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Preliminary Report

Preliminary Prospective and Randomized Study of Highly Purified Polynucleotide vs Placebo in Treatment of Moderate to Severe Acne Scars

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Abstract

Background: Managing acne scars is a challenge and therapies are divided into nonsurgical and surgical. Highly Purified Technology Polynucleotides (PN-HPT) is a compound that contains a mixture of DNA polymers of different lengths. Numerous studies have shown that PN-HPT also serves as an energy source, thus influencing cellular growth and cell vitality.

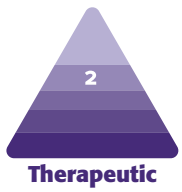
Objectives: The authors aimed to assess the improvement in dermal quality and acne scars after PN-HPT vs placebo according to Antera 3D and the patient responses to the patient satisfaction questionnaire after a comparison of pretreatment and posttreatment photographs at 1 and 3 months.

Methods: Included were women aged 30 to 50 years with grade 3 to 4 moderate-to-severe atrophic scars according to the Goodman classification; nonsmokers; and had not had active acne during the past 5 years. Ten patients (PN-HPT group) were treated with 4.0 mL of PN-HPT, and 10 patients (control) were treated with 4.0 mL of normal saline. All medical treatments were performed in a double-blinded manner; neither the injection doctor nor the patient knew if the PN-HPT or the placebo was being administered.

Results: Twenty women who fit the inclusion criteria were enrolled in this study. Only patients in the PN-HPT group improved significantly at 1 and 3 months after treatment compared with baseline.

Conclusions: This prospective and randomized study showed that PN-HPT in monotherapy was safe and effective treatment for atrophic scar acne compared with placebo. Prospective and randomized studies will be necessary to investigate the clinical effectiveness in a larger cohort of patients and for a longer follow-up.

Level of Evidence: 2



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The most common sequelae of the severe inflammatory process of acne is scarring, which is prevalent in more than 90% of adolescents.[1,2] Scars are characterized by a loss or damage of tissue; vary in morphological characteristics such as shape, size, and depth; and are graded as various types such as rolling, boxcar, and ice pick.[3]

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Furthermore, they can be erythematous at the early stage or may become purplish or pigmented as they develop.[4,5] In most patients, acne scars are mixed types and distributed around the face. A standard tool for assessing atrophic scars is the 4-grade Goodman and Baron classification.[6]

Managing acne scars is a challenge, and therapies are divided into nonsurgical and surgical. The former includes anti-acne agents, chemical peels, microneedling, ablative and non-ablative fractional and nonfractional lasers, and microfused ultrasound alone or associated with fractional CO_2 laser resurfacing.[7-25] The latter include subcision.[26-28] Platelet-rich plasma has been employed as noninvasive procedure for atrophic acne scar.[29,30] Furthermore, microneedling and erbium-YAG laser associated with autologous growth factors produced statistically significant improvement in validated outcomes over microneedling, subcision, or erbium-YAG laser alone.[31,32]

Highly Purified Technology Polynucleotides (PN-HPT) (Mastelli srl, Sanremo, Italy) is a compound that contains a mixture of DNA polymers of different lengths. PN-HPT is obtained from male salmon trout gonads through purification and high-temperature sterilization. PN-HPT has been utilized in aesthetic medicine as biorevitalizer in monotherapy and associated with CO_2 laser for stretch marks, in dermatology for healing of venous ulcers of the lower limbs, in postmenopausal women as biorevitalizer for labia majora, and in orthopedy for intra-articular osteoarthropathy knee treatment.[33-38] The goal of this prospective and randomized study was to assess the safety and effectiveness of PN-HPT monotherapy for treating moderate-to-severe atrophic acne scars.

METHODS

This study was conducted in accordance with the ethical principles of the 1975 Declaration of Helsinki and is consistent with Good Clinical Practice principles.

Study Population

Inclusion criteria included Caucasian women aged 30 to 50 years with grade 3 to 4 moderate-to-severe atrophic scars according to the Goodman classification; nonsmokers; had not had active acne during the past 5 years; and had never undergone any dermatological or aesthetic medical treatments for acne scars.

Exclusion criteria included men; Asian and African women; patients younger than 30 or older than 50 years; smokers; with mild atrophic scars according to the Goodman classification (grade 1-2); underwent nonsurgical or surgical face treatment for acne scars; were pregnant; had systemic or local illnesses that might affect wound healing; had severe solar elastosis or scarring; had a history of keloid formation or hypertrophic scars; utilized anticoagulant or antiplatelet medications; or had a history of autoimmune disease or chronic drug or alcohol abuse.

Study Design

Ten patients (PN-HPT group) were treated with 4.0 mL of PN-HPT (Plinest, Mastelli, Sanremo, Italy), and 10 patients (control group) were treated with 4.0 mL of normal saline. A total of 30 microdrops (0.1-0.2 mL at each injection point) were delivered subdermally on the face by a 30-G, 13-mm needle. Patients did not receive any posttreatment medication and were asked not to massage the treated areas. The treatment was repeated after 3 weeks.

All medical treatments were performed in a double-blinded manner; neither the injection doctor (first author A.A.) nor the patient knew if the PN-HPT or the placebo was being administered.

The study was conducted from January to March 2020.

Randomization

The randomization was conducted utilizing computer software (Filemaker Pro, FileMaker Inc., Santa Clara, CA). Two separate databases were created: a patient database that listed basic information such as patient name, age, and gender; and a randomization database with data on which patients had been registered in the trial along with their treatment allocations.

The database also included a “coin flip” programming code for randomization. The computer randomly generated a number between 0 and 1 and assigned patients to treatment with PN-HPT if it was less than 0.5 and otherwise to the control. The randomization database was password protected so that it was accessible only by the primary investigator, second author of the study (F.A.), and a nominated computer technician.

The injection doctor (A.A.) accessed the patient database, typed in the patient’s name and details, and then utilized an icon to randomize the patient. This led to a dialog box asking the doctor to confirm that the patient was eligible. After the “okay,” the patient’s information was automatically sent to the randomization database. The result of randomization (patient allocated to PN-HPT or control) was stored and sent back to the patient database where the injection doctor could see it. Because the injection doctor could not access or modify the secure, password-protected randomization database, there was no way to predict a patient’s allocation before registration to the trial or to change it afterwards.

Table 1. Patient Satisfaction Questionnaire (PSQ)

		Score
Not satisfied	I look worse than before	0
	I cannot see any difference before and after. My relatives do not notice any difference.	1
	I can see minimal difference before and after. My relatives do not notice any difference.	2
	I can see moderate difference before and after. My relatives notice minimal difference.	4
Moderately satisfied	I can see moderate difference before and after. My relatives notice moderate difference.	6
Satisfied	I can see good difference before and after. My relatives notice moderate difference.	8
Very satisfied	Beyond my expectations. All my relatives notice a great improvement.	10

Assessment of Efficacy and Tolerability

Digital macrophotographs were taken to ensure reproducibility in terms of positioning and lighting with a Nikon camera (Nikon Corporation, Tokyo, Japan; D7100, 12.0 megapixels, AF-5 micro Nikkor 60 mm, close-up 4D–62 mm + Nital Macro Lighting Spider). Furthermore, assessments were made by Antera 3D (Miravex Limited, 11 St. Stephen's Green, Dublin 2, Ireland), which provides qualitative and quantitative analysis of wrinkles, texture, and hemoglobin. Antera 3D has been proven to be effective and has been validated in the treatment of wrinkles and for analyzing texture.[39-41]

Finally, patients were provided with a patient satisfaction questionnaire (PSQ) developed in-house by our working university group and utilized for the first time in this prospective study to measure patient satisfaction after a comparison of pre- and posttreatment photographs at the 1- and 3-month follow-up (Table 1). The PSQ was anonymous. The questionnaire was administered by email, and all patients returned the questionnaire. Follow-up was performed after 1 and 3 months after the second treatment. Results at 1 and 3 months were compared with baseline, and results at 3 months were compared with results at 1 month.

Study Endpoints

The first study endpoint was the improvement in dermal quality and acne scars after PN-HPT vs placebo according to Antera 3D at 1 and 3 months of follow-up.

The second endpoint was patient responses to PSQ after a comparison of pre- and posttreatment photographs at 1 and 3 months of follow-up.

Assessment of Safety

The patients' faces were examined for evidence of any acute response, such as erythema or edema, and the days after any minor or major side effects were recorded.

Statistical Analysis

Data analysis was performed employing the Statistical Package for the Social Sciences Windows version 13.0 (SPSS, Chicago, IL). Descriptive statistics for quantitative continuous variables were the mean and standard deviation after confirmation of a normal distribution. Normality assumptions were demonstrated with histograms, Q-Q plots, skewness, and kurtosis as well as Kolmogorov/Smirnov and Shapiro-Wilk tests. Descriptive statistics for qualitative categorical variables were performed with frequencies. A *t* test was utilized to compare continuous variables among groups. The χ^2 test and Fisher's exact test were employed to compare nominal variables. All *P* values were considered significant if below 0.05.

RESULTS

Twenty women (average age 36.3 ± 5.48 years; range, 30-44 years) who attended our medical centers of aesthetic medicine in Milan and fit the inclusion criteria were enrolled in this study. Before treatment, they were asked to stop utilizing any cosmetic creams, make-up, or sunscreen creams and to avoid exposure to the sun for 4 weeks before and during the study period. Patients were informed about the study treatment protocol, signed a consent form, and were divided into 2 homogeneous groups (Table 2). They received the full treatment according to the study design. No minor or major side effects were reported during the study, and all of the patients completed the follow-up after 3 months.

Results in the PN-HPT Group

According to Antera 3D, wrinkles ($P < 0.05$) and skin texture ($P < 0.05$) improved significantly at 1 and 3 months after treatment compared with baseline. Hemoglobin increased by 13% after 1 month and by 19% after 3 months,

Table 2. Patient Data

		Group 1	Group 2	
Patient	20	10	10	
Age (y)	36.3 ± 5.48 (range, 32-49)	37.4 ± 3.29	38.6 ± 2.68	N.S.
BMI (kg/m^2)	22.8 ± 1.03	23.2 ± 1.0	21.9 ± 1.3	N.S.
Ethnicity (Caucasians)	20	10	10	N.S.
Fitzpatrick	3.15 ± 0.44	3.2 ± 0.62	3.8 ± 0.8	N.S.
Wrinkles		41.1 ± 1.18	40.2 ± 1.8	N.S.
Texture		37.1 ± 0.69	36.8 ± 1.3	N.S.
Hemoglobin		12.08 ± 1.9	11.25 ± 0.9	N.S.

N.S., not significant.

Table 3. Result: Group 1

Group 1	Before	1 mo		3 mo	
Wrinkles	41.1 ± 1.18	33.14 ± 1.33	$P < 0.05$	29.24 ± 1.1	$P < 0.05$
Texture	37.1 ± 0.69	27.79 ± 0.74	$P < 0.05$	22.75 ± 0.54	$P < 0.05$
Hemoglobin	13.06 ± 1.17	14.16 ± 1.12	N.S.	16.26 ± 1.13	N.S.
PSQ	12.08 ± 1.9	5.26 ± 0.88	$P < 0.05$	4.92 ± 1.12	$P < 0.05$

N.S., not significant; PSQ, patient satisfaction questionnaire.

but the difference was not statistically significant ($P > 0.05$). Based on the PSQ data, patients were moderately satisfied at 1 month and satisfied at 3 months posttreatment (Table 3) (Figures 1A and B; 2A and B; 3A and B; 4A and B; and 5A and B).

Results in the Control Group

According to Antera3D, wrinkles ($P > 0.05$), skin texture ($P > 0.05$), and hemoglobin ($P > 0.05$) failed to improve at 1 and 3 months after treatment. Based on the PSQ data, patients were not satisfied either at 1 and 3 months posttreatment (Table 4).

Comparative Results Among Groups

According to Antera 3D, wrinkles and skin texture improved significantly more in the PN-HPT group ($P < 0.05$) compared with the control group at 1 and 3 months after treatment. No difference was recorded for hemoglobin ($P > 0.05$) (Table 5).

DISCUSSION

For the treatment of atrophic acne scars, a wide range of methods have been proven to be effective for improving and reducing their appearance as the high intensity focused ultrasound.[42-44] PN-HPT is a compound that contains a mixture of DNA polymers of different lengths. PN-HPT is obtained from the sperm of trout salmon by an extraction process in which purification and high-temperature sterilization procedures are performed to obtain a pure active product.

Numerous studies have shown that PN-HPT injection can be employed in clinical settings to treat wounds and ulcers.[36] PN-HPT has a consolidated utilization in the aesthetic field from skin rejuvenation to stretch marks and vulvo-vaginal biorevitalization; recently, specific guidelines in their utilization have been implemented.[33-35,37] To the best of our knowledge, no controlled study has yet investigated the efficacy and safety of PN-HPT in monotherapy for atrophic acne scars. We included in the study only young Caucasian women and excluded other groups to avoid ethnicity bias. We compared the PN-HPT group with a homogeneous control group at 1 and 3 months.

By utilizing Antera 3D, we demonstrated changes in the quality of the dermis and in particular qualitative and quantitative analyses of wrinkles, texture, and hemoglobin. Our study showed that PN-HPT monotherapy significantly improved wrinkles and skin texture and reduced clinical evidence of atrophic acne scars compared with placebo at 1 and 3 months. Furthermore, the increase in the hemoglobin level suggested that PN-HPT improved the vascularity of the targeted tissue, but this difference was not statistically significant. This may

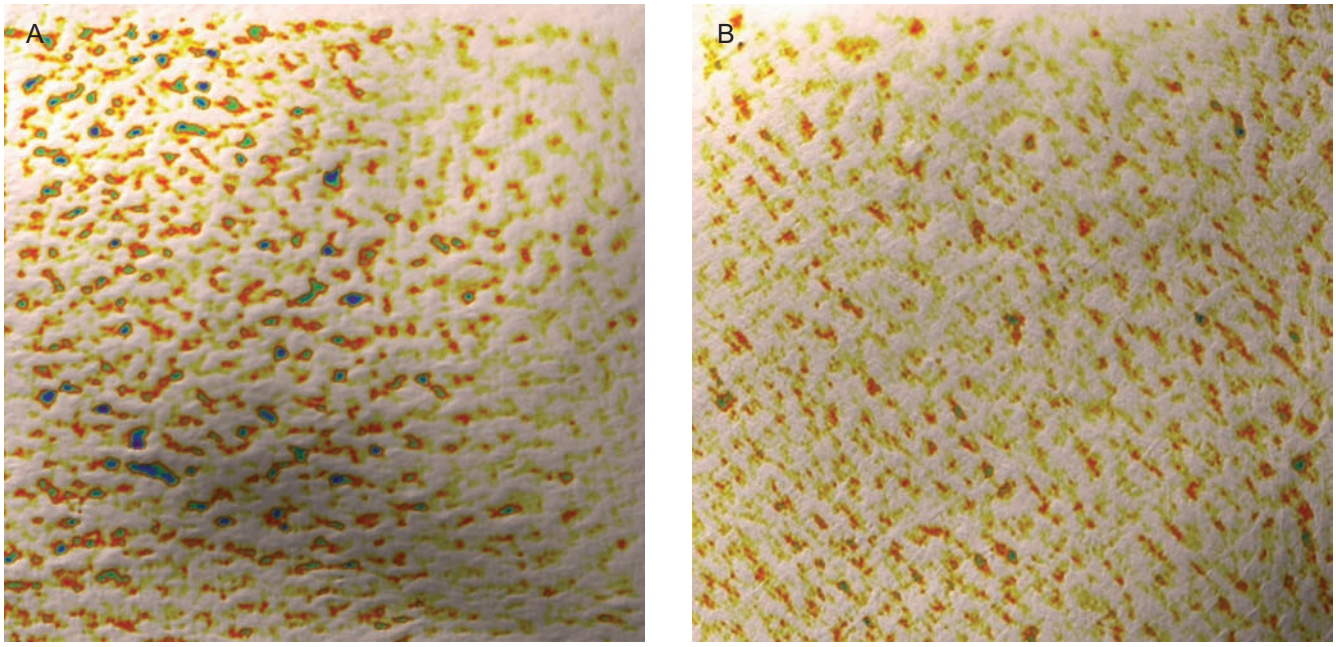

Figure 1. This 43-year-old female patient (A) with Antera 3D wrinkle pretreatment and (B) 6 months posttreatment with Highly Purified Technology Polynucleotides™.

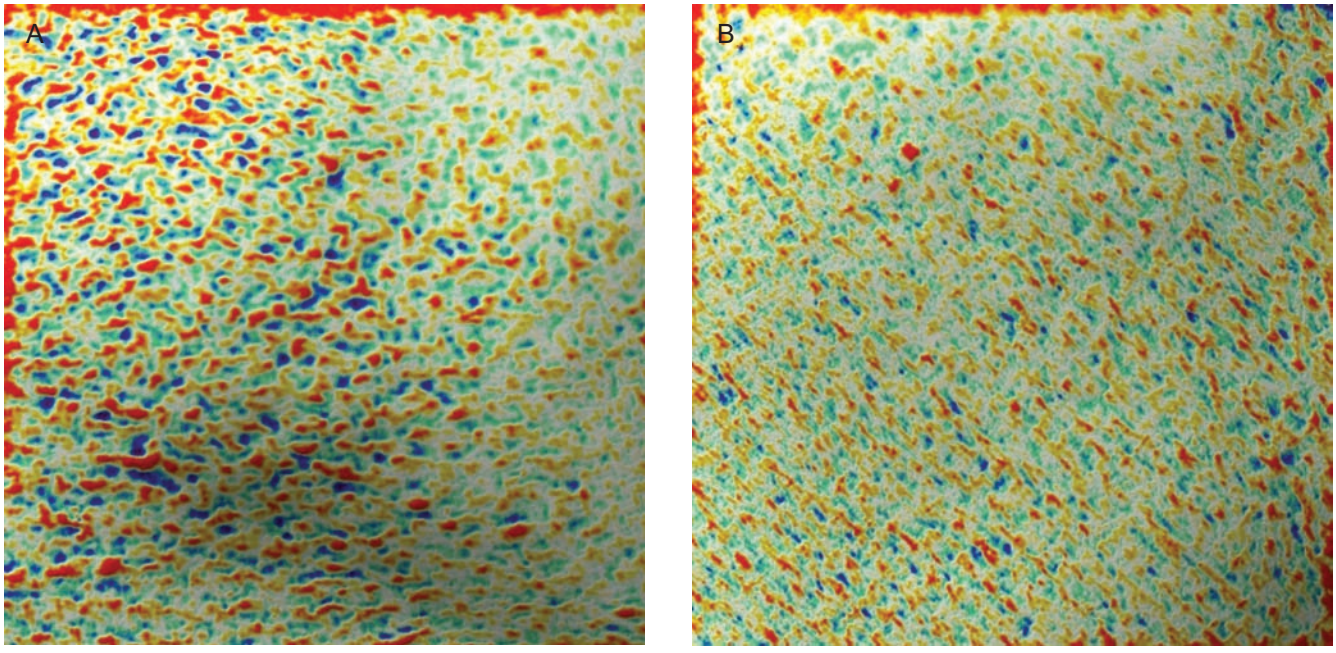

Figure 2. This 43-year-old female patient (A) with Antera 3D texture pretreatment and (B) 6 months posttreatment with Highly Purified Technology Polynucleotides.

be explained by the lack of specificity and the power of Antera 3D to analyze this issue in a small cohort of patients.

Finally, the PSQ reflected the clinical results. In fact, at 1 and 3 months posttreatment, only patients in group 1 treated with PN-HPT were satisfied compared

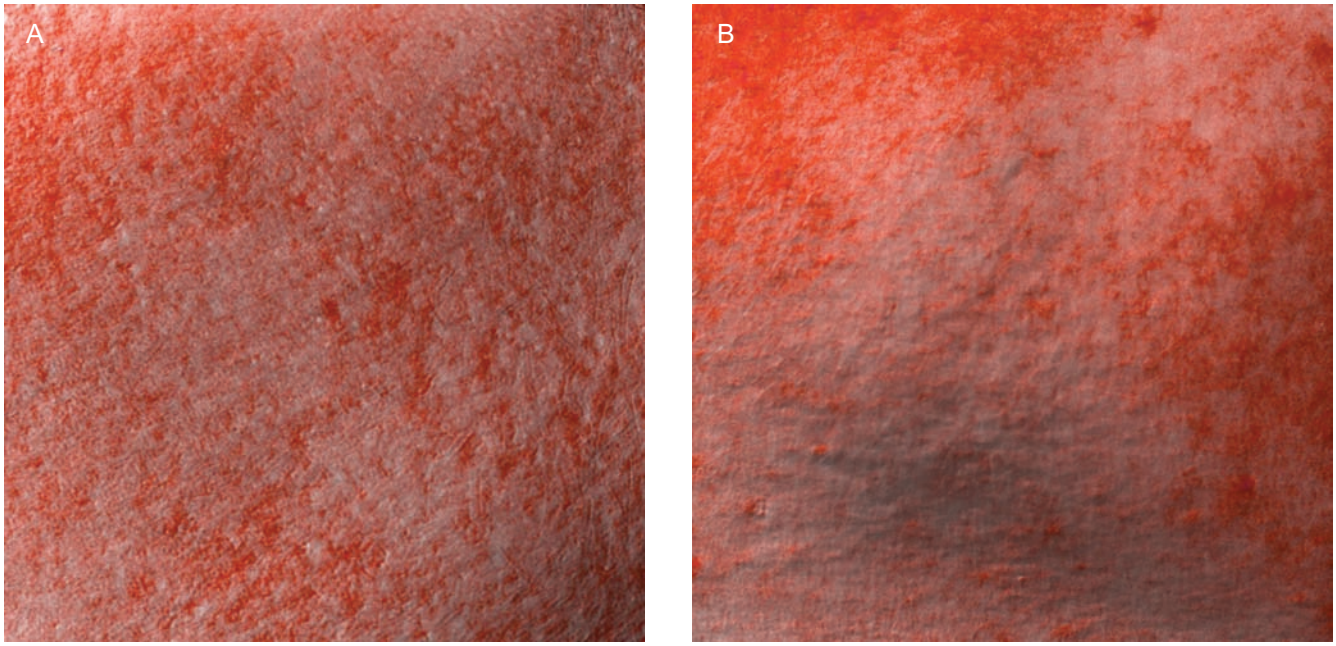

Figure 3. This 43-year-old female patient (A) with Antera 3D hemoglobin pretreatment and (B) 6 months posttreatment with Highly Purified Technology Polynucleotides.

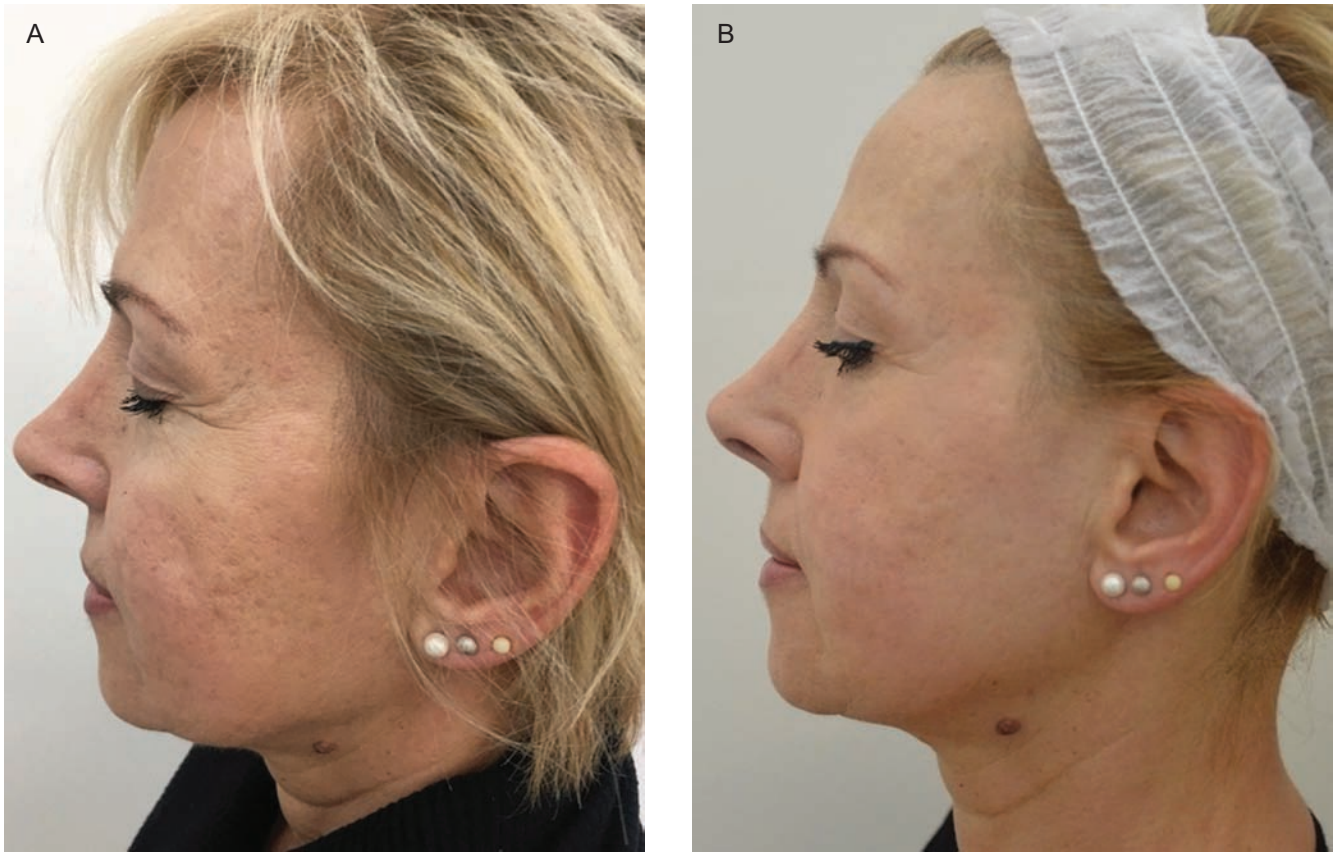

Figure 4. This 52-year-old female patient (A) pretreatment and (B) 6 months posttreatment with Highly Purified Technology Polynucleotides.

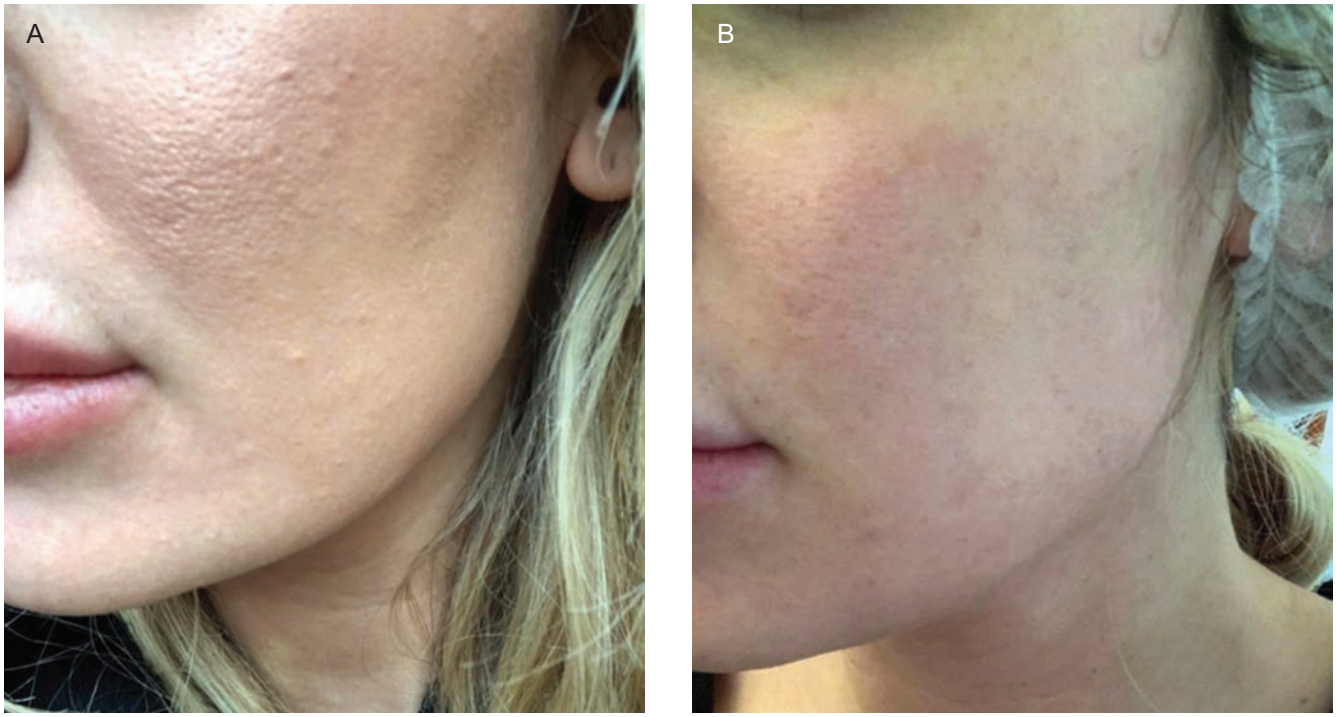

Figure 5. This 36-year-old female patient (A) pretreatment and (B) 6 months posttreatment with Highly Purified Technology Polynucleotides.

Table 4. Result: Group 2

Group 2	Before	1 mo		3 mo	
Wrinkles	40.2 ± 1.8	39.6 ± 1.1	N.S.	39.2 ± 0.8	N.S.
Texture	36.8 ± 1.3	36.43 ± 0.87	N.S.	35.8 ± 1.1	N.S.
Hemoglobin	12.35 ± 1.2	11.55 ± 0.8	N.S.	12.4 ± 1.6	N.S.
PSQ	11.25 ± 0.9	10.6 ± 1.2	N.S.	12.2 ± 1.1	N.S.

N.S., not significant; PSQ, patient satisfaction questionnaire.

Table 5. Comparative Result Among Groups

Wrinkles	Group 1	Group 2	
Before	41.1 ± 1.18	40.2 ± 1.8	N.S.
1 mo	33.14 ± 1.33	39.6 ± 1.1	$P < 0.05$
3 mo	29.24 ± 1.1	39.2 ± 0.8	$P < 0.05$
Texture			
Before	37.1 ± 0.69	36.8 ± 1.3	N.S.
1 mo	27.79 ± 0.74	36.43 ± 0.87	$P < 0.05$
3 mo	22.75 ± 0.54	35.8 ± 1.1	$P < 0.05$
Hemoglobin			
Before	13.06 ± 1.17	12.35 ± 1.2	N.S.
1 mo	14.16 ± 1.12	11.55 ± 0.8	N.S.
3 mo	16.26 ± 1.13	12.4 ± 1.6	N.S.

N.S., not significant.

with the placebo group. No complications were recorded after the treatments; this indicates that PN-HPT in monotherapy is safe. We believe that the results of this study are promising. In fact, PN-HPT alone has been proven effective as a derma bio-stimulant. Furthermore, PN-HPT costs 10% to 15% lower compared with other bio-stimulating agents available on the market.

The present study has some limitations. First, the number of patients enrolled in this study was small; however, we found it difficult to enroll more patients with our strict inclusion criteria. Second, the follow-up stopped at 3 months posttreatment, so we could not observe the stability of the achieved results after 6 and 12 months. For

these reasons, we stress that we have only conducted a preliminary study and further randomized prospective studies are needed.

CONCLUSIONS

Our prospective and randomized study showed that PN-HPT in monotherapy was safe and effective treatment for atrophic scar acne compared with placebo. Prospective and randomized studies will be necessary to investigate the clinical effectiveness in a larger cohort of patients and for a longer follow-up.

Disclosures

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