Sidra Tariq Butt

Master's thesis

Effect of botulinum toxin treatment of the calf muscles on balance and muscle activation during walking in children with Cerebral Palsy

Master's thesis in Physical Activity and Health
Movement Science
Supervisor: Karin Roeleveld
August 2023

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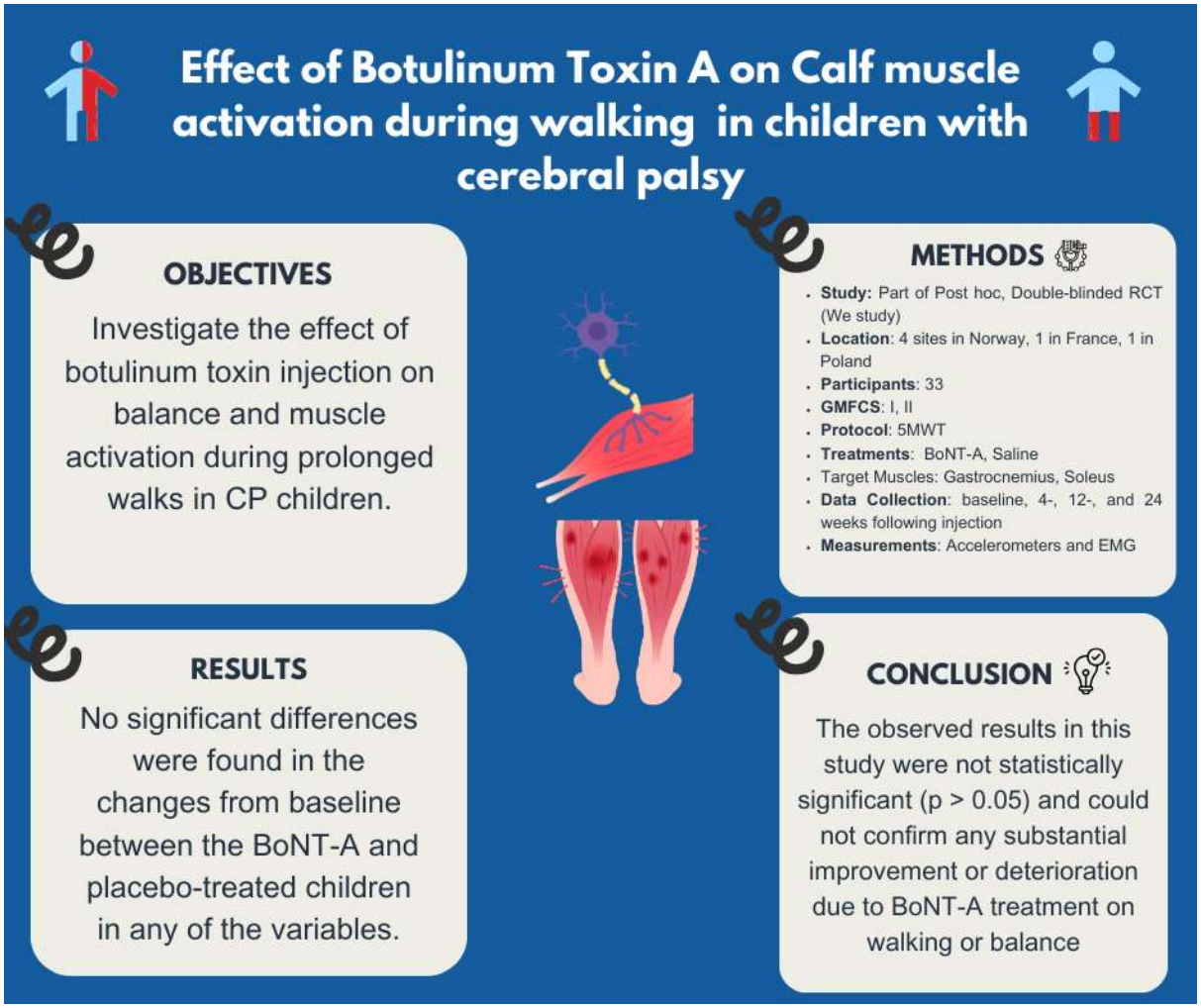
Infographic



By: Sidra Tariq Butt

Acknowledgment

I want to express my gratitude and appreciation to all those who contributed to completing this research thesis.

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Lastly, I sincerely thank my family for their unconditional love, constant encouragement, and unwavering belief in me.

Dedications

In memory of my mother.

Abstract

Background: Cerebral palsy children face walking difficulty due to spasticity and balance problems during prolonged walking. Botulinum Toxin A injections in Gastrocnemius and Soleus show reduced muscle spasticity. However, their effectiveness in improving balance during prolonged walks has yet to be fully established.

Aim: The objective of this study was to evaluate the effect of BoNT A injection on balance and muscle activation in cerebral palsy patients during prolonged walking.

Method: This study is based on a double-blinded placebo control RCT, with 59 participants named as WE study, which was carried out in France, Poland, and Norway. Thirty-three children from the WE study were included in this study, with a 5-minute walk test data where they were instructed to move back and forth at their normal speed for 5 minutes in a 25-meter-long hallway. They wore two EMG electrodes at the gastrocnemius and soleus and four Axivity AX3 activity monitors (back, thigh, right, and left foot) throughout the test. Distance walked was recorded with a measuring wheel. Data was gathered at four different time points: pre-treatment (baseline), at four weeks, at 12 weeks, and 24 weeks following treatment. The participants were analyzed through EMG and accelerometers for muscle activation and balance. RMS-EMG, walking speed, step detection, and sample entropy were the outcome variables calculated from the EMG and acceleration signals captured by the accelerometers, respectively. Statistical analysis was done through S.P.S.S. using a linear mixed model and Wilcoxon signed-rank test.

Results: No significant differences were found in the changes from baseline between the BoNT-A and placebo-treated children in any of the variables.

Conclusion: Our study could not confirm a significant improvement or deterioration due to BoNT-A treatment on walking or balance.

Keywords: BoNT A, cerebral palsy, balance, muscle activation, prolonged walk

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Abbreviations

CP: Cerebral Palsy

GMFCS: Gross motor function classification system

BoNT-A; Botulinum toxin injection A

5MWT: 5-minute walk test

RCT: Randoied control trial

EMG: Electromyography

V, ML, AP: vertical, medio-lateral, anterio-posterior

RMS: Root mean square

1 Introduction

1.1 Cerebral Palsy

Cerebral Palsy is the most common cause of motor disorders in children due to a defect or damage to the immature brain[1]. It can also arise from brain lesions before or around birth[2, 3]. CP is a term that encompasses a group of permanent movement and posture disorders resulting in activity limitation. Along with motor issues, individuals with cerebral palsy may experience challenges related to sensation, perception, cognition, communication, and behavior. Although the brain damage is non-progressive, the symptoms might be progressive and cause life-long neurological deficits, resulting in severe motor impairments[4]. The primary brain area damaged during this condition is the motor area that controls voluntary movements of the body. Sensory damage to the brain results in disrupted sensations and perception issues. Cognition impairment and behavioral problems may arise in cerebral palsy cases[5, 6].

Most of the children with cerebral palsy experience significant challenges related to the neuro-muscular-skeletal system, such as muscle contractures, discoordination, spasticity, increased co-contraction, decreased selective motor control, muscle weakness, and bony malformations as permanent severe impairments[7]. Impaired walking, posture, mobility, and balance dysfunction are key hurdles for CP children who can ambulate independently, resulting in physical handicaps and participation restrictions in routine activities during childhood 7]. This situation significantly affects parents, families, and the child's health[8].

Most children with CP usually complain about poor balance and difficulty walking because they get fatigued during activities of daily life. This fatigue can lead to abnormal walking patterns and muscle imbalance[9, 10]. The prevailing notion suggests that the tiredness experienced by children with cerebral palsy (CP) is primarily influenced by muscle fatigue in their lower limbs[11]. Muscle Fatigue is defined as any exercise-induced reduction in force generation capacity[12]. Approximately 70% of children experience restrictions during walking, such as impaired balance[13], reduced walking capacity[14], decrease in walking speed (1.04m/s) as compared to typically developing children (1.13 m/s)[15], and high energy cost[16], contributing to muscle fatigue[17].

1.2 Gross Motor Function Classification System and Cerebral Palsy

GMFCS classifies cerebral palsy on children's functional status at different levels, and we used GMFCS I and II in this study[18]. GMFCS is the most salient and extensively used scale to assess a child's current gross motor functional level. It was established to cater to the requirement for a standardized classification system of movement limitations in cerebral palsy[19].

It consists of five levels of classification, ranging from level I to level V. Self-started walking, standing, and sitting abilities are the basic functional units of GMFCS, and children at levels I and II are marked as independent walkers. At level III, they can walk with assistive devices; at level IV, children can walk through small distances with an assistive device or supervision 20]. At Level V, all motor functions are restricted, and it is not easy to hold the body in an antigravity posture[20]. As a system to measure a child's functional status and discover a range of issues, this scale has been adopted worldwide[21-23].

The GMFCS, developed by Palisano et al[18], primarily focuses on voluntary movements, and special attention is paid to core muscles and trunk control during sitting and walking 20]. The categorization of motor functional levels in the (GMFCS) considers various factors, such as functional limitations, use of assistive devices, quality of movement, and clinically significant signs. The primary purpose of the GMFCS classification system is to evaluate a child's motor function abilities and limitations in typical settings, including school, home, and community environments. It is important to note that when using the GMFCS to classify a child's functional status, judgments regarding prognosis and potential future abilities should not be factored into the classification process[20].

1.3 Management of CP

For CP management, different approaches have been employed based on the extent of the condition. One standard method involves conservative measures such as splints, braces, and casts[24]. Physical therapy and rehabilitation after casts and braces help maintain muscle tone and improve strength, coordination, and balance. It also helps to enhance the fitness level and quality of life[25]. Besides drug therapy and surgical treatment, intramuscular injection of BoNT-A for chemo-denervation[18, 26].

1.4 BoNt-A and CP

A frequently applied treatment of muscle spasticity in children with CP to improve walking is the administration of intramuscular botulinum toxin (BoNT-A) in the affected muscles**.** BoNT-A is a neuromuscular paralyzing agent that inhibits the release of the neurotransmitter acetylcholine (that causes muscle contractions) at the neuromuscular junction, resulting in a temporary reduction in muscle tone by chemo denervation[27, 28]. It is considered the safest of its seven serotypes (A-G). Anaerobic bacteria named Clostridium Botulinum produces this toxin. It is commercially prepared, sold under different names, and widely used to treat spasticity and reduce secondary complications due to muscle contractures[29]. The dosage and muscle site to be injected should be appropriately selected for every child according to body weight and type. Inappropriate doses may cause unwanted side effects or the absence of valuable impacts[29]. The muscle activity is crucial in clinical response after administrating BoNT-A in CP children, especially concerning the foot muscles. After injection, the BoNt-A toxin is selectively taken up through the process of endocytosis and blocks the release of acetylcholine, reducing muscle activity in the injected muscle[30]. This phenomenon is reversible, and the body takes around 90 days to recover the blocked terminal by sprouting. The injection's clinical effect lasts 12 to 16 weeks[31]. The BoNT-A benefits gait by minimizing muscle activity and reducing spasticity[32].

Management of muscle fatigue and spasticity with BoNT-A is the safest evidence-based clinical practice, as identified by the literature. It has been used to reduce spasticity since 1993[33]. Existing literature provides substantial evidence regarding the significant effects of BoNT-A injections in the calf muscles. The results demonstrate notable improvements in walking patterns and ankle range of motion[34-36]. Furthermore, studies have documented reduced muscle tone and length following BoNT-A treatment[37, 38]. In a study by Engstorm et al., 14 children were taken from which 9 showed improved walking and ceased toe walking over a 12-month follow-up period. The study indicates that BoNT-A has beneficial long-term effects on muscle activity and gait patterns[39]. A prospective cohort of 94 cerebral palsy children applied eight botulinum injections in total (2 injections in each muscle) in Gastrocnemius, Soleus, adductors, and hamstrings. The assessment was conducted both before and after three months after the application of BoNT-A. Across all muscles, a decrease in muscle tone was observed. Notably, the most significant reduction in tone was documented in the gastrocnemius

muscle. Moreover, the administration of BoNT-A does not affect the range of motion or prevent contracture development in the lower limbs[40]. Molenaers et al. reported that the administration of BoNT-A started at an early age improved the overall motor function in CP children. The short-term beneficial effects of BoNT-A on functional improvement in gait are well-documented in the literature; however, there is a need to investigate the long-term impact[33].

1.5 Problem statement

BoNT-A injection is used extensively in various research to reduce spasticity, but still, we need more research about its functional effects on walking and muscle activation. Therefore, more research is required to close the information gap regarding the impact on functional walking, balance, and muscle exhaustion.

1.6 Aim

The present study aims to investigate the effect of botulinum toxin injection (BoNT-A) on balance and muscle activation during prolonged walks in CP children.

2 Materials and methods

2.1 Methods

This study includes post hoc analyses of the double-blinded, placebo-control RCT named WE study organized in clinical settings of four sites in Norway, one in France, and one in Poland. The participants were randomly allocated on a 1:1 ratio for treatment selection between BoNT-A and saline (placebo)injections in the calf muscles.

In this RCT, 59 young children aged between 4 and 17 years identified with unilateral or bilateral spastic cerebral palsy at level I or II according to the gross motor function classification system (GMFSC) participated. The inclusion criteria were indications for BoNT-A treatment in the calf muscles by their responsible medical doctor, Participants able to take verbal instructions, and the GMFCS level must be I or II. Exclusion criteria were participants with a history of orthopedic surgery in the preceding two years and those who received BoNT-A injection in the lower limb in the last six months. We included 33 participants from the WE study with EMG and acceleration data in this investigation during the 5-minute walk test. Ethical approval under project number 2013/1195 was granted by the Regional Committee of Ethics in Medical Research (REK) in the included countries. Parents of participating children procured written informed consent before participating in the study[15].

2.2 Protocols

The study procedure relied on the 5MWT Walking Test.

For the 5MWT, participants were instructed to move back and forth at their normal speed for 5 minutes in a 25-meter-long hallway. They performed heel drops as directed to synchronize the accelerometers and EMG electrodes before the test.

2.3 Data Collection

Data was gathered at four separate intervals: (baseline) prior to injection, 4, 12, and 24 weeks after injection, labeled as (B, P1, P2, and P3).

2.4 Equipment and Measurements

A research team member walked alongside the child, using a measuring wheel to measure the walking distance. The team manually recorded the starting and ending times. Additionally, an accelerometer was attached to the wheel to monitor the walking speed during the 5MWT.

Triaxial accelerometers (Axivity AX3, Newcastle, U.K.) were used to measure the gait cycle during walking and were fixed to the skin on the lower back (central at the lumbar vertebrae 3), thigh, right forefoot, and left forefoot (Figure 1). Accelerometers had a sampling frequency of 200 Hz and a sample range of ±8 g. Acceleration was measured in the vertical (V), mediolateral (ML), and anterior-posterior (AP) directions by AX3 monitor. AX3 software (Omgui version 1.0.0.28) was used to configure and download log data[15].

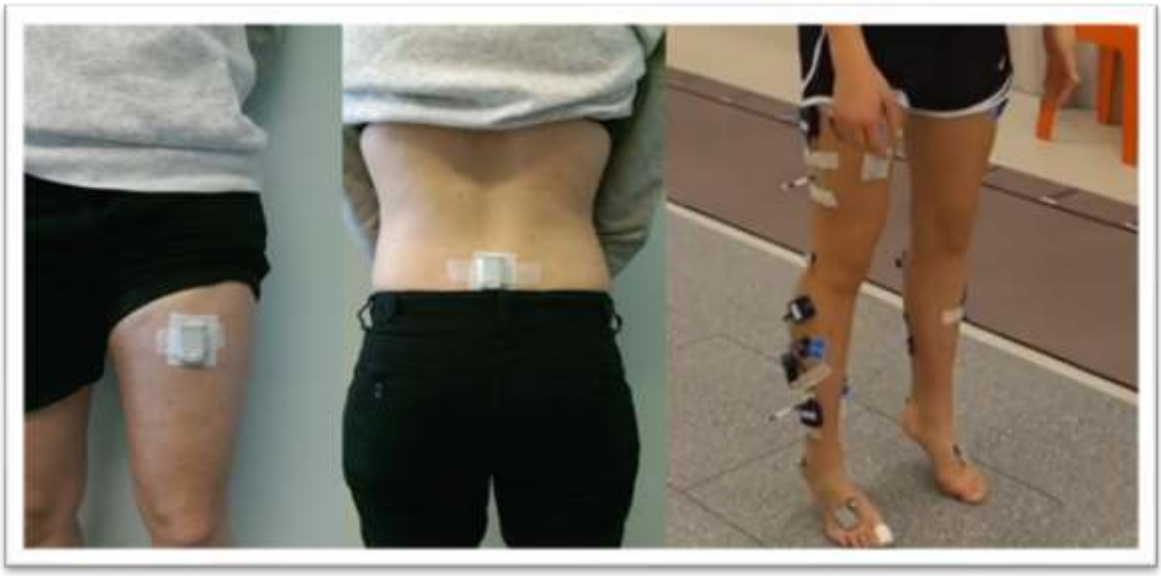


Figure 1: Placement of AX3 activity monitor on mid-thigh and Lower back (L3) right forefoot, left forefoot, and E.M.G. electrodes on Rectus Femoris, Semitendinosus, Tibialis Anterior, Gastrocnemius, and Soleus.

Surface EMG was measured by placing wireless EMG (in Trondheim, Myon, Schwarzenberg, Switzerland) electrodes over gastrocnemius medialis and soleus sampled at 2000hHz to investigate muscle activation. According to the guidelines for non-invasive assessment of muscles, skin was prepared before placing electrodes. Participants were asked to perform heel drops before starting and finishing walking tests to synchronize acceleration and EMG signals.

2.5 Data analyses

The Mathworks' MATLAB version R2022a was used to analyze the data. EMG and acceleration data were initially synchronized by detecting heel drop spikes before the regular walking signals.

Before further analyses, raw data signals were visually inspected to ensure the quality of the signals. The EMG data were then high pass filtered at 20Hz to remove movement artifacts.

2.6 Variables

From the all-time series, the median value of the whole 5MWT was taken to represent the average value, automatically omitting outliers.

The median from the first and last minute without the first and last 10 seconds was used to calculate the percentage (%) change using the formula (100*(end-start)/start) from beginning to end during the 5MWT(Figure 2).

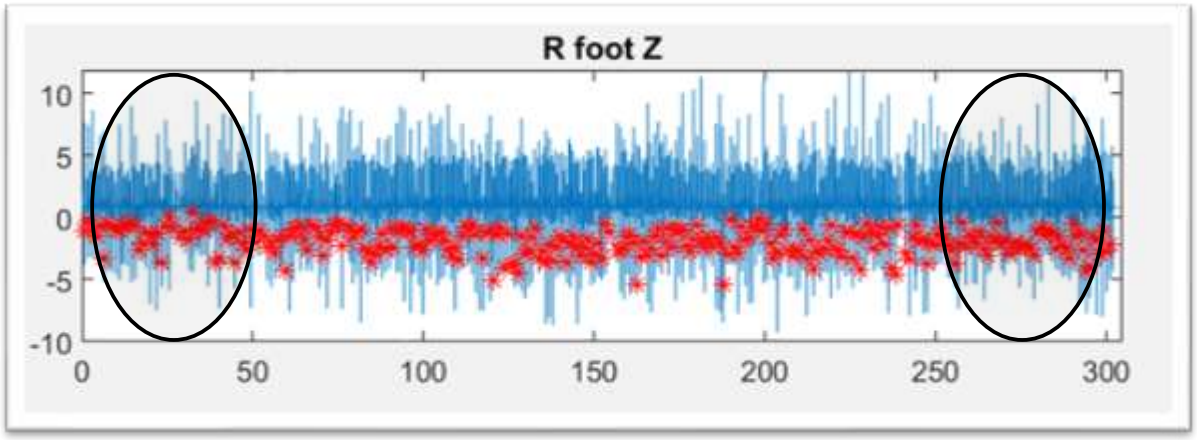


Figure 2: 5 Minute walk test for the variable calculation, circles represens the time interval(s) used for calculation of %age change

2.7 Walking speed

Instantaneous walking speed (m/s) was estimated from the accelerometer on the wheel, using each cycle of a wheel (Figure 3) with a circumference of 1m.

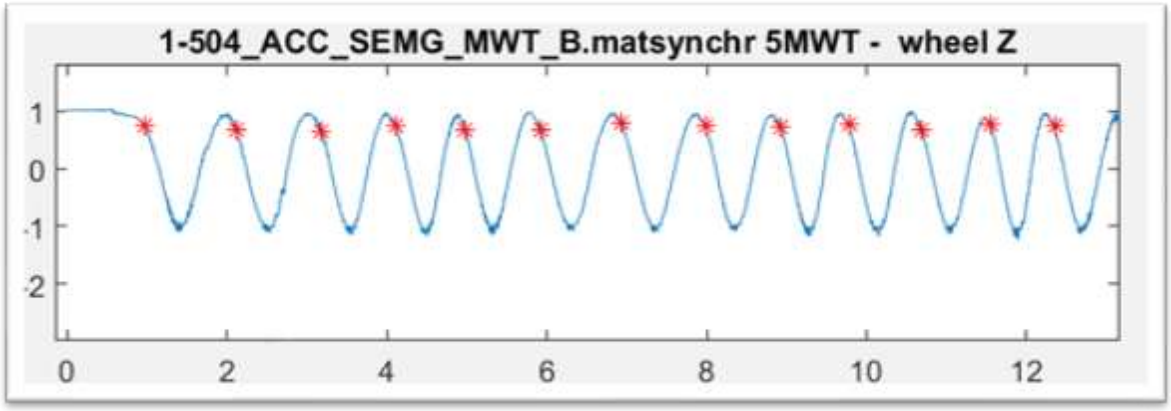


Figure 3: Accelerometer on the wheel, each red star represents a complete cycle, and speed (m/s) was calculated for each cycle where time (s) is on the x-axis and velocity (m/s) is on the y-axis.

The identification of gait cycles for both feet was achieved by detecting the impact through peak detection in the raw acceleration signals of right and left foot accelerometers.

2.8 Sample Entropy

Walking balance and trunk stability were assessed using a back accelerometer's acceleration signals in the x,y, and z axes (Figure 4).

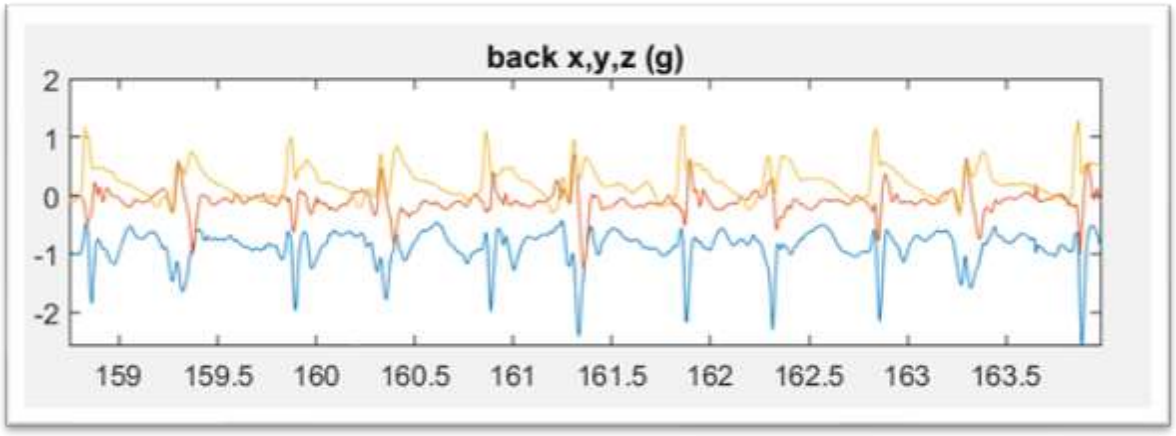


Figure 4: Acceleration signal of back accelerometer at Mediolateral, Vertical, and Anterior-posterior aspect where time is at the x-axis and acceleration at the y-axis.

The number of steps per minute was calculated by detecting the impact (peak) in the acceleration signal of the foot accelerometer during each gait cycle (Figure 5).

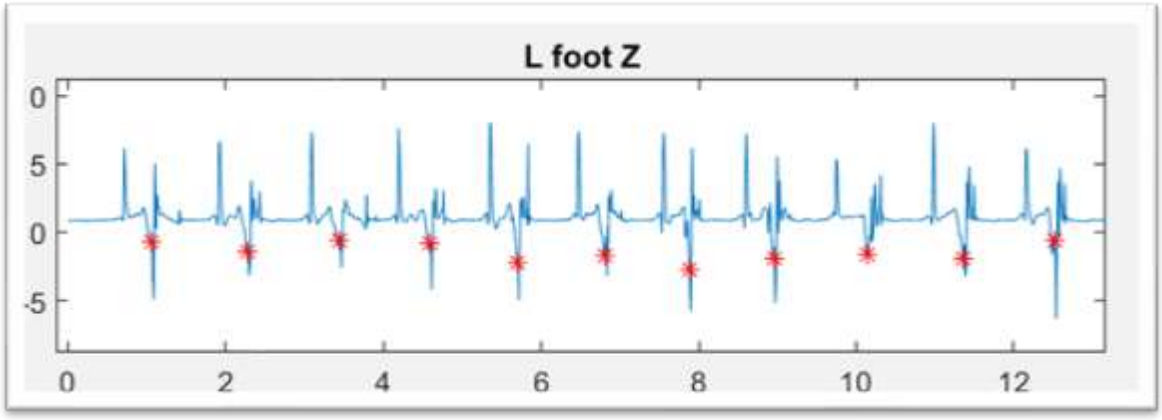


Figure 5 Step detection from peaks in acceleration signal in each gait cycle.

The (R) foot accelerometer served as the initial step detection reference, with the (L) foot accelerometer used in cases where steps were absent from the (R) foot.

Upon detecting each step, the following variables were calculated:

2.8.1 Spectral Entropy

The instantaneous spectral Entropy was calculated to access the trunk movements while walking from the back accelerometer signal in M-L, A-P, and vertical directions through MATLAB-function *pentropy*.

2.8.2 Standard deviation

The standard deviation of acceleration signals was calculated to estimate the energy while walking (Figure 6).

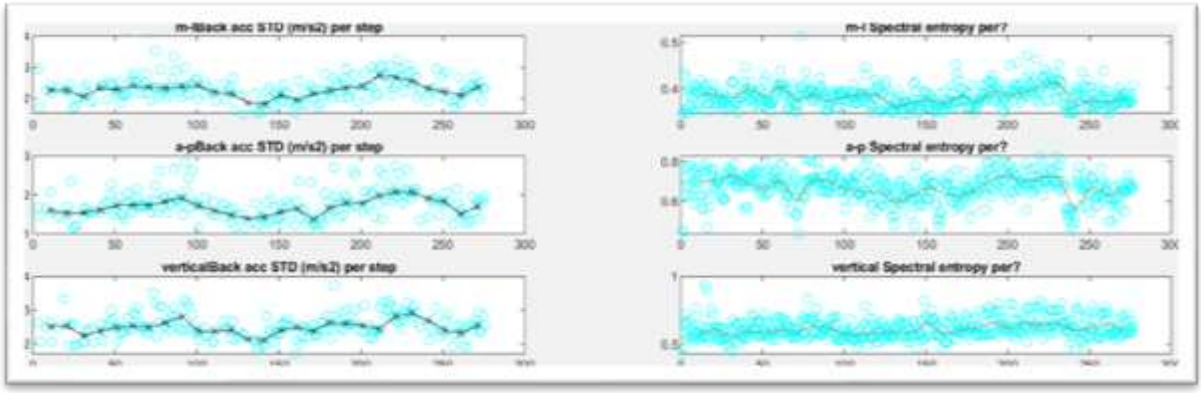


Figure 6: *Acceleration signal indicating Entropy, the black line shows the median value in 10-sec epochs during the 5MWT.*

2.8.3 RMS of EMG signals

We calculated the root mean square (RMS) in microvolts for each gait cycle from EMG signals of the gastrocnemius and soleus muscles to detect the extent of muscle activation while walking after treatment (Figure 7).

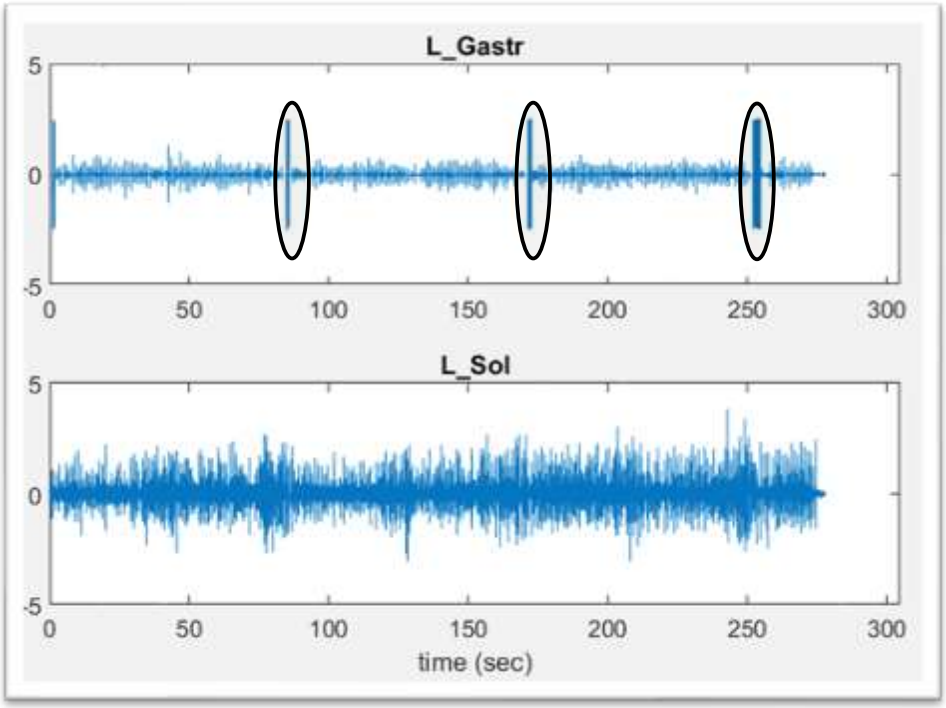


Figure 7: EMG signal showing extent of muscle activation, time (s) is at the x.axis, and EMG-RMS (uv) of G.M. and S is at the y-axis. Encircled lines in (L) gastrocnemius signals are cable artifacts, and L signals show the noise in the signal.

All the variables from MATLAB were initially transferred to Excel (Version 2305 Build 16.0.16501.20074) and subsequently imported into SPSS for further analysis. Descriptive statistics were performed to describe participants' characteristics between the two treatment groups (Saline and BoNt-A). The Shapiro-Wilk test and histograms assessed the normality of the data with a normal distribution curve. A linear mixed model was used to analyze normally distributed variables where fixed factors were time points (B, P1, P2, P3) and treatments (BoNt-A, Saline), whereas subjects were considered as random factors. Moreover, the interaction was between treatments (Saline, BoNT-) over time in two treatment groups. Furthermore, Wilcoxon signed-rank tests were used for variables that were not normally distributed.

The data was grouped as BoNT-A and Saline and exhibited in boxplots, which present the median value in the plot's center and a box containing the middle 50% of the observations (the interquartile range). Two whiskers on the top and bottom of the box, respectively, present the top and bottom extreme values. Outliers are indicated above or below the whiskers and are eliminated from the median and interquartile range calculations. In some cases, where data was missing or E.M.G. was short, we adopted the variable-by-variable approach to exclude those specific cases. Statistical analyses were conducted using IBM SPSS Statistics Version 28, with a significance level set at $p < 0.05$.

3 Results

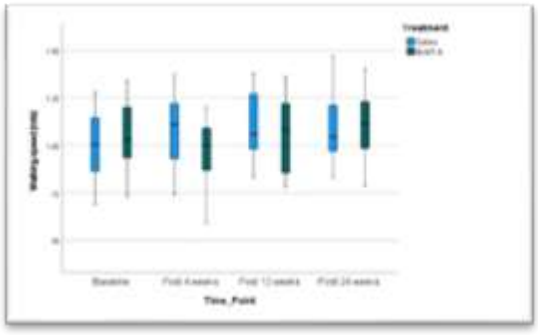
Out of the 33 children included in this study, 16 were treated with Saline as a placebo and 17 with BoNT-A. The characteristics of the participants can be found in Table 1.

Table 1: Characteristics of CP(Cerebral palsy) Children

Table:1 Characteristics of CP Children n=33		
Participants characteristics	Saline Placebo N=16	BoNT-A N=17
Sex(F/M)	7/9	5/12
GMFCS Level (I/II)	15/1	15/2
Age (y: mo)	8.8(±2.9)	8.4(±3.8)
Height (cm)	125.9(±15.2)	130.4(±20.7)
Weight (Kg)	26.9(±8.5)	29.9 (±16.0)
B.M.I. (Kg/m2)	16.6(±2.4)	16.6(±2.6)
CP unilateral (1)/Bilateral (2)	14/2	15/2

3.1 Walking Speed (m/s) and Estimated Frequency of steps/s

a



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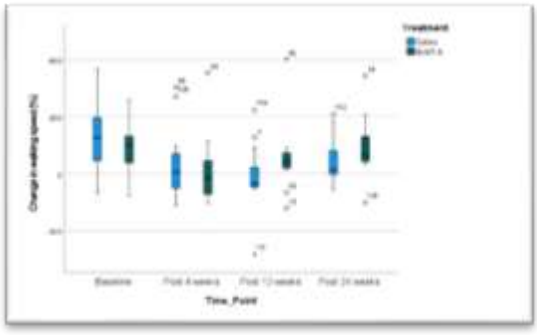

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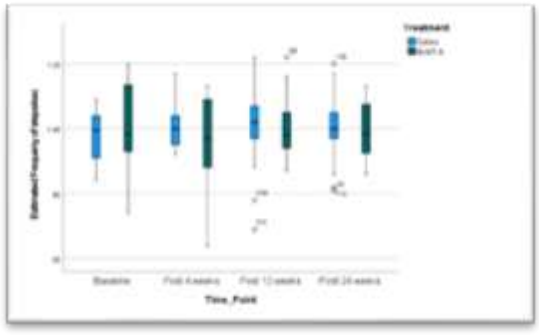


Figure 8 :Walking speed (m/s) and estimated frequency of steps, figures (8a & 8c) show the average walking speed (m/s) and estimated frequency of steps (Steps/sec) during the whole 5MWT, whereas Figure (8b) shows the percentage (%) change in walking speed from the first to the last minute, excluding the first and last 10 seconds of the 5MWT.

The participants had a walking speed of roughly 1 m/s during the 5MWT.

The average walking speed and estimated number of steps/s during the whole 5MWT are presented in (Figures 8a and 8c), and the percentage (%) change in walking speed from the first to the last minute, excluding the first and last 10 seconds of the 5MWT in Figure (8b)

In average walking speed (Figure 8a), both the saline and botox groups walked at the same speed; however, after four weeks following the treatment, there was a minor increase in the average walking speed of the saline group and a slight decrease in the botox group, respectively. At post 12 and 24 weeks, the saline group walked at the same speed, but there was a slight increase in the walking speed of children who got BoNt-A injection. However, none of those differences reached statistical significance.

The percentage (%) change in walking speed (Figure 8b) demonstrates that both groups increased their walking speed during the 5MWT at baseline. However, they walked at a more constant speed at post-four weeks, followed by a slight increase in walking speed at post-12 and 24 weeks.

The average cadence (estimated number of steps per minute) in 5MWT in both groups presented in Figure (8c) demonstrated minimal to no variation between the step frequency of both treatment groups at all four time points. The percentage change for estimated step

frequency is not included in the results due to unknown and unidentified errors in the data. Additionally, the statistical analysis has yet to reveal any significant findings.

3.2 Standard Deviation of Acceleration at ML, AP, and V aspect

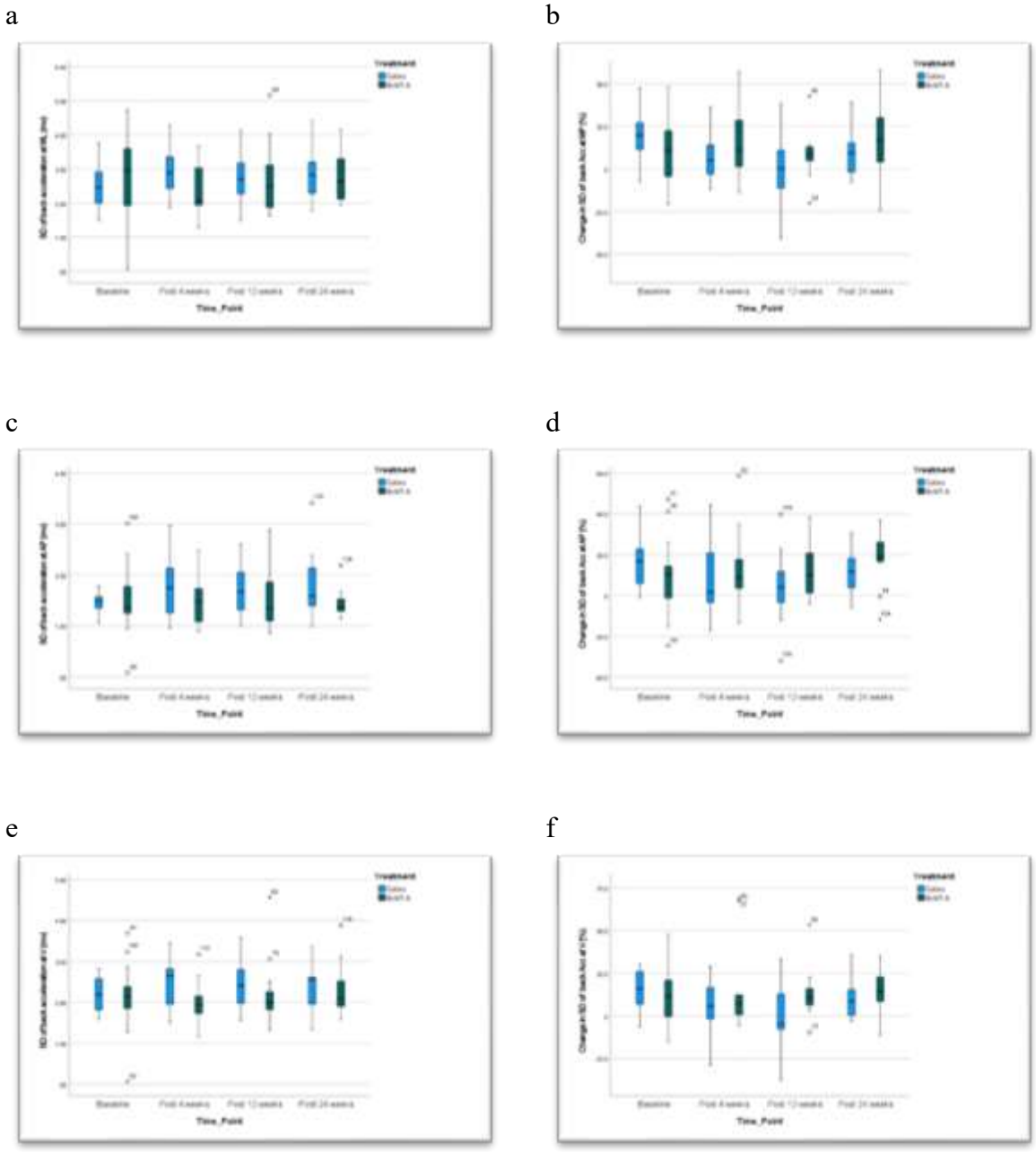


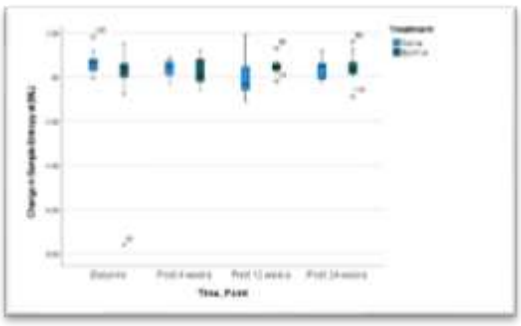
Figure 9: Standard deviation of acceleration, left-hand column (Figures 9a,9c, and 9e) in the preceding panels shows the average Standard deviation of back acceleration signals in three planes at all four time points. At the same time, the right column (Figures 9b,9d,9f) shows the percentage (%) change in acceleration from the initial to the last minute of 5MWT, excluding the first and last 10 seconds.

While examining the boxplots in (Figures 9a,9c, and 9e), there was no discernible increase or decrease in the average standard deviation (S.T.D.) of back signals in all three planes over time between both treatment groups. The majority of the data points cluster around the median value. Although there were few extreme values, they did not indicate significant patterns or trends to depict the variability and stability of gait.

The percentage (%) change in S.D. of back acceleration signals at the initial and final minute of 5MWT in (Figures 9b,9d, and 9f) showed a bit high standard deviation in the saline group at baseline, as compared to post 4-,12 and 24 weeks in all the three planes. At the same time, the botox group did not demonstrate any noticeable increase or decrease in S.D., and it was almost identical at four time points. No statistically significant difference was observed in the acceleration signals of the back when comparing the treatment groups over time.

3.3 Sample entropy of back signal at ML, AP, V Aspect

a



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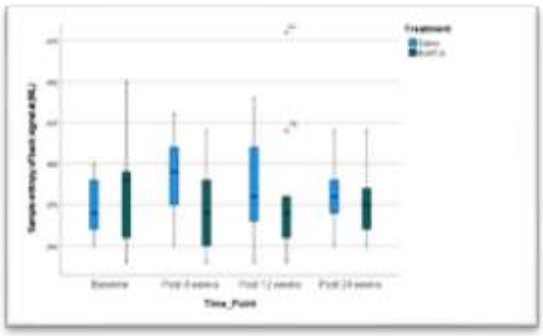

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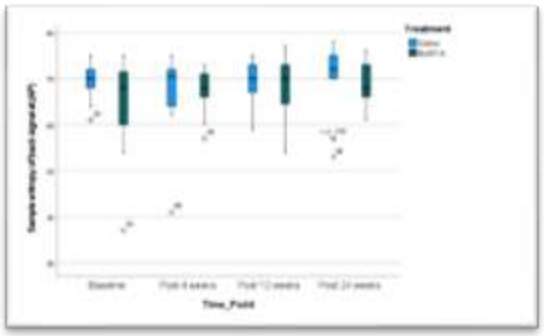

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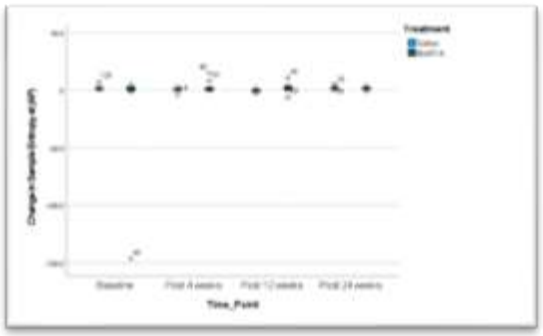


e f

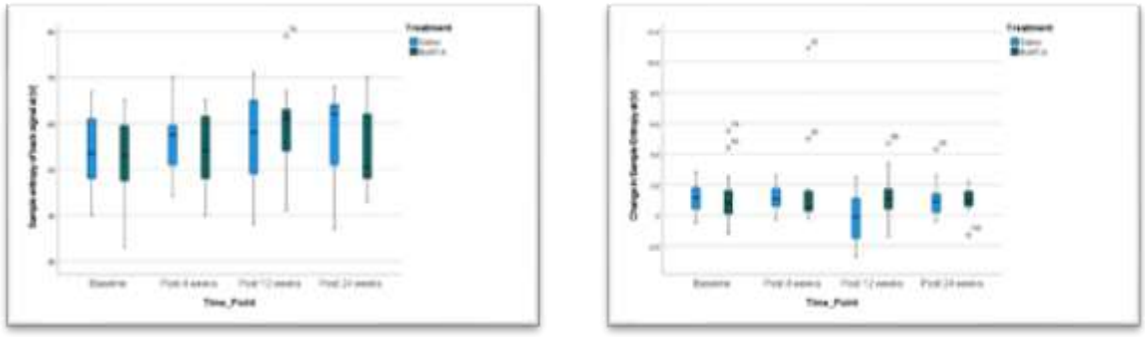
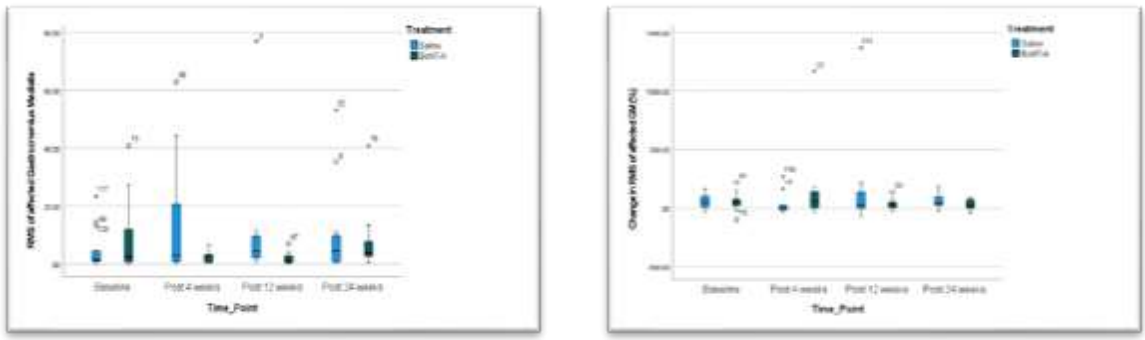


Figure 10: Sample Entropy, The left-hand column (Figures 10a,10c, and 10e) in the preceding panels shows the average Sample Entropy of back acceleration signals in 5MWT in three planes at all four time points. The (%) change in sample Entropy of the acceleration signal from the start to the end of 5 MWT is presented in (Figures 10b,10d, and 10f) on the right-hand side. Abbreviations such as Mediolateral (ML), Anteroposterior (AP), and Vertical (V) are abbreviations

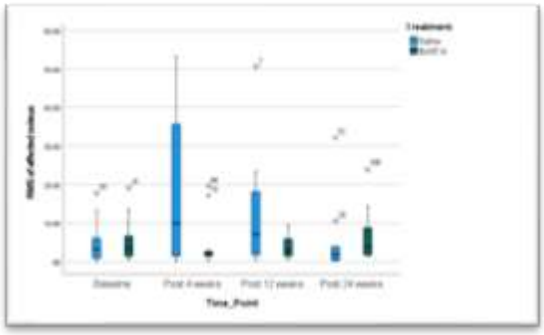
When analyzing all six boxplot figures in (Figures10a,10b,10c,10d,10e,10f), there was no noticeable increase or decrease in the average and percentage (%) change of sample entropy of back signal, which would suggest any irregularity or improvement in balance for either group. No clear pattern of increase or decrease was observed at any of the time points in the Mediolateral (ML), Anteroposterior (AP), and Vertical (V) planes, and all the results from statistical analysis using a linear mixed model were statistically insignificant.

3.4 RMS of affected Gastrocnemius Medialis and Soleus

a b



c



d

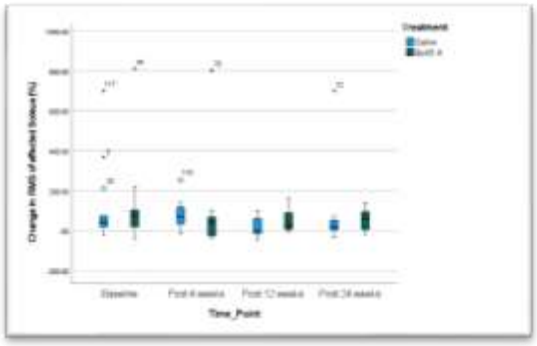


Figure 11: Average and Percentage change in RMS-EMG: (a & c) shows the average RMS-EMG amplitude (uv) during 5 M.W.T., whereas (b & d) present the percentage (%) change in root mean square of E.M.G. amplitude from the start to the end of 5MWT for treated gastrocnemius and soleus muscles.

The average RMS-EMG per gait cycle for the gastrocnemius and soleus muscles varied between 3 and 10 uV, as presented in (Figures 11a and c). There were no statistical differences between groups and time points. However, the lowest average with the lowest variation can be seen at P1 and P2 for the gastrocnemius and at P1 for the soleus in the BoNT-A group.

Regarding the percentage change in RMS during 5MWT in (Figure 11b), gastrocnemius showed minimal activity change at baseline, P2, and P3, whereas it had slightly increased activity at P1 in the botox group. In contrast to the botox group, the gastrocnemius of the saline group showed the lowest increased activity at P1 and had marginal activity change at baseline, P2, and P3. However, the percentage change for the soleus muscle demonstrated in Figure (11d) shows observable muscle activity change at the baseline, which slightly decreased at post-4 weeks, further decreased at post-12, and increased at post-24 weeks in the botox group. The muscle activation change in the soleus was similar at the baseline level and post 24 weeks but shows slight increase at post 24 weeks.

When comparing both groups, a slight difference in muscle activity can be observed in both muscles. Both treatment groups had extreme values in the average and percentage change of RMS. amplitude; these extremes do not indicate any significant overall trends.

4 Discussion

This study aimed to investigate the impact of botulinum injections on calf muscle activation and balance during walk. It was assessed using surface EMG and Accelerometer over 5 minutes using a 5-minute walk test. This study aimed to determine the potential improvement in gait and balance following treatment with Botox injections. Additionally, the secondary objective was to determine whether muscle fatigue in the calf muscles decreases or whether there are any enhancements in walking speed after administering the botulinum toxin injections.

The study included two treatment groups: one received a saline injection (placebo), and the other received the primary botulinum toxin treatment to investigate the treatment effects over time. The results obtained from these two groups were subsequently evaluated and compared. Upon conducting an analysis and applying various statistical tests to the outcome variables, the study's primary findings indicated statistically insignificant results for all variables. This study analyzed the participants at the maximum interval of 24 weeks after treatment; no significant change was seen in muscle activation and walking speed ($p>0.05$) in the group receiving BoNT-A treatment compared to the group receiving saline injections. Thus, our study could not confirm a significant improvement or deterioration due to BoNT-A treatment.

The average walking speed over the course of 5 MWT for both the treatment groups was between 1 and 1.25 m/s, similar to the walking speed reported in other studies with patients with similar characteristics[41-43]. Although not statistically significant, the saline group had somewhat lower average walking speed at baseline. In contrast, the BoNT-A group had the lowest average speed four weeks after treatment, which would collide with the expected peak effect of the toxin. Both groups increased their walking velocity during the 5MWT at baseline but kept a more stable velocity at the other test points, especially 4 and 12 weeks post-treatment. This could be attributed to children's learning behavior, or another possibility could be that some children may not have followed the instructions. All gait variables are dependent on walking speed[44]. The mostly stable walking speed during the 5MWT and between the test periods allows a direct comparison of the gait variable changes over time without velocity as a possible confounder.

Like walking speed, our results indicate no discernible change in the amplitude of trunk acceleration (vertical, medial-lateral, or anterior-posterior) in either of the treatment groups,

but previous studies indicated higher trunk acceleration while walking, which shows trunk instability and poor balance in CP children[43]. Trunk acceleration signals offer valuable information about the variability, stability, complexity, and irregularity of gait and back movements during walking[45]. These irregularities can be associated with neuromuscular or movement disorders. Health professionals can assess gait abnormalities by utilizing trunk-worn sensors, enabling them to design more effective treatment programs. By understanding and monitoring these signals, healthcare providers can gain insights into the underlying causes of gait abnormalities and develop tailored interventions accordingly[46].

The impact of BoNT-A on muscle activation during the 5MWT was evaluated using EMG. No statistically significant differences were observed, but the average EMG amplitude in both the gastrocnemius and soleus showed slight activity at baseline. Muscle activation was lowest after 4 and 12 weeks of injection in gastrocnemius and after four weeks in soleus. There was little activation at post-24 weeks in gastrocnemius and at post-12 and 24 in soleus, which confirms the working mechanism of the treatment. The results indicate slightly increased muscle activation during the 5MWT, but that was not considered, and all the results were statistically nonsignificant.

Although our study did not observe a significant improvement in gait, existing literature demonstrates substantial improvements in gait analysis and walking velocity in children with CP. For instance, Sarioglu et al. found substantial progress in gait after applying botulinum toxin type A (BoNT-A) injection, particularly in the gastrocnemius muscle, with substantial improvements observed four weeks post-treatment[47]. Another study by Sutherland et al. focused on the gastrocnemius muscle and suggested that using Botox effectively managed dynamic foot deformities and was deemed safe for children with cerebral palsy. This study utilized a double-blind, placebo-controlled design[48].

Additionally, Koman et al. conducted a double-blind, placebo-controlled trial to investigate the impact of botulinum toxin on the lower extremities in children with cerebral palsy. Their study involved 114 children with cerebral palsy, and they evaluated the participants over three months using observational gait analysis, nerve conduction studies, and ankle dorsiflexion assessments. The researchers reported positive outcomes regarding gait improvements and partial denervation of muscles due to botulinum toxin treatment[49].

Our study's participants had an average age of 8 years. Wissel et al. conducted a study on the effects of botulinum toxin treatment in people over and under seven. Their findings suggested that starting botulinum toxin treatment at a young age can result in long-term improvements in dynamic function and corrected abnormalities[50]. The age factor could have affected the less favorable outcomes seen in our investigation. Most studies in the current literature have yet to establish the best age to begin botulinum toxin treatment. However, T Ubhi et al. found no significant age effects in their investigation. It is worth noting that age-related alterations in children's problems can occur[50].

Balaban and colleagues' study conducted in America found that the botox injection in the gastrocnemius muscle positively affected walking patterns, improved spasticity, and increased ankle range of motion. The results are compatible with the study's findings regarding walking improvement but not with the findings regarding spasticity[51]. Another study examined how botox affected gait characteristics and muscle activation in kids with cerebral palsy. The study showed that walking, gait characteristics, and muscle activation had improved[52]. Even though the literature supports the effect of botulinum injection A on muscle tone, it is interesting to find out if it reduces muscle activity and how it can improve walking with partially or fully paralyzed muscles.

5 Conclusion

In conclusion, the observed results in this study were not statistically significant ($p > 0.05$). They could not confirm a significant improvement or deterioration due to BoNT-A treatment on walking or balance. It is worth noting that the small sample size in this study may have contributed to these inconsistent findings. The current study is exploratory, emphasizing the need for additional research to corroborate recent results in the broader population of CP patients.

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