Intramuscular Neural Distribution of the Sartorius Muscles: Treating Spasticity With Botulinum Neurotoxin

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Research Article

Keywords: botulinum neurotoxin, spasticity, sartorius muscle, Sihler’s staining

DOI: https://doi.org/10.21203/rs.3.rs-129928/v1

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Abstract

This study aimed to detect the idyllic locations for botulinum neurotoxin injection by analyzing the intramuscular neural distributions of the sartorius muscles. A altered Sihler’s staining was conducted on sartorius muscles (15 specimens). The nerve entry points and intramuscular arborization areas were measured as a percentage of the total distance from the most prominent point of the anterior superior iliac spine (0%) to the medial femoral epicondyle (100%). Intramuscular neural distribution were densely detected at 20–40% and 60–80% for the sartorius muscles. The result suggests that the treatment of sartorius muscle spasticity requires botulinum neurotoxin injections in particular locations. These locations, corresponding to the locations of maximum arborization, are suggested as the most safest and effective points for botulinum neurotoxin injection.

Introduction

Spasticity is a main contributor to functional loss in patients with impaired central nervous system, such as in stroke, cerebral palsy, multiple sclerosis, traumatic brain injury, spinal cord injury, and others. Sartorius muscle, as a hip and knee flexor, is one of the commonly involved muscles, and long-lasting spasticity of the muscle results in abnormalities secondary to muscle hyperactivity, affecting lower levels of functions, such as impairment of gait. For ambulatory patients, hip and knee flexor spasticity results in crouched gait, which needs much more strength than in people with normal hip and knee extension. Since hip and knee flexor spasticity occurs in conjunction, surgical and medical treatments are required in sartorius muscle to improve the posture.

Presently, botulinum neurotoxin (BoNT) injection is among the most secure and effective approaches for relieving spasticity. Since its consequences depend on the amount, the recommended BoNT levels should be sufficient in sartorius muscle at the area of neuromuscular junctions. However, BoNT overdose may cause the neurotoxin to spread to adjacent muscles and cause undesirable paralysis. Thus, in order to lessen the adverse effects and to maximize its clinical efficacy, BoNT must be injected near the neural arborized areas. Numerous studies have revealed the anatomical location of neuromuscular junctional areas of many different muscles. The consequence of neural arborized area-targeted injection, in which most neuromuscular junctions are located, has been established in clinical studies on biceps brachii and psoas major muscles. Intramuscular injection in neural arborized areas of biceps brachii and psoas major resulted in a much higher volume reduction than the control.

Studies that explain BoNT injection locations by navigating intramuscular neural distribution with naked eye dissection have limits, such as perceiving minuscule nerves and the likelihood of neural damage. Moreover, earlier studies have pronounced trouble in precisely detecting the tiny nerves. These restrictions can be disregarded when proceeding with Sihler’s staining, which is a whole-mount nerve staining that changes the muscle translucent while stained nerves.
Sihler’s staining is an efficient method to precisely demonstrate the intramuscular nerve distribution without damaging the nerve itself, and offers a discrete and comprehensive outline of the nerve distribution.

The objective of this study was to determine the intramuscular nerve distribution of sartorius muscle using Sihler’s staining and to suggest BoNT injection points for treating hip and knee flexor spasticity.

**Results**

**Location of Nerve Entry Points**

In all cases, the sartorius muscle was innervated by the femoral nerve. The mean entry point of the motor nerve penetrating the muscle was 1 to 3. Four cases had three nerve entry points, nine cases had two nerve entry points, and two cases had one nerve entry point. The distance of all nerve entry points was located at 10–30% from the anterior superior iliac spine (0%) to the medial femoral epicondyle (100%).

**Intramuscular Arborization Patterns**

Eleven out of 15 sartorius muscles had the most nerve arborizations appear at two regions, located at proximal 20–40% and distal 60–80%. Three had most nerve arborization at proximal 30–40% and distal 60–80%, and one had it at proximal 30–40% and distal 60–70%. All specimens had the most distally located intramuscular nerve ending at around 80–90% of each muscle (Fig. 1).

**Discussion**

The sartorius muscle, the lengthiest muscle in the human anatomy, running over both hip and knee joints. It is a superficially and anteriorly located muscle of the thigh and diagonally runs from the anterior superior iliac spine to the medial side of the proximal tibia at the pes anserine. The insertion of the sartorius muscle is the superior medial part of the tibial bone, close to the medial tibial tubercle. The conjoined tendon of gracilis, semitendinosus, and sartorius muscles is known as the pes anserinus. The sartorius muscle is innervated by the femoral nerve, provided by the nerve roots L2 to L4. The nerve entry point, where the nerve pierces the muscle, was at its proximal end.

The most prevalent spasticity patterns in the lower extremities are hip flexion and adduction, knee flexion, equinovarus foot, and big toe hyperextension that impedes the gait of affected patients. Since the sartorius is the only hip and knee flexor muscle, it is commonly targeted for BoNT injections to ease spasticity. BoNT injection in the sartorius muscle is not only for spasticity treatment, as many studies have performed BoNT injection to effectively prevent muscle contractures in total hip and knee arthroplasties and other surgeries. Awaad et al. reported the functional contribution of BoNT injection into the sartorius muscle in the treatment of postoperative stiff hips. Hamdy et al. used BoNT in femoral lengthening surgery and concluded that it improved the post-operative pain reduction. On the contrary, Park et al. reported that BoNT had no significant impact on pain reduction after...
lengthening osteotomy. However, their study had the limitation of using smaller doses than Hamdy et al. and had targeted only the proximal part of the sartorius muscles with no clear injection points.

Moreover, the sartorius muscle is a superficially located muscle that is highly suitable for many applications in free muscle transfer and local transposition in the field of reconstructive plastic surgery. However, it was less used than the other flaps due to the lack of anatomical studies on its intramuscular neurovascular distribution. Obeid et al. assessed 170 patients who underwent sartorius muscle flap for treating groin wounds, and reported that sartorius muscle flaps had low perioperative reintervention rate but had high complication rate. The neurovascular anatomy is credible, and surgical delay could expand the neural supply and extend the arc of rotation of the local flap.

The major therapeutic effects of BoNT are by impeding muscle contractions at the neuromuscular junction and cutting off the vicious cycle of pain. However, there is still a risk of damaging the nerve trunks when BoNT was not injected into the neural arborized area, even if it is minimally invasive compared to surgical procedures. Likewise, substantial BoNT injections have resulted in the formation of antibodies which lowers its effectiveness. Consequently, to increase the effectiveness and reduce the negative effects, a smaller amount of BoNT needs to be injected directly into the neural arborized areas, where most neuromuscular junctions are assumed to be located. Its efficacy was recognized in a clinical study on psoas major and biceps brachii muscles. Intramuscular neural arborized area-targeted injection in these studies resulted in a higher reduction in muscle volume than conventional injection methods.

Currently, there is no standardized injection point for BoNT treatment of the sartorius muscle. The amount of BoNT should be adequate to introduce a sufficient toxin level in the arborized area of neural distribution. This study used the Sihler’s staining method, which provides a possible solution to resolve the limitations of manual dissection. The application of Sihler’s staining to sartorius muscle will facilitate accurate and thorough understanding of the neural distribution. This study performed Sihler’s staining to reveal the intramuscular neural distribution of the sartorius muscle in order to determine the most effective and safest BoNT injection point, and guide neural distributions for sartorius flap surgery.

**Methods**

**Subjects**

Informed consent and approval were obtained from the families of the cadavers before the dissections were performed. All cadavers used in this study were legally donated and approved from ethics committee of the Surgical Anatomy Education Center, Yonsei University College of Medicine (approval code 20 - 009; approval date: May 5th, 2020). Sixteen sartorius muscles from 10 cadavers (five women and five men with a mean age of 74.2 years; range, 63–84 years) were dissected to explore nerve entry points, and Sihler’s staining was performed to detect intramuscular neural distribution.
The 16 sartorius muscles were harvested from 10 cadavers (five men and five women) and Sihler's staining was done. This procedure requires multiple stages to acquire the image of the intramuscular neural distribution. We conducted this procedure with some modifications. After the staining procedures, the sartorius muscles were equally divided into 10, each represented division of 10%, respectively to the total length. The origin of the sartorius muscles, nerve entry points, and intramuscular neural arborized locations were measured as percentages from the anterior superior iliac spine (0%) to the medial femoral epicondyle (100%) (Fig. 3).

The stages of the Sihler's staining of the sartorius muscle are described in Fig. 4.

**Modified Sihler's Staining**

Fixation stage: The extracted sartorius muscles underwent fixation for 30 days in a container filled with 10% unneutralized formalin. The formalin solution was replaced each time it turned hazy.

Maceration and depigmentation stages: Once fixation was completed, the sartorius muscles were placed in flowing water for 1 h. Next, the sartorius muscles were positioned in a 3% aqueous potassium hydroxide solution with hydrogen peroxide for two weeks.

Decalcification stage: The macerated sartorius muscle was immersed in a container filled with Sihler's solution I, which is composed of glycerin, aqueous chloral hydrate, and glacial acetic acid, for three days.

Staining stage: Sufficiently decalcified sartorius muscles were later stained by placing them for a day in Sihler's solution II, composed of glycerin, acetic acid, and aqueous chloral hydrate.

Destaining stage: Stained sartorius muscles were immersed for 3 to 5 hours in Sihler's solution I once again for sartorius muscle tissue destaining. The immersion was terminated before the nerves were destained.

Neutralization stage: Destained sartorius muscle was prepared in flowing water for 30 min. Afterwards the muscles were immersed in 0.05% lithium carbonate solution for 30 min.

Clearing stage: Neutralized sartorius muscles were placed in containers with increasing level of glycerin concentration within five days. Throughout this procedure, the concentration level was progressively increased in 20% increments to 40–100%.

**Abbreviations**

botulinum neurotoxin (BoNT), fixation (FX), maceration and depigmentation (MD), decalcification, staining (ST), and clearing (CL).

**Declarations**
Acknowledgements

The authors sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially increase mankind's overall knowledge that can then improve patient care. Therefore, these donors and their families deserve our highest gratitude. The authors thank Eun-Byul Yi from Eonbuk elementary school for illustrations. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP) (NRF-2020R1A2B5B0100223811). This work was supported by the Korea Medical Device Development Fund grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, the Ministry of Food and Drug Safety) (Project Number: 202012D01)

Author Contribution

KHY: research concept, study design, and writing of the manuscript

JHL: research concept and study design

KKS: literature review and data collection

HJK: reviewing/editing a draft of the manuscript, Supervise the manuscript

Declarations

Not applicable

Funding

Not applicable

Conflicts of interest/Competing interests

The author(s) declare no competing interests.

References

1 Barnes, M. P., Kent, R. M., Semlyen, J. K. & McMullen, K. M. Spasticity in multiple sclerosis. Neurorehabilitation and neural repair 17, 66-70 (2003).

2 Rosales, R. L. et al. Botulinum toxin injection for hypertonicity of the upper extremity within 12 weeks after stroke: a randomized controlled trial. Neurorehabilitation and neural repair 26, 812-821, doi:10.1177/1545968311430824 (2012).

3 Rameckers, E. A., Speth, L. A., Duysens, J., Vles, J. S. & Smits-Engelsman, B. C. Botulinum toxin-a in children with congenital spastic hemiplegia does not improve upper extremity motor-related function over
rehabilitation alone: a randomized controlled trial. *Neurorehabilitation and neural repair* **23**, 218-225, doi:10.1177/1545968308326629 (2009).

4 Lam, T. I. *et al.* Beneficial effects of minocycline and botulinum toxin-induced constraint physical therapy following experimental traumatic brain injury. *Neurorehabilitation and neural repair* **27**, 889-899, doi:10.1177/1545968313491003 (2013).

5 Sun, S. F. *et al.* Combined botulinum toxin type A with modified constraint-induced movement therapy for chronic stroke patients with upper extremity spasticity: a randomized controlled study. *Neurorehabilitation and neural repair* **24**, 34-41, doi:10.1177/1545968309341060 (2010).

6 Diserens, K. *et al.* Effect of repetitive arm cycling following botulinum toxin injection for poststroke spasticity: evidence from FMRI. *Neurorehabilitation and neural repair* **24**, 753-762, doi:10.1177/1545968310372138 (2010).

7 Won, S. Y. *et al.* Intramuscular nerve distribution pattern of the adductor longus and gracilis muscles demonstrated with Sihler staining: guidance for botulinum toxin injection. *Muscle & nerve* **46**, 80-85, doi:10.1002/mus.23273 (2012).

8 Hsu, T. S., Dover, J. S. & Amdt, K. A. Effect of volume and concentration on the diffusion of botulinum exotoxin A. *Archives of dermatology* **140**, 1351-1354, doi:10.1001/archderm.140.11.1351 (2004).

9 Kinnett, D. Botulinum toxin A injections in children: technique and dosing issues. *Am J Phys Med Rehabil* **83**, S59-64, doi:10.1097/01.phm.0000141131.66648.e9 (2004).

10 Lepage, D., Parratte, B., Tatu, L., Vuiller, F. & Monnier, G. Extra- and intramuscular nerve supply of the muscles of the anterior antebrachial compartment: applications for selective neurotomy and for botulinum toxin injection. *Surgical and radiologic anatomy : SRA* **27**, 420-430, doi:10.1007/s00276-005-0012-9 (2005).

11 Chantelot, C. *et al.* Innervation of the medial epicondylar muscles: an anatomic study in 50 cases. *Surgical and radiologic anatomy : SRA* **21**, 165-168 (1999).

12 Hwang, K., Jin, S., Hwang, S. H., Lee, K. M. & Han, S. H. Location of nerve entry points of flexor digitorum profundus. *Surgical and radiologic anatomy : SRA* **29**, 617-621, doi:10.1007/s00276-007-0260-y (2007).

13 Roberts, C., Crystal, R. & Eastwood, D. M. Optimal injection points for the neuromuscular blockade of forearm flexor muscles: a cadaveric study. *Journal of pediatric orthopedics. Part B* **15**, 351-355 (2006).

14 Sheverdin, V. A. *et al.* Extra- and intramuscular nerves distributions of the triceps surae muscle as a basis for muscle resection and botulinum toxin injections. *Surgical and radiologic anatomy : SRA* **31**, 615-621, doi:10.1007/s00276-009-0490-2 (2009).
15 Gracies, J. M. et al. Botulinum toxin dilution and endplate targeting in spasticity: a double-blind controlled study. *Arch Phys Med Rehabil* **90**, 9-16 e12, doi:10.1016/j.apmr.2008.04.030 (2009).

16 Van Campenhout, A., Verhaegen, A., Pans, S. & Molenaers, G. Botulinum toxin type A injections in the psoas muscle of children with cerebral palsy: muscle atrophy after motor end plate-targeted injections. *Res Dev Disabi* **34**, 1052-1058, doi:10.1016/j.ridd.2012.11.016 (2013).

17 Lee, J. H., Lee, B. N., An, X., Chung, R. H. & Han, S. H. Location of the motor entry point and intramuscular motor point of the tibialis posterior muscle: for effective motor point block. *Clinical anatomy* **24**, 91-96, doi:10.1002/ca.21062 (2011).

18 Oddy, M. J., Brown, C., Mistry, R. & Eastwood, D. M. Botulinum toxin injection site localization for the tibialis posterior muscle. *Journal of pediatric orthopedics. Part B* **15**, 414-417, doi:10.1097/01.bpb.0000228387.94065.ff (2006).

19 Yi, K. H. et al. Anatomical guide for botulinum neurotoxin injection: Application to cosmetic shoulder contouring, pain syndromes, and cervical dystonia. *Clin Anat*, doi:10.1002/ca.23690 (2020).

20 Yi, K. H. et al. Effective botulinum toxin injection guide for treatment of cervical dystonia. *Clin Anat* **33**, 192-198, doi:10.1002/ca.23430 (2020).

21 Yi, K. H. et al. Neuromuscular structure of the tibialis anterior muscle for functional electrical stimulation. *Surg Radiol Anat* **39**, 77-83, doi:10.1007/s00276-016-1698-6 (2017).

22 Yi, K. H. & Kim, H. J. Is variation in posterior tibial veins a risk factor for deep-vein thrombosis? *Clin Anat*, doi:10.1002/ca.23691 (2020).

23 Yi, K. H. et al. Intramuscular Neural Distribution of Rhomboid Muscles: Evaluation for Botulinum Toxin Injection Using Modified Sihler's Method. *Toxins (Basel)* **12**, doi:10.3390/toxins12050289 (2020).

24 Yi, K. H., Lee, H. J., Lee, J. H., Lee, K. L. & Kim, H. J. Effective botulinum neurotoxin injection in treating iliopsoas spasticity. *Clin Anat*, doi:10.1002/ca.23670 (2020).

25 Yi, K. H. et al. Intramuscular nerve distribution pattern of ankle invertor muscles in human cadaver using sihler stain. *Muscle Nerve* **53**, 742-747, doi:10.1002/mus.24939 (2016).

26 Rha, D. W., Yi, K. H., Park, E. S., Park, C. & Kim, H. J. Intramuscular nerve distribution of the hamstring muscles: Application to treating spasticity. *Clin Anat* **29**, 746-751, doi:10.1002/ca.22735 (2016).

27 Won, S. Y. et al. Intramuscular communicating branches in the flexor digitorum profundus: dissection and Sihler's staining. *Surgical and radiologic anatomy : SRA* **32**, 285-289, doi:10.1007/s00276-010-0634-4 (2010).
28 Yang, H. M. et al. Course and distribution of the lingual nerve in the ventral tongue region: anatomical considerations for frenectomy. *The Journal of craniofacial surgery* 20, 1359-1363, doi:10.1097/SCS.0b013e3181ae42fa (2009).

29 Buckland, A. et al. Neurovascular anatomy of sartorius muscle flaps: implications for local transposition and facial reanimation. *Plast Reconstr Surg* 123, 44-54, doi:10.1097/PRS.0b013e3181904bc6 (2009).

30 Johnson, C. A., Burridge, J. H., Strike, P. W., Wood, D. E. & Swain, I. D. The effect of combined use of botulinum toxin type A and functional electric stimulation in the treatment of spastic drop foot after stroke: a preliminary investigation. *Archives of physical medicine and rehabilitation* 85, 902-909 (2004).

31 Seyler, T. M. et al. Botulinum toxin type A injections for the management of flexion contractures following total knee arthroplasty. *J Surg Orthop Adv* 17, 231-238 (2008).

32 Bhave, A. et al. Botulinum toxin type A injections for the management of muscle tightness following total hip arthroplasty: a case series. *J Orthop Surg Res* 4, 34, doi:10.1186/1749-799X-4-34 (2009).

33 Awaad, Y., Rizk, T., Abak, A. A., Kabiruddin, K. & Khoshhal, K. I. Botulinum toxin-A in postoperative pediatric stiffness. *Journal of Taibah University Medical Sciences* 8, 187-191, doi:https://doi.org/10.1016/j.jtumed.2013.06.001 (2013).

34 Hamdy, R. C. et al. Safety and Efficacy of Botulinum Toxin A in Children Undergoing Lower Limb Lengthening and Deformity Correction: Results of a Double-blind, Multicenter, Randomized Controlled Trial. *J Pediatr Orthop* 36, 48-55, doi:10.1097/BPO.0000000000000398 (2016).

35 Hamdy, R. C. et al. Botulinum toxin type A injection in alleviating postoperative pain and improving quality of life in lower extremity limb lengthening and deformity correction: a pilot study. *J Pediatr Orthop* 29, 427-434, doi:10.1097/BPO.0b013e3181aad628 (2009).

36 Park, H. et al. Is Botulinum Toxin Type A a Valuable Adjunct During Femoral Lengthening? A Randomized Trial. *Clin Orthop Relat Res* 474, 2705-2711, doi:10.1007/s11999-016-5018-6 (2016).

37 Obeid, T. et al. Sartorius Muscle Flaps: Perioperative Outcomes Based on Surgical Specialty. *Ann Vasc Surg* 43, 226-231, doi:10.1016/j.avsg.2017.01.003 (2017).

38 Tepper, S. J. Treatment of headache pain with botulinum neurotoxins. *Pain Pract* 4 Suppl 1, S38-46, doi:10.1111/j.1533-2500.2004.04013.x (2004).

39 Ondo, W. G., Vuong, K. D. & Derman, H. S. Botulinum toxin A for chronic daily headache: a randomized, placebo-controlled, parallel design study. *Cephalalgia* 24, 60-65, doi:10.1111/j.1468-2982.2004.00641.x (2004).
40 Troost, B. T. Botulinum toxin type A (Botox) in the treatment of migraine and other headaches. *Expert Rev Neurother* **4**, 27-31, doi:10.1586/14737175.4.1.27 (2004).

41 Liem, R. S. & Douwe van Willigen, J. In toto staining and preservation of peripheral nervous tissue. *Stain technology* **63**, 113-120 (1988).

42 Won, S. Y. *et al.* Intramuscular innervation patterns of the brachialis muscle. *Clinical anatomy* **28**, 123-127, doi:10.1002/ca.22387 (2015).

43 Yang, H. M., Won, S. Y., Kim, H. J. & Hu, K. S. Sihler staining study of anastomosis between the facial and trigeminal nerves in the ocular area and its clinical implications. *Muscle & nerve* **48**, 545-550, doi:10.1002/mus.23875 (2013).

44 Yang, H. M., Won, S. Y., Lee, Y. I., Kim, H. J. & Hu, K. S. The Sihler staining study of the infraorbital nerve and its clinical complication. *The Journal of craniofacial surgery* **25**, 2209-2213, doi:10.1097/01.scs.0000436676.43949.19 (2014).

45 Won, S. Y. *et al.* Trabecular bone ratio of mandible using micro-computed tomography in Korean. *The Journal of craniofacial surgery* **21**, 920-924, doi:10.1097/SCS.0b013e3181d87a42 (2010).

46 Won, S. Y. *et al.* Extra- and intramuscular nerve distribution patterns of the muscles of the ventral compartment of the forearm. *American journal of physical medicine & rehabilitation / Association of Academic Physiatrists* **89**, 644-652, doi:10.1097/PHM.0b013e3181d8a116 (2010).

**Figures**
Figure 3

Sartorius muscles were harvested from the anterior superior iliac spine (0%) to the medial femoral epicondyle (100%).