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Original article

A CLINICAL INVESTIGATION OF HYALURONIC ACID FORTIFIED WITH AMINO ACIDS FOR ADDRESSING FACIAL AGING

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ABSTRACT

Within the field of aesthetic medicine, various methodologies have been employed to combat skin aging, particularly in the facial region. One such approach involves the utilization of hyaluronic acid to enhance water retention and support extracellular matrix integrity. This research aims to clinically and histologically assess the impact of combining low molecular weight hyaluronic acid fragments with amino acids (HAAM) on revitalizing facial skin through intradermal microinjections. A cohort of twenty female participants, with an average age of 45 falling within the range of 35 to 64, was included in this investigation. Among them, eight were in the postmenopausal stage, while twelve were in their childbearing years. Mesotherapy was employed to administer HAAM products to the patients for 4 section. The outcomes from the current investigation revealed that applying hyaluronic acid with fragments of 20 to 38 monomers, along with amino acids through the dermal injection technique, leads to an enhancement in the visual appearance of the treated

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patients' facial features. The clinical evaluation highlighted the significant influence of subcutaneous HAAM infiltration on both the dermis, improve pH levels, sebum production, skin hydration and enhance the overall clinical presentation of the facial region.

KEYWORDS: *Skin imperfections, Cutaneous biostimulation, Hyaluronic acid fragments, Aesthetic enhancement.*

INTRODUCTION

The human skin serves a dual role, acting as both a protective barrier and a mediator of metabolic and informational exchanges between the body and its external environment. Unfortunately, various external factors like ultraviolet radiation and internal stressors such as endocrine-metabolic conditions, coupled with the inevitable passage of time, can lead to a series of functional and structural changes in the skin. These alterations can compromise its integrity to varying degrees (1). This can manifest as issues like uneven pigmentation, lack of moisture, reduced elasticity, and microvascular transformations. These concerns can become particularly pronounced in critical areas like the face and décolleté, potentially becoming significant aesthetic concerns (2-4).

To counteract these skin imperfections, especially the emergence of wrinkles and related issues, the concept of "cutaneous biostimulation" has been proposed in the field of cosmetic procedures. This approach involves injective techniques aimed at revitalizing the skin's normal structure and functions. This is achieved through targeted interactions with the fibroblasts that form the skin and the surrounding extracellular matrix (5).

The current study investigates the potential of hyaluronic acid, alongside select organic and inorganic substances, in preventing and treating common skin imperfections. The primary objective is to clinically evaluate the impact of introducing low molecular weight hyaluronic acid fragments combined with amino acids (referred to as HAAM) through intradermal microinjections on the rejuvenation of facial skin.

MATERIALS AND METHODS

A total of twenty women, with an average age of 45 and ranging from 35 to 64, were enrolled in this study. Among them, eight were in the menopausal phase, while twelve were in the childbearing age range. The research took place within a private medical practice setting. The investigation was conducted in collaboration with a private, multi-specialty medical practice located in Montesilvano and Modena, Italy. The study adhered fully to ethical principles, including the guidelines set forth in the World Medical Association Declaration of Helsinki (6), as well as the additional requirements specified by Italian law.

Each participant provided informed consent prior to undergoing the prescribed procedure. Notably, none of the participants were aware of the specific condition being addressed through the treatment. All patients exhibited common characteristics, including generalized rhytidosis, inadequate skin hydration, and diminished sebum production. These conditions were observed under circumstances of dryness and low oxygen levels, and there were no modifications to their dietary habits during the course of treatment. Individuals who had made alterations to their diets, were pregnant, had a history of heavy smoking (20 cigarettes per day), had a history of allergic and/or irritant contact hand dermatitis, were afflicted with systemic diseases, or had psychiatric disorders were excluded from participation. After anamnestic information collection and physical evaluation, the patients were submitted to pH measurement, assessment of sebometry and hydrometry; photography in the glabella, and malar-cheek regions. All patients received the HAAM products (SKIN-B® Italfarmacia, Rome, Italy) by mesotherapy technique, for 8 weeks providing a treatment on a weekly basis with the use of 2.5 cc syringes and 13 mm 30G needles. The solution was inoculated into the deep layer of the dermis with a suitable amount of at least 0.2 / 0.3 mL in the cutaneous points every 15 days, for 4 times.

Statistical analysis

The study data were analyzed by the statistical software package Graphpad 8 (Prism, San Diego- CA USA) through a special designed form. The D'Agostino & Pearson test was conducted for the normal distribution assessment and visually

represented by the QQ-distribution plot. The paired t-Student test was conducted to measure the significance of the study variables differences before and after the treatment. The level of significance was considered for $p<0.05$.

RESULTS

All participants successfully completed the follow-up period. Treatment involving the use of injectable HAAM medical devices yielded favorable outcomes, visually captured through photographs. This was manifested by a noticeable reduction in periorbital and frontal wrinkles, an enhancement in skin texture, an increase in skin radiance and firmness, along with volumetric improvements. These immediate enhancements contributed to a visibly healthier and more aesthetically pleasing skin appearance (Fig. 1).

Physical examinations indicated a noteworthy enhancement in dermal appearance of various facial regions including the glabella and malar-cheek regions. This improvement was noted in the metrics of pH levels, sebum production, and skin hydration when compared to the baseline measurements (Table I).

Ultimately, the study successfully achieved its intended objective, specifically showcasing that the application of medical devices containing hyaluronic acid fragments spanning 20 to 38 monomers via injection techniques led to a noticeable aesthetic enhancement in the facial features of the treated patients. The improvement primarily observed within the first month. Importantly, this enhancement was statistically significant (Fig. 2, Table I).

DISCUSSION

The findings of the present study underscore that employing medical devices containing hyaluronic acid fragments spanning 20 to 38 monomers, coupled with amino acids through dermal injections, leads to a discernible enhancement in the aesthetic quality of treated patients' facial features. The utilization of HAAM treatment appears to have a tangible impact on

Table I. *Summary of the effectiveness before and after the biomodulation treatment (mean, standard deviation).*

	pH-metry		Sebometry [µg/cm2/min]		Hydration [g/m²/h]	
	Before	After	Before	After	Before	After
Mean	4.81	5.81	39.8	59.1	57.5	67.9
SD	±0.95	±1.40	±2.5	±3.3	±4.8	±3.7
Lower 95% CI	4.36	5.15	38.66	57.6	55.3	66.3
Upper 95% CI	5.26	6.46	41.00	60.6	59.8	69.6
p- value	p=0.03		p<0.01		p<0.01	

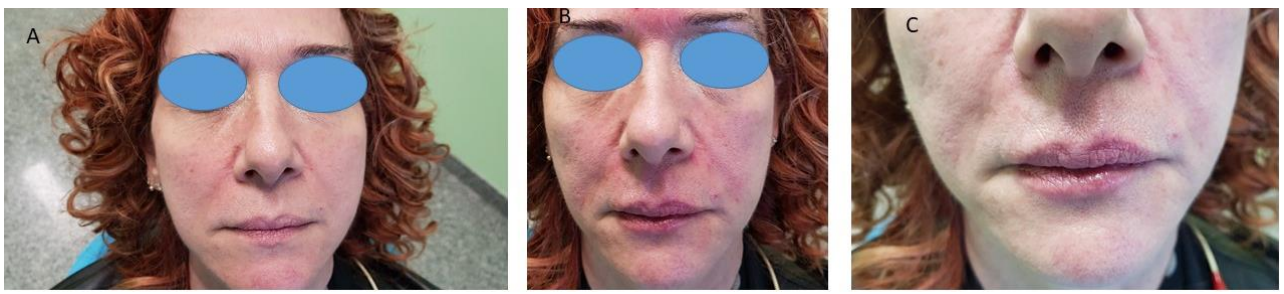

Fig. 1. ***A.*** *Before treatment.* ***B.*** *Immediately after treatment with the injectable medical devices of the HAAM was associated with a mild and transient erythema.* ***C.*** *After 2 months favourable results are visible at a photographic level.*

epidermal hydration, potentially indicating an increased availability of water for regular physiological processes (7).

Hyaluronic acid stands out as the predominant glycosaminoglycan within the extracellular matrix, attaining its peak concentration in highly hydrated tissues like the vitreous fluid and the umbilical cord (8). Enzymatic activity by hyaluronidases fragments this acid, subsequently triggering processes like angiogenesis, tissue remodeling, and cellular turnover (9, 10). The length of these hyaluronate fragments (HF) directly influences their biological effects. A greater number of receptor sites exposed to longer HF reduces the likelihood of ligand detachment from the cell surface. A gradual increase in HF length, transitioning to 20-22 monomers, correlates with a substantial surge in receptor avidity, which then triples (11, 12). An HF containing 20 monomers, composed of two hexamers separated by an octamer with a probable favorable conformation (e.g., helicoidal), each binding to a CD44 receptor, achieves an optimal state (13).

The integration of hyaluronic acid fragments with amino acids (HAAM) aptly meets the requirements of contemporary physiological biomodulation. These HAAM complexes effectively achieve their objective of redirecting metabolic and informational flows to restore the morpho-functional balance in imperfect skin. This concept draws a parallel to the role of musical instruments within an orchestra, altering sound or tone to achieve a harmonious effect (14).

Multiple studies conducted both in vivo and in vitro have reported that amino acids serve as stimulants for protein synthesis (15, 16).

The incorporation of amino acids helps in maintaining a stable pH within the extracellular matrix.

Furthermore, these amino acids could potentially be harnessed for the synthesis of bioactive peptides (17). During this process, primary amino acids like glycine, L-proline, and L-alanine play a crucial role in synthesizing collagen polypeptide chains. Meanwhile, glycine, L-valine, L-alanine, and L-proline are vital for elastin synthesis (18). Subsequently, branched-chain amino acids like L-valine, L-leucine, and L-isoleucine are indispensable for promoting the synthesis of actin, a contractile protein present in myofibroblasts derived from fibroblasts (19). Serine, a targeted amino acid in post-translational kinase/phosphatase modulation processes, serves as a precursor for arachidonyl-serine, an endocannabinoid recently discovered for its capacity to stimulate endothelium repair and angiogenesis (20). Lastly, cysteine serves as a

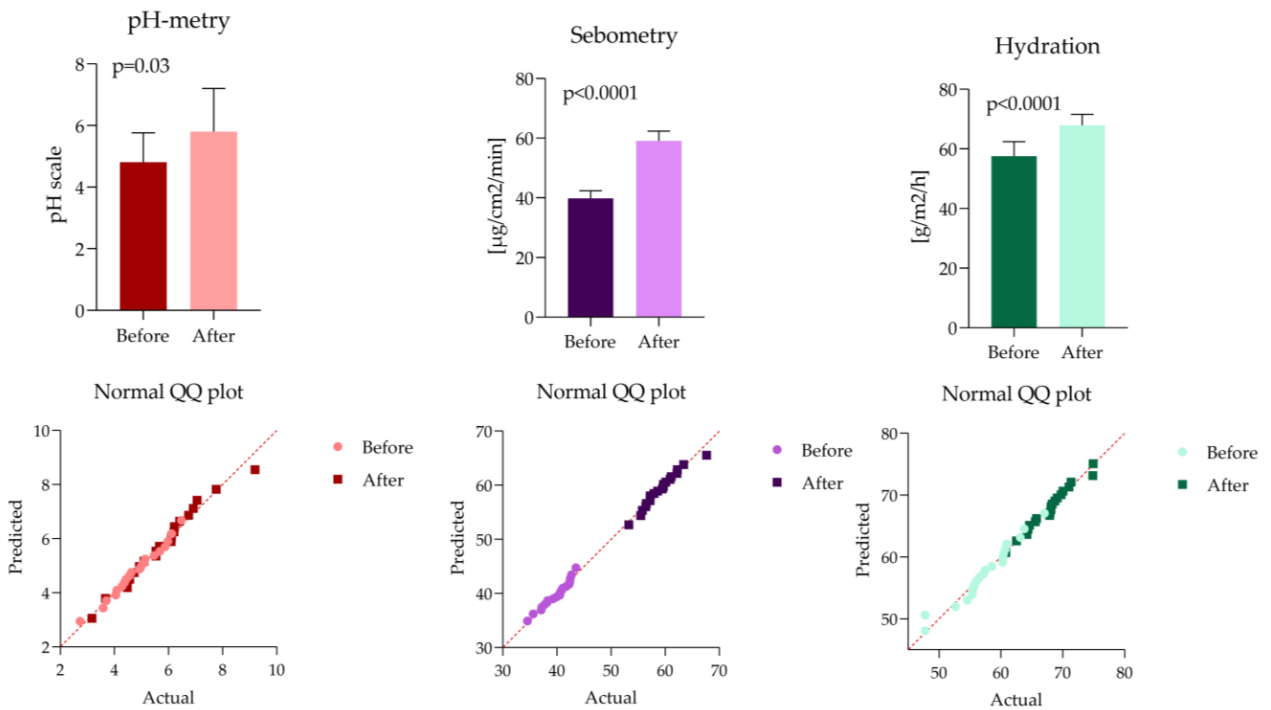

Fig. 2 *The bar graphs show the number of pH-metry, sebometry and tissue hydratation, before and after the treatment (paired t-Student test).*

precursor for glutathione, the most potent intracellular antioxidant (21, 22). Its role is pivotal in modulating the impact of reactive oxygen species (ROS), as an excess of ROS could be detrimental to cellular repair processes (23).

In summary, the utilization of HAAM through injection technique improve pH levels, sebum production, skin hydration and enhances the aesthetic appearance of the treated patients' facial features.

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