

**“VICTOR BABES” UNIVERSITY OF MEDICINE AND PHARMACY  
TIMISOARA**

**FACULTY OF GENERAL MEDICINE  
DISCIPLINE OF GENERAL SURGERY**

**CASTIGLIONE LUCA**



# **DOCTORAL THESIS**

**EXPERIMENTAL INVESTIGATIONS OF BOTULINUM TOXIN  
IN GENERAL SURGERY**

Scientific Coordinator

**PROF. UNIV. DR. HABIL. MURARIU MARIUS**

**Timisoara**

**2024**

# TABLE OF CONTENTS

PERSONAL CONTRIBUTIONS	VI
LIST OF ABBREVIATIONS	VII
LIST OF FIGURES	VIII
ACKNOWLEDGEMENTS	IX
INTRODUCTION	X

## GENERAL PART

CHAPTER 1: HISTORY AND BACKGROUND OF BOTULINUM TOXIN	1
CHAPTER 2: BOTULINUM TOXIN STRUCTURE AND PROPERTIES	6
CHAPTER 3: MECHANISM OF ACTION	13
CHAPTER 4: CLINICAL APPLICATIONS	26

## SPECIAL PART

I. STUDY 1: ASSESSMENT OF QUALITY OF LIFE IN PATIENTS WITH CHRONIC ANAL FISSURES: A 1-YEAR FOLLOW-UP STUDY BEFORE AND AFTER BOTULINUM TOXIN (BOTOX) INJECTION	33
I.1 INTRODUCTION	
<b>Error! Bookmark not defined.3</b>	
I.1.1 BACKGROUND	
<b>Error! Bookmark not defined.</b>	
I.1.2 PURPOSE OF THE RESEARCH	
<b>Error! Bookmark not defined.5</b>	
I.2 MATERIALS AND METHODS	
<b>Error! Bookmark not defined.6</b>	
I.2.1 RESEARCH DESIGN AND ETHICAL CONSIDERATIONS	
<b>Error! Bookmark not defined.6</b>	
I.2.2 INCLUSION AND EXCLUSION CRITERIA	36
I.2.3 VARIABLES AND PROCEDURES	37
I.2.4 SURVEYS EMPLOYED	38
I.2.5 STATISTICAL ANALYSIS	40
I.3 RESULTS	41
I.4 DISCUSSIONS	47
I.4.1 LITERATURE FINDINGS	47
I.4.2 STUDY LIMITATIONS	51
I.5 CONCLUSIONS	52
II. STUDY 2: LONGITUDINAL ASSESSMENT OF FACIAL HYPERHIDROSIS MANAGEMENT: EVALUATING THE EFFICACY AND QUALITY OF LIFE IMPROVEMENTS FOLLOWING BOTULINUM TOXIN THERAPY AT BASELINE, 6 MONTHS, AND 1 YEAR	54
II.1 INTRODUCTION	54
II.1.1 BACKGROUND	54
II.1.2 PURPOSE OF THE RESEARCH	55
II.2 MATERIALS AND METHODS	56
II.2.1 RESEARCH DESIGN AND ETHICAL CONSIDERATIONS	56
II.2.2 INCLUSION AND EXCLUSION CRITERIA	57

II.2.3 VARIABLES AND PROCEDURES	58
II.2.4 SURVEYS EMPLOYED	59
II.2.5 STATISTICAL ANALYSIS	61
II.3 RESULTS	62
II.4 DISCUSSIONS	68
II.4.1 LITERATURE FINDINGS	68
II.4.2 STUDY LIMITATIONS	73
II.5 CONCLUSIONS	74
III. STUDY 3: ASSESSING BOTULINUM TOXIN EFFECTIVENESS AND QUALITY OF LIFE IN AXILLARY HYPERHIDROSIS: A ONE-YEAR PROSPECTIVE STUDY	76
III.1 INTRODUCTION	76
III.1.1 BACKGROUND	76
III.1.2 PURPOSE OF THE RESEARCH	77
III.2 MATERIALS AND METHODS	78
III.2.1 RESEARCH DESIGN AND ETHICAL CONSIDERATIONS	78
III.2.2 INCLUSION AND EXCLUSION CRITERIA	79
III.2.3 VARIABLES AND PROCEDURES	80
III.2.4 SURVEYS EMPLOYED	81
III.2.5 STATISTICAL ANALYSIS	82
III.3 RESULTS	84
III.4 DISCUSSIONS	90
III.4.1 LITERATURE FINDINGS	90
III.4.2 STUDY LIMITATIONS	94
III.5 CONCLUSIONS	95
IV. FINAL CONCLUSIONS AND FUTURE PERSPECTIVES	97
BIBLIOGRAPHY:	99
ANNEX	

**Error! Bookmark not defined.**

# **STUDY 1: ASSESSMENT OF QUALITY OF LIFE IN PATIENTS WITH CHRONIC ANAL FISSURES: A 1-YEAR FOLLOW-UP STUDY BEFORE AND AFTER BOTULINUM TOXIN (BOTOX) INJECTION.**

## **CONTEXT**

Botox works by inhibiting acetylcholine release, causing temporary muscle paralysis. In treating anal fissures, Botox injections into the anal sphincter reduce spasms, improve blood flow, and aid healing. Studies show Botox's effectiveness in healing fissures and reducing symptoms, with a lower risk of incontinence compared to surgery. Despite evidence of Botox's effectiveness, its long-term impact on quality of life, including psychological, social, and functional aspects, remains under-explored. The recurrent pain and discomfort from fissures can lead to anxiety, social isolation, and reduced life satisfaction, making it vital to evaluate the quality of life to understand Botox's benefits and limitations fully.

This study aims to conduct a year-long analysis of life quality in patients with anal fissures treated with Botox. It hypothesizes that Botox injections significantly and sustainably improve patients' quality of life over a year. The study will assess changes in physical symptoms, psychological well-being, social functioning, and overall life satisfaction before and after Botox treatment, providing a comprehensive view of its long-term efficacy in managing anal fissures.

## **SUMMARY OF FINDINGS**

The study encompassed 113 patients, predominantly middle-aged (40–65 years), making up 77.9% of the group, with a mean age of 38.1 years. Young adults (18–39 years) constituted 22.1% of the sample. There was a slight male majority (54%) and most participants lived in urban areas (69.9%). A significant portion (70.8%) were in relationships or married. In terms of medical history, 18.6% had a Charlson Comorbidity Index (CCI) greater than 2, and the average disease duration was 10.3 months. Most fissures were located posteriorly (79.6%), and prior to the study, two-thirds had received medical treatment while a third had undergone surgery.

One year post-Botox treatment, 78.8% of patients experienced fissure healing, 14.2% had recurrences, and 7.1% reported incontinence. This reflects the effectiveness of Botox in treating anal fissures. The study also monitored the impact of treatment on patients' quality of life across various domains using the WHOQOL-BREF survey. Scores in the physical and mental domains showed significant improvements one month after treatment, which were mostly sustained over 12 months. Similarly, the social and environmental domains exhibited notable score increases post-treatment, despite slight declines at the 12-month mark.

Coping strategies of patients also shifted significantly post-treatment. Disengagement strategies decreased notably from 72.1% to 30.2% one month after treatment and remained lower over 12 months. Conversely, engagement strategies increased post-treatment, as did problem-focused coping, indicating a positive shift in coping mechanisms. The use of emotion-focused strategies decreased significantly, aligning with the overall trend of improved coping post-Botox treatment.

The study also assessed physical and mental health using the SF-36 survey. Physical health scores improved significantly one month after treatment and were sustained over the year. Mental health also showed improvement post-treatment, with a slight decrease at the 12-month mark. The total score for overall health status and quality of life followed a similar trend, improving significantly post-treatment and remaining higher than the pre-treatment score after 12 months.

The treatment's impact extended beyond health scores. Physical discomfort decreased significantly, and pain management improved post-treatment. Scores for daily activity impact, mood, emotional well-being, and social engagement all showed significant improvements,

especially notable at 6 months post-treatment. These results indicate that Botox treatment not only aids in physical healing but also enhances overall quality of life in multiple aspects.

Figure 1 – Longitudinal assessment of WHOQOL-BREF survey results.

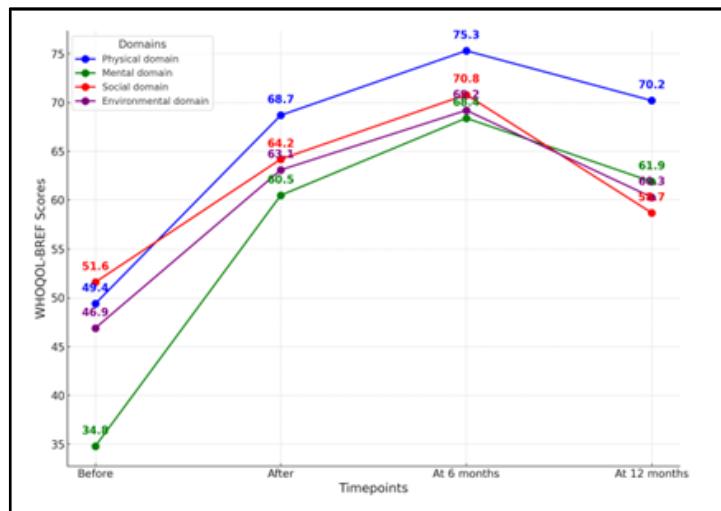
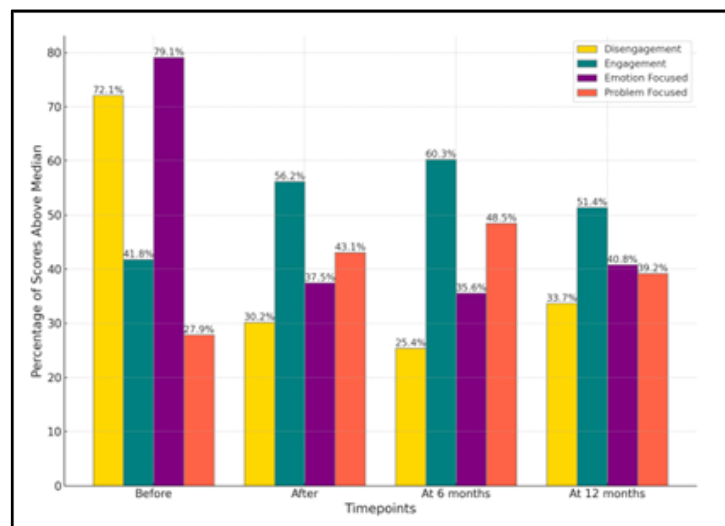


Figure 2 – Longitudinal assessment of COPE-60 survey results.



## CONCLUSIONS

In conclusion, the study conclusively demonstrates that Botulinum toxin (Botox) injections significantly improve the quality of life for patients with chronic anal fissures, with these benefits persisting over a one-year period. The enhancements were particularly notable in both physical and mental health aspects, as evidenced by marked improvements across various domains of WHOQOL-BREF and SF-36 surveys. These findings highlight the effectiveness of Botox not only in alleviating physical symptoms of anal fissures but also in substantially enhancing patients' mental and social well-being, thereby supporting its use as a long-term treatment strategy for this condition.

## **STUDY 2: LONGITUDINAL ASSESSMENT OF FACIAL HYPERHIDROSIS MANAGEMENT: EVALUATING THE EFFICACY AND QUALITY OF LIFE IMPROVEMENTS FOLLOWING BOTULINUM TOXIN THERAPY AT BASELINE, 6 MONTHS, AND 1 YEAR.**

### **CONTEXT**

Facial hyperhidrosis, marked by excessive facial sweating, is a globally prevalent condition that causes substantial social, emotional, and psychological challenges. Often underdiagnosed, it adversely affects quality of life, leading to embarrassment, social withdrawal, and diminished self-esteem. Its causes are diverse, involving overactive sympathetic nerves and can be idiopathic or secondary to other medical conditions, making it a widespread issue in dermatology and neurology.

Traditional treatments for facial hyperhidrosis, such as topical agents, oral medications, and iontophoresis, typically provide only partial and temporary relief. More invasive methods like sympathectomy, although effective, come with potential complications. Consequently, there's growing interest in minimally invasive yet effective solutions, with Botulinum toxin (Botox) emerging as a promising therapy. Botox works by inhibiting acetylcholine release at neuromuscular junctions, reducing sweating and improving comfort and social interactions. Its efficacy in mitigating hyperhidrosis symptoms, combined with a favorable safety profile, has been well-documented.

Despite Botox's proven effectiveness in treating hyperhidrosis, its long-term impact on patients' quality of life remains under-explored. Quality of life encompasses physical, psychological, and social well-being, all of which can be significantly affected by the persistent discomfort and social stigma of facial hyperhidrosis. Therefore, a comprehensive understanding of Botox's benefits and limitations requires assessing its impact on quality of life. This study aims to conduct a year-long analysis of quality of life in patients with facial hyperhidrosis treated with Botox, evaluating changes in physical symptoms, psychological well-being, social interactions, and overall life satisfaction pre- and post-treatment.

### **RESULTS**

The study of 77 patients assessing Botulinum toxin therapy for facial hyperhidrosis revealed a youthful average age of 32.5 years, with a slight female predominance (54.5%) and a majority residing in urban areas (61.0%). Regarding relationship status, 67.5% were in a relationship or married. In terms of employment and education, most were employed (64.9%) or studying (19.5%). The average symptom duration was 18.2 months, and hyperhidrosis severity, measured by the Hyperhidrosis Disease Severity Scale (HDSS), was evenly distributed among participants, half experiencing less severe symptoms and the other half more severe.

The treatment showed significant improvements in quality of life aspects, according to a longitudinal assessment. The severity of facial sweating decreased substantially post-treatment, and this improvement was sustained over a year. Sweating management, impact on daily activities, and self-confidence all showed significant improvements post-treatment, suggesting that Botulinum toxin therapy effectively mitigates the impacts of hyperhidrosis on daily life and boosts self-confidence. The physical and mental domains of quality of life also saw improvements, with the mental domain exhibiting a notable positive change, indicating a substantial and sustained positive impact on mental well-being.

Further analysis highlighted significant improvements in the social domain of quality of life, with scores increasing post-treatment and remaining high at 12 months. However, the environmental domain did not show significant changes, suggesting that the treatment mainly affected social aspects of patients' lives. Additionally, there was a substantial decrease in average scores from baseline to post-treatment in life quality, symptom severity, and sweat production, indicating consistent improvements in these areas following therapy.

Correlation analysis revealed strong negative correlations between the Dermatology Life Quality Index (DLQI) total score and both hyperhidrosis severity (HDSS) and sweat production, indicating that reduced severity and sweat production lead to improved quality of life. Positive correlations in the WHOQOL domains also showed that improved mental and social quality of life is related to lower severity of hyperhidrosis and less sweat production. Regression analysis further confirmed these relationships, suggesting that younger patients and those with better physical and mental health as per WHOQOL surveys tend to have a better dermatological quality of life.

Figure 3 – Correlation analysis.

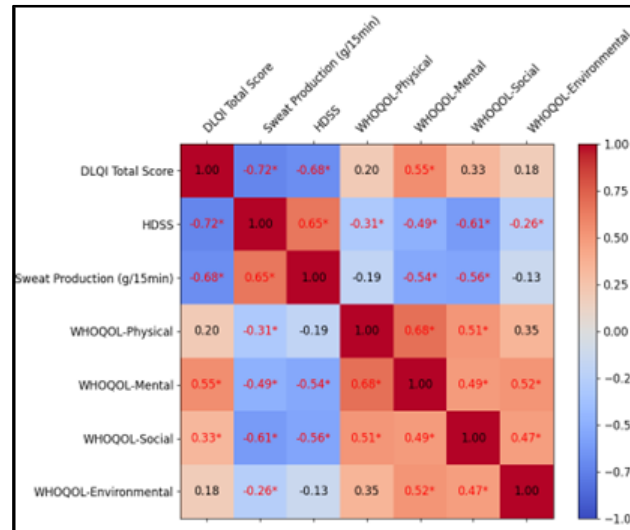
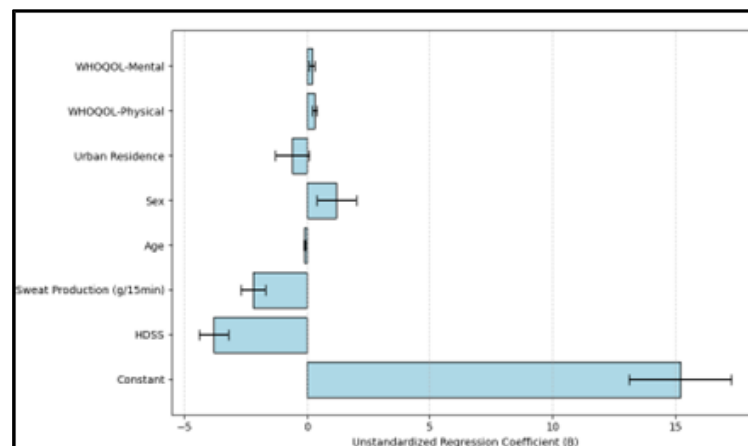


Figure 4 – Regression analysis for DLQI total score.



## CONCLUSIONS

The study conclusively demonstrates that Botulinum toxin therapy is a highly effective long-term treatment for facial hyperhidrosis, significantly improving patients' quality of life. Over a one-year period, substantial reductions were observed in the Hyperhidrosis Disease Severity Scale (HDSS) scores, Dermatology Life Quality Index (DLQI) scores, and sweat production, with these improvements sustained at 12 months. Notably, the treatment positively impacted both physical symptoms and psychological well-being, as evidenced by improved WHOQOL-BREF scores in mental, physical, and social domains. The strong negative correlations identified between DLQI total scores, HDSS, and sweat production underscore the comprehensive efficacy of Botulinum toxin in addressing the multifaceted challenges posed by facial hyperhidrosis.

## **STUDY 3: ASSESSING BOTULINUM TOXIN EFFECTIVENESS AND QUALITY OF LIFE IN AXILLARY HYPERHIDROSIS: A ONE-YEAR PROSPECTIVE STUDY.**

### **CONTEXT**

Axillary hyperhidrosis, marked by excessive sweating in the underarm area, brings significant discomfort and social challenges, impacting psycho-social and emotional well-being. Despite not being life-threatening, it leads to social embarrassment, anxiety, and occupational limitations. The condition is caused by overactive eccrine sweat glands, influenced by cholinergic sympathetic nerves, and is characterized by sweating that exceeds thermoregulatory needs, indicating an autonomic nervous system imbalance.

Current treatments for axillary hyperhidrosis include topical agents, iontophoresis, and surgery, each varying in effectiveness, invasiveness, and side effects. Botulinum toxin, a neurotoxin from *Clostridium botulinum*, stands out as a non-invasive option, working by inhibiting acetylcholine release and thus reducing sweat production. This treatment has shown effectiveness in reducing sweat and improving patients' quality of life. However, while its immediate effects on sweat reduction are clear, the long-term psychosocial impacts require further exploration, as quality of life in axillary hyperhidrosis involves emotional, social, and functional aspects.

This study aims to evaluate the long-term effectiveness of botulinum toxin therapy in reducing sweat production in patients with axillary hyperhidrosis and its impact on various aspects of their lives over a year. The hypothesis is that this treatment will not only sustainably reduce sweat production but also significantly enhance psychological well-being, social interactions, and daily functioning. The research will involve quantitative measurements of sweat reduction at different intervals and qualitative assessments of the treatment's evolving impacts on patients' quality of life.

### **RESULTS**

The study analyzed 81 patients with axillary hyperhidrosis, averaging 29.3 years in age, with a fairly even age distribution across different groups. Gender-wise, there was a higher proportion of women (58%) than men (42%). The majority of participants resided in urban areas (59.3%), and in terms of relationship status, most were in a relationship or married (64.2%). The average duration of symptoms was 12.6 months, with a slight skew towards more severe cases of hyperhidrosis.

Over the course of the study, there was a significant reduction in the severity and management of underarm sweating, indicating an improvement in condition management. The impact on daily activities decreased, showing an easing of daily challenges, while mood and emotional well-being saw a slight, non-significant increase. Social engagement improved, but personal relationships and professional or educational activities experienced a decline. Sleep quality saw a modest improvement, and overall, there was a notable enhancement in the quality of life.

In the physical domain, participants reported minor fluctuations in their well-being, with a modest variation in the physical quality of life over the treatment period. The mental domain exhibited more pronounced improvements, suggesting a sustained positive impact on mental well-being. The social domain showed significant improvements, indicating a considerable enhancement in social aspects of quality of life. In contrast, the environmental domain experienced steady improvements throughout the study, indicating a positive influence on the environmental dimension of the patients' quality of life.

Substantial improvements were observed across various metrics following Botulinum toxin therapy. The Dermatology Life Quality Index (DLQI) total score, which assesses the impact of hyperhidrosis on life quality, showed a marked decrease, indicating an enhancement in life quality. The Hyperhidrosis Disease Severity Scale (HDSS) and sweat production also



saw significant reductions, reflecting an improvement in the severity of hyperhidrosis symptoms. Correlation analysis revealed that decreased severity of hyperhidrosis and reduced sweat production are associated with improved quality of life. Regression analysis further confirmed these relationships, suggesting that younger patients and those with better physical and mental health tend to have a better dermatological quality of life.

Figure 5 – Correlation analysis.

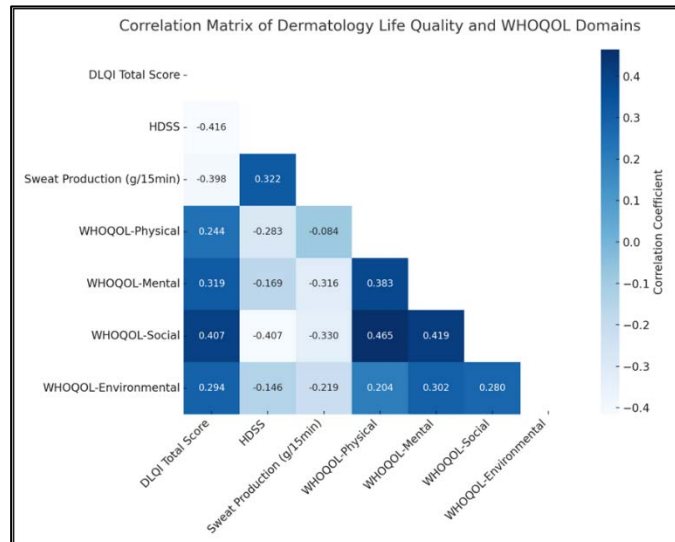
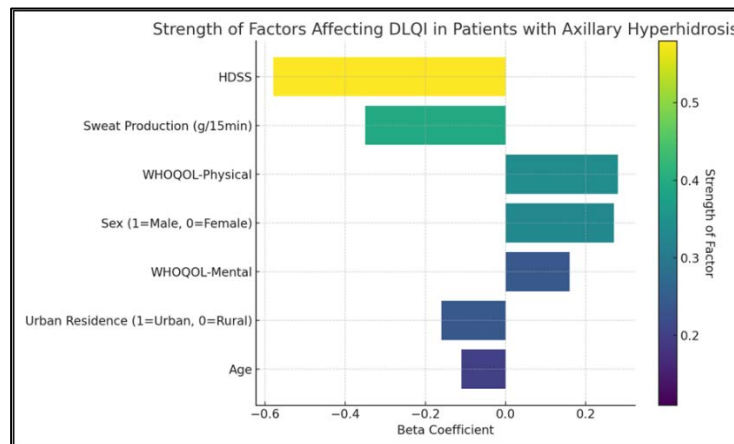


Figure 6 – Regression analysis for DLQI total score.



## CONCLUSIONS

Botulinum toxin therapy proved effective in reducing sweat production and improving the quality of life in axillary hyperhidrosis patients over a one-year period. These improvements were statistically significant in both physical and psychosocial domains. The study highlights the potential long-term benefits of Botox therapy for hyperhidrosis.