Int. J. Morphol.,
38(4):975-982, 2020.

Recommendation of the Best Site Based on the Distribution Pattern of Extramuscular and Intramusular Nerves for Gluteal Muscle Injection

Recomendación del Mejor Sitio para la Inyección del Músculo Glúteo Basado en el Patrón de Distribución de los Nervios Extramusculares e Intramusculares

Junxi Wu[1]; Yanzhen Cai[1]; Ai Cao[1]; Yu Bi[1]; Xiangnan Hu[2] & Shengbo Yang[2]

WU, J.; CAI, Y.; CAO, A.; BI, Y.; HU, X. & YANG, S. Recommendation of the best site based on the distribution pattern of extramuscular and intramuscular nerves for gluteal muscle injection. *Int. J. Morphol., 38(4)*:975-982, 2020.

SUMMARY: To reveal the extra- and intramuscular nerve distribution patterns of the gluteus maximus, medius, and minimus, and to provide guidance for gluteal muscle injection in order to avoid nerve injury. Ten adult and 10 child cadavers were used. The superior and inferior gluteal nerves innervating the gluteus maximus, medius, and minimus were dissected, exposed, and sutured in-situ on the muscle. The three gluteal muscles were removed, and the distribution patterns of the intramuscular nerves were revealed by modified Sihler's nerve staining. The nerve distribution pattern was returned to the corresponding position in the body, and the patterns in the four quadrants of the buttock were analyzed. There were 3–12 extramuscular nerve branches of the gluteus maximus, medius, and minimus. After entering the muscle, these nerve branches arborized and anastomosed to form an arc-shaped, nerve-dense zone. The nerve distribution was most dense in the inferomedial region of the superolateral quadrant and the inferolateral region of the superomedial quadrant of the buttocks. The nerve distribution was relatively dense in the inferolateral region of the superolateral quadrant, and the medial region of the inferomedial quadrant. An arc-shaped, nerve-sparse zone in the superolateral and superomedial quadrants near the lower iliac crest accounted for about two-fifths of the two quadrants' limits. The arc-shaped, nerve-sparse zone in the superolateral quadrant is the preferred injection site, and the superomedial quadrant near the lower iliac crest is also recommended as a gluteal intramuscular injection region, free from nerve injury.

KEY WORDS: Gluteal muscle injection; Sihler's staining; Nerve distribution pattern; Injection nerve injury; Intramuscular injection site.

INTRODUCTION

The buttock is a common site for intramuscular injection in adults and children as it has faster absorption rates than other routes (Elgellaie *et al*., 2018). This injection is administered into the gluteus maximus, medius, and minimus muscles. Clinically defined methods for the gluteal intramuscular injection site include: the cross-localization method (four quadrants), the dorsogluteal site (four quadrants), the ventrogluteal site, and Clark's point (Small, 2004; Li *et al*., 2019; Nakajima *et al*., 2020). Among them, the superolateral quadrant of the first two methods is the most clinically administered site (Arslan & Özden, 2018). However, these methods have limitations, which can cause pain or unpleasant complications, such as muscle atrophy caused by nerve injury, gluteal fibrosis and gluteal muscle contracture, granuloma formation, among others (Barry *et al*., 2013; Rai *et al*., 2017; Alves *et al*., 2018).

Drugs can be injected into the gluteus maximus and/or gluteus medius and gluteus minimus during gluteal muscle injection. To avoid complications caused by intramuscular injection, many studies have investigated the movement and localization of superior and inferior gluteal vessels and nerves (Brown *et al*., 2015; Kaya *et al*., 2015; Coskun *et al*., 2016). However, there are more reports of injection-related sciatic nerve injury (Brown *et al*.; Kaya *et al*.; Geyik *et al*., 2017). In fact, incidences of medications mistakenly injected into the blood vessels and damaging the main nerve are often avoidable, but common, possibly due to underdeveloped

[1] Department of Clinical Medicine, Zunyi Medical University, Zunyi, Guizhou Province, People's Republic of China.
[2] Department of Anatomy, Zunyi Medical University, Zunyi, Guizhou Province, People's Republic of China.
The authors Junxi Wu and Yanzhen Cai contributed equally.

medical technology or accidents (Mishra & Stringer, 2010). However, the distribution pattern of small, extra, intramuscular nerves is often overlooked. Injection of medication into these nerves can cause discomfort and complications due to nerve injury.

Therefore, this study aims to investigate the distribution pattern of extra- and intramuscular nerves of the gluteus maximus, medius, and minimus by performing gross anatomy and modified Sihler's staining. These techniques will reveal regions with dense nerve distribution and regions with sparse nerve distribution, which can be used to recommend the most suitable injection site. This information will improve the quality of gluteal muscle injections and reduce the incidence of complications.

MATERIAL AND METHOD

Specimens and ethics. The study was performed on ten formalin fixed Chinese adult cadavers (5 men and 5 women) ranging from 35 to 75 (64.3±7.4) years old, and ten Chinese children cadavers (5 men and 5 women) ranging from 5 to 15 (9.2±3.4) years old. No neuromuscular disease history or gluteal injuries were found. Cause of death for adult donors included: cancer, cardiovascular disease, and cerebrovascular accidents. Cause of death for child donors included: drowning, traffic accidents, pneumonia, cardiovascular disease, and cancer. The cadaver donors were not members of any vulnerable groups. Written informed consent was provided, free of charge, by all donors or immediate family. The study was approved by the Ethics Committee of our school in advance (Grant No.: #2016-1-006).

Gross Anatomy. Because the superolateral quadrant of the buttock is commonly administrated in clinical gluteal muscle injection, this study delimits it by the cross localization method (the vertical line through the apex of the iliac crest is the longitudinal reference line, i.e. the L line; the transverse line through the vertex of the anal cleft is the horizontal reference line, i.e. the H line) for convenience of description. All cadavers were placed in the prone position, the skin and subcutaneous fat of the buttocks were removed, anatomized, and the gluteus maximus, medius, and minimus muscles were exposed layer-by-layer. Fishing line was sewn to mark the junction of the L line, H line, and gluteal muscle edge. Next, we cut the origin of muscle on the iliac wing and the sacrum, rotated it outward and downward, exposing the superior and inferior gluteal nerves. We then carefully combed the branches of the two nerves to the nerve entry points. Then these nerve branches were sutured in situ to the muscles. Finally, these muscles were removed from the gluteal trochanter, iliotibial tract, and trochanter femoris for intramuscular nerve staining.

Modified Sihler's staining. According to the Sihler's intramuscular nerve staining method reported previously, the muscles were treated as follows (Tang *et al*., 2018): In short, Depigmentation: 3 % aqueous potassium hydroxide with the addition of 3 % hydrogen peroxide for 3–5 weeks, until the specimen becomes well bleached and translucent. Decalcification: Macerated specimens are shifted to Sihler's solution I (1:2:12 = glacial acetic acid: glycerin: 1 % aqueous chloral hydrate) for 4-5 weeks until the shrinked muscles could stretch and restore. Staining: Specimens are placed in Sihler's Solution II (Ehrlich's hematoxylin: glycerin: 1 % aqueous chloral hydrate = 1:2:12) for 4 weeks. Destaining: Stained specimens are placed into Sihler's solution I for 6-20 hours, an ideal result is muscles stained purple, nerve branches stained black. Neutralization: Specimens are placed in 0.05 % lithium carbonate solution for approximately 2 hours. Clearing: The specimens are then cleared with increasing concentrations of glycerin (40 %, 60 %, 80 %, and 100 %) for 1 week. We observed the distribution patterns of the extra- and intramuscular nerve branches using an X-ray box, and then we photographed and drew the pattern diagram.

Analysis of Sihler's staining results. The L- and H-lines were drawn on the gluteal structure pattern using the cross-localization method. The muscle and nerve pattern were then restored to the corresponding position of the gluteal structure pattern according to the position marked by the fishing line. Then the nerve distribution patterns of the gluteal superolateral, superomedial, inferolateral and inferomedial quadrants were analyzed.

RESULTS

Gross anatomy observation. Turning over the gluteus maximus, the inferior gluteal nerve emerged below the piriformis muscle and further divided, at the medial-central region underneath the muscle, into a superior and an inferior branch. Before entering the muscle, the superior nerve branch often divided into 3 primary branches; the inferior branch divided into 7-9 primary branches (Fig. 1). The superior gluteal nerve also divided into two nerve branches after it emerged from the suprapiriform foramen, one of which innervated the gluteus medius, and divided into 3-4 primary branches in the deep surface before entering the muscle. The other branch traveled outward and innervated the gluteus minimus muscle and tensor fascia lata, which divided into 4-5 primary branches on the superficial surface before entering the muscle (Figs. 2 and 3).

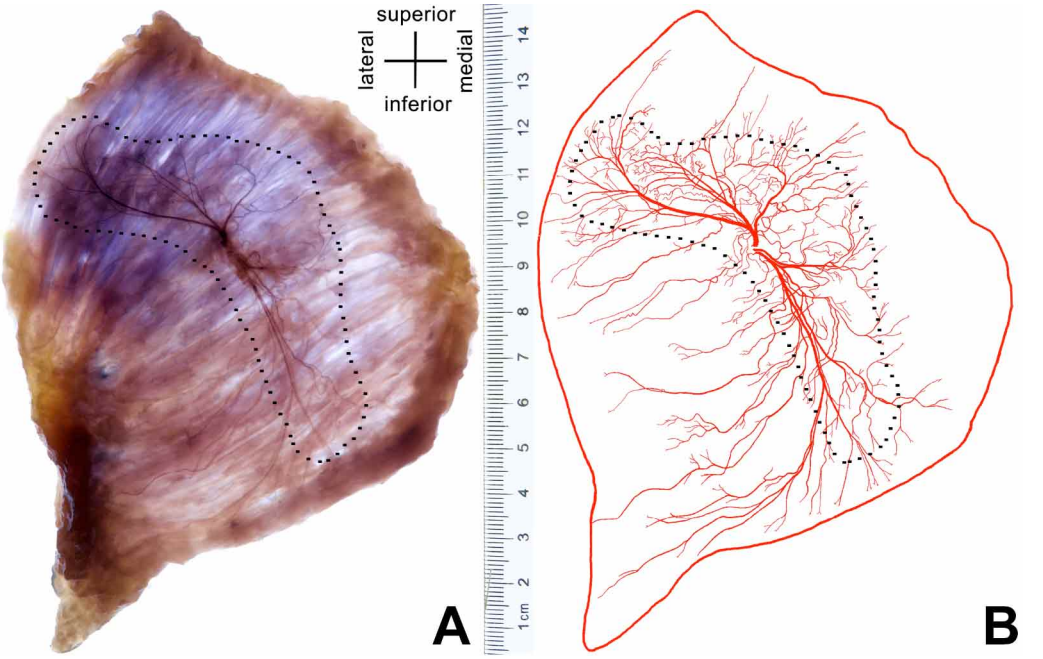

Fig. 1. Extra- and intramuscular nerve distribution pattern of the right gluteus maximus muscle (deep view). 1A: Sihler's staining; Nerve dense zone is in the black frame; 1B: Schematic drawing of 1A showing the distribution of the nerves.

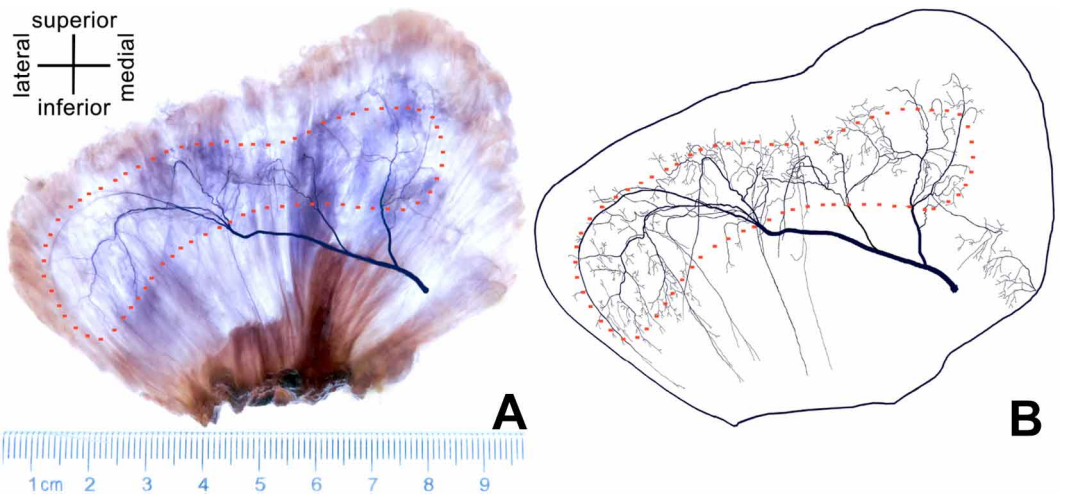

Fig. 2. Extra- and intramuscular nerve distribution pattern of the right gluteus medius muscle (deep view). 2A: Sihler's staining; Nerve dense zone is in the red frame; 2B: Schematic drawing of 2A showing the distribution of the nerves.

Sihler's staining results. There was no significant difference in the pattern of nerve distribution between adults and children, men and women, and gluteal muscles on the left and right sides.

Gluteus maximus muscle: The primary branch of the superior trunk of the inferior gluteal nerve entered the muscle and traveled from superomedial, superior, and superolateral regions to the superficial surface of the muscle. There are various numbers of branches arborizing along the way. All of these branches anastomosed in the middle of the muscular parenchyma forming an arc-shaped band of dense nerve branches, which innervated the upper half of the muscles. The primary branch of the inferior trunk of the inferior gluteal nerve projected lateral branches to each side after entering the muscle. These branches traveled as arches and sent arborized branches to form a dense zone of nerves distributed through the lower half of the muscle (Fig. 1).

Gluteus medius muscle: The primary branches of the gluteus medius nerve branch entered the muscle and traveled in asuperior, superolateral, and inferolateral direction, respectively. Among the branches, the superolateal and inferolateral primary branches often divided into secondary branches, which turned downward, and gradually reached the superficial surface of the muscle. There was obvious anastomosis between the branches of each secondary branch in the middle and upper part of the muscle, which formed an arc-shaped nerve-dense zone (Fig. 2).

Gluteus minimus muscle: The primary branches of the nerve innervating the gluteus minimus entered the muscle and travelled toward the origin and insertion of the muscle respectively, reaching the deep surface of the muscle, and the branches along the way formed "Y", "U", and "O" shaped anastomoses. An approximately arced nerve distribution zone can also be seen in the muscle (Fig. 3).

An holistic observation of the distribution of the three gluteal muscles and their nerves. Distribution of muscles: The gluteus medius and gluteus minimus were mostly covered by the gluteus maximus, except for the superolateral quadrants, where they were mostly not covered by the gluteus maximus. Additionally, the gluteus medius covered the gluteus minimus. The gluteus maximus was mainly located on the medial side of the L-line, with a small part located on the top and bottom of the H-line lateral to the L-line; while the gluteus medius and gluteus minimus were mainly located on the lateral side of the L-line and above the H-line (Fig. 4).

Distribution of nerves: The distribution of nerves in the inferomedial area of the superolateral quadrant was very dense. The nerves in this area were overlapped by the secondary nerve branches innervating the gluteus maximus and their arborized branches; some nerve trunks, primary branches, secondary branches, and arborized branches innervated the gluteus medius and the gluteus minimus.

Distribution of nerves in the inferolateral area of the superolateral quadrant is relatively dense. Those nerves in this area were overlapped by some secondary branches innervating the gluteus medius and their arborized branches; some nerve trunks, primary branches, secondary branches, and arborized branches innervated the gluteus minimus.

Distribution of nerves in the superomedial and superolateral areas of the superolateral quadrant was sparse, with less nerve distribution closer to the iliac crest. Under the iliac crest, there was an arc-shaped, nerve-sparse zone, which accounted for about two-fifths of the superolateral quadrants' limits.

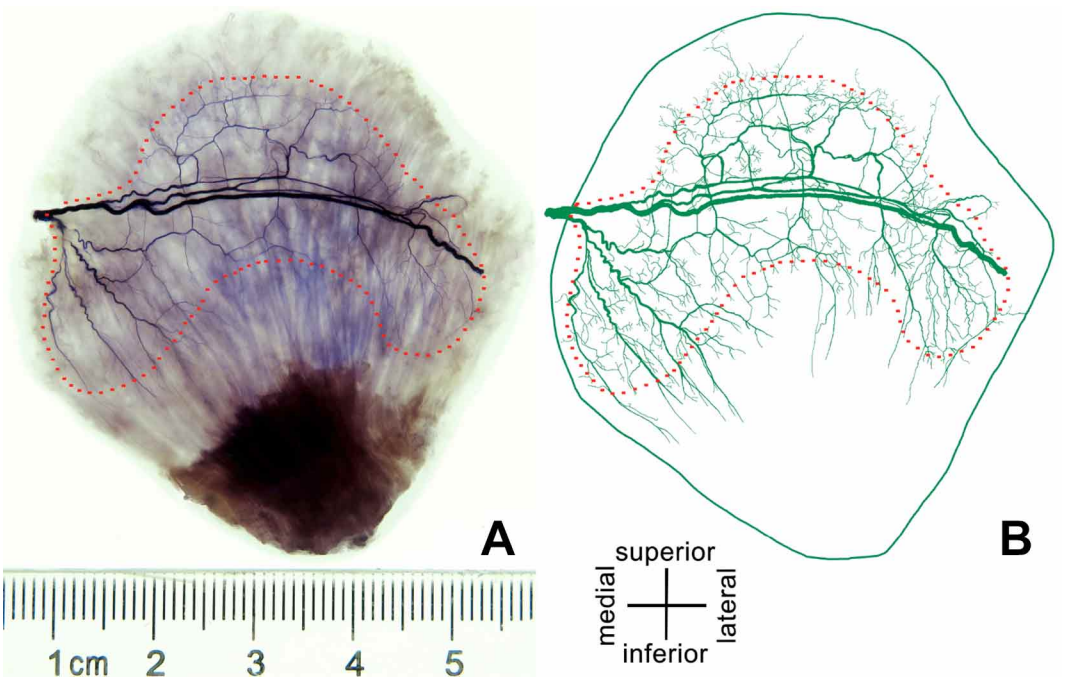

Fig. 3. Extra- and intramuscular nerve distribution pattern of the right gluteus minimus muscle (deep view). 3A: Sihler's staining; Nerve dense zone is in the red frame; 3B: Schematic drawing of 3A showing the distribution of the nerves.

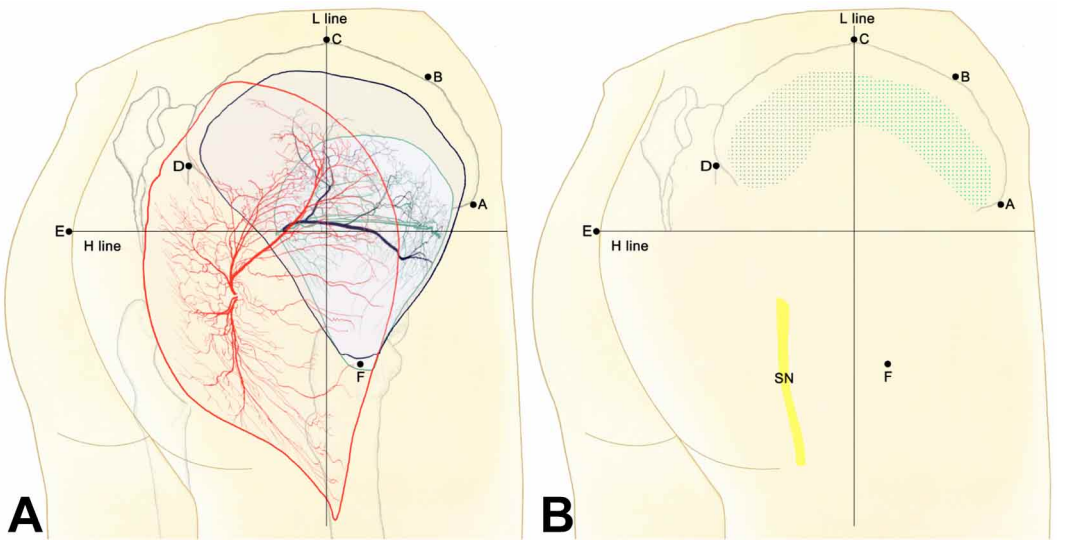

Fig. 4. Schematic diagram of the overall nerve distribution of the gluteal muscle and suitable intramuscular injection site. 4A. The whole-number nerve distribution pattern of the gluteus maximus, gluteus medius, and gluteus minimus. Red represents the range and nerve of gluteus maximus, dark blue represents the range and nerve of gluteus medius,and green represents the range and nerve of gluteus minimus. A = anterior superior iliac spine, B = iliac tubercle, C = the apex of the iliac crest, D = posterior superior iliac spine, E = the vertex of the anal cleft, F = the greater trochanter. L line = the vertical line through the apex of the iliac crest; H line = the horizontal line through the vertex of the anal cleft. 4B. The schematic diagram of the optimum area for gluteal muscle injection. SN = Sciatic nerve. Gray-green color represents the practicable site of intramuscular injection, and light green color represents the best injection site.

The distribution of nerves in the inferolateral area of the superomedial quadrant (near the center of the gluteal region) was very dense. Besides the secondary branches innervating the three muscles and the following branches, there were also some primary branches innervating the gluteus maximus, as well as some nerve trunks and primary branches that innervated the gluteus medius and minimus. The distribution of nerves in the inferomedial area of the superomedial quadrant (near the sacrum) was relatively sparse, with only a portion of the secondary and following branches in the gluteus maximus; the superomedial and superolateral areas of the superomedial quadrant lacked substantial nerve innervation, especially the two-fifths area near the iliac crest. The medial area of the inferomedial quadrant had nerve branches only in the gluteus maximus, which were distributed relatively densely. The upper part of the lateral area of the inferomedial quadrant (near the center of the gluteal region), although the three muscles overlap, had relatively sparse distribution of nerves, and the lower part of the lateral area had only a few nerve branches in the gluteus maximus. The superomedial part of the inferolateral quadrant (near the center of the gluteal region) contained a fewsmall branches in the three gluteal muscles; the nerves in the superolateral part consisted of a large number of secondary branches and the following branches innervated the gluteus medius and minimus (Fig. 4).

DISCUSSION

Revealing the distribution of extramuscular and intramuscular nerve distribution patterns in the gluteus. Gluteal muscle injections are given more than 12 billion times a year world-wide (Alves *et al*.). Approximately 20 % of injection-related peripheral nerve injuries occur in the buttocks (Yeremeyeva *et al*., 2009). However, there are more concerns and reports of injection-related sciatic nerve injuries (Brown *et al*.; Kaya *et al*.; Geyik *et al*.). In fact, any injection can result in neurotoxicity , even if 0.1 ml of saline is injected into the nerve, significant axonal degeneration can result (Barry *et al*.; Emir *et al*., 2016). Direct injection of the nerve may prevent injection of the drug due to the immediate onset of symptoms. However, chronic neurological damage due to drug infusion may not be detected (Jung Kim & Hyun Park, 2014; Emir *et al*.) Therefore, to avoid complications caused by gluteal muscle injection, it is important to reveal the nerve distribution pattern of the above three muscles for the selection of a reasonable site for gluteal muscle injection in the clinic.

Analysis of the intramuscular injection site of the gluteal in the past definition and application. There are many methods to define the muscle injection site of the buttocks.

In the dorsogluteal site method, the buttocks are divided into four quadrants, and the superolateral quadrant is the injection site, defined as: from the highest point of the ilium crest to the gluteal sulcus as vertical line, through the midpoint of the vertical line as a horizontal line (Mishra & Stringer). The injectable range of the superolateral quadrant is large, which avoids the thick sciatica and the inferior gluteal nerve trunk of the inferomedial quadrant and is the most commonly used method in clinical practice at present. According to the study of Brown *et al*., if the injection is too low and too inward, the superior gluteal nerve and blood vessels distributed in the lower region of the superolateral quadrant, as well as the sciatic nerve in the inferomedial quadrant, will be damaged by varying degrees (Geyik *et al*.). Hesby suggested that to correctly evaluate the accurate location marks and boundaries of the target injection area, the injection site must be completely exposed (Nicoll & Hesby, 2002). In this way, the cross method is more conducive to the protection of privacy and easy injection. To avoid nerves and blood vessels, Von Hochstetter proposed the ventrogluteal site method, used as follows: the practitioner places a palm on the patient's great trochanter, points the index finger to the anterior superior iliac spine and the middle finger to the iliac crest to form a v-shaped area, where no thick nerves or blood vessels pass through the interposition, which is the injection site (Zelman, 1961). Some scholars think that this method is superior to the dorsogluteal site method (Arslan & Özden), but others think that this method is too complicated, with an increased risk of pricking the user's finger during an injection (Wynaden *et al*., 2006). de Meneses & Marques (2007) argued that the ventrogluteal method could not always maintain a proportional relationship between the size of the practitioner’s hand and the patient's gluteus, which would result in inaccurate injection sites. They proposed the center of gravity of a triangle formed by connecting three points of the greater trochanter of the femur, the anterior superior iliac spine, and the posterior margin of the iliac tubercle as the injection point. Recently, researchers compared the blood vessels and nerves in the v-shaped and triangular areas mentioned above through ultrasound examination, and more supported de Meneses' method (Coskun *et al*.). Clark´s Method also recommended the point that is defined as the anterior one-third between theanterior and posterior superior iliac spine should be the injection point (Nakajima *et al*.). However, the injection site of the latter three methods is only one injection point, and repeated injection to the same site will increase the risk of nerve, vascular, and muscle injury, which is not suitable for patients requiring repeated injections (Small).

Recommendation of the best location of gluteal intramuscular injection based on the distribution of extra and intramuscular nerves. The above-mentioned injection site is defined mainly by the consideration of extramuscular blood vessels and nerve distribution, while the distribution of intramuscular nerves has not been considered. In fact, for blood vessels, if the injector pulls back the piston for 5 to 10 seconds, it can be determined whether the syringe contains blood (Mraz *et al*. 2018). However, nerve injury can occur from injection even if the technique is correct. Coskun *et al*. believe that this may be related to small nerve injury. Injection injury of the superior gluteal nerve is less common than the sciatic nerve, but simple injury of the superior gluteal nerve has been reported for a long time. Injection injuries often cause atrophy of the gluteus medius, gluteus minimus, and tensor fascia lata, persistent and painful contracture of gluteus medius, Trendelenburg gait (faltering gait), among others (Obach *et al*., 1983). These complications may be due to injury of the intramuscular tiny nerves. The results of this study show that the three muscles in the inferomedial area of the superolateral quadrant of the buttocks are arranged in an overlapping manner, and the nerve distribution is very dense (Fig. 4). If the medication is injected into or near this area, the medication will cause neurotoxic damage, which may be the cause of the above-mentioned complications.

According to our results, the arc-shaped, nerve-lacking zone in the superolateral and superomedial quadrant near the lower iliac crest, which accounts for about two-fifths of the two quadrants’ limits, can be recommended as a gluteal intramuscular injection area free from nerve injury. However, taking into consideration injection convenience and privacy protection, the development of the gluteus medius is better than the gluteus maximus for those who have not learned to walk, and for thin people (Kaya *et al*.; Yapucu Günes¸ *et al*., 2016). We prefer to recommend the arc-shaped, nerve-lacking zone in the superolateral quadrant as the best injection site. The methods of Von Hochstetter and de Meneses are complex and have few clinical applications. Additionally, complications such as gluteal muscle atrophy and tensor fascia lata paralysis have been reported (Obach *et al*.; Müller-Vahl, 1985). As can be seen from Figure 4, the distribution of extra- and intramuscular nerves is quite dense in the injection area defined by Von Hochstetter and de Meneses, and the probability of nerve injury during medication injection is great. Considering Clark's method, the distribution of intra- and extramuscular nerves at the injection point is also dense, and there is a risk of neurotoxic injury. The gluteus maximus and gluteus medius are overlapping in the arc-shaped, nerve-lacking zone of the superomedial quadrants, which can be used for injection of large doses of medication.

Overall, this study clearly and holistically demonstrated the pattern of nerve distribution associated with gluteal muscle injection. In particular, the distribution pattern

of intramuscular nerves in the gluteus maximus muscle, with larger and thicker muscle mass, was successfully revealed, which provided further guidance for the selection of the site of gluteal muscle injection that is free from nerve injury. However, no variant individuals were found in the study, perhaps due to the small number of study specimens. Despite this, the authors hope that the results will be translational and applicable, to reduce the incidence of complications of gluteal muscle injection.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 31660294, 31540031) and the Innovation Project for University Students of Zunyi Medical University (No. ZY2016-3702).

WU, J.; CAI, Y.; CAO, A.; BI, Y.; HU, X. & YANG, S. Recomendación del mejor sitio para la inyección del músculo glúteo basado en el patrón de distribución de los nervios extramusculares e intramusculares. *Int. J. Morphol., 38(4)*:975-982, 2020.

RESUMEN: El objetivo de este trabajo fue revelar los patrones de distribución nerviosa extramusculat e intramuscular de los músculos glúteo máximo, medio y mínimo y proporcionar orientación para la inyección en la región glútea con el propósito de evitar lesiones nerviosas. Se utilizaron diez cadáveres adultos y diez niños. Los nervios glúteos superior e inferior que inervan a los músculos glúteo máximo, medio y mínimo fueron disecados, expuestos y suturados *in situ* en el músculo. Se extirparon los tres músculos glúteos y se revelaron los patrones de distribución de los nervios intramusculares mediante la tinción nerviosa de Sihler modificada. El patrón de distribución nerviosa se devolvió a la posición correspondiente en el cuerpo y se analizaron los patrones en los cuatro cuadrantes de la región glútea. Se encontraron 3 a 12 ramos nerviosos extramusculares de los músculos glúteo máximo, medio y mínimo. Después de ingresar al músculo, estas ramas nerviosas se arborizaron y anastomizaron para formar una zona densamente nerviosa en forma de arco. La distribución nerviosa fue de mayor densidad en la región inferomedial del cuadrante superolateral y en la región inferolateral del cuadrante superomedial de la región glútea. La distribución nerviosa era relativamente densa en la región inferolateral del cuadrante superolateral y en la región medial del cuadrante inferomedial. Una zona en forma de arco en los cuadrantes superolateral y superomedial y con escasa inervación, cerca de la cresta ilíaca representaba una parte de los límites de los dos cuadrantes. La zona de poca inervación en forma de arco en el cuadrante superolateral es el sitio de inyección preferido, y el cuadrante superomedial próximo a la cresta ilíaca también se recomienda como una región de inyección intramuscular glútea, libre de lesión nerviosa.

PALABRAS CLAVE: Inyección del músculo glúteo; Mancha de Sihler; Patrón de distribución nerviosa; Lesión del nervio de inyección; Sitio de inyección intramuscular.

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Corresponding author:
Shengbo Yang
Department of Anatomy
Zunyi Medical University
6 West Xufu Road
Xinpu Developing Zones
Zunyi 563099
CHINA

E-mail: yangshengbo8205486@163.com

Received: 16-02-2020
Accepted: 25-03-2020