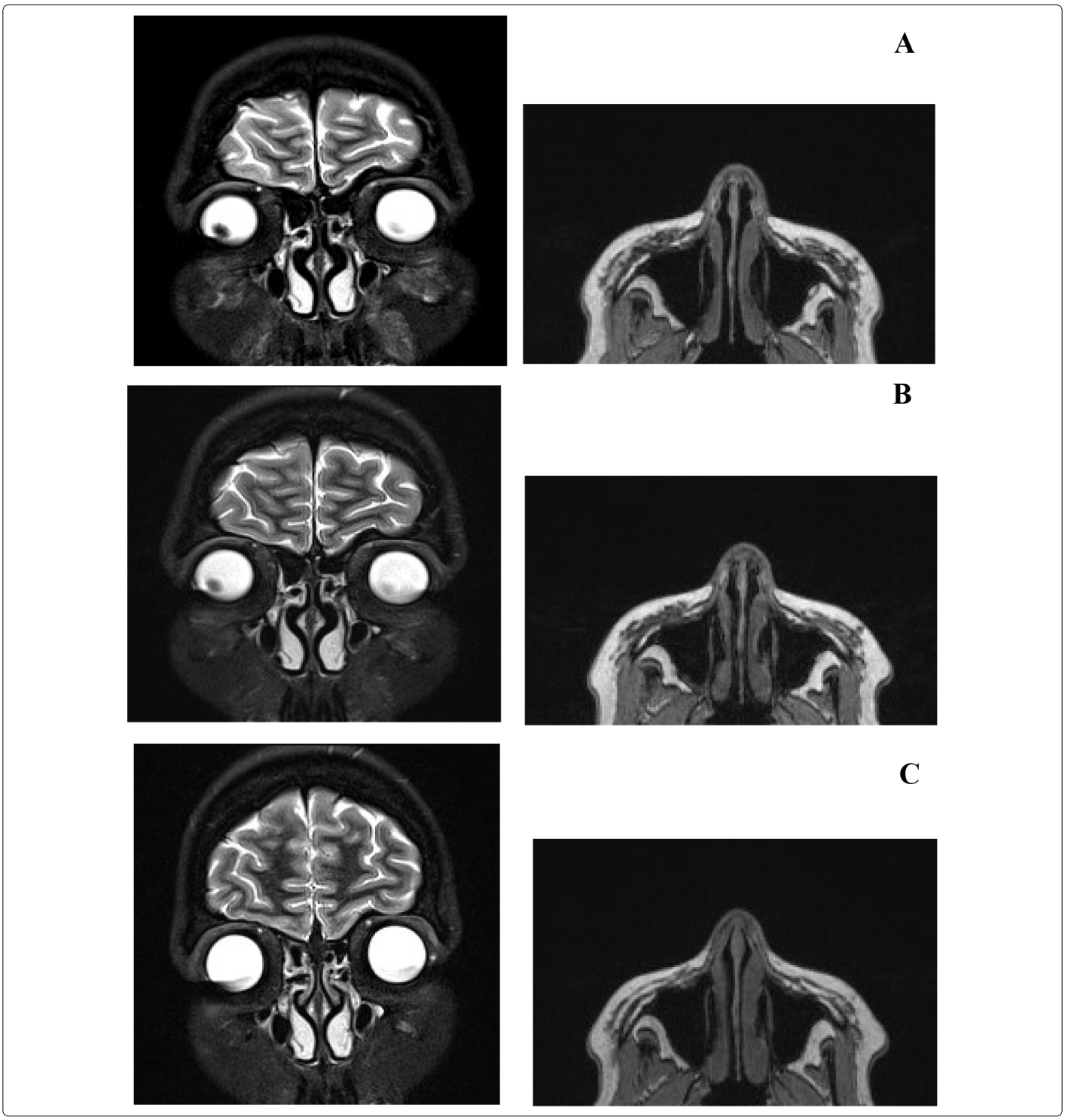


Figure 7. MRI of the maxillofacial region. (A) MRI in the coronal and axial projections 3 days after the injection of CaHA-based drugs. MRI images demonstrate symmetrical distribution of the injected drugs as a "depot" in the middle third of the face in the periosteal region at the level of the zygomatic bones on the right and left. (B) MRI in the coronal and axial projections 1 month after the injection of CaHA-based drugs. MRI images demonstrate symmetrical distribution of the injected drugs in the middle third of the face in the periosteal region at the level of the zygomatic bones on the right and left. (C) MRI in the coronal and axial projections 12 months after the injection of CaHA-based drugs. MRI images demonstrate almost complete biodegradation of the gel carrier of CaHA-based drugs in the middle third of the face in the periosteal region at the level of the zygomatic bones on the right and left.

Evaluation of effectiveness based on the results of histomorphological examination

Comparative assessment of histological and histochemical changes: Initial analysis of the pathohistological characteristics of skin biopsies revealed epidermis with signs of atrophy and acanthosis. The dermis is represented by connective tissue with a small number of microvessels and lymphohistiocytic elements scattered in the perivascular tissue. Small layers of adipose tissue of varying degrees of severity were discernible in the biopsies. When stained for elastic according to Weigert, elastic fibers were found in small quantities, fragmented, in the form of individual fibers in the perivascular and periglandular tissue in the deep layers of the dermis (*Figure 7*).

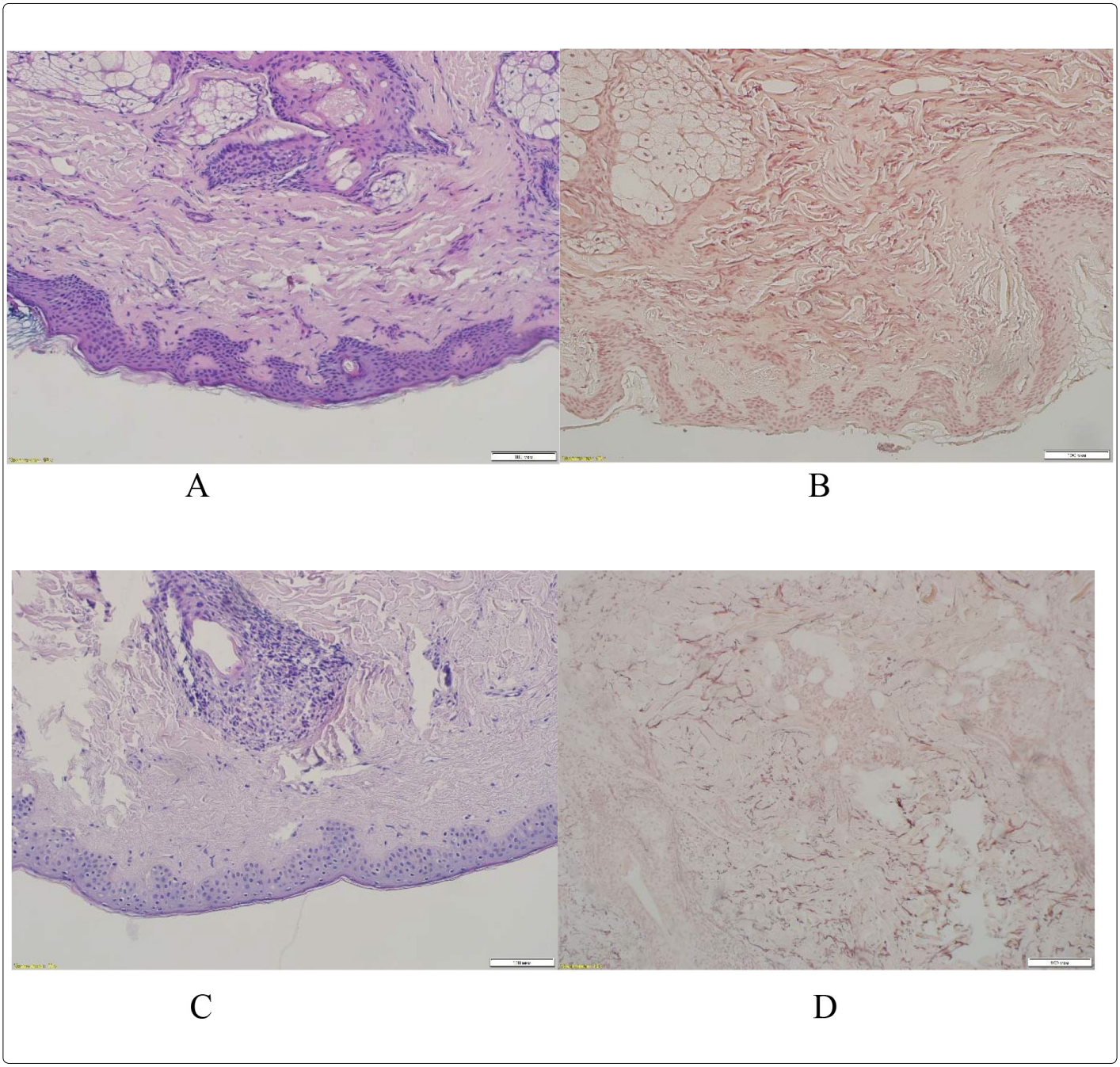
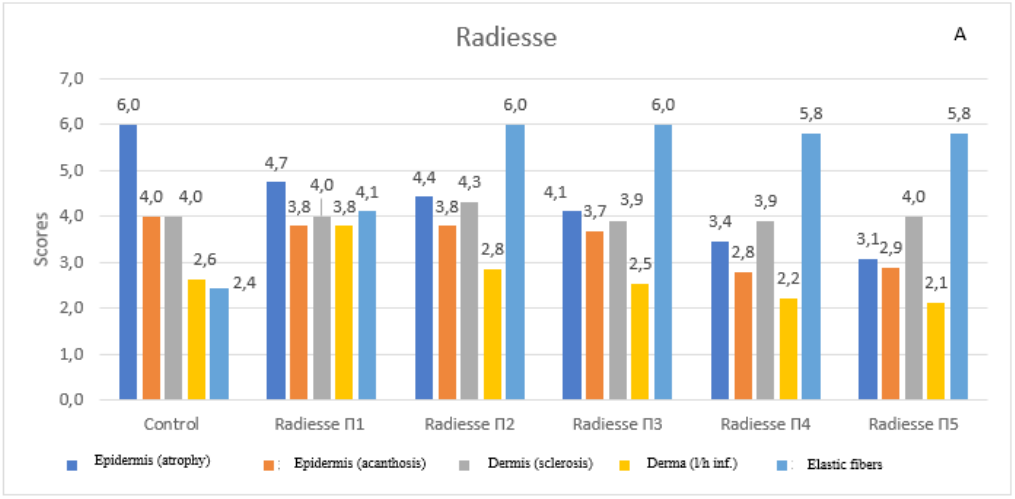
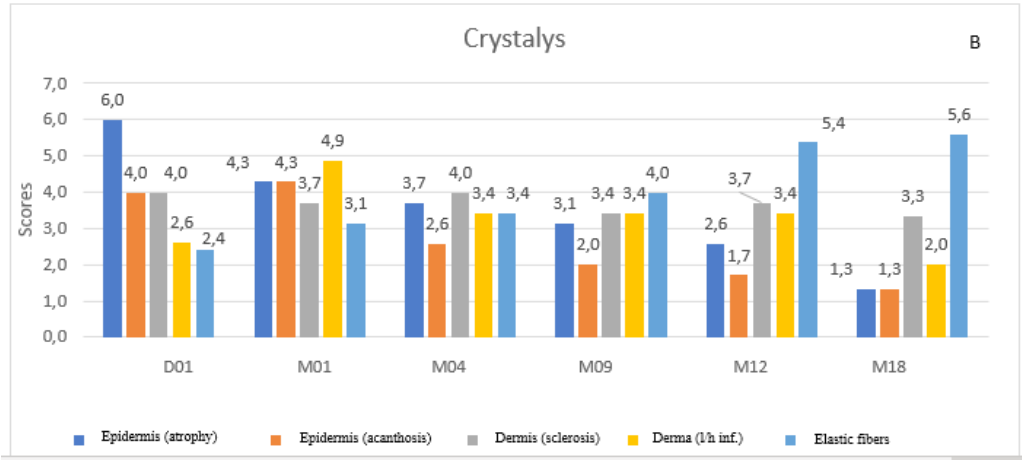


Figure 8. Histological characteristics of women's skin before using dermal fillers. Epidermal atrophy with moderate acanthosis, lymphohistiocytic infiltration of the dermis, a small number of microvessels (A, C) and elastic fibers (B, D). Hematoxylin and eosin staining (A, C) and Weigert's elastic stain (B, D).

Comparative analysis of histomorphological characteristics of skin biopsy samples allowed to establish that the studied medicinal products have a remodeling effect on the skin. At the same time, a decrease up to the disappearance of signs of epidermal atrophy, acanthosis, an increase in the concentration of elastic fibers in the dermis were noted. In the first months after the injection of the studied medicinal products, an increase in lymphohistiocytic infiltration in the dermis was noted and their spheroid deposits were found in the form of a productive inflammatory reaction with giant multinucleated cells of foreign bodies (*Figure 8*).

During the study, some differences in pathomorphological changes between the compared medicinal products were found. Thus, according to the parameter of disappearance of atrophic changes in the epidermis and acanthosis, the best parameters were found in Facetem and Crystalys after 9 months compared to Radiesse, after correction with which the disappearance of atrophy and acanthosis was observed after 12 months. Accumulation of elasticity in the dermis was noted 1 month after correction with Radiesse and Crystalys and 4 months after correction with Facetem filler.The disappearance of the deposition of globules of medicinal products with a productive inflammatory reaction was observed 4 months after the correction of Radiesse and Facetem and 9 months after the correction of Crystalys.





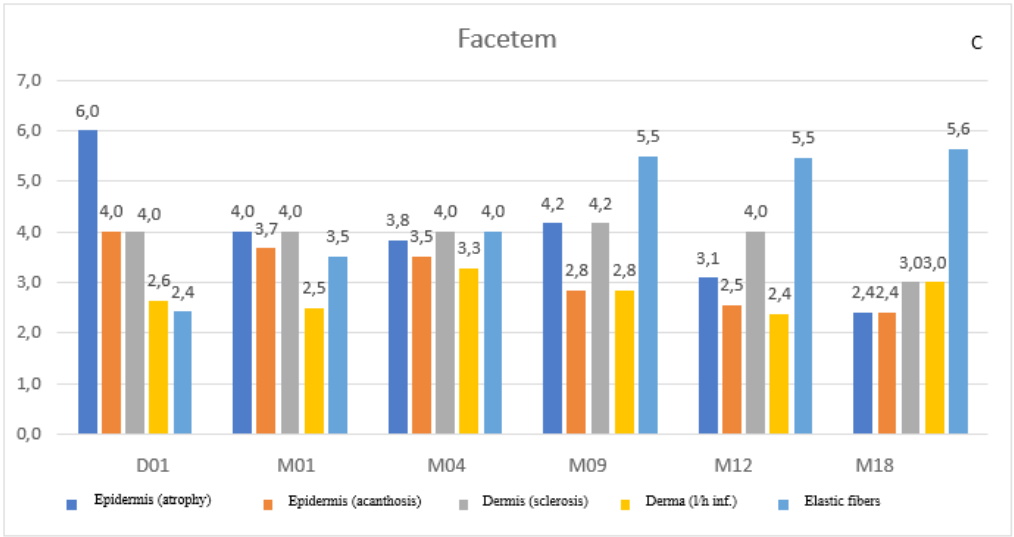


Figure 9. Comparative analysis of histomorphological characteristics of skin biopsies before (D01), after 1 month (M01), after 4 months (M04), after 9 months (M09), after 12 months (M12) and after 18 months (M18) after the injection of (A) Radiesse, (B) Crystalys, (C) Facetem.

Comparative immunohistochemical changes

At the first stage, before the injection of the medicinal products, immunohistochemical characteristics indicated similar changes in the study participants. Collagen type I was thin intertwined fibers located mainly in the subepithelial and superficial layers of the dermis. Collagen type III was represented by thicker intertwined fibers with a similar location in the dermis, and its amount was comparable to the amount of collagen type I (2 points). Membrane protein CD34 was determined as areas of stained cytoplasm in individual vascular endothelial cells (2 points). Protein Ki-67 was detected in a small part of proliferating epithelial cells, proliferation was 4% in the epidermis (*Figure 9*).

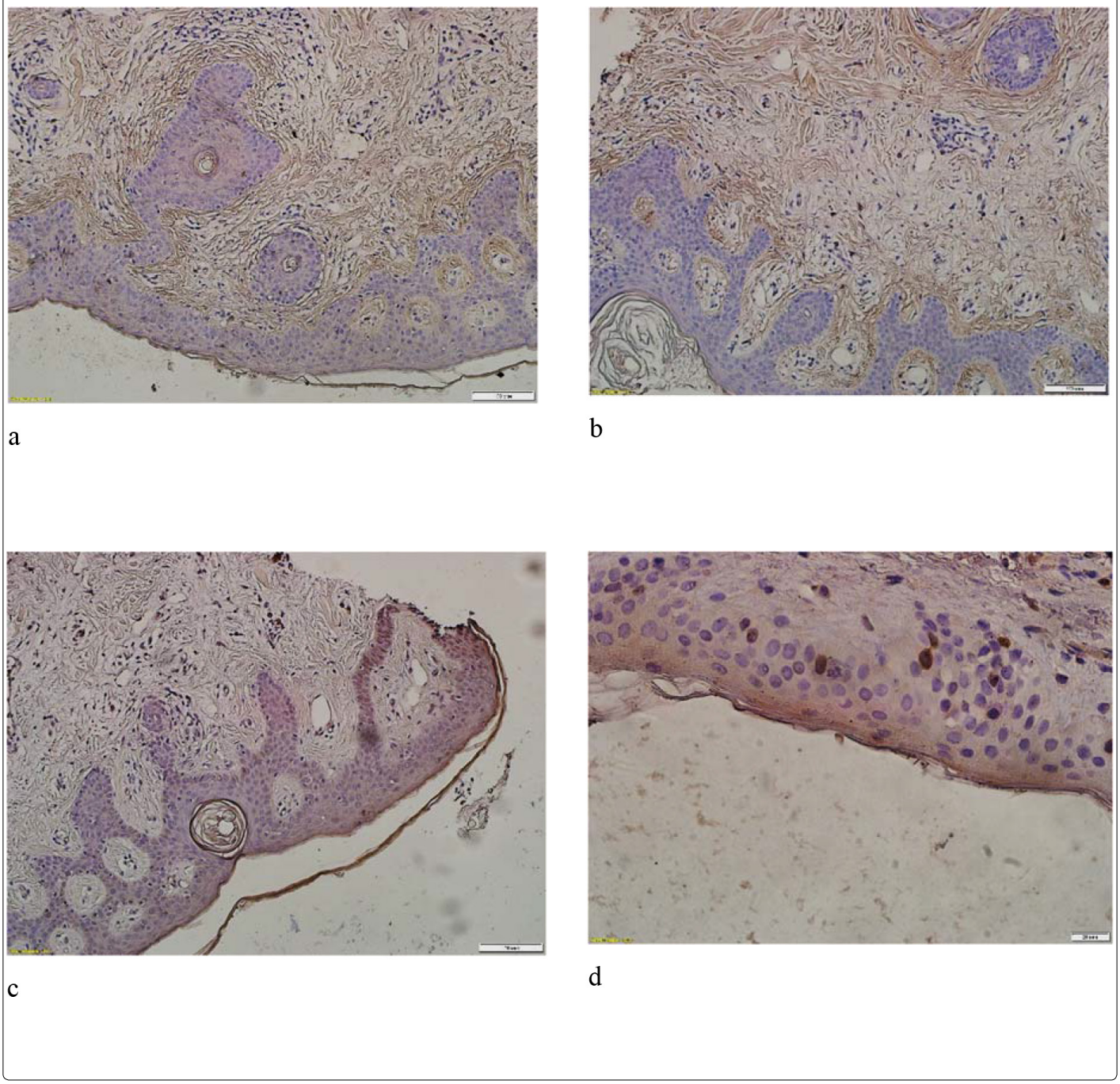
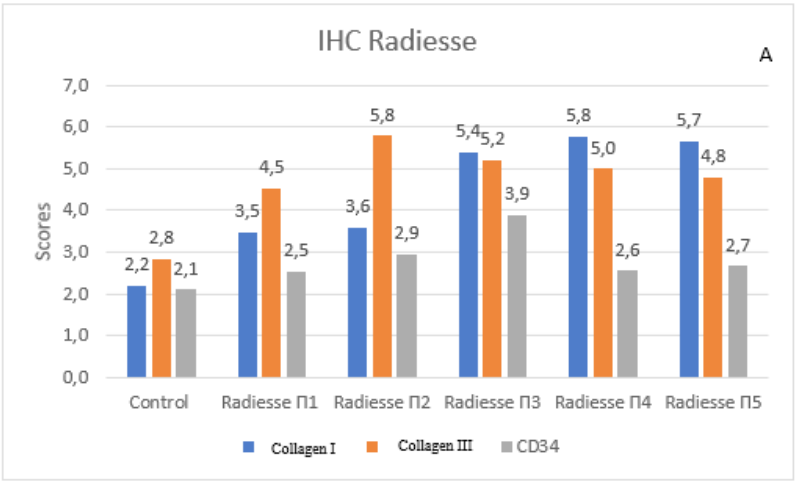
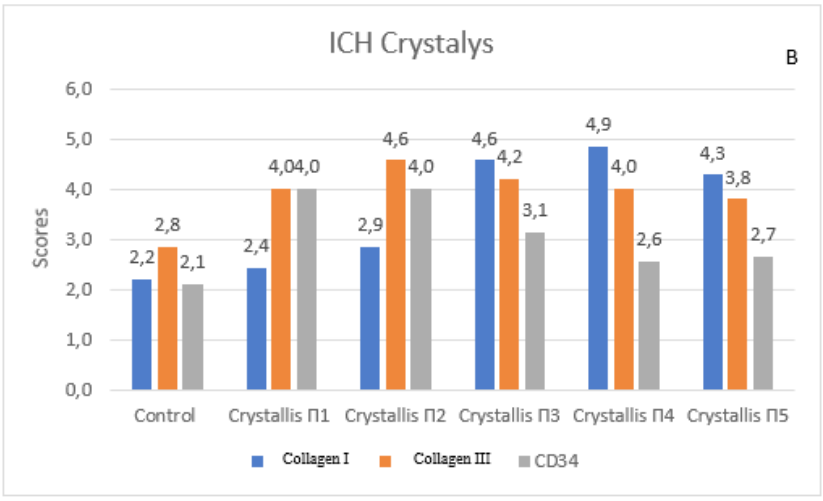


Figure 10. Immunohistochemical characteristics of women's skin before application of dermal fillers. Low content of collagen type 1 (a) and type 3 (b), a small number of microvessels with CD34+ endothelium in the dermis, individual proliferating Ki-67+ squamous epithelial cells in the epidermis. Immunoperoxidase reaction with DAB

Immunohistochemical analysis of volunteers' skin biopsies revealed that with the injection of Radiesse and Crystalys, Facetem fillers, collagen types 1 and 3 accumulate in the tissues with a dynamic restructuring of their mutual ratio, with a predominance of collagen type 1 over collagen type 3 (6.0 points, $p < 0.05$) and new formation of microvessels in the dermis (2.0 points). At the same time, the level of proliferative activity is detected unchanged in the epidermal cells and ranges from 3% to 5.6%.

Comparative analysis of immunohistochemical tissue characteristics for such features as accumulation of collagen types 1 and 3 showed that Crystalys and Facetem have an equal effect, while the effect of Radiesse is distinguished by an earlier accumulation of collagen type 3, starting from the 4th month of the study. According to the CD34 parameter, Crystalys is characterized by an earlier increase in angiogenesis, the effect was observed already 1 month after the procedure (*Figure 10*).





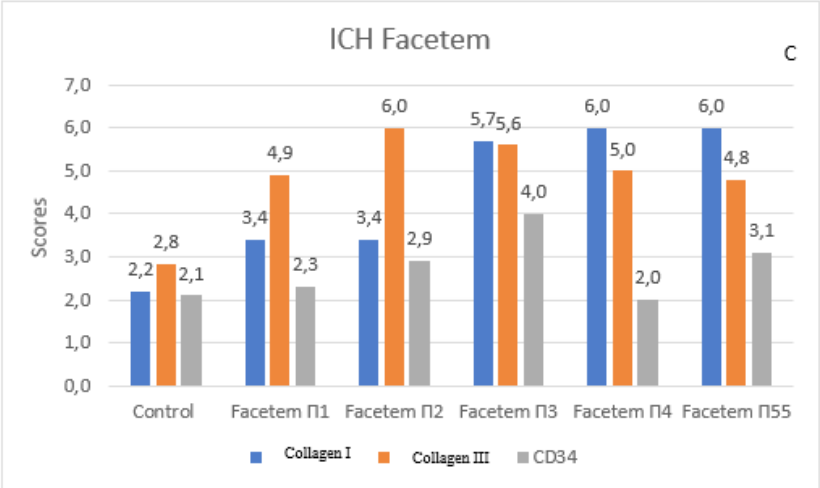


Figure 11. Comparative analysis of immunohistochemical parameters of skin biopsies before, after 1 month (P1), after 4 months (P2), after 9 months (P3), after 12 months (P4) and after 18 months after the injection of (A) Radiesse, (B) Crystalys, (C) Facetem.

Safety assessment results

The study reported cases of erythema, ecchymosis and petechial hemorrhage at the injection site (in 6 patients), isolated hematomas that resolved spontaneously within a short period after injection (in 3 patients), and pain and sensitivity at the injection site that resolved spontaneously some time after injection (in 8 patients). No additional AEs or serious AEs were observed during the 18-month observation period.

4. Discussion

CaHA-based products consist of microspheres of synthetic calcium hydroxyapatite suspended in a carrier gel. The transformation of CaHA-based products after administration into the soft tissues of the face can be divided into two phases. During the first phase, CaHA microspheres are evenly distributed at the injection site due to the soluble carrier gel, which provides instant correction of the volume

and shape of the soft tissues of the face[14]. During the second phase, the carrier gel is gradually absorbed [14], leaving microspheres of synthetic CaHA in the area of drug administration, which stimulate the production of the body's own (endogenous) collagen and dermal fibroblasts [14, 15, 16]. According to X-ray and CT studies, many patients who received injections of calcium hydroxyapatite-based drugs show visible correction results that last for a long time [17]. Thus, it can be assumed that the stable cosmetic effect resulting from the use of CaHA is associated not only with the presence of calcium hydroxyapatite microspheres in soft tissues, but also with the production of collagen.

The aim of this work was a comparative clinical, histomorphological and immunohistochemical assessment of the efficacy and safety of using Radiesse, Crystalys and Facetem in patients with the severity of NLF at rest from grade II and higher on the 5-point Merz scale.

This split-face study involving patients divided into two groups demonstrated independent remodeling effects at the injection site of the study medicinal products Radiesse, Crystalys and Facetem. According to the MRI study, effective correction of the volume and shape of the soft tissues of the face was noted after the use of the study drugs. Preservation of the correction of the volume and shape of the soft tissues of the middle third of the face after biodegradation of the gel carrier of the CaHA-based drugs after 12 months of the study can be regarded as an indirect sign of collagenogenesis, while a more pronounced result was recorded at the injection site of the Radiesse medicinal product compared to Crystalys and FaceTeam drugs. The results of the assessment of histomorphological and immunohistochemical changes showed that in the injection zone there is a decrease up to the disappearance of signs of epidermal atrophy, acanthosis, an increase in the concentration of elastic fibers in the dermis, as well as the accumulation of collagen types 1 and 3 with a dynamic restructuring of their content, with a predominance of collagen type 1 over collagen type 3 and new formation of microvessels in the dermis. At the same time, the level of proliferative activity without changes is detected in the cells of the epidermis and ranges from 3% to 5.6%. During the comparative analysis, some differences in pathomorphological changes between the compared drugs were found. Thus, according to the parameter of disappearance of atrophic changes in the epidermis and acanthosis, the improvement indicators were recorded after the use of all the studied drugs, while when using Facetem and Crystalys, these changes were recorded at the 9th month of the study, after using Radiesse - after 12 months. After using Radiesse, higher and earlier rates of stimulation of elastin production were recorded compared to Crystalys and Facetem, with positive (compared to the control) dynamics persisting up to 12 months ($p<0.05$). Also, after using Radiesse and Facetem, higher rates of stimulation of collagen types 1 and 3 were noted in the injection area, starting from the first stage (1 month) to the final stage (18 months).

Thus, in this study, both general patterns and some features of the action of the studied medicinal products Radiesse, Crystalys and Facetem were established. General patterns include skin remodeling with a decrease, up to complete disappearance, of atrophy and acanthosis of the epidermis, with the accumulation of elastic fibers and collagens of types 1 and 3, with a predominance of type 1, and improved neoangiogenesis. The features of the action of the studied drugs include higher rates of stimulation of elastin production already in the early stages of the study after the use of Radiesse with the preservation of positive cumulative dynamics up to 12 months. According to the collagen indicators at the injection site of Radiesse and Facetem, an earlier increase in the amount of collagen type 3 was noted compared to the use of Crystalys. The normal ratio of collagen types 1 and 3 with a predominance of collagen type 1 occurs already by 9 months after the use of all the studied medicinal products. Drug residues after injections in the dermis in the form of globules were detected for 4 months after the injection of Radiesse and Facetem, and longer after the injection of Crystalys (left) for up to 18 months.

5. Conclusions

Four As a result of the study, Radiesse, Crystalys and Facetem medicinal products demonstrated clinical efficacy, high safety profile and good tolerability of the contour plastic procedure using them. These conclusions were made based on the results of MRI of the maxillofacial area, assessment of histomorphological and immunohistochemical changes in tissues after injections, 3D photography, questionnaires on the GAIS scale and clinical examination of patients during the entire study period.

The studied CaHA-based preparations in standard form with a single periosteal injection showed effective correction of the volume and shape of the soft tissues of the face in all patients; during the MRI study at control time points, a stable increase in the thickness/volume of the soft tissues of the middle third of the face was noted compared to the MRI study data performed before the injection of the above medicinal products. Preservation of the correction of the volume and shape of the soft tissues of the middle third of the face after biodegradation of the carrier gel of Radiesse, Crystalys, Faceteam, confirmed by the results of MRI, can be regarded as an indirect sign of collagenogenesis. At the same time, after the use of the medicinal product Radiesse, according to MRI data, greater efficiency was noted compared to the medicinal products Crystalys and Faceteam.

Based on the results of the assessment of histomorphological and immunohistochemical changes in the tissues after injections of Radiesse, Crystalys and Facetem in general, it can be concluded that all the studied medicinal products have a remodeling effect on the skin. The features of the studied medicinal products include earlier and more pronounced stimulation of elastin production after using

Radiesse with positive dynamics maintained for up to 12 months; earlier stimulation of type 3 collagen synthesis after using Radiesse and Facetem; higher rates of accumulation of types 1 and 3 collagens with dynamic restructuring of their content and new formation of microvessels in the dermis after using Radiesse.

Clinical evaluation of efficacy and safety showed a high level of satisfaction with the results of the procedures performed by both the doctor and the patient.

6. Additional Information

Merz Pharmaceuticals GmbH, Frankfurt am Main (Germany), provided financial support for the preparation of this publication. None of the authors received any financial compensation for their work on the content and writing of this publication. All listed authors approved the article before submission for publication.

7. CRediT Author's Contribution Statement

Ya. A. Yutskovskaya — conceptualization, methodology, project management, writing the draft version of the article; **E. A. Kogan**— research, study and analysis of sources; revision and editing of the article.

8. Conflict of Interest

Dr. Ya. A. Yutskovskaya is a consultant and researcher for Merz Pharmaceuticals. Dr. E. A. Kogan declares that there is no conflict of interest in this work.

References

1. Lemperle G, Morhenn V, Charrier U. Human histology and persistence of various injectable filler substances for soft tissue augmentation. *Aesthetic Plast Surg*. 2003; 27(5): 354-66. doi: 10.1007/s00266-003-3022-1

2. Pavicic T. Calcium hydroxylapatite filler: an overview of safety and tolerability. *J Drugs Dermatol*. 2013; 12(9): 996-1002. PMID: 24002146

3. Kunzler C, Hartmann C, Nowag B, Shah R, El-Banna R, Backfisch S, et al. Comparison of Physicochemical Characteristics and Biostimulatory Functions in Two Calcium Hydroxyapatite-Based Dermal Fillers. *J Drugs Dermatol*. 2023; 22(9): 910-916. doi: 10.36849/JDD.7684

4. Aguilera SB, McCarthy A, Khalifian S, Lorenc ZP, Goldie K, Chernoff WG. The Role of Calcium Hydroxylapatite (Radiesse) as a Regenerative Aesthetic Treatment: A Narrative Review. *Aesthet Surg J*. 2023; 43(10): 1063-1090. doi: 10.1093/asj/sjad173

5. Zakharov NA, Polunin IA, Polunin KE, et al. Calcium hydroxyapatite for medical applications. *Inorg Mater*. 2004; 40(6): 641-648. doi: 10.1023/B:INMA.0000032000.83171.9f

6. Silvers SL, Eviatar JA, Echavez MI, et al. Prospective, open-label, 18-month trial of calcium hydroxylapatite (Radiesse) for facial soft-tissue augmentation in patients with human immunodeficiency virus–associated lipoatrophy: one-year durability. *PlastReconstr Surg*. 2006; 118 (Suppl):34S. doi: 10.1097/01.prs.0000234847.36020.52

7. Zennifer A, Senthilvelan P, Sethuraman S, et al. Key advances of carboxymethyl cellulose in tissue engineering & 3D bioprinting applications. *CarbohydrPolym*. 2021; 256: 117561. doi: 10.1016/j.carbpol.2020.117561

8. Priya G, Madhan B, Narendrakumar U, et al. *In Vitro* and *In Vivo* evaluation of carboxymethyl cellulose scaffolds for bone tissue engineering applications. *ACS Omega*. 2021; 6(2): 1246-1253. doi: 10.1021/acsomega.0c04551

9. Tsvi Segal, Ana Schwartz, Konstantin Konfino. Post Marketing Study of Safety and Efficacy of Crystalys, a Calcium Hydroxyapatite Based Filler for Facial Soft Tissue Augmentation.

10. E.S. Bagnenko, S.A. Povzun. Histomorphodynamics of changes in the skin resulting from the injection of fillers for the purpose of revitalization. Cosmetics and Medicine Special Edition. 2021. No. 4.

11. E.S. Bagnenko, S.A. Povzun. Anisomorphism of calcium hydroxyapatite particles in some fillers as a possible factor in the instability of the results of their use: an electron microscopic study. *Injection methods in cosmetology*. 2018; (2): 7–13.

12. G.M. Mogilnaya, Yu.V. Kovalchuk, E.V. Fomicheva, et al. Injectable implant Facetem and the reaction of the dermis to its injection. *Modern problems of science and education*. 2021; No.5. doi: 10.17513/spno.31070

13. E.S. Bagnenko, S.A. Povzun. Comparative experimental study of the surface-active properties of Radiesse and generics. *Injection methods in cosmetology*. 2017; (4): 30–34.

14. Yutskovskaya Y, Kogan E. Improved neocollagenesis and skin mechanical properties after injection of diluted calcium hydroxylapatite in the neck and décolletage: a pilot study. *J Drugs Dermatol*. 2017; 16: 68-74.

15. Mayer R, Lightfoot M, Jung I. Preliminary evaluation of calcium hydroxylapatite as atransurethral bulking agent for stress urinary incontinence. *Urology*. 2001; 57(3): 434-438. doi: 10.1016/s0090-4295(00)01098-0

16. Marmur ES, Phelps R, Goldberg DJ. Clinical, histologic and electronmicroscopic findings after injection of a calcium hydroxylapatite filler. *J Cosmet Laser Ther*. 2004; 694: 223–226. doi: 10.1080/14764170410003048

17. Berlin AL, Hussain M, Goldberg DJ. Calcium hydroxylapatite filler forfacial rejuvenation: a histologic and immunohistochemical analysis. *Dermatol Surg*. 2008; 34(Suppl 1): S64–S67. doi: 10.1111/j.1524-4725.2008.34245.x