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Research Article - Human Anatomy Case Report

Aberrant innervation of the lateral abdominal muscles by direct branch of L4 nerve

Cameron Schmidt[1], Vlad Voin[1,*], Joe Iwanaga[1], Marios Loukas[2], Rod J. Oskouian[3], R. Shane Tubbs[1]

[1] Seattle Science Foundation, Seattle, WA
[2] St. George's University School of Medicine, Grenada, West Indies
[3] Swedish Neuroscience Institute, Seattle, WA

Abstract

Surgical approache through the posterior abdominal wall used for nephrectomy or other access to contents of the retroperitoneal space requires to be cognizant of the regional nerve supply to the posterolateral abdominal wall muscles. We herein report a, to our knowledge, previously undescribed direct branch from the L4 spinal nerve that formed a plexus with regional nerves to then innervate the lateral abdominal wall musculature. Such a nerve variant should be considered by the surgeon who operates this region.

Key words

Anatomy, posterior abdominal wall, lumbar plexus, oblique muscles.

Introduction

Muscles of the abdominal wall serve a number of roles, including flexion and rotation of the trunk, abdominal protection, and forced expiration (Urquhart et al., 2005). The lateral abdominal wall is composed of three paired muscles: the external oblique (EO), internal oblique (IO), and transversus abdominis (TrA). These three muscle overlie one another in the lateral abdomen and become aponeurotic medially (Hebbard et al., 2010).

These muscles traditionally receive innervation from spinal nerves T6 – L1 (Yang et al., 2003; Rozen et al., 2008; Hebbard et al., 2010). We report a cadaveric dissection where L4 was found to be innervating muscles of the anterolateral abdomen. We believe this variation to be previously unreported in the literature.

Case Report

During routine dissection of the posterior abdominal wall via a posterior approach, a large, direct branch of the L4 spinal nerve was found to travel laterally and innervate the lateral abdominal wall muscles (Figs. 1 and 2). The specimen was from an 84-year-old male died from heart failure. The branch had a horizontal course and distally joined into a neural plexus formed by T12-L2 spinal nerves. A lateral branch from L3 was not observed. The branch from L4 was approximately 5 cm in length and 1.2

* Corresponding author. E-mail: vvoin@outlook.com

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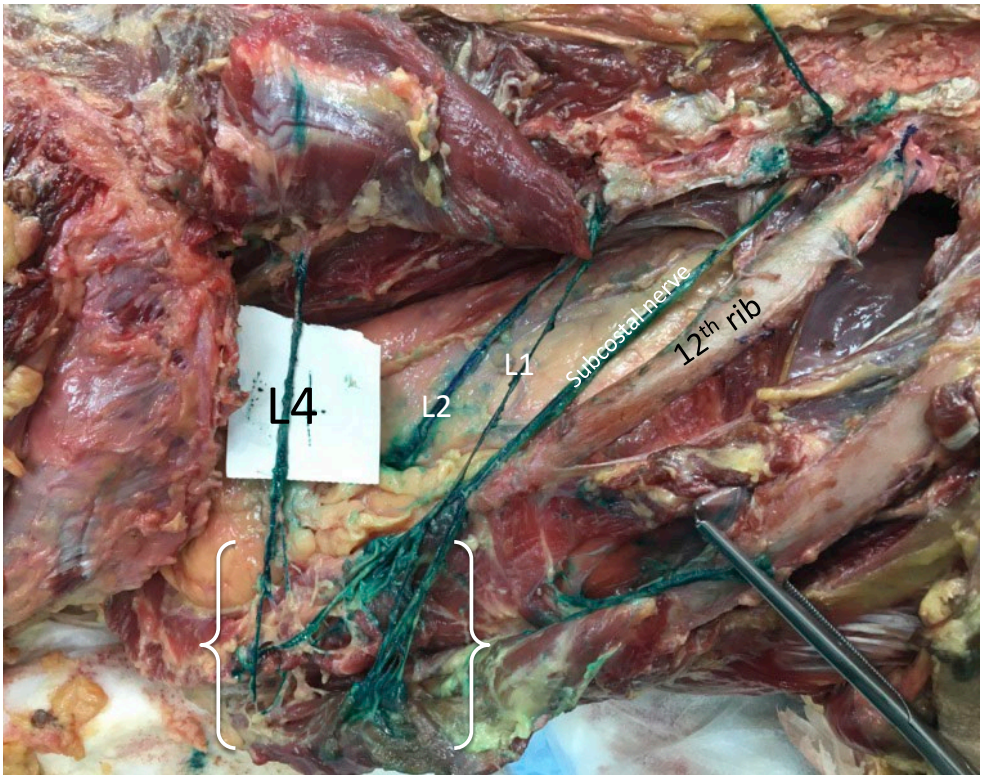

Figure 1. Posterior dissection of the right lumbar region. The subcostal nerve is seen just inferior to the 12th rib. L1 and L2 are seen coursing over the dissector tool. The L4 branch is seen at the arrow.

mm in diameter. The plexus created by it and contributions from T12-L2 formed just distal to the tip of the rib and provided branches to the EO, IO, and TrA muscles. The L4 branch did not innervate any other muscle or structure in this region. No other grossly visible anatomical variations were identified in this specimen. Particularly, no direct L4 branch to the lateral abdominal wall muscles was found on the left side.

Discussion

Strongest and most superficial, the EO arises from attachments at the lower margins of ribs 4 through 12 travelling in an infero-anterior direction (Yang et al., 2003; Urquhart et al., 2005). Upper and middle fibers, interdigitating with the serratus anterior, terminate in the anterior aponeurosis, while lower fibers, interdigitating with the latissimus dorsi muscle, attach at the iliac crest (Yang et al., 2003). A smaller and thinner muscle, the IO, lies deep to the EO, arising from the iliopectineal arch (Yang et al., 2003). Above the iliac crest, fascicles of the IO are oriented superomedially, while taking on a horizontal orientation below the iliac crest. Posterior fibers of the IO travel in

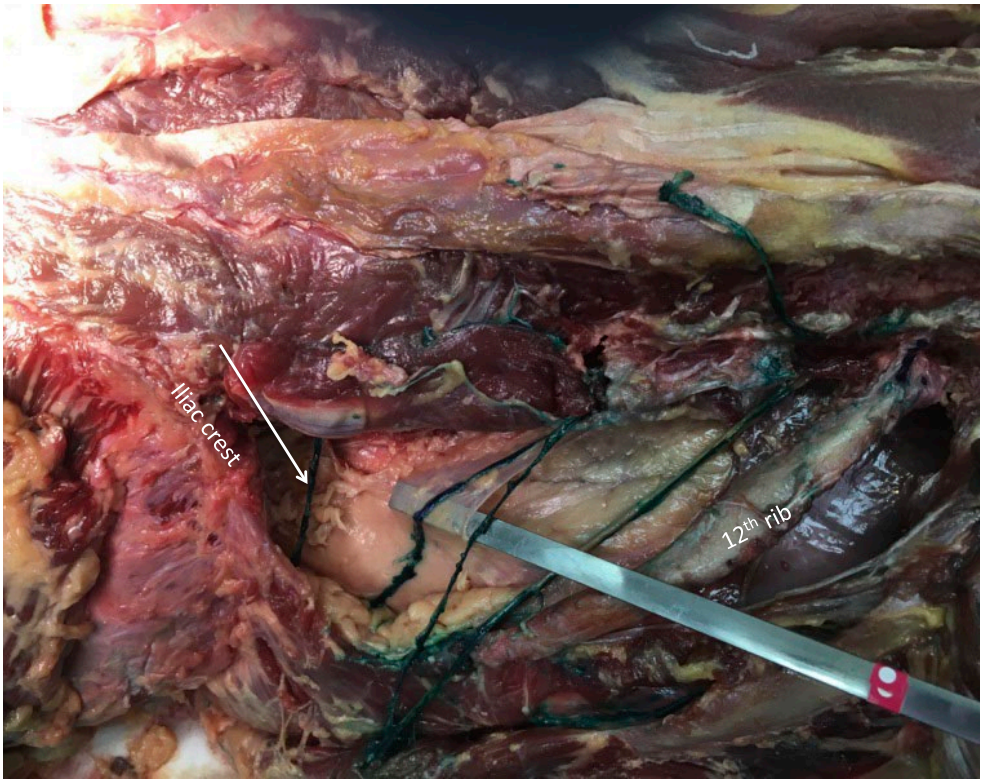

Figure 2. Closer view of Figure 1. Note the nerve plexus (brackets) formed between T12-L2 and the L4 nerve contributions.

a superior-anterior direction to insert on the lower 3 or 4 ribs. Still other fibers travel in an anterior direction becoming aponeurotic medially (Yang et al., 2003). The deepest and thinnest of the anterolateral abdominal muscles is the TrA.

The lateral abdominal wall receives segmental innervation from ventral rami of the lower six thoracic spinal nerves, including the intercostal nerves (T7-11) and subcostal nerve (T12), as well as the iliohypogastric and ilioinguinal nerves, derived from the ventral rami of L1 (Duchateau et al., 1988; Yang et al., 2003; Rozen et al., 2008). T7-L1 all run together with their associated blood vessels in a tissue plane between the IO and TrA, the transversus abdominis plane (TAP) (Davies et al., 1932; Rozen et al., 2008; Jankovic et al., 2009; Hebbard et al., 2010; Sviggum et al., 2012). T6-9 enter the TAP between the anterior axillary line and the midline, progressing anteriorly (Rozen et al., 2008). Sensory nerves branch laterally out of the plane in cutaneous terminal branches (Sviggum et al.. 2012). Within the lateral abdominal wall, each muscle and skin segment is innervated by at least two spinal nerves (Davies et al., 1932).

Any surgeon operating in this abdominal region should be aware of this possible variation. The TAP is of clinical relevance, given its importance in abdominal anesthetic procedures. The posterior TAP block involves the injection of local anesthetic

into the TAP in the lateral abdominal wall (Rozen et al., 2008; Jankovic et al., 2009; Hebbard et al., 2010). The TAP block represents one of a number of abdominal trunk blocks, including the ilioinguinal-iliopypogastric and rectus sheath block, used for pain control after abdominal surgery (Sviggum et al., 2012). In addition, the neuroanatomy of this region holds importance in abdominal wall flaps (Yang et al. 2003; Rozen et al. 2008). Oblique muscle flaps are used for a number of purposes, but frequently play a role in reconstructive surgery of the breast (Tansatit et al. 2006). The identification of all intramuscular neurovasculature is important in avoiding damage, which can lead to denervation, with additional complications including herniation, abdominal wall weakness, and abdominal bulges.

In conclusion, in the present paper we report what is believed to be the first case of lateral abdominal wall innervation by L4. As the quadratus lumborum muscle is also innervated by L4, such a variant innervation of the oblique muscles might suggest a common embryological muscle origin.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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Review - Basic and Applied Anatomy

Aortic arch branching pattern variation: its incidence on a 20030 cases review

Caryn Recto, Maria Boddi, Jacopo Junio Valerio Branca, Gabriele Morucci, Alessandra Pacini, Massimo Gulisano, Ferdinando Paternostro*

Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Abstract

Variations in the branching pattern of the aortic arch are clinically relevant because of the direct influence that their presence can have on the success of cardio-vascular procedures, neck or thorax surgery, trauma management or intensive care. In most cases these anatomical variations are asymptomatic and considered clinically benign, but some particular aortic branching patterns have been associated with surgical complications or with vascular diseases in non-surgical patients. The main objective of this work was to study the frequency of variation of the aortic arch branching pattern in a wide and varied population on the basis of literature reports. The aortic arch branching pattern of 20,030 cases reported by 40 anatomical or radiological studies were analyzed. 84,52% of the studied population had a three branches pattern and 14,65% had a two branches pattern. The four primary arteries were seen arising directly from the aortic arch in 0,81% of the cases and only 0,02% had them all arising from a common trunk.

Key words

Aortic arch, branching pattern, aortic variations, bovine arch.

Key to abbreviations

BCT: brachiocephalic trunk
LBT: left brachiocephalic trunk
LCCA: left common carotid artery
LECA: left external carotid artery
LICA: left internal carotid artery
LSA: left subclavian artery
LVA: left vertebral artery
RBT: right brachiocephalic trunk
RCCA: right common carotid artery
RSA: right subclavian artery
RVA: right vertebral artery
Tima: thyroid ima artery

* Corresponding author. E-mail: ferdinando.paternostro@unifi.it

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Introduction

Several anatomical variations of the aortic arch related to the different origin of carotid, subclavian, vertebral and thyroid arteries are known. They have been variously described by autoptic studies or by second-level imaging exams. In most cases these anatomical variations are asymptomatic, but some patterns of aortic arch branching have been associated with a broad spectrum of pathologies, such as peripheral and/or central nervous system vascular diseases or aortic aneurysms dissection (Lu et al., 2015; Gudbrandsson et al., 2016; Maiti et al., 2016; Shang et al., 2016).

The embolic spread of atherosclerotic plaques is a possible complication during aortic arch interventions, leading to a higher impairment of brain perfusion if both common carotid arteries arise from an unique trunk (Herrera et al., 2013; Cordova et al., 2011).

An atypical origin of the left vertebral artery (LVA) can be wrongly described by radiologists, predisposing to surgical or endovascular complications (Huapaya et al., 2015; Hu et al., 2009) or to excessive/unnecessary treatments if erroneously considered obstructed (Goray et al., 2005).

When dysphagia cannot be explained by usual cause, dedicated exams should be performed to search for a right subclavian artery (RSA) arising from the left side of the aortic arch. This variation, usually asymptomatic, can sometimes cause dysphagia, especially when it develops an aneurismatic dilatation. Some authors also suggest that intensive care patients should be screened for an aberrant RSA before nasogastric tube long term placement due the extreme unfavorable prognosis in case of the development of an esophageal fistula (Fazan et al., 2003). Others suggest that if an aberrant RSA is diagnosed during aortic arch repair, corrective surgery should be considered to prevent complications and further disease (Fazan et al., 2003, Feugier et al., 2003; Inzunza and Burdiles, 2010). An aberrant RSA also determines a non-recurrent right laryngeal nerve, which translates into a higher risk of nerve's injury during otorhinolaryngeal and endocrinological surgery of the neck (Inzunza and Burdiles, 2010).

The reduced number of fixation points found in some arch patterns determinates a concentration of energy in the arising point of the artery during blunt trauma, leading to arterial dissection or transversal section (Dumfarth et al., 2015). Patients with these anatomical variations might need particular attention in the immediate follow-up, mainly those in which both carotid arteries share a common trunk, where an hyperextension mechanisms of trauma could lead to a complete compromise in brain perfusion (Cordova et al., 2011).

Because of all these reasons, it is highly advisable to know and consider the possible anatomical variations of the aortic arch in clinical practice. The correct recognition of the main anatomical variations could have direct positive effects on endovascular treatments and in diagnostic or therapeutic procedures.

Materials and methods

The key words "aortic branching pattern", "aortic anatomical variations" and "aortic variations" were introduced in search engines such as PubMed, SciELO and EMBASE. They were also used directly in several anatomical and medical journals such as Scholar Science Journals (www.ssjournals.com), Romanian Journal Of Mor-

phology and Embryology (www.rjme.ro), African Journals Online (www.ajol.info), Hindawi Publishing Corporation (www.hindawi.com), International Journals in Medical and Health Reseach (www.ijmhr.org), Asian Pacific Journal of Health Sciences (www.apjhs.com), Firenze University Press (www.fupress.net), International Journal of Experimental and Clinical Anatomy (www.anatomy.org.tr), American Journal of Roentgenology (www.ajronline.org), Via Medica Journals (www.journals.viamedica.pl), Impact Journals (www.impactjournals.us), Revista Argentina de Anatomía Clínica (www.anatclinar.com.ar).

The selected articles presented an accurate anatomical description of the branching pattern, obtained either by dissection or imaging studies performed in an adult healthy population. As some of these studies lacked a clear anatomical description, the authors were contacted to provide further anatomical details.

Exclusion criteria were case-report studies, studies with less than 20 cases descriptions, and studies for which the anatomical description was unavailable or incomplete.

We found 20,081 cases of aortic arch branching variations reported by 39 cohort studies and one case-control study.

Fifty-one cases out of 20.081 were excluded because they described either anatomical anomalies of the arch (i.e.: double or right sided aortic arch, aortic coarctation) or previously known congenital heart disease. Finally, 20.030 cases were included for the statistical analysis.

Results

If we just consider the four primary arteries - *i.e.* left common carotid artery (LCCA), right common carotid artery (RCCA), right subclavian artery (RSA) and left subclavian artery (LSA) - 84.52% of the population has a 3 branch pattern and 14.65% has a two branch pattern, independently of the order in which they arise, the combination of the primary arteries or the presence of secondary arteries (Table 1).

In a minority of the cases (0.81%) the four primary arteries could be seen originating independently from the arch, whereas in a 0.02% of the analyzed cases they will all arise from a common trunk.

The prevalence of the normal anatomical disposition in our review resulted to be 80.0%. In the literature the prevalence of this condition varies in a wide range, going from 63.5% (Budhiraja et al., 2013; Tapaia et al., 2015) to 97.4% (Sunitha and Narasinga Rao, 2012).

Table 1. Number of primary arteries arising directly from the aortic arch

Number of primary arteries	Number of cases	Percentage
1	3	0.02
2	2935	14.65
3	16929	84.52
4	163	0.81
Total	20030	100.00

Table 2. Totality of the described patterns and its prevalence.

	N	%
One branch patterns		
1: BCT + LCCA + LSA	2	0.010
1: BCT + LCCA + LSA 2: LVA	1	0.005
Two branches patterns	N	%
1: BCT + LCCA 2: LSA*	2025	10.100
1: BCT + LCCA 2: LVA 3: LSA	42	0.210
1: BCT + LCCA 2: LSA 3: LVA	5	0.030
1: BCT + LCCA 2: LVA + LSA	1	0.005
1: BCT + LCCA 2: LSA 3: RVA	1	0.005
1: BCT + LCCA 2: Suprascapular artery 3: LSA	1	0.005
1: oc BCT + LCCA 2: LSA	764	3.810
1: oc BCT + LCCA 2: LVA 3: LSA	28	0.140
1: BCT + LICA 2: LECA 3: LSA	1	0.005
1: BCT 2: LBCT	63	0.310
1: BCT 2: LBCT 3: left coronary artery	1	0.005
1: BCT 2: Tima 2: LBCT	1	0.005
1: RCCA + LCCA 2: RSA + LSA	2	0.010
Three branches patterns	N	%
1: BCT 2: LCCA 3: LSA**	16023	80.000
1: BCT 2: LCCA 3: LVA 4: LSA	752	3.750
1: BCT 2: LCCA 3: LSA 4: LVA	13	0.070
1: BCT 2: LCCA 3: oc LVA + LSA	3	0.020
1: BCT 2: LCCA 3: LSA 4: RVA	3	0.020
1: BCT 2: RVA 3: LCCA 4: LSA	2	0.010
1: BCT 2: Tima 3: LCCA 4: LSA	38	0.190
1 : BCT 2 : RCCA 3 : double LVA 4 : LSA	1	0.005
1: oc RSA + RCCA 2: LCCA 3: LSA	3	0.020
1: RSA 2: RCCA + LCCA 3: LSA	13	0.070
1: LCCA + RCCA 2: LSA 3: RSA	68	0.340
1: RCCA + LCCA 2: LVA 3: LSA 4: RSA	2	0.010
1: LSA 2: BCT 3: LCCA	3	0.020
1: BCT 2: LSA 3: LCCA	3	0.020
1: RCCA 2: LICA 3: LECA 4: LSA 5: RSA	1	0.005
1 : RSA 2 : RCCA 3 : LBT	1	0.005

The classically known "bovine arch" refers to a common origin of the right brachiocephalic trunk (RBT) and the LCCA. In our review, the bovine arch was described in

	N	%
Four branches patterns	N	%
1: RSA 2: RCCA 3: LCCA 4: LSA	43	0.210
1: RSA 2: RCCA 3: LCCA 4: LVA 5: LSA	8	0.040
1: RCCA 2: LCCA 3: LSA 4: RSA	108	0.540
1: RCCA 2: LCCA 3: LVA 4: LSA 5: RSA	3	0.020
1: RCCA 2: LCCA 3: LVA 4: Tima 5: LSA 6: RSA	1	0.005
Total	20030	100,000

* bovine arch, ** normal anatomy.
oc: ostium. RCCA: right common carotid artery. LCCA: left common carotid artery. LVA: left vertebral artery. RVA: right vertebral artery. LSA: left subclavian artery. RSA: right subclavian artery. LICA: left internal carotid artery. LECA: left external carotid artery. Tima: thyroid ima artery. BCT: brachiocephalic trunk. LBT: left brachiocephalic trunk (LCCA + LSA).

10.1% of the cases, being the variation most frequently found. Other studies reported its prevalence in a range from 0% (Davivongos and Sangiampong, 1986; Alsaif and Ramadan, 2010; Bhattarai and Poundel, 2010; Demertzis et al., 2010; Inzunza and Burdiles, 2010; Ogeng'o et al., 2010; da Silva, 2012; Fazal et al., 2012; Yuksekkaya et al., 2012; Budhiraja et al., 2013; Acar et al., 2013; Herrera et a., 2013; Rekha and Senthilkumar 2013; Durai Pandian et al., 2014; Karacan et al., 2014; Lale et al., 2014; Maheria et al., 2014; Makhanya et al., 2004; Bhatia et al., 2015; Ergun et al., 2015; Nurefşan et al., 2015; Tapaia et al., 2015) up to 33.3% (Ergun et al., 2015). Table 2 to show the patterns described and its prevalence.

The presence of a RSA as the last branch emerging from the arch, also known as arteria lusoria, was described in 0.91% of the reviewed cases. In 96.2% of these cases the aberrant RSA was the sole anatomical variation, while in 3.8% the lusoria artery was accompanied by secondary arteries arising directly from the arch.

In the literature, an aberrant RSA was described in a range that goes from 0% (Davivongos and Sangiampong, 1986; Zamir and Sinclair, 1990; Fazan et al., 2003; Natsis et al., 2009; Alsaif and Ramadan, 2010; Bhattarai and Poundel, 2010; Demertzis et al., 2010; Indumathi et al., 2010; Inzunza and Burdiles, 2010; Ogeng'o et al., 2010; Fazal et al., 2012; Mata-Escolano et al., 2012; Patil et al., 2012; Budhiraja et al., 2013; Herrera et a., 2013; Rekha and Senthilkumar 2013; Shakeri et al., 2013; Vučurević et al., 2013; Maheria et al., 2014; Rea et al., 2014; Ajit and Amarnath 2015; Bhatia et al., 2015; Ergun et al., 2015; Tapaia et al., 2015; Jalali et al., 2016) to 2.25% (Nurefşan et al., 2015). Table 3 shows the aberrant RSA prevalence.

The LVA was the secondary artery most frequently found arising directly from the aortic arch. While in the present review it was found in 4.3% of the cases, its prevalence in the literature varies from 0% (Makhanya et al., 2004; Fazal et al., 2012) to 15.4% (Budhiraja et al., 2013).

The thyroid ima artery (Tima) was the second most frequent secondary artery, representing 0.20% of the cases in the present work. In the literature its prevalence ranges from 0% (Davivongos and Sangiampong, 1986; Zamir and Sinclair, 1990; Fazan et al., 2003; Makhanya et al., 2004; Nayak et al., 2006; Il-Young et al., 2008;

Table 3. Aberrant RSA prevalence.

	Number of cases	Percentage
Aberrant RSA as the only variation	176	0.88
Aberrant RSA + secondary arteries	7	0.03
Total number of cases with an aberrant RSA	183	0.91

Table 4. Secondary arteries prevalence.

Secondary artery	Total number of cases	Percentage
LVA	860	4.300
TIma	40	0.200
RVA	6	0.030
LICA/LECA	2	0.010
Left coronary	1	0. 005
Suprascapular	1	0. 005
Total	910	4.500

***** one patient presented more than one secondary artery arising directly from the AA (LVA+TIma) and was considered in each one of the single categories.

Berko et al., 2009; Alsaif and Ramadan, 2010; Bhattarai and Poundel, 2010; Demertzis et al., 2010; Indumathi et al., 2010; Inzunza and Burdiles, 2010; Ogeng'o et al., 2010; da Silva, 2012; Mata-Escolano et al., 2012; Patil et al., 2012; Sunitha and Narasinga Rao, 2012; Yuksekkaya et al., 2012; Acar et al., 2013; Budhiraja et al., 2013; Ergun et al., 2013; Herrera et a., 2013; Rekha and Senthilkumar 2013; Lale et al., 2014; Maheria et al., 2014; Rea et al., 2014; Ajit and Amarnath 2015; Bhatia et al., 2015; Shakeri et al., 2013; Ergun et al., 2015; Nurefşan et al., 2015; Tapaia et al., 2015) up to 2.2% (Vučurević et al., 2013).

The rest of the secondary arteries, when not isolated cases, were very infrequently found, as seen in Table 4.

Discussion

Over the last years growing evidence was collected that anatomical variations of aortic arch branching may be associated with a broad spectrum of pathologies, such as peripheral and/or central nervous system vascular diseases or aortic aneurysms dissection.

Even if variations of aortic arch branching are commonly asymptomatic and considered clinically benign, in recent years some patterns (like that classically known as "bovine arch" or variants in which the LVA or an aberrant RSA originate directly from the arch) have been linked to a higher rate of thoracic aortic disease when

compared to general population. A role as potential anatomic biomarkers or as a risk factor for future development of thoracic aortic disease has been suggested by some authors (Dumfarth et al., 2015).

The knowledge of the exact morphology of anatomical organization of aortic arch branching in each subject should be an essential information to be acquired for the correct planning of surgical interventions (Ried et al., 2015; Spear et al., 2016;Yang et al., 2016), being relevant not only for vascular surgeons but also for general and thoracic ones (Thors et al., 2014; Ried et al., 2015; Jalali Kondori et al., 2016), clinical physicians (Fujita et al., 2015; Lu et al., 2015) and radiologists (Jalali Kondori et al., 2016; Maiti et al., 2016; Wilbring et al., 2016).

The aortic arch branching pattern influences not only the technical procedure *per se* but also reconstruction, catheterization phases and brain perfusion strategies. Indeed, patients with anatomical variations are reported to have higher rates of direct interventions on aortic arch (Dumfarth et al., 2015) and increased neurological complications during simple procedures such as carotid stenting, due to additional technical difficulties (Faggioli et al., 2007).

An incidental injury of an unrecognized LVA arising from the aortic arch during surgery or endovascular procedures can lead to hemorrhagic or permanent neurologic complications (Hu et al., 2009; Huapaya et al., 2015).

Anatomical variations can also represent an obstacle in radio-diagnostics, especially when imaging techniques are applied in adverse situations such as emergencies and trauma (Wilbring et al., 2016). In other cases, an artery might be erroneously considered obstructed during radiological studies either because it eludes catheterization or because it is not found in the normally expected area, leading to diagnostic mistakes and eventually unnecessary or excessive treatments.

The aberrant RSA, independently of the number or type of the other branches, was present in 0,91% of the cases. These numbers should be evoked before long term nasogastric tube placement and during aortic arch repair surgery, otorhinolaryngological and endocrinological neck's surgery or study of dysphagic patients.

Emergency room physicians and trauma surgeons should consider the presence of two branches patterns in trauma patients who show unexpected neurological clinical signs and evolution due to a complete brain perfusion hampering (Feugier et al., 2003; Cordova et al., 2011).

Anatomical variations of aortic arch branching may also alter data interpretation during hemodynamic procedures because pattern of blood flow, pressure waves and velocity profiles are highly influenced by and correlated with the vascular morphology. If the anatomical disposition of vessels is not taken into account, measures can be misinterpreted and cause wrong diagnosis (Babu and Sharma, 2015; Flores et al., 2016).

Conclusions

This work includes, as far as we know, the highest number of cases and the widest ethnical representation on the addressed issue. Its results should be taken into account in the different situations described.

The normal anatomy still remains the most frequent pattern. The two branches patterns are less frequent, but they're found in almost 15% of the population. Four

branches patterns were found in almost 1% of the evaluated cases, while patterns in which all the primary arteries arose from a common trunk are almost anecdotal.

Variants in which the LVA or an aberrant RSA originate directly from the aortic arch have low prevalence but must be known because of the clinical relevance of its potential complications (Shakeri et al., 2013; Rea et al., 2014).

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Research Article - Human Anatomy Case Report

Unilateral absence of Casserio's nerve and a communicating branch to the median nerve. An additional variant of brachial flexors motor innervation

Francesca A. Pedrini, Giulia A. Mariani, Ester Orsini, Marilisa Quaranta, Stefano Ratti, Lucio Cocco, Lucia Manzoli*, Anna Maria Billi

University of Bologna, Department of Biomedical and Neuromotor Sciences, Division of Anatomy

Abstract

Anomalies of the brachial plexus including the distribution of the nerves as well as its terminal branches in the upper limb have been largely described in the literature. In this case report we describe a further variant of brachial plexus formation identified during routine anatomical dissection of the right upper limb of a 62-year-old Caucasian female cadaver. On the right side no musculocutaneous nerve was identified, the median nerve was formed as expected but a short extra branch communicating between the lateral cord and the medial head of the median nerve appeared. Coracobrachialis muscle was innervated by a direct branch from the lateral cord, while biceps brachialis and brachialis muscles were reached by collaterals of the median nerve. Moreover, in the distal half of the upper limb, the median nerve contributed to the innervation of the lateral aspect of the forearm skin via the lateral cutaneous nerve of the forearm. In order to analyze this specific variant relevance we compared it with all the similar previous reported cases, trying to explain the embryological bases of the variant. The knowledge of anatomical variations of peripheral nerves is pivotal not only for surgeons, radiologists and anesthesiologists that may operate on the axilla, but also for every medical doctor to understand inexplicable clinical signs.

Key words

Median nerve, musculocutaneous nerve, brachial plexus, anatomical variation, lateral cord.

Introduction

During the third week of gestation the peripheral nervous system begins to develop. The upper limb buds are visible by days 26-27. The brachial plexus appears as a single radicular cone in the upper limb of embryos 34-35 days old. The main branches grow distally and divide into dorsal and ventral primary rami. Dorsal primary rami innervate dorsal axial musculature, vertebral joints and the skin of the back. Ventral primary rami innervate ventral body wall and the limbs forming the major nerve plexuses (Moore et al., 2015). The brachial plexus is formed by the anastomoses between the ventral rami of the spinal nerves from C5 to T1 with a variable contribution from C4 and T2. It supplies the innervation for thorax and shoulder girdle muscles, articulations and skin of the upper limb. The brachial plexus is the most variable part of the peripheral nervous system, prevalence of variations is reported

* Corresponding author. E-mail: lucia.manzoli@unibo.it

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between 12.8% and 53% (Pandey et al., 2007; Johnson et al., 2010; Pacholczak et al., 2011). The musculocutaneous nerve has frequent variations and they were discussed extensively even in very early articles (Kerr, 1918; Hovelaque, 1927; Ferner, 1938). Its variants are present in 6.25% of the cases; among them its absence seems to be rare, ranging in human subjects from 1.4% to 11.2% as reported in some studies (Choi et al., 2002; Beheiry, 2004; Chitra, 2007; Guerri-Guttenberg and Ingolotti, 2009; Budhiraja et al., 2011). In this case report, we focus on both musculocutaneous and median nerves anatomy in order to characterize an additional anatomical variation we found during cadaveric dissection.

Materials and methods

A cadaver of an adult Caucasian female was properly embalmed following the routine procedure of our anatomical dissecting laboratory. The axilla and the arm regions of right upper limb were meticulously dissected: namely, a skin incision along the line between the jugular notch and xiphisternal joint was made and then it was continued following the inferior border of the mammary gland until the median axillary line. After the reflection of skin and subcutaneous tissue the pectoralis major and minor were overturned to access the axilla, where the brachial plexus and the axillary artery were surrounded by the axillary sheath and fat. To improve the dissection of the arteries and nerves, the axillary vein was removed. Unusual communication and distribution of median nerve and musculocutaneous nerve were identified. The same anatomic preparation was subsequently performed on the left upper limb to study and compare the brachial plexus anatomy.

All the figures were digital, realized using Adobe Photoshop cs5 and Adobe Illustrator cs6.

Results

During a gross anatomy course for undergraduate medical students we observed a particular disposition of the nerves fibers that supply the flexor of the arm on the right upper limb of a 62-year-old Caucasian female cadaver. The musculocutaneous nerve, as reported in literature, should arise solely from the lateral cord. On the right side, it was absent. All the flexor muscles of the arm were supplied by branches from the median nerve except the coracobrachialis muscle that was innervated by a direct branch from the lateral cord (Fig. 1). Using the coracoid process as a reference point, we measured brachial flexor branches position: the nerve to the coracobrachialis muscle arose 5.6 cm distal to the coracoid process, the origin of the fibers to biceps brachii muscle was at 13.6 cm and the branch to the brachialis muscle at 17.2 cm. Below the last branch to brachialis, the median nerve gave off the lateral cutaneous nerve of the forearm that passed beneath the biceps brachialis muscle and went to the anterior aspect of the forearm (Fig. 1). Comparing arm flexor size among the two sides we did not encounter any variation in number of heads, length or mass volume. The trunks of the plexus were normal on both sides of the body, while on the right side we observed another variant at the origin of the median nerve, namely an extra con-

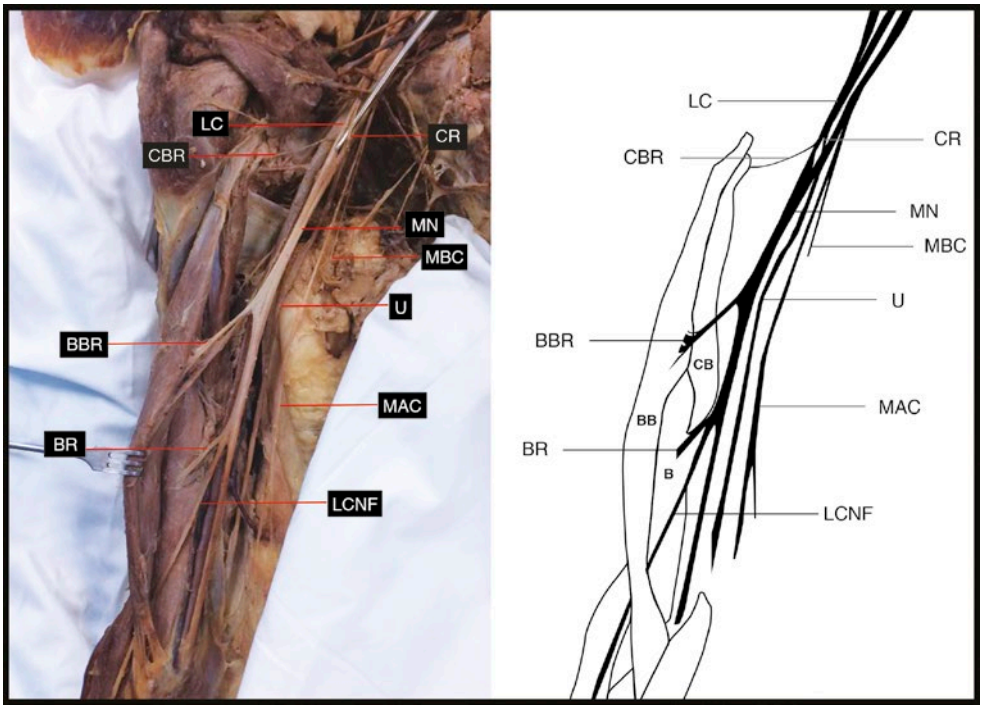

Figure 1. Absence of musculocutaneous nerve and innervation of the flexor muscles, except coracobrachialis, by branches of the median nerve. CB: coracobrachialis muscle; BB: biceps brachialis muscle; B: brachialis muscle; LC: lateral cord; MN: median nerve; CR: communicating branch; CBR: coracobrachialis ramus (nerve arising directly from the lateral cord); BBR: biceps brachii ramus (nerve); BR: brachialis ramus (nerve); MBC: medial brachial cutaneous nerve; MAC: medial antebrachial cutaneous nerve; U: ulnar nerve; LCNF: lateral cutaneous nerve of the forearm.

nection between the lateral cord and the median head of the medial nerve (Figs. 1, 2). We did not encounter any vascular abnormality in this case. We performed the same dissection on the left upper limb to study if the same variation was present. On dissecting the left axilla no anatomical variants were noted.

Discussion

Musculocutaneous anatomical variations

The course of the musculocutaneous nerve and its relationship with the coracobrachialis muscle was first observed by the Italian anatomist Giulio Cesare Casserio (1561-1616), student of the anatomist Girolamo Fabrici d'Acquapendente from Padua. It is one of the two terminal branches of the lateral cord, the other one is the lateral root of the median nerve. Musculocutaneous nerve primarily hosts fibers from the fifth and sixth cervical nerves (Standring, 2015), in 50% - 80% of individuals the

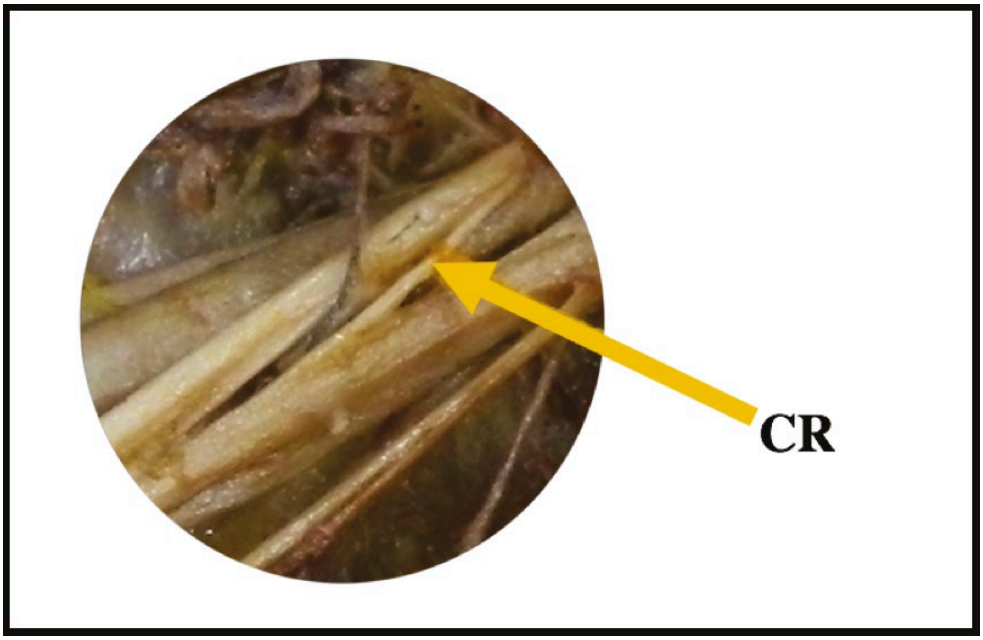

Figure 2. CR: communicating branch between the lateral cord and the medial root of the median nerve.

fourth and seventh cervical nerves may also contribute (Kerr, 1918). Its origin usually occurs at the level of the third part of axillary artery, where the lateral cord is lateral to the vessel (Standring, 2015); it arises solely from the lateral cord in 88.75% cases but it may also receive a contribution from the medial and the posterior cords (Kerr, 1918). Musculocutaneous nerve descends lying on the subscapularis muscle and subsequently passes through coracobrachialis muscle, which it innervates. Then it gives branches to biceps brachii and brachialis muscles and terminates as the lateral cutaneous nerve of forearm, deep to the biceps brachii, before emerging lateral to it and running down the lateral aspect of the forearm (Standring, 2015).

Failure of nerve fibers to divide into the common anatomic form often leads to abnormal branch patterns. Several kinds of variations of the musculocutaneous nerve have been reported, among them: musculocutaneous and median nerve sharing a communicating branch, muscular branches to brachial flexors transposed to median nerve and the absence of the musculocutaneous nerve (Le Minor, 1990; Venieratos and Anagnostopoulou, 1998; Adiguzel, 2000; Prasada Rao and Chaudhary, 2001; Choi et al., 2002; Song et al., 2003; Budhiraja et al., 2011; Pacholczak et al., 2011; Jeon et al., 2013; Parchand and Patil 2013; Sarkar and Saha, 2014; Gümüsburun and Hayashi et al., 2017).

The specific pattern we found is not belonging to any type described in the current literature.

Le Minor (1990) classified musculocutaneous and median nerve variations into five types based on the positional relationship between musculocutaneous nerve and coracobrachialis muscle (Fig. 3a). Type I (normal): there are no communicating

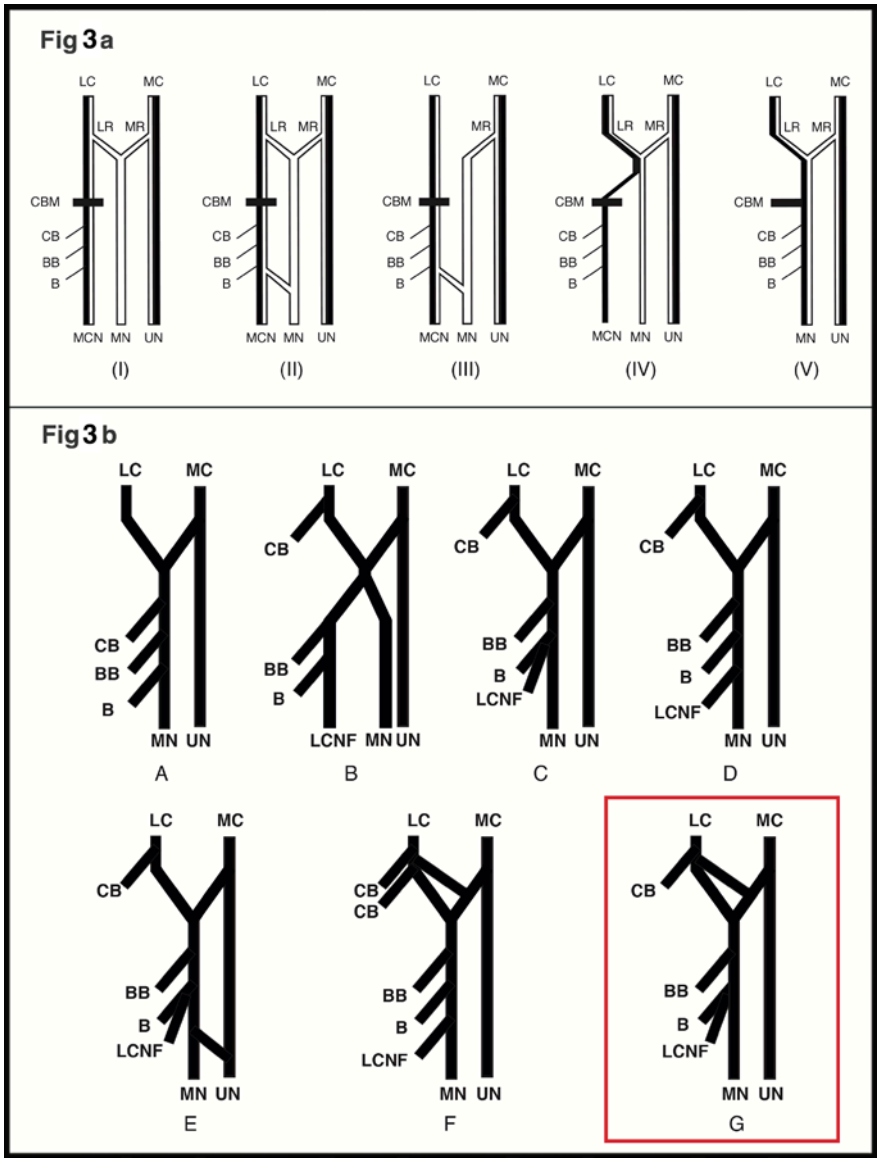

Figure 3. a) Classification of variations of the median nerve and the musculocutaneous nerve according to Le Minor (1990). CBM: position of the coracobrachialis muscle; CB: coracobrachialis ramus (nerve); BB: biceps brachii ramus (nerve); B: brachialis ramus (nerve); LR: lateral root forming the median nerve; MR: medial root of the median nerve; LC: lateral cord; MC: medial cord; MCN: musculocutaneous nerve; MN: median nerve; UN: ulnar nerve. b) Musculocutaneous nerve absence: cases where the transposition of the arm flexors innervation was similar to our case. A: Type V of Le Minor classification (1990); B: Prasada Rao and Chaudhary (2001); C: Budhiraja et al., (2011); D: Sarkar and Saha (2014); E: Gumusburun and Adiguzel (2000); F: Song et al., (2003); G: present case. LC: lateral cord; MC: medial cord; CB: coracobrachialis ramus (nerve); BB: biceps brachii ramus (nerve); B: brachialis ramus (nerve); MN: median nerve; UN: ulnar nerve; LCNF: lateral cutaneous nerve of the forearm.

rami between musculocutaneous nerve and median nerve, as described in classic textbooks. Type II: this pattern is similar to the normal but an extra ramus connects the musculocutaneous nerve and the median nerve while it gives the branch for the brachialis muscle. Type III: fibers from the lateral root follow the musculocutaneous nerve distally and leave it to unite with the medial head of the median nerve only after the musculocutaneous nerve originates rami to coracobrachialis, biceps brachii and brachialis muscles. Type IV: the musculocutaneous nerve arises from the median nerve as a proper and independent nerve after the origin of the median nerve. Type V: the musculocutaneous nerve is absent. Its fibers run into the median nerve along its course. All the brachial flexors are innervated by median nerve rami. Our finding is not corresponding to any of the Le Minor (1990) types described, however the type that is more similar to our case is type V (Fig. 3a).

Several authors found variants with absence of musculocutaneous nerve that do not have the same morphology as Type V of Le Minor classification (Budhiraja et al., 2011; Sarkar and Saha, 2014). In their cases all the arm flexors where innervated by rami originating from the median nerve and the coracobrachialis ramus arose from the lateral cord of the brachial plexus (Fig. 3b). Gümüsburun and Adiguzel (2000) described a similar pattern but there was also an extra anastomosis between the ulnar nerve and the median nerve (Fig. 2b). Prasada Rao and Chaudhary (2001) reported a case where musculocutaneous nerve was absent and arm flexors were innervated by two main branches: the coracobrachialis ramus arising from the lateral cord, and a ramus innervating the other arm flexors arising after the fusion of the medial and the lateral roots of the median nerve (Fig. 3b). Another description in the current literature shows an appearance similar to our variant but still different: two branches were identified inserting into the coracobrachialis muscle instead of just one (Song et al., 2003).

Recently, Hayashi et al. (2017) proposed a novel classification of muscolocutaneous nerve variations, nevertheless it does not include our specific pattern morphology.

Allocating musculocutaneous nerve variations into the already described classifications may be challenging. Lack of a clear and complete definition in current classifications leads to problems such as having three nomenclatures for the same anatomical variation (Guerri-Guttemberg and Ingolotti, 2009). Choi et al. (2002) refer as musculocutaneous nerve fused with the median nerve the condition that other author name musculocutaneous nerve absence (Nakatani et al., 1997; Gümüsburun and Adigüzel, 2000; Prasada Rao and Chaudhary 2001). Choi et al. (2002) and Buch-Hansen (1955) describe as musculocutaneous nerve absence a variation that occurs only when all the branches arising from the median nerve into the arm are sensitive rami. Hence, difference between musculocutaneous nerve absence and fusion needs to be clarified. Furthermore a complete musculocutaneous nerve classification including all the already published anatomic variants needs to be structured. Considering all these classification and nomenclature issues it is hard to quantify the real prevalence of musculocutaneous nerve absence. However, it is clear that musculocutaneous nerve absence belong to the most rare variations among brachial plexus variability (Guerri-Guttemberg and Ingolotti, 2009).

Thereafter, as nerves are called with their specific name due to their course of innervation and not from their origin (Song et al., 2003), it is reasonable to consider that in the present case report the median nerve was a combined nerve and the musculocutaneous nerve was absent on the right upper limb.

Embryological bases

Anatomical variations of the brachial plexus can be a consequence of aberrant development of the trunks, divisions and cords. An explanation of the different morphology may be achieved by understanding normal embryological development. Brachial plexus growth begins at the 34th to 35th day of intrauterine life when regional expression of Hox D genes leads to the development of forelimb muscle from the mesenchyme of paraxial mesoderm. Here, paraxial mesoderm differentiates into dermatomes, sclerotomes and myotomes. The last ones enlarge rapidly both dorsally and ventrally and divide into a dorsal epaxial portion and ventrolateral hypaxial portion. At this time, neural crest cells growing out of the neural tube make contact with the cells of the corresponding myotome, as the developing nerves begin to split into a dorsal primary ramus and a ventral primary ramus connected to corresponding portions of the myotome (Morgan and Tabin, 1994; Moore et al., 2015; Sadler, 2015). During further development, the nerve grows inside the muscle and follows it during any successive migration. This connection once established will be maintained during further development. The distribution area of developing axons is regulated by several factors: the signaling between the neuronal growth cones and the mesenchymal cells, the expression of specific chemoattractants and chemorepulsants (brain derived neurotrophic factor, C-kit ligand, netrin-1, netrin-2 etc) and specific circulatory factors overproduced at the brachial plexus's time of fission (Larsen, 2001; Catala and Kubis, 2013). The constituents of the extracellular matrix play a crucial role in the control of neural crest cell migration, in particular fibronectin, laminin, and tenascin. These molecules are recognized by integrins which are surface receptors expressed by neural crest cells (Catala and Kubis, 2013). After careful investigation of the complex developmental process driving the formation of brachial plexus, it is not surprising that anatomical variations of the brachial plexus are common. Alterations in these process lead to anomalies in the nerve supply to muscles (Prasada Rao and Chaudhary, 2001).

Clinical correlations

In the present case musculocutaneous nerve fibers ran into the median nerve and the musculocutaneous nerve was absent. Absence of the musculocutaneous nerve indicates that most of the nerve fibers of C5 and C6 passed via the lateral root to the median nerve and then distributed to muscles of the anterior compartment of the upper arm as well as anterolateral cutaneous area of the forearm through the branches of the median nerve (Moore et al. 2015). This variation does not lead to paralysis of the arm flexors neither to hypoesthesia of the lateral surface of forearm, since motor and sensory fibers can arise from other nerves (Budhiraja et al., 2011).

Lesions of the median nerve in cases where the musculocutaneous nerve is absent and its fibers are transposed to the median nerve would lead to unexpected clinical signs. In addition to the normal median nerve palsy related symptomatology, it will result in the paralysis of the brachialis, coracobrachialis and biceps brachii muscles. It would also produce a weak flexion at the elbow and a weak supination when the elbow is flexed. Loss of sensation or burning dysesthesia of the lateral aspect of the forearm could also follow (Gillingham and Mack 1996).

Being aware of musculocutaneous nerve absence is clinically relevant for surgeons, orthopedists and anesthetists performing treatment on the upper limb (Prasada Rao and Chaudhary, 2001; Budhiraja et al., 2011). During flap dissection, shoulder reconstruction, axillary lymph node dissection and surgical procedures around the shoulder/axillary area accidental nerve damages may happen, especially if the surgeon is familiar with the normal anatomic course of the nerve and its regional relationship, but not aware of all the possible variants that may be present (Flatow et al., 1989; Budhiraja et al., 2011; Hayashi et al., 2017). Information about the course and the topography of the musculocutaneous nerve is important also for anesthetic axillary plexus block, where a selective block of the musculocutaneous nerve is necessary to achieve a successful anesthetic axillary plexus block. It could not be achieved in case of unknown musculocutaneous nerve absence (Kjelstrup et al., 2017).

Compliance with ethical standards

Informed consent was obtained from all individuals who had a right to express it according to Italian law.

Conflict of interest

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Research Article - Basic and Applied Anatomy

Internal jugular vein fenestration: a rare but possible event. A case report and review of the literature

Ferdinando Caranci[1,*], Enrico Tedeschi[2], Giuseppe Leone[2], Vincenzo Giugliano[3], Andrea Elefante[2], Aldo Bruno[4], Luigi Califano[5], Roberta De Vizia[2], Francesco Briganti[2], Attilio Varricchio[6], Luca Brunese[1]

[1] Department of Medicine and Health Sciences, University of Molise, Campobasso, Italy
[2] Unit of Neuroradiology, Department of Advanced Biomedical Sciences, Federico II University of Naples, Italy
[3] Unit of Radiology/Neuroradiology, GEPOS Clinic, Telese Terme (BN), Italy
[4] Vascular Surgery Division, GEPOS Clinic, Telese Terme (BN), Italy
[5] Audiology and Phoniatry Division, Gaetano Rummo Hospital, Benevento, Italy
[6] Diagnostic and Surgical Upper Respiratory Tract VideoEndoscopy Unit, San Gennaro Hospital, Naples, Italy

Abstract

While fenestration and duplication are relatively common in the arteries, they are extremely rare in the venous compartment: internal jugular vein fenestration has been reported occurring in 0.4% of unilateral neck dissections. Familiarity with these morphological anomalies is important for the radiologist and for the surgeon to prevent neurovascular injury, especially in neck surgery and interventional catheterization. We present the case of a patient harboring a fenestration of the left internal jugular vein, diagnosed by magnetic resonance angiography, and a systematic review of the literature. To our knowledge, from 1985 until 2016 only 36 patients (including the present) were diagnosed as having an internal jugular vein morphological anomaly. Out of 36 patients, only 11 (30,5%) were diagnosed using radiological imaging; the high rate of intraoperative diagnoses (22/36, 62,5%) is likely related to the limited use of diagnostic imaging or to misdiagnosis/misinterpretation of a relatively unknown and rare morphological anomaly. A contrast enhanced computed tomography or magnetic resonance angiography should be considered in case of vascular procedures in a patient with known internal jugular vein anomaly.

Key words

Duplication, fenestration, internal jugular vein, magnetic resonance angiography.

Introduction

The jugular venous system constitutes the primary venous drainage of the head and neck, in a pattern common to human and other species (Williams, 1995; Mancini et al., 2015).

It is known that the internal jugular vein (IJV) presents high variability in its flow rate and cross-sectional area (Cocozza et al., 2016), whereas fenestration and duplication are extremely rare, compared to the arterial compartment: IJV fenestration has been reported occurring in 0.4% of unilateral neck dissections (Prades et al., 2002).

Familiarity with these morphological anomalies is important for the radiologist and the surgeon to prevent neurovascular injury, especially in neck surgery and interventional catheterization.

* Corresponding author. E-mail: ferdinando.caranci@unimol.it

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We present the case of a patient harboring a fenestration of the left IJV, diagnosed by magnetic resonance (MR) angiography (MRA), and a systematic review of the literature.

Case report

A 35 year-old-man with the diagnosis of Méniere's disease was admitted to investigate the coexistence of chronic cerebrospinal venous insufficiency and eventually planning of IJV percutaneous transluminal angioplasty.

Magnetic resonance imaging was performed with a 1.5 T clinical MR system (Magnetom Essenza Siemens), by using 3D contrast-enhanced MR angiography/venography imaging of the neck (TE 1.49 ms; TR 3.88 ms; flip angle 25°; slice thickness 1.20 mm; FOV 340 mm, FOV PHASE 75%), with maximum intensity projection reconstructions.

Contrast medium administration for time-resolved MR venography was performed using an automatic contrast-injector with intravenous 1 mmol/ml gadobutrol (Gadovist 1.0, Schering AG, Switzerland), 0.1 mmol/kg at 2 mL/s beginning simultaneously with the start of the sequence, followed by 20 mL bolus of saline at the same rate.

Magnetic resonance angiography and maximun intensity projection reconstructions showed that the left IJV emerged as a single trunk from the jugular foramen, then split into two parts, anterior and posterior, after descending for about 6.5 mm from the base of the skull. Both parts then rejoined to form a single trunk in the lower part of the neck, before joining the subclavian vein to form the brachiocephalic vein (Figure 1).

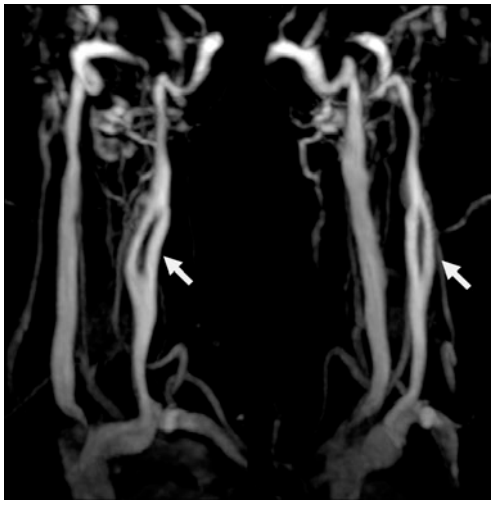

Figure 1. Magnetic Resonance Angiography (oblique views). Splitting of the internal jugular vein into two parts (arrows), then rejoining to form a single trunk in the lower part of the neck before joining the subclavian vein to form the brachiocephalic vein.

Discussion

The true incidence of IJV fenestration is difficult to assess since most duplicated or fenestrated IJVs are discovered as incidental findings (Cvetko, 2015) and because the terms duplication, partial duplication, and fenestration have been used interchangeably, although a duplication is defined as two distinct vessels with separate origins and no distal convergence, while fenestration is a division of a vessel into distinctly separate channels, each with its own endothelial and muscularis layers, while the adventitia may be shared (Parmar et al., 2005): it refers to a 'window-like opening' in the IJV, with a typical 'eye-of-the-needle' appearance (Dwonie et al., 2007). IJV fenestration are extremely rare, occurring in a recent study in 0.4% of unilateral neck dissections (Prades et al., 2002).

The primary blood vessels of head and neck consists embriologically of close meshed capillary plexus, drained on each side by the precardinal (anterior cardinal) vein, at first continuous cranially with a transitory primordial hind brain vein which is soon replaced by the primary head vein to become continuous with the precardinal vein.

The etiology of IJV duplication-fenestration is still unclear. Three hypothetical explanations have been suggested. The vascular theory, most commonly adopted, is based on the paucity or absence of the IJV muscular layer. The neural hypothesis assumes that the IJV anomaly depends on the altered position of the spinal accessory nerve in relation to the transverse process of the atlas, which can lead to the duplication of the developing IJV. The bony hypothesis suggests that variation in the ossification of the bony bridges of the jugular foramen causes venous duplication; this theory does not explain the relation of the spinal accessory nerve to the duplicated IJV - see below (Sylaidis et al., 1997; Guerra et al., 2000; Gardiner et al., 2002; Alaani et al., 2005; Striano et al., 2005).

To our knowledge, analyzing the literature using Medline database (www.ncbi.nlm.nih.gov/pubmed), from 1985 until 2016 only 36 patients including the present were diagnosed as having a IJV morphological anomaly (Table 1). Out of these 36 patients, 32 (about 89%) had unilateral anomaly, 4 (11%) bilateral anomaly. Out of 40 IJV anomalies, 21 (about 52,5%) were duplication, 19 (47,5%) were fenestration; 25 (62,5%) were on the left, 13 (32,5%) were on the right, 2 were not defined in the report. Out of 36 patients, 22 (about 61%) were diagnosed during neck surgery, only 11 (30,5%) using imaging, and 3 (8,5%) at cadaveric dissection.

Overall, morphological anomalies of the IJV are described more often as unilateral, on the left side, and in almost equal percentage in terms of fenestration and duplication.

Most duplications and fenestrations occur in the upper third of the IJV (Bachoo and Evans, 2014); in the case presented here, the fenestration was observed in the middle of the IJV.

Regarding to the way of detection, the high rate of intra-operative diagnoses (22/36, 62,5%) is likely related to the limited use of diagnostic imaging or to misdiagnosis/misinterpretation of a relative unknown and rare morphological anomaly (Caranci et al., 2015). Colour-Doppler ultrasonography and computed tomography angiography were the technique most often used for the diagnosis, while MRA was used only in 1 paper (Rossi et al., 2001) before our report. Only 3/36 (8,5%) were found in cadaveric dissections.

Table 1. Internal jugular vein anomalies reported in cronological order.

Authors	N.° of patients and anomalies	Morphological anomaly	Side	Diagnosis method
Som et al., 1985	1 unilateral	duplication	right	Imaging
Sylaidis et al., 1997	1 unilateral	fenestration	right	surgery
Guerra et al., 2000	1 unilateral	duplication	right	surgery
Rossi et al., 2001	1 bilateral	duplication fenestration	right left	Imaging
Gardiner et al., 2002	1 unilateral	fenestration	left	surgery
Prades et al., 2002	3 unilateral	fenestration fenestration fenestration	left left right	surgery
Towbin et al., 2004	2 unilateral	fenestration fenestration	left left	Imaging
Turan-Odzemir et al., 2004	1 unilateral	duplication	right	Imaging
Alaani et al., 2005	1 unilateral	fenestration	left	surgery
Nayak 2006	1 unilateral	fenestration	left	cadaveric dissection
Downie et al., 2007	1 bilateral	duplication duplication	left right	cadaveric dissection
Gonzalez-Garcia et al., 2007	1 unilateral	duplication	left	surgery
Iseri et al., 2007	1 unilateral	fenestration	left	surgery
Uecker et al., 2007	1 unilateral	duplication	left	surgery
Coca Pelaz et al., 2008	1 bilateral	duplication duplication	left right	surgery
Colella et al., 2008	1 unilateral	duplication	right	surgery
Wong et al., 2010	1 bilateral	duplication duplication	left right	Imaging
Atalar et al., 2012	1 unilateral	fenestration	left	Imaging
Kapre et al., 2012	1 unilateral	fenestration	left	surgery
Radak et al., 2012	1 unilateral	duplication	right	Imaging
Thakur at al, 2012	1 unilateral	fenestration	not reported	surgery
Kayashima et al., 2013	1 unilateral	duplication	left	Imaging
Ayoub et al., 2014	1 unilateral	duplication	left	surgery
Bachoo et al., 2014	1 unilateral	duplication	left	surgery
Torres et al., 2014	1 unilateral	fenestration	left	Imaging
Cvetko et al., 2015	1 unilateral	fenestration	left	cadaveric dissection
Moreno-Sànchez et al., 2015	1 unilateral	fenestration	1 right	surgery
Pegot et al., 2015	1 unilateral	fenestration	left	surgery
Contrera et al., 2016	3 unilateral	2 fenestration, 1 duplication	2 left 1 right	surgery
Sidana et al., 2016	1 unilateral	duplication	not reported	surgery
Present report 2017	1 unilateral	fenestration	left	Imaging

The IJV is also a radiological landmark (Pegot et al., 2015). It is important for the radiologist to be familiar with the anatomical variations of these veins, in order to avoid misinterpretation and misidentification [31]: IJV anomalies have been sometimes mistaken as laryngoceles or branchial cleft cysts (Caranci et al., 2015).

Also, their knowledge is essential before performing head and neck surgery, oncological surgery, percutaneous catheterization or when planning IJV percutaneous transluminal angioplasty for chronic cerebrospinal venous insufficiency (Briganti et al., 2004, 2013, 2016), in order to avoid severe clinical consequences. In neck dissections, IJV anomalies could greatly increase the risk of bleeding, or make complete clearance of lymph nodes impossible, particularly if the patient has previously been treated with radiation. The IJV is also often used as a recipient vein for a free flap and in some cases fenestrations could increase operative difficulties and morbidity (Pegot et al., 2015).

The IJV is a common site for insertion of a central venous line; in case of a fenestration, difficulties in insertion of the catheter could cause vascular injury with cervical bleeding or hematoma. The anatomical relation of the IJV to the contiguous structures, particularly the accessory nerve, can change depending of the presence or absence of a fenestration or duplication. In fact, the spinal accessory nerve usually passes superficial to the IJV, so any tissue that is superficial to this landmark can be dissected during neck surgery with no risks. In duplicated or fenestrated IJV, the spinal branch of the accessory nerve is always reported to pass deep to the anterior branch of the duplicated internal jugular vein and superficial to the posterior branch (Parmar et al., 2005), or rarely deep to both posterior and anterior branches of the vein (Alaani et al., 2005).

Consideration of contrast enhanced computed tomography (CTA) or MRA is warranted if a patient with known IJV anomaly is undergoing interventional procedures involving major vascular structures (Caruso et al., 2002; Thakur et al., 2012; Torres et al 2014).

Patients with IJV bifurcation may have a higher prevalence of additional vascular abnormalities. Like in arteries, where duplication is associated with aneurysm formation, duplication of the IJV is usually reported in association with phlebectasia, a congenital dilation of the jugular venous system. Phlebectasia is a local fusiform, soft, non-pulsatile swelling in the cervical region which increases during Valsalva maneuver (Som et al., 1985; Rossi et al., 2001; Prades et al., 2002). IJV duplication associated with phlebectasia was reported in a 2 year old girl studied by color-Doppler ultrasound and MRA (Rossi et al., 2001).

In our patient no phlebectasia was assessed; moreover, he didn't find symptoms related to IJV anomaly. This is consistent with past reports, although there have been cases of patients presenting with neck swelling, dyspnoea, and dysphagia (Wong et al., 2010); moreover, IJV anomalies raises the possibility for deep venous thrombus formation secondary to changes in flow velocities (Cvetko, 2015).

In conclusion, IJV is a rare vascular anomaly that may have significant clinical consequences. Familiarity with these morphological anomalies is important for the radiologist and for the surgeon to prevent neurovascular injury, especially in neck surgery and interventional catheterisation. Given the advances and wider availability of imaging examinations worldwide in the last decades, it is expected that a growing number of cases will be identified (Torres et al., 2014; Cocozza et al., 2016).

Disclosures

All authors declare that they have no involvement, financial or otherwise, that might potentially pose conflict of interest.

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Research Article - Histology And Cell Biology

Relationships between seasonal (spring, summer, autumnal) thermal variations and cell proliferation in heterothermic vertebrates, as revealed by PCNA expression in the brain of adult *Triturus carnifex*

Vito Margotta[1,*], Claudio Chimenti[2]

[1] Dipartimento di Biologia animale e dell'Uomo, Università "La Sapienza", Roma, Italia
[2] Dipartimento di Biologia e Biotecnologie Charles Darwin, Università "La Sapienza", Roma, Italia

Abstract

Inspired both by the literature reports and our previous findings on the question if a seasonal cycle alone, consisting of temperature and photoperiod variations, might impact on or activate natural proliferative fluctuations or unmask a latent spontaneous proliferative power in adult brain of poikilothermal Anamnia (fresh water, earth-dwelling) and Amniota (terrestrial), consequently allowing for encephalic reparative and even regenerative potentialities, an investigation has been carried on in normal adult brain of *Triturus carnifex* caught in nature in spring, summer, autumn. Cells immunostained for PCNA, *i.e.* cycling cells, were found scattered ("matrix cells") in the olfactory territories, where they appeared scarce in spring, more frequent in summer, noticeable in autumn; also, immunostained cells were found clustered in "matrix areas", also named *zonae germinativae dorsales* and *ventrales*, in the telencephalic hemispheres: few clusters in spring, an intermediate condition in summer, frequent cell groups in autumn. These results reveal an increasing trend in proliferation from spring, through summer, to autumn. This scenario was appreciable in the forebrain, mainly in the olfactory and telencephalic districts, which is the typical site of stem cells. Signs of potential proliferative activity are well appreciable in the urodele Amphibians, which are the best provided among vertebrates with reparative and regenerative power and possess the richest endowment of dormant cells susceptible to be recruited to proliferation.

Key words

Seasonal influence, neural matrix cells/areas, *Triturus*.

Introduction

In vertebrates, among the great amount of investigations testifying the plasticity, now well known, of the brain in adult poikilothermal Anamnia (fresh water, like Teleosts, earth-dwelling, like Amphibians) and heterothermic Amniota (terrestrial, like lacertilian Reptiles), the literature refers a handful of observations about whether seasonal cycle, including temperature and photoperiod variations, alone or coupled with various experimental approaches, might activate natural proliferative fluctuations or unmask a latent spontaneous proliferative power at the level of adult brain, conse-

* Corresponding author. Home address: Via Vigna Stelluti n. 30, 00191, Roma, Italy

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quently making evident some encephalic reparative and even regenerative potential. This potential is most pronounced in Urodeles Amphibians, intermediate in Teleosts, lower in Anuran Amphibia, most limited in lacertilian Reptiles.

Kirscke (1967) always takes the credit for a wide, systematic study on such issue in adult non-mammalian vertebrates ranging from Teleosts to Birds, passing through urodelan and anuran Amphibia and lacertilian Reptiles. This author must be acknowledged for the identification of the sites and features of the cells responsible of proliferative events which appear linked to the survival of stem cells in adults. These cells are capable of self-reproduction and can start cycling again giving rise to descendants which may evolve into neuronal or glial cells.

These normally quiescent cells, small and basophilic, are remnants of the embryonic neural layer responsible of central nervous system histogenesis (Kahle,1951; Fujita, 1963; Kirsche, 1967) and their number decreases during life from the embryonic to adult age through - if present - the larval stages.

The number of these neural-like cells varies among vertebrates. It is higher in lower than in higher vertebrates and depends on the different encephalic districts: cycling cells are easy to find in the anterior portion of the brain (olfactory bulbs/peduncles, telencephalon), but not in the diencephalon, in contrast they are absent from the *cerebellum* (except in Teleosts); as regards the *truncus cerebri*, they cannot be easily found in the midbrain (except in Teleosts) nor in the *medulla oblongata*.

These cycling cells appear scattered ("matrix cells") in the olfactory districts, otherwise clustered, often layered, in circumscribed areas ("matrix areas", or *Matrixzonen* of Kirsche, 1967) in the telencephalic hemispheres, at the dorsal and ventral edges of the lateral surface of each semi-lunar shaped ventricle which are named *zonae germinativae dorsales* and *ventrales*, respectively, which are extended antero-posteriourly. The cells in the former areas are exhausted before those in the latter areas, which is also generally wider and richer in cells (Kirsche, 1967).

In detail, matrix cells appear more frequently in relationship with the peri-ventricular grey matter or within the brain tissues, while matrix areas are typically located among the ependymal cells and in the sub-ependymal layer lining each encephalic cavity.

The knowledge on these putative precursor or perhaps stem cells has expanded through a great amount of observations derived from experimental procedures such as brain-injury, ablation of encephalic portions sometimes with subsequent heterotopic heterotransplantation (even of the whole brain), *in vitro* culture. In those studies several techniques: first classical histology, then autoradiography and immunohistochemistry targeting proliferation-related enzymes, seldom electron microscopy.

Past investigations in brain-injured or normal adult *Rana esculenta* (Minelli et al., 1982) and in brain-damaged adult *Podarcis hispanica* (Ramirez et al., 1997) have raised the question if seasonal cycle alone, that is made of temperature and photoperiod variations, might impact encephalic proliferative fluctuations. Since we have recently investigated this same question by immunohistochemistry in adult normal *Rana bergeri* (now synonymous of *R. esculenta*: Capula, 2000) (Margotta and Chimenti, 2017, 2018), we have now extended the observations to normal adult *Triturus carnifex*, addressing the presence and localization of proliferating cell nuclear antigen (PCNA: Miyachi et al., 1978), a reliable marker of cycling cells (see Margotta and Chimenti, 2016).

Materials and methods

Normal adult *Triturus carnifex* - ascertained following Bonifazi (2000) - of both sexes involved in the actual research originated from three captures, performed on purpose, in the wild near Rome: the first in spring (environmental temperature varying between 12°C to 18°C), the second in summer (environmental temperature varying between 14°C and 24°C), the third in autumn (temperature varying between 8°C to 18°C). The newts were sacrificed under anaesthesia with tricaine methanesulfonate (Ms 222 from Sandoz, Holzkirchen, Germany: 1:1000). The head was cut off and after partial disarticulation of the cranial bones it was fixed in Bouin's fluid and then transferred to 80% ethyl alcohol, where the brain was removed under a stereomicroscope. The tissue was dehydrated through graded ethyl alcohol, cleared in histolemon and embedded in plastic paraffin under *vacuum*. Transverse, 8 μm thick serial sections were cut in antero-posterior direction with a rotary microtome.

For immunohistochemistry the sections, upon removal of paraffin and hydratation, were soaked in isotonic, 0.01 mol/litre phosphate buffered saline, pH 7.4 (PBS), incubated in 3% H_2O_2 in methanol for 30 min to block endogenous peroxidase, washed in PBS, incubated in 20% normal horse serum to block unspecific binding sites and incubated overnight at 4 °C in a monoclonal antibody against PCNA (PC10 mouse IgG from Sigma-Aldrich, St. Louis, Missouri), diluted 1:1000 with PBS plus 1% normal horse serum. Negative control sections were incubated with non-immune mouse IgG instead of the primary monoclonal. The bound antibodies were detected using secondary horse anti-mouse biotinylated antibodies (Vector, Burlingame, California), diluted 1:100 with PBS plus 1% normal horse serum, for 1 h at room temperature, and avidin-biotin-peroxidase complex (ABC Kit, Vector), 30 min at room temperature. Peroxidase was detected with 3-3'-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich) 1 mg/ml, plus 1% $NiSO_4$ and 0.017% H_2O_2 in 0.05 mol/litre Tris-HCl, pH 7.6. Slides were dehydrated and mounted with Entellan (Merck, Darmstadt, Germany). The specificity of the immunostaining was tested by replacing the primary antibody with non-immune goat serum.

Results

In the olfactory bulbs immunoreactive PCNA-positive cells were present scattered among the ependymal cells lining the symmetrical falciform-shaped ventricles, at times in the sub-ependymal layer. That appeared rarely in "spring" specimens (Fig. 1a), in a more pronounced manner in "summer" individuals (Fig. 1b), in a noticeable amount in "autumn" samples (Fig. 1c).

In the telencephalic hemispheres (each including a wide, irregular, falciform-shaped vertical cavity) among the ependymal epithelial cells and in the peri-ventricular grey matter layer at the level of the latero-dorsal edges and latero-ventral-medial bottom some clustered labelled cells were positioned at the surface of the ventricles, sometimes associate to other scattered stained cells. There were few clusters in "spring" specimens (Fig. 2a), an intermediate amount in "summer" individuals (Fig. 2b) and many in "autumn" samples (Fig. 2c). These cells occurred in the sites where the *zonae germinativae dorsales* (Figs. 2a, 2b, 2c) and *ventrales* (Figs. 2a, 2b, 2c)

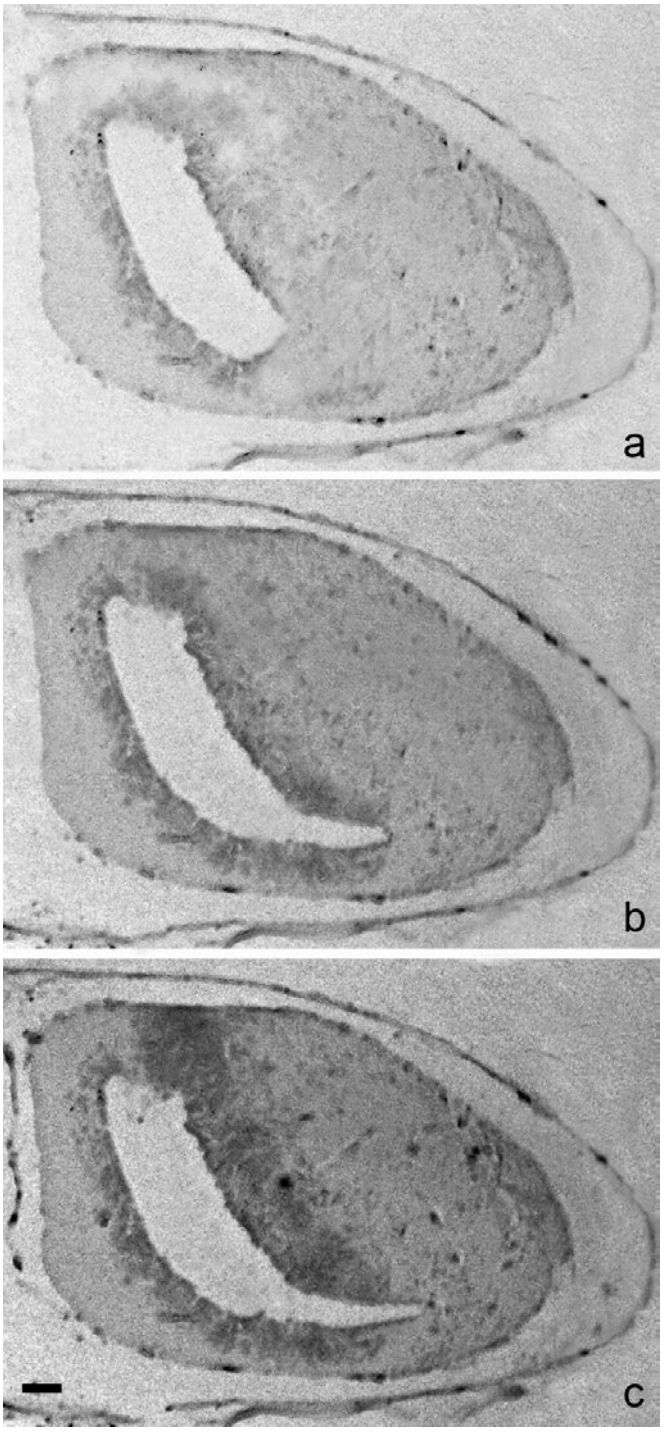

Figure 1. Transverse sections of olfactory bulbs of normal adult *Triturus carnifex*. Labelling appear mainly scattered in the ependyma, rarely in the sub-ependyma, around the ventricles. PCNA-positivity was scanty in "spring" specimens (a), intermediate in "summer" specimens (b) and abundant in "autumn" specimens (c). PCNA immunocytochemistry without nuclear conterstaining. Calibration bar = 50 µm.

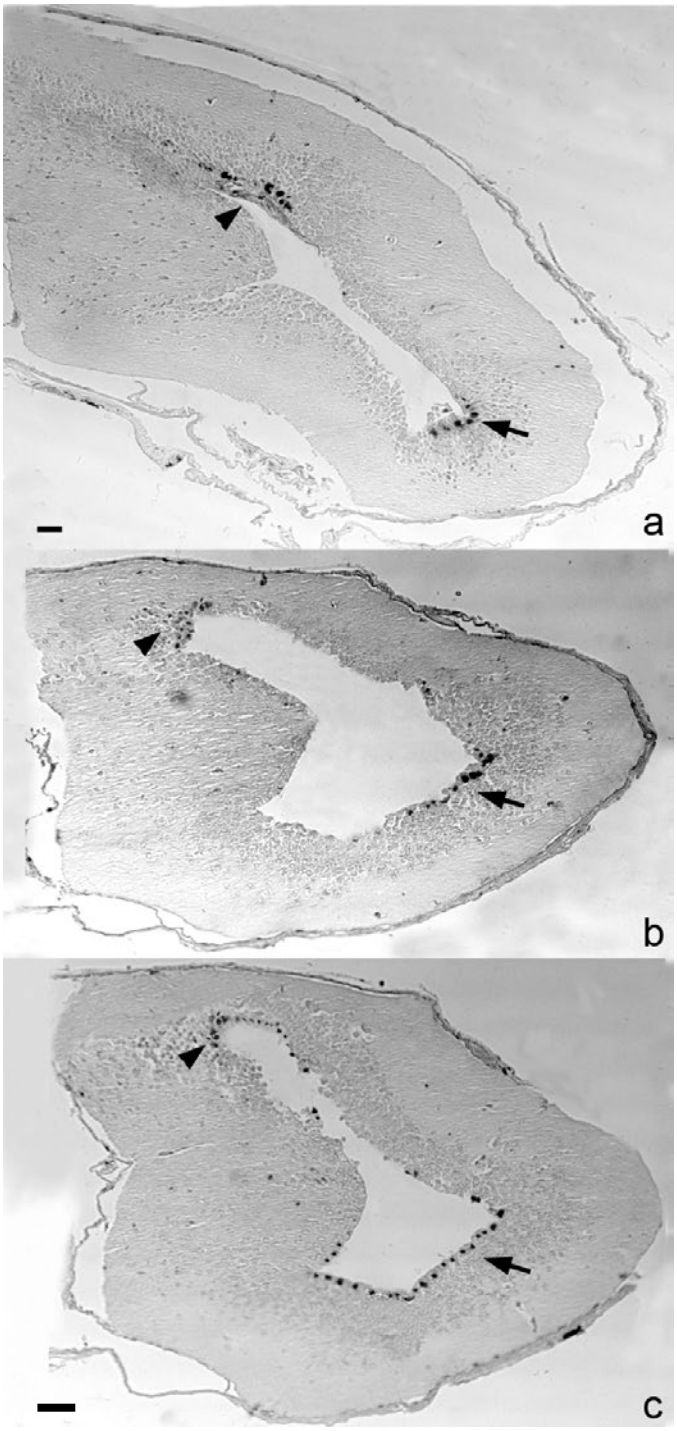

Figure 2. Transverse sections of telencephalic hemispheres of normal adult *Triturus carnifex*. PCNA-positive grouped and scattered cells appear in the ependymal and periependymal layers at the latero-dorsal edges (*zonae germinativae dorsales*, arrowheads) and at the bottom (*zonae germinativae ventrales*, arrows) of the lateral ventricles: labelled cells were scanty in "spring" specimens (Fig. 2a), some more in "summer" specimens (Fig. 2b) and numerous in "autumn" specimens (Fig. 2c). PCNA immunocytochemistry without nuclear conterstaining. Calibration bar = 50 µm.

are known to be located. Both *zonae germinativae* were extended antero-posteriorly, but the *zonae ventrales* were provided with more PCNA-labelled cells than the *zonae dorsales*.

In the diencephalon of "spring", "summer" and "autumn" individuals, weakly stained cells appeared in the ependyma and in the peri-ventricular grey matter around the narrow IIIth ventricle; pronounced immuno-positivity could be identified dorsally and ventrally where the symmetrical habenular ganglia and the impair preoptic and infundibular recesses, respectively, are located, without appreciable differences among "spring", "summer" and "autumn" samples.

In the midbrain the immuno-reaction was pale and hard to see in all samples, while in the remaining brain districts lying behind no labelling was traceable in any specimen.

Discussion

Numberless researches on plasticity of normal adult brain of vertebrates have clearly stated that the entity of proliferative phenomena in such organisms vary depending on the place in the systematic scale and structural complexity, so these events appear be limited in some poikilothermal, anamniotic and amniotic, groups. They depend on the survival of stem cells among the adult brain tissues, which appear scattered ("matrix cells") or clustered, often layered, in circumscribed areas ("matrix areas"): the first are found mainly in the olfactory, diencephalic, cerebellar and medullary districts, the latter in other regions (telencephalic, mesencephalic) limited to circumscribed areas: the *zonae germinativae*. Such quiescent cells make their appearance most frequently within the peri-ventricular grey matter (the matrix cells) and, among the ependymal cells (the matrix areas).

Investigations have addressed the question if seasonal cycle by itself, with variations only of temperature and photoperiod, might activate encephalic proliferative fluctuations or unmask latent spontaneous proliferative potentialities, otherwise hidden, consequently favouring reparative and even regenerative activity. This activity is most pronounced in urodele Amphibians, the vertebrates richest in putative stem cells, and decreases through Teleosts and anuran Amphibia down to lacertilian Reptiles.

From the evaluation of spontaneous fluctuations in the number of PCNA labelled cells in adult *T. carnifex* it has emerged that in the olfactory districts scattered labelled cells appeared occasionally in "spring" conditions, some more in "summer" conditions and numerous in "autumn" conditions, at the level of the ependyma and peri-ventricular grey matter. In the telencephalic hemispheres, the *zonae germinativae dorsales* and *ventrales* were identifiable as clusters of stained cells, which were few in "spring" conditions, in intermediate amount in "summer" conditions and most abundant in "autumn" conditions, mainly among the ependymal cells and rarely in the peri-ventricular grey matter.

Summarizing, these results reveal an increasing proliferation trend from spring through summer to autumn in the forebrain, precisely in the olfactory and telencephalic portions which are the typical sites of matrix cells/areas, while the putative proliferating cells diminished harshly or totally disappeared in the more caudal districts in all conditions.

Studies have also been published regarding both brain-injures and few normal brain of adult *R. esculenta* (Minelli et al., 1982) and brain-damaged adult *P. hispanica* (Ramirez et al., 1997). Minelli et al. (1982) observed by autoradiography an increase in proliferating cells in autumn with respect to spring and then a strong reduction in winter. Coherently, Ramirez et al. (1997), also by immunocytochemistry, noticed a proliferative peak in summer and stated that: "cold (winter) temperature prevented migration of the newly generated immature neurons", an observation possibly correlated with an involvement of radial glial cells in that migration (for details see Margotta and Morelli, 1997).

The seasonal gap in the studies of Minelli et al.(1982) and Ramirez et al.(1997) has been filled for the normal adult brain of *R. bergeri* by Margotta and Chimenti (2017, 2018), of *P. sicula* by Margotta and Chimenti (in press) and of *T. carnifex* by the actual investigation. We have considered unnecessary to repeat observations on "winter" specimens given the findings for this season of Minelli al. (1982) and Ramirez et al. (1997), which should reasonably apply also to newts.

In adulthood besides in different taxonomic species, the immunocytochemical patterns described here for *T. carnifex* seem to be in substantial agreement with those in different other species (frogs, lizards) including the findings of Minelli et al. (1982), related to spring and autumn, and of Ramirez et al. (1997), related to summer.

In experimental conditions similar to present ones, an increase in proliferation from spring through summer to autumn has been described for the adult brain of normal earth-dwelling Anamnia and terrestrial Amniota, including *R. bergeri* in spring, autumn (Margotta and Chimenti, 2017) and summer (Margotta and Chimenti, 2018). This report informs on *T. carnifex* and attention is worth being devoted also to *P. sicula* (Margotta and Chimenti, in press).

The previous and present findings on encephalic proliferative capacity in normal adult poikilothermal Anamnia (fresh water, like Teleosts, and earth-dwelling, like Amphibians) and in heterothermic terrestrial Amniota (like lacertilian Reptiles) may explain the different extent of reparative or regenerative events obtained by previous authors, if one admits that a role in this respect may also be played - among others - by the type of experimental stress applied (traumatic, surgical, thermal).

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Research Article - Histology and Cell Biology

Effect of cigarette smoke and treatment with relaxin on guinea pig skin

Angela Silvano*, Silvia Nistri, Laura Calosi, Paolo Romagnoli

Department of Experimental and Clinical Medicine, University of Florence, Italy

Abstract

Cigarette smoking causes microvascular dysfunction and skin aging. Relaxin, primarily but not exclusively involved in reproduction, has connective tissue among its targets. Within a project on the interference of relaxin with the effects of smoke on guinea pigs, we examined the skin response to those stimuli. Adult guinea pigs were exposed to cigarette smoke daily for 8 weeks, and some of them were treated also with relaxin, 1 or 10 μg/die. Controls were treated with relaxin vehicle alone. The skin was analyzed by light and electron microscopy and histochemistry for mast cells and the collagen specific chaperonin Hsp47. The epidermis appeared unaffected by any treatment. In the superficial dermis, smoke led to a decrease in mast cell number and intensity of astra blue staining, suggestive of granule discharge. Relaxin caused further significant reduction in mast cell number. In the superficial and deep dermis, the staining intensity of Hsp47 positive cells, assumed as active fibroblasts, increased upon smoke. The staining intensity decreased gradually in the superficial dermis upon relaxin, reaching significance after treatment with 10 μg/die relaxin, while in the deep dermis it decreased significantly upon treatment with 1 μg/die relaxin and underwent further, significant increase with 10 μg/die relaxin. The results suggest that relaxin can enhance skin mast cell secretory response, possibly antagonizing nicotine induced vasoconstriction and, depending on dose and localization of responding cells, can counteract the profibrotic stimulus of smoke on dermal fibroblasts.

Key words

Hsp47, mast cells, collagen fibres, elastic fibres, skin, relaxin.

Abbreviations

PBS: Phosphate buffered saline
Hsp47: heat shock protein 47
RLX: relaxin
TRITC: trimethylrhodamine.

Introduction

Guinea pig is a useful model for allergic diseases (Morimoto et al., 2014), especially those depending on hypersecretion of factors by mast cell (Ashoori et al., 1996), and for pulmonary damage from inhaled agents (Papi et al., 1999). The skin of guinea

* Corresponding author. E-mail: angela.silvano@hotmail.it

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pig has been taken as a model to study hypertrophic scars (Aksoy et al., 2002), evaluate the effect of laser treatments on tissue elasticity (Seckel et al., 1997) and test the toxicity of some substances (Korani et al., 2011). It is similar to human skin, however thinner and with Langerhans cells containing fewer Birbeck granules than in humans (Sueki et al., 2000).

Cigarette smoke exerts multiple negative effects on human skin (Yin et al., 2000; Rajagopalan et al., 2016). It affects pathways related to oxidative stress, cell repair and tissue homeostasis, causes microvascular dysfunction (Rossi et al., 2014), induces fibroblast senescence *in vitro* (Yang et al., 2013) and causes premature skin ageing *in vivo* (Morita, 2007); it also negatively affects dendritic cells of the immune system (Nouri-Shirazi and Guinet, 2003).

Relaxin (RLX), initially known for its effects on reproduction and pregnancy, acts on numerous targets including the cardiovascular and respiratory systems and tegument. The availability of human recombinant RLX and the achievements on its biological effects, especially on connective tissue remodelling and cardiovascular physiopathology, have sparkled the interest of clinicians to better explore its therapeutic potential (Bani et al., 2009). RLX has revealed a potent antifibrotic activity which mainly relies on down-regulation of collagen production and of myofibroblast differentiation and increased collagen degradation (Samuel et al., 2007). Its administration has been investigated for the therapy of systemic sclerosis (Samuel et al., 2007; Bennett, 2009) and has been proposed for the treatment of keloids (Lee et al., 2012) . In a swine model, RLX enhanced skin extensibility (Kibblewhite et al., 1992) without significantly affecting dermal thickness (Samuel et al., 2003).

Recent research in our laboratory has investigated the pulmonary and vascular response of guinea pig to cigarette smoke inhalation and the influence of RLX on that response (Pini et al., 2016). Within the framework of that research, we have addressed if smoke stimulates a skin response in guinea pigs and if RLX influences that response.

Material and methods

Reagents

Relaxin (recombinant human H2 RLX, or serelaxin) was kindly provided by the RRCA Relaxin Foundation (Florence, Italy). Kentucky Reference cigarettes 3R4F, each containing 11 mg total particulate matter, 9.4 mg tar and 0.73 mg nicotine, were obtained from the Kentucky Tobacco Research Council (Lexington, KY). Unless otherwise specified, the other reagents used for the experiments were from Sigma-Aldrich (Milan, Italy).

Animals and treatment

Male Hartley albino guinea pigs, average weight 830 g, were bought from Harlan (Correzzana, Italy). Animal handling and use complied with the European Community guidelines for animal care (2010/63/EU) and were approved by the Committee for Animal Care and Experimental Use of the University of Florence. The animals were housed on a 12 h light/dark cycle at 22°C room temperature and had free access to

food and water. The experiments were designed to minimize pain and the number of animals used.

Experimental groups were as follows. (1) Control animals treated with RLX vehicle alone (N = 4). (2) Animals exposed daily to cigarette smoke for 8 weeks (N = 6). (3) Animals exposed daily to cigarette smoke for 8 weeks and treated with RLX 1 μg/d (N = 5). (4) Animals exposed daily to cigarette smoke for 8 weeks and treated with RLX 10 μg/d (N = 4).

The animals were exposed to cigarette smoke in a chamber (2.5 litres), similar to a vacuum desiccator equipped with an open tube for cigarette positioning at one end and a vacuum-connected tube and stopcock at the opposite end, modified from Das et al. (2012). To each group of smoke-exposed animals, five 3R4F reference cigarettes were administered daily. Each cigarette was fitted on the inlet tube and lit; then, a puff of cigarette smoke was introduced into the chamber containing the animals by applying a mild suction (4 cm water for 20 s). The guinea pigs were exposed to the smoke for further 40 s. After a pause of 60 s during which the chamber was opened and ventilated with fresh air, a second puff was administered with the same procedure. The gap between each of the 5 cigarettes/day was 1 h.

Relaxin was dissolved in phosphate buffered saline, pH 7.4 (PBS), and was given by continuous subcutaneous infusion using osmotic minipumps (Alzet, Durect Corporation, Cupertino, CA). The pumps were implanted on the back upon anaesthesia (intraperitoneal ketamine hydrochloride, 100 mg/kg of body weight, and xylazine, 15 mg/kg) one day before starting the exposure to cigarette smoke and were filled with 60 or 600 μg RLX to deliver the daily dose of the drug for the whole duration of the experiment.

At the end of the treatment, the animals were anesthetized as indicated before and sacrificed by decapitation, and tissue samples were collected for analyses.

Light microscopy and histochemistry

Skin samples were fixed in phosphate buffered formaldehyde, pH 7.2 (Immunofix, Bio-Optica, Milan, Italy), for 24 h and embedded in paraffin. Sections about 5 μm thick were stained with haematoxylin and eosin, astra blue (Bani et al., 2006) (Fluka, Buchs, Switzerland), trimethylrhodamine (TRITC)-conjugated avidin (6.25 ng/ml, at 37°C for 1 h) to show mast cell granules (Tharp et al., 1985), picrosirius red to show collagen fibres, Gomori's paraldehyde-fuchsin for elastic fibres. Stained non fluorescent slides were observed under a Microstar IV (Reichert, Depew, NY) or a BA310E microscope (Motic, Hong Kong), both equipped with a T900107 digital photocamera (Tiesselab, Milan, Italy) served by dedicated software (BELView, Bel Engineering Informer Technologies, Madrid, Spain). Fluorescent slides were mounted wet with Fluoromount (Diagnostic BioSystems, Pleasanton, USA), observed in an Axioskop microscope equipped for epifluorescence (Zeiss, Oberkochen, Germany) and captured with an Axio Vision 4 digital system (Zeiss).

To demonstrate the expression of heat shock protein 47 (Hsp47), an intracellular collagen-specific chaperonin (Sluijter et al., 2004), sections were soaked in PBS, followed by citrate buffer (pH 6.0) for 20 min at 95°C to expose antigenic sites and then let to cool to room temperature. Nonspecific binding sites were blocked with 10 ng/mL bovine serum albumin in PBS for 30 min at room temperature, with the addi-

tion of 0.5% triton X-100 (Sigma, Milan, Italy). The primary antibody (StressGen Biotechnologies Corp., Victoria, British Columbia, Canada) was applied 1:100 overnight at 4°C; a secondary, goat polyclonal anti-rabbit TRITC-labelled secondary antibody (Sigma-Aldrich; 1:50) was applied for 2 h at room temperature. Omission of primary antibody or substitution with irrelevant ones were used as negative controls. Microscopic observation was as specified above.

Morphometry

In slides stained with picrosirius red and with paraldehyde fuchsin, the intensity of staining per microscopic field was measured with Image J for Windows (NIH, Bethesda, MD) on 6 to 10 microscopic fields per animal at magnification x400. The area of each field was 0,051 mm^2. Each animal was assumed as a sample unit.

The cells labelled with avidin were counted on 20 microscopic fields (magnification x400, field area 0.036 mm^2) of superficial dermis and as many of deep dermis per animal. The cells labelled for Hsp47 were counted on 15-20 microscopic fields per dermal layer and per experimental condition, distributed among all the animals of each group. Each animal was assumed as a sample unit for statistics.

Since the intensity of fluorescent staining of mast cells with TRITC-conjugated avidin was almost always maximal, the amount of substances stored in mast cell granules was evaluated on slides stained with astra blue. Stained cells were delimited by hand, and their section surface area and average stain intensity were measured by Image J (NIH) software; the product of average stain intensity by cell section surface area was assumed as the total amount of label taken up by each cell. The intensity of astra blue staining represents the amount of granules per cell; its decrease indicates degranulation. To evaluate cells immuno-stained for Hsp47, photomicrographs taken at magnification x400 (1388 x 1040 pixel, reproduced tissue surface area 220 x 160 μm) were made binary and the threshold was selected manually so that only labelled cells were visible against the background. The surface area and mean fluorescence intensity of every cell were measured through Image J (NIH) software and multiplied by each other: the product was assumed as the total fluorescence intensity of that cell. For these analyses, each cell was considered a sample unit for statistics.

Electron microscopy

Skin samples were fixed in 2% formaldehyde and 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, osmicated and embedded in epoxy resin. Sections were stained with gadolinium acetate (Nakakoshi et al., 2011) (Electron Microscopy Sciences, Hatfield, PA) and either lead citrate or bismuth subnitrate, and observed in a Jeol JEM 1010 electron microscope (Tokyo, Japan) at 80 kV. Photomicrographs were taken with a MegaView III digital camera (Soft Imaging System, Muenster, Germany) served by a dedicated software (AnalySIS, Soft Imaging Software, Muenster, Germany).

Statistics

The data are reported as mean ± standard error of the mean (SEM). Differences among groups were evaluated with one-way ANOVA followed by Student–New-

man–Keuls multiple comparison test, using GraphPad Prism 2.0 statistical program (GraphPad Software, San Diego, CA, USA). $P<0.05$ was considered significant.

Results

Light microscopy

The skin tissue structure was similar among all groups. The epidermis was thin and with shallow rete pegs, the dermis contained hair follicles, sebaceous glands and a few sweat glands (Fig. 1).

Collagen fibres were thin and interwoven in the superficial dermis, of intermediate thickness in the middle dermis where they were interspersed with adnexa, and arranged in thick bundles in the deep dermis. Quantitative analysis of picrosirius red staining showed no significant differences in the density of collagen fibres among the experimental groups (Fig. 1a-d).

Paraldehyde-fuchsin stained elastic fibres were sparse and thin in the superficial dermis and numerous, relatively thick and interwoven in the middle layer, where they formed a mesh around adnexa. In the deep dermis, elastic fibres were rare and approximately as thick as those in the middle layer (Fig. 1e-h). No significant differences were shown by morphometry among the experimental groups.

Mast cells were significantly more numerous in the superficial dermis than in the intermediate and deep tissue layers. The number of mast cells decreased upon smoking, although not significantly. This number further decreased upon RLX, attaining values significantly lower than controls in the superficial dermis at any RLX dose; this decrease was significantly more marked upon 10 μg/d RLX than upon 1 μg/d RLX (Fig. 2a).

The intensity of mast cell staining with astra blue was significantly reduced by smoking in the superficial dermis. This modification was not significantly affected by RLX at either dosage (Fig. 2b, 3).

Immunohistochemistry

The number of Hsp47 positive cells was slightly higher in the superficial than in the deep dermis. In the deep dermis, it increased upon smoking, an effect that was inhibited by RLX 1 μg/d but not by RLX 10 μg/d (Fig. 2c, 4). However, the differences did not reach significance.

The fluorescence intensity per Hsp47 labelled cell in control animals was higher, albeit not significantly, for the deep than the superficial dermis; upon smoking the fluorescence intensity per cell increased significantly in both dermal layers. RLX counteracted in part this increase, with different dose-response relationship between dermal layers. In the superficial dermis the inhibition was progressively more marked with the dose and fluorescence values significantly lower than controls were attained with 10 μg/d. In the deep dermis the increase was significantly inhibited already by RLX 1 μg/d while RLX 10 μg/d produced a significant increase of fluorescence intensity over that upon smoke (Fig. 2d, 4).

Hsp47 positive cells were larger in the deep than in the superficial dermis in all experimental conditions, but the difference was not significant in control conditions.

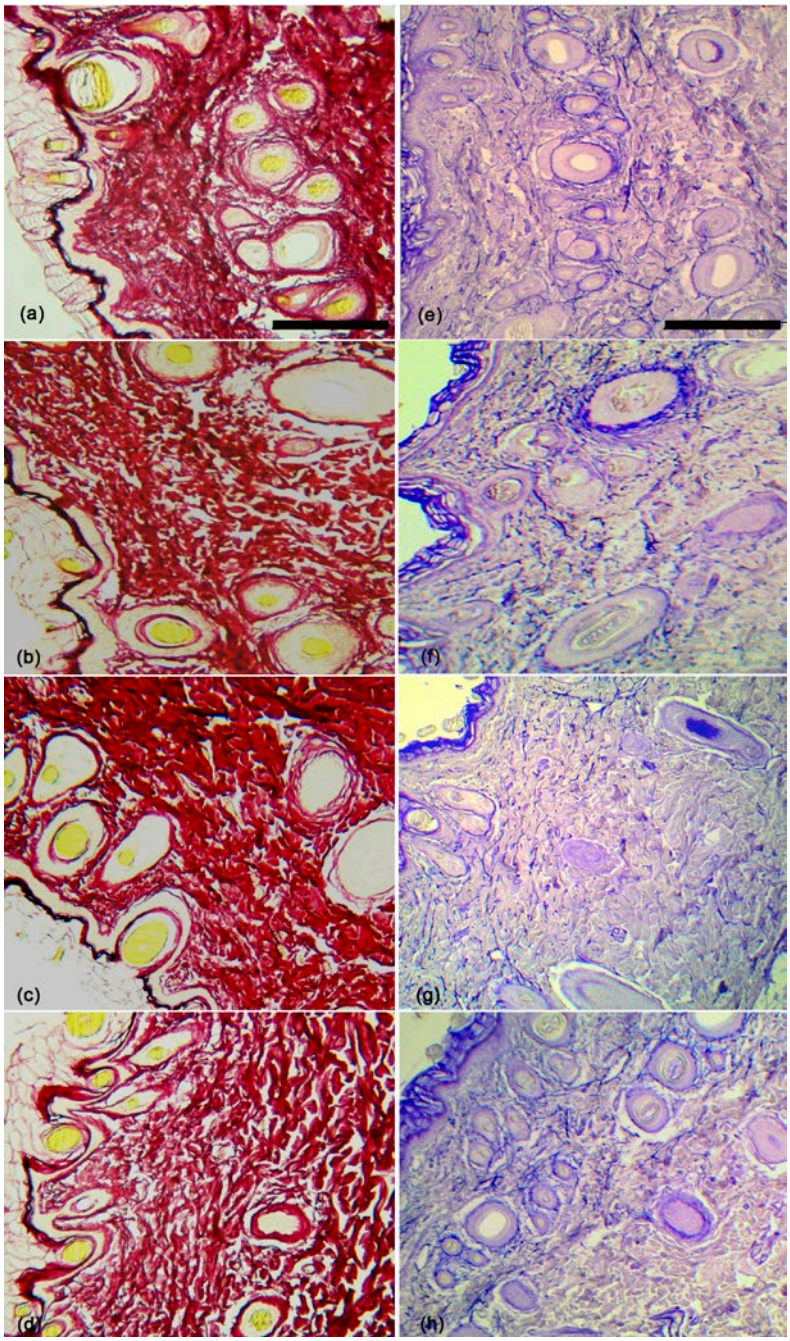

Figure 1. Guinea pig control skin stained with: (a-d) picrosirius red for collagen fibres; (e-h) paraldehyde fuchsin for elastic fibres. (a-e) Control; (b-f) Smoke; (c-g) Smoke plus RLX 1µg/d; (d-h) Smoke plus RLX 10µg/d. Scale bar = 170 µm.

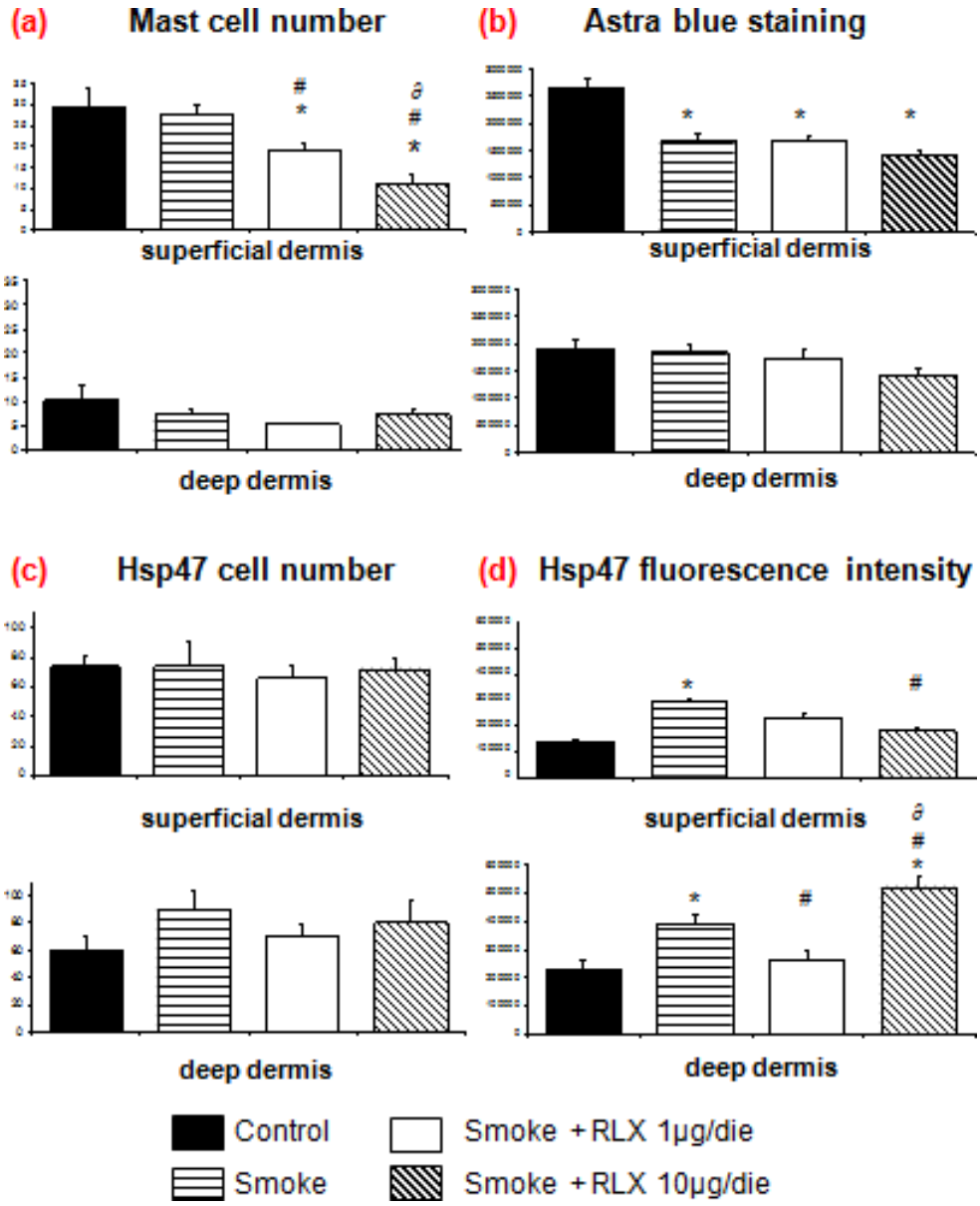

Figure 2. Cell counts and staining intensity. (a) Mast cell number per mm2 of skin section surface area. (b) Astra blue staining intensity of mast cells, in arbitrary units. (c) Hsp47 positive cell number per mm2 of skin section surface area. (d) Fluorescence intensity of Hsp47 positive cells, in arbitrary units. RLX: relaxin (at the indicated dose in µg/d). Symbols indicate significance ($p<0.05$) between experimental conditions, within a same layer: (*) compared with control; (#) compared with smoke; (∂) compared with RLX 1µg/d. For mast cell number and astra blue staining, control values in the deep dermis were significantly different ($p<0.05$) from those in the superficial dermis.

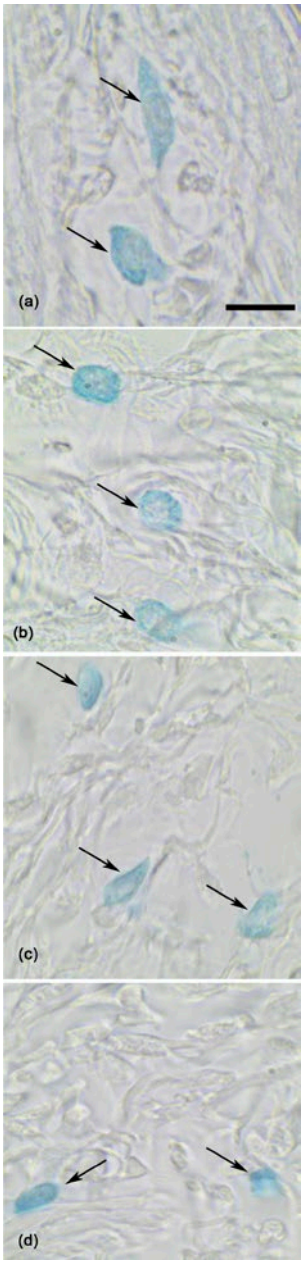

Figure 3. Astra blue stain of mast cells. Example of staining for the superficial dermis (a-d). a: Control. b: Smoke. c: Smoke plus relaxin 1µg/d. d: Smoke plus relaxin 10 µg/d. Scale bar = 20 µm.

Significant differences were induced by treatment in the deep dermis: smoke led to cell enlargement over controls, that was only slightly inhibited by RLX 1 μg/d (not significant) and on the contrary markedly increased upon RLX 10 μg/d ($p<0.05$ versus control and RLX 1 μg/d) (Fig. 5).

Electron microscopy

The epidermis appeared thin, with one layer of basal cells, one or two layers of prickle cells and one layer of granular cells, topped by a few layers of horny cells. Langerhans cells were located between the basal and prickle cell layers, had a small, ovoid body and few Birbeck granules with lightly marked inner structure; some Birbeck granules were bent, some had at one end a pale, tubular or slightly dilated portion (Fig. 6).

The dermis contained few cells of different types: fibroblasts, macrophages with well developed lysosomes, and mast cells with granules having a reticulated inner structure. In the basement membrane, the reticular layer lying under the lamina densa was thin and poor in fibrils. Blood vessels and fine nerves with non-myelinated fibres were identified in the superficial dermis; a few myelinated nerve fibres were found in nerves further away from the epidermis; the dermis also contained arrectores pili muscles.

The electron microscopic structure of the epidermis and dermis did not appear affected by any treatment.

Discussion

Normal guinea pig skin

The findings on the normal guinea pig skin were coherent with those reported by Sueki et al. (2000). It is possible that epidermal Langerhans cells have fewer Birbeck granules than those in human epidermis, because the guinea pig skin is protected by a fur, as suggested by data from hairless rat (Itagaki et al., 1995).

In the dermis, mast cells were more numerous in the superficial than in the deep part of the tissue, conceivably in relation with the different density of blood microvessels to which mast cells are associated. Many Hsp47 positive cells were seen sparse in the dermis. Hsp47 can be assumed as a marker of active fibroblasts

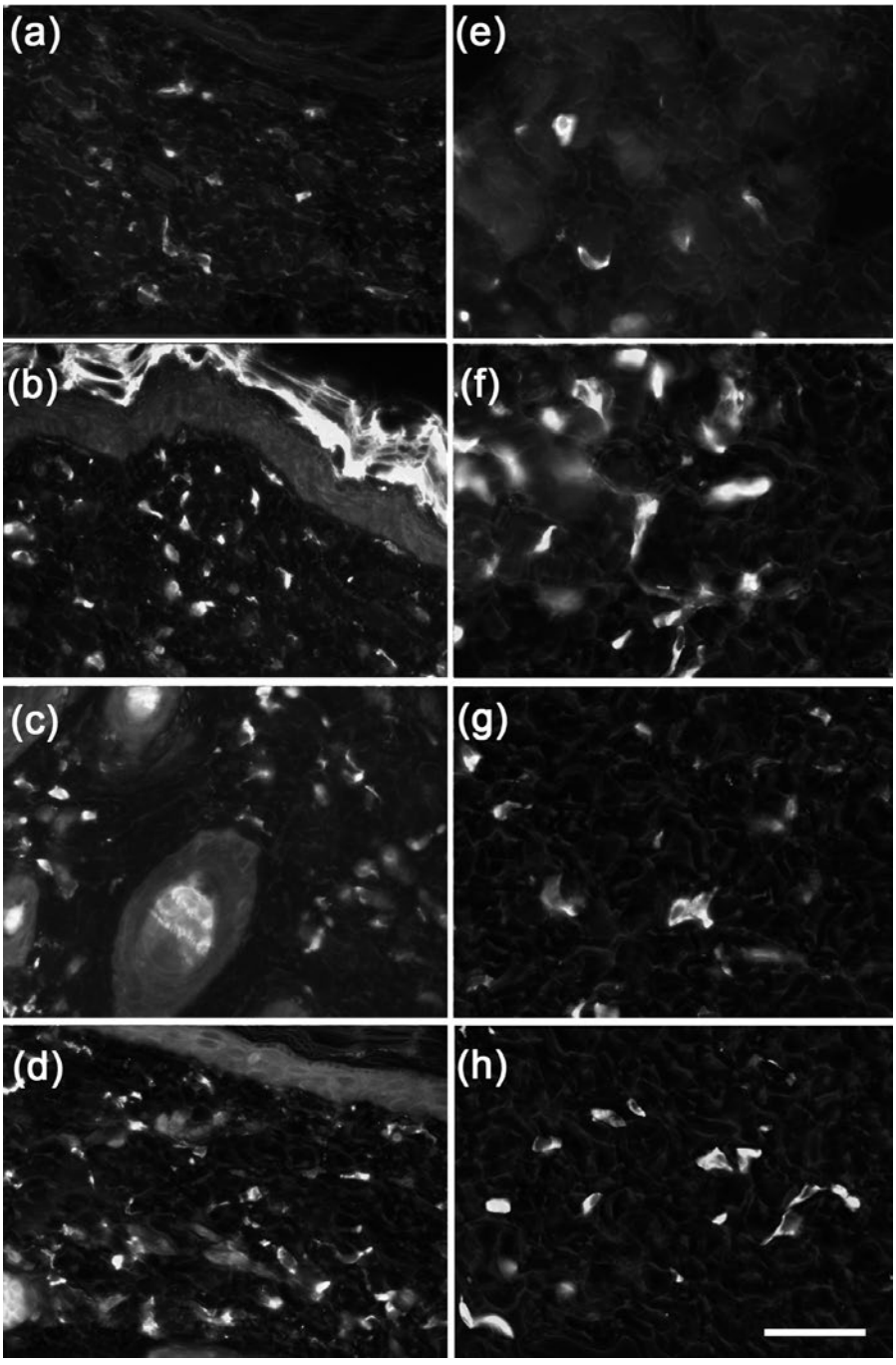

Figure 4. Hsp47 positive cells in superficial dermis (a-d) and deep dermis (e-h). a, e: Control. b, f: Smoke. c, g: Smoke plus relaxin 1µg/d. d, h: Smoke plus relaxin 10 µg/d. Scale bar = 40 µm.

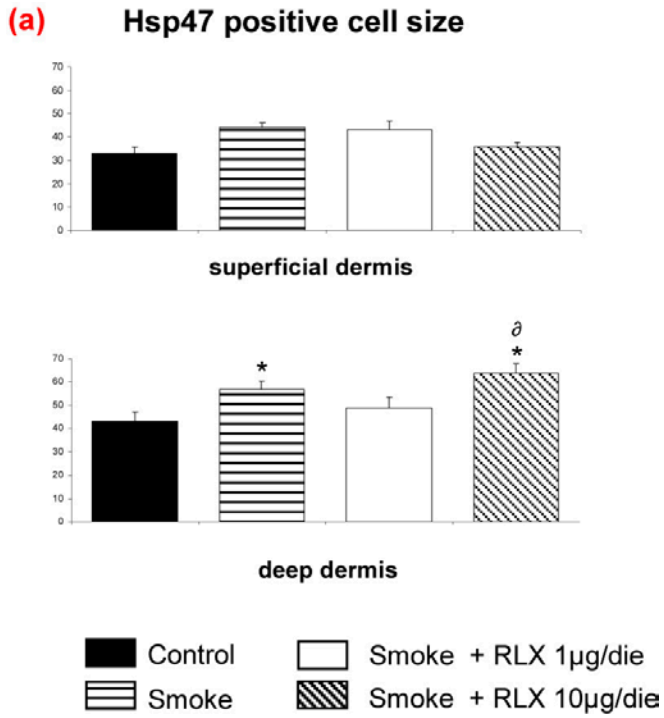

Figure 5. Size of Hsp47 positive cells in µm2. In the deep layer Hsp47 positive cells have a larger size than the Hsp47 positive cells in the superficial dermis in all experimental conditions. Symbols indicate significance ($p<0.05$) between experimental conditions, within a same layer: (*) compared with control; (∂) compared with relaxin 1µg/d. The size of cells in the deep dermis of the animals exposed to cigarette smoke and those exposed to smoke plus relaxin 10 µg/d was significantly larger than that of the cells of superficial dermis in the same conditions.

(Mehta et al., 2005). This chaperonin does not seem to have been an object of study in the guinea pig skin until now.

Effects of smoke on guinea pig skin

Smoke did not lead to changes in the epidermis, while it appeared to affect the dermis. Mast cells underwent reduction in number and in the intensity of astra blue staining, which depends on the amount of cytoplasmic granules. Both these parameters indicate active secretion of mast cell granules, because highly degranulated cells are no more recognizable histologically, and hint that mast cell secretion was increased by smoking. Components of cigarette smoke, such as nicotine, cause vasoconstriction of microvessels in the skin, which may lead to decreased moisture content and dryness, and poor wound healing (Mosely et al., 1977; Rossi et al., 2014). Increased mast cell degranulation in the superficial dermis may represent an effort to counteract vasoconstriction in the superficial dermis.

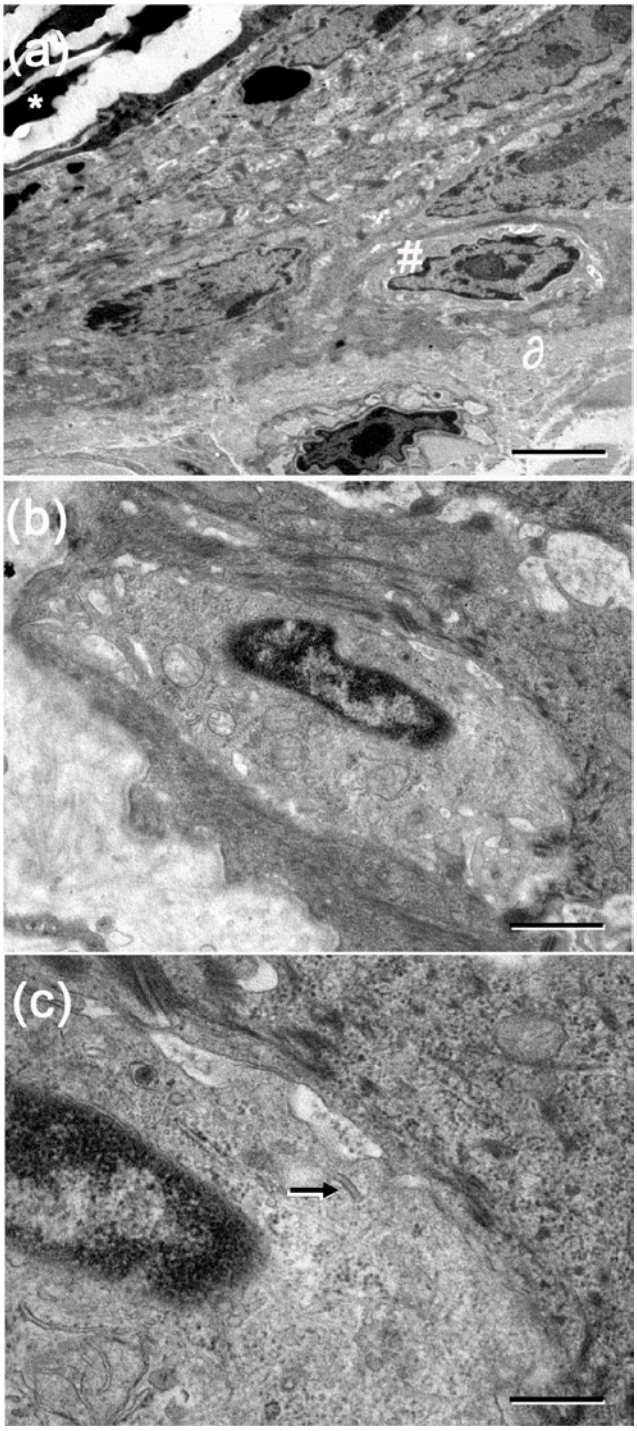

Figure 6. Electron microscopy. (a): Overview of epidermis from a smoke exposed guinea pig. The horny layer is at the upper left corner. The asterisk (*) indicates the granular layer. The hashtag (#) indicates a Langerhans cell. ∂ indicated the dermis. Scale bar = 2 µm. (b): Higher magnification of a Langerhans cell. Scale bar = 1 µm. (c): Detail of the cell of panel (b), showing a small Birbeck granule (arrow). Scale bar = 500 nm.

Smoke led to increase in the staining intensity of Hsp47 positive cells, assumed as active fibroblasts, at all dermal levels. The increase in production of this chaperonin was paralleled by an increase in cell size, especially in the deep dermis. *In vitro* studies had shown that skin fibroblasts undergo widespread damage if exposed to cigarette smoke extract, with inhibition of cell viability and proliferation (Rossi et al., 2014). The present results suggest that skin fibroblasts are not damaged by smoke *in vivo*, at least in this guinea pig model, at variance with smoke extract effect *in vitro*: this discrepancy may depend on differences in composition and concentration of smoke-derived substances that reach the tissue through the bloodstream upon smoke inhalation. Smoke tar does not reach the skin while carbon monoxide and nicotine do. This may affect cells both directly (through a receptor mediated mechanism) and indirectly (through vasoconstriction and damage to microcirculation). Fibroblasts express the α7-nicotinic receptor in humans and the α3-nicotinic receptor in mice (Arredondo, 2003). The stimulation of nicotinic receptor *in vitro* and *ex vivo* leads to reduced proliferation of fibroblasts (Xanthoulea et al., 2013). Studies *in vitro* have shown alternatively an increase (Chamson et al., 1992) or a decrease in collagen production by fibroblasts upon treatment with tobacco smoke extracts (Yin et al., 2000). *In vivo* the effect of tobacco smoke extract on mouse skin depends on the way of administration: at variance with topical or intradermal treatment, systemic (intraperitoneal) administration seems to have no effect on skin collagen bundles (Yang et al., 2013). These discrepancies give strength to *in vivo* animal models as suitable tools to study what may happen in clinics.

The effects of smoking on guinea pig skin are partially counteracted by RLX.

Our study indicates a partial protective effect of RLX on skin injury in the animals exposed to cigarette smoke, because the further reduction in number of dermal mast cells induced by RLX upon cigarette smoke exposure may be assumed as a sign of degranulation, hence of enhanced secretion of vasodilator and vasoprotective agents by these cells. In other organs RLX has an opposite effect on mast cells than that observed in the present study on the skin: in fact, it inhibits peritoneal and heart mast cell degranulation and histamine release *in vivo* (Masini et al., 1994; Nistri et al., 2008). These opposite effect of RLX could be related to heterogeneity of mast cells settled in different anatomical sites and tissues, as described in the literature (Dwyer et al., 2016). Therefore. RLX may be partially protective against cigarette smoke effects on the skin, but not on visceral organs.

Relaxin counteracted the increase in the staining intensity of Hsp47 positive fibroblasts in the superficial dermis in a dose dependent manner, RLX at low dose also counteracted such an increase in the deep dermis whereas at the latter level the higher dose of RLX stimulated a paradoxical increase in Hsp47 staining intensity over control values. This suggests a down-regulation of collagen production by low dose RLX, which fits well with its well-known anti-fibrotic properties. It can be speculated that the inconspicuous differences found in dermal collagen fibres depend on the fact that 8 weeks were insufficient to cause extensive remodelling in the skin tissue not directly exposed to the noxious effects of smoke, assuming that major modifications of collagen fibres usually take place in the long time. The paradoxical effect of the higher RLX dose may be explained by RLX receptor down-regulation by excess

ligand because of dimerization and negative cooperativity between receptor molecules (Svendsen et al., 2009). The difference between the superficial and the deep dermis in the fibroblast response to RLX administration upon cigarette smoke hints to a tissue specificity in the response to RLX, which needs to be taken into consideration when planning to use this molecule in a clinical setting.

Relaxin has not proven effective in the clinics to reverse established alterations of systemic sclerosis (Khanna et al., 2009), despite relaxin is able to interfere with mechanisms of fibrosis in several organs (McVicker and Bennett, 2017; Samuel et al., 2017). Those data and the present results suggest that an influence of possible clinical relevance can be expected during the initial, dynamic phase of any fibrotic process (be it scarring, systemic sclerosis or else), before fibrosis has established.

In conclusion, smoking leads to an increase in fibroblast activity in the guinea pig skin, as indicated by Hsp47 enhanced expression, and RLX can counteract this effect depending on the dose and the localization of responding cells. Guinea pig mast cells undergo modifications suggesting secretion of their granules after smoke and even more upon administration of RLX, which may represent a protective reaction against the vasoconstrictor effect of nicotine. This study also showed that guinea pig fibroblasts and mast cells behave differently between the superficial and the deep dermis. The presence of histological and functional compartments in the skin should be taken into account when planning and evaluating experiments and clinical treatments on this tissue in guinea pigs and possibly in other species including humans.

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Conflict of interest

The senior author is Editor-in Chief of the Italian Journal of Anatomy and Embryology.

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Research Article - Basic and Applied Anatomy

Penetrating chest injury in a case of situs inversus totalis

Nasirudeen Oladipupo Ajayi[1,2], Lelika Lazarus[1], Kapil Sewsaran Satyapal[1,*]

[1] Department of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Westville Campus, Durban, South Africa
[2] Benedictine Hospital, Nongoma, KwaZulu-Natal, South Africa

Abstract

Situs inversus totalis is a congenital condition characterized by the transposition of the thoracic and abdominal organs to the opposite side of the body. *Situs inversus totalis* is typically asymptomatic, however, these individuals are susceptible to certain defects and malformations such as vascular anomalies and mal-positioned viscera, which can result in a shortened lifespan. There are reports on penetrating abdominal injury in patients with *situs inversus*. In addition, the presence of pathology of thoracic and abdominal organs in *situs inversus* patients have been reported. However, from the literature reviewed, there is a paucity of reports on penetrating chest injuries in patients with *situs inversus*. Hence, we present a case of a patient who presented with a stab chest with *situs inversus totalis* and a brief discussion on the embryology of the condition.

Key words

Chest injury, situs inversus totalis, embryology.

Introduction

Thoracic and abdominal organs such as the lungs, atria, liver, spleen and stomach are commonly asymmetric. The position of these organs relative to the midline is described by the term situs solitus (Gindes et al., 2007). Historically, a case of the reversal of the liver and the spleen was reported by Fabricius in 1606 and the transposition of the viscera was first recognized by a clinician in 1824 (Cleveland, 1926). Situs inversus totalis (SIT) is a congenital condition characterized by the transposition of the thoracic and abdominal organs to the opposite side of the body (Nakano et al., 2017). This anomaly can be inherited through an autosomal recessive trait, however, the precise genetic cause is indeterminate (Nursal et al., 2001, Suh 2017). SIT can exhibit dextrocardia which is characterized by the reversal of the chambers of the heart (Suh 2017). In 90-95% of cases presenting dextrocardia with SIT, the heart is structurally normal (Marta et al., 2003). This is in contrast to dextrocardia with situs solitus referred to as dextroversion, which reports a higher frequency in structural defects (Marta et al., 2003). SIT is also characterized by abdominal organs such as the stomach and spleen located on the right side, as well as the liver and gallbladder located on the left side of the midline (Suh, 2017).

* Corresponding author. E-mail: satyapalk@ukzn.ac.za

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Situs inversus totalis can vary in incidence from 1 in 5000 to 20 000 individuals, and this is possibly as a result of different diagnostic methods (Oms and Badia, 2003; Uludag et al., 2017). People presenting with SIT are typically asymptomatic, however, these individuals are susceptible to certain defects and malformations such as vascular anomalies and mal-positioned viscera, which can result in a shortened lifespan (Sugawara et al., 2001; Giuliani et al., 2017). The anatomical variations presented in this condition can cause significant technical difficulties during surgical treatment, regardless of there being no obvious abnormalities in the functioning of the transposed organs (Uludag et al., 2017). The difficulty in diagnosis and surgical management of patients with SIT is a result of the contralateral disposition of the viscera (Oms and Badia, 2003). Procedures such as ultrasonography or auscultation of the heart during physical examinations may contribute to the accurate diagnosis of SIT (Nursal et al., 2001).

The occurrence of complete situs inversus has no gender or racial disparity. It is most often an isolated and accidental event occurring in an individual and rarely runs in families (Supriyah et al., 2013). There are reports on penetrating abdominal injury in patients with situs inversus. In addition, the presence of pathology of thoracic and abdominal organs in situs inversus patients have been reported. However, from the literature reviewed, there is paucity of reports on penetrating chest injury in a patient with situs inversus. Hence, we present a case of stab chest in a patient with SIT and a brief discussion on the embryology of situs inversus.

Case report

A 32 year old male presented to the emergency room of a rural hospital in South Africa approximately 10 hours after sustaining a stab wound to the chest. On examination he was found to have 3 cm deep laceration inferior to the middle third of the left clavicle. On clinical examination, he was not in respiratory distress; the trachea was centrally located, there was good air entry bilaterally on auscultation of the lungs. He was hemodynamically stable. Blood pressure and laboratory blood results were within normal limits. He had an erect chest x-ray which showed clear lung fields without hemo-pneumothorax but an incidental finding of dextrocardia and the location of the gastric bubble on the left of the abdomen was observed (Figure 1). The laceration was irrigated, cleaned and sutured. He was subsequently admitted for observation due to the depth of the laceration. An ultrasound of the abdomen revealed the transposition of the abdominal organs with a right-sided spleen. The liver and gall bladder were left-sided with the liver having a normal echotexture; the gall bladder presented with no calculi. A repeat chest x-ray 24 hours after admission to the hospital revealed a collapsed left lung with a hemo-pneumothorax (Figure 2). The patient was administered with a left thoracostomy tube for the management of the left hemo-pneumothorax and about 200 ml of blood were drained. A post-thoracostomy chest x-ray revealed the expansion of the left lung with visible left costophrenic angle (Figure 3). The thoracostomy tube was removed and the patient was counseled and discharged without any complication.

Table 1. Incidence of SIT.

Author (year)	Incidence of SIT
Applegate et al. (1999)	0.01% of general population
Nursal et al. (2001)	1:5000 20 000 hospital admissions
Sugawara et al. (2001)	0.025% to 0.005% of general population
Marta et al. (2003)	1:10 000 births
Oms and Badia (2003)	1: 5000 to 20 000 people
McKay and Blake (2005)	1:5000 – 20 000 people
Kouwenhoven et al. (2007)	1:10 000 of general population
Ramling and Dakshayani (2014)	1:10 000 live births
Giuliani et al. (2017)	1:5000 to 1:10 000 adults
Juma et al. (2017)	1-2 per 10 000 individuals
Nakano et al. (2017)	0.005% - 0.01% live births
Segel (2017)	6000 – 8000 live births
Suh (2017)	1:10 000 to 50 000 persons
Uludag et al. (2017)	1:5000 to 20 000 people

Discussion

Anatomic asymmetry is established during embryogenesis, and the left-right axis is established during early embryological development (Marta et al., 2003). Positioning of the organs and determining their asymmetries is orchestrated by a cascade of signal molecules and genes such as LEFTY, NODAL, IV (inversus viscerum), HAND, ZIC3, SHH, ACVR2B, and PITX2 genes (Marta et al., 2003; Sadler 2015). The gene PITX2 is responsible for determining left-sidedness and if expressed ectopically can lead to laterality defects (Sadler, 2015). The genes that influence the development of the right side are not as clearly described, however, the transcription factor SNAIL is thought to control the genes responsible for establishing right-sidedness (Sadler, 2015).

Situs inversus totalis is a laterality defect that occurs during embryological development (Uludag et al., 2017). This can be a result of random developmetal events, genetic or environmental factors (Catana and Apostu, 2016; Segel, 2017). Suh (2017) suggested that the development of SIT is due to immobility of the nodal cilia which inhibits the flow of extra-embryonic fluid during embryogenesis. Trulioff et al. (2017) suggested that the assumption of two types of cilia can provide a possible explanation for the role of cilia in SIT. This hypothesis assumes that there are movable primary cilia which generate and rotate extra-embryonic fluid flow, as well as immobile cilia which serve as mechanoreceptors of the flow and can influence the expression of the genes responsible for left-sided development (Trulioff et al., 2017).

Many people with SIT are oblivious of their uncommon anatomy until they seek medical attention for an unrelated condition (Supriyah et al., 2013). In the majority of cases (90-95 %), the presence of situs inversus with dextrocardia and normal anatomic relationship of the great vessels is associated with normal cardiac structure and

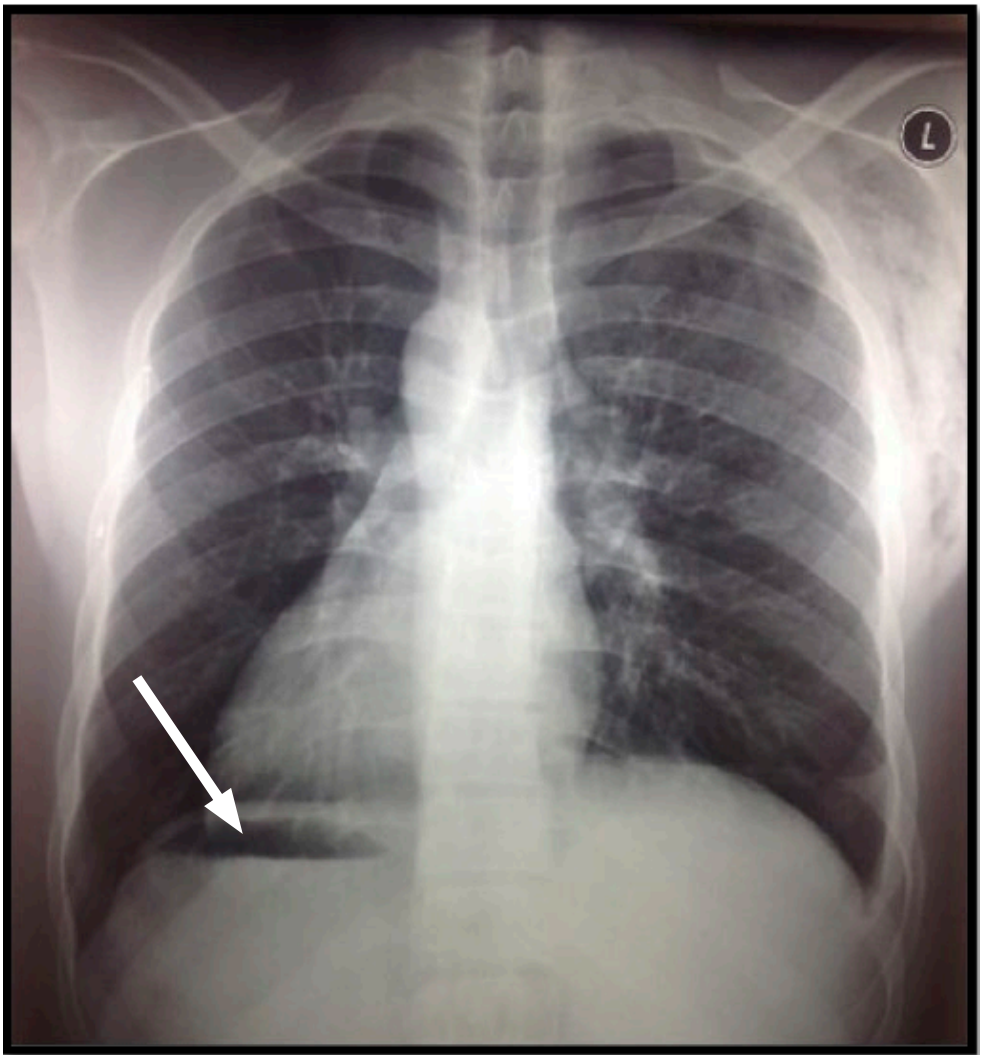

Figure 1. Chest X-ray (posteroanterior view) showing heart in the right hemithorax and the gastric bubble on the right (white arrow).

function. However, congenital cardiac disease such as tetralogy of Fallot or pulmonary artery atresia and interatrial or interventricular septal defects are not uncommon (5-10% of cases) (Marta et al., 2003). Therefore, most patients with situs inversus totalis live a normal life.

The presence of the combination of situs inversus, dextrocardia, bilateral cystic bronchiectasis and chronic sinusitis confirms the diagnosis of Kartagener's syndrome in these patients. (Mohan et al., 2007). Situs inversus may be associated with other congenital anomalies such as duodenal atresia, asplenism, multiple spleens, ectopic

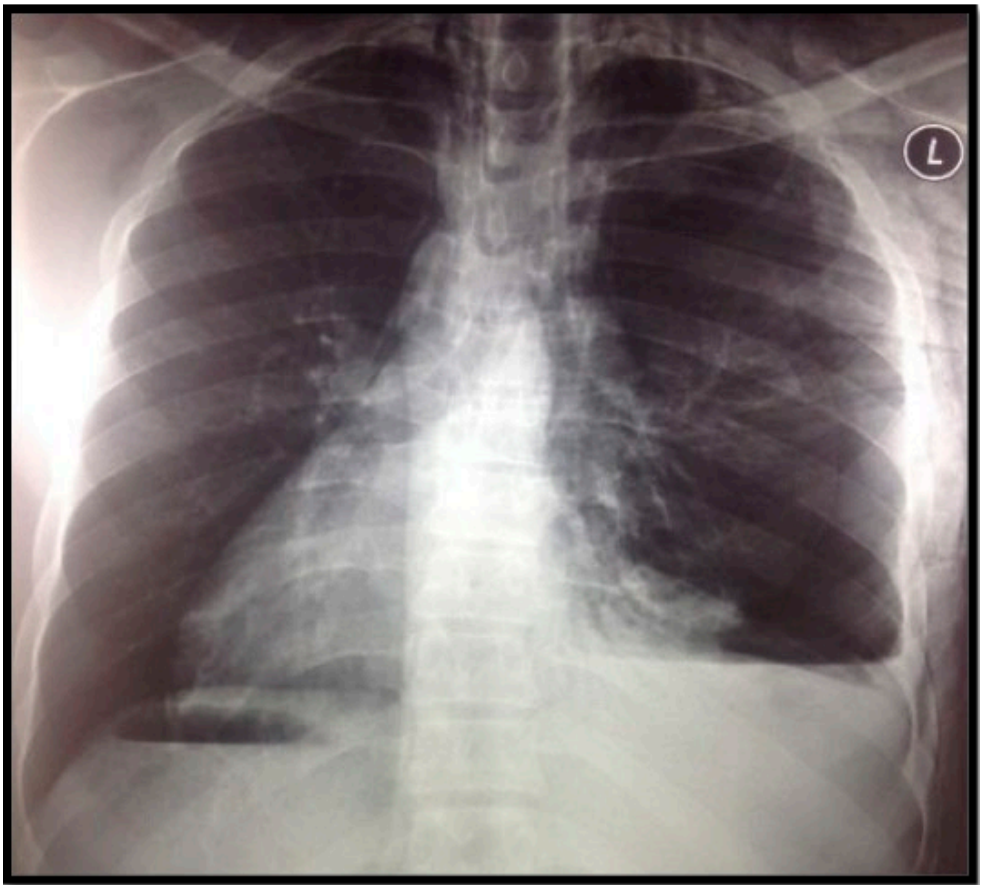

Figure 2. Chest X-ray (posteroanterior view) showing left hemo-pneumothorax.

kidney, horseshoe kidney and various pulmonary and vascular abnormalities (Kumar et al., 2014). On abdominal ultrasound, the patient in this case report had the liver and spleen transposed and there were no gross anomaly of the abdominal organs.

Penetrating trauma in the proximity of major vessels may result in vascular injury and the prompt detection of such injuries will facilitate early treatment and minimize complications (Williams et al., 2007). The clinical presentation in patients with situs inversus differs from what is found in most patients because the organs are a mirror image of the usual anatomy. The presence of situs inversus may prevent the occurrence of severe vascular injury or may be deleterious in some cases. In the present case report, the location of the stab to the left side of the chest could have affected some vital structures such as the arc of the aorta or the heart. However, the presence of dextrocardia and 'inversion' of the thoracic organs may have protected the patient from serious vascular injury had the penetration been very deep or lower in the thoracic cavity. Therefore, in cases where the clinical presentation does not correlate with

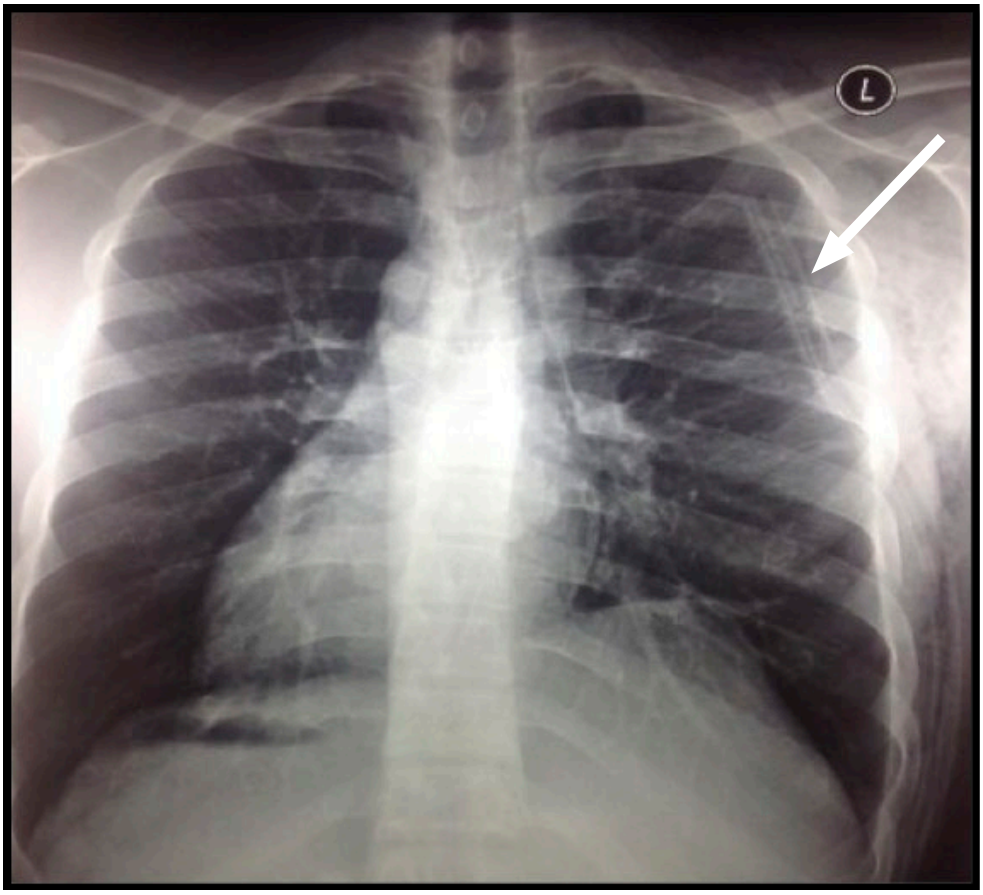

Figure 3. Chest X-ray (posteroanterior view) showing re-expansion of the left lung and resolution of the hemo-pneumothorax with the chest tube (white arrow) in-situ.

the location of the injury or suspected pathology, an unusual anatomy of the thoracic or abdominal organs should be suspected.

The patient in this report was successfully managed with a thoracostomy tube drainage for his hemo-pneumothorax which does not differ to the management for a situs solitus patient. Surgical procedures can be performed successfully in situs inversus, however, such operations are technically challenging (Ahmad et al., 2015). The identification of situs inversus is important for preventing surgical complications that could result from the inability to recognize the reversed anatomy (Casanova et al., 2008). Due its rarity, situs inversus may complicate organ transplantation procedures as donor organs will most likely come from situs solitus donors (Supriyah et al., 2013).

Conclusion

Penetrating thoracic injury in a situs inversus patient could be safely managed as in patients with situs solitus. The 'inversion' of the thoracic organs maybe of an advantage as it may preclude severe vascular or organ injury.

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Research Article - Embryology

A comparative study of placental morphometry in diabetic and normal mothers in a tertiary care hospital of West Bengal

Sarmistha Chakraborty[1], Santanu Bhattacharya[2,*]

[1] Demonstrator, Department of Anatomy, Calcutta National Medical College, Kolkata-14, West Bengal, India
[2] Associate Professor, Department of Anatomy, Coochbehar Govt. Medical College & Hospital, West Bengal, India

Abstract

Among the hundred cases ranging from 19-38 years, fifty placentae were from normal uncomplicated pregnancies and the rest from diabetic mothers. In case of normal pregnancies, the average (±standard deviation) placental weight, volume, thickness, diameter, area and circumference were 513.3 (±53.13) g; 437.4 (±59.8) cm^3,1.79 (±0.24) cm, 17.5 (±1.52) cm, 242.19 (±40.21) cm^2, 54.95 (±4.78) cm respectively. In case of diabetic mothers, the corresponding values were 579.2 (± 44.3) g, 503.9 (± 46.11) cm^3, 2.45 (±0.49) cm, 17.56 (±1.57) cm, 243.96 (±41.41) cm^2, 55.14 (±4.93) cm. The average birth weight of baby and foeto-placental ratio were 2.55 (±0.25) kg and 4.97(±0.5) respectively in normal cases and 3.42 ± 0.26 kg and 5.91 ± 0.33 respectively in cases of diabetic pregnancy. Among the different parameters the birth weight of baby was the best predictor of the placental morphometric parameters while body weight and age of mother were poor predictors.

Key words

Pregnancy, diabetes, placenta.

Introduction

Diabetes mellitus (Type 1, Type 2 and gestational) is now a major health concern in our society. Pregnancy complicated by diabetes mellitus has been associated with alteration in placental anatomy and physiology (Fletcher,1981). Although transplacental glucose flux flow is limited and independent of glucose transporter availability, transport of essential and non-essential amino acids, and expression of genes involved in lipid transport and metabolism are significantly affected by diabetes mellitus (Diamant et al., 1982).

According to the Centres for Disease Control and Prevention, from 1980 to 2005, the crude incidence of diagnosed diabetes mellitus increases from 3.3 per 1000 to 7.4 per 1000. Studies suggest that the prevalence of diabetes mellitus among women of childbearing age is increasing due to more sedentary lifestyles, changes in diet, and the virtual epidemic of childhood and adolescent obesity that is presently involving our country (Park, 2005).

The placenta is a membranous vascular organ of female mammals except monotremes and marsupials. It develops during pregnancy from the chorion of the embryo

* Corresponding author. E-mail: dr.santanubhattacharya@gmail.com

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and decidua basalis of the maternal uterus and connects the foetus with the maternal uterine wall (Sadler, 2010). The human placenta is aptly described as "discoid" (due to the shape), "haemochorial" (due to direct contact of the maternal blood with chorion), "deciduate" (as same amount of maternal tissue is shed during parturition) and "villous" (labyrinthine) structure (Sadler, 2010). The placenta usually remains attached to the upper part of the posterior wall of uterus. It has a rough, shaggy looking maternal surface, which is mapped out into 15-20 cotyledons separated by intervillous septae and a smooth, shiny foetal surface covered by chorion and amnion. The site of insertion of umbilical cord either centrally, marginally or eccentrically (Cunningham et al., 2010).

Placenta is the mirror of maternal and foetal status and the most accurate record of the infant prenatal experience. After delivery, if the placenta is examined minutely it provides much insight into the prenatal health of the baby and the mother (Cunningham, 2010).

The aim of the present work is to improve the knowledge about gross and morphometric changes of diabetic placenta in West Bengal population.

Materials and methods

After acquiring local ethical committee approval and informed patient consent, the materials of the present study i.e. placentae were collected at random from pregnancy cases attending the Department of Obstetrics and Gynaecology, NRS Medical College and Hospital, Kolkata, during the period one year. All the cases included in the study were booked cases according to WHO, in each case antenatal check-up was done routinely.

The placentae collected from cases included in this study were broadly divided into two groups.

Group A: Normal uncomplicated pregnancy, 50 cases;

Group B: Pregnancy complicated with diabetes mellitus (Type I, Type II and Gestational diabetes mellitus), 50 cases.

Each group comprised of patients with known last menstrual period), expected date of delivery, blood sugar levels, body weight and gestational period ranging from 36 to 40 weeks.

The following parameters were measured or computed: Weight, volume, thickness, diameter, area and circumference of placenta and cord insertion site.

An electronic weighing device was used to measure the weight. The volume of the placenta was determined by water displacement method. The diameter of the placenta was measured with a measuring tape. For measuring the thickness, a thin long graduated needle was inserted at the centre, at the margin and midway between the centre and margin and the average of the three reading was taken as the thickness of the placenta.

The area (A) was computed from quation $A = \pi \times (\text{maximum diameter})/2 \times (\text{minimum diameter})/2$. The circumference (P) was computed from $P = \pi \times (\text{maximum diameter} + \text{minimum diameter})/2$. To estimate the cord insertion site, the minimum distance between the site of cord insertion and the margin of the placenta was measured and denoted as 'x'; assuming the placenta to be a perfect circle, the mean

radius 'r' was obtained and then the insertion site was worked out as x/r x 100 and expressed in per cent.

Body weight and age of mother at the time of delivery, birth weight of baby and with gestational age were also recorded.

The data were tabulated in a Microsoft Excel spread sheet and analysed by Epi-info 3.5.1 software (Centres for Disease Control and Prevention (CDC), Atlanta, GA).

Results

One hundred cases were included in the study, 50% of which were diabetic. Among the normal pregnancies, six babies were born at preterm and 44 were born at term and among the diabetic mothers only one had undergone preterm delivery, the rest of the mothers delivered at term.

The mothers included in this study were from different age groups starting from 19 years to 38 years with average value and standard deviation of 27.92 and 5.08 years respectively; the commonest observed age was 24 years. The mean gestational age was 37.17 weeks (range: 32-39 weeks) with standard deviation 1.08 weeks. The commonest gestational age was 37 weeks.

There was a great variation in the body weight of the mothers, ranging from 50 to 79 kg with average weight and standard deviation of 65.68 kg and 7.98 kg respectively. The most frequently observed weight was 72 kg. The average body weight of baby was 2.983 kg with standard deviation of 0.508 kg. The commonest observed body weight of baby was 2.500 kg, ranging from 1.600 to 4,000 kg.

Table 1 shows the analyzed parameters of normal and diabetic mothers and their babies; significant differences in the average body weight of mothers and in the birth weight of babies were observed between normal and diabetic mothers (P value < 0.05).

Among the different parameters, significant differences were found for placental weight, volume and thickness, birth weight of baby and foeto-placental ratio; the results are shown in Table 2.

Table 3 shows the different types of insertion of umbilical cord. Medial eccentric variety was the commonest type of insertion of umbilical cord on the foetal surface of placenta.

The correlation among different parameters are shown in Table 4. Baby weight at birth was the best. Baby weight at birth was the best predictor of placental morphometric parameters and in contrast body weight and age of mother were poor predictors for those parameters.

Table 5 shows the linear regression equations for all placental morphometry parameters. The coefficient of determination, r^2, allows to determine how certain one can be in making predictions from a certain model/graph and denotes the strength of the linear association.

Gestational age at delivery did not show any significant correlation with placental morphometry parameters in case of diabetic mothers though it showed a moderate correlation with placental diameter, placental circumference, placental area and foeto-placental ratio in normal uncomplicated pregnancy (Table 4).

Table 1. Parameters of normal and diabetic mothers.

Parameters		Mean	Range	Mode	Standard deviation	*p*
Age of mother (years)	Normal	28.68	19-38	26	5.4415	not significant
	Diabetic	27.16	19-36	23	4.6174	
Body weight (kg)	Normal	63.8	50-76	55	8.3910	< 0.05
	Diabetic	67.56	54-79	70	7.1462	
Gestational age at delivery (weeks)	Normal	37.34	32-39	38	1.5066	not significant
	Diabetic	37.0	36-38	37	0.2020	
Birth weight of baby (kg)	Normal	2.5480	1.6-3.1	2.5	0.2557	<0.01
	Diabetic	3.4180	3-4	3.5	0.2651	

Table 2. Morphometric parameters of placenta from normal and diabetic pregnancy.

Parameters	Normal pregnancy (n=50)			Diabetic pregnancy (n=50)			*p*
	Mean	Range	SD	Mean	Range	SD	
Placental weight (g)	515.3	350-600	53.13	579.2	500-675	44.3	<0.001
Placental volume (cm^3)	437.4	300-530	59.8	503.9	410-570	46.11	<0.001
Placental thickness (cm)	1.79	1-2.5	0.24	2.45	1.8-3.2	0.49	<0.001
Placental diameter (cm)	17.5	12-20	1.52	17.56	12-20	1.57	not significant
Placental area (cm^2)	242.19	113.04-314	40.21	243.96	113.04-314	41.41	not significant
Placental circumference (cm)	54.95	37.7-62.8	4.78	55.14	37.7- 62.8	4.93	not significant
Birth weight of baby (kg)	2.55	1.6-3.1	0.25	3.42	3-4	0.26	<0.001
Foeto-placental ratio	4.97	3.2-5.71	0.5	5.91	5-6.36	0.32	<0.001

Table 3. Insertion of umbilical cord on the foetal surface of placenta.

No. of cases	Central	Eccentric		
		Medial	Lateral	Marginal
Normal (n = 50)	14 (28%)	22 (44%)	12 (24%)	2 (4%)
Diabetic (n = 50)	20 (40%)	23 (46%)	6 (12%)	1 (2%)
TOTAL (n = 100)	34 (34%)	45 (45%)	18 (18%)	3 (3%)

Discussion

Relevant studies of different anomalies of placenta in relation to its morphometry are discussed sequentially.

Brody and Frenkel (1953) reported that, in 70% of premature labour, the cord was inserted marginally into the placenta. Aherne and Dunhill (1966) described relation

Table 4. Correlation coefficients (r) of baby weight at birth, body weight of mother during delivery, gestational age at delivery and age of mother with morphometric parameters of placenta. Strong to moderate correlation is indicated in bold.

Parameters	Baby weight at birth (kg)		Body weight of mother at delivery (kg)		Gestational age at delivery (weeks)		Age of mother (years)	
	Normal (n = 50)	Diabetic (n = 50)	Normal (n = 50)	Diabetic (n = 50)	Normal (n = 50)	Diabetic (n = 50)	Normal (n = 50)	Diabetic (n = 50)
Placental weight	**0.50**	**0.74**	0.00	0.00	0.10	0.00	0.00	0.14
Placental volume	**0.42**	**0.72**	0.00	0.10	0.22	0.00	0.00	0.28
Placental thickness	**0.46**	**0.44**	0.14	0.14	0.28	0.00	0.00	0.10
Placental diameter	**0.56**	**0.54**	0.17	0.00	**0.35**	0.00	0.00	0.10
Placental circumference	**0.57**	**0.54**	0.17	0.00	**0.35**	0.00	0.00	0.10
Placental area	**0.54**	**0.54**	0.17	0.00	**0.32**	0.00	0.00	0.10
Foeto-placental ratio	**0.50**	**0.39**	0.00	0.00	**0.42**	0.10	0.10	0.17

Table 5. Linear regression equations between placental morphometric indices and baby weight at birth.

Parameters	Baby weight at birth (kg)	
	Normal (n = 50)	Diabetic (n = 50)
Placental weight (kg)	r^2 =0.25 =252.311+103.214×Baby weight	r^2 =0.54 =158.654+123.039×Baby weight
Placental volume (cm^3)	r^2 =0.18 =186.369+98.521×Baby weight	r^2 =0.52 =75.643+125.295×Baby weight
Placental thickness (cm)	r^2 =0.21 =0.685+0.435×Baby weight	r^2 =0.20 = -0.362+0.825×Baby weight
Placental diameter (cm)	r^2 =0.31 =8.993+3.339×Baby weight	r^2 = 0.29 =6.746+3.164×Baby weight
Placental circumference (cm)	r^2 =0.32 =28.158+10.517×Baby weight	r^2 =0.29 =21.167+9.939×Baby weight
Placental area(cm^2)	r^2 =0.29 =25.364+85.097×Baby weight	r^2 =0.29 =-44.615+84.427×Baby weight
Foeto-placental ratio	r^2 =0.24 =2.500+0.970×Baby weight	r^2 =0.15 =4.278+0.477×Baby weight

between birth weight of babies, placental area and volumes in normal infants. They also obtained a reduction in the placental volume of abnormally small infants (350 ± 65m). Benirschke and Driscoll (1967) described that velamentous insertion of cord is associated with foetal malformation.

Younoszai and Haworth (1969) established that infants with smaller than normal placentae for their gestational ages also were found to suffer from intrauterine growth retardation. Thomson et al. (1969) found that the placenta of diabetic women tends to be heavier than that of the non-diabetic women. These placental were also paler in colour due to villous oedema and the placental weight and size are directly proportional to birth weight of the babies. Foetal macrosomia, congenital malformations and intrauterine growth retardation are commonly seen in poorly controlled diabetes (Fletcher, 1981). Benirschke and Kaufman (2000) proposed that the placenta is the most accurate record of the infant's prenatal experience. They suggested that if the placenta is minutely examined after delivery it may provide much insight into the prenatal life of the neonate and maternal complications during pregnancy if any. Complications like hypertension, diabetes mellitus of the mother developing during present pregnancy also causes several relevant changes in placenta.

The placenta in pregnancy complicated with diabetes is generally larger than normal and has numerous structural abnormalities that are likely to have a role, resulting in disturbances of foetal growth and development (Singer, 1984). Normal placenta at term has a thickness of about 2.5 cm to 3 cm. If it is less than 2.5 cm it may be associated with pre-eclampsia, intra- uterine growth restriction etc. and thickness greater than 4 cm is usually found in case of maternal diabetes and intrauterine foetal infections (Kaplan, 1996).

According to Park (2005) the diabetic pregnancy is characterized by numerous disturbances in the foetal growth and development. Majumdar et al. (2005) showed in their study that maternal factors causing small weight placenta are also related with small birth weighted baby. Jansson et al. (2006) suggest that altered placental function may be a mechanism contributing to foetal over growth in diabetic pregnancies.

There have been many reports in the literature of placental weights and foeto-placental ratios at different gestational ages. It is well known that in normal preterm and term infants there is a direct relation between the birth weight of babies and the weight of the placentae The chorionic villous surface area is dependent upon the placental weight and infant weight in turn is related to the chorionic surface area. After the 36^{th} week placental weight increases due to hyperplasia of fibrous tissue. Placentae weighing over 600 g are usually found in gestational diabetes (Cunningham at al., 2010).

Conclusion

In diabetic mothers all the placental morphometric parameters (weight, volume, thickness, diameter, area, and circumference) tend to become higher in comparison to the normal placentae. Birth weight of the new born of diabetic mother is strongly associated with placental weight and volume and significantly higher than the baby of normal mother. The present study depicts the actual scenario of West Bengal population where incidence of diabetes is growing day by day. Therefore, further research to study these changes in vivo would be worthwhile in future.

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Research Article - Education in Anatomy and Embryology

The anatomical representation of the human body: From epistemological examples deriving from medical history to morphometric imaging performed with the laser scanner technique

Giacomo Gelati, Ferdinando Paternostro*, Andrea Alberto Conti, Giovanni Orlandini

Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Abstract

The anatomical illustration of the human body is a topic rich in epistemological elements in the course of medical history. Since ancient times concerns about the real correspondence of the scientific and/or artistic representation of human anatomy with the original one arose. First of all, a static two-dimensional representation, the one typical of drawings, was not able to get the depth and complexity of dynamic three-dimensional anatomical morphology. In addition, the epistemic issue that a post-mortem illustration could not somehow correspond to living structures was present even in the past. For a long time the anatomical representation of the human body has been attracting the interest of medical doctors, artists, scholars and philosophers as a fact-finding technique of dissection of corpses preparatory to curative surgical practice in the living body. With regard to that, in the Western world the sixteenth century is often seen as the golden age of normal and macroscopic human anatomy. Major steps in the evolution of the anatomical discipline are the switch from the "normal" to the "pathological" area during the seventeenth century and the transition from the macroscopic to the microscopic level in the eighteenth century; that is true also from an illustrative and iconographic point of view. The tradition of setting up three-dimensional models for the study of the human body dates back to the eighteenth century too. Today's research techniques in the field of anatomical images are so advanced that they allow the full conformity of human representation, the continuous availability of preserved images, the complete multi-dimensionality of the rendering and the complete dynamism of the whole view. In this context, laser scanner could be the ideal tool to create a new Atlas of Human Anatomy composed of models which are rotatable, observable from every perspective, absolutely faithful to reality, analysable as in a real dissection and carefully measurable.

Key words

Anatomy, history of medicine, epistemology, research methods, laser scanner, morphometry.

The anatomical illustration of the human body throughout history of medicine and scientific progress is a fascinating topic rich in epistemological elements.

Since ancient times concerns about the real correspondence of the scientific and/or artistic representation of human anatomy with the original one arose (Carlino, 2000). First of all, a static two-dimensional representation, the one typical of drawings, was not able to get the depth and complexity of dynamic three-dimensional

* Corresponding author. E-mail: ferdinando.paternostro@unifi.it

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anatomical morphology. In addition, the epistemic issue that a post-mortem illustration, even if accurate, could not correspond to living structures and to morphological vitality was present even in the past (Conti and Conti, 2010).

For a long time the anatomical representation of the human body has in any case been attracting the interest of philosophers, artists and medical doctors (Conti, 2011).

In the IV century BC the Greek philosopher and logician Aristotle from Stagira (384-322 BC) dealt with the anatomical illustration of female and male structures and, some decades later, Herophilos of Chalcedon (335-280 BC) and Erasistratus of Ceos (305-250 BC), the founders of the medical school of Alexandria (Egypt), performed some of the first autopsies (Cosmacini et al., 1996). These two anatomists began to practice the dissection of animals in a systematic way and they were also pioneers in the anatomical study of the human body (Porter, 1995).

In the II century AD the physician Galen of Pergamon (129-201 AD) dealt with the schematic illustration of the human body in his "De usu partium". Galen was a great expert of the anatomy of various animals, from pigs to monkeys, from dogs to goats, and set a new anatomical descriptive standard which influenced the study of the discipline until Renaissance (French, 1993).

Between the XIII and the XIV centuries, the scholar and precursor of French surgery Henri de Mondeville (1260-1320 AD) developed illustrative plates of advanced anatomical interest and he used to employ these plates as a didactic tool during his lessons. Iconography was essential for the anatomical study, in particular when the direct visualization of human body structures was not formally possible (for religious or legal reasons) or technically feasible (because of the scarce availability of corpses). In this context, the physician and anatomist Mondino de' Liuzzi (1270-1326 AD) used human cadavers for his medical lessons (anatomical lessons) for the first time in Italy and ordered to place the corpses of two women in a University classroom (Feher, 1989). The bodies were placed in horizontal position to be dissected, as it was appropriate in the educational and institutional environment of the University, and no more in vertical position, as pork butchers used to do when they slaughtered pigs (Grmek, 1998).

This change in the position of the body to be dissected (etymologically, to undergo "anatomy" means to undergo "cut", "dissection") represented a major cognitive and procedural innovation during the Middle Ages in the Western world, pioneering the Renaissance anatomical revolution of Andreas Vesalius. Vesalius (1514-1564 AD), a Flemish physician and a very young lector in surgery and anatomy in Padua, determined a paramount change of paradigm in the anatomical field, challenging the work of Galen in a systematic way, also thanks to an innovative methodology of morphological representation (Lippi and Baldini, 2000). "Tabulae Anatomicae Sex" (1538 AD) are among the first scientific illustrations explicitly conceived for University students and they were useful to the critic reconsideration of Galenic writings (Siraisi, 1990). The anatomical plates masterfully drawn by the German painter and engraver Johannes Stephan von Kalkar (1499-1545 AD), which accompanied the text "De Humani Corporis Fabrica" (1543 AD), the most important work of Vesalius, still today represent a milestone in the two-dimensional human anatomical representation, since they referred to positions (although they were static and two-dimensional) inspired to the dynamic posturology of the living body (O'Malley, 1964).

Some historians of medicine consider the XVI century as the golden age of normal and macroscopic human anatomy, interpreted as a fact-finding technique of dissection of

corpses preparatory to curative surgical practice in the living body (Pazzini, 1947). Successive steps of the history of the anatomical discipline were the switch from the "normal" (that is the study of human morphology in healthy subjects) to the "pathological" area during the XVII century and the transition from the macroscopic to the microscopic level in the XVIII century, and this was relevant also from an illustrative and iconographic point of view (Richardson, 1987). Even the tradition of setting up three-dimensional models for the study of the human body dates back to the XVIII century and it is fundamental at the anatomical representative level too. With regard to that, the leather-covered puppets prepared by the Scottish physician William Smellie (1697-1763 AD) were essential for the progress of anatomical-clinical education (Porter, 1997). Smellie was a pioneer in the obstetrical-gynaecological field with his fundamental work "Treatise on the Theory and Practice of Midwifery" (1752 AD) (Premuda, 1957).

The Italian ceroplastics deserves to be mentioned in the anatomical eidetic-educational framework (Musajo Somma, 2007). In the century of Enlightenment the morphological representative tradition in medicine was in fact enriched by an original and innovative contribution, coming from Bologna and Florence, specifically anatomical ceroplastics, the art of re-creating whole bodies or single anatomical parts through wax modelling (Orlandini and Paternostro, 2010). Ceroplastics worked as a bridge between the two-dimensional illustration and the three-dimensional representation of the human body, anticipating the scientific imaging of the XX century, thanks to the simultaneous attention both for details and for the subject as a whole.

The photographic representation of anatomy is worthy of note. Since the invention of the technique, photography allowed to fix the image of anatomical preparations with high fidelity to particulars, bypassing the artistic representation by the author, apart from accuracy.

Even if frames can be layered and assembled in sequence, they remain static, so they offer a limited possibility of interaction with the observer.

The advent of information technology and of graphic synthesis techniques has allowed to realize four-dimensional human virtual models. They can be rotated according to the three spatial axes and, thanks to this, they can be observed from every point of view. They allow to visualize the whole body or to emphasize the particular structures we are interested in: it is possible to zoom in on muscles, vessels, nerves, on their topographic relation; it is possible to observe the organs in situ or isolated. It is even allowed to observe cavities, organs or the whole body dissected according to sagittal, frontal or transverse planes. For example, muscles which are characterized by a very complex organization can be visualised singularly or all together and even their dynamic action can be showed thanks to some animations. It is also possible to resect superficial anatomical structures, in order to reach deeper ones and vice versa. The possibility of observing the examined object both intact and dissected from every point of view, according to the rotation perspective, definitely represents a big help. However, the representations obtained through virtual graphic synthesis are very far from reality, apart from the accuracy, since they are schematizations. It is fundamental to emphasize the fact that these reproductions lack real colour and real light.

The gap between the iconographic representation and the existing thing is not eliminable and will always remain. However, today, a particular application of laser technology, the laser scanner, allows to reduce this gap to minimal levels, with a quick and easy acquisition process.

Laser scanners generate a cloud of points of the examined object. Each point is identified through exact coordinates. Besides, the photos of the same object can be layered on the 3D model deriving from cloud. The result is a virtual model that reconstructs the real object, faithfully corresponding for morphology and colours, rotatable, observable from every perspective and measurable (Gelati and Tanga, 2015).

The laser scanner was born as a measuring instrument in industry and it maintains such feature in the anatomical field, allowing to quantify with high precision the area of the organs, the diameters, the volumes, and so on, with an accuracy of ±25μm. The anatomical preparation is literally "immortalized", up to under-millimetre details, where a naked eye is ineffective. The image thus obtained allows to observe and measure the object forever.

The marriage between the ancient dissection techniques and the modern laser scanner technologies allows the realization of a lot of innovative and revolutionary applications. For physicians, non-medical staff and for any researcher in the field of human body studies, Human Anatomy represents a great part of the basis of the whole professional knowledge. At the stage of university education the dissection of a fresh cadaver represents the most effective and preferable approach, however, the use of an atlas cannot be renounced during personal study on anatomical books and treaties.

The process of acquisition of anatomical images conceived by the first of the present authors and protected by patent, takes place through a series of existing instruments: computerized tomography (CT), 3D surface scanner and high resolution cameras; the combination of the images through a process of scale superimposition allows to obtain a three-dimensional object which can be analysed at the same time both on the surface and in depth, according to sagittal, frontal or transverse planes. Moreover the finished product can be processed in order to be observed even in 3D through different types of glasses and visors: anaglyph glasses, polarized glasses, active glasses with shutter, virtual reality visors, augmented reality visors.

Taking a human cranium as a model, let us illustrate in detail the process leading to the obtainment of a complete model.

Computerized Tomography (CT): the CT images allow to observe and to analyse in depth the object under examination, according to the sequential sagittal, frontal, or transversal planes. The scanned images can be processed through specific software, in order to obtain a 3D reconstruction, which offers a global three-dimensional overview of the anatomical structure and it can still be observed according to cross sectional planes at the same time (Figure 1).

Non-contact surface scanning: the non-contact surface scanning of the examined object can be made through different types of scanners, such as laser scanner or structured-light scanner. In our case laser scanner has generated a cloud of points of the examined object. Each point is identified by specific coordinates and starting from this cloud, laser scanner reconstructs a three-dimensional model of the scanned surface, which is characterized by very high quality and definition: the technologies that are available nowadays can reach an accuracy of micron (Figure 2).

High definition photography: the object under examination can be photographed in controlled conditions (particularly referring to the ambient light conditions, through flashes, lights, etc.), in order to obtain high resolution images, absolutely faithful to real colours and real light, with the possibility of zooming in on the smallest anatomical details. Then it is possible to superimpose the high definition photos

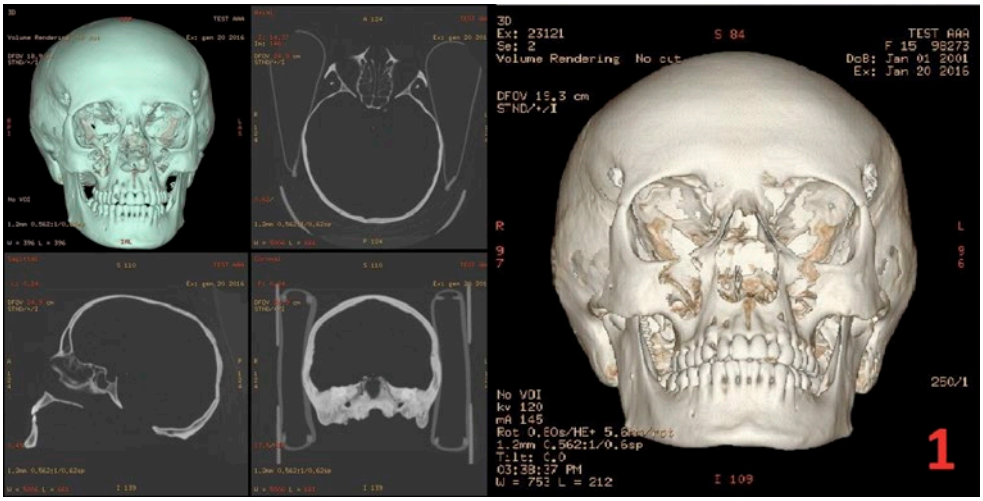

Figure 1. Computerized Tomography (CT) of a human skull.

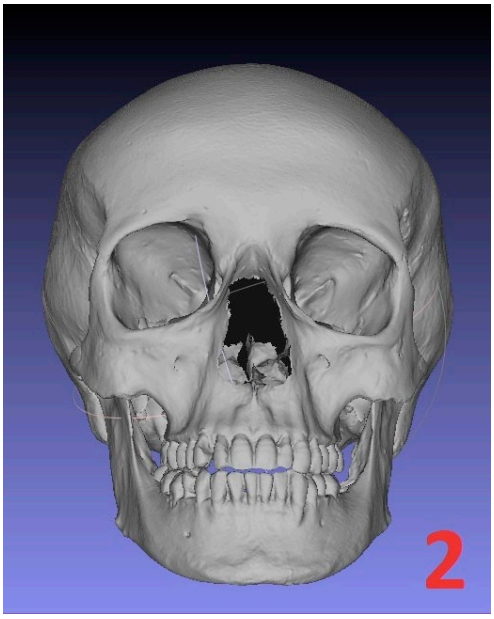

Figure 2. Three-dimensional interactive model of the surface of the same skull as figure 1, derived from the cloud of points, obtained by the laser scanner (see text for the complete explanation).

Figure 3. High definition photography of the same skull as figure 1: the object under examination can be photographed in controlled conditions (particularly referring to the ambient light conditions, through flashes, lights, etc.), in order to obtain high resolution images, absolutely faithful to real colours and real light, with the possibility of zooming in on the smallest anatomical details.

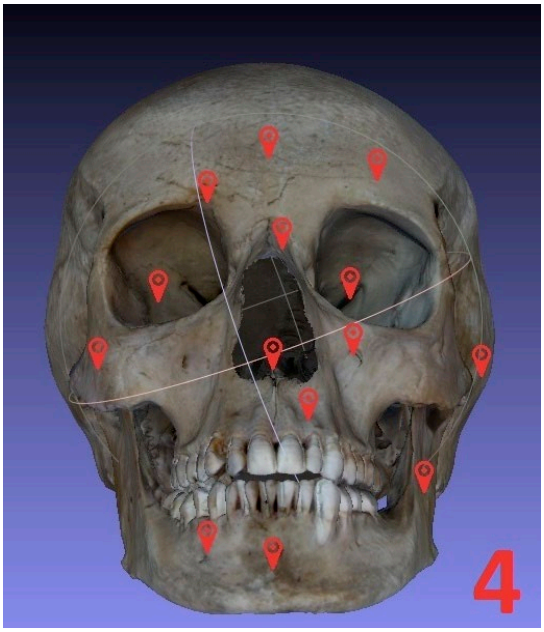

Figure 4. Scale superimposition of the 3D reconstruction from CT images, of the three-dimensional model from the non-contact surface scanning and of the high definition photos of the skull of preceding figures.

to the three-dimensional model of the cranium surface, obtained through laser scanner, in order to give real colour and real light to the model (Figure 3).

A scale superimposition of the 3D reconstruction from CT images, of the three-dimensional model obtained from the non-contact surface scanning and of the high definition photos leads to the complete finished model, which can be analysed both on the surface and in depth. The aforesaid model can work as an Anatomy Atlas if some hyperlinks are added in order to tag the anatomical details (Figure 4).

More models of the same anatomical structure made through the aforesaid procedure in different moments of the dissection, can be overlaid and organized in sequence, in order to obtain a new model (much more interactive and dynamic) which allows the observer to eliminate some structures from the anatomical preparation and/or to add some others, as in a real dissection. It is important to emphasize the possibility of measuring the anatomical model with the highest authenticity and precision (±25 μm in accuracy).

There is a big dimensional/informational gap between the world of 3D laser scanner models and the world of watercolour tables, of photos and of three-dimensional models of virtual graphic synthesis. The digital anatomical preparations obtained with laser scanner techniques can be observed and measured with a resolution which is much higher than human eye's potentialities. As a consequence, the informational content of these figures is beyond the physiological limits of our naked eye: only after enlargement the under-millimetre structures become analysable. This is made possible

by the billions of points identified by specific coordinates which make up the models. They can be processed and managed only through powerful computers existing today. In this case the observer has the opportunity of analysing no more a schematization, but a model made through an objective instrumental data acquisition process, not falsifiable, highly corresponding in colours, in light and in morphology, from the macroscopic aspect to the under-millimetre details, which are visible only after enlargement.

Laser scanner could be the ideal tool to create a new Atlas of Human Anatomy composed of multi-dimensional models which are rotatable, observable from every perspective, absolutely faithful to reality, analysable as in a real dissection and carefully measurable.

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Review - Basic and Applied Anatomy

Ansa pancreatica. Review of the literature

Kympouris Sotirios*, Filippou Dimitrios, Skandalakis Panagiotis

Department of Anatomy and Surgical Anatomy, Medical School, National and Kapodestrian University of Athens, Athens, Greece

Abstract

Ansa pancreatica is a reversed S-shaped pancreatic duct arising from the main pancreatic duct of Wirsung and ending at or near the minor duodenal papilla. Described for the first time in 1961, it is a rare anatomic variant of the pancreatic ducts system and is characterized by the absence of the accessory duct of Santorini. It probably serves as a counter measure after the accessory duct obliteration, in order to maintain sufficient pancreatic juice drainage. The literature concerning ansa pancreatica seems to be rather poor, compared to other anatomic variants and congenital anomalies of the pancreatic ducts. We tried to define the ansa pancreatica incidence among general population and highlight the possible differences between different populations, and to define its possible correlation with pancreatitis. The existing data correlate ansa pancreatica with recurrent acute pancreatitis and pancreatitis in alcoholics. Despite the lack of extended data, ansa pancreatica is a rare anatomic variant, proven to play an important role in certain clinical conditions.

Key words

Ansa pancreatica, pancreatitis, pancreas, anatomy of pancreas, pancreatic ducts.

Introduction

Various congenital anomalies and anatomic variants of the pancreas and the pancreatic duct patterns have been described in the literature (Lehman, 2000; Türkvatan et al., 2013). These conditions have been highlighted and studied, especially since magnetic resonance cholangiopacreatography (MRCP) and endoscopic retrograde cholangiopacreatography (ERCP) play a substantial role in the diagnosis, treatment and follow up of pancreaticobiliary diseases.

Developmental malformations and anatomic variants of the pancreas and the pancreatic ducts include: variations in the course of the main pancreatic duct (sigmoid, descending, loop etc.), variations of the configuration, duplication anomalies, anomalous pancreaticobiliary ductal junction, pancreas divisum (fusion related), annular pancreas (migration related), ectopic pancreatic tissue, pancreatic agenesis or hypoplasia of the dorsal pancreas or accessory pancreatic lobe (Türkvatan et al., 2013).

Ansa pancreatica (Fig. 1) is a very rare fusion related pancreatic duct variation, in which the duct of Santorini forms a reversed S shape and connects with a side branch of the duct of Wirsung (Mortele et al., 2006). It was first described by Dawson and Langman (1961) in an anatomical and radiological study of cadaveric subjects. This condition has been proposed as a potential cause of pancreatitis, especially in alcoholics (Tanaka et al., 1991, 1992).

* Corresponding author. E-mail: sotiriskympouris@hotmail.com

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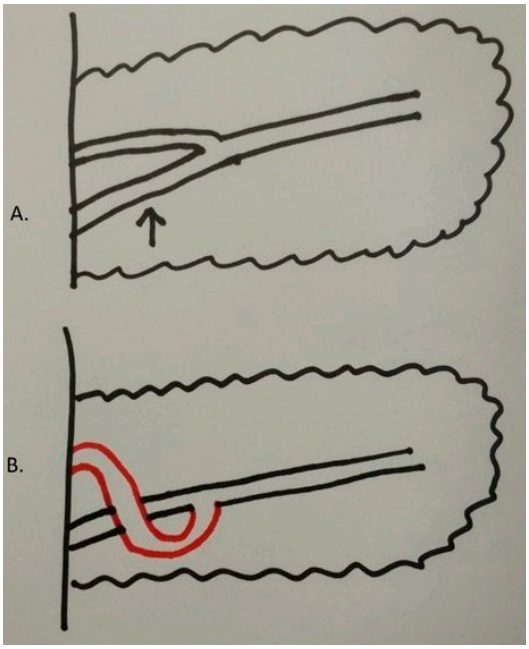

Figure 1. (A) Common pancreatic ductal anatomy with dominant Wirsung duct (black arrow) (B) Ansa pancreatica (red duct) arising from and passing in front of the main pancreatic duct

The aim of this paper is to review the literature about the prevalence of ansa pancreatica and put emphasis on the potential clinical implications of such a rare pancreatic duct anatomic variation.

Materials and methods

Pubmed and journals outside Pubmed were searched in the internet using ansa pancreatica as a search term. The literature found was rather poor and heterogenous, so no exclusion criteria were set, as it was tried to include as much information as possible concerning ansa pancreatica. Approximately twenty papers were found on the whole.

Results

There are a few anatomic, radiologic and clinical studies in the literature concentrating on the pancreatic ductal patterns but only a recent one, to the author's knowledge, focuses on ansa pancreatica (Hayashi et al., 2016). The studies are based either on anatomic-cadaveric examination or MRCP-ERCP reports. In the study of Dawson and Langman (1961), where ansa pancreatica was originally described, this duct

Table 1. Studies reporting ansa pancreatica incidence.

Authors/Date	Subjects examined	Methods of examination	Country	Incidence of ansa pancreatica
Langman and Dawson (1961)	120	Cadaveric and radiologic	Canada	17%
Kamisawa et al. (1998)	213	ERCP	Japan	13.6%
Adibelli et al.(2016)	1158	MRCP	Turkey	1.2%
Hayashi et al. (2016)	660	MRCP	Japan	1.2%
Narayanan and Shabna C. (2017)	50	Cadaveric	India	2%
Liessi et al. (2010)	300	MRCP after secretin injection	Italy	1%

ERCP: endoscopic retrograde cholangiopacreatography
MRCP: magnetic resonance cholangiopacreatography

variation was found in 17% of the subjects (21/120 cases). It was an anatomic study based on fresh specimens, enhanced by the injection of a contrast medium and radiographic imaging. In 66% of the cases with ansa pancreatica, this duct was obliterated at its junction with the duodenum, despite a visible minor papilla.

Using dye injection during ERCP and interpreting dynamic images, Kamisawa et al. (1998) reported an incidence of 13.6% in 213 patients with diagnosed or suspected pancreaticobiliary disease. The incidence of an impervious minor papilla in these cases was almost 80%, even higher than reported Dawson and Langman (1961).

These reported high values of incidence of ansa pancreatica are in dispute with studies based on MRCP imaging (Table 1.).

In a Turkish population based, retrospective study of 1158 MRCPs of people with suspected biliary or pancreatic disease, the ansa pancreatica incidence was found to be 1.2% (Adibelli et al., 2016). This study tried to establish the connection between pancreatic ducts variations and the modified for MRCP Cambridge classification system of chronic pancreatitis (Choueiri et al, 2010). In the results, ansa pancreatica was associated with higher score and thus, might be considered a relevant factor to the onset of chronic pancreatitis (Adibelli et al., 2016).

Another retrospective, Italian population study based on the interpretation of 300 dynamic MRCPs (before and after secretin administration) of patients with suspected or known pancreatic disease, resulted in an incidence of 1% for ansa pancreatica (3/300) (Liessi et al., 2010). A cadaveric study of the pancreatic ductal system of 50 subjects in an Indian population in 2017 revealed a 2% incidence rate of ansa pancreatica (Narayanan and Shabna, 2017).

Ansa pancreatica has also been associated, in case reports, with idiopathic acute pancreatitis (Bhasin et al., 2006), acute pancreatitis (Jarrar et al., 2013) and pancreatitis complicated with walled off pancreatic necrosis (Jagielski et al., 2017). According to Ishii et al., (1998) approximately 7% of patients with ansa pancreatica present with at least an episode of acute pancreatitis.

In 2016, the first attempt to determine the non-biased prevalence of ansa pancreatica and its correlation with pancreatitis was made, with a retrospective study based on a Japanese population (Hayashi et al., 2016). 663 community based subjects and 85 subjects with episode of acute pancreatitis (of which 18 with recurrent pancreatitis) underwent medical examination, blood tests and examination by magnetic resonance imaging-MRCP. The accessory pancreatic duct was clearly visualized in 587 case in the community group and in 73 cases in the pancreatitis group (not statistically significant difference). The ansa pancreatica incidence was 0.85% in community group subjects, 1.81% (1/55) in patients with single episode of pancreatitis and 11.1% (2/18) in patients with recurrent acute pancreatitis. In this study, a statistically significant correlation between ansa pancreatica and the onset of acute recurrent pancreatitis was established. Ansa pancreatica was also found to be more prevalent in patients with alcoholic pancreatitis, but no exact and significant correlation could be achieved.

Among all the studies found, either based on cadaveric examination, radiographic evaluation (ERCP-MRCP) or a combination of methods, no statistically significant connection was detected between ansa pancreatica and the demographic characteristics of the subjects examined.

Discussion

During fetal life, the two pancreatic buds (ventral and dorsal) along with their respective ductal systems, fuse in order to form the adult pancreas. This happens by asymmetric rotation of the duodenum and a 180 degree counterclockwise rotation of the ventral pancreatic bud. The ventral duct will fuse with the dorsal duct in the cephalic portion of the pancreas and give birth to the main pancreatic duct of Wirsung that drains at the major duodenal papilla. The majority of the pancreatic juice drainage will happen through this duct. The dorsal duct system will form the accessory pancreatic duct of Santorini, which drains into the duodenum through the minor papilla (Fig. 2) (Flati and Andrén-Sandberg , 2002).

Kamisawa et al. (1997) reported a 59% of healthy subjects with an impervious minor papilla, while Dawson and Langman (1961) reported a very high proportion (80-90%) of patent accessory ducts in fetuses and infants. Taking these data into account might lead to the conclusion that minor papilla obliteration happens later in adult life.

Ansa pancreatica is an anatomic variant of the pancreatic ductal system, characterized by a blocked minor papilla in the majority of cases (Dawson and Langman, 1961; Kamisawa et al., 1998), along with obliteration of the accessory duct at the extremity near its junction with the main pancreatic duct. The accessory duct is replaced by a hook shaped duct which serves as a communication between ventral and dorsal duct systems. This duct is formed by the proximal part of the dorsal duct, the lower branch of the ventral duct and the lower branch of the dorsal duct (Fig. 3). It ends at or near the minor papilla. It is merely a hypothesis that this procedure serves as a counterweight to the accessory duct or minor papilla obliteration, in order not to disturb pancreatic drainage.

The verification of the actual anatomy of the pancreatic ducts has been made possible since ERCP has entered everyday medical practice. MRCP serves as an equally

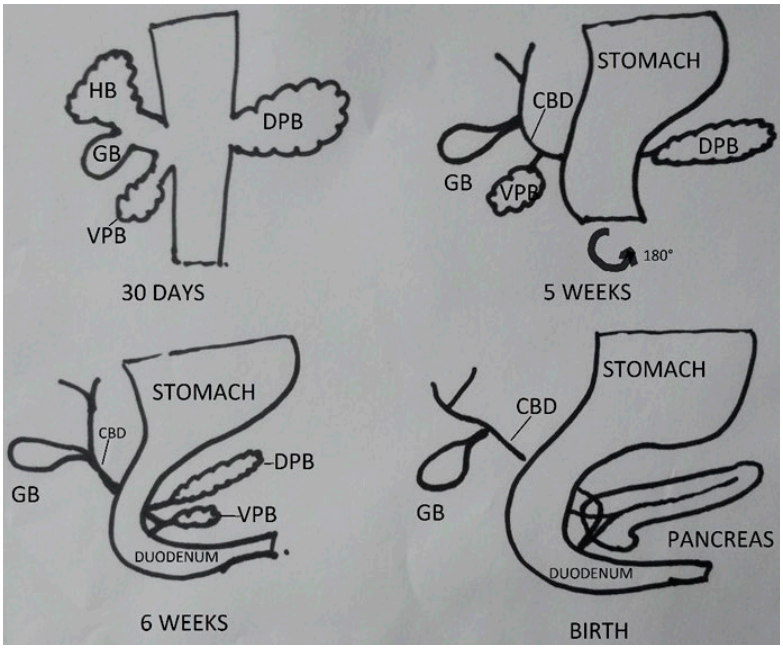

Figure 2. Embryologic development of the pancreas and the pancreatic ducts. HB: hepatic bud; GB: gallbladder; VPB: ventral pancreatic bud; DPB: dorsal pancreatic bud; CBD: common bile duct.

accurate tool (Bret et al., 1996; Bülow et al., 2014), with the advantage of not being invasive. However, in MRCP, ansa pancreatica is often depicted as a faint duct, compared to ERCP. This can be probably explained because of the absence of an injected radiologic agent that results into higher intraductal pressure (Tamura et al., 2006). The magnetic resonance imaging before and after the administration of secretin and the design and conduction of retrospective studies might be a good idea in order to inspect and perhaps increase MRCP's sensitivity in detecting pancreatic ductal variations, such as ansa pancreatica.

The studies found in the literature describing the incidence of ansa pancreatica are few and equivocal (Table 1). More and larger studies are needed, even in healthy individuals, in order to accurately determine ansa pancreatica incidence. Interestingly, no difference was found between men and women, however race might be important. A remarkable deviance in results was noticed between studies based on different national groups.

Throughout the literature, several case reports and studies have proposed the connection between ansa pancreatica and pancreatitis. Only recently, solid statistically significant evidence has been provided to support this correlation (Hayashi et al., 2016). Not only this, but also a relationship between alcohol, ansa pancreatica and pancreatitis has been highlighted. This study might be a great motive for new, anatomic based studies of alcoholic pancreatitis. It is also a big question, whether all individuals with pancreatitis should undergo pancreatic ductal anatomy examination

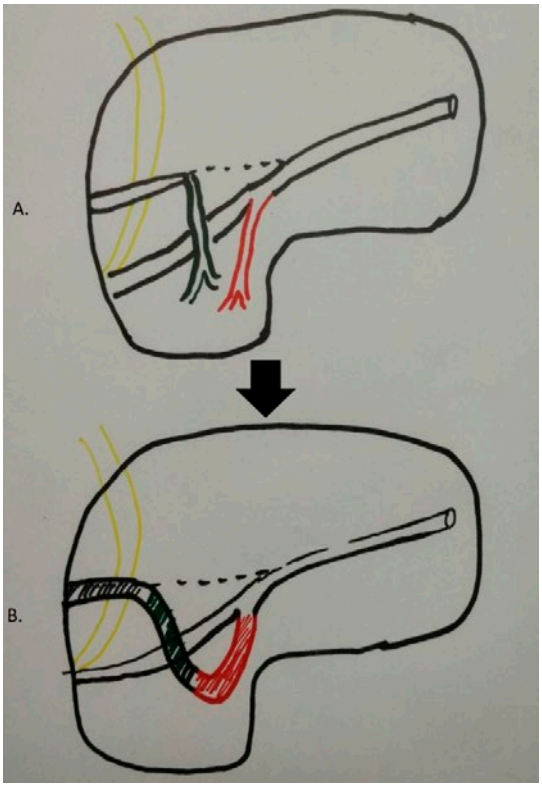

Figure 3. (A) Formation of ansa pancreatica, dashed line: obliterated accessory duct, (B) Ansa pancreatica formed, red component: lower branch of ventral duct, green component: lower branch of dorsal duct, black component: proximal accessory duct, yellow duct: common bile duct

with either ERCP or MRCP, as anatomic variants of pancreatic ducts are in the majority of cases asymptomatic and undiagnosed (Lehman, 2000).

It is indisputable that further studies of randomized nature are needed, in order to more precisely define the exact risk of pancreaticobiliary disease occurrence, in patients with this rare pancreatic duct anatomic variant. Physicians and especially surgeons often come across with anatomic variations that either are asymptomatic or cause pathology. Ansa pancreatica is a bold example of how aberrant anatomy could predispose to certain pathologic conditions. Firm knowledge of common and variant anatomy is a formidable tool in the hands of physicians, irrespectively to their specialty or expertise.

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Review - Basic and Applied Anatomy

Management of daily physical activity and diabetic foot prevention

Piergiorgio Francia[1,*], Giulia Iannone[2], Ferdinando Paternostro[1], Ugo Santosuosso[1], Massimo Gulisano[1]

[1] Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy
[2] ANIMO Association, Florence, Italy

Abstract

Even if physical activity plays a key role within diabetic foot treatment its use and the results obtained from this treatment seem to be still limited. Nowadays, new and even more advanced technologies for the long term daily physical activity monitoring are available and they are radically changing some aspects of physical activity such as its amount, features and monitoring. In spite of the past, the several electronic devices that are currently available can be integrated into routine care and provide essential information for management to both the healthcare providers and patients. In particular, since the end of the last century, an increasing number of studies have applied the movement monitoring to patients at risk or with history of ulceration. The questionnaires have been progressively replaced with modern technologies such as accelerometers or complex multisensory devices able to objectively measure the physical activity performed. The data collected through the use of such devices can allow a better assessment of patient's condition and provide useful information for the definition of a more complete treatment protocol. Daily physical activity monitoring devices provide to the Diabetes Units information on the typology, quantity, distribution and intensity of the daily physical activity performed by each patient concurring to the prevention of foot ulcers that represent the most dreadful diabetes complications. The different functions and modes of operation of monitoring devices can be integrated to provide a more comprehensive and intelligent monitoring system that provide valuable information on patients' ongoing health status and the physical activity performed during daily life. These devices can manage in real time or even in remote the physical activity performed in addition to calculate that to be performed in the following hours. As a result, they contribute to improve patients' lifestyle and reduce the costs for the treatment of such complications. The aim of this review is to define and emphasize the role of a long term daily physical activity monitoring in the prevention of diabetic foot ulcers.

Key words

Diabetic foot prevention, daily physical activity, life style, accelerometer, movement monitoring, exercise.

Introduction

Diabetes mellitus (DM) is a metabolic disorder that causes mortality and morbidity whose incidence is increasing faster than in the past and it has been estimated that by 2035 over 500 million people will be affected worldwide (Guariguata et al.,

* Corresponding author. E-mail: piergiorgiofrancia@libero.it

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2014; NCD Risk Factor Collaboration, 2016). Sedentary and incorrect lifestyle negatively affect patients with diabetes, inducing the occurrence of chronic complications in addition to hinder their treatment (Brazeau et al., 2008; Healy et al., 2008; Colberg et al., 2010). Of the long-term complications that can affect DM patients, diabetic foot ulcers are the most ominous and dreaded, since they affect not only the patient's mobility and overall well-being, but they can increase morbidity and mortality (Apelqvist et al., 2008; van Schie, 2008; Bakker et al., 2016).

The yearly incidence of diabetic foot ulcer in the DM population is around 4% in developed countries and even higher in developing countries while the lifetime risk of a patient to develop a foot ulcer is about 25% (Prompers et al., 2008; Bakker et al., 2016).

The complexity of the multifactorial pathogenesis of diabetic foot ulcer makes difficult its prevention (Pound et al., 2005; Prompers et al., 2008; Apelqvist, 2012). The timely consideration of this complexity plays a key role in guaranteeing proper treatment through physical activity (figure 1). It is well known that physical activity is a milestone in patient therapy aimed at achieving metabolic control and prevention of the major diabetes complications (Balducci et al., 2006, Smith et al., 2006; Colberg et al., 2010; Kluding et al., 2012; Vinik, 2016).

In this article "physical activity" means body movement generated by muscle contraction while "exercise" means physical activity aimed at improving fitness or functional/motor deficits. Unstructured physical activity means "non-exercise" or daily life activities (Colberg et al., 2010; Umpierre et al., 2011, Colberg, 2017). Structured and unstructured physical activity can be performed in an adapted and scheduled way in order to prevent diabetic foot.

Innovations in electronic healthcare are revolutionizing the involvement of both specialists and patients in the modern healthcare system by extending the capabilities of monitoring devices (Appelboom et al., 2014).

These devices have the potential to change the way healthcare is currently being managed. Moreover, healthcare information exchange will make it easier for any diabetes service provider to access the relevant information and provide better and informed point-of-care solutions (Baig et al., 2017).

The aim of this review is to arouse specialists' attention on the role of the proper long term daily physical activity monitoring and management as preventive measures against diabetic foot ulcers.

Daily Physical Activity Monitoring (DPAM) and foot ulcer prevention

Patients monitoring systems are emerging as effective tools for the prevention, early detection and management of chronic conditions as those induced by diabetes mellitus.

These devices are able to continuously monitor free-living patients and consequently expedite treatments. They are easily managed and are becoming increasingly accurate and reliable for patient care (Appelboom et al., 2014; Baig et al., 2017).

Among wearable patient monitoring systems, physical activity performed can be evaluated by pedometer, accelerometers, and gyroscopes (Maluf and Mueller, 2003; Armstrong et al., 2004; Appelboom et al., 2014; Brazeau et al., 2015; Majumder et al., 2017).

The different systems and monitoring techniques can be integrated to provide a more comprehensive system for measuring other parameters (i.e. body-temperature, posture, etc…) as modern complex monitoring systems have already provided (Mathie et al., 2004; Waaijman et al., 2013; Dasanayake et al., 2015; Baig et al., 2017).

Daily physical activity monitoring could play an important role in diabetes management even for those patients affected by foot ulcer and it can positively affect several aspects of diabetes disease: from glycemic control to foot plantar pressure. The data collected on the daily physical activity performed allow the improvement of patient's treatment through a proper organization and management of structured or unstructured physical activity (Maluf and Mueller, 2003; Armstrong et al., 2004; Lim et al., 2015; Ding and Schumacher, 2016).

Daily physical activity monitoring and major diabetes-induced risk factors

A first key step in preventing diabetic foot is the timely knowledge and management of all major risk factors in addition to an accurate clinical picture of each patient.

Diabetic foot lesions frequently occur in patients who show two or more risk factors (Apelqvist et al., 2008). Diabetic peripheral neuropathy and peripheral vascular disease negatively affect mobility and a patient's quality of life (Prompers et al., 2008; van Schie, 2008; Francia et al., 2014; Vinik, 2016). More than half of patients with type 2 DM are affected by diabetic peripheral neuropathy, that can progressively induce motor dysfunction preceded by sensory deficits (Balducci et al., 2006; Smith et al., 2006; Apelqvist, 2012; Kluding et al., 2012; Vinik, 2016).

The neuromuscular problems (i.e. muscle weakness, reduced endurance, and loss of coordination) that may occur in patients with diabetes can worsen or lead to abnormalities in the biomechanics of the foot and of the whole body, in dynamic as well as static postures (Mueller et al., 1994; Uccioli et al., 1995; Francia et al., 2014, 2015; Sartor et al., 2014;Toosizadeh et al., 2015). These impairments can also result in abnormal foot rollover and plantar pressures which significantly increase the risk of painless foot ulcer (Sawacha et al., 2009; Apleqvist, 2012; Francia et al., 2014). In these patients the use of DPAM can help specialists to define a proper exercise therapy protocol indicated for daily life.

In addition to diabetic peripheral neuropathy, even peripheral arterial disease plays an important role in the development of foot ulcers, and can also negatively affect healing (Apelqvist et al., 2008; Bakker et al., 2016). It has been observed that about 50–60% of all diabetic foot ulcers are ischemic or neuroischemic (Prompers et al., 2008). This condition can induce different functional limitations: minor gait speed and walking distance, resting pain, and claudication (Stewart et al., 2002; Collins et al., 2011; Francia et al., 2014). However, even in the presence of this complication, DPAM can help provide better patient management.

The presence of foot deformities and the importance of avoiding foot and leg trauma are other major risk factors to be considered in the physical activity management of diabetic patients (Apelqvist et al., 2008; Apelqvist, 2012; Bakker et al., 2016; Anichini et al., 2017).

Today it is known that general joint mobility of the lower limbs, usually evaluated at the ankle and foot joints (subtalar and first metatarsophalangeal), can signifi-

cantly decrease in subjects with diabetes (Delbridge et al., 1988; Francia et al., 2017). It is even known that limited joint mobility can contribute to the development of foot deformities, and these structural abnormalities may be harmful and trigger abnormal forces on the foot's plantar surface (Fernando et al. 1991; Zimny et al. 2004; Francia et al., 2017).

It has been demonstrated that targeted exercise therapy protocols can improve muscle strength, joint mobility, flexibility, and balance, in addition to abnormalities in gait speed and walking distance (Anichini et al., 2005; Allet et al., 2010; Morrison et al., 2010; Song et al., 2011; Sartor et al., 2014; Francia et al., 2015). Likewise, the proper management of daily life activities can help overcome some limitations connected to the physical activity practice such as the patient's vulnerability and limited compliance, difficulty in performing the protocols routinely and for prolonged periods, feelings of tiredness, and the fear of hypoglycemia (Thomas et al., 2004; Brazeau et al., 2008; Francia et al., 2014; Lim et al., 2015; Ding and Schumacher, 2016). In this context, DPAM can monitor and help manage the patient's lifestyle, their physical activity and other things such as their adherence to medical prescriptions (Waaijman et al., 2013).

Daily physical activity monitoring and foot plantar pressure management

Physical activity is paradoxically an important element of therapy but, at the same time, stressful for feet. The early studies of this paradoxical "risk factor" focused on analyzing gait quality and foot plantar pressures. Consequently, during the 20th century, and especially at the end of the 1970s, the importance of investigating the qualitative or quantitative aspects of human movement for the prevention of foot ulcers has aroused more attention (Stokes et al., 1975; Ctercteko et al., 1981; Cavanagh et al., 1993; Sawacha et al., 2009). The assessment of daily life physical activity performed by patients with and without risk of diabetic foot ulcer has been carried out by different methods (questionnaires, pedometer and accelerometer; Table 1). The use of even more advanced sensors and systems for the assessment of DPAM started to be applied to diabetic foot units since the current century (Table 1) (Armstrong et al., 2001a; Armstrong et al., 2001b; Maluf and Mueller, 2003; Lemaster et al., 2003).

The DPAM of daily life style can be of great importance for its possible correlation with the total plantar pressure exerted on the feet. It can also highlight potentially harmful lifestyles such as those tending to concentrate or increase plantar foot stresses up to a dangerous degree, and help patients to modify their daily lifestyle in order to prevent ulcers (Armstrong and Boulton, 2001; Armstrong et al., 2004; Kshamata et al., 2012).

This estimation on long period can be nowadays possible considering together the results of DPAM and the patients foot pressure in static and dynamic conditions evaluated by the use of devices as baropodometry, in-shoe pressure sensors or instrumental walkways with a force platform (Table 1) (Maluf and Mueller, 2003; Kanade et al., 2006; Kshamata et al., 2012; Sawacha et al., 2009).

The "Three cornerstones" of physical activity management

Supervised exercise therapy can be important at the beginning of treatment in patients at higher risk of ulcer for the improvements that allows achieving in balance,

Table 1. Studies on the use of daily physical activity monitoring (DPAM) in diabetes settings; key to abbreviations at the end of table.

Study (year)	General purpose	Study type	Sample size	Duration and equipment	Results and conclusion
Armstrong et al. (2001b)	To compare the effectiveness of three off-loading modalities to heal neuropathic foot ulcerations	Prospective longitudinal study	63 patients with DM and plantar foot ulcers	12 weeks. Pedometer	Patients treated with total-contact casts were significantly less active than those treated with half-shoe. There was not a significant difference in activity between patients treated with the total-contact casts and those treated with the removable cast walkers
Armstrong et al. (2001a)	To evaluate the magnitude and location of patients activity level	Prospective longitudinal study	20 DM patients at high risk	1 week. Activity monitor	Patients were most active during late morning and mid-afternoon hours. At home the patients used the physician-approved shoes less than outside home
Maluf and Mueller (2003)	To compare the amount of weight-bearing activity and estimate of cumulative plantar tissue stress	Cross-sectional study with matched groups	20 DPN patients with and without history of foot plantar ulcer, 10 non-diabetic control subjects	1 week. Two-dimensional accelerometers and in-shoe pressure measurement	Patients with diabetes and a history of previous ulcers may be susceptible to plantar tissue injury even at relatively low levels of cumulative tissue stress
Lemaster et al. (2003)	To determine whether weight bearing activity increased the risk of foot ulcer	Prospective longitudinal cohort study	400 patients with DM and a prior history of foot ulcer	2 years. 24-h activity questionnaire	Increased weight-bearing activity did not increase the risk of foot re-ulceration
Armstrong et al. (2004)	To evaluate the role of activity in the development of neuropathic foot ulceration	Prospective longitudinal study	100 DM patients at high risk	>25 weeks (or until ulceration). Accelerometer/ pedometer	Patients with diabetes who develop ulceration may actually have a lower overall daily activity than their non ulcerated counterparts, but the quality of that activity may be more variable

Study (year)	General purpose	Study type	Sample size	Duration and equipment	Results and conclusion
Kanade et al. (2006)	To explore plantar loading of the surviving foot following unilateral trans-tibial amputation	Cross-sectional study with matched groups	21 patients with DPN and trans-tibial amputation; 21 patients with DPN without history of ulceration	8 consecutive days. Stepwatch Activity Monitor and in-shoe pressure measurement system	Adaptations in gait and level of walking activity affect the plantar pressure distribution and ultimately the potential risk of ulceration to the surviving foot
Najafi et al. (2010)	To monitor spontaneous daily physical activity and examine both walking and standing activities	Prospective longitudinal study	13 patients with DPN	2 days. Body-worn sensor	Patients with DPN spent 13.5% of time in standing and 6.1% in walking. Walking may cover little of a person's daily physical activity and hence might not be representative of what the subject is doing during daily life activities
Van Schie et al. (2011)	To evaluate the validity of the Step Activity Monitor for assessing physical activity and the relation with the self-reported physical activity.	Prospective longitudinal study	24 patients with DPN	2 days. Step Activity Monitor, Step Watch 3, and International physical activity Questionnaire	Step Activity Monitor was shown to be a valid tool to assess physical activity
Waaijman et al. (2013)	To objectively assess adherence to wearing prescribed custom-made footwear	Randomized controlled trial	107 DPN patients with a recently healed plantar foot ulcer	7 consecutive days. Temperature-based monitor and ankle-worn activity monitor	Adherence to wearing custom-made footwear is insufficient, particularly at home where patients exhibit their largest walking activity. This low adherence is a major threat for reulceration

Study (year)	General purpose	Study type	Sample size	Duration and equipment	Results and conclusion
Lim et al., (2015)	To investigate the effect of an individualized multidisciplinary u-health care service combined with exercise monitoring and dietary feedback on glucose control	Randomized controlled trial	100 T2DM patients assigned to a self monitored blood glucose group or u-healthcare group	6 months. Glucometer and an activity monitor that automatically transferred test results to a hospital-based server	The HbA_{1c} level was significantly decreased in the u-healthcare group compared with the self monitored blood glucose group
Brazeau et al. (2015)	To determine if there was an inverse relationship between sitting and step counts in a diabetes cohort	prospective cohort study	The cohort included 198 T2DM adults	14 days. Pedometer, International physical activity Questionnaire	There was a low correlation between sitting time and step counts
Dasanayke et al. (2015)	To develop a method to detect the onset and end of exercise	Research study	16 adults with T1DM	2 days. Diary, accelerometer, heart rate monitor, and continuous glucose monitor	The method identified the onset and end of exercise in approximately 5 minutes, with an average blood glucose change of only -6 mg/dL
Kluding et al. (2017)	To determine the impact of an intense lifestyle intervention on neuropathy progression and quality of life	Randomized controlled trial	140 type 2 DM patients with peripheral neuropathy	18 months of supervised exercise training. 7 day of actigraphy based counseling to reduce sedentary behavior	An intensive lifestyle intervention may be a sustainable, clinically effective approach for people with DPN that improves patients outcomes and can have an immediate impact on patient care
Jao et al. (2017)	To evaluate the accuracy of two physical activity monitors	Cross-sectional study	31 patients with history of foot ulcer	14 weight-bearing and non-weight-bearing activities. Two physical activity monitors	There was an important difference in accuracy of weight-bearing activities between the physical activity monitors.

DFU: diabetic foot ulcer; DM: Diabetes Mellitus; DPN: diabetic peripheral neuropathy; GPS: Global Positioning System; HbA_{1c}: glycated hemoglobin; T1DM: type 1 diabetes mellitus; T2DM: type 2 diabetes mellitus; u-healthcare: ubiquitous healthcare.

strength and gait (Goldsmith et al., 2002; Allet et al., 2010; Morrison et al., 2010; Sartor et al., 2014). During exercise therapy periods, the patient's functional abilities and quality of movement should be checked (as well as lifestyle changes) so that instructions can be promptly adapted according to the new needs identified. These evaluations should be periodically repeated according to the patient's needs (Otterman et al., 2011; Sartor et al., 2014; Francia et al., 2015).

It is important to underline that all due precautions must be taken in the management of a patient's daily physical activity designed to prevent the occurrence or facilitate the recovery of risk factors for ulcers (Otterman et al., 2011; Francia et al., 2014; Kluding et al., 2015).

Besides leg trauma and falls, the disuse/overuse of muscles and connective tissues must be avoided (Abate et al., 2013; de Jonge et al., 2015). The abnormal balance, posture and gait biomechanics that patients at risk can exhibit, in addition to the presence of foot deformities, may lead to overuse of some lower limb structures (i.e. muscle and connective tissue) while others cannot be involved during daily physical activity and undergo disuse (Francia et al., 2014; de Jonge et al., 2015). In particular, the overuse of the foot and leg structures (i.e. Achilles tendon or plantar fascia) is especially feared because it can contribute to the development of foot ulcer (Giacomozzi et al., 2005; de Jonge et al., 2015).

Once again, the results of postural and gait analysis can provide useful information on the management of daily physical activity. It is important to assess the at-risk patient's muscle strength, joint mobility, balance and posture, as carried out in some studies, and to manage daily physical activity considering further functional deficits, by directing it to the prevention of ulcers.

Such management of the "three cornerstones" of physical activity (long term physical activity monitoring, posture-gait analysis and muscle strength/joint mobility assessments) may ensure that the results achieved by an exercise therapy program are more long-lasting in patients (Figure 1).

Daily physical activity monitoring and glycemic control

Diabetic foot prevention begins with proper care of the patient at the time of diagnosis through treatment aimed at achieving good metabolic control (figure 1). Most treatment involves patient education sessions on the role and importance of an active lifestyle (Colberg et al., 2010; Umpierre et al., 2011). However, as well as nutrition, even physical activity can induce considerable variations in glycemic levels in patients with diabetes. This effect can limit metabolic control, and become a barrier to exercise, especially in patients with type 1 diabetes, making physical activity a risk factor for glycemic control (Brazeau et al., 2008; Colberg et al., 2013). One goal to pursue in the management of a patient's physical activity is to improve blood glucose control in addition to a better peripheral insulin action and improvement in the body mass index (Colberg et al., 2010, 2015; Umpierre et al., 2011).

If on the one hand, in order to maintain glycemic control, a balance between insulin dosage and food intake is required to maintain a proper glycemia during and after physical activity (Loprinzi et al., 2013; Colberg et al., 2015), on the other hand, the type and duration of the ensuing physical activity can be defined for pursuing this goal (Francia et al., 2018).

Table 2. Pros and cons of Daily Physical Activity Monitoring (DPAM).

Pros	Cons
Being informed of the lifestyle and daily physical activity performed	Device availability and limitations
Estimate foot plantar pressure and its daily distribution	Time and cost for processing the data collected
Monitoring of the effects of the interventions carried out	Patient's compliance: - Willingness to wear the device for enough time and repeat the evaluation several times if necessary - Does not change his/her own lifestyle during the monitoring - Fills in a log book to record activities and also those performed without wearing DPAM systems - Synchronizes the devices for remote monitoring
Remote patient monitoring	Knowing patient's functional, postural and movement characteristics before modifying daily lifestyle
Being aware of seasonal and daily variations of physical activity level	Differences in the results achieved by different devices
Multi-parametric monitoring	Limitations in the accurate recognition of each kind of complex movement (small steps, cycling, etc.)
	Indirect evaluation of foot plantar pressure
	Type of activities not allowing the use of DPAM devices (i.e. immersions)

Daily physical activity monitoring means that patients with diabetes have to be informed about physical activity performed and provides indications about what is to be performed calculated on the basis of data collected during long term monitoring. As a result, patients are more aware of managing appropriate food-liquid intake and/or drug therapy to achieve good metabolic control (Armstrong and Boulton, 2001; Colberg et al., 2013; Kluding et al., 2017).

It has also been suggested that the evaluation of physical activity performed between main meals, in addition to that during 24 h, can enable patients to better orient themselves in their choices regarding glycemia management (Francia et al., 2016). The use of devices for the continuous monitoring of glycemia or carbohydrate intake adds useful information to patients and specialists for the management of DM (Armstrong and Boulton, 2001; Lim et al., 2015; Ding and Schumacher, 2016; Colberg, 2017).

However, specialists and the ensuing patients must be aware that vigorous or prolonged physical activity may have significant acute effects on glycemic fluctuations encountering difficulties in their management (Colberg et al., 2013; Yardley et al., 2013). In comparison to structured physical activity, daily life movement can usually be performed at light or moderate intensity so as not to excessively modify blood

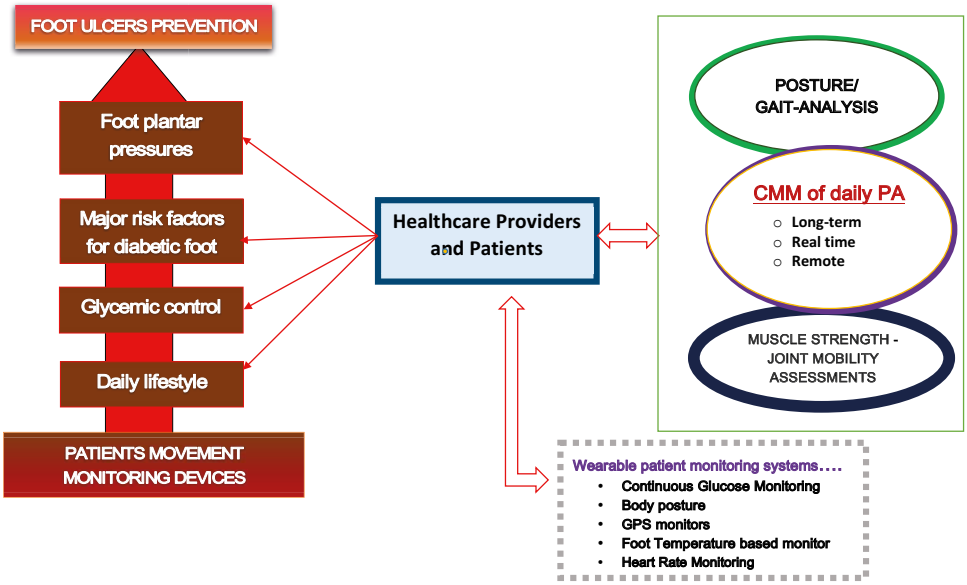

Figure 1. "Three cornerstones" for the prevention of diabetic foot ulcer. DPAM (daily physical activity monitoring) provides targeted intervention at several levels (footplantar pressure, glycemic control and other major risk factors of diabetic foot) aimed at preventing the development of foot ulcers.

glucose values (Umpierre et al., 2011; Yardley et al., 2013). The improvement in glycemic control over time can be attained with DPAM, with knowledge of the type, duration, intensity, and distribution of the activity performed. Each of these parameters can be set up or modified in order to achieve proper glycemia (Armstrong and Boulton, 2001; Colberg et al., 2013; Ding and Schumacher, 2016).

Discussion

The complex etiopathogenesis of diabetic foot ensures that to date the role played by exercise therapy in the primary or secondary prevention of foot ulcers is not yet fully understood.

It has been demonstrated that most of the motor and functional deficits in DM patients significantly improve after short exercise therapy training periods and can achieve almost the level of healthy control group performance (Dijs et al., 2000; Allet et al., 2009; Nicolucci et al., 2012; Francia et al., 2015).

Patients can perform most of these activities as home-based exercise programs (Dijs et al., 2000; Collins et al., 2001; Goldsmith et al., 2002; Sartor et al. 2014) including weight-bearing and/or non-weight-bearing exercises according to the patient's needs (Dijs et al., 2000; Allet et al., 2009; Mueller et al., 2013). However, to date, little attention has been paid to the role of exercise in the prevention of postural and biomechanical deficits that can induce dangerous foot plantar pressure distribution (De

Leòn Rodriguez et al., 2013; Francia et al., 2014; Sartor et al., 2014).

The real preventive impact on foot ulcers of exercise therapy which is not systematically included in the treatment of patients with diabetic foot cannot be defined. In this context, information on the daily physical activity performed by patients can be useful within the preventive measures against foot ulcers (Maluf and Mueller, 2003; Van Schie et al., 2011).

Daily physical activity monitoring provides targeted intervention at several levels in the process leading to the development of foot ulcers in diabetic patients (figure 1). Starting from providing information for metabolic control, DPAM can also show the amount of daily stress exerted on the foot (Kanade et al., 2006; Connelly et al., 2013; Lim et al., 2015).

This approach may also be able to determine what constitutes an unhealthy lifestyle, seasonal changes in leisure-time or working hours in addition to the location of activities so as to enhance patient management. For a full comprehension of these parameters, it can be appropriate to repeat measurements several times a year (Pivarnik et al., 2003; Ding and Schumacher, 2016). Patients with diabetes could benefit from a continuous movement monitoring because the best way to monitor patient is through understanding their interaction with daily activities allowing them to continue monitoring themselves outside the hospital with an accurate assessment of the data collected (Appelboom et al., 2014).

Daily physical activity monitoring can help to evaluate the effect of protective foot devices since it provides information adherence to prescriptions in addition to all kinds of activities performed (Armstrong et al., 2001b; Waaijman et al., 2013). However, the use of devices for DPAM evaluations involves costs, not only for buying the equipment but due to the involvement of specialized personnel and time for processing the data collected (Table 2).

It is important that DPAM covers all 24 hours in at-risk patients. In fact, irregular monitoring can easily hinder data collection regarding the physical activity performed, since only a few minutes are needed to significantly modify the daily physical activity evaluation.

The use of modern devices can help overcoming the barriers to the diffusion of physical activity and reducing negative effects diabetes-induced to patients' attendance at sports or physical activities (Colberg et al., 2015).

Although patients cannot wear a device continuously, they can fill out a log book or diary to record the activities performed when the device is not worn. Special forms can help the patient to accurately register the activities performed (Armstrong et al., 2001b; Najafi et al., 2010; Dasanayake et al., 2015).

Drawbacks to the use of new technology for DPAM include the difficulty in assessing, understanding and managing the lifestyle of patients at risk. This may explain the currently limited use of such methods, despite their promising start (Armstrong and Boulton, 2001; Armstrong et al., 2001a).

Conclusions

The proper management of daily life activities, well-organized in quantity, intensity, type, and distribution, monitored by the use of new DPAM devices, can be a

winning element in showing the potentialities of physical activity in the treatment of patients with diabetes. This approach can ensure better metabolic control, concur in the prevention and treatment of diabetic foot ulcers as well as give more opportunities for modifying the patient's lifestyle. The timely knowledge of each patient's health condition and his/her compliance, in addition to the cost, the time needed for processing data and information on the physical activity performed, are essential requirements for the diffusion of this management approach.

The reliability of patients monitoring by DPAM promotes a prevention-centered culture despite a widespread medicalization. Although the assessment of daily lifestyle in at-risk patients is still limited, the use of DPAM and functional tests following the protocol can provide valuable information for the definition of a more complete patients' assessment and development of care pathways.-

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Letter - Basic and Applied Anatomy

Median occipital fossa: is it really a sign of crime or simply an anatomical variant?

Domenico Chirchiglia[1,*], Pasquale Chirchiglia[1], Rosa Marotta[2]

Departments of [1]Neurosurgery and [2]Health Sciences, University Of Catanzaro, Italy

Abstract

Anatomical variants are alterations of the form, thickness, length, width, position of organs and anatomic structures that can cause problems of a functional nature. They can be vascular, bony, muscular and more. They represent anomalies that may cause disturbances or do not cause changes in functions. The criminologist Cesare Lombroso had correlated the anatomical variations to the criminal tendency. The most emblematic case was that related to the alleged brigand Villella, on which Lombroso, performing the autopsy, found in the skull the so-called medial occipital fossa or a third dimple. He stated that the median occipital dimple was considered a sign of atavism, the expression of the criminal. In fact all the anatomical variants described by Lombroso are variations of normality. No one ever said that anatomical variants were a sign of crime. In conclusion, anatomical variants cannot be related to crime tendency, therefore the debated median occipital fossa is only and simply an anatomical variant.

Key words

Anatomy, anatomical variants, median occipital fossa, Cesare Lombroso.

Dear Editor,

The occipital bone is the main bone of the occiput, lower part of the skull. It is trapezoidal in shape and overlies the occipital lobes of the cerebrum. The base of the skull in the occipital bone contains the foramen magnum, which allows the passage of the spinal cord. From front to back there is the basilar part, at the sides of the foramen magnum are the lateral parts, and the back is the squamous part. The inner surface of the occipital bone forms the base of the posterior cranial fossa. The median internal occipital crest travels behind it to the internal occipital protuberance, and serves as a point of attachment to the falx cerebri.

The inner surface of the occipital bone presents four fossae or depressions. At the midpoint where the depressions intersect is the internal occipital protuberance. Near the center of the outer surface of the squamous part of the occipital there is a prominence, the external occipital protuberance. The criminologist Cesare Lombroso claimed to have studied the skull of 452 criminals and to have found in 16 of them the substitution of the internal occipital prominence with a dimple, which he called the median occipital fossa.

* Corresponding author. E-mail: chirchiglia@unicz.it

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"The median occipital dimple that replaces the lower half of the normal internal occipital ridge is between its two branches that spread towards the occipital foramen: it is almost always missing in the orangutan and the gorilla and it is constant in the other primates and inferior mammals (monotremes, marsupials, carnivores, rodents, etc.), in which the medium-developed cerebellum is reclining. In the inferior human races, particularly in the Aymara of America, in which it is found in 40%, in the madmen and in the degenerates it is present in greater proportions than in the normal civilized man and corresponds to a hypertrophy of the cerebellum worm (cerebellum median). It was from me for the first time noticed in the skull of a thief, certainly Villella, and I also established the atavistic meaning (Lombroso, 1876).

The Lombrosian theories on atavism and morpho-anthropology today have a purely historical value. Physiognomy and craniometry are currently revisited critically. Regarding the anomaly of the median occipital dimple, which according to Lombroso was present in the delinquents, it remains a historical finding. In fact, there is no trace in the literature of the anomaly found by Lombroso. The scientific works of some interest concerning anatomical variants of the occipital bone include misalignment between the internal occipital crest and internal occipital prominence (D'Costa et al., 2009; Kim and Ahmad, 2016).

Duplication of the falx of the cerebellum, occipital sinus and internal occipital crest were found (3), as well as a paracondylar process of the occipital bone (Narayanan et al., 2014). Another research showed an anomalous internal occipital crest causing hydrocephalus (Pozzati et al., 1979). Other studies have been conducted on the variations of the occipital condyle concerning length, width, shape, calibration (Mc Call et al., 2010). Anatomical variants are a non-rare condition. The above described researches confirm this. In none of these researches a combination of anomaly and tendency to crime have been described. Today, thanks to the increasingly innovative means of diagnostic investigation, the discovery of anomalies and anatomical variants is made easier. Anatomy has been used to formulate bizarre and even unthinkable hypotheses as in the case of Lombrosian thought. Time has shown that there is no correlation between anatomy criminal behaviour. The median occipital fossa was even questioned about its existence. Lombroso claimed to have found a fair number of criminals who had the median occipital fossa, considering this the sign of crime. No one then followed these theories, giving more interest to anatomical and physiological knowledge. There are many factors that come into play in the genesis of anatomical abnormalities, but certainly a normal human morphology can contain anatomical variants. The brigand Villella who was the object of Lombroso's study apparently had the median occipital fossa and thus the latter represented for that author the mark of the criminal. It is more reasonable to assume that the medial occipital fossa is only an anatomical variant and has no connection to delinquency. Morpho-anthropology had a certain following outside Europe, in particular in the United States. The singular fact was that Lombroso's theories were postulated in an era in which there was a great scientific activity, with discoveries in the anatomical and physiological fields. Anatomy is the study of the human body, which is in an advanced stage of knowledge.

There are however some variations in morphology compared to normal. Anatomical variants may be found in vascular districts, such as celiac tripod, superior mesenteric artery, vertebral artery, circle of Willis: the anomalies in origin and course of

the coronary arteries have a prevalence of 0.85%. Also the sciatic nerve has significant variations regarding its topography and division. A high level of sciatic nerve division is a relatively frequent phenomenon, and these variants can be found in its terminal branches, anywhere in the thigh or pelvis. In the latter case, its branches or even the entire nerve can emerge either below the piriformis muscle, or above it, or through it. The anatomic variant near the root of the nerve may be unilateral or bilateral and often leads to compression of the nerve with consequent piriformis syndrome.

In short, the presence of anatomical variants of body organs always offers new knowledge about human anatomy. Thus, the duplication of the cerebellar falx as the presence of the median occipital fossa must remain in the sphere which is due to them, that is, simple anatomical variants, and a criminal is not such for those anomalies.

Conflict of interest

To the best of our knowledge, there is no possible conflict of interest for any author, nor have there been sources of financial support.

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Research Article - Basic and Applied Anatomy

Can the vertical jump height measure the lower limbs muscle strength?

Gabriele Mascherini[1], Mario Marella[1], Paolo Bosi[2], Marta Radini[2], Paolo Spicuglia[2], Massimo Gulisano[1], Piergiorgio Francia[1,*]

[1] Department of Clinical and Experimental Medicine, University of Florence, School of Human and Health Sciences, Florence, Italy
[2] Laboratory for Motor Science Applied to Medicine, University of Florence, Florence, Italy

Abstract

The vertical jump is frequently used for the functional evaluation of athletes and non-sporting subjects. The jump height is often used as an indicator of lower limbs strength. The aim of this study was to verify the presence of a relationship between the maximum height reached and muscle parameters expressed during the vertical jump. In 22 healthy males practicing recreational physical activity (age, mean ± standard deviation: 22.5±1.2 years; body mass: 72.8±13.2 kg; body height: 177.1±7.0 cm) and in 15 female volley players (age: 16.5±0.4 years; body mass: 64.4± 8.4 kg; body height: 175.5±7.9 cm), Jump Height (cm), Muscle Strength (N/kg) and Power (W/kg) were recorded during the jump tests. In the healthy males group, jump height was correlated with muscle power: $r = 0.33$, $p>0.05$; a higher correlation resulted between muscle strength and power: $r = 0.62$, $p<0.01$. In the female volleyball players group, only the muscle strength and power showed a correlation: $r = 0.54$, $p<0.05$. It is therefore possible to confirm that the jump height reached during a vertical jump does not provide clear information on the strength of the lower limbs. At the same time, an improvement in muscular strength of the lower limbs does not guarantee an increase in jump height. Several parameters should be evaluated at the same time for a correct functional assessment of athletes and healthy non-sporting subjects.

Key words

Accelerometer, motor skills, muscle power, counter movement jump.

Introduction:

Jumping is an activity of many sports, both team and individual. Ziv and Lidor (2010) reported that the maximum height achieved by an athlete in this activity is currently a functional evaluation parameter frequently used by professionals. In particular, athletic trainers use the maximum jump height as an indirect parameter for assessing the strength and muscle power of the lower limbs (Markovic et al, 2004).

During a competitive season, this value is considered by researchers and trainers as means to verify the effectiveness of the training program (Harman et al., 1991). In addition, in youth sports this type of assessment is also used, along with the anthropometric evaluations, for determining athletes' attitudes to certain sports (Moss et al. 2015).

* Corresponding author. E-mail: piergiorgiofrancia@libero.it

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Tests provided by literature for the evaluation of the vertical jump are the Squat Jump (SJ) and the Counter Movement Jump (CMJ). The squat jump (SJ) is defined as a jump that is performed from a squatting position. A counter movement jump (CMJ) is defined as a jump performed by a subject starting in an upright position, who squats down to a pre-determined height and then immediately jumps up from that position. The CMJ is more of a short pre-loading of the muscle-tendons followed by an immediate contraction: this feature makes the CMJ more natural and therefore closer to the activities required during sport (Finni et al., 2010).

Gathercole et al. (2015) underlined that an analytical approach is needed to assess the performance of CMJs. In particular, it is necessary to describe other variables, *e.g.* those of the concentric phase of the jump, such as peak power and strength, in addition to the maximum height achieved.

In fact, it has been demonstrated that there are no correlations between the maximum isometric and dynamic muscle strength of the lower limbs and the jump height in CMJ (Nuzzo et al., 2008).

Devices for this evaluation have been available for a long time with specific characteristics, which allow not only the quantitative evaluation of the jump height but also the qualitative one of the power and muscle strength expressed during all the phases of the vertical jump (Harman et al. 1991). This method should be also applicable to populations with different characteristics such as subjects of different gender, either practicing sport or recreational physical activity (RPA), *i.e.* the activity typically associated with structured and organized activities aimed at wellbeing without competing or participating to sport competitions or training (Ma et al., 2016) or practicing sports compared to sedentary ones.

The aim of this study was to verify the relationship between the jump height and the parameters of muscle strength and power measured during a vertical jump by devices that can be worn in two groups of subjects with different sport habits.

Material and methods

Participants

Twenty-two healthy males practicing recreational physical activity (age, mean ± standard deviation: 22.5±1.2 years; body mass: 72.8±13.2 kg; body height: 177.1±7.0 cm) and 15 females practicing volleyball(age: 16.5±0.4 years; body mass: 64.4± 8.4 kg; body height: 175.5±7.9 cm). None of the participants had pathological or traumatic history at lower limbs. The local ethics committee approved the experimental protocol, and all subjects provided the written informed consent before starting the study protocol.

Testing procedures

The measurements were carried out at the Laboratory of Motor Sciences Applied to Medicine, University of Florence, Italy. All participants were asked to avoid strenuous exercise on the day before the assessments and any additional resistance training in the 72 hours before being tested.

Athletic performance was evaluated by the standard functional performance test, the CMJ test, using the Accelerometer Free Power Jump Next (Sensorize, Rome, Italy).

After 15 minutes warm-up, subjects were expected to reach their maximal jump height within 3 counter movement jumps. This test involves a single jump starting from an upright position with hands on hips and with counter movement. The test consists in the following phases:

- From a standing position the subjects were instructed to maintain their hands on the iliac crest to avoid a different variance resulting from arm swing;
- Subjects squatted to the point they considered an optimal depth (approximately one-third of a full squat);
- Subjects jumped vertically to the maximal height;
- They landed in normal flexion and lasted in a neutral position for 1-2 seconds.

This test allows to determine the height achieved from the center of gravity. Jump height was assessed by measuring the flight time during the CMJ and the highest jump height was used for further evaluations.

Additional parameters evaluated were muscle strength (N/kg) and power (Watt/kg) collected from the acceleration data.

Statistical analysis

Descriptive statistics is given as mean, standard deviation, minimum and maximum value. To establish a relationship between the three variables describing the vertical jump, the Pearson correlation test was used; $p< 0.05$ and $p<0.01$ were recorded separately and considered as significant..

Data analysis was performed using the Statistical Package for the Social Sciences software version 21 (SPSS Inc, Chicago, IL).

Results

Table 1 shows the mean of the maximum values achieved by males practicing recreational physical activity and performing the vertical jump test (height = 49.5 ± 3.5 cm; strength = 20.3 ± 4.6 N/kg; power = 38.1 ± 5.2).

Relationships between the three variables were the following:

- Jump height vs muscle strength: $r = 0.14$, not significant;
- Jump height vs power: $r = 0.33$, not significant;
- Muscle strength vs power: $r = 0.62$, $p<0.01$.

While, on one hand, the vertical height increased, on the other the strength and muscle power did not show an equal increase.

Table 2 shows the mean of the maximum values achieved by females volleyball players performing the vertical jump test (height = 44.4 ± 9.2 cm; strength = 12.4 ± 3.7 N/kg; power = 32.1 ± 6.6).

Relationships between the three variables were as follows:

- Jump height vs muscle strength: $r = 0.33$, not significant;
- Jump height vs power: $r = 0.33$, not significant;
- Muscle strength vs power: $r = 0.54$, $p<0.05$.

Table 1. Average results of height, strength and power during jumping tests performed by healthy male subjects.

Healthy male	Jump height (cm)	Strength (N/kg)	Power (Watt/kg)
Subjects	22	22	22
Mean	49.5	20.3	38.1
St. Dev.	3.5	4.6	5.2
Minimum	43.0	13.4	28.1
Maximum	55.0	29.4	47.2

Table 2. Average results of height, strength and power during jumping tests performed by female volleyball players.

Female volley players	Jump height (cm)	Strength (N/kg)	Power (Watt/kg)
Subjects	15	15	15
Mean	44.4	12.4	32.1
St. Dev.	9.2	3.7	6.6
Minimum	22.6	11.1	21.9
Maximum	55.5	24.6	44.9

In this case also, when the vertical height increased, muscle strength and power did not show an increase.

Discussion

The vertical jump provides information on motor skills (jumping height) and dependant skills (muscle strength). The goal of this study was to verify the presence of a relationship between strength and/or muscle power and the jump height, in addition to determining if height could be not only an indicator of an ability but also of a dependant skill.

Based on the results gained in two distinct groups of subjects we can confirm that the jump height achieved during a vertical jump does not provide information on the strength of the lower limbs. At the same time, an improvement in muscular strength of the lower limbs does not guarantee an increase in jumping height: in fact, the studies carried out by Hatze (1988) on the biomechanics of the CMJ show that the increase in strength and muscle power does not bring about any improvement in skills. This is also due to minor changes that occur during the strength training on the biomechanics of gesture (Hatze, 1988). It is therefore necessary to use strength and muscle power training models, sport-specific, respecting the timing and degrees of freedom of the trained motor skill.

Therefore, since these two aspects of the science of training are not directly linked to each other during a vertical jump, collecting more parameters is needed to carry

out a better functional evaluation both in athletes and in healthy non-sporting subjects (Gathercole, 2015).

It is important to use statistically significant indicators both at the beginning of the training period and during the competitive season to correctly set up a training program and verify its ongoing effectiveness

At the same time, it is important to know the limits and reliability of the tests and devices used during functional evaluation of both athletes and healthy non-sporting subjects.

Conductivity carpets and photocells estimate jump height based on the flight time, but cannot provide precise information on strength and muscle power (Dugan et al., 2004).

A direct assessment is therefore necessary for this dependant skill during the vertical jump.

Devices available to directly measure strength/ muscle power are the following:

- Accelerometer, wearable device by recording the acceleration during the phases of the motor gesture,
- Strength platform, by recording the ground reaction force exerted by the foot in contact with the platform.

Scientific literature considers the strength platform a gold standard in the evaluation of the CMJ (Cordova and Armstrong, 1996). However, this method is currently expensive and requires the use of devices difficult to move, hindering the possibility of making multiple evaluations during a competitive season (Cordova and Armstrong, 1996). Other methods for assessing the jumping height are the result of flight time's integration with other parameters closely correlated to the force expressed such as body acceleration during jumping. Therefore, several easily moving, reliable and repeatable methods have been validated to correctly evaluate CMJ by an inertial measurement unit (MacDonald et al., 2016). Recently, smartphones applications using their own accelerometer for this assessment have been produced (Stanton et al., 2017). All this availability of devices needs to be carefully monitored because system errors may arise during the interpretation of the results gained: devices used have to always be the same in case of sequential evaluations because they are not interchangeable (Hilmersson et al., 2015). This is the case of an evaluation at the beginning of the training, followed by one in progress and another at the end.

Conflict of interest:

The authors declare no potential conflict of interest and no financial support.

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Research Article - Basic and Applied Anatomy

Effects of static and dynamic stretching on upper limb explosive, isometric and endurance strength, in male volleyball players

Alessandra di Cagno[1], Giuseppe Calcagno[2], Andrea Buonsenso[2], Enzo Iuliano[2], Giovanni Innocenti[3], Marina Piazza[3,*], Giovanni Fiorilli[2]

[1] Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy
[2] Department of Medicine and Health Sciences, University of Molise, Campobasso, Italy
[3] Department of of Experimental and Clinical Medicine ,Section of Anatomy and Histology, University of Florence, Florence, Italy

Abstract

The purpose of this study was to investigate the acute effects of both static and dynamic stretching on upper limb strength and to assess whether a cross-over inhibitory effect occurred during the time in which this effect may appear. Eighteen male volleyball players (aged 21.50, standard deviation 3.12 years) underwent the experimental protocol organized in in two sessions, one of static and the other of dynamic stretching for upper body muscles. Participants performed three specific strength tests: Ball Throwing, Maximum Voluntary Isometric Contraction, and Push-Up, to assess explosive, isometric and endurance strength respectively, at baseline (T0) and 10, 20 and 60 minutes after the static stretching and dynamic stretching sessions. The Ball Throwing results showed significant differences between the two stretching protocols ($F_{1,14} = 4.967$; $p = 0.043$; $\eta p2 = 0.262$), among the 5 time measures ($F_{4,58} = 7.476$; $p < 0.001$; $\eta p2 = 0.348$), and for the interaction Protocol × Time (F4,58 = 8.258; $p < 0.001$; $\eta p2 = 0.371$). Maximum Voluntary Isometric Ccontraction scores showed significant differences among the time measures ($F_{4,58} = 4.015$; $p = 0.006$; $\eta p2 = 0.223$) and for the interaction Session × Time ($F_{4,58} = 2.625$; $p = 0.044$; $\eta p2 = 0.158$). At the Push-Up test significant differences were found only among the time measures ($F_{4,58} = 5.634$; $p = 0.001$; $\eta p2 = 0.287$). Static stretching may adversely affect upper limb endurance strength, whereas no changes in isometric and explosive strength were found. Dynamic stretching did not have a detrimental effect on upper limb endurance strength, whereas it may improve isometric and explosive strength.

Key words

Sport performance, shoulder, range of motion, resistance.

Introduction

Good shoulder flexibility and strength are essential components in volleyball performance, especially in the most explosive movements such as spike, stroke with jump and overhead movements. Shoulder range of motion positively affects the power and consequently the efficacy of these technical skills (Liu and Andersson, 2008).

Stretching is used by athletes and recommended to improve performance (Kay and Blazevich, 2009), to prevent injuries (Andersen, 2005), and to decrease soreness (Arampatzis et al., 2001).

* Corresponding author. E-mail: marina.piazza@unifi.it

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Several previous studies have established that acute static stretching (SS) has an adverse effect on maximal performances (Dallas et al., 2014; Leone et al, 2014), suggesting that neuromuscular inhibition may be the mechanism responsible for muscular impairment, rather than changes in muscle stiffness (Ryan et al., 2008). Viscoelastic stress relaxation after SS produces both mechanical and structural tissue alterations (passive muscle stiffness reduction), that affect muscle-tendon complex force transmission and consequently decreases the muscle force production (Moran et al., 2009). Moreover, a reduction in motoneuron pool excitability decreases the muscle performance (da Silva et al., 2015). Previous studies strongly recommended not performing SS immediately before a maximal strength performance (Behm et al., 2006). Impairment was estimated to persist for up to 60 minutes (Knudson et al., 2001). The duration of single SS exercise is also responsible for loss of strength performance: a duration of 30 sec or less, showed no detrimental effect on performances that required maximal strength and power (di Cagno et al., 2010). Several authors showed that dynamic stretching (DS) does not negatively affect performance and may improve some body physical skills (McMillian et al., 2006; Yamaguchi et al., 2007). Few studies have examined the effects of stretching on upper body muscles and found controversial findings. Evetovich et al. (2003) and Leone et al. (2014) provided evidence for a decrease in strength production and sport performance. Knudson et al. (2001) and Torres et al. (2008) did not find any negative effect following previous SS in the upper limb maximal force production and power tasks. Jelmini et al. (2018) demonstrated that acute SS negatively affects the rate of force generation more than peak force, due to a neural inhibitory mechanism.

The aim of the present investigation was to determine whether, in volleyball athletes, isometric, isotonic and explosive strength were negatively influenced by an acute bout of SS and DS. We hypothesised that an acute bout of SS would decrease the strength muscle performance, whereas DS would improve or would have no effect on the type of strength output tested in this study.

Material and methods

Participants

Eighteen adults male volleyball players (aged 21.50 ± 3.12 years old) underwent the study procedures. Participants were recruited in two different volleyball clubs competing in the national championships. In order to be enrolled in the study, participants had to meet the following inclusion criteria: (1) participation in at least 80% of the training sessions and in the competitions of their own club, (2) at least 5 years of volleyball practice, and (3) no injury occurred in the last year. The following exclusion criteria were applied: presence of injury or disease (temporary or not) influencing the experimental protocol execution and the testing session and use of medicine influencing neuromuscular functioning, resistance performance and/or muscular characteristics (e.g. elasticity, stiffness, or contractility). The study was designed and conducted in conformity with the Declaration of Helsinki. All participants gave their informed written consent.

Procedures

The procedures were designed in order to evaluate the respective effects of SS and DS on explosive, isometric, and endurance strength. The experimental protocol consisted of two sessions, performed in two non-consecutive days of the same week, two days apart each other. Pre and post intervention specific strength tests consisted in: Ball Throwing test (BT), Maximum Voluntary Isometric Contraction test (MCIV), and Push-Up test (PU), that assessed explosive, isometric and endurance strength respectively.

Three repetition of each strength test (BT, MVIC and PU) were performed at baseline (T0), immediately after the stretching protocols (T1), and at 10 (T10), 20 (T20) and 60 (T60) minutes after each stretching protocols. The tests and the stretching protocols used in the experimental sessions are described in detail in the following paragraphs.

Strength tests

The three strength tests (BT, MVIC and PU) were completed in 90 sec, with 10 sec rest between each test and the next. This procedure was used in both sessions (SS and DS) and in the repeated measures (T0, T1, T10, T20 and T60), to reduce the influence of each test on the next. In fact, the fatigue produced by each test could influence the result of the subsequent tests. However, the proper evaluation of the immediate acute effects of stretching did not allow longer rest periods or multiple attempts. The execution of the tests in the same order, instead of randomised order, was used to favour the homogenous overestimation or underestimation of the test scores. Authors tolerated the systematic error of measurements, due to the impossibility to eliminate the interferences among the tests.

Ball throwing

This text was performed to evaluate the upper limb explosive strength. The test consisted in throwing the heavy medicine ball (Dynamax Inc. Dallas, TX 3 kg, 65 cm in diameter), sitting on the floor, the back oriented vertically against a back support, with legs crossed, knees flexed at 90°. Participants were secured to a support with an elastic strapping, placed around the trunk at mid-chest level under the axillae. This position and mode of stabilization minimized trunk movements during the put. Tacking the ball with both hands from behind the head, participants threw the ball ahead, using an explosive forward movement. Participants completed 3 medicine ball puts, and the greater distance was considered for the analysis.

Maximum voluntary isometric contraction

This test was performed to evaluate the maximum isometric contraction of shoulder extensor muscles, assessed when participants were in the same position in which they usually impact the ball during the spike. In sitting position, the participants had to pull, with the outstretched arm, a dynamometer handle, keeping a shoulder abduction of ≈140° and a horizontal adduction of ≈30°. The core and the trunk of the participants were stabilized during the test.

Push-up

This test was used to evaluate the muscular endurance strength of the participants (Vossen et al., 2000; Battaglia et al., 2013). The PU was performed in a prone position. Each subject lifted the body, raising the arms and leaving the feet in touch with the ground, and, without pausing, changed direction to return in starting position. In this test, the participants had to perform the largest number of complete and correct push-up in 30 sec. The maximum number of correct lifts that each subject made in 30 second was considered for the analysis.

Stretching sessions

Static stretching session

Static stretching consisted of a slow passive manoeuvre until the maximum range of motion was attained, in a position in which subjects reported a feeling of maximal stretch but no discomfort or pain. The participants performed two sets of three stretch repetitions of 30 sec each (2 sets x 3 rep x 30 sec), with a 10 sec rest between repetitions and a 15-sec rest between sets (Behm et al., 2004). They did not warm-up prior to stretching. Participants performed three type exercises as showed in the following paragraphs. All stretches were performed in standing position. The investigator helped and controlled each stretch of the subjects to ensure consistency in stretching procedures. The total time under stretch was 180 sec for each exercise.

In the first exercise the subject extended the right arm across the upper chest with the forearm, roughly parallel with the floor, then he pressed the left hand against the outside of the right elbow (posterior deltoid and under scapular muscle stretching). In the second exercise the subject placed one arm behind the head and tried to touch the opposite shoulder blade with the hand. The investigator placed a hand on the elbow of the stretched arm and began the stretch, pushing the elbow across the subject's body toward the opposite shoulder (triceps muscle stretching). In the third exercise the subject, standing in front of the investigator with the investigator grasping the elbow joins, abducted the shoulder and extended the arms to a position that was below parallel to the ground. The investigator pushed the arms together to stretch the pectoralis major and anterior deltoid muscles.

Dynamic stretching session

Dynamic stretching consisted of moving the limbs actively with a controlled slow to moderate velocity until maximum range of motion. The participants performed 10 repetitions of 6 sec each (3 sec in the ascendant phase and 3 sec in the descendent phase) for each of the 6 different exercises. Dynamic stretching consists of function based exercises through a full range of motion, which use sport-specific movements to prepare the athletes for practice and competition. Dynamic shoulder stretch included external and internal arm rotation, abduction, adduction, flexion and extension movements.

Statistical analysis

The analysis of variance for repeated measures (RM-ANOVA) was performed on the scores obtained from each resistance test. The RM-ANOVA was performed assuming the two sessions for BETWEEN FACTORS analysis (Session: SS vs. DS) and the 5 repeated measures for WITHIN FACTORS analysis (Time: T0 vs. T1 vs. T10 vs. T20 vs. T60). The distance covered in the BT, the isometric strength, measured with the MVIC (in Newton), and the number of push-ups, performed in the PU test, were used as dependent variable for the analysis, and analysed separately. Due to the 5 repeated measures in the time factor, paired comparisons were performed when a significant F was observed, using Bonferroni post-hoc test.

The results are reported as mean ± standard deviation. The alpha test level for statistical significance was set at 0.05. The analysis was performed using SPSS statistical software package version 22 (IBM, Armonk, NY, USA).

Results

For BT, significant values were found for the differences between the two stretching protocols ($F_{1,14} = 4.967$; $p = 0.043$; $\eta_p^2 = 0.262$) and among the 5 time measures ($F_{4,58} = 7.476$; $p < 0.001$; $\eta_p^2 = 0.348$), and for the interaction Protocol × Time ($F_{4,58} = 8.258$; $p < 0.001$; $\eta_p^2 = 0.371$). Analysis performed on MVC scores showed significant differences among the time measures ($F_{4,58} = 4.015$; $p = 0.006$; $\eta_p^2 = 0.223$) and significant interaction Session × Time ($F_{4,58} = 2.625$; $p = 0.044$; $\eta_p^2 = 0.158$), but no differences between the two protocols ($F_{1,14} = 2.921$; $p = 0.109$; $\eta_p^2 = 0.173$). Concerning the PU, significant differences were found only among the time measures ($F_{4,58} = 5.634$; $p = 0.001$; $\eta_p^2 = 0.287$), whereas no differences were found between the two session ($F_{1,14} = 1.420$; $p =$

Table 1. Duration-dependent effects of acute static and dynamic stretching on explosive, isometric and endurance strength in volleyball players (mean ± standard deviation).

	T0	T1	T10	T20	T60	Significance (p<0.05)
Static Stretching						
BT (m)	4.43 ± 0.43	4.28 ± 0.44	4.38 ± 0.5	4.4 ± 0.5	4.54 ± 0.46	None
MVC (kg)	11.77 ± 1.92	10.44 ± 1.75	10.87 ± 2.79	11.12 ± 2.49	11.63 ± 2.51	None
PU (n)	21.88 ± 1.46	22.75 ± 2.82	22.5 ± 2.93	20 ± 2.98	18 ± 4.63	T1 vs. T60
Dynamic Stretching						
BT (m)	4.43 ± 0.43	4.92 ± 0.39	5.14 ± 0.5	5 ± 0.4	4.98 ± 0.64	T0 vs. T1, T10, T20 e T60
MVC (kg)	11.77 ± 1.92	12.41 ± 2.33	14.01 ± 1.88	13.22 ± 2.62	13.96 ± 3.12	T0 vs. T10
PU (n)	21.88 ± 1.46	22.13 ± 3.23	23.38 ± 3.81	22.5 ± 5.21	22.25 ± 5.28	None

BT= Ball Throwing; MCV=Maximum Voluntary Contraction; PU= Push-Up.
T0, T1, T10, T20 and T60= assessment of upper limb strength at baseline (T0), immediately after Static and Dynamic Stretching (T1) and after 10 (T10), 20 (T20) and 60 minutes (T60).

0.253; $\eta_p^2 = 0.092$) and no significance for the interaction Session × Time ($F_{4,58} = 2.163$; $p = 0.085$; $\eta_p^2 = 0.134$). The detailed scores and the post-doc analysis are showed in Table 1.

Discussion

The purpose of this study was to investigate the acute effects of both static and dynamic stretching (SS and DS) on upper limb strength and to assess whether a cross-over inhibitory effect occurred during the time in which this effect may appear. It was hypothesized that an acute bout of SS would adversely affect maximum voluntary isometric contraction, endurance and explosive strength in the stretched upper limbs.

The main findings were a significant difference between the effects of the SS and DS protocols on explosive strength (BT), as highlighted also by the significant interaction Protocol × Time. After SS no significant changes in BT test, used to assess explosive strength, were found. These results agree with other studies in which it was reported that there were no SS effects on upper limb muscular strength or power (Knudson et al., 2004; Torres et al., 2008; Molacek et al., 2010). In the present study DS improved BT performance within 20 minutes, in contrast with preview studies, in which no changes in upper limb explosive strength were found after DS. Torres et al. (2008) did not find any increase in upper body performance following DS, with the exception of the lateral throw. These conflicting results may depend by the different type of stretching protocols applied (Kay and Blazevich, 2012). Faigenbaum et al. (2005) and Yamaguchi and Ishii (2005) hypothesized that the improvements in strength after DS may be due to a post-activation potentiation effect on performance, increasing the rate of cross-bridge attachments, which allows a greater number of cross bridges to form. The BT improvement after DS is important for volleyball performance in which the players need to develop force rapidly and at high velocity (Leone et al., 2014; Piazza et al., 2014).

Regarding the duration dependent effects of stretching on muscle strength endurance, no significance changes after DS were found, whereas a significant decrease between T1 and T60 was found following SS in accordance with the results of Nelson et al. (2005). The impairment may be attributable to different mechanisms consequent to SS, as motor unit fatigue state prior to the initiation of endurance task, a decrease in the motor units available for activation (Fowles et al, 2000), and/or a decrease in blood flow during the time in which muscles are being stretched, with lower oxygen available. Moreover, the partial ischemia elevates the level of metabolites within the muscles (Poole, 1997). Finally, the altered Ca++ kinetics, due to SS, may determine a 63% decrease of twitch tension (Armstrong et al., 1999).

No changes in isometric strength were found as result of SS. The duration dependent effects showed a little decrease at T1-T20, reaching the baseline value at T60. Preview studies reported that, after 15 minutes of recovery from SS, the decrease in isometric strength was due to intrinsic mechanical properties of the stretched muscles, rather than neural factors (Fowles et al., 2000). The length-tension relationship and the plastic deformation of connective tissue, altered by muscle elongation, impaired the maximal force-producing capacity and decreased the stiffness of the complex muscle-tendon (Nelson et al., 2001). In the present study, the little decrease

in isometric strength may be attributable also to the angle-torque relationship used during isometric shoulder muscle action, chosen at 30°. A preview study highlighted that the stretching induced force deficit was most evident at shorter muscle lengths (Mc Hugh and Johnson, 2006). The DS elicited a significant improvements in isometric strength until T10. This result may be due to an increase in electromyography activity after DS, that counteract a loss of force production related to the altered length-tension relationship. Moreover, an increase in temperature due to DS may have improved the compliance of both contractile and non-contractile tissues in the muscles (Taylor et al., 1995). In contrast with these findings, Evetovich et al. (2003) reported no changes electromyography but an increase in mechanomyography amplitude for the biceps brachii after stretching and suggested that neither SS no DS decreased muscle activation but both SS and DS increased muscle compliance.

Further investigations are needed to explain why upper and lower-body strength performances respond differently to an acute bout both of SS and DS. For example the stretching-induced effect increases the compliance but does not change the muscle activation in the biceps brachii, whereas in the vastus lateralis and rectus femoris muscles it decreases the muscle activation but causes no change in muscle compliance (Evetovich et al., 2003; Cramer et al., 2006; Kirialinis et al., 2015). Future studies may assess whether structural architectural differences among upper and lower limbs determine different acute responses to stretching. The difficulty in determining volume and intensity levels of each static and dynamic stretch regimen limits the application of the results of the present study on this topic. A limitation of this study is that the physical condition of the participants was not controlled, especially the stiffness of the tissues which regulates their flexibility profiles.

In conclusion, in the present study SS adversely affected the upper limb endurance strength, whereas no changes in isometric and explosive strength after an acute bout of SS were found. The DS did not have a detrimental effect on upper limb endurance strength, whereas it can improve isometric and explosive strength. Therefore, DS rather than SS should be proposed before volley performance to maintain or increased muscle strength and power performance of the upper limbs.

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