Malar augmentation with Hyaluronic acid enriched with glycine and proline: a clinical evaluation

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The aging process causes skin modification and wrinkle formation with an alteration of the face harmony and imperfections. The aim of the present investigation was to evaluate a cross-linked hyaluronic acid enriched with glycine and proline for zygomatic- malar region infiltrations. A total of twenty healthy female patients were treated for zygomatic hypotonia. The level of patients and surgeon satisfaction was evaluated by global aesthetic improvement scale (GAIS) at baseline, immediately after the procedure and at 6 months from the treatment. The healing phase was uneventful, and no complications were reported in the perioperative and follow-up periods. No significant differences were reported between patients and surgeon GAIS score ($p<0.05$). A significant difference of GAIS score was reported immediately after the procedure and at 6 months if compared to the baseline ($p<0.05$). The cross-linked hyaluronic acid enriched with glycine and proline is a useful biomaterial for zygomatic- malar augmentation with no significant local complications and a high stability and satisfaction level of the procedure.

Genetic, actinic, and environmental factors influence face aging, which is characterized by wrinkle formation, with atrophic changes that produce facial fat and bone loss and skin sagging and influences the youthful appearance of men and women (1, 2).

Aging mostly involves the facial area and is characterized by skin changes, sagging and volume loss. All facial tissues including skin, fat, muscle and bone are involved in the change process which is visible on the surface. So facial aging involves the tissues of support including bone tissue and for this reason bone loss effects the appearance of the skin (3, 4). A recent study clearly demonstrates that the aging of the face is primarily one of bone loss (5) and changes in bones and the facial skeleton have a major affect (6).

Facial bone anatomy is complex, in its suitability to serve a multitude of functions. The teeth are important for soft tissue support and it is known that retrusion of the maxilla can occur in the fully edentulous patient (7). Craniofacial bones alter the facial morphology into one that is instantly recognizable as an aged face (7). The midface bones are formed by the maxilla, by the body and arch of the zygoma (8). In a recent study it has been clearly demonstrated that midface retrusion does occur with aging also in dentulous patients (9, 10), and that the maxilla observed in aging patients is more evident than the zygoma (5), with resorption and loss of

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deep medial cheek fat, resulting in diminishing of the malar projection and width, and reduction of the aesthetic ‘‘ogee curve’’ of the mid face. Such patients often benefit from volumetric augmentation to enhance their skeletal support.

Different techniques have been proposed for soft or hard tissue augmentation (5, 11). The facial soft tissues can be addressed by a resurface technique through laser (12, 13) or atmospheric plasma (14) and chemical peeling. These techniques are nonsurgical cosmetic medicine and are exponentially more commonly used than surgical techniques (15). The absorbable injectable filling agents are largely used in the facial region (16, 17), and the safety of these products substantially contribute to their widespread use. For this reason, they are used in simple, short and rapid procedures in aesthetical facial rejuvenation for tissue enlargement and improvement of aesthetic appearance of the skin (18, 19). Different temporary fillers are such as collagen, agarose (20) and hyaluronic acid (21) are widely used for improving the skin’s contour and reducing depressions in the skin (17, 22). Different HA fillers have been developed for soft tissue augmentation, but it works by simply filling spaces without producing new collagen (23).

The aim of the present study was to evaluate malar augmentation treated by cross-linked hyaluronic acid enriched with glycine and proline with a hypothesis that HA mixed with glycine and proline has slow resorption and therefore a bio-stimulating effect on fibroblasts (24).

MATERIALS AND METHODS

The present clinical study was based in a private practice in Montesilvano (Italy), in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki and the additional requirements of Italian law.

A total of twenty healthy female patients were treated with a mean age 41±3 (age range: 33-48 years old) affected by zygomatic hypotonia, and reduced volume, who were admitted for aesthetic treatment and experimental research. The deflated zygoma increase the formation a nose-labial fold. All subjects signed an informed consent for the clinical study and were treated by zygomatic augmentation procedure. Exclusion criteria included active skin disease, history of autoimmune diseases, irritation, or inflammation in the target areas of injection. Other exclusion criteria included breastfeeding or pregnancy, poor general health, known hypersensitivity or allergy to the treatment components, previous permanent filler treatments in the area, or resorbable fillers in the area in the previous 12 months.

Zygomatic Augmentation Procedure

Cross-linked hyaluronic acid sodium hyaluronate (26 mg) and 2.5% of amino acids, glycine and proline, in sterile buffered water (Italfarmacia, Rome, Italy), was used in this study. The zygomatic skin area was covered with an anesthetic cream containing prilocaine and lidocaine (Emla, AstraZeneca, Sweeden) for blocking nerve signals to reduce discomfort during infiltration of the filler. It was applied 20 to 40 minutes before the treatment, removed with gauze and the skin was disinfected with chlorhexidine 0.2% immediately before the injections. The patients’ mouths were rinsed with a chlorhexidine digluconate solution 0.2% for 2 minutes. Also, infiltrative anesthesia of the infraorbital nerve was performed with an intraoral approach with Articaine® (Ubistesin 4% - Espe Dental AG Seefeld, Germany) associated with epinephrine 1:100.000.

The Hinderer model was used for treating the patients and to determine the position of the needle tip, thereby ensuring correct placement of the filler (Fig. 1). Two intersecting lines were designed to identify the zygomatic-malar region, one from the lateral canthus to ala and one from the tragus to the labial commissure, the Ha was placed in the upper outer quadrant. So, the upper outer quadrant was the point of needle insertion, to 2-4 mm along the skin until it touched the bone, at this point, while removing the needle the HA was delivered in the preperiosteal plane determining soft-tissue augmentation with retrograde linear threading technique following the fan technique. Once the needle was in deep between the pre periosteal plane, under the malar bone plane, the filler was delivered during withdrawal of the needle, creating a canal-shaped pattern and delivery of the filler was interrupted immediately before complete withdrawal of the needle.

The filler was injected by a transcutaneous approach through a 25G needle, length 13 mm, to provide

a zigomatic augmentation and the application was administered following a retrograde technique following fan application. The needle entered the skin at an angle of 70°-90° to the surface. The HA was be injected slowly, in a retrograde manner with a microbolus technique (25). The HA was gently moulded to smoothness through manual pressure so as not to predispose the patient to ecchymoses. This technique creates an expansion and elevation of the overlying soft tissue envelope, resulting in a more natural and aesthetically pleasing appearance. An aliquot of between 0.6-1 ml of filler was placed for each malar area (~1 syringes) until a clinical effect was visible and the total volume injected was recorded.

All the procedures were performed by a single aesthetic surgeon. No massage was performed, we recommended intermittent local application of ice for ten minutes, no patients needed oral cortisone or anti-inflammatories. All patients were told to sleep in a supine position with the head upward to reduce water collecting around the implant and to drink a lot of water for 2-3 days after the treatment.

Patients were additionally photographed in frontal and oblique views at a distance of 1m, with standardized zoom and automatic focus, allowing for a clear image of the malar area. Before, immediately after the filler treatment and after 6 months, standardized photographs were again taken, and the patient and the surgeon satisfaction were measured by means of a validated Global Aesthetic Improvement Scale (GAIS):

- Grade 5: Excellent (completely satisfied with the result)
- Grade 4: Very good (very satisfied with the result)
- Grade 3: Satisfactory (although a slight improvement is seen, an additional correction is required)
- Grade 2: Indifferent (no changes)
- Grade 1: Unsatisfied (the patient´s condition is worse than before the procedure)

Percentage change in malar area and change in subjective patient scores were compared between before and after treatment.

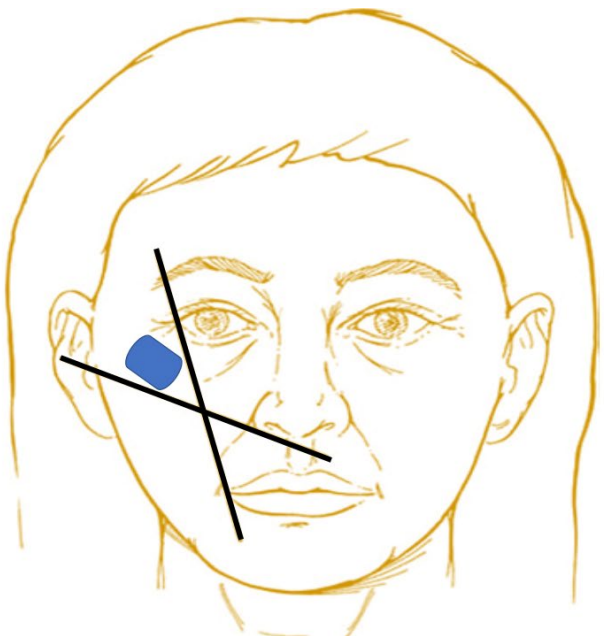

Fig. 1. *Hinderer model used for treating the patients and to determine the position of the needle tip, thereby ensuring correct placement of the filler (blue area).*

Statistical analysis

The sample size to demonstrate the relative overall improvement after HA filler injection was calculated using a software for determining the number of patients to achieve statistical significance for validated Global Aesthetic Improvement Scale (GAIS) (26). The planned enrolment was 20 subjects, assuming a drop-out rate of 10%. The sample size was calculated for dichotomous variables (yes/no effect) by the incidence effect (Before treatment: 85%; After treatment:15%); the alpha error was set at 0.05 and power was 90%. The optimal sample size for the evaluation was 16 patients per group. Numerical results are presented as the ±SD means of all the experiments. The study data was statistically analyzed by the dedicated software package Graphpad 6 (Prism, San Diego CA- USA). The normality distribution was tested by the Kolmogorov-Smirnov analysis. The Student t-test was conducted for a statistically significant comparative evaluation between the study groups. The level of significance was set at $p<0.05$.

RESULTS

No drop-out was recorded. The effect on the malar volume augmentation was evaluated using Global Aesthetic Improvement Scale (GAIS). GAIS was evaluated after HA filler injection compared to before injection, by the subjects (Fig. 2). Erythema was present to a minimal extent immediately after filler treatment and no edema was recorded at day 1 (Fig. 3-4).

The results immediately and six months after the treatment showed a mean patient satisfaction score respectively 4.2±0.3 and 3.4±0.4, while the surgeon satisfaction index was 4.1±0.4 after the procedure and 3.1±0.2 at 6 months (Fig.5). A statistically significant difference was detected from baseline to immediately after treatment ($p<0.01$) and baseline to 6 months ($p<0.05$). All subjects showed at least 2 grade improvement in GAIS score after HA filler injection. The analysis of patient satisfaction after the last follow-up clearly demonstrated good results. There were no serious adverse events observed, such as outbreaks of herpes, ecchymosis, hyperpigmentation, hypopigmentation, erythema, itching, pain, infectious processes nor scarring or allergy. Only one patient reported swelling of the malar region which recovered completely within 5 days after the HA filler injection. No statistical differences were detected between patients and surgeon Global Aesthetic Improvement Scale (GAIS) at baseline, immediately after the treatment and at 6 months (Fig. 5).

Comparative analysis of photographic documentation showed that after 6 months the volume obtained was diminished, but the patients were still satisfied with these results. Decrease in the increase

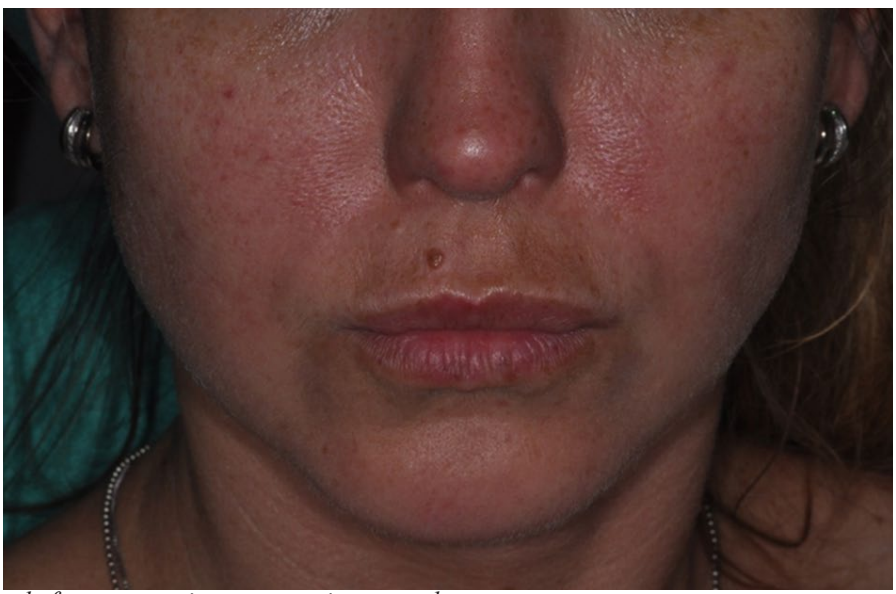

Fig. 2. *Frontal view before zygomatic augmentation procedure.*

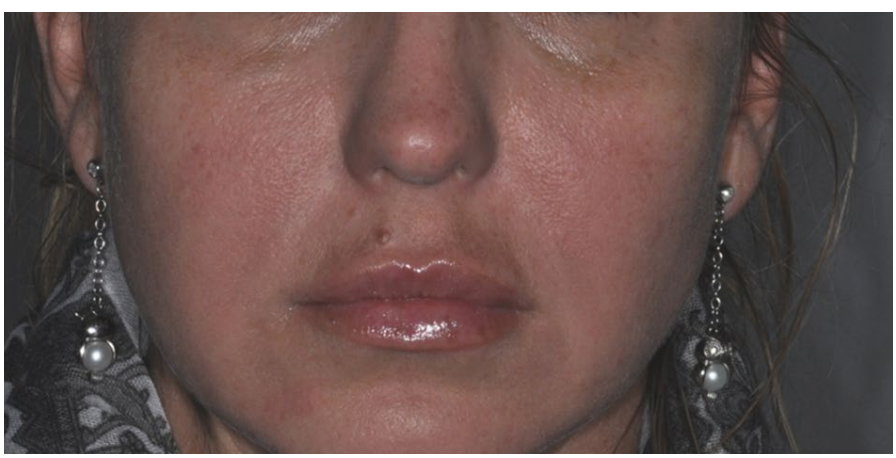

Fig. 3. *Frontal view immediately after zygomatic augmentation with cross-linked hyaluronic acid sodium hyaluronate (26 mg) and 2.5% of amino acids, glycine and proline.*

in volume of the malar region was observed by only two patients, that might need another treatment. In one patient an asymmetry was observed, and a new treatment was scheduled after one week.

A mild swelling and prominence of the implant were observed due to immediate local tissue response. In the following day, no bruising was recorded and to reduce this incidence needle repositioning for another passage was avoided. Macroscopically, a clinical effect with an appreciable change in malar region was recorded immediately after the procedure. Hyaluronic Acid deposits were still visible at the 6 months follow-up.

DISCUSSION

The outcome of this study showed a good aesthetical result with great satisfaction of the patients and the surgeon. An augmentation of the malar area after use of the hyaluronic acid filler with a rejuvenation of the malar area resulted in a more youthful appearance. These results were persistent until six months, confirming that the improved performance of volume augmentation of the malar are due to HA filler enriched with glycine and proline.

Several clinical studies have described the therapeutic use of molecular enriched HA for skin regeneration and wound healing (27). A recent research showed that HA fragments associated with amino acids produce stimulation of fibroblasts and increase collagen protein (24). In the present study we have chosen to add the amino acid in the HA because collagen is a protein with a combination of amino acids, glycine (Gly), proline (Pro) and

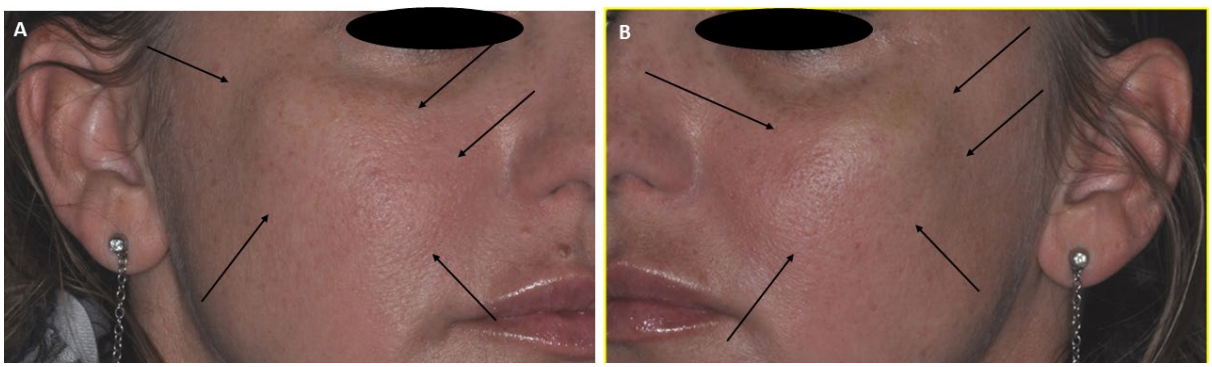

Fig. 4. *¾ view 12 months after the zygomatic augmentation.* ***A)*** *It can be noted a local improvement in right zygomatic volume (Arrows).* ***B)*** *After treatment evident a local improvement in left zygomatic volume (Arrows).*

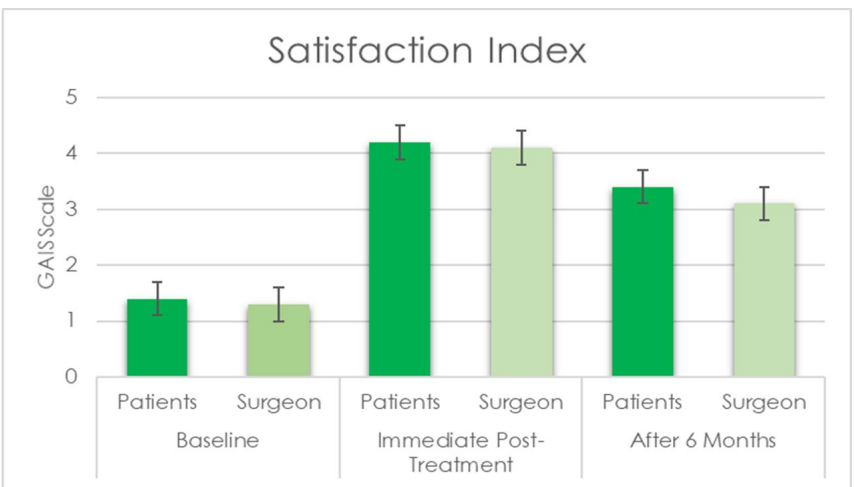

Fig. 5. *Bar graphs of the Global Aesthetic Improvement Scale before treatment and at six months after treatment.*

hydroxyproline (Hyp) the extremely repeated units that are responsible for the triple-helical structure (28). Glycine is the most dominant unit in collagen and helps the formation and stabilization of collagen structure through hydrogen bonding (29). The HA used in the present study is safe, able to produce an immediate and lifelong volumetric effect with a natural appearance.

HA filler has shown encouraging clinical results in terms of texture, softness, and quality of skin pattern. It is the most used filler in clinical practice and has been exponentially used in the past two decades for facial soft tissue augmentation lost for congenital, acquired, or senile issues. Face rejuvenation with HA is an easy technique, effective for the treatment of all small skin wrinkles, being able to improve the texture and elasticity. It is considered a long-lasting, resorbable filler, and is highly biocompatible (30, 31). The results indicate that HA enriched by glycine and proline can create a lasting soft tissue augmentation in the malar area. However, some minor and major complication have been reported after use of the filler.

Minor complications of early onset such as swelling, bruising, erythema overcorrection, surface irregularities, filler visibility are relatively common. The major complications occur secondary to cannulation of the vessels (32). In the malar area there is the vascular danger zone of the infraorbital area (33), this arterial is not located in the infiltration area used in the present study, in fact Henderer's lines were used to individualize the injection area. The fat pad gives volume to the malar area, it is a triangular shaped area with base at the nasolabial fold and apex at the malar eminence in the youthful face. During face aging there is a loss of skin elasticity and weakening of the septae, that extend from the superficial musculoaponeurotic system (SMAS) to the malar fat (9). This event leads to apoptosis and forward descent of the skin and malar fat pad and creates a nasolabial fold. The filler used can rebuild facial harmony, balance, and beauty. Malar volume and contour restoration using HA fillers have become a valuable addition to the aesthetic surgeon's armamentarium because they are quite fast to perform, have little down time, and result in a high rate of patient satisfaction.

One major adverse compliant in malar augmentation is malar edema because it can persist for 6 to 8 months and only minimally responds to methylprednisolone and massage (34).

To avoid this adverse event, it is important to limit the filler quantity, and by placing the HA filler deep and laterally to the malar septum at the immediate preperiosteal level (34). In the present study the HA was delivered in a preperiosteal plane on the malar area bone, placing small boluses of filler, following a fanning technique from a single access point. HA injecting over the periosteum has the advantage of having the bulk of the correction under the muscles, avoiding the ugly effect of too much moving volume during mandible movement when most of the volume is placed in the superficial subcutaneous or skin layer. With exponential increase in the popularity of non-invasive techniques, it is very important to approach patients seeking rejuvenation enhancement and/or with a gentle modality, respectful of their anatomy and age. Temporary fillers satisfy many cosmetic patients' needs and guarantee an immediate and long-term result and Hyaluronic acid gels are the most widely used soft tissue fillers for volume replacement.

Given that an increased demand on the market for soft tissue augmentation fillers is reported, safety represents a fundamental property for all medical devices and products. The effectiveness of the present study reported that the HA with glycine and proline is capable of malar augmentation, with no clinical response, and an excellent tolerability of the treatment.

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