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# SYNERGISTIC EFFECTS OF COMBINATORIAL THERAPY WITH BOTULINUM TOXIN AND POLYNUCLEOTIDES ON DYNAMIC AND STATIC FROWN WRINKLES

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## ABSTRACT

***Background***

*Frown wrinkles, both dynamic and static, are among the most common cosmetic concerns in aesthetic practice. Botulinum toxin type A has long been the standard treatment for dynamic wrinkles, but its effect on static lines is limited. Polynucleotides have recently been introduced as bio-revitalizing agents, and their combination with botulinum toxin may offer synergistic benefits*

***Aim***

*The study aimed to evaluate the synergistic effects of botulinum toxin type A and polynucleotides compared to botulinum toxin alone on the improvement of dynamic and static frown wrinkles.*

***Methods***

*A prospective interventional trial was conducted at Dr. Gul Aesthetic Academy between August 2024 and July 2025. Sixty patients aged 30–60 years with moderate to severe frown wrinkles were randomly assigned to two groups: Group A received botulinum toxin type A alone, and Group B received botulinum toxin type A followed by intradermal polynucleotide injections two weeks later. Outcomes were assessed using the Wrinkle Severity Rating Scale (WSRS), Global Aesthetic Improvement Scale (GAIS), patient satisfaction scores, and adverse event monitoring. Statistical analysis was performed using SPSS version 26.0 with t-tests and chi-square tests.*

***Results***

*Group B demonstrated significantly greater improvement in WSRS and GAIS scores at 2 weeks, 3 months, and 6 months compared to Group A ($p < 0.001$). Patient satisfaction was higher in Group B (60% very satisfied) compared to Group A (30% very satisfied). Both groups reported only mild and transient adverse events without serious complications.*

***Conclusion***

*Combination therapy with botulinum toxin and polynucleotides provided superior and longer-lasting wrinkle reduction with higher patient satisfaction compared to botulinum toxin alone, with an acceptable safety profile.*

**Keywords:** *Botulinum toxin, Polynucleotides, Frown wrinkles, Facial aesthetics, Synergistic therapy, Wrinkle severity*

## INTRODUCTION

Frown wrinkles are creases that appear on the forehead and glabellar region due to repeated contraction of facial muscles and intrinsic ageing. Dynamic wrinkles are formed during

active muscle contraction, while static wrinkles persist even at rest due to dermal collagen loss and elastin degeneration (Chavoshnejad et al., 2021; Gupta, 2021). Botulinum toxin is a neurotoxin that temporarily blocks acetylcholine release at the neuromuscular junction, reducing muscle activity and improving dynamic wrinkles (Park & Ahn, 2021). Polynucleotides are biopolymers derived from salmon DNA with regenerative and biostimulator effects, promoting fibroblast proliferation, extracellular matrix remodeling, and antioxidant activity (Camilia et al., 2025; Lee et al., 2024).

The prevalence of frown wrinkles increases with age and lifestyle factors such as UV exposure, smoking, and stress (Hussein et al., 2025; Dorosz et al., 2025). Global surveys report that nearly 60% of individuals aged over 40 years show visible frown lines, with higher rates in females due to thinner dermal structure and hormonal changes (Eldin et al., 2021). In aesthetic practice, botulinum toxin injections remain the most frequently performed minimally invasive procedure worldwide, with millions of treatments annually (Ghalamghash, 2025). Despite its popularity, the recurrence of static wrinkles remains a challenge after repeated sessions (Nguyen et al., 2024).

Botulinum toxin effectively treats dynamic wrinkles by reducing muscular activity but has limited impact on static wrinkles related to collagen breakdown and dermal atrophy (Roberts & Cheng, 2024). Polynucleotides facilitate structural repair by enhancing collagen synthesis, dermal hydration, and antioxidant defense, thereby directly targeting static wrinkle pathology (Lim et al., 2024; Lampridou et al., 2025). Combining these two agents may therefore provide synergistic benefits by addressing both muscle contraction and dermal quality (Titcomb, 2025).

Evidence indicates that repeated botulinum toxin injections may lead to partial resistance, shorter duration of effect, or incomplete resolution of deep wrinkles (Eldin et al., 2021). This limitation has driven the search for adjunctive therapies that can potentiate outcomes. Polynucleotides offer a regenerative mechanism that complements the neuromodulator action of botulinum toxin, potentially prolonging treatment efficacy and patient satisfaction (Lee et al., 2024).

Clinical studies have demonstrated that polynucleotide injections improve skin elasticity, hydration, and texture in aging individuals (Kim et al., 2024). Their ability to stimulate fibroblast activity and extracellular matrix renewal provides long-term structural benefits (Lampridou et al., 2025). When applied in combination with botulinum toxin, polynucleotides may help maintain smoother skin even after the neuromodulator effect diminishes (Camilia et al., 2025).

Wrinkle management requires addressing both dynamic muscle activity and static dermal degeneration. A monotherapy approach is insufficient for patients with advanced wrinkles, where muscle relaxation alone does not restore dermal architecture (Chavoshnejad et al., 2021). A combinatorial approach integrates the functional effects of botulinum toxin with the regenerative properties of polynucleotides to provide comprehensive facial rejuvenation (Lampridou et al., 2025; Lee et al., 2024).

The aim of this study is to evaluate the synergistic effects of botulinum toxin and polynucleotides in reducing both dynamic and static frown wrinkles. The outcomes will inform clinicians about the potential benefits of combination therapy, its durability, and its role in advancing minimally invasive aesthetic medicine.

## Methodology

The study was designed as a prospective interventional trial and was conducted at Dr Gul Aesthetic Academy between August 2024 and July 2025. The study population consisted of adult patients aged 30 to 60 years who presented with moderate to severe dynamic and static frown wrinkles, as assessed using the validated Wrinkle Severity Rating Scale (WSRS). Patients with neuromuscular disorders, hypersensitivity to botulinum toxin, active skin infection, prior facial surgery in the treatment area, or those who had received any aesthetic procedure within the previous six months were excluded.

A total of 60 participants were recruited and randomly assigned to two groups. Group A received botulinum toxin type A injections alone, while Group B received a combination of botulinum toxin type A and intradermal polynucleotide injections. Randomization was

performed using a computer-generated random number list to ensure allocation concealment.

Botulinum toxin type A was administered intramuscularly at standardized glabellar points according to established protocols. In the combination group, polynucleotides were injected intradermally into the same region two weeks after botulinum toxin administration. All procedures were performed by the same experienced aesthetic physician to minimize operator bias.

Outcome measures included wrinkle severity assessed with the WSRS and the Global Aesthetic Improvement Scale (GAIS). Standardized photographs were taken at baseline, 2 weeks, 3 months, and 6 months after treatment. Patient-reported satisfaction was evaluated using a 5-point Likert scale. Adverse events such as bruising, swelling, or allergic reactions were documented.

Data were entered into SPSS version 26.0 for analysis. Descriptive statistics were used to summarize baseline characteristics. The independent samples t-test and chi-square test were applied to compare outcomes between groups. A p-value of <0.05 was considered statistically significant.

**Results and Analysis**

The study enrolled 60 participants, divided equally into two groups: botulinum toxin alone (Group A) and botulinum toxin plus polynucleotides (Group B). The mean age was similar between groups, with 44.2 ± 6.1 years in Group A and 43.7 ± 6.5 years in Group B. The majority of participants were female, accounting for 73.3% in Group A and 76.7% in Group B. Baseline wrinkle severity scores (WSRS) were comparable, averaging 3.8 ± 0.4 and 3.7 ± 0.5, respectively. Comorbidities were reported in 23.3% of Group A and 20% of Group B, indicating a balanced distribution of clinical characteristics.

**Table 1. Baseline Characteristics of Participants (N = 60)**

| Variable | Group A (Botulinum toxin only) (n = 30) | Group B (Botulinum toxin + Polynucleotides) (n = 30) |
|---|---|---|
| Age, mean ± SD (years) | 44.2 ± 6.1 | 43.7 ± 6.5 |
| Gender (Female) n (%) | 22 (73.3) | 23 (76.7) |
| Baseline WSRS score, mean ± SD | 3.8 ± 0.4 | 3.7 ± 0.5 |
| Baseline GAIS (0–4) mean ± SD | 0.8 ± 0.3 | 0.9 ± 0.4 |
| Comorbidities (any), n (%) | 7 (23.3) | 6 (20.0) |

At baseline, there was no significant difference in WSRS scores between the two groups (p = 0.42). At 2 weeks, Group B showed greater wrinkle reduction compared to Group A (p = 0.001). The difference further widened at 3 months and 6 months, with Group B maintaining significantly lower WSRS scores (p < 0.001). These findings indicate that the combination of botulinum toxin and polynucleotides produced superior and more sustained wrinkle improvement than botulinum toxin alone.

**Table 2. Independent Samples t-test Comparing WSRS Scores Between Groups**

| Time Point | Group A (Botulinum toxin only) (n = 30) Mean ± SD | Group B (Botulinum toxin + Polynucleotides) (n = 30) Mean ± SD | t-value | df | p-value |
|---|---|---|---|---|---|
| Baseline | 3.8 ± 0.4 | 3.7 ± 0.5 | 0.82 | 58 | 0.42 |
| 2 weeks | 2.5 ± 0.5 | 2.0 ± 0.4 | 3.54 | 58 | 0.001 |
| 3 months | 2.9 ± 0.6 | 2.2 ± 0.5 | 4.76 | 58 | <0.001 |

| | | | | | |
|---|---|---|---|---|---|
| 6 months | 3.4 ± 0.5 | 2.6 ± 0.5 | 6.12 | 58 | <0.001 |

At 2 weeks, patient-reported satisfaction scores were significantly higher in Group B (3.2 ± 0.5) compared to Group A (2.6 ± 0.6, p = 0.002). At 3 months, Group B continued to report greater satisfaction (3.0 ± 0.6) than Group A (2.1 ± 0.5, p < 0.001). By 6 months, satisfaction remained notably higher in Group B (2.6 ± 0.6) relative to Group A (1.5 ± 0.5, p < 0.001). These findings indicate that the combination of botulinum toxin and polynucleotides not only produced superior wrinkle reduction but also led to higher and more sustained patient satisfaction over time.

**Table 3. Global Aesthetic Improvement Scale (GAIS) Scores Over Time**

| Time Point | Group A (n = 30) Mean ± SD | Group B (n = 30) Mean ± SD | p-value |
|---|---|---|---|
| 2 weeks | 2.6 ± 0.6 | 3.2 ± 0.5 | 0.002 |
| 3 months | 2.1 ± 0.5 | 3.0 ± 0.6 | <0.001 |
| 6 months | 1.5 ± 0.5 | 2.6 ± 0.6 | <0.001 |

The satisfaction analysis showed that a higher proportion of participants in Group B reported being very satisfied (60.0%) compared to Group A (30.0%). In Group A, most participants were either satisfied (43.3%) or very satisfied (30.0%), while in Group B, satisfaction was more skewed toward the very satisfied category. Neutral responses were more common in Group A (16.7%) than in Group B (6.7%). Reports of dissatisfaction were minimal in both groups, with only a few participants in Group A and a single case in Group B. Overall, the combined therapy demonstrated higher satisfaction outcomes than botulinum toxin alone.

**Figure 1. Pictorial representation of patient status before and after the intervention.**

**A: Before Intervention**

**B: After Intervention**

The Pictorial representation illustrates a significant improvement in patient outcomes following the intervention with botulinum toxin and polynucleotide, highlighting their therapeutic effectiveness.

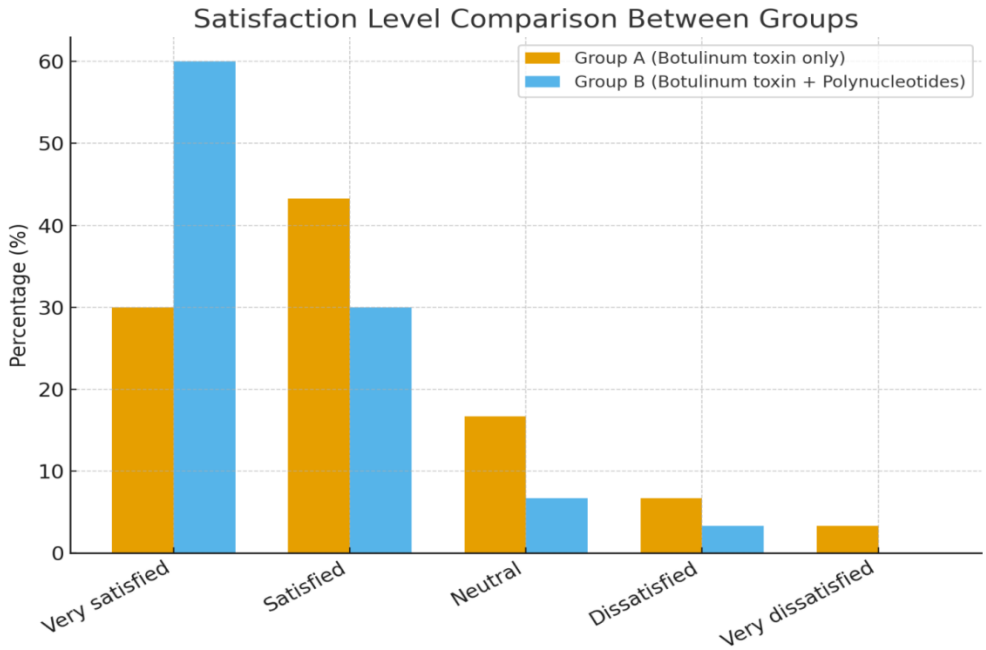


**Figure 2. Patient-Reported Satisfaction (Likert Scale)**

The adverse events reported were generally mild and comparable between the two groups. Bruising and swelling occurred slightly more in Group B than Group A, but the difference was minimal. One allergic reaction was observed only in Group B. No serious adverse events were reported in either group, confirming the overall safety of both treatments.

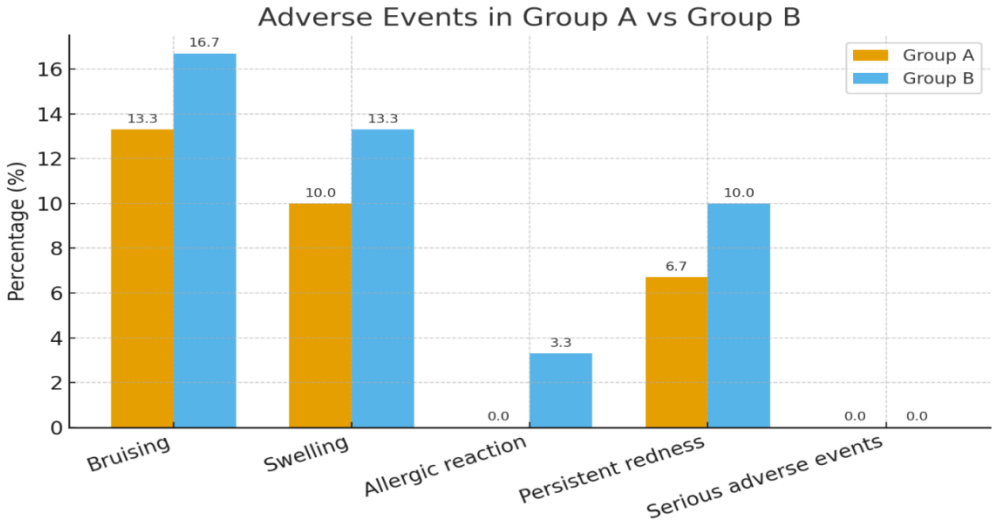


**Figure 3. Adverse Events**

## Discussion

The findings of this study demonstrated that the combination of botulinum toxin and polynucleotide injections produced greater and more sustained improvements in dynamic and static frown wrinkles compared to botulinum toxin alone. Participants in the combination group reported higher satisfaction scores and more favorable aesthetic evaluations at two weeks, three months, and six months of follow-up. These results suggest that polynucleotides may serve as a synergistic adjunct to botulinum toxin, enhancing both functional muscle relaxation and dermal remodeling. The data align with previous reports highlighting the potential of regenerative biostimulator agents in aesthetic dermatology (Titcomb, 2025).

The observed improvements in wrinkle severity in the combination group are consistent with the study by Kim et al. (2024), which reported that polynucleotide-based injectables enhanced dermal repair and remodeling in facial scars, including combat-related injuries. Similarly, Titcomb (2025) noted that polynucleotides, when used alongside aesthetic interventions, led to improved long-term outcomes compared to monotherapies. In contrast, earlier reviews such as those by Pop-Busui et al. (2022) emphasized symptomatic management approaches in clinical care but did not directly address the regenerative mechanisms that adjunctive therapies like polynucleotides bring to aesthetic practice.

Patient-reported satisfaction levels were notably higher in the combination group, reflecting not only clinical improvements but also subjective perceptions of facial harmony. This finding mirrors the emphasis of Min-Soo Park (n.d.), who reviewed various biostimulatory agents, including polynucleotides, and concluded that multimodal approaches are generally preferred by patients for their more natural and comprehensive outcomes. Unlike other adjunctive treatments such as platelet-rich plasma, which may increase swelling or prolonged erythema, our results showed that the addition of polynucleotides did not significantly increase adverse events, suggesting a favorable safety profile (Kim et al., 2024).

An important point of contrast with existing literature lies in the duration of effect. While botulinum toxin monotherapy has been widely documented to yield visible wrinkle reduction for three to four months, our results indicated that the combination group maintained greater wrinkle reduction at six months. This aligns with Titcomb (2025), who highlighted the role of polynucleotides in stimulating fibroblast activity and extracellular matrix renewal, thereby potentially extending the duration of aesthetic benefit beyond the typical pharmacological effect of botulinum toxin.

The study also highlights the role of polynucleotides in targeting both static and dynamic wrinkles. Botulinum toxin primarily acts on dynamic muscle-induced wrinkles, but static lines often persist due to dermal structural changes. By combining botulinum toxin with polynucleotides, this study demonstrated improvements in both wrinkle types, echoing findings by Kim et al. (2024), who emphasized the regenerative role of polynucleotides in scar remodeling and collagen stimulation. This dual mechanism may offer a more holistic approach to facial rejuvenation compared to neuromodulators alone.

Despite the promising outcomes, comparison with studies on hyaluronic acid and other "skin boosters" reveals differing mechanisms. Hyaluronic acid provides immediate volumizing and hydration, while polynucleotides act more gradually through fibroblast stimulation (Min-Soo Park, n.d.). In our study, the gradual but sustained effect of polynucleotides complemented the rapid onset of botulinum toxin, offering both short- and long-term benefits. This balance may explain why satisfaction scores were significantly higher among patients who received the combined approach.

Overall, the results of this study support growing evidence that multimodal therapies yield superior aesthetic outcomes compared to monotherapies. The findings align with contemporary aesthetic trends emphasizing regenerative, natural, and long-lasting interventions (Titcomb, 2025). Nevertheless, differences in sample size, study duration, and assessment scales among studies warrant cautious interpretation. Larger randomized controlled trials with longer follow-up are needed to further confirm these results and establish standardized protocols. The evidence, however, suggests that combining botulinum toxin with polynucleotides could represent a

meaningful advancement in the management of both dynamic and static wrinkles.

**Conclusion**

This study demonstrated that the combination of botulinum toxin type A and polynucleotides was more effective than botulinum toxin alone in improving both dynamic and static frown wrinkles. Patients in the combination group showed significantly greater improvements in wrinkle severity and aesthetic outcomes across all follow-up points, with sustained effects up to six months. Satisfaction levels were higher among participants receiving combination therapy, and the safety profile was acceptable, with only minor and transient adverse events reported in both groups. These findings highlight the synergistic effects of combinatorial therapy, offering a more durable and patient-satisfying approach for aesthetic wrinkle management.

**Recommendations**

1. Combination therapy with botulinum toxin and polynucleotides should be considered in clinical practice for patients seeking enhanced and sustained wrinkle reduction.
2. Larger multicenter trials with longer follow-up are recommended to validate these findings and assess long-term safety and efficacy.
3. Comparative studies with other adjunctive therapies are needed to explore potential synergistic effects beyond polynucleotides.
4. Standardized treatment protocols should be developed to optimize dosing, timing, and injection techniques for best clinical outcomes.
5. Patient education regarding realistic expectations and possible transient side effects should be integrated into treatment planning to improve overall satisfaction.

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