Exogenous Intake of Fluorides in Caries Prevention: Benefits and Risks

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ABSTRACT
Caries incidence and prevalence have decreased significantly over the last few decades due to widespread use of fluoride. However, an increase in the prevalence of dental fluorosis has been reported in both fluoridated and non-fluoridated communities. Care must be taken to ensure that a balance between the optimal fluoride preventive effect at the individual and community level and minimal risk of dental fluorosis is maintained. This review describes the main sources of fluoride intake that have been identified: fluoridated drinking water, dietary fluoride supplement, and topical forms comprising toothpastes, rinses, gels and varnishes. The cited data were taken from meta-analytic studies and reports from Cochrane database systematic reviews up to December 2019. Efficiency, but safety, of topically applied fluorides in individual home care is dependent on the degree of compliance of individuals/parents and on the level of competence of providers of preventive counselling. The broad spectrum of these resources allows individualization of fluoride prevention based on risk analysis of caries attack and taking into consideration other preventive measures.

KEYWORDS
fluoride; systemic and topical fluoride prevention; dental caries; dental fluorosis

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INTRODUCTION

For over 60 years, dental professionals have been attempting to control caries with fluoride and its various inorganic and organic compounds (1). In the middle of the 20th century, it was believed that fluoride had to be incorporated into dental enamel during pre-eruptive stage of development to achieve its maximum protective effect. Ingestion of fluoride in the early years of life was considered essential for a full range of fluoride benefits to be achieved. It was assumed that incorporation of fluoride in the hydroxyapatite crystal during tooth formation and mineralization resulted in a permanent or long-lasting resistance to dental caries (2). However, it has been found that the fluoride-rich surface layer of the enamel, which was a result of systemic fluoride administration, was gradually lost because of insufficient post-eruptive maturation of enamel surface, and was unable to keep significant long-lasting protection against caries (3).

The recent view of our understanding of how fluoride works has been changed. The evidence suggests that the cariostatic effect of fluoride is mostly by its topical rather than systemic effect (3). This effect might be even greater when combined with tooth brushing with fluoride toothpastes. Based on both in vitro and in vivo studies it has been demonstrated that fluoride has a direct topical influence on the dynamic de- and re-mineralization processes that occur at the interface between the tooth surface and the adjacent dental biofilm (4).

FLUORIDE BENEFITS AND CONCERNS

Fluoride appears to provide its benefit when present at the plaque fluid during the caries challenge. Its effectiveness depends on how frequently it is administered in the oral cavity, and the mechanism of fluoride's topical anti-caries effect depends on the mode of application, its chemical formulation and, especially its concentration. Our current understanding of how fluoride affects tooth mineral and makes it more resistant in acidic environment has been reviewed extensively (5–7). The mechanism of action of fluoride includes the inhibition of demineralization on crystal surface, enhancement of remineralization on crystal surface, and inhibition of bacterial activity (8).

The concept of the topical application of fluorides became widely accepted as a key factor in successful caries prevention without significant ingestion of fluoride. Fluorides are also effective as therapeutic agents in non-invasive caries treatment for the inactivation or arrest of caries lesion (9, 10).

The concerns about the prevalence of dental fluorosis considered as the most frequently discussed risk of implementation of fluoride, has mostly been related to the use of fluoride supplements, especially during the first 6 years of life (11). Early exposure to fluoride toothpaste might also be a risk factor due to ingestion of toothpaste by small children (12, 13). The most important risk factor for dental fluorosis is the total amount of fluoride consumed from all sources during the critical period of tooth development. This critical period is the late secretion-early maturation stage of pre-eruptive dental enamel (13, 14).

WHICH INDIVIDUALS ARE AT RISK?

Small children under the age of 4 years are considered to be at risk of dental fluorosis of permanent incisors and permanent first molars. This period of life is known as the period of calcification and maturation of these teeth (15). The longitudinal Iowa study found that exposure to fluoride during the first 3 years of life was the most important for fluorosis development on the permanent maxillary incisors (16). The use of fluoride must be therefore carefully monitored and balanced. The prevention of early childhood caries on one hand and minimize the risk of dental fluorosis on the other hand should always been considered. The amount of fluoride intake from all potential sources has been calculated as 0.05–0.07 mg F/body weight/day as an optimal intake of fluoride, safe with respect of mild form of dental fluorosis and effective enough in caries prevention (15, 17, 18). Special attention should be given to the use of topically applied fluoride gels and solution, because of the inadequate control of the swallowing.

In pre-school children up to 6 years may develop dental fluorosis of posterior teeth (premolars and second molar). Nevertheless, it presents less of an aesthetic problem, which needs to be weighed against the marked benefit of fluorides in caries prevention of primary and young permanent teeth. The risk of dental fluorosis in school children older than 6 years is very small (19).

SOURCES OF FLUORIDE INTAKE

Concern about dental fluorosis and its potential impact on quality of life should be taken into consideration. Therefore, all forms of fluoride intake from all sources should be calculated, especially in children. Fluoride prevention and prophylaxis against dental caries is commonly divided to topical and systemic. Topical agents as fluoridated toothpaste, fluoride gels, solutions and varnishes, are applied onto to enamel surface of erupted teeth. Systemic application (water, milk, salt, supplements) of fluorides brings fluoride compounds to GIT, where they are absorbed and in blood way they get into hard dental tissues of developing teeth. The less amount of fluoride gets into saliva and oral environment. Topically applied fluorides can be unintentionally swallowed and therefore increase the alimentary ingested fluorides.

FLUORIDATED DRINKING WATER

Community water fluoridation (CWF) was introduced over 70 years ago as a public health measure to prevent and control caries at a population level. A great advantage of CWF it that it benefits all residents in a community, regardless age, socio-economic status, education, oral hygiene practice or access to routine dental care (8). CWF is

also a cost-effective method of delivering caries prevention to a large population (20). The fluoride concentration of water in CWF programs typically ranges from 0.5 to 1.1 mg L^{-1} (ppm F). A recent Cochrane review estimated that the initiation of CWF reduced caries levels by 35% in the primary dentition and 26% in the permanent dentition of children. The review also found that CWF led to a 15% increase of children with caries-free primary teeth and a 14% increase of children with caries-free permanent teeth, compared to children without water fluoridation (21). CWF has its place in economic disadvantaged communities with low level of health literacy. After all, fluoridated water is nowadays consumed by a half of billions of people worldwide (21).

Several recent reviews on the impact of fluoridated water on human health have been published (21–23). Positive effect of CWF is significant also when combined with other forms of fluoridation. No reliable evidence for any health risk associated with the use of fluoridated water at the recommended level was found. The mild form of dental fluorosis is the only unwanted effect associated with CWF. The Cochrane review of water fluoridation estimated the 12% prevalence of dental fluorosis of aesthetic concern at fluoride levels of 0.7 mg L^{-1} (ppm F) in drinking water (21).

There is the evidence of increasing consumption of bottled drinking water during the last two decades in form of spring water or mineral water. Alimentary consumed water in any form represents almost 80% of alimentary ingested fluoride (24). Some of mineral waters contain fluoride in similar concentration as recommended amount of fluoride in CWF and therefore this source of fluoride should be considered as a significant alimentary fluoride intake.

FLUORIDATED MILK

Milk fluoridation has been reported to be successful in dental caries prevention, particularly in children as a community preventive programs in form of milk snacks (23, 25). This community measure is supported by WHO in all global documents focused on caries prevention (26). The fluoridated milk is distributed in 200 ml packs containing 5 ppm fluoride, what corresponds to 1 mg of fluoride in the pack. The effect of fluoridated milk has been evaluated on the groups of children in Great Britain, Hungary, China, Peru and Thailand. The results of the meta-analytic study from these countries published in Cochran review reported 43–85% reduction of caries incidence in permanent dentition, but less significant results in primary dentition (27). Repeated studies on Bulgarian children have confirmed 40% caries reduction in primary dentition when the beginning of fluoridated milk consumption was at 3–3.5 years. Additionally, these studies have found the comparable effect of fluoridated milk with fluoridated water if number of daily doses exceeds 160 per year (28). Recently fluoridated milk is extensively used in Russian Federation, Great Britain, China and Bulgaria. The administration of fluoridated milk in children older than 3 years of age is regarded as the safe preventive method from the point of view of its ratio on total alimentary fluoride intake (26). No potential adverse effect of fluoridated milk was found (27). A high concentration of fluoride in milk (5 mg L^{-1}) is needed for two reasons: children did not drink the beverage throughout the day and calcium in the milk complexes with fluoride, which would reduce its availability for topical benefit (27).

FLUORIDATED SALT

Fluoridated salt was firstly introduced in Switzerland in the middle of the last century on the basis of good experience with iodized salt. The number of successful clinical studies resulted in recommendation of fluoridated salt as the systemic forms of caries prevention (29), (30). The fluoride content in salt varies from 250–350 mg F/kg in most countries, where this method was approved for the individual home use. The meta-analytic studies in school children lasting more than three years reported the significant caries decline in permanent dentition compared to control (non-fluoridated salt group) with Odds ratio from –2.13 to –4.22 (31, 32). The effect of fluoridated salt on primary dentition in pre-school children is not significant. The concern of excessive alimentary intake of fluoride from salt with the combination of other forms of systemic administration of fluorides is low in the view of the fact that recent dietary advice lead to minimize the exposure to salt from diet in small children (33).

FLUORIDE SUPPLEMENTS (TABLETS, LOZENGES AND DROPS)

Fluoride supplements were first introduced to provide systemic fluoride in areas where water fluoridation was not available. Supplements contain a measured amount of fluoride, typically 0.25 mg, 0.5 mg, or 1.0 mg, usually as sodium fluoride, acidulated phosphate fluoride, potassium fluoride, or calcium fluoride (34). There are few data on the extent to which these products are used, but they are known to have widespread use as a caries preventive for children. The post-eruptive efficacy of fluoride supplements has been demonstrated in school children, though the original rationale for their use, i.e. pre-eruptive uptake by developing enamel to form a more resistant tooth is no longer tenable. Additionally, when using them, care should be taken that the products have sufficient substantive topical effect in oral cavity (34). The recommendation of use of fluoride supplements varies from country to country. The most of experts has very restrained view on fluoride supplements and they recommend this form of caries preventive method in children older than three years of age with high caries risk because of risk of dental fluorosis and low parental compliance in regular, daily administration of the supplements (35, 36). The systematic reviews published by Ismail AI, 2008 evaluated the results of 20 longitudinal studies. They concluded the effect of fluoride tablets in the reduction of caries incidence in permanent dentition up to 43% but with the mild risk of dental fluorosis (OR 1.8–2.2). The preventive effect of fluoride supplements in primary dentition is still controversial because of

low level of evidence and confirmed risk of dental fluorosis in permanent dentition (11). The results from the panel of experts, entitled "European view of fluoride supplementation" reached the consensus on the necessity 1) to indicate fluorides supplements administration strictly to the back ground fluoride intake from food and beverages, 2) to start with fluoride supplementation (if necessary) at the age of 3 years, 3) to take fluoride supplementation by no means as a wide spread preventive measure (34).

FLUORIDE TOOTHPASTE

Fluoride toothpastes were introduced in the late 1960s and early 1970s and their rapid and remarkable increase on the market share was followed by massive reduction in dental caries seen in many countries over the past 40 years (37). Tooth brushing with fluoride toothpaste is close to an ideal public health method being convenient, inexpensive, culturally approved and widespread. Within the meaning of fluoride caries prevention, tooth paste is understood as a carrier of fluoride which increasing the fluoride concentration in oral cavity during the tooth brushing, increasing the overall amount of fluoride in oral environment what leads to enhancing remineralization, inhibiting demineralization and some metabolic processes of oral bacteria (38). The number of systematic reviews confirmed the positive effect of fluoride toothpaste in the last decades (38–43). The systematic review of Twetman reported the 24% decline of caries incidence in children (39). The meta-analytic study of 76 published randomized studies in children and adolescents published by Walsh et al. 2010 confirmed the 19–27% increasing caries reduction with the arising fluoride concentration in the tooth pastes (43). There is a substantial concern that small children during the tooth brushing with fluoride tooth paste swallow some paste with a subsequent risk of fluorosis (41). Fluoride toothpaste may be responsible for up to 80% of the "optimal" total daily intake of fluoride in children below 3 years of age (44). Therefore, parents must be strongly advised to assist and supervise tooth brushing until at least 7 years of age. The amount of tooth paste applied onto the tooth brush must be age-related and parents of pre-school children should not allow them to do it alone. There is a little evidence of effectiveness of the toothpaste with fluoride concentration less than 1000 ppm F in caries prevention in older pre-school (4–5 years) and school children particularly in those in caries risk (43). The recommended fluoride concentration in tooth pastes is presented in table 1.

FLUORIDE GELS, RINSES, VARNISHES

Except of tooth pastes, other topically applied agents are considered effective especially in children at high caries risk, including children with special oral health care needs or under orthodontic treatment particularly when permanent teeth start to erupt. Fluoride rinses recently recommended as the supplementary measure in caries prevention contain 200–900 ppm fluoride in form of sodium fluoride or aminfluoride. The lower concentration, usually 225–250 ppm fluoride is recommended for daily use, those with 900 ppm fluoride are used weekly, in caries risk children older than 8 years of age. Children are advised to use them after the tooth brushing to prolong the increased amount of fluoride in oral cavity. They are not recommended in children below 6 years of age because of risk of swallowing what may lead to increased fluoride plasma levels and mild risk of dental fluorosis. The several meta-analytic studies have been reported and the effect of fluoride rinses have been clinically studied (44–46). Twetman et al., 2004 evaluated the data from 24 studies and found the 29% reduction of caries increment both in case of daily rinse containing 225 ppm fluoride and weekly rinse containing 900 ppm fluoride (47). It can be concluded that rinses are reported as effective in permanent teeth (20–50% caries reduction) but no data are available in primary teeth (45). Fluoride gels in caries prevention are used more than 50 years. Formerly they were used for professional application in dental office, recently are also recommended for home use as 2% sodium fluoride, 1.23% acidulated fluorophosphates and combination of 0.25% aminfluoride + 1% sodium fluoride. Several meta-analytic studies have confirmed their preventive effect in permanent dentition ranging from 28–37% in reduction of caries increment (46, 48, 49). The effect of gels in primary teeth is uncertain. Especially, in pre-school children, the risk of ingestion and subsequent dental fluorosis should be weighed against the potential caries-preventive benefit. Fluoride varnishes contain 1–5% sodium fluoride (22,600 ppm F) as resin or synthetic formula. They are for professional use only. Varnish as the only high fluoride topical agent can be used in both pre-school and school children with the caries reduction 50–70% (50). They are applied on clean and dry enamel surface 3–4 per year particularly in high caries risk children. They serve also as the prophylactic agent in initial subsurface lesions (9). There is a moderate evidence of its efficacy with minimal risk of dental fluorosis, because once it sets to hard, it cannot be swallowed (44).

Tab. 1 Recommended use of fluoride toothpaste in children.

Age of the child	Fluoride concentration (ppm F)	Frequency	Amount of toothpaste (g)	Size
First tooth up to 2 years	1000	Twice daily	0.125	Grain of rise
2–6 years	1000*	Twice daily	0.25	Pea
Over 6 years	1450	Twice daily	0.5–1.0	Up to full length of the brush

* In caries risk children 1000+ ppm F is recommended based on the caries risk assessment.

Adopted from: (19)

CONCLUSIONS

This literature review presents the evidences supporting the current strategy of fluoride use in caries prevention and its potential risk, particularly in children. Where fluoride is used in conjunction with other fluoride vehicles, the cumulative fluoride exposure must be taken into consideration for children less than 6 years of age. Care must be taken to ensure that a balance between the optimal preventive effect and minimal risk of dental fluorosis is maintained. On the other hand the recent recommendations of fluoride use are as safe as can be, but dependent on the degree of compliance of individuals, respectively parents of children and on the level of competence of providers of preventive counselling. The broad spectrum of these resources allows individualization of fluoride prevention based on risk analysis of caries attack and taking into consideration other preventive measures.

Based on the scientific evidence and current literature data, authors strongly support the recommended use of fluoride in paediatric population and adopt them by both paediatric and paediatric dentistry community.

AUTHOR CONTRIBUTIONS

RKI, VR, FK and VM equally contributed to the design of the review, RKI, VR and FK writing the original draft, VM revised the draft.

DECLARATION OF INTEREST STATEMENT

All authors declare that they have no conflict of interest regarding the publication of this manuscript.

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Innervation Patterns of the Pronator Teres Muscle and Their Possible Role in Neurotization: A Systematic Review of Cadaveric Studies

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ABSTRACT

Background: Contrary to the classic anatomical description, many recent studies have reported wide variations in branching patterns and location of motor branches that are supplying the pronator teres muscle. To understand these variations and their implications in surgical procedures of the nerve transfers, a systematic review was performed on the innervation of pronator teres muscle from cadaveric studies.

Methods: A systematic literature search was performed in databases such as Medline, PubMed, Google Scholar, SciELO, ScienceDirect, Cochrane reviews and orthopedics textbooks using the search terms "pronator teres nerve branches"; AND "number" OR "location" OR "length" OR "diameter" yielded 545 article links. Articles were evaluated according to PRISMA guidelines.

Results: A total of twenty cadaveric studies including 648 branches have registered 52.9% of two branch innervation pattern followed by 31.3%-single branch pattern; 13.5%-three branch pattern; 1.7%-four branch pattern, and 0.4%-five branch patterns, respectively. Of the 403 branches studied for their location in relation with the humeral intercondylar line, most branches were located distal to the line (50.3%), followed by 32.7% (proximal to it) and 16.8% at the line, respectively. The distance of branches located proximal and distal to humeral intercondylar line was in the range of 1.25–10 cm, and 1.1–7.5 cm, respectively. The mean length and diameter of nerves reported were 4.37 ± 2.43 cm, and 1.5 mm, respectively.

Conclusions: Our data defined the morphometrics of nerve branches and they often met the required diameter for neurotization procedures. Our findings also demonstrated that the morphometrics, branching pattern and their location vary between populations and this information is very vital for surgeons during the nerve transfers.

KEYWORDS

pronator teres; innervation; morphometrics; neurotization

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INTRODUCTION

Median nerve (Mn) is one of the important branches arising from C5–T1 roots via medial and lateral cords of the brachial plexus. After its formation, Mn descends through the arm along with brachial artery to reach the cubital fossa. Here it is closely related to the pronator teres muscle (PTM). PTM is a fusiform muscle that has a humeral head and an ulnar head. Humeral head extends from the medial epicondyle of humerus and medial intermuscular septum, and an ulnar head that originates from the coronoid process of the ulna. Both heads run downwards and merge to form a common flexor tendon, which inserts at the mid-portion of the lateral surface of radius (1, 2). The usual anatomical description is that the median nerve passes between two-headed PTM and gives a branch to it before entering the forearm (Fig. 1a and 1b). This branching site could be at a variable distance proximal to the elbow joint (3). Contrary to the classic description, many recent studies have reported variability in the motor branches of Mn that are supplying PTM. And these branches are located at a variable distance either proximal or distal to the humeral intercondylar line (HIL) or at the level of HIL (4–9). In cases, where more than one branch to the PTM is present, these remain as an effective alternative surgical option for nerve transfer procedures (5, 8, 10, 11). To this, a recent experimental study treating lower brachial plexus injury in rats confirmed that the PTM branches seem to be a better donor than the other nerves in their electrophysiological and histological examination (12).

Although a lot of clinical information is available on their role in nerve transfer procedures, the basic anatomical details facilitating the identification of these nerve branches is not so comprehensively reported in the literature. Despite their clinical importance, there is no accurate data available on the number, location and morphometrics of the nerves in association with ethnicity, gender and side. Therefore, we critically reviewed the available literature regarding the innervation patterns of PTM and quantitatively interpreted the pooled data. We hope that this quantitative data may give a broader perspective to surgeons in avoiding iatrogenic injuries to these nerve branches and harvesting them safely for neurotization procedures.

MATERIALS AND METHODS

SEARCH STRATEGY AND INCLUSION-EXCLUSION CRITERIA

A thorough search was made mainly using the electronic databases such as Medline, PubMed, Google Scholar, SciELO, ScienceDirect, Cochrane reviews and orthopedics textbooks. The keywords for search used were as follows; [("pronator teres nerve branches") AND "number OR location OR length OR diameter")]. To arrive at a standard dataset, we have strictly confined our search criteria to the cadaveric studies by excluding all the case reports, case series, letter to editor and brief communications from other clinical sources. The mean pooled data on PTM branches,

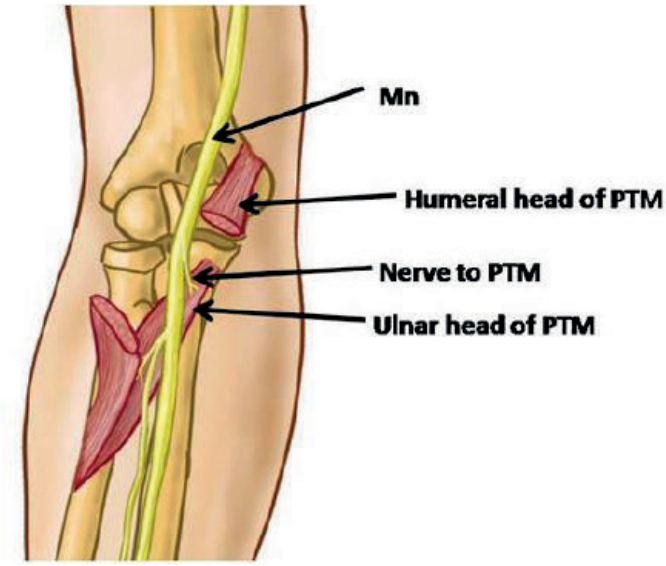

Fig. 1a Schematic diagram showing the pronator teres muscle with its innervation.

Mn: median nerve; PTM: pronator teres muscle.

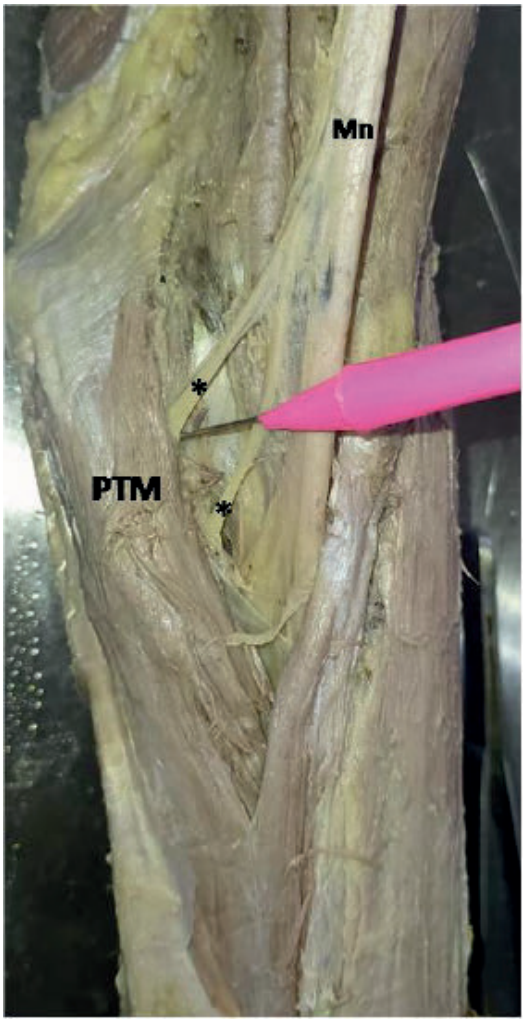

Fig. 1b A cadaveric dissection showing the pronator teres muscle with its innervation (two branch pattern).

* Showing the PTM branches. Mn: median nerve; PTM: pronator teres muscle.

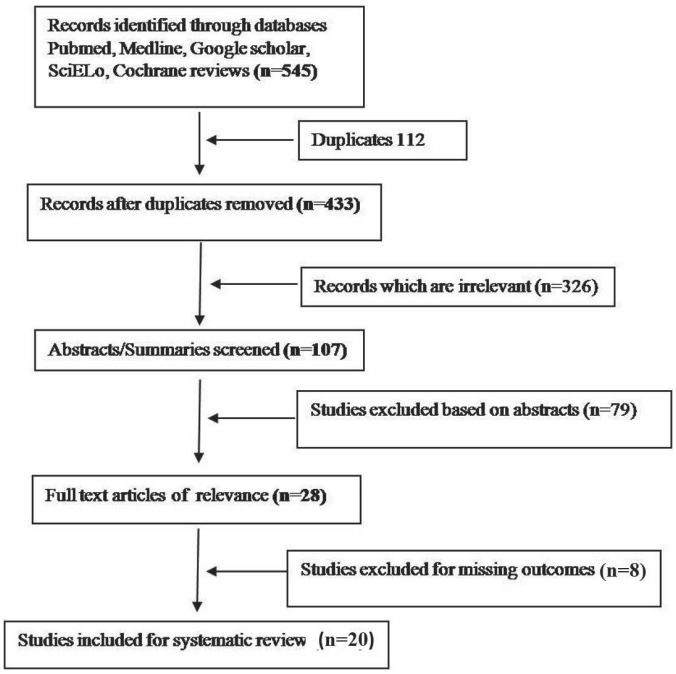

Fig. 2 Showing the application of selection criteria as per PRISMA guidelines.

their location with regards to HIL and their side and gender-based values were set to be the outcomes of our study. The references of the included articles were checked, and duplicates were deleted. We have not set restrictions on date or language of the studies. Initial screening on titles and abstracts of the articles were done to obtain the full-text articles (Fig. 2). The data collection was done using the guidelines of Preferred Reporting Items for Systematic reviews and MetaAnalyses (PRISMA) checklist (13). Search strategy was carried out independently by two authors and no conflict was noticed.

STATISTICAL ANALYSIS

Extracted data included the country of study, sample size, number of the PTM branches and their length, the diameter and location. The mean ± SD were calculated for all the outcomes independently by two observers and no conflict was noticed. Then the mean values of all the outcomes were analyzed as per the population using SPSS Version 23.0 (USA).

RESULTS

A total of twenty cadaveric studies met the inclusion criteria (Fig. 2). All the limbs are of adults with an age ranging from 20–89 years. Five studies (4, 8, 10, 17, 22) reported their gender distribution with 69 males and 56 females from the total sample. Six studies (7, 17, 21, 23, 25, 26) have reported data on 159 left and 154 right limbs. Tables 1 and 2 show the characteristics of limbs with PTM and their branches.

Tab. 1 Characteristics of the included studies.

Studies (year)	Region	Sample size of limbs	Type of study	Age (Years)	Male	Female	Left	Right
Sunderland, 1978 [14]	UK	20	Cadaveric	NR	NR	NR	NR	NR
Fuss and Wurzl, 1990 [15]	Austria	50	Cadaveric	NR	NR	NR	NR	NR
Gunther et al., 1992 [16]	USA	20	Cadaveric	NR	NR	NR	NR	NR
Canovas et al., 1998 [4]	France	10	Cadaveric	70–85	4	6	NR	NR
Chantelot et al., 1999 [17]	France	50	Cadaveric	NR	20	30	25	25
Tung and Mackinnon, 2001 [5]	USA	31	Cadaveric	NR	NR	NR	NR	NR
Alves et al., 2004 [18]	Brazil	18	Cadaveric	NR	NR	NR	NR	NR
Safwat and Abdel-Meguid, 2007 [6]	Saudi Arabia	23	Cadaveric	NR	NR	NR	NR	NR
Demirci et al., 2007 [19]	Turkey	34	Cadaveric	NR	NR	NR	NR	NR
Tubbs et al., 2011 [10]	USA	20	Cadaveric	60–89	12	8	NR	NR
Pushpalatha et al., 2011 [20]	India	50	Cadaveric	NR	NR	NR	NR	NR
Bindurani et al., 2013 [21]	India	50	Cadaveric	20–50	NR	NR	26	24
Yang et al., 2014 [22]	China	30	Cadaveric	NR	18	12	NR	NR
Gupta et al., 2015 [23]	India	24	Cadaveric	NR	NR	NR	12	12
Olewnik et al., 2017 [7]	Poland	50	Cadaveric	NR	NR	NR	26	24
Basanagouda and Halagatti, 2017 [24]	India	62	Cadaveric	NR	NR	NR	NR	NR
Caetano et al., 2018 [8]	Brazil	30	Cadaveric	NR	15	NR	NR	NR
Gaikwad et al., 2018 [25]	India	39	Cadaveric	NR	NR	NR	20	19
Bertelli et al., 2020 [9]	Brazil	32	Cadaveric	NR	NR	NR	NR	NR
Vantmuri and Joshi, 2020 [26]	India	100	Cadaveric	25–70	NR	NR	50	50

NR: not reported.

Tab. 2 Outcomes of different studies on the number of branches to PTM.

Studies (year) & Region	Gender/ side	Number of PTM branches				
		One	Two	Three	Four	Five
Sunderland (1978) – UK [14]	Overall	7	14	0	0	0
Fuss and Wurzl (1990) – Austria [15]	Overall	14	22	10	4	0
Gunther et al. (1992) – USA [16]	Overall	1	6	3	0	0
Canovas et al. (1998) – France [4]	Overall	0	10	0	0	0
Chantelot et al. (1999) – France [17]	Overall	28	13	1	0	0
Tung and Mackinnon (2001) – USA [5]	Overall	8	16	5	1	0
Alves et al. (2004) – Brazil [18]	Overall	NS	NS	NS	NS	NS
Demirci et al. (2007) – Turkey [19]	Overall	4	19	10	1	0
Safwat and Abdel-Meguid (2007) – Saudi Arabia [6]	Overall	0	23	0	0	0
Tubbs et al. (2011) – USA [10]	Overall	NS	NS	NS	NS	NS
Pushpalatha et al. (2011) – India [20]	Overall	34	14	2	0	0
Bindurani et al. (2013) – India [21]	Overall Right Left	28 16 12	18 7 11	4 1 3	0 0 0	0 0 0
Yang et al. (2014) – China [22]	Overall	NS	NS	NS	NS	NS
Gupta et al. (2015) – India [23]	Overall Right Left	5 4 1	12 9 3	5 3 2	2 2 0	0 0 0
Olewnik et al. (2017) – Poland [7]	Overall	7	26	10	0	0
Basanagouda and Halagatti (2017) – India [24]	Overall	47	13	2	0	0
Caetano et al. (2018) – Brazil [8]	Overall	9	21	0	0	0
Gaikwad et al. (2018) – India [25]	Overall Right Left	1 0 1	9 3 6	21 11 10	3 1 2	3 2 1
Bertelli et al. (2020) – Brazil [9]	Overall	0	32	0	0	0
Vantmuri and Joshi (2020) – India [26]	Overall Right Left	10 3 7	75 40 35	15 7 8	0 0 0	0 0 0

PTM: pronator teres muscle; NS: not specified from their respective studies.

OUTCOMES ON THE NUMBER OF PTM BRANCHES

Of 648 branches studied from twenty studies have registered 52.9% of two branch innervation pattern) followed by 31.3% (for single-branch pattern); 13.5% (for three- branch pattern); 1.7% (for four-branch pattern) and 0.4% (for five-branch pattern), respectively. Pooled data of the branching pattern are listed in Tables 2 and 3. The percentage of single branches was in the range of 0–53.8% varying from population to population. The Saudis followed by Turkish population had the lowest single branches. The highest value was found among French population. The two-branch pattern was in the range of 43.6–100%. The Indian followed by Austria and French populations had the lowest double branches while the highest value was found in Saudi population. In case of three branching pattern, Saudi, Brazilian and British followed by French population had the lowest triple branches. The highest value was found among Turkey and Poland populations. Whereas the percentage of four branching pattern was low in almost all the populations in the range of 0–2.9%. Only one study (25) reported five branches in three specimens (2 right and 1 left). 211 branches reported from four studies (21, 23, 25, 26) have analyzed the branching pattern in relation with side and no statistically significant differences ($P > 0.05$) found between right and left branching patterns. None of the studies have analyzed branching pattern in relation with sex (Table 2). Two studies from USA (10) and China (22) are not included in data as they did not mention the number of branches explicitly as per our search.

Tab. 3 Showing population wise percentages of the branches to PTM.

Country	% Single branches	% Double branches	% Triple branches	% Four branches	% Five branches
Saudi Arabia [6]	0	100	0	0	0
Turkey [19]	11.7	55.8	29.4	2.94	0
Brazil [8, 9, 18]	14.5	85.4	0	0	0
Poland [7]	16.2	60.4	23.2	0	0
USA [5, 10, 16]	22.5	55	20	2.5	0
Austria [15]	28	44	20	8	0
UK [14]	33.3	66.6	0	0	0
India [20, 21, 23–26]	38.6	43.6	15.1	1.54	0.9
France [4, 17]	53.8	44.2	1.92	0	0

PTM: pronator teres muscle.

OUTCOMES ON THE BRANCHING PATTERN OF PTM BRANCHES IN RELATION WITH HIL

Ten of twenty studies comprising 403 PTM branches have reported their location in relation with HIL (Table 4). Of 403 branches studied, majority (203 branches – 50.3%) located distal to HIL. Whereas 132 out of 403 branches (32.7%) located proximal to HIL, followed by 68 of 403 branches (16.8%) at the level of HIL. The French population reported zero percentage of branches located proximal to HIL while Indians having the highest percentage (55.9%). The American (0%) followed by French (1.4%) and Brazilian population (5.8%) reported the lowest branches at HIL while Indians having the highest (86.7%). Regarding branches located distal to HIL, it was observed that the British population had low percentage (3.9%) while the Indian population having the highest (50.2%) (Table 4). None of the studies have analyzed branching pattern in relation with sex (Table 4). Only one study (23) conducted on 52 branches from Indian population did explicitly analyze distance of the branches from HIL in relation with side and no significant differences ($P > 0.05$) found between right and left sides (Table 4).

Tab. 4 Outcomes of different studies on anatomical location of the branches with reference to HIL.

Studies (year) and Region	Type of study	Gender/ Side	Number of branches		
			Proximal to HIL	At HIL	Distal to HIL
Sunderland, 1978 – UK [14]	Cadaveric	Overall	8	4	8
Canovas et al., 1998 – France [4]	Cadaveric	Overall	0	1	9
Tung and Mackinnon, 2001 – USA [5]	Cadaveric	Overall	7	0	24
Alves et al., 2004 – Brazil [18]	Cadaveric	Overall	14	NR	7
Safwat and Abdel-Meguid, 2007 – Saudi Arabia [6]	Cadaveric	Overall	23	0	23
Gupta et al., 2015 – India [23]	Cadaveric	Overall Right Left	7 4 3	27 12 15	18 11 7
Basanagouda and Halagatti, 2017 – India [24]	Cadaveric	Overall	33	0	29
Caetano et al., 2018 – Brazil [8]	Cadaveric	Overall	14	NR	7
Gaikwad et al., 2018 – India [25]	Cadaveric	Overall	26	32	55
Bertelli et al., 2020 – Brazil [9]	Cadaveric	Overall	NR	4	23

HIL: humeral intercondylar line; NR: not reported.

Fourteen studies that presented population-wise data on the distance of branches located proximal and distal to HIL is shown in Table 5. The branches located proximal and distal to HIL in the range of 1.25–10 cm, and 1.1–7.5 cm, respectively (Fig. 3). None of the studies reported the distances in relation with sex.

Tab. 5 Outcomes on the distance of PTM nerve branches from HIL.

Studies (year) and Region	Distance of branches from HIL (cm)	
	Proximal	Distal
Sunderland, 1978 – UK [14]	7.0	2.3
Fuss and Wurzl, 1990 – Austria [15]	3.5	5.5
Gunther et al., 1992 – USA [16]	4.5	3.5
Canovas et al., 1998 – France [4]	7.1	2.4
Tung and Mackinnon, 2001 – USA [5]	1.25	1.35
Alves et al., 2004 – Brazil [18]	4.9	1.5
Bindurani et al., 2013 – India [21]	1.31	1.2
Yang et al., 2014 – China [22]	NR	3.87
Gupta et al., 2015 – India [23]	10.0	3.0
Olewnik et al., 2017 – Poland [7]	NR	5.3
Basanagouda and Halagatti, 2017 – India [24]	5.0	7.5
Caetano et al., 2018- Brazil [8]	6.8	2.8
Gaikwad et al., 2018 – India [25]	2.5	1.1
Bertelli et al., 2020 – Brazil [9]	1.5	3.8

PTM: pronator teres muscle; HIL: humeral intercondylar line; NR: not reported.

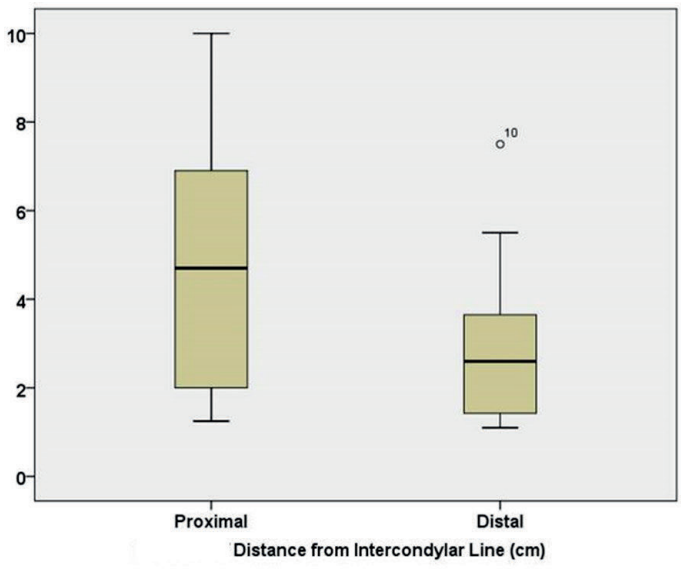

Fig. 3 Showing the distance of PTM branches located proximal and distal to HIL.

HIL: humeral intercondylar line.

OUTCOMES ON THE MEAN LENGTH AND DIAMETER OF PTM BRANCHES

Six studies (5, 8–10, 18, 22) reported the mean length and three studies (8–10) reported the diameter of PTM branches. The mean length of proximal PTM branches reported from two studies (5, 9) was 2.45 ± 0.25 cm, ranged from 2.2 ± 0.8 cm (USA) to 2.7 ± 8 cm (Brazil). The mean length of distal branches was 3.35 ± 0.45 cm, ranged from 2.9 ± 1.0 cm (USA) to 3.8 ± 1.5 cm (Brazil). Four studies (8, 10, 18, 22) reported the mean length (without specifying the proximal and distal branches) as 5.85 ± 2.38 cm, ranged from 3.6 cm (USA) to 9.6c m (China). Overall, mean length of the nerves from all reported studies is 4.37 ± 2.43 cm. Only one study from Brazil (9) reported the mean diameter of proximal and distal PTM branches and these were 1.4 ± 0.4 mm and 1.3 ± 0.4 mm, respectively. In two reported studies (8, 10) the mean diameter (without specifying the proximal and distal branches) was 1.5 mm. None of the studies analyzed length and diameter of the nerves in relation with sex and side (Table 6).

Tab. 6 Outcomes of different studies on morphometry of the PTM branches.

Studies (year) and Region	Type of study	Gender/ Side	Nerve length (cm)	Nerve diameter (mm)
Tung and Mackinnon, 2001 – USA [5]	Cadaveric	Overall	2.2 ± 0.8 (P); 2.9 ± 1.0 (D)	NR
Alves et al., 2004 – Brazil [18]	Cadaveric	Overall	6.2	NR
Tubbs et al., 2011 – USA [10]	Cadaveric	Overall	3.6	1.5
Yang et al., 2014 – China [22]	Cadaveric	Overall	9.64 ± 0.71	NR
Caetano et al., 2018 – Brazil [8]	Cadaveric	Overall	4.0 ± 1.2	1.5 ± 0.6
Bertelli et al., 2020 – Brazil [9]	Cadaveric	Overall	2.7 ± 8 (P); 3.8 ± 15 (D)	1.4 ± 0.4 (P); 1.3 ± 0.4 (D)

P: proximal branch; D: distal branch; NR: not reported.

DISCUSSION

Our study revealed considerable variations in the innervation pattern of PTM contrary to the classic description of anatomical textbooks. We found majority of the branches (52.9%) are two in number and most of the branches are located distal to HIL (50.3%) from the reported studies. Knowledge on the number and location of branches is very important for clinicians in planning the appropriate type of electrostimulation in rehabilitation process to

restore the motor function (6, 27). It is very surprising to see the data on number of PTM branches in relation with gender; side is very vague and quite often not reported in the published studies. The French followed by American population having a low percentage of branches located proximal to HIL. The American followed by French and Brazilian populations having the lowest branches at HIL while Indians having the highest. Whereas the British having low percentage of branches distal to HIL while Indians having the highest (Table 4). We firmly believe that the above comparison and knowledge of knowing their population wise distances from the HIL (Table 5) can be of help while undertaking the surgical intervention procedures associated with pronator teres syndrome, pronator teres rerouting or neurotomy etc.

The quantitative anatomical studies investigating PTM nerve morphometrics are found to be relatively rare. This finding is unexpected given the widespread use of PTM nerves in neurotization procedures. During neurotization procedures, surgeons usually face a challenge in searching an adequate nerve which has an appropriate length and diameter. From our analysis, we found that the length of PTM nerves ranging from 2.2–9.6 cm (Table 6). Predominantly, studies reported measuring the length of PTM branches from their point of origin (from Mn) to the point where they enter the muscle. Our observation of wide variation in the length of branches is up to 7.4 cm. The nerve length is shown to vary between populations. Our analysis reported the nerve length is shortest in Americans while Chinese having the longest. Despite using the same anatomical landmarks for measurements in both studies, they are seen to have significantly different values (Table 6). The reason for this wide variation could be the differences in usage of embalmed vs. fresh cadavers or the length of limb or age of the cadaveric sample. Our data shows that the branches are in enough length to reach the important motor nerves such as anterior interosseous nerve (AIN), radial nerve branches to extensor carpi radialis brevis (ECRB), extensor carpi radialis longus (ECRL) and posterior interosseous nerve (PIN). Caetano et al. (8) based on findings in 12 limbs reported that one of the PTM branches is too long to be connected to the PIN distal to the emergence of the nerve to supinator muscle.

We analyzed the mean diameter of PTM branches from 82 limbs of three studies (Table 6). The mean diameter of the proximal and distal branches of PTM reported from studies is: 1.45 ± 0.5 mm; 1.3 ± 0.4 mm, respectively. Two studies (8, 10) on 50 limbs have reported the mean diameter of nerve as 1.5 mm without specifying the proximal and distal measurements. The reported mean diameter of these nerves is almost compatible and corresponding to the mean diameters of AIN, ECRL and ECRB. Few studies have reported the mean diameter of AIN as 1.6 mm (28); 1.7 mm (8) and 2.0 mm (29), respectively. The mean diameter of PTM branches from our analysis is almost corresponding to 94%; 88% and 75% of the mean diameter of AIN from the above reported studies. In addition to AIN, the mean diameter of branches for the ECRL (1.5 ± 0.6 mm) and ECRB (1.4 ± 0.7 mm) reported by Caetano et al. (8) are also corresponding to 100% and 90% of the diameter of PTM branches observed by us. On the other hand, studies have also reported the diameter of PIN as 3.0 ± 0.5 mm (8, 30) and the compatibility of PTM branches to PIN from these reports seems to be 50%. Our analysis on the above compatibilities further supported by a recent histomorphometric study, wherein, the PTM branches are seen to have an average of 646 and 599 myelinated fibers in both proximal and distal branches, respectively. These myelinated fibers are more or less close to the 548 and 457 fibers of ECRB reported (9, 31). The above comparison of diameter differences and nerve fibers might give an explicit idea about selecting the donor nerve for neurotization procedures. It may not be possible to get a 100% accurate donor nerve with identical structure of the recipient nerve at both macro and microarchitectural properties. Few papers published in the past on animal experiments have demonstrated that the axonal multiplication between donor and recipient was 1 : 3 (32) and at least 30% of the original motor neurons are required to achieve normal muscle function (33). Therefore, the donor nerve must have at least 30% of the number of axons of the receptor nerve (34). And the quantitative data which we have procured from the literature is clearly supporting the assumption that the PTM branches are compatible (50–100%) to all the nearby motor nerves.

In conclusion, our pooled data demonstrated that the innervation patterns of PTM branches vary between populations in terms of their number, location, and morphometry. These variations between number, length, diameter, and their placement in relation with HIL could be a result of anatomical dissections they have performed on embalmed vs. fresh cadavers. Although such procedural bias could exist, the larger pooled data of our study could give a standard dataset about their morphometry and we firmly believe that this data is of help for surgeons in comparing donor and recipient sites pre-operatively.

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AUTHOR CONTRIBUTIONS

BKP: Conceptualized and designed the study, reviewed the literature, compiled all the data, drafted the manuscript. MVRS: Independently reviewed the literature, involved in thorough checking of all the data and references. Both authors approved the final manuscript submitted for publication.

COMPETING INTERESTS

The authors declare that they have no competing interests associated with this manuscript.

ABBREVIATIONS

AIN: Anterior Interosseous Nerve
ECRB: Extensor Carpi Radialis Brevis,
ECRL: Extensor Carpi Radialis Longus
HIL: Humeral Intercondylar Line
Mn: Median nerve
PIN: Posterior Interosseous Nerve
PTM: Pronator Teres Muscle

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The Effect of *Lactobacillus casei* on Experimental Porcine Inflammatory Bowel Disease Induced by Dextran Sodium Sulphate

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ABSTRACT

Background: Gastrointestinal injury caused by dextran sodium sulphate (DSS) is a reliable porcine experimental model of inflammatory bowel disease (IBD). The purpose of this study was to evaluate the effect of probiotic *Lactobacillus casei* DN 114001 (LC) on DSS-induced experimental IBD.

Results: Eighteen female pigs (Sus scrofa f. domestica, weight 33–36 kg, age 4–5 months) were divided into 3 groups (6 animals per group): controls with no treatment, DSS, and DSS + LC. LC was administered to overnight fasting animals in a dietary bolus in the morning on days 1–7 (4.5×10^{10} live bacteria/day). DSS was applied simultaneously on days 3–7 (0.25 g/kg/day). On day 8, the pigs were sacrificed. Histopathological score and length of crypts/glands (stomach, jejunum, ileum, transverse colon), length and width of villi (jejunum, ileum), and mitotic and apoptotic indices (jejunum, ileum, transverse colon) were assessed.

DSS increased the length of glands in the stomach, length of crypts and villi in the jejunum and ileum, and the histopathological score of gastrointestinal damage, length of crypts and mitotic activity in the transverse colon. Other changes did not achieve any statistical significance. Administration of LC reduced the length of villi in the jejunum and ileum to control levels and decreased the length of crypts in the jejunum.

Conclusions: Treatment with a probiotic strain of LC significantly accelerated regeneration of the small intestine in a DSS-induced experimental porcine model of IBD.

KEYWORDS

dextran sodium sulphate; experimental inflammatory bowel disease; *Lactobacillus casei* DN 114001; pigs

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FOOTNOTE

Preliminary results of this study were presented as a poster at the 26th United European Gastroenterology Week, Vienna, October 20–24, 2018 (abstract published in UEG J 2018; 6, Suppl 1: A654) and Digestive Disease Week, San Diego, CA, USA, May 18–21, 2019 (abstract published in Gastroenterology 2019; 156, No 6 Suppl 1: S623–S624).

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BACKGROUND

The aetiology and pathogenesis of inflammatory bowel disease (IBD) comprise genetic susceptibility, various environmental factors (including infectious agents and xenobiotics), and abnormal immune response to intestinal microbiota (1). Both ulcerative colitis (UC) and Crohn's disease (CD) are associated with a reduced microbial diversity (2). Thus, the use of probiotics could be beneficial as they increase microbial diversity, which may subsequently improve the balance and function of intestinal microbiota (3, 4). The possible therapeutic role of probiotics and/or synbiotics has been evaluated in several studies; however, no indisputable final conclusions were achieved (5, 6).

The European Crohn's and Colitis Organisation has stated that there is no evidence to suggest that probiotics are beneficial for the maintenance of remission in CD (7). According to the Cochrane Database, there is insufficient evidence currently to draw any conclusion regarding the efficacy of probiotics for induction and maintenance of remission and prevention of post-operative recurrence of CD (8–10). In contrast, probiotic VSL#3 (a mixture of eight strains, including bifidobacteria, lactobacilli and Streptococcus thermophilus) can improve therapeutic response and maintenance of remission in UC patients (11). The probiotic VSL#3 has been shown to prevent pouchitis within the first year after surgery. According to Magro et al., after achieving remission in chronic pouchitis by treatment, VSL#3 can maintain the remission (12). Another probiotic strain that was found possibly beneficial to maintain the remission in UC is *Escherichia coli Nissle*. However there is no evidence on the efficacy of other probiotics regarding UC (13). The Cochrane Database reviews stated that conventional therapy combined with a probiotic does not improve overall remission rates in patients with mild to moderate UC (14–15). The effects of antibiotics, probiotics and other interventions for treating and preventing pouchitis are uncertain (16). Further studies are indispensable so that conclusive inference on the efficacy of probiotics in UC and CD can be made (4).

The experimental model of colitis induced by dextran sodium sulphate (DSS) in mice was proposed in mid 90s (17–19). DSS-induced mucosal injury also represents a suitable and reliable experimental porcine model of IBD (20–22). Pigs can be used in various preclinical experiments due to their relatively similar gastrointestinal physiology compared to that of humans (23–24), including the porcine intestinal microbiome (25–27). In our previous projects, we studied the effect of probiotic *Escherichia coli Nissle* on bacteriocin production and indomethacin-induced gastrointestinal injury in experimental pigs (28, 29). *Escherichia coli Nissle* alone provided a significantly favourable trophic effect on the colonic mucosa. By contrast, indomethacin and probiotics administered together led to the worst outcome on the porcine stomach, small and large bowel ("anti-synbiotic" effect), and bacteriocin production (28, 29). On the other hand, lactobacilli can ameliorate indomethacin-induced intestinal injury (30). Additionally, lactobacilli possess a protective effect against DSS-induced experimental colitis in mice (31–34). The purpose of this study was to evaluate the effect of probiotic *Lactobacillus casei* DN 114001 (LC) on a DSS-induced experimental porcine model of IBD.

METHODS

ANIMALS

Eighteen experimental adult female pigs (Sus scrofa f. domestica, hybrids of Czech White and Landrace breeds; weight: 33–36 kg, mean 34.3 ± 1.0; age 4–5 months) were enrolled into the study. The animals were purchased from a certified breeder (Stepanek, Dolni Redice, Czech Republic; SHR MUHO 2050/2008/41). The pigs were housed in an accredited vivarium (temperature 21 ± 1 °C, 12 hour light/dark cycle; Faculty of Military Health Sciences, Hradec Kralove, Czech Republic). All animals were fed with standard assorted A1 food (Ryhos, Novy Rychnov, Czech Republic) of equal amounts twice a day and had free access to drinking water. The acclimatization period was 21 days before the experiment.

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies (35). Animals were held and treated in conformity with the European Convention for the Protection of Vertebrate Animals (36) and in accordance with the ARRIVE Guidelines (37).

STUDY DESIGN

The animals were divided into 3 groups: controls with no treatment (n = 6), DSS (n = 6) and DSS + LC (n = 6). LC was administrated to overnight fasting animals in a single dietary bolus in the morning on days 1–7 (4.5×10^{10} live bacteria/day). DSS (molecular weight 40 kDa; purchased from Sigma-Aldrich, St. Louis, MO, USA) was applied simultaneously in another dietary bolus on days 3–7 (0.25 g/kg/day). On day 8 (after 24 hours of fasting), the pigs were anaesthetized (intramuscular ketamine, Narkamon, Bioveta, Ivanovice na Hane, Czech Republic, dose 20 mg/kg; and azaperone, Stresnil, Jansen Pharmaceutica, Beerse, Belgium, dose 2 mg/kg), and sacrificed by exsanguination. Immediate autopsy was performed and specimens for structural and morphometric analysis were collected, including the stomach (middle part of the gastric body), and the middle parts of the jejunum, ileum and transverse colon. The samples were immediately fixed with 10% neutral buffered formalin (Bamed, Ceske Budejovice, Czech Republic). There were no adverse events in any experimental group.

STAINING OF SAMPLES

The formalin-fixed samples were routinely processed. This included dehydration, embedding into paraffin (Paramix, Holice, Czech Republic), preparation of 5 µm thick tissue sections using microtome model SM2000 R (Leica, Wetzlar, Germany), rehydration, staining with haematoxylin-eosin (Sigma-Aldrich), final dehydration, and mounting into an aqueous-free mounting medium DPX (Sigma-Aldrich).

HISTOPATHOLOGICAL SCORE

Stained samples were evaluated using a BX-51 microscope (Olympus, Tokyo, Japan). The histopathology score (from 0 to 11) was measured according to Appleyard and Wallace by summation of scores for loss of mucosal architecture, cellular infiltration, muscle thickening, crypt abscess formation, and goblet cell depletion (Table 1) (38). Evaluation of all samples was performed by one person.

Tab. 1 Histopathology score of gastrointestinal damage (ref. 38).

Parameter	Score
loss of mucosal architecture	0, 1, 2, 3 (absent, mild, moderate, severe)
cellular infiltration	0, 1, 2, 3 (absent, mild, moderate, extensive)
muscle thickening	0, 1, 2, 3 (absent, mild, moderate, extensive)
crypt abscess formation	0 or 1 (absent or present)
goblet cell depletion	0 or 1 (absent or present)

LENGTH OF CRYPTS AND GLANDS AND LENGTH AND WIDTH OF VILLI

The length of crypts/glands (all segments) and length and width of villi (small intestine only) were assessed by BX-51 microscope equipped with image analysis software ImagePro plus 7 (Media Cybernetics, Rockville, MD, USA). For this analysis, 20 randomly selected glands and 20 randomly selected crypts and villi per animal were measured under 80× and 200× magnification, respectively.

EVALUATION OF MITOTIC AND APOPTOTIC ACTIVITIES

In crypts, mitotic and apoptotic activity were measured under 400× magnification and published as apoptotic and mitotic indices. The definition of an apoptotic cell and calculation of both indices were according to previous work done by Pejchal et al. (39).

STATISTICS

Kruskal-Wallis test with multiple pairwise comparisons was used for statistical analysis (IBM SPSS Statistics, version 24; IBM Corp., Armonk, NY, USA). Differences were considered significant when $p < 0.05$.

ETHICS APPROVAL

The Project was approved by the Institutional Review Board of the Animal Care Committee of the University of Defence (Record Number 14922006), Faculty of Military Health Services, Hradec Králové, Czech Republic.

RESULTS

DSS treatment increased the length of gastric glands by 12% ($p < 0.001$), the length of villi and crypts in the jejunum by 10% ($p = 0.023$) and 41% ($p < 0.001$) respectively, the length of villi and crypts in the ileum by 16% ($p = 0.047$) and 23% ($p < 0.001$) respectively, and the histopathological score in the colon from 0 (controls) to 3.80 ± 1.3 ($p = 0.007$), which was associated with increased length of crypts and mitotic activity by 57% and 158% respectively (Table 2). Administration of LC reduced the length of villi in the jejunum and ileum to control levels. It also decreased the length of crypts in the jejunum by 13% when compared with DSS-treated animals (Table 2). Minor to moderate inflammatory changes were found over the small and large intestine (Figures 1–3).

Tab. 2 Average values of histopathological score, morphometric parameters and apoptotic and mitotic indices in the stomach, jejunum, ileum, and transverse colon (mean ± SEM).

		Controls	DSS	DSS + LC
	Stomach			
Histopathological score		0 ± 0	0 ± 0	0 ± 0
glands	length (μm)	1000 ± 28	1118 ± 19 †	1102 ± 17 †
	Jejunum			
Histopathological score		0 ± 0	0.6 ± 0.4	0.4 ± 0.4
villi	length (μm)	293 ± 14	308 ± 13 †	282 ± 16 ‡
	width (μm)	196 ± 11	191 ± 10	189 ± 10
crypts	length (μm)	312 ± 9	440 ± 13 †	385 ± 15 †‡
	apoptotic index (%)	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
	mitotic index (%)	0.7 ± 0.2	0.8 ± 0.3	0.7 ± 0.3
	Ileum			
Histopathological score		0 ± 0	0.2 ± 0.4	0.6 ± 0.7
villi	length (μm)	251 ± 13	292 ± 19 †	245 ± 14 ‡
	width (μm)	186 ± 9	187 ± 12	195 ± 12
crypts	length (μm)	282 ± 9	347 ± 12 †	323 ± 11 †
	apoptotic index (%)	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1
	mitotic index (%)	1.3 ± 0.6	1.0 ± 0.3	1.1 ± 0.3
	Transverse colon			
Histopathological score		0 ± 0	3.8 ± 1.3 †	3.6 ± 1.2 †
crypts	length (μm)	421 ± 10	660 ± 19 †	660 ± 18 †
	apoptotic index (%)	1.0 ± 0.4	0.8 ± 0.3	0.7 ± 0.4
	mitotic index (%)	1.2 ± 0.3	3.1 ± 1.6 †	2.4 ± 1.0 †

† Significant differences between control and DSS or control and DSS + LC groups: $p \leq 0.05$.

‡ Significant differences between DSS and DSS + LC groups: $p \leq 0.05$.

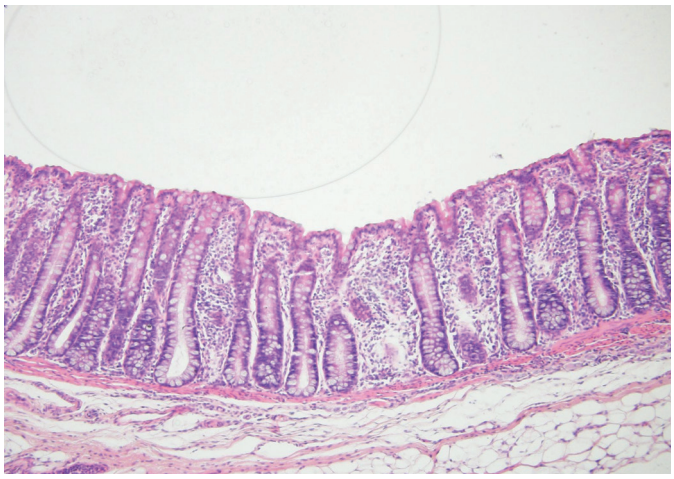

Fig. 1 Control sample of the porcine transverse colon stained with haematoxylin-eosin at 100fold original magnification. No pathology can be observed.

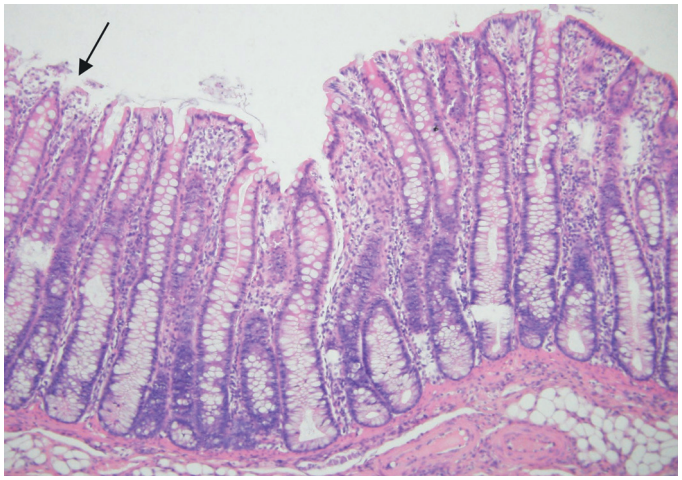

Fig. 2 DSS-treated sample of the porcine transverse colon stained with haematoxylin-eosin at 100fold original magnification. Slight mucosal oedema with acute inflammatory infiltrate and prolonged crypts can be observed. Arrow indicates mucosal erosion.

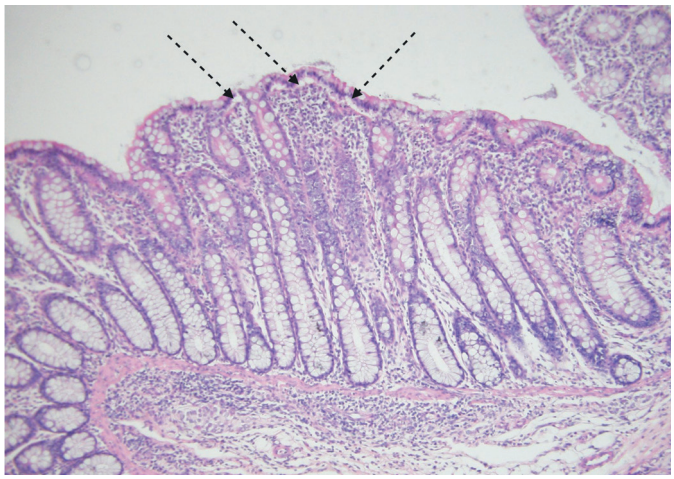

Fig. 3 DSS and Lactobacillus casei treated sample of the porcine transverse colon stained with haematoxylin-eosin at 100fold original magnification. Slight subepithelial (dashed arrows) and mucosal oedema with an in inflammatory infiltrate and prolonged crypts can be observed.

DISCUSSION

Our current study brought new important insight into experimental IBD. To our best knowledge, this is the first study of LC in a DSS-induced porcine experimental model of IBD. DSS is able to induce not only colonic but also small intestinal injury. The lengths of jejunal villi and small intestinal and colonic crypts were significantly taller in the DSS group compared to controls and the DSS+LC group. The histopathology score and mitotic index were increased significantly only in the porcine colon.

Knowledge on a number of species of the genus *Lactobacillus* has broadened considerably during the past 15 years. More than two hundred species are currently recognized (40). Some probiotic lactobacilli have been used for decades, and several species are clearly characterized by their anti-inflammatory effect (41–43). Nonetheless molecular mechanisms underlying the probiotic impact have as yet not been fully understood (40). An ameliorating and/or preventive impact of lactobacilli in murine DDS-induced colitis has been found in several studies (44–47). This effect may be explained by inhibition of excessive activation of the NF-κB pathway (44, 45), suppression of TNF-α-mediated apoptosis of intestinal epithelial cells (48), by activation of epidermal growth factor receptor (49), down-regulation of neutrophilic infiltration (in the case of incomplete toll-like receptor 4 complex signalling) (46), or by down-regulation of T follicular helper cells (50).

Even a lysate of non-living probiotic lactobacilli can prevent severe inflammation by improving the integrity of the intestinal barrier, and/or by modulation of the murine gut microenvironment (51–53). *Lactobacillus casei* decreases caecal and colonic inflammatory scores (41, 47). It can also prevent body weight loss in experimental animals in DSS-induced murine colitis (47, 54).

Vetuschi et al. (55) and Araki et al. (56) found increased apoptosis and decreased proliferation of epithelial cells that might lead to a breakdown of the epithelial barrier function. The authors concluded that this could facilitate the mucosal invasion of intraluminal microorganisms in DSS-induced murine colitis (56). Chae et al. found that lactic acid bacteria can reduce both colitis-induced and NF-κB-mediated apoptosis of intestinal epithelial cells in mice (48). We did not find any significant difference in apoptosis in our current porcine study. However, the mitotic index of the colonic mucosa was significantly higher in the DSS group. It is surprising that the apoptotic index did not change in any segment of the investigated gastrointestinal tract. However, apoptosis is a very complex event which is regulated by both pro-apoptotic and anti-apoptotic components. Survivin, an anti-apoptotic protein has been studied extensively in cancer patients, but little knowledge exists about this inhibitor of apoptosis in IBD patients. It has been reported that levels of survivin are increased in lamina propria T-cells in patients with CD, which leads to an anti-apoptotic effect of the T cells (57). Mennigen et al. found that the probiotic mixture VSL#3 (also containing lactobacilli) protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis (58). Other studies with VSL#3

in murine DSS-induced colitis found also a beneficial effect of probiotics improving ileal microbiota composition (59, 60). The impact of DSS on the entire gastrointestinal tract depends on three variables: molecular weight of DSS, daily dose and cumulative dose of DSS. In our current study, only minor to moderate inflammatory changes were found over the small and large intestine. Differing doses of DSS have been recommended (from 0.25 to 1 g/kg/day) to induce experimental IBD. We intentionally decided for a lower dose. Experimental animals (mouse, rat, pig) may express different sensitivity to DSS. In addition, particular batches of DSS may differ in their grade of toxicity. That is why we recommend conducting preliminary testing of a particular batch of DSS on control animals so that the dose can be adjusted accordingly (our unpublished data).

We are aware of possible limits of our current study. The project was designed as an acute one, lasting eight days only. Longer duration could reveal additional findings, especially possible apoptotic changes of the intestinal epithelial cells.

Probiotics may have a positive impact on intestinal inflammatory changes through their interaction directly with the immune system or indirectly through the modulation of gut microbiota (61). Further studies, both experimental and clinical, are needed to understand this process in detail. Only thus, possible clinical applications may be possible.

CONCLUSIONS

Treatment with the probiotic strain LC significantly accelerated regeneration of the small intestine in a DSS-induced experimental porcine model of IBD.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Unintentional Opioid Overdoses Treated at University Clinic of Toxicology-Skopje in a Nine-Year-Period

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ABSTRACT

Background: The aim of this study was to assess the epidemiological profile of unintentional opioid overdoses, the prevalence and number of psychotropic substances involved in opioid overdoses.

Methods: This was a descriptive study, in which 180 participants were enrolled, and covered a nine-years-period. For collecting data was used the National patient electronic system "My term". The variables as gender, age, duration of opioid dependence, number of overdoses, type of substance, number of antidote ampoules, duration of hospitalization were analyzed. Severity of poisoning was made by using the Poison severity score.

Results: Opioid overdose cases were significantly higher among males than females. Mean age with standard deviation (SD) was 32.23 ± 6.71 years. Mean years (±SD) of duration of opioid use disorder was 11.60 ± 5.89 years. The most commonly used primary substance was methadone in 68.89% and heroin in 31.11% cases. Twenty patients were treated with mechanical ventilation because of the severe respiratory depression. Poison severity score was moderate in 51.11%, severe in 45.56% and fatal in 3.33% of the cases.

Conclusion: Most of the cases, predominantly males used one or two substances. The combination of methadone and benzodiazepine was most frequently used and the most common way was by injecting the abused substances. In most of the subjects PSS score was moderate and severe with no differences between genders.

KEYWORDS

opioids; overdose; Naloxone; Flumazenil; poison severity score

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INTRODUCTION

Opioid use disorders, fatal and nonfatal opioid-related overdoses (OODs) are significant public health problems. Accurate identification of OODs is essential to quantify the burden of the problem, evaluate risk-reduction strategies, monitor population-level outcomes, and improve prevention and quality of care (1).

Drug overdose continues to be a major cause of death, especially among young people in Europe, with recent data showing that it accounts for more than 3.4% of all deaths among Europeans between the ages of 15 and 39 (Eurostat, 2013). European countries have implemented a variety of approaches in their attempt to reduce overdose deaths at the national level using evidence-based interventions drawing on an understanding of individual and environmental risk factors. The type of substance used, the route of administration and the health of the user all have an impact on the risk of overdose. Most overdose deaths are linked to the use of opioids, primarily the injection of heroin (2).

Methadone is well established as an effective treatment of patients with opioid use disorder. However, the risk of sedation and respiratory depression with improper methadone dosing remains. Despite precautions for methadone prescription such as the dispensing of methadone under daily witnessed supervision in unstable patients and regular urine drug screen testing to verify compliance, fatalities associated with methadone have increased markedly across numerous jurisdictions in recent years (3).

Drug overdose mortality has reached unprecedented levels in the United States. Over the past two decades, drug overdose has more than tripled to become the leading cause of injury deaths in the US, outnumbering deaths from motor vehicle accidents and homicides according to data from the Centers for Disease Control and Prevention (CDC) / National Center for Health Statistics (NCHS). The epidemic shows no signs of leveling off: drug overdose mortality continued to rise through 2017, amounting to over 70,000 deaths in that year and increasing by 16 percent per year between 2014 and 2017 (4).

Compared with fatal heroin overdoses, the phenomenon of nonfatal overdose has been largely overlooked, apart from early reports based on intravenous drug user (IDU) surveys. Given that such surveys are subject to sampling bias, caution is required when attempting to determine the prevalence and characteristics of nonfatal overdoses from these samples. As such, the utilization of ambulance data may play an important role in determining the prevalence of nonfatal overdose. In addition, many heroin users who survive an overdose do not seek medical assistance (5).

Aims: 1. To assess the epidemiological profile of unintentional opioid overdoses over a nine-year period.

MATERIAL AND METHODS

DESIGN OF THE STUDY

This study has a descriptive design comprising a nine-year-period (2011–2019), and included a total of 180 patients with drug overdose, either illicit or prescribed opioids.

University Clinic of Toxicology is part of the biggest national tertiary care center, the Clinical campus "Mother Teresa" in Skopje, Republic of North Macedonia. This Clinic functions as an emergency center for internal diseases, which also includes the Toxicology department. Patients with opioid use disorder (OUD) come into the Institution/Hospital for one of the following reasons: overdoses with illicit and prescribed drugs, clinical examination and treatment of somatic diseases despite continuing opioid use, detoxification, withdrawal symptoms, or initiation of OUD treatment with the substitute buprenorphine. All participants underwent an interview and a complete clinical examination performed by the University Clinic of Toxicology specialists in internal medicine.

The National patient electronic system "My term" was used for collecting data. The variables: gender, age, duration of opioid dependence, number of overdoses, type of substance, number of antidote ampoules, duration of hospitalization were analyzed. Assessment of patient severity of poisoning was made by using the Poison severity score (PSS, Persson H et al., 1998) with the following score: none – 0 (no symptoms or signs related to poisoning); minor – 1 (mild, transient and spontaneously resolving symptoms); moderate – 2 (pronounced or prolonged symptoms); severe – 3 (severe or life-threatening symptoms); fatal – 4 (death).

SAMPLE

We analyzed patients with unintentional opioid overdoses who were treated as out/inpatients. The inclusion criteria were: 1. Overdoses with illicit and prescribed opioid drugs identified by a combination of three signs and symptoms referred to as the "opioid overdose triad": pinpoint pupils, unconsciousness, respiratory depression. 2. Positive response to Naloxone, 3. History of patient positive for opioid use disorder. Exclusion criteria were the following: alternative diagnosis (e.g., trauma or infection), non-opioid drug poisoning. This study was in accordance the etic protocol for use of electronic data from the National electronic medical system provided to the University Clinic of Toxicology.

DATA ANALYSES

Data was statistically analyzed with the SPSS software package, version 22.0 for Windows (SPSS, Chicago, IL, USA). The qualitative series were processed by determining the coefficient of relations, proportions, and rates, and were shown as absolute and relative numbers. Quantitative series were analyzed with measures of central tendency (average, median), as well as with dispersion measures (standard deviation, standard error). Pearson Chi square test and Fischer exact test were used to determine the association between certain attributive dichotomies. Difference test was used for comparison of proportions. A two-sided analysis with a significance level of $p < 0.05$ was used to determine the statistical significance.

RESULTS

During the period of 9 years (2011–2019), we registered a total of 180 unintentional opioid overdose cases, and there were significantly more cases among males than among females. The highest proportion of unintentional overdoses, 26 (14.44%), was observed in 2014 and 2016 and the lowest, 7 (3.89%), in 2011. In 2015/16 we did not register any case of unintentional opioid overdose among females. The mean age of males was 32.51 ± 6.42 years and of females 29.79 ± 6.70, with no significant differences between genders (p = 0.0683). Also, there were no significant gender differences (p = 0.1784) related to the duration of opioid use disorder, which was 11.80 ± 5.95 vs. 9.89 ± 5.19 years in males and females, respectively. The number of previous overdoses were significantly more present among males compared to females (p = 0.0203), with maximum of 5 vs. 3 years, respectively (Table 1).

Tab. 1 General characteristics of the sample of unintentional opioid overdoses (2011–2019).

Parameters	Gender			p
	Male	Female	Total	
Sample				
N (%)	161 (89.44%)	19 (10.56%)	180 (100%)	[1]p = 0.0001*
Year of unintended opioid overdoses				
2011	6 (85.71%)	1 (14.29%)	7 (3.89%)	[1]p = 0.0001*
2012	24 (88.89%)	3 (11.11%)	27 (15%)	[1]p = 0.0001*
2013	20 (95.24%)	1 (4.76%)	21 (11.67%)	[1]p = 0,0001*
2014	18 (69.23%)	8 (30.77%)	26 (14.44%)	[1]p = 0.0061*
2015	23 (100%)	0 (0%)	23 (12.78%)	–
2016	26 (100%)	0 (0%)	26 (14.44%)	–
2017	17 (80.95%)	4 (19.05%)	21 (11.67%)	[1]p = 0.0001*
2018	18 (94.74%)	1 (5.26%)	19 (10.56%)	[1]p = 0.0001*
2019	9 (90%)	1 (10%)	10 (5.56%)	[1]p = 0.0001*
Age				
± SD Min/Max Median (IQR)	32.51 ± 6.42 20/55 32 (28–37)	29.79 ± 6.70 15/47 29 (27–32)	32.23 ± 6.71 15/55 32 (28–36)	Z = 1.8226; p = 0.0683
Duration of addiction (years)				
± SD Min/Max Median (IQR)	11.80 ± 5.95 2/32 11 (8–15)	9.89 ± 5.19 2/27 9 (8–11)	11.60 ± 5.89 2/32 10 (8–14)	Z = 1.3454; p = 0.1784
Number of overdoses				
± SD Min/Max Median (IQR)	2.04 ± 0.99 1/5 2 (1–3)	1.47 ± 0.71 1/3 1 (1–2)	1.98 ± 0.98 1/5 2 (1–3)	Z = 2.3207; p = 0.0203*

[1] Difference test; Mann-Whitney U test = Z; * significant for p < 0.05

The number of substances used for unintentional opioid overdoses was one in 104 (57.78%) cases, two in 67 (37.22%), and three in 7 (3.89%) cases. Four and five substances were used only by 1 (0.56%) person each. Regarding gender, reported use of one or two substances was 90 (55.9%) vs. 62 (38.51%) in males and 14 (73.68%) vs. 5 (26.32%) in females, respectively. The use of three and more substances was found only among male subjects.

The most commonly used primary substance for unintentional opioid overdose was methadone in 124 (68.89%) and heroin in 56 (31.11%) cases. Slow releasing morphine, Tramadol, alcohol, buprenorphine, amphetamine, and cocaine were found to be used only by male patients. The most frequently used combination was methadone and benzodiazepine (n = 59) and the most common way was by injecting the abused substances (Table 2).

We found no significant gender differences related to treatment, duration of hospitalization, and mechanical ventilation (p = 0.2559 vs. p = 0.1861 vs. p = 0.3911). Males were significantly more often treated with Naloxone compared to females (p = 0.0162), but this was not the case in the treatment with Flumazenil. The mean value of Naloxone antidote was 1.37 ± 0.74 mg (min/max: 0.04/4.4 mg). The mean value of Flumazenil antidote was 0.73 ± 0.3 mg (min/max: 0.5/1.5 mg). PSS score was moderate in 92 (51.11%) and severe in 82 (45.56%) of the cases with no percentage differences between the genders for p = 0.1320 vs. p = 0.2060, consequently. We found fatal PSS score among 6 (3.33%) male cases. Four patients received the methadone and benzodiazepine combination; the fatal outcome in one patient was as a result of using heroin, methadone, Tramadol and cocaine combination, and the last patient had fatal outcome as a result of methadone, benzodiazepine and alcohol combination (Table 2).

Tab. 2 Substances used and treatment of cases with unintentional opioid overdoses (2011–2019).

Parameters	Gender			p
	Male N = 161	Female N = 19	Total N = 180	
Substance				
Heron	48 (28.81%)	8 (42.11%)	56 (31.11%)	X2 = 1.198; df = 1; p = 0.2737
Methadone	113 (70.19%)	11 (57.89%)	124 (68.89%)	X2 = 1.190; df = 1; p = 0.2736
Tramadol	5 (3.11%)	0 (0%)	5 (2.78%)	–
Benzodiazepine	57 (35.40%)	5 (26.32%)	62 (34.44%)	X2 = 0.622; df = 1; p = 0.4305
Slow releasing morphine	1 (0.62%)	0 (0%)	1 (0.56%)	–
Alcohol	4 (2.48%)	0 (0%)	4 (2.22%)	–
Buprenorphine	1 (0.62%)	0 (0%)	1 (0.56%)	–
Amphetamine	2 (1.24%)	0 (0%)	2 (1.11%)	–
Cocaine	3 (1.90%)	0 (0%)	3 (1.69%)	–
Treatment				
Outpatient	80 (49.68%)	12 (63.16%)	92 (51.11%)	X2 = 1.2909; df = 1; p = 0.2559
Inpatient	81 (50.32%)	7 (36.84%)	88 (48.89%)	

Parameters	Gender			p
	Male N = 161	Female N = 19	Total N = 180	
Duration of hospitalization (days)				
± SD Min/Max Median (IQR)	3 ± 2.67 1/17 2 (2–3)	2 ± 0.01 2/2 2 (2–2)	2.93 ± 2.59 1/17 2 (2–3)	Z = 1.3222; p = 0.1861
Number of Naloxone (ampoules 0.4 mg/ml)				
± SD Min/Max Median (IQR)	3.41 ± 1.91 1/11 3 (2–4)	2.32 ± 1.11 1/5 2 (2–3)	3.29 ± 1.87 1/5 2 (2–3)	Z = 2.4045; p = 0.0162*
Number of Flumazenile (ampoules 0.5 mg / 5 ml)				
± SD Min/Max Median (IQR)	1.46 ± 0.60 1/3 1 (1–2)	1.50 ± 0.58 1/2 1.5 (1–2)	1.47 ± 0.59 1/3 1 (1–2)	Z = -0.2074; p = 0.8357
Poisoning severity score – PSS				
moderate – 2	79 (49.06%)	13 (68.42%)	92 (51.11%)	[1]p = 0.1320
severe – 3	76 (47.21%)	6 (31.58%)	82 (45.56%)	[1]p = 0.2060
fatal – 4	6 (3.73)	0 (0%)	6 (3.33%)	–
Mechanical ventilation				
Yes	19 (11.80%)	1 (5.26%)	20 (11.11%)	[2]p = 0.3911
No	142 (88.20%)	18 (94.74%)	160 (88.89%)	

Pearson Chi-square test = X2; Mann-Whitney U test = Z; [1] Difference test; [2] Fisher exact test; * significant for p < 0.05

DISCUSSION

In this study we analyzed 180 patients with opioid overdose over of a nine-year-period. Of the total number of patients, 86 were inpatients, and the rest were treated in outpatient setting. The male population was predominant 89.44%. The mean age of the participants was 32.23 ± 6.71. The youngest was a female patient – fifteen years old and she experienced a heroin overdose. The oldest patient was a 55-year-old male. He overdosed with methadone and benzodiazepines intravenously.

Findings from one study of New South Wales, Victoria, Western Australia, and the Australian Capital Territory, reported gender distribution 70/30, with patients twice as likely to be males (6). This is consistent with other reports that showed that the heroin-using population is predominantly male. It was also reported that the mean age of heroin users was approximately 30 years (6). More recently, there has been some indication that the average age of users and the average age of initiation is decreasing (6). Warner-Smith et al. in their study noted that the decline in the average age of initiation does not necessarily indicate a corresponding decline in the mean age of heroin users (7). In studies reported by Darke and Loxley the average age of the male population was 27.7 years and of females 26 years (5). Ambulance data from Western Australia are similar to Queensland data, with a 2-year age difference between males (27.5 years) and females (25.6 years) (5). Previous studies have also found this approximate 2-year difference between the sexes (5). In our study there was a 2.7 years difference between males 32.51 ± 6.42 and females 29.79 ± 6.70. Males, older people and people with low socio-economic status are at higher risk of opioid overdose than women, as well as young people and people with higher socio-economic status (8).

Research has identified patient characteristics and prescribing practices that increase the risk of prescription opioid-related overdose and death (9). Patients with psychiatric disorders, and those using benzodiazepines or illicit drugs have higher risk of opioid-related overdose and death (9). Higher doses of prescription opioids are also associated with more overdose deaths (10, 11). Likewise, long-acting opioids are associated with an increased risk of non-fatal overdose (12). Among long-term opioid users in Medicaid, pharmacy shopping and overlapping prescriptions are associated with an increased risk of overdose (13). Additionally, for patients on opioid substitution therapy in England and Wales, methadone had a relative risk of overdose death of 6.23 when compared to buprenorphine. Among injection drug users in British Columbia, prescription opioid abuse was independently associated with overdose (14). Even in this study the most commonly used primary substance for unintentional opioid overdose was methadone in 124 (68.89%) and heroin in 56 (31.11%) cases. Of these, 86 patients were with prescribed methadone substitution therapy, and the remaining patients supplied drugs on the "black market". The combination of methadone and benzodiazepine was most frequently used. In our study out of total heroin overdoses (n = 56), three patients combined heroin with Diazepam. Fifty-nine patients made a combination of methadone (overdose) and benzodiazepines. All patients administered substances intravenously. Liang in his study reported that persons at highest risk of overdose (adjusted hazard ratios of 2–3) received a daily MED of ≥100 mg regardless of the total dose or a daily MED of 50 to 99 mg with a high total MED >1,830 mg (15). In our study the mean value of the prescribed methadone dose was 90 mg.

A study conducted by Fox included 109 patients (35.5%) who had met criteria for severe respiratory depression. Ninety patients received Naloxone alone, 9 underwent endotracheal intubation alone, and 10 received both Naloxone and endotracheal intubation (16). Recently, in one study the authors examined insurance claims to derive risk factors for overdose or opioid-induced respiratory depression. They found that among various comorbidities examined, a history of a substance use disorder was closely associated with development of opioid-induced respiratory depression, with an odds ratio of 12.7 (5).

In our study antidote Naloxone was administered in all 180 patients, and Flumazenil was administered in 59 patients. Twenty patients were treated with mechanical ventilation because of the severe respiratory depression and six patients had fatal outcome.

LIMITATIONS OF THE STUDY

This study included only subjects who came to the clinic, and thus the generalizability of the results may be limited. There were six fatal outcomes, and therefore it was not sufficient to give information about the risk of mortality. In addition, in our study, we focused on unintentional

overdoses, but the data in the survey were self-reported by the patients. Substance users who experience an overdose are usually polydrug users; it is very difficult to distinguish which and how many drugs were used before an overdose episode.

CONCLUSION

Opioid overdose was predominant in the male population. There were no significant differences between genders related to treatment opioid overdose, duration of hospitalization, and mechanical ventilation and duration of opioid use disorder. The number of previous overdoses was significantly higher in the male population. Most of the study participants, predominantly males used one or two substances, and rarely more substances. The most commonly used primary substance for unintentional opioid overdose was methadone and heroin. The combination of methadone and benzodiazepine was most frequently used, and the most common way was by injecting the abused substances. There were no significant gender differences related to treatment, duration of hospitalization, and mechanical ventilation. In most of the subjects PSS score was moderate and severe with no differences between genders.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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AUTHOR CONTRIBUTIONS

N.S. designed the study, managed the literature searches and analyses. A. B. obtained data. V. V. S. undertook the statistical analysis and all the authors discussed the results. All authors revised and approved the final manuscript.

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Genotype Associations with the Different Phenotypes of Atopic Dermatitis in Children

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ABSTRACT

This study deals with detecting the associations of atopic dermatitis' (AD) phenotypes in children: alone or combined with seasonal allergic rhino-conjunctivitis (SARC) and/or perennial allergic rhinitis (PAR), and/or with bronchial asthma (BA) with single nucleotide polymorphisms (SNP) of filaggrin (*FLG*), thymic stromal lymphopoietin (*TSLP*) and orsomucoid-like-1 protein 3 (*ORMDL3*) genes.

Male and female pediatric patients aged from 3 to 18 years old were recruited into the main (AD in different combinations with SARC, PAR, BA) and control groups (disorders of digestives system, neither clinical nor laboratory signs of atopy). Patients were genotyped for SNP of rs_7927894 *FLG*, rs_11466749 *TSLP*, rs_7216389 *ORMDL3* variants.

Statistically significant associations of the increased risk were detected of AD combined with SARC and/or PAR and AD combined with BA (possibly, SARC and/or PAR) with C/T rs_7927894 *FLG*and T/T rs_7216389 *ORMDL3* genotypes. Genotype C/C rs_7927894 *FLG* significantly decreases the risk of AD combined with SARC and/or PAR by 2.56 fold.

Several genotypes' associations had a trend to significance: C/C rs_7216389 *ORMDL3* decreases and C/T rs_7216389 *ORMDL3* increases the risk for developing AD alone phenotype; A/G rs_11466749 *TSLP* decreases the risk of AD combined with BA (possibly, SARC and/or PAR) phenotype development.

KEYWORDS

atopic dermatitis; children; genotype; phenotype; associations; filaggrin; thymic stromal lymphopoietin; orsomucoid1-like protein 3

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INTRODUCTION

Atopic dermatitis is a common, chronic, relapsing, inflammatory skin disease that primarily affects young children with an underlying inherited tendency to produce immunoglobulin E (IgE) antibodies in response to minute amounts of common environmental proteins (1), basically of food origin. AD clinically manifests with skin itching, skin lesions, dryness or oozing and rash of a multiple morphology and localization. It can induce the development of other atopic disorders (AtD) in children: seasonal allergic rhino-conjunctivitis (SARC), perennial allergic rhinitis (PAR) and bronchial asthma (BA). Such the phenomenon of AD progression into other AtD is defined as atopic march (AM) (2). Within different models of atopy progression there is an evident linkage between AD and AtD in the upper and lower airways (2, 3). Still, some studies directly point out at the oversimplification of AM hypothesis as an approach for interpreting the progression of AtD in children – there is being introduced the phenomenon of personalized AD phenotypes in combination with SARC and/or PAR, and/or BA (4, 5).

It was demonstrated that pathogenesis of AD and other AtD is preceded by a genetic predisposition and the set of major candidate-genes involved is being updated at the current moment (6). The recent studies yielded an evidence that genes to have such associations are: filaggrin (*FLG*) (2, 7, 8), thymic stromal lymphopoietine (*TSLP*) (8–10), orsomucoid1-like protein 3 or sphingolipid biosynthesis regulator 3 (*ORMDL3*) (11). Despite that *FLG* mutations are being studied longitudinally with much data collected so far (2, 6, 8), still there is a lack of studies on detection of the role of particular single nucleotide polymorphisms (SNP) of *FLG*, *TSLP* and *ORMDL3* genes in pathogenesis of AD and its phenotypes at pediatric patients. Accordingly, the associations and their character – increasing or decreasing – of the mentioned genes SNP with the risk of developing respective AD phenotypes need a novel elucidation.

Given the aforesaid, study goal was to detect the associations of different AD phenotypes with SNP rs_7927894 *FLG*, rs_11466749 *TSLP*, rs_7216389 *ORMDL3* in children.

MATERIALS AND METHODS

We had recruited 95 patients into the main group suffering the AD alone (n = 47) and in different combinations: a cohort of AD combined with either SARC and/or PAR (n = 38), AD combined with BA (possibly, with SARC and/or PAR) (n = 10). The patients were aged from 3 to 18 years old, age median had been 8 years old (LQ–HQ: 5–11). They were being recruited at Department of pediatrics 1 and medical genetics of SE "Dnipro medical academy of Health Ministry of Ukraine", in-patient and out-patient departments of the Allergy Centre of MNCE "Clinical hospital of the emergency care" of Dnipro City Council". The inclusion criteria consisted of: age 3–18 years old, the officially established diagnosis of AD, AD with SARC, AD with PAR, AD with SARC and/or PAR, AD with BA (possibly, SARC and/or PAR), elevated serum total IgE (>100 IU/ml). The exclusion criteria comprised absence of skin or airways' AtD's specific clinical signs, not elevated serum total IgE (<100 IU/ml).

The control group consisted of 80 patients not suffering from AtD recruited at the Department of pediatric gastroenterology of the MNCE "City clinical hospital #1" of Dnipro City Council". The children were aged from 3 to 18 years old and had been suffering the following diseases of gastro-intestinal tract: functional dyspepsia, chronic gastritis, peptic ulcer, gastro-esophageal reflux disease, functional disorders of the biliary system. The inclusion criteria were as follows: no clinical signs of AD, SARC, PAR or BA at the moment of enrollment into the study as well as in case history, not elevated IgE (<100 IU/ml).

Patients of all the groups had undergone the buccal swab, the material then had been consequently stored within a temperature range from –18° to –32° centigrade in the freezer; afterwards the material had been studied by genotyping using the method of allele discrimination analysis based on polymerase chain reaction in real time (qPCR). The genotyping was carried out on the Applied Biosystems 7500 Fast Real Time PCR System (12) using rs_7927894, rs_11466749, rs_7216389 TaqMan® allelic discrimination assays: C__3243267_10, C__29062108_10 and C__31152869_10 respectively.

All the patients had the informed consent duly filled in (signed by their parents or legal representatives). All the study was performed according to the Declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, October 2000), and the procedures have been approved by the local ethics committee of the SI "Dnipropetrovsk medical academy of HM of Ukraine".

To verify the difference of relative values statistical significance, we had applied the Pearson's chi-squared test ($\chi 2$) and Fischer' s exact test, two-tailed (FET, for small values, $n < 5$), verified by the p-value level ($p < 0.05$). For detection of associative relationship between the values of the main and control groups we applied the Pearson' s contingency coefficient (rc). To calculate relative risks of AD phenotypes' association with SNP we applied the Odds ratio (OR) value with the respective 95% confidence interval (95% CI), the significance having been evidenced by p-value level ($p < 0.1$).

RESULTS

There was received the following age-gender distribution of the patients in the main and control groups (Tables 1, 2).

As it can be distinctly seen from Table 1, we detected the prevalence of male atopic patients compared to females in the most of cohorts of the main group, excluding the AD combined with BA (possibly, SARC and/or PAR) cohort.

In the age distribution the most frequent age interval was 7–11 years old in all the main group cohorts, therefore in the control group there had been detected the prevalence of patients aged 12–18 years old.

Tab. 1 The gender distribution among patients of the main and control groups.

	MAIN GROUP, COHORTS				
GENDER	AD	AD +SARC / AD + PAR / AD + SARC +PAR	AD + BA (SARC and/or PAR)	AD PHENOTYPES TOTAL	CONTROL GROUP
Patients total (N)	47	38	10	95	80
MALE, N	27	25	4	56	47
MALE, %	57.4%	65.8%	40.0%	58.9%	58.8%
FEMALE, N	20	13	6	39	33
FEMALE, %	42.6%	34.2%	60.0%	41.1%	41.2%

Tab. 2 The age distribution in patients of the main and control groups.

AGE, YEARS OLD, N/%	MAIN GROUP, COHORTS				
	AD	AD +SARC / AD + PAR / AD + SARC +PAR	AD + BA (SARC and/or PAR)	AD PHENOTYPES TOTAL	CONTROL GROUP
0–3, N	6	1	0	7	2
0–3, %	12.8%	2.6%	0.0%	7.4%	2.5%
4–6, N	18	8	0	26	13
4–6, %	38.3%	21.1%	0.0%	27.4%	16.2%
7–11, N	16	17	6	39	23
7–11, %	34.0%	44.7%	60.0%	41.0%	28.8%
12–18, N	7	12	4	23	42
12–18, %	14.9%	31.6%	40.0%	24.2%	52.5%
Me, years (LQ–HQ),	6 (4–10)	9 (7–12)	11 (10–12)	8 (5–11)	12 (9–15)

ASSOCIATIONS OF AD ALONE WITH SNP rs_7927894 *FLG*, rs_11466749 *TSLP* AND rs_7216389 *ORMDL3*

The data in Table 3 show no statistically significant association of the AD alone phenotype development risk with any of the genes-candidiates. Still, trends to statistical significance with this phenotype were detected (p-value between 0.05 and 0.1): with C/T rs_7216389 *ORMDL3* genotype – the maximal risk (OR = 2.14 (95% CI 0.98, 4.65)), and with C/C rs_7216389 *ORMDL3* genotype – minimal risk (OR = 0.41 (95% CI 0.16, 1.04)).

Tab. 3 Associations and frequency of SNP rs_7927894 *FLG*, rs_11466749 *TSLP* and rs_7216389 *ORMDL3* with AD alone phenotype in children.

Cohorts	Genotypes SNP rs_7927894 *FLG*		
	C/C	C/T	T/T
Main group	36.2%	46.8%	17.0%
Control group	47.5%	32.5%	20.0%
Statistical significance by Pearson χ2-test	p > 0.05	p > 0.05	p > 0.05
	Genotypes SNP rs_11466749 *TSLP*		
	A/A	A/G	G/G
Main group	55.3%	42.6%	2.1%
Control group	56.3%	40.0%	3.7%
Statistical significance by Pearson χ2-test (* FET, two-tailed)	p > 0.05	p > 0.05	p > 0.05*
	Genotypes SNP rs_7216389 *ORMDL3*		
	C/C	C/T	T/T
Main group	14.9%	72.3%	12.8%
Control group	30.0%	55.0%	15.0%
Statistical significance by Pearson χ2-test	p = 0.0557	p = 0.0526	p > 0.05
OR (95% CI)	0.41 (0.16; 1.04)	2.14 (0.98; 4.65)	
PCC (rc)	–0.170	0.172	

ASSOCIATIONS OF AD COMBINED WITH SARC AND/OR PAR PHENOTYPE WITH SNP rs_7927894 *FLG*, rs_11466749 *TSLP* AND rs_7216389 *ORMDL3*

Data obtained shows that patients carrying C/C rs_7927894 *FLG* genotype have significantly decreased risk by 2.56 fold of AD onset (OR = 0.39 (95% CI 0.17, 0.92); rc = –0.202; p < 0.05); patients carrying C/T rs_7927894 *FLG* genotype have the significantly increased risk by 2.57 fold (OR = 2.57 (95% CI 1.1, 5.67); rc = 0.217; p < 0.05) and carriers of T/T rs_7216389 *ORMDL3* genotype – increased risk by 3.31 fold (OR = 3.31 (95% CI 1,34; 8,14); rc = 0,246; p < 0.01) for developing the AD combined with SARC and/or PAR phenotype.

Tab. 4 Associations and frequency of SNP rs_7927894 FLG, rs_11466749 *TSLP* and rs_7216389 *ORMDL3* with AD combined with SARC and/or PAR phenotype in children.

Cohorts	Genotypes SNP rs_7927894 *FLG*		
	C/C	C/T	T/T
Main group	26.3%	55.3%	18.4%
Control group	47.5%	32.5%	20.0%
Statistical significance by Pearson χ2-test	p < 0.05	p < 0.05	p > 0.05
OR (95% CI)	0.39 (0.17; 0.92)	2.57 (1.16; 5.67)	0.39
PCC (rc)	–0.202	0.217	
	Genotypes SNP rs_11466749 *TSLP*		
	A/A	A/G	G/G
Main group	60.5%	31.6%	7.9%
Control group	56.3%	40.0%	3.7%

Cohorts	Genotypes SNP rs_11466749 *TSLP*		
	A/A	A/G	G/G
Statistical significance by Pearson χ2-test (* FET, two-tailed)	p > 0.05	p > 0.05	p > 0.05*
	Genotypes SNP rs_7216389 *ORMDL3*		
	C/C	C/T	T/T
Main group	18.4%	44.7%	36.9%
Control group	30.0%	55.0%	15.0%
Statistical significance by Pearson χ2-test	p > 0.05	p > 0.05	p < 0.01
OR (95% CI)			3.31 (1.34;8.14)
PCC (rc)			0.246

ASSOCIATIONS OF AD COMBINED WITH BA (POSSIBLY, SARC AND/OR PAR) WITH SNP rs_7927894 *FLG*, rs_11466749 *TSLP* AND rs_7216389 *ORMDL3*

The evidence is obtained of a statistically significant increased risk by 4.85 fold within C/T rs_7927894 FLG genotype carriers (OR = 4.85 (95% CI 1.16, 20.27), rc = 0.245) and decreased risk trending to significance by 5.88 fold A/G rs_11466749 TSLP genotype carriers (OR = 0.17 (95% CI 0.02, 1.38), rc = −0.196) with developing the aforesaid AD phenotype (Table 5).

Summarizing the dataset obtained, genotypes with the significant risk and candidiate genotypes with a trend to significance of developing the different AD phenotypes are provided with the respective OR (Fig. 1).

Tab. 5 Associations and frequency of SNP rs_7927894 *FLG*, rs_11466749 *TSLP* and rs_7216389 *ORMDL3* with AD combined with BA (possibly, SARC and/or PAR) phenotype in children.

Cohorts	Genotypes SNP rs_7927894 *FLG*		
	C/C	C/T	T/T
Main group	30.0%	70.0%	0.0%
Control group	47.5%	32.5%	20.0%
Statistical significance by FET, two-tailed	p > 0.05	p < 0.05	p > 0.05
OR (95% CI)		4.85 (1.16; 20.27)	
PCC (rc)		0.245	
	Genotypes SNP rs_11466749 *TSLP*		
	A/A	A/G	G/G
Main group	80.0%	10.0%	10.0%
Control group	56.3%	40.0%	3.7%
Statistical significance by FET, two-tailed	p > 0.05	p = 0.0806	p > 0.05
OR (95% CI)		0.17 (0.02; 1.38)	
PCC (rc)		−0.196	
	Genotypes SNP rs_7216389 *ORMDL3*		
	C/C	C/T	T/T
Main group	30.0%	50.0%	20.0%
Control group	30.0%	55.0%	15.0%
Statistical significance by FET, two-tailed	p > 0.05	p > 0.05	p > 0.05

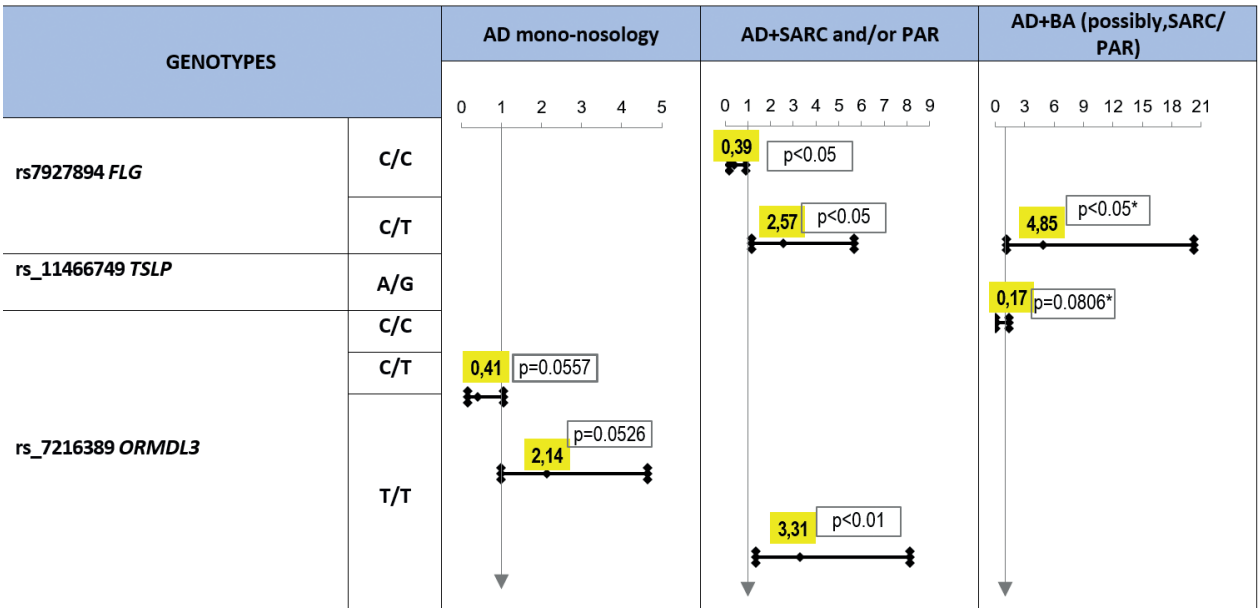

Fig. 1 Risk (OR (95% CI)) of different AD phenotypes' development within different genotypes of SNP rs_7927894 *FLG*, rs_11466749 *TSLP*, rs_7216389 *ORMDL3* (significance by Pearson's χ2-test (* by FET, two-tailed).

DISCUSSION

Significant association by 2.57 fold ($p < 0.05$) with developing AD combined with SARC and/or PAR phenotype was detected with the carriage of genotype C/T rs_7927894 *FLG* and by 3.31 fold ($p < 0.01$) with genotype T/T rs_7216389 *ORMDL3* (PCC between 0.217 and 0.246 ($p < 0.05$)). Along with that, carriage of the genotype C/C rs_7927894 *FLG* does significantly decrease the risk of the mentioned AD phenotype by 2.56 fold (OR = 0.39; $p < 0.05$). This suggests a novel approach towards the genetic background of AD phenotypes compared to prevailing studies on FLG null loss-of function mutations (6, 13).

Statistically significant association of AD combined with BA (possibly, SARC and/or PAR) phenotype with the carriage of genotype C/T rs_7927894 *FLG* was detected with the increased risk (OR = 4.85; $p < 0.05$) which is even higher than in relevant studies of *FLG* gene variants' associations with the risk of developing AD with BA (7, 14).

Results obtained which need to be confirmed in further studies – are the genotypes' s trending to significance associations with the risks of developing different AD phenotypes (p-value between 0.05 and 0.1). Thus, AD alone phenotype is by 2.44 fold less likely to develop within the carriers of genotype C/C rs_7216389 *ORMDL3* (OR = 0.41) and by 2.14 fold more likely to develop within the carriers of genotype C/T rs_7216389 *ORMDL3* (OR = 2.14). Result obtained for A/G rs_11466749 variant of *TSLP* gene – which had been found in association with AD development in recent studies (7, 14) – is that AD combined with BA (possibly, SARC and/or PAR) is by 5.88 fold less likely to develop within it' s carriers (OR = 0.17). This paves the way for further studies of AD phenotypes' genetics.

CONCLUSIONS

AD phenotypes' development is significantly associated with the genotypes C/T rs_7927894 of *FLG* gene and T/T rs_7216389 of *ORMDL3* gene.

Children carrying genotype C/T rs_7927894 of *FLG* gene are exposed to a significantly by 2.57 fold increased risk of developing AD combined with SARC and/or PAR and significantly by 4.85 fold increased risk of developing AD combined with BA (possibly, SARC and/or PAR) phenotypes.

Children carrying genotype T/T rs_7216389 of *ORMDL3* gene are exposed to the significantly by 3.31 fold increased risk of developing AD combined with SARC and/or PAR phenotype.

Children carrying genotype C/C rs_7927894 of *FLG* gene have the significantly by 2.56 fold decreased risk of developing AD combined with SARC and/or PAR phenotype.

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Iatrogenic Fracture of the Lower Jaw: A Rare Complication of Lower Molar Extraction

Radovan Mottl[1,*], Martina Kunderová[1], Radovan Slezák[1], Jan Schmidt[1]

ABSTRACT

Iatrogenic mandible fracture is a rare complication of a tooth extraction with an incidence between 0.0033–0.0034%. This study retrospectively analyzes a total of 8 patients who underwent lower molar extraction associated with mandible fracture during tooth removal in the period from April 2006 to March 2019. The assessed parameters were age and sex of patients, method of tooth extraction, side distribution of fracture, type of extracted tooth, the position of a lower third molar, presence of bone pathological lesion formed in connection with a tooth, displacement of bone fragments, and sensory impairment in the innervation area of the mental nerve. The position and impaction of the lower third molars were evaluated according to Pell and Gregory's classification and Winter's classification. One fracture was left-sided, and 7 fractures were right-sided. In 6 cases, Winter's extraction elevator was used. In 7 patients, the mandible fracture was treated surgically by performing stable osteosynthesis with the plates and screws. One patient was treated conservatively. This work analyzes the causes of iatrogenic mandible fractures and provides recommendations to reduce the risk of their occurrence.

KEYWORDS

Iatrogenic fracture; lower jaw; tooth extraction; dental elevator; complications

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INTRODUCTION

Extraction of lower permanent molars is a routine surgical procedure. In most cases, the reason for the tooth removal is tooth decay and its complications (pulpitis, apical periodontitis), acute or chronic pericoronitis, less often periodontal disease, and orthodontic treatment (1). However, complications can occur during or after the procedure. The most common non-specific complications of lower molar extractions include alveolar osteitis (alveolitis sicca dolorosa), early or late bleeding after extraction, hematoma, soft tissue contusion, collateral edema, or prolonged healing of the extraction wound. Some complications are rather specific, occurring mainly in association with the extraction of lower third molars that are also called wisdom teeth. Due to the localization of lower third molars, the surgical procedure may lead to an injury of the neurovascular bundle in the canalis mandibulae manifested by bleeding from the mandibular canal or inferior alveolar nerve damage.

A rare and specific complication is also an iatrogenic fracture of the lower jaw, which can occur not only during the operation itself but in the period after the operation as well (2, 3). The aim of this work is a retrospective evaluation of the frequency and circumstances of this surgical complication in individuals registered at the Dental Clinic, Charles University, Faculty of Medicine in Hradec Králové and University Hospital Hradec Králové in the years 2006–2019.

MATERIAL AND METHODS

The retrospectively evaluated group consisted of a total of 8 patients who underwent lower molar extraction associated with mandibular fracture during the surgery in the period from April 2006 to March 2019. The assessed parameters were age and sex of patients, method of tooth extraction, side distribution of fracture, type of extracted tooth, the position of a lower third molar on the extraoral panoramic radiograph (orthopantomogram), presence of tooth related bone pathological lesion formed (intraosseous odontogenic cyst, chronic periodontitis), displacement of bone fragments, and sensory impairment in the innervation area of the mental nerve. The position and impaction of the lower third molars were evaluated according to the Pell and Gregory's classification (Fig. 1) and Winter's classification (Fig. 2) (4, 5). Preoperative radiographs were available in 6 patients.

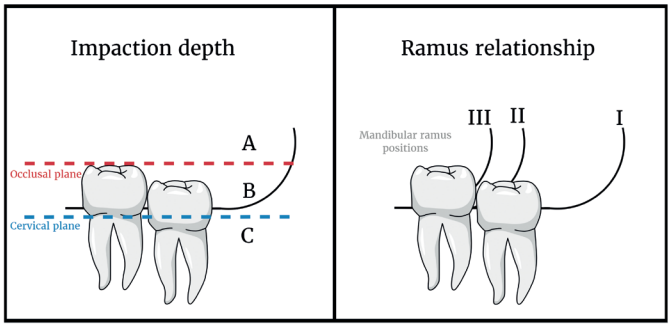

Fig. 1 Pell and Gregory's classification of lower third molars position.

RESULTS

In 6 patients, the extraction was performed under local anesthesia – 3 of them underwent the extraction in private practices of general dentists, and 3 individuals in the Dental Clinic, University Hospital Hradec Králové. In 2 patients, the extraction was performed under general anesthesia in the Dental Clinic, University Hospital Hradec Králové. In 7 cases, the lower third molar was extracted, and in one case, the lower ankylotic partially erupted second molar was extracted (Fig. 3). The mean age of the patients was 53.2 years and the median age was 54.5 years (range 36–77 years). The sex distribution of the study sample was 2 males and 6 females. 7 fractures were right-sided, and one fracture was left-sided. 4 fractures were associated with recurrent inflammatory conditions, i.e., pericoronitis, in the anamnesis, and in 3 cases, the presence of a tooth related bone pathological lesion (one odontoma and two dentigerous cysts) was detected in the period before the surgery. In 3 patients, the distoangular position of the lower third molar was determined according to Winter's classification. In 6 cases, Winter's extraction elevator was used to remove the tooth, among other instruments. In 6 patients, jaw fragments were displaced, followed by a unilateral sensory impairment, i.e., paresthesia, located in the innervation area of the mental nerve. In 7 patients, the mandible fracture was treated surgically by performing stable osteosynthesis with the plates and screws. In one patient, it was treated conservatively with intermaxillary fixation. Fractures were healed without complications in all patients, and the patients were followed for two years. The sensitive innervation was fully recovered only in two patients within two years after the procedure.

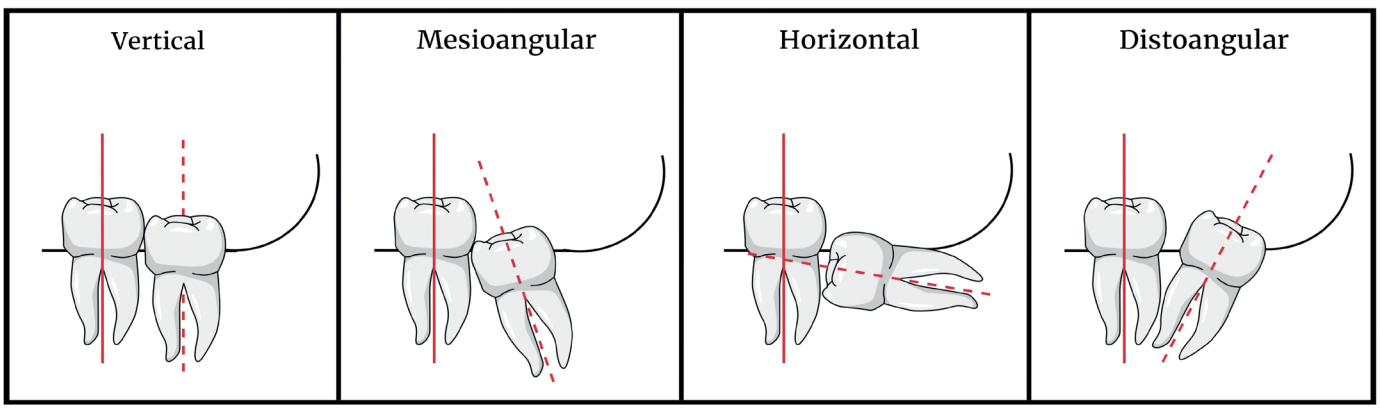

Fig. 2 Winter's classification of lower third molars position.

Figures 4–6 demonstrate the situation before and after the tooth extraction, and after the treatment in three patients. For this illustration, the most common panoramic radiographs, i.e., orthopantomograms, were used (Fig. 4a–c, 5a–c, 6a–b).

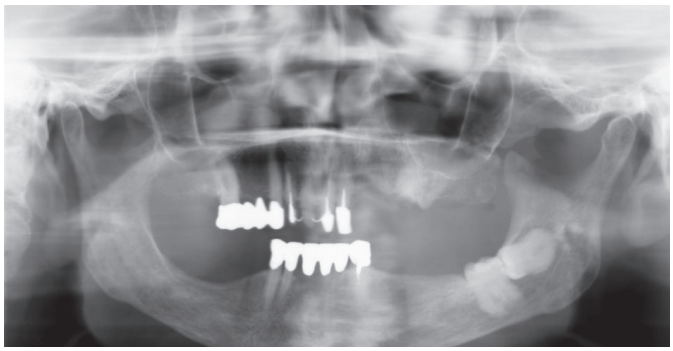

Fig. 3 Panoramic radiograph before the extraction of the tooth 37.

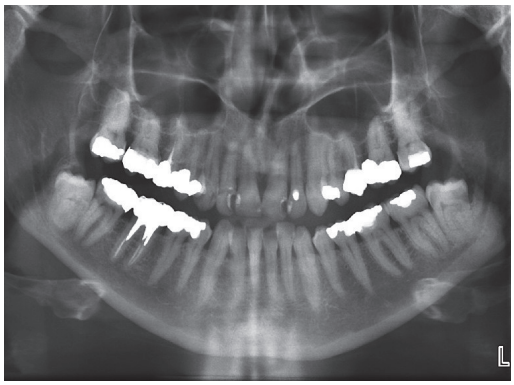

Fig. 4a Panoramic radiograph before the extraction of the tooth 48 in patient No. 3.

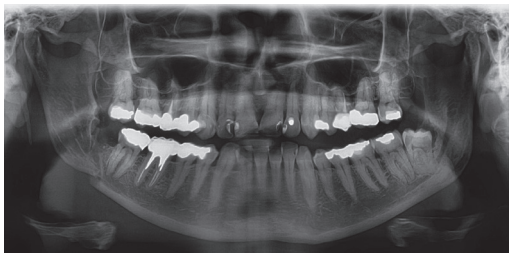

Fig. 4b Panoramic radiograph demonstrating the mandibular fracture after the right-sided lower third molar extraction in patient No. 3.

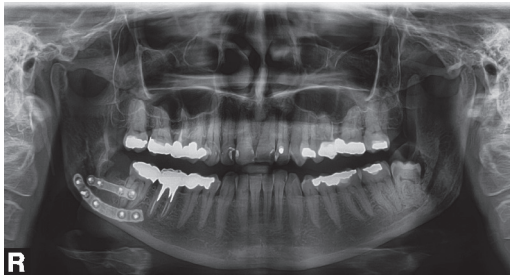

Fig. 4c Panoramic radiograph demonstrating the osteosynthesis of the mandibular fracture after the right-sided lower third molar extraction in patient No. 3.

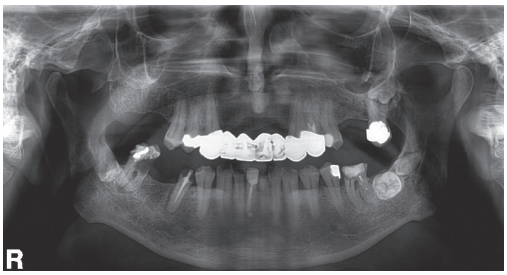

Fig. 5a Panoramic radiograph before the extraction of the teeth 48 and 47 in patient No. 5.

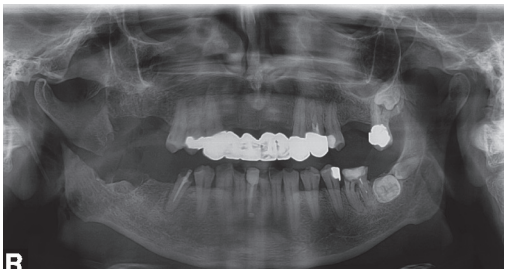

Fig. 5b Panoramic radiograph demonstrating the mandibular fracture after the right-sided lower molars extractions in patient No. 5.

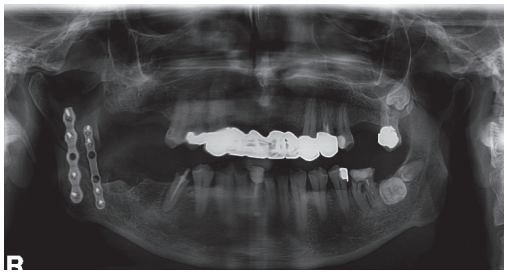

Fig. 5c Panoramic radiograph demonstrating the osteosynthesis of the mandibular fracture after the right-sided lower third molars extractions in patient No. 5.

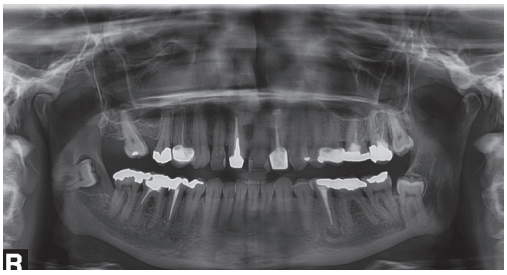

Fig. 6a Panoramic radiograph before the extraction of the tooth 48 in patient No. 7.

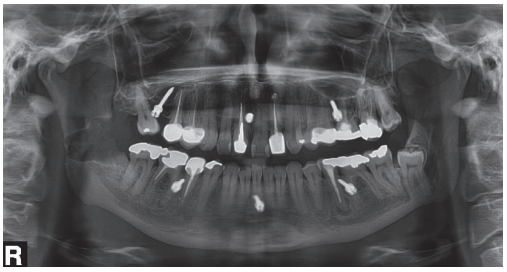

Fig. 6b Panoramic radiograph demonstrating the mandibular fracture after the right-sided lower third molar extraction in patient No. 7. after insertion of IMF screws and intermaxillary fixation with rubber rings.

The results are summarized in Table 1.

Tab. 1

Patient Nr.	Age	Gender	Side	Tooth	Presence of the pathological lesion	Localization	Dislocation	Sensory impairment	Tooth position according to Pell and Gregory's classification
1	38	W	R	48	Yes	Angle	Yes	Yes	not classified
2	38	M	R	48	No	Angle	Yes	Yes	C II.
3	53	W	R	48	No	Angle	Yes	Yes	B II.
4	77	W	L	37	No	Body	Yes	Yes	not classified
5	66	M	R	48	No	Ramus	Yes	No	C I.
6	62	W	R	48	No	Angle	Yes	Yes	B I.
7	36	W	R	48	Yes	Ramus	No	No	C III.
8	55	W	R	48	Yes	Angle	Yes	Yes	C I.

DISCUSSION

The area of the mandibular angle (*angulus mandibulae*) is an area with lower resistance to fractures. It is due to the fact a transition zone between the toothed and non-toothed part of the mandible is formed here. Additionally, the presence of the third lower molar, which is often incompletely erupted and impacted in the bone, further contributes to bone weakening. By cause of these conditions, approximately 75% of the iatrogenic mandibular fractures are associated with the lower third molar removal (6). In other parts of the mandible, iatrogenic fractures are also possible. However, in these localisations, iatrogenic fractures are disproportionately rare and associated with other significant bone weaknesses (retained canines and premolars, large bone cysts, benign and malignant tumors, conditions after marginal resection of the mandible, or some bone diseases).

Fracture of the mandible occurring during or after the lower molar extraction is a rare complication of this surgical procedure. According to literature data, the incidence of mandible fractures during tooth extractions ranges from 0.0033 to 0.0034%, while the incidence of fractures occurring after tooth extractions is 0.0042–0.0049% (7–10). According to Ethunandan et al. is the ratio 1 : 2.7 (11).

Extractions of the lower wisdom teeth are most often performed on patients under 25 years of age. However, in this age group, iatrogenic mandible fractures occur very rarely (11). The findings of our study, in conclusion with the findings of other authors, confirm that a significant risk factor for the occurrence of this complication is older age, with the risk increasing from approximately the age of 36 (12–14). This fact is explained by an age-related decrease in bone elasticity, narrowing of the periodontal space leading to tooth ankylosis, and also by a more frequent occurrence of pathological lesions in the jawbones, especially odontogenic cysts (15, 16). In the group of our study, the mean age of patients with iatrogenic mandible fracture was 53.2 years, and the median was 54.5 years. Such age distribution is only slightly higher than in other studies (9, 12).

Regarding sex distribution, perioperative iatrogenic mandibular fractures are more frequently reported in women, with a ratio of 1.3 : 1. In contrast, postoperative iatrogenic mandibular fractures are reported to be more frequent in men, with a ratio of 3.9 : 1 (11, 13, 17, 18). In our study, 75% of the perioperative iatrogenic mandibular fractures were observed in female subjects. Although our results indicating higher frequency in females correspond with the findings of other authors, the quantitative outcome differs. Such a disparity may be attributed to the limited number of subjects involved in our study.

The presence of an intraosseous pathological lesion was observed in three cases – one benign odontogenic tumor, i.e., odontoma, and two odontogenic dentigerous cysts. In 4 cases, the pericoronary sac enlarged by more than 2 mm was visible on the radiograph.

Some authors reported an unequal side distribution of iatrogenic mandibular fractures with a higher incidence on the left side (6, 17, 19, 20). This phenomenon may be explained due to more difficult access to the operating field for surgeons with right-hand dominance, which is more frequent in the population. However, the data of our study do not match with their findings as 7 of the fractures were localized on the right side.

Another risk factor for the development of an iatrogenic fracture of the mandible is the impaction level of the extracted tooth. This risk factor is associated not only with lower third molars, but generally with any tooth. According to Izuka et al., the percentage ratio comparing the height of the lower third molar to the remaining height of the mandibular bone measured on the orthopantomogram ranges between 44–84% (12). Some authors reported on this ratio exceeding 50% to be associated with a higher risk of mandible fracture (6, 21, 22). The work of Joshi et al. relates 54% of iatrogenic mandibular fractures to the removal of lower third molars fully impacted in bone (6). In our study, the ratio of tooth height to the ratio of remaining bone height was more than 50% in 5 patients, i.e., 62.5% of the study subjects. Interestingly, the study of Reitzik et al. demonstrates the differences in force leading to the mandible fracture in primate

(*Cercopithecus aethiops*) jaws with various levels of third molar eruption. Mandibles with unerupted third molars were found to be significantly weaker compared to the mandibles with erupted third molars. The force required to break the jaw with the impacted third molar was at the level of 60% of the force required to break the jaw with erupted third molars (23).

The position of lower third molars is also a factor that affects the risk of complications development. According to Winter's classification, the most common position is mesioangular, which is approximately 45% of all cases, and the least common is the distoangular position, which occurs in the range of 5–12.8% (11, 24). The latter is considered to be the most difficult position for tooth removal and it is also associated with the highest risk of fracture (25). According to Pell and Gregory's classification, the highest risk of jaw fracture is related to the tooth position class B or C and type II or III (12, 19). The findings of our work reflect the same outcome, as the disto-angular position of the tooth was found in 50% of the patients involved in our study. Class B or C was detected in 6 patients, with no preoperative X-ray available in the remaining two patients.

An additional factor increasing the risk of fracture is a history of soft tissue inflammation around the tooth crown, such as pericoronitis (10, 14). In our study, a total of 4 patients (50%) reported a history of chronic pericoronitis associated with the extracted tooth.

According to some professional authorities, the risk of any complications resulting from tooth removal is higher if the extraction is performed by personnel with less than three years of experience in the field (26–28). However, this topic remains controversial as other authors reported on it with different conclusions (10, 21).

A total of 7 patients underwent surgical reposition followed by stable osteosynthesis with plates and screws under general anesthesia. Only one patient was treated conservatively using intermaxillary fixation. Some authors report the conservative approach to be chosen more often. However, these works do not provide more detailed information about fractures (6, 11). In 7 cases of our study, the fractures were complicated with a severe displacement of bone fragments, and stable osteosynthesis with plates and screws was necessary.

The use of Winter's extraction elevator and similar instruments is also considered a significant risk factor for jaw fracture (14). The results of our study correspond with these findings as Winter's extraction elevator was used in 6 of 8 patients. Thus, we consider it relevant to comment on this outcome in detail.

The extraction elevator is a paired tool used mainly to extract tooth roots from their beds. We distinguish several types of these instruments, and the most commonly used are called Winter's (Figure 7) and Barry's (Figure 8) elevators. Although they are useful for root extraction, their use for other purposes, including the extraction of lower third molars, is controversial. In principle, elevators are levers and these simple tools amplify an input force to provide a greater output force. Since the resulting moment of force acting on both arms of the lever is given by the magnitude of the force multiplied by the length of the arm, it is thus inversely proportional to the length of the arms (29). In Winter's extraction elevator, the handle and the working end are arms of the lever. As the average length of the handle is 90 mm and the average length of the working end is 8–9 mm, the force applied at the working side is approximately 10 times higher. As evidenced by examples from clinical practice and also by a number of studies published on this topic, the mechanically weakened area of the mandible angle may not always withstand the action of such a force (30, 31). Mandible fracture risk factors include age over 37 years, the presence of bone pathological lesions, tooth impaction, and the high ratio of tooth height in relation to the remaining bone height (9). If this ratio is greater than 50%, and if the roots of the tooth overlap or are close to the mandibular canal, it is necessary to consider whether tooth removal is necessary. If so, extra care must be taken during the extraction as the jaw fracture risk is impending (12, 32). Such a risk is imminent if elevators are used for tooth extraction in older patients where ankylosis is more frequent (33). If nerve damage or an increased risk of lower jaw fracture due to tooth position is anticipated, coronectomy is also a possible option. This procedure is recommended in patients older than 25 years but is contraindicated in cases of the horizontal position of the lower third molar or if the molar is closely related to a tumor or a cyst (34, 35)

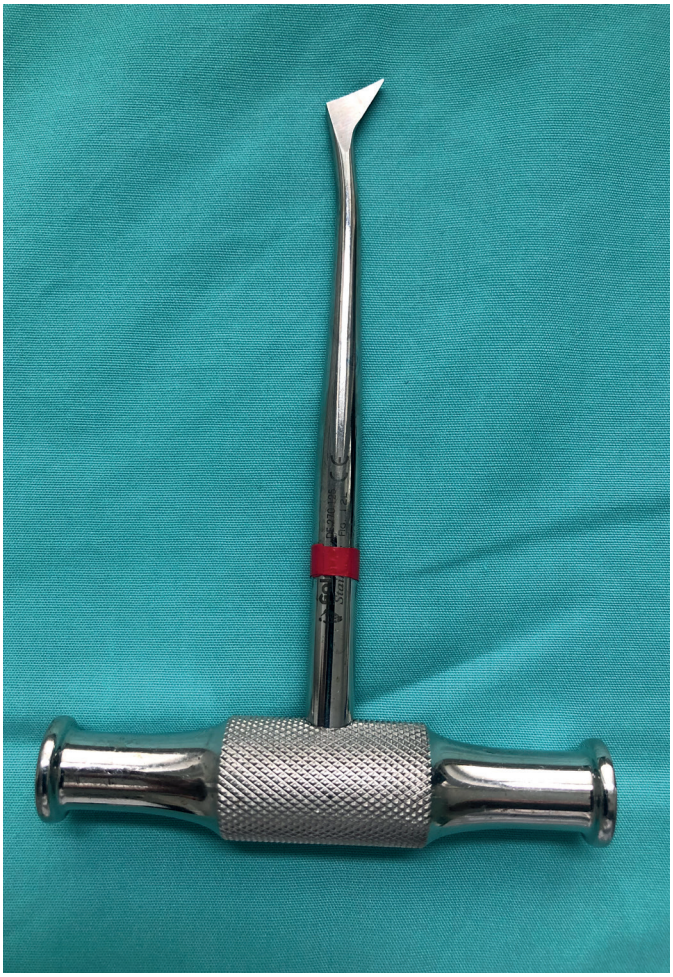

Fig. 7 Winter's elevator.

Fig. 8 Barry's elevator.

CONCLUSION

Before the extraction of any lower molar, and especially before the extraction of a lower third molar, the surgeon must always take into account any and all factors that may increase the risk of a mandibular fracture. It is necessary to have a preoperative X-ray, preferably an orthopantomogram. In some cases, e.g., intimate proximity of the tooth roots to the mandibular canal or unclear anatomy of the roots, cone-beam computed tomography (CBCT) targeting the mandibular angle region and the lower third molar position is recommended. Such an examination provides a 3D reconstruction of the roots and adjacent tissues arrangement. To prevent further complications, any excessive bone loss during the extraction should be avoided. It is advisable to remove the tooth in pieces to avoid any excessive bone drilling and especially preserve the area of *linea obliqua externa*.

Special care should be taken when using dental elevators. These tools allow the surgeon to exert a great force, which, if used improperly, can lead to jaw fracture. This was demonstrated even in our study as 6 of 8 (75%) iatrogenic mandible fractures resulted from the use of Winter's elevator during the tooth removal. Thus, the exerted force shall be always applied very carefully after all risk factors have been considered.

Prior to the extraction, all patients should be made aware of the eventual risks, including the mandible fracture. A soft diet should be recommended for at least 3–4 weeks to every patient who underwent a complicated extraction.

The extraction of lower permanent molars, especially lower third molars, is considered to be a very difficult and risky procedure within dentoalveolar surgery. Taking this into consideration, if all recommendations and guidelines are followed in the preoperative examination and operative procedure, the risk of an iatrogenic fracture of the lower jaw remains very low.

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Rasch Validation of the LVQOL Scale

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ABSTRACT

Aim: This study proceeds to rigorously examine and validate the Low Vision Quality-of-Life Questionnaire (LVQOL) on a Greek population of ophthalmic patients employing Rasch measurement techniques.

Methods: It is a prospective observational study of 150 cataract patients and 150 patients with other ophthalmic diseases, all followed longitudinally for a period of two months pending surgical or other corrective therapy, after which they were administered the LVQOL for a second time.

Results: The original 25-item LVQOL demonstrated high reliability and validity, excellent measurement precision and ordered response category thresholds. A small number of items carry an acceptable level of measurement error while three items had some differential functioning for gender, Age and underlying disorder that did not exceed the established thresholds.

Conclusions: This validation study is the first to employ Rasch measurement to examine the validity of the LVQOL and it supports its use with no changes to the original structure. The LVQOL can be employed in a large range of ophthalmic diseases and reliably assess improvements in quality-of-life following phacoemulsification surgery or any other intervention.

KEYWORDS

quality of life; LVQOL; Rasch validation; cataract; phacoemulsification

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INTRODUCTION

The Low Vision Quality-of-Life Questionnaire (LVQOL) has been constructed by Wolffsohn et al (1) as an index of functional impairment designed to a vision-specific quality-of-life assessment tool that can reliable be utilized to measure the outcome of low-vision rehabilitation, help with evaluation of current rehabilitation strategy, eventually leading to the improvement of the offered services while securing and enhancing funding within managed care plans. It has been translated in several languages including Dutch (2), Spanish (3), Chinese (4), Thai (5), Korean (6) and Tamil (7), demonstrating excellent reliability and validity when examined with the classical test theory. A systematic review of vision-related quality of life questionnaires concluded that the LVQOL was one of the two best questionnaires for use in low vision patients (8).

The more modern approaches to assessing the psychometric properties of a quality-of-life instrument involve item response theory and particularly the Rasch model. Item response theory relates to the measurement of a latent construct from a number of items in a questionnaire, included parameters describing characteristics of the individual items and was built around the premise that respondents and items could be placed on the same quantitative latent continuum (9).

The item response theory "Rasch" model is a probabilistic mathematical method that has been employed to assess the psychometric properties of an instrument and its' measurement quality against an established framework of precision criteria (10), while transforming ordinal test responses into interval-level scores thus reducing measurement noise, increasing precision and statistical power to test the hypotheses with a smaller sample size. Rasch models have become a method of choice for examining the validity of an assessment instrument (11).

The only version of the LVQOL that has been tested with an item response theory approach is the Dutch one (12) and was deemed satisfactory although two items (item 1 and 24) were removed because of differential item functioning, meaning that the interpretation of the original questionnaire differed by subgroups of the research population and that it was influenced by some confounding factors.

The principal aim of this study is to assess a Greek version of the LVQOL using Rasch analysis and test its applicability in patients suffering from ophthalmic disease including cataract and other causes.

METHODS

ETHICS

License for the validation of a Greek version of the LVQOL was obtained by the original author on the 18th of November 2018. Institutional Review Board approval was obtained for this study by the Ethics board of the Aristotle University of Thessaloniki (approval ref. number 4.139/17.7.2019) and all procedures adhered to the tenets of the Declaration of Helsinki. Data collection took place between September 2019 and February 2020.

STUDY DESIGN

Selection and Description of Participants

This is a prospective observational study of 300 patients who were treated for vision problems in the outpatient services of the 2nd Department of Ophthalmology, Aristotle University of Thessaloniki. The patients were longitudinally followed for two months, during which they received appropriate treatment depending on their underlying disease. A full list of the underlying disorders for the full sample is presented in Table 1. The largest sub-cohort of 150 consecutive patients underwent phacoemulsification

Tab. 1 Underlying disorders.

Disorder	Frequency	%	Age in years (mean/SD)	Males (N/%)	Females (N/%)	Visual acuity at beginning (mean/SD)	Visual acuity at end (mean/SD)
Cataract	150	50.0	73.67 (7.93)	86 (57.3%)	64 (42.7%)	0.497 (0.195)	0.213 (0.162)
Age-related macular degeneration	30	10.0	74.13 (6.23)	18 (60%)	12 (40%)	0.505 (0.29)	0.412 (0.31)
Glaucoma	24	8.0	69.04 (8.47)	13 (54.2%)	11 (45.8%)	0.391 (0.41)	0.306 (0.46)
Ectropion	18	6.0	71 (7.5)	15 (83.3%)	3 (16.7%)	0.232 (0.21)	0.211 (0.23)
Proliferative diabetic retinopathy	16	5.3	67.56 (10.16)	11 (68.8%)	3 (31.3%)	0.584 (0.34)	0.534 (0.328)
Canalicular obstruction	13	4.3	73.46 (6.1)	5 (38.5%)	8 (61.5%)	0.203 (0.44)	0.185 (0.39)
Blepharitis	13	4.3	74.92 (5.34)	7 (53.8%)	6 (46.2%)	0.198 (0.38)	0.174 (0.51)
Central vein occlusion	10	3.3	72.8 (4.1)	3 (30%)	7 (70%)	0.811 (0.91)	0.791 (1.1)
Dry eye	10	3.3	72.6 (6.77)	8 (80%)	2 (20%)	0.231 (0.75)	0.184 (0.81)
Retinal vein occlusion	5	1.7	69 (4.06)	2 (40%)	3 (60%)	0.654 (1.2)	0.588 (1.1)
Ptosis	5	1.7	71.6 (4.56)	3 (60%)	2 (40%)	0.194 (0.47)	0.186 (0.68)
Keratoconus	4	1.3	61.5 (2.88)	4 (100%)	–	0.535 (0.04)	0.36 (0.04)
Fuch's endothelial dystrophy	2	0.7	51 (2.82)	–	2 (100%)	0.75 (0.07)	0.65 (0.1)
Total	300	100.0					

surgery and was comprised of 86 men (57.3%) with a mean age of 73.84 years and a standard deviation (SD) equal to 8.55 years, and 64 women (42.7%) with a mean age of 73.45 years (SD = 7.05 years). The combined disorders group was comprised of 89 men (59.3%) with a mean age of 72.16 years (SD = 7.74 years) and 61 women (40.7%) with a mean age of 72.1 years (SD = 7.95 years). Exclusion criteria for all patients were the existence of other comorbid eye diseases, any complications related to their treatment and any previous ophthalmic disease that is associated with low vision.

TECHNICAL INFORMATION

All patients were initially handed out a brief demographics questionnaire that included information on their gender, age, marital status, living arrangements and comorbid health issues that necessitated continuous medical care. The patients were required to fill in the 25-item Low Vision Quality-of-Life Questionnaire (LVQOL), a vision-specific quality-of-life assessment tool designed to be used in a clinical setting in order to evaluate low-vision rehabilitation strategy and management (1). The LVQOL's 25 items are graded in a Likert-type scale ranging from five (having no difficulty with the item because of their vision) to one (having a great difficulty with the item because of their vision) or as 0 (item could no longer be performed because of their vision). Other options are the "not relevant", if the item in question was not relevant to a patient in his/her daily life; these answers are given an average score so that those individuals who scored more items irrelevant did not have a lower summed questionnaire score and therefore an apparently worse quality of life. The total summed score ranges between zero (a low quality of life) and 125 (a high quality of life). The LVQOL was measured twice, the second instance being two months after the initial one. In addition to the questionnaires, the patients' best corrected visual acuity was also measured pre- and post-surgery with the Early Treatment Diabetic Retinopathy Study (ETDRS) charts.

STATISTICS

The validation process started with translating the items from English to Greek by two medical doctors who are fluent in both languages and the initial draft was revised by a panel of experts for clarity. The draft was then translated back into English and compared with the original English version to identify any discrepancies between the two versions, which were then again revised by the panel. The final draft was then tested in twenty patients for comprehension before being handed out to the patient groups.

All subsequent Rasch measurements were carried out with the aid of the statistical package Winsteps (13). Five assumptions and properties of the model were examined to assess the validity of the Greek version of the LVQOL with Rasch modeling (14, 15) including:

Measurement Precision

Measurement precision refers to how the questionnaire performs as an instrument of measurement. It is estimated with the person and item separation statistics. Separation is the signal-to-noise ratio in the data. Person separation indicates how efficiently a set of items is able to separate those persons measured while item separation indicates how well a sample of people is able to separate those items used in the questionnaire. A low Person Separation Index (PSI) implies that the instrument may not be sensitive enough to distinguish between high and low performers and more items may be needed while a low Item Separation Index (ISI) implies that the person sample is not large enough to confirm the construct validity of the instrument (15). A PSI of 1.5 represents an acceptable level of separation, an index of 2.00 represents a good level of separation, and index of 3.00 represents an excellent level of separation (16). A PSI > 2.0 and a person reliability (PR) score >0.8 are generally considered to be the minimum requirements for satisfactory discrimination of at least three strata of participants level of the trait (i.e., vision functioning) (14, 15).

Unidimensionality

Unidimensionality is prerequisite for construct validity since it refers to whether a questionnaire measures only a single underlying trait (i.e., visual functioning) and it is assessed in Rasch measurement by examining the item fit statistics and with a principal component analysis (PCA) of the residuals. Item fit relates to how well the responses meet the test requirements and ultimately how well the items fit the construct. The item fit statistics are expressed in mean square statistics and there are two types of fit statistics, infit and outfit (15). According to established criteria (17), mean fit values ranging between 0.5–1.5 are productive for measurement, values over 1.5 are unproductive for construction of measurement, but not degrading, values under 0.5 are less productive for measurement, but not degrading and values over 2 denote an item that distorts or degrades the measurement system. To test for local independence the method of choice is the conduct of a PCA of the residuals, a process in which we scan for patterns in the part of the data that does not accord with the Rasch measures. If this is the case then there is a possibility that a secondary dimension is present that may distort measurement and the unidimensionality assumption is violated. When 60% of the variance in the PCA of the residuals is explained by the raw data then this is an indication of unidimensionality since there is little noise to form a pattern (14). Residuals in PCA are grouped in contrasts and if the first contrast has an eigenvalue of >2.0 then this is considered as evidence that a second contrast is being measured by the questionnaire (14).

Category Threshold Order

The response categories for the items in a questionnaire should ideally be used in an orderly fashion; this requires that the category definitions are clear and distinct to one another and the number does not exceed the range that the respondents can distinguish or is smaller than the nuances

Tab. 2 Fit statistics for the Low Vision Quality-of-Life Questionnaire items.

LVQOL item	MODEL		INFIT		OUTFIT		EXACT MATCH	
	Measure	S.E.	MNSQ	ZSTD	MNSQ	ZSTD	Observed %	Expected %
LVQOL 16	-3.02	0.10	1.88	7.87	1.54	3.33	54.8	63.6
LVQOL 10	1.02	0.08	1.81	8.39	1.74	7.52	41.8	49.5
LVQOL 9	0.15	0.08	1.63	6.71	1.66	6.97	40.5	48.9
LVQOL 11	0.56	0.08	1.59	6.40	1.61	6.52	44.8	48.7
LVQOL 13	-0.90	0.08	1.31	3.51	1.27	2.95	46.2	51.3
LVQOL 14	-0.74	0.08	1.18	2.08	1.16	1.83	47.5	50.8
LVQOL 15	-1.52	0.08	1.16	1.89	1.06	0.66	51.5	53.8
LVQOL 3	0.92	0.08	0.94	-0.71	0.98	-0.28	43.8	49.3
LVQOL 8	0.26	0.08	0.98	-0.19	0.98	-0.22	49.5	48.6
LVQOL 12	0.07	0.08	0.98	-0.19	0.96	-0.47	45.5	48.9
LVQOL 22	-1.15	0.08	0.95	-0.55	0.98	-0.24	45.8	52.7
LVQOL 5	0.56	0.08	0.94	-0.74	0.92	-0.98	51.8	48.7
LVQOL 21	0.56	0.08	0.79	-2.83	0.92	-1.03	59.9	48.7
LVQOL 7	0.19	0.08	0.90	-1.27	0.87	-1.68	47.5	48.8
LVQOL 17	-0.74	0.08	0.89	-1.41	0.90	-1.17	52.2	50.8
LVQOL 19	0.69	0.08	0.90	-1.35	0.87	-1.63	48.8	48.8
LVQOL 23	-0.52	0.08	0.84	-2.13	0.87	-1.57	49.5	50.4
LVQOL 6	0.52	0.08	0.86	-1.89	0.84	-2.06	45.5	48.7
LVQOL 4	0.56	0.08	0.75	-3.55	0.77	-3.13	54.8	48.7
LVQOL 18	0.37	0.08	0.77	-3.15	0.74	-3.62	54.2	48.9
LVQOL 24	-0.29	0.08	0.77	-3.06	0.76	-3.23	57.2	50.2
LVQOL 20	0.43	0.08	0.72	-3.92	0.73	-3.72	52.8	48.8
LVQOL 1	1.28	0.08	0.69	-4.43	0.69	-4.12	58.9	50.6
LVQOL 25	0.01	0.08	0.67	-4.76	0.68	-4.41	56.5	49.0
LVQOL 2	0.72	0.08	0.52	-7.42	0.53	-7.20	62.5	48.8
Mean	0	0.08	1.02	-0.3	1.00	-0.4	50.6	50.2
P.SD	0.93	0.01	0.35	4.0	0.32	3.6	5.7	3.0

S.E. = Standard Error, MSNQ = Mean Square, ZSTD = Z – standardized, P.SD = Population Standard Deviation, LVQOL = Low Vision Quality-of-Life Questionnaire

of the category that we are trying to ascertain (18). If there is disordering, then some answers are significantly more likely than others or even unlikely.

Targeting

Targeting refers to how far the average or modal measure is from the center of the item calibrations, denoting how persons of higher or lower ability (i.e. visual functioning) will be able to relate to the items that are offered and respond meaningfully (19). Perfect targeting would have a difference in means equal to zero logits and poor targeting over two logits while a value between 0.5 and 1 logit indicates very good targeting (20). A person-item map visualizes targeting by placing on two sides of the same continuous line the participant scores on the Rasch-calibrated questionnaire and the relative difficulty of each of the questionnaire items, showing whether the items adequately cover the range of person ability and whether there is any overlap in questions.

Differential Item Functioning

Differential item functioning (DIF) indicates whether subgroups are responding in a different pattern than the rest of the sample despite having equal levels of the assessed trait (15). In order to ascertain clinically important differential item functioning two conditions had to be satisfied at the same time, a Welsh's test statistically significant p-value ($P < .05$) and a contrast value of >0.64 logits. If both conditions were satisfied it would indicate that the interpretation of the questionnaire differs by group and that it is influenced by confounding factor(s).

Comparative statistics

Gender differences on age and the LVQOL score were assessed with Mann-Whitney tests. The difference in LVQOL scores pre and post operation was assessed with a paired samples t-test. All comparative statistics were calculated using the SPSS statistical package, version 25.

RESULTS

RASCH ANALYSIS

Measurement Precision and Unidimensionality

In our sample the LVQOL questionnaire had a PSI = 4.43 and a PR = 0.95 which are excellent values. There were however four items exhibiting a mean square statistic (MNSQ) higher than 1.5 but none higher than 2. Those four items were item 16, item 10, item 9 and item 11. The PCA had had 62.1% of raw variance explained by the measures and the unexplained variance by the first contrast of the residuals was 1.82 eigenvalue units. The reliability of the LVQOL is assessed with two measurements, Cronbach alpha's coefficient equals 0.959, while the more accurate Rasch measurement methodology offers a model reliability upper estimate of 0.99 and a 'real' reliability lower estimate of .95. In every case, reliability of the LVQOL is excellent.

CATEGORY THRESHOLD ORDER

Figure 1 presents the probability of a specific response selection after one considers the item being answered plotted against person item measure in logits, meaning the overall attitude measure of the respondent. Each LVQOL response category is most probable for some combination of person measures and item measures, with an increased probability for the first and last response categories, depending on the person item measure.

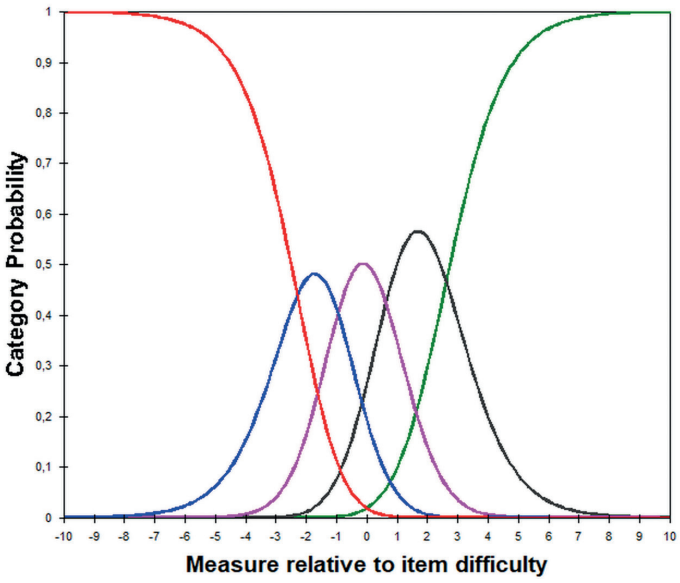

Fig. 1 Category probability curves for the LVQOL questionnaire, demonstrating the operation of the five-point Likert-style response categories. All response categories have a range along the ability score where they are most likely to be chosen over the other responses.

TARGETING

The LVQOL had excellent targeting, with a difference between the person and item means on the person-item map equal to 0.06.

PERSON-ITEM MAP

The person-item map in Figure 2 displays the participant scores on the Rasch-calibrated questionnaire and the relative difficulty of each of the questionnaire items. On the left side of each Wright Map the mean (M) and two standard deviation points (S = one SD and T = two SD) are shown for each patient's vision functioning. Participants with the highest level of vision-related quality of life are located at the top of the figure while those with the lowest are found at the bottom. On the right side of the map, the mean difficulty of the items (M) and two standard deviation points (S = one SD and T = two SD) for the items are shown, where 'mean difficulty' refers to the mean possibility of answering positively the item, an item being 'more difficult' when less participants answer it positively. Items are grouped between the range of +1 to −1 SD from mean ability denoting that there is less discriminating ability for persons of high or low visual functioning, although there is spacing between the items indicating little redundancy. Item 16 appears to break the pattern being situated at more than 2 SD below mean patient ability. The mean ability of the patients (M on the left side of the scale) is identical to the mean difficulty of the items (M on the right side of the scale) denoting an excellent level of item understanding.

DIFFERENTIAL ITEM FUNCTIONING

Differential item functioning for gender, age and underlying disorder was examined for the LVQOL. Gender was included because there are differences between the genders with regards to the usual activities that they perform and value the most, hence it is possible that they would place a differential emphasis on the items of the questionnaire that are more closely related to their everyday needs. Age has a direct impact on visual functioning but also the activities that the patients are expected to perform since the higher the age, the higher the chance of comorbid disease that limits general functionality. We divided the sample into two subsamples for this DIF analysis, those patients up to and including 70 years of age, since they comprised one third of the total sample and those aged over 70.

Table 3 presents the summary of the examination of the LVQOL items for differential item functioning by gender, age and disorder (cataract or other). Results indicated a number of instances where items met the statistical significance for differential functioning (Welch's test p-value $P < 0.05$), especially when examining differential item functioning by disorder but in every case the contrast effect size was lower than .64 denoting that the difference in functioning between the subgroups was negligible. These items were item 11 and 13 for gender, items 5, 16 and 24 for age and items 4, 6, 7, 10, 16, and 23 for the underlying disorder.

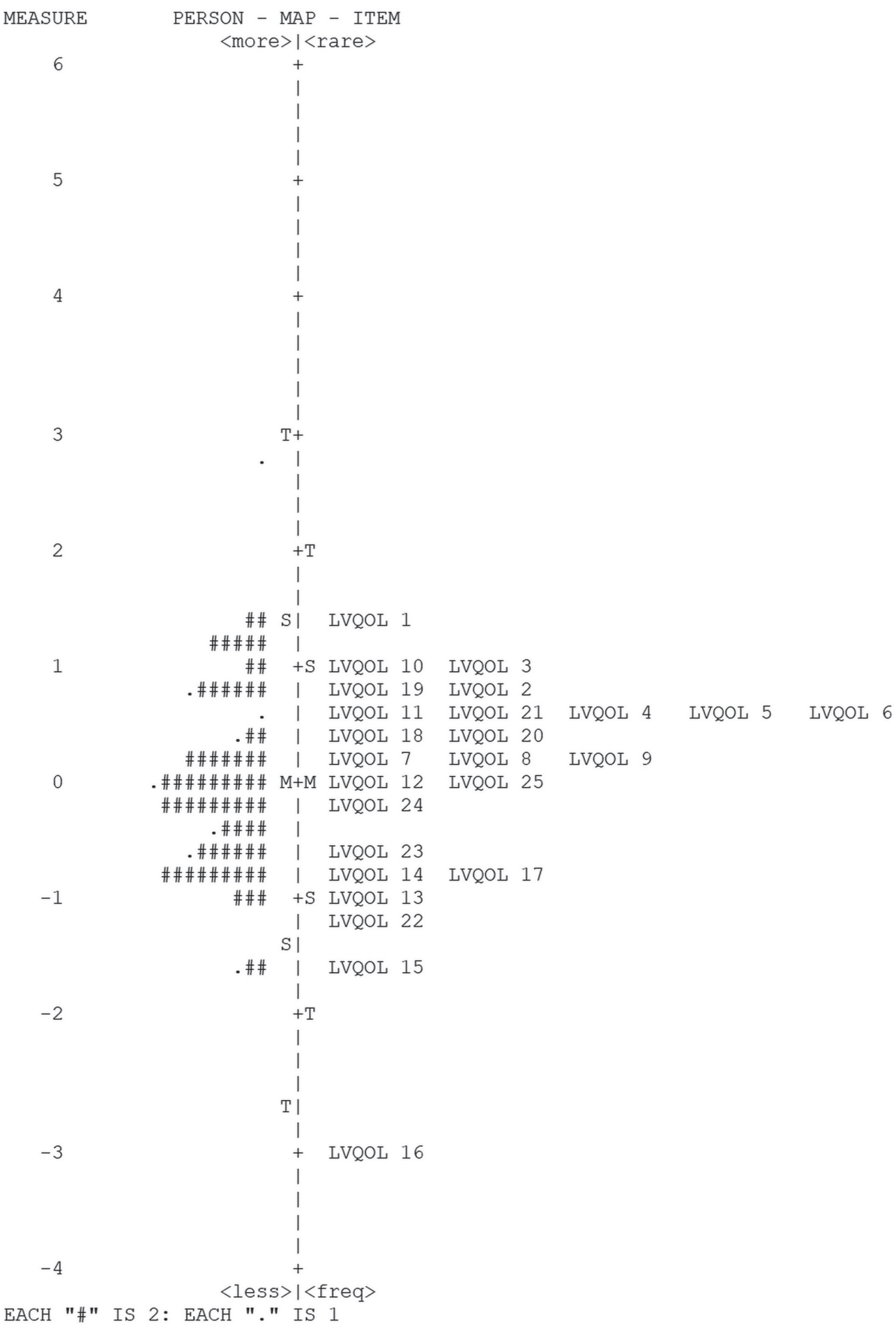

Fig. 2 Wright map for the LVQOL questionnaire. Items of the questionnaire are marked LVQOL1-25, M = mean difficulty of the items, S = one standard deviation, T = two standard deviations.

Tab. 3 Differential Item Functioning by gender, age and disorder.

Original LVQOL item	DIF by gender				DIF by age				DIF by disorder			
	Male	Female	Contrast	Welch's test p-value	≤70 years	>70 years	Contrast	Welch's test p-value	Cataract	Other	Contrast	Welch's test p-value
LVQOL 1	1.21	1.40	−0.19	0.231	1.28	1.28	0	1.00	1.37	1.21	0.16	0.312
LVQOL 2	0.72	0.72	0	1.00	0.68	0.74	−0.06	.727	0.72	0.72	0	1.00
LVQOL 3	0.92	0.92	0	1.00	0.92	0.92	0	1.00	1.03	0.80	0.23	0.145
LVQOL 4	0.56	0.59	−0.03	0.850	0.51	0.60	−0.09	.586	0.72	0.41	0.31	0.045
LVQOL 5	0.60	0.51	0.10	0.529	0.35	0.69	−0.34	.033	0.56	0.56	0	1.00
LVQOL 6	0.46	0.59	−0.13	0.410	0.54	0.52	0.03	.870	0.67	0.36	0.31	0.044
LVQOL 7	0.23	0.13	0.10	0.518	0.19	0.19	0	1.00	0.34	0.03	0.31	0.046
LVQOL 8	0.21	0.33	−0.12	0.440	0.26	0.26	0	1.00	0.32	0.20	0.12	0.452
LVQOL 9	0.18	0.10	0.08	0.611	0.08	0.19	−0.11	.497	0.21	0.08	0.13	0.389
LVQOL 10	1.10	0.90	0.20	0.199	1.02	1.02	0	1.00	0.84	1.21	−0.37	0.018
LVQOL 11	0.70	0.38	0.32	0.042	0.45	0.63	−0.19	0.238	0.42	0.71	−0.28	0.066
LVQOL 12	0.14	−0.03	0.17	0.277	0.05	0.07	−0.02	0.887	0.11	0.03	0.08	0.624
LVQOL 13	−1.08	−0.65	−0.42	0.010	−0.85	−0.93	0.08	0.633	−0.94	−0.85	−0.09	0.578
LVQOL 14	−0.81	−0.64	−0.17	0.300	−0.88	−0.65	−0.23	0.172	−0.88	−0.59	−0.29	0.068
LVQOL 15	−1.43	−1.64	0.21	0.215	−1.54	−1.52	−0.03	0.869	−1.56	−1.48	−0.08	0.636
LVQOL 16	−2.99	−3.08	0.09	0.665	−3.35	−2.86	−0.49	0.030	−3.28	−2.76	−0.52	0.012
LVQOL 17	−0.84	−0.61	−0.23	0.154	−0.64	−0.81	0.17	0.307	−0.72	−0.77	0.05	0.740
LVQOL 18	0.31	0.46	−0.15	0.338	0.46	0.32	0.14	0.375	0.35	0.39	−0.04	0.786
LVQOL 19	0.72	0.67	0.05	0.750	0.69	0.69	0	1.00	0.80	0.59	0.22	0.165
LVQOL 20	0.43	0.45	−0.02	0.892	0.43	0.43	0	1.00	0.51	0.35	0.16	0.304
LVQOL 21	0.65	0.43	0.22	0.158	0.62	0.53	0.09	0.568	0.68	0.44	0.24	0.121
LVQOL 22	−1.12	−1.19	0.07	0.691	−1.12	−1.15	0.03	0.869	−10.24	−10.06	−0.18	0.276
LVQOL 23	−0.55	−0.49	−0.06	0.718	−0.35	−0.63	0.28	0.090	−0.69	−0.34	−0.35	0.027
LVQOL 24	−0.33	−0.22	−0.11	0.475	−0.08	−0.41	0.32	0.045	−0.39	−0.18	−0.21	0.176
LVQOL 25	0.01	0.01	0	1.00	0.13	−0.06	0.19	0.243	−0.11	0.13	−0.23	0.131

DIF = Differential Item Functioning, LVQOL = Low Vision Quality-of-Life Questionnaire

ADDITIONAL EXAMINATIONS OF THE LVQOL RELIABILITY, CONTENT AND CONCURRENT VALIDITY

Table 4 presents the results from the application of the LVQOL per disease and per gender, pre and post treatment. Results indicate that the cataract group had statistically significantly lower quality of life than the combined group of other diseases before treatment (Mann-Whitney Z = 2.096, P = 0.036), while the increase in quality-of-life post treatment led to it being significantly higher post treatment Mann-Whitney Z = 3.55, $P < 0.001$. There was no difference in quality of life between the genders or between those older than 70 and younger than 70 years of age ($P > 0.05$).

Tab. 4 Results from the application of the Low Vision Quality-of-Life Questionnaire in the research sample.

Estimated person measure (total LVQOL mean score / standard deviation)		Before treatment by gender		Total by disorder
		Male	Female	
Before treatment	Cataract	75.69 (16.86)	69.32 (17.39)	72.98 (17.32)
	Other	77.46 (18.82)	80.9 (20.51)	78.86 (19.53)
	All	76.59 (17.85)	74.97 (19.77)	
After treatment	Cataract	97.59 (22.88)	95.15 (22.12)	96.55 (22.51)
	Other	88.49 (19.88)	90.2 (20.11)	89.28 (19.93)
	All	92.96 (21.83)	92.84 (21.21)	

When comparing the cataract group to the other diseases group, there was a significant difference in the increase of visual functioning post treatment for the cataract group (Mann-Whitney Z = 5.5, $P < 0.001$) that coincided with the relative increase in quality of life (Mann-Whitney Z = 6.479, $P < 0.001$). Apparently, the larger gain from treatment for cataract patients leads to a direct increase in their vision related quality of life that surpasses that of patients with other ophthalmic diseases.

We examined the difference in LVQOL scores pre- and post-surgery in the cataract patients' group, assuming that a corrective surgery would carry a positive effect onto the visual functioning of the patient so as to test content validity. A paired-sample t-test returned a statistically significant difference between quality-of-life pre- and post-cataract surgery assessed with the LVQOL, $t(149) = 13.238$, $P < 0.001$. In order to ascertain concurrent validity, we examined the correlation between the scores on the LVQOL and the visual acuity pre- and post-surgery. Results indicated that the LVQOL score after surgery correlated with the improvement between visual acuity pre- and post-surgery, Spearman's rho $r(s) = 0.681$, $P < 0.001$.

DISCUSSION

The examination of the LVQOL questionnaire with Rasch measurement demonstrated high reliability and validity with a small number of items that carry an acceptable level of measurement error. Those were items 16, 10, 9 and item 11. Item 16 in particular appeared to be separate from the grouping of items in the Wright map; this item queries the subjects as to how well their eye condition been explained to them. This is unrelated to the visual impairment per se, and it is detached from the other group of items in the questionnaire itself. However, it does provide useful input for the researcher and the clinician as to a possible source of vision-related anxiety and skipping it would impoverish the trove of information that the LVQOL provides. Since the item's MNSQ did not exceed the two-unit threshold the decision was made to retain it in the Greek version of the LVQOL. This item had statistically significant differential item functioning for age and underlying disorder, denoting that older patients with more complex diseases may require extra assistance in understanding their predicament. Obviously, this information would not be available now for the clinician if the item was omitted from the questionnaire.

Items 9, 10 and 11, also had an MSNQ higher than 1.5 but lower than 2; these questions relate to depth of vision (item 9) and moving outside on the street unaided without being hindered by small obstacles (items 10 and 11). These items could be alternatively consolidated into a single item in future research, however changing the structure of a questionnaire that has been widely employed worldwide in this specific form has the minus of reducing comparability between different studies.

A limitation of this study is the non-stratified sample that cannot be considered representative for the general Greek patient population; however, there is no reason to assume that the study population may differ to a significant extent from the average population examined in the outpatient services of an ophthalmic department. Also, the differences between diagnoses have been considered with cataract as the main diagnosis and the other diagnoses considered as a single group, due to their lower number. Future studies can replicate the findings in other diagnoses with larger sample sizes for each diagnosis.

These limitations however are offset by the validation process of this study. The examination of the differential item functioning by gender, age and disorder was essential practice in order to demonstrate that the LVQOL is reliable and valid across genders, age range and underlying eye disorder. Our sample had two large subpopulations; the first one was indicative of the demographics of cataract patients that are referred for phacoemulsification since these patients were consecutive and not selected with bias. The second subpopulation was comprised from patients with twelve distinct eye disorders that can lead to low vision, their relative frequency is indicative of how common they are compared to one another among patients who are referred to outpatient services. There was no difference in the validity of the LVQOL among these patient groups; this finding has not been tested in other cultural validations so far in the literature and is unique to this study.

Cataract phacoemulsification surgery offers immediate positive results to the patient and it was expected that the improvements in quality-of-life, as was the case here. The magnitude of improvement in visual acuity determined the improvement in quality-of-life as well. Successful cataract

surgery leads to beneficial results in the patient's quality of life and the documentation of this fact with a valid questionnaire, as the LVQOL, can be very important for the funding of ophthalmological departments in the new era of managed care. The introduction of modern surgical equipment and related procedures can be thus justified in practical terms of overall patient improvement and satisfaction. The importance of cataract surgery in particular is demonstrated in a recent study where the current COVID-19 pandemic did not affect patients' decision to visit a hospital for cataract surgery (21). Any decisions to limit the provision of these important patient services should therefore be weighted against the considerable benefits that they infer to the patients' lives.

CONCLUSION

In conclusion, our validation study has resulted in the acceptance of the Greek version of the LVQOL as a valid research tool. The employment of the Rasch measurement model has resulted in identification of a number of items that are not ideally suited for the questionnaire yet do not degrade the measurement system and have been retained for their clinical value and compatibility with other versions of the questionnaire that have been employed worldwide. The LVQOL can be employed in a large range of ophthalmic diseases and reliably assess improvements in quality-of-life following phacoemulsification surgery.

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APPENDIX

THE LOW VISION QUALITY-OF-LIFE QUESTIONNAIRE (LVQOL) ENGLISH VERSION

Distance Vision, Mobility and Lighting GRADING

How much of a problem do you have:	**None**	**Moderate**			**Great**		
With your vision in general	5	4	3	2	1	x	n/a
With your eyes getting tired (e.g. only being able to do a task for a short period of time)	5	4	3	2	1	x	n/a
With your vision at night inside the house	5	4	3	2	1	x	n/a
Getting the right amount of light to be able to see	5	4	3	2	1	x	n/a
With glare (e.g. dazzled by car lights or the sun)	5	4	3	2	1	x	n/a
Seeing street signs	5	4	3	2	1	x	n/a
Seeing the television (appreciating the pictures)	5	4	3	2	1	x	n/a
Seeing moving objects (e.g. cars on the road)	5	4	3	2	1	x	n/a
With judging the depth or distance of items (e.g. reaching for a glass)	5	4	3	2	1	x	n/a
Seeing steps or curbs	5	4	3	2	1	x	n/a
Getting around outdoors (e.g. on uneven pavements) because of your vision	5	4	3	2	1	x	n/a
Crossing a road with traffic because of your vision	5	4	3	2	1	x	n/a
Adjustment **Because of your vision, are you:**	**No**	**Moderately**			**Greatly**		
Unhappy at your situation in life	5	4	3	2	1	x	n/a
Frustrated at not being able to do certain tasks	5	4	3	2	1	x	n/a
Restricted in visiting friends or family	5	4	3	2	1	x	n/a
	Well		**Poorly Not explained**				
How well has your eye condition been explained to you	5	4	3	2	1	x	

Reading and Fine Work **With your reading aids / glasses, if used, how**							
much of a problem do you have:	**None**	**Moderate**			**Great**		
Reading large print (e.g. newspaper headlines)	5	4	3	2	1	x	n/a
Reading newspaper text and books	5	4	3	2	1	x	n/a
Reading labels (e.g. on medicine bottles)	5	4	3	2	1	x	n/a
Reading your letters and mail	5	4	3	2	1	x	n/a
Having problems using tools (e.g. threading a needle or cutting)	5	4	3	2	1	x	n/a

Activities of Daily Living **With your reading aids / glasses, if used, how**							
much of a problem do you have:	**None**	**Moderate**			**Great**		
Finding out the time for yourself	5	4	3	2	1	x	n/a
Writing (e.g. cheques or cards)	5	4	3	2	1	x	n/a
Reading your own hand writing	5	4	3	2	1	x	n/a
With your every day activities (e.g. house-hold chores)	5	4	3	2	1	x	n/a

ΕΡΩΤΗΜΑΤΟΛΟΓΙΟ LOW VISION QUALITY-OF-LIFE QUESTIONNAIRE (LVQOL)

Μακρινή όραση, Κινητικότητα και Φωτισμός ΒΑΘΜΟΛΟΓΗΣΗ

Πόσο σοβαρό πρόβλημα έχετε:	**Καθόλου**		**Μέτριο**		**Μεγάλο**		
Γενικά με την όραση σας	5	4	3	2	1	x	δ/ε
Με την κόπωση των ματιών σας (π.χ. το να μπορείτε να διεκπεραιώσετε συγκεκριμένο έργο σε σύντομο χρονικό διάστημα)	5	4	3	2	1	x	δ/ε
Με την όραση σας τη νύχτα μέσα στο σπίτι	5	4	3	2	1	x	δ/ε
Με το να έχετε αρκετό φως για να μπορείτε να δείτε	5	4	3	2	1	x	δ/ε
Με λάμψεις (π.χ. να σας τυφλώσουν τα φώτα των αυτοκινήτων ή ο ήλιος)	5	4	3	2	1	x	δ/ε
Βλέποντας πινακίδες στο δρόμο	5	4	3	2	1	x	δ/ε
Βλέποντας τηλεόραση (παρακολουθώντας την εικόνα)	5	4	3	2	1	x	δ/ε
Βλέποντας κινούμενα αντικείμενα (π.χ. αυτοκίνητα στο δρόμο)	5	4	3	2	1	x	δ/ε
Με την εκτίμηση του βάθους ή της απόστασης των αντικειμένων (π.χ. προσπαθώντας να πιάσετε ένα ποτήρι)	5	4	3	2	1	x	δ/ε
Βλέποντας σκαλοπάτια ή γωνίες στα κράσπεδα	5	4	3	2	1	x	δ/ε
Να κινηθείτε σε εξωτερικούς χώρους (π.χ. σε ανώμαλα πεζοδρόμια) λόγω της όρασης σας	5	4	3	2	1	x	δ/ε
Να διασχίσετε ένα δρόμο όπου κυκλοφορούν αυτοκίνητα λόγω της όρασης σας	5	4	3	2	1	x	δ/ε
Προσαρμογή σε συνθήκες ζωής Λόγω της όρασης σας, είστε:	**Όχι**		**Αρκετά**		**Σημαντικά**		
Δυστυχής με τη κατάσταση σας στη ζωή	5	4	3	2	1	x	δ/ε
Απογοητευμένος με την αδυναμία σας να κάνετε κάποιες εργασίες	5	4	3	2	1	x	δ/ε
Περιοριστεί μόνο στις επισκέψεις σε φίλους ή συγγενείς	5	4	3	2	1	x	δ/ε
	Καλά		**Ελάχιστα**		**Καθόλου**		
Πόσο καλά σας έχει εξηγηθεί το πρόβλημα με τα μάτια σας	5	4	3	2	1	x	

Ανάγνωση και λεπτές εργασίες Εφόσον χρησιμοποιήσετε τα βοηθήματα ανάγνωσης/ γυαλιά σας, πόσο σημαντικό πρόβλημα έχετε να	**Καθόλου**		**Μέτριο**		**Μεγάλο**		
Διαβάσετε μεγάλους χαρακτήρες (π.χ. επικεφαλίδες εφημερίδων)	5	4	3	2	1	x	δ/ε
Διαβάσετε το κείμενο σε εφημερίδες και βιβλία	5	4	3	2	1	x	δ/ε
Διαβάσετε ετικέτες (π.χ. σε μπουκάλια φαρμάκων)	5	4	3	2	1	x	δ/ε
Διαβάσετε την αλληλογραφία και τα e-mail σας	5	4	3	2	1	x	δ/ε
Αντιμετωπίσετε προβλήματα με τη βοήθεια εργαλείων (π.χ. να περάσετε μία κλωστή ή να την κόψετε)	5	4	3	2	1	x	δ/ε

Δραστηριότητες της καθημερινότητας Εφόσον χρησιμοποιήσετε τα βοηθήματα ανάγνωσης / γυαλιά σας, πόσο σημαντικό πρόβλημα έχετε να	**Καθόλου**		**Μέτριο**		**Μεγάλο**		
Βρείτε ο ίδιος τι ώρα είναι	5	4	3	2	1	x	δ/ε
Γράψετε κάτι συγκεκριμένο (π.χ. επιταγές ή κάρτες)	5	4	3	2	1	x	δ/ε
Διαβάσετε τα δικά σας γράμματα	5	4	3	2	1	x	δ/ε
Με τις καθημερινές σας δραστηριότητες (π.χ. δουλειές στο σπίτι)	5	4	3	2	1	x	δ/ε

Reference Values for Cardiopulmonary Exercise Testing in Young Male Slovak Athletes

Filip Olekšák[1], Pavol Dvoran[1,*], Ľubica Jakušová[1], Peter Ďurdík[1], Matúš Igaz[1], Peter Bánovčin[1]

ABSTRACT
Background: The reference values of young athletes for cardiopulmonary exercise testing are lacking. Expert opinions encourage production of local values specific for certain population.
Patients and methods: The study population consisted of 136 healthy male caucasian athletic children and adolescents coming from one specific football school in northern Slovakia. Exercise testing with continuous electrocardiography was performed, and ventilatory parameters, oxygen uptake (VO_2), and carbon dioxide (CO_2) production were measured continuously with a respiratory gas analysis system.
Results: Peak VO_2max/kg was changing very little across the childhood, whereas the peak work rate, heart rate and O_2Pulse were. Linear regression analysis showed a significant effect of age on VE/VCO_2.
Conclusion: This work provides a reference values for the most important cardiopulmonary variables that can be obtained during cardiopulmonary exercise testing in athletic children.

KEYWORDS
cardiopulmonary exercise testing; reference values; athletic children

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INTRODUCTION

Stress tests are among the most popular non-invasive diagnostic methods in cardiological evaluation and evaluation of functional capacity of the organism. In the paediatric population, cardiopulmonary exercise testing (CPET) is considered valid but neglected diagnostic tool whose indications and location in the clinical setting are still pending for proper application (14). Adding the function of evaluation of expired air to regular stress test we get a direct insight into the functioning of oxygen transport from air to mitochondria and also into its metabolism in the body directly under stress. The way and rate of response of the organism to increasing bout of exercise can be applied in clinical practice not only to evaluate the functional parameters of athletes but also to correctly interpret the risk stratification in patients with congenital or acquired heart, lung, muscle or metabolic diseases (12). Growing amount of information about this examination and its relevance to clinical practice could become a starting point for its more frequent application in paediatric practice.

Regular pre-participation examinations in athletes should consist of physical examination and 12 lead ECG. Preparticipation screenings are meant to prevent sudden cardiac deaths (3). Athletes (in paediatrics their parents) very often ask for more thorough examination in order to gain more information about the state of their health. CPET is ideal tool to evaluate whole body state of health as it is golden standard among examinations documenting aerobic fitness of an individual.

Cardiopulmonary exercise testing differs in many aspects from the tests performed in adults (2). Results gained by CPET are dependent on the will to perform and on motivation of examined patient. To be able to evaluate one's health and level of functional capacity certain level of exhaustion must be achieved. Young athletes are much more often highly motivated, and performance results gained from their examinations are above any reference values for populations of non-athletes. On the other hand, their other than performance results are very similar to healthy non-athletes. As the physiological responses to exercise change during growth and development, appropriate paediatric reference values are essential for an adequate interpretation of the cardiopulmonary exercise test (14). Some parameters yielded from CPET in athletes of young age are incomprehensible due to lack of reference values and clinical implications. Reference values for CPET parameters may change over time and should be regularly updated or validated (14). Considering prognostic value of CPET in many diseases we can anticipate same use of this tool in training process of young athletes. Data provided by CPET might be of high value in screening for unknown underlying disease that might be aggravated by strenuous exercise.

Normal values for CPET are already published and represent a set of normal values for specific population coming from specific region with different anthropometric and cultural characteristics (16). For an adequate interpretation of CPET, the normal range of variety of CPET parameters is essential. In many studies, however, only mean or median value for the population is provided. Expert review (14) recommends reporting lower and upper limit of normal. The use of 80% of predicted as lower limit of normal should be abandoned. Instead, a Z-score should be used with a lower and upper limit of normal of –1.96 SD and +1.96 SD, respectively (14).

METHODS

The study population consisted of 136 healthy male Caucasian athletic children and adolescents coming from one specific football school in northern Slovakia (Table 1). They were recruited as healthy male athletic population of children and adolescents. Athletes were examined prospectively in the period between July 2018 and December 2019. We excluded all children with a history of acute illness within 3 weeks, history of chronic disease or smoking. Every athlete underwent spirometry (Geratherm Respiratory GmbH, Germany), 12 lead ECG (BTL Cardiopoint, Czech Republic) and orthostatic blood pressure test (Omron M3, Japan) and only those with physiological findings were included. All of the athletes were highly active individuals with professional coaching, exercising for more than two hours more than 3 times a week. All children came to the hospital by car, or they came by bike or walking if their residence was within 10 min away from the hospital. Patients were informed to consume light meal at latest 1 hour before testing and to come properly hydrated.

Tab. 1

Age group	8–10	11–14	15–19
Age	9.7 ± 1.5	12.5 ± 2	16.3 ± 2.3
Height	140.9 ± 11.3	155.0 ± 18.9	178.0 ± 13.3
Weight	31.0 ± 8.3	41.0 ± 17.8	67.0 ± 13.2
BMI	16.2 ± 2.4	17.4 ± 3.6	20.8 ± 3.3
BSA	1.1 ± 0.2	1.4 ± 0.4	1.8 ± 0.2
n	46	48	42

CPET was performed in upright position using treadmill (ITAM ERT-100, Poland) with breath-by-breath respiratory gas analysis (Geratherm Respiratory GmbH, Germany). Subjects breathed through a face mask of appropriate size and through a low impedance turbine volume transducer for measurement of expiratory volume and expiratory gas concentrations. CPET results were interval averaged (every 30 s), reported peak value represents mean value of all data collected during the final stage (if longer at least 30 s). CPET was performed with the personalized incremental ramp protocol to maximal exhaustion which was achieved between the 8th and 12th minute of exercise (6). The blood pressure was measured every three minutes until peak exercise and then every 2 minutes until full recovery. All participants were verbally encouraged to exercise to exhaustion. Cut-off values for maximal exertion were established as RER greater than 1.10, breathing rate more than 40/min and/or plateau in oxygen consumption. Before testing all equipment was calibrated according to the instructions of the manufacturer.

Resting HR was measured after at least 3 min in supine position before exercise testing and was calculated as number or RR intervals on ECG over 1 minute. Heart rate was measured by continuous 12-lead ECG during resting, warm up, peak exercise and recovery phase. Peak HR (HRpeak) was defined as the highest HR achieved during exercise. HR was recorded at 1. and 2. min after cessation of the exercise, and HR recovery (HRR1, HRR2) was calculated as the difference between HRpeak and the HR in first and second minute. VO_2max is defined as plateau in oxygen consumption with increase less than 2 ml/kg/min with increase of 10% work rate (6). VO_2peak is then defined as the highest achieved VO_2 in settings of not achieving VO_2max and is calculated as the mean of two highest consecutive values of 15-s averages of VO_2 (2). The VE/VCO_2 slope was obtained by linear regression analysis of whole measured dataset of exercise data before achieving respiratory compensation point. Peak work rate (WRpeak) was measured in absolute values and as weight adjusted value. The point of initiation of anaerobic metabolism (anaerobic threshold AT, ventilatory anaerobic threshold VAT) is defined as nonlinear elevation in production of CO_2 to consumption of O_2. VO_2 vs WR ($\Delta VO_2/\Delta WR$) was measured as the slope obtained by linear regression analysis of VO_2 (mL/min) versus WR (W). For ventilatory parameters we obtained minute ventilation, tidal volume, breathing frequency and breathing reserve (BR) at AT and peak exercise.

STATISTICS

Statistical analysis was performed with SPSS 26.0 (SPSS, Inc., Chicago, Illinois, USA). Values are presented as mean values and mean values ± 1.96 standard deviation. The effect of age on the measured parameters was determined by linear regression analysis.

RESULTS

We examined 136 boys with an age range 8–18. Subject characteristics are shown in Table 1. In order to obtain clear data capable of direct use we divided study group into subgroups according to age. Results are presented as mean value and lower and upper limit using 1.96 SD (standard deviation). We provide data that has been measured resting on treadmill, at AT and peak exercise. All participants performed CPET without complications and were able to adhere to chosen protocol. Table 2 presents CPET data and Table 3 presents regression equations for chosen parameters. There were no noted ECG abnormalities during exercise testing. According to our findings resting heart rate (71/min vs. 65/min vs. 61/min) and peak heart rate (190/min vs. 188/min vs. 180/min) declined with age in highly active children. (Figure 3) On the other hand, resting O_2Pulse (2.22 ml/beat vs. 2.68 ml/beat vs. 3.99 ml/beat) and peak O_2Pulse (8.27 ml/beat vs. 11.62 ml/beat vs. 19.41 ml/beat) raised with age. Resting breathing rate remained unchanged during whole childhood (19/min vs. 19/min vs. 18/min) whereas resting tidal volume and minute ventilation raised. VO_2max raised with age (48.29 ml/min/kg vs. 51 ml/min/kg vs. 52.81 ml/min/kg). VO_2/WR remained unchanged with age (10.28 ml/min/W vs. 10.20 ml/min/W vs. 10.32 ml/min/W). VE/VCO_2 as a marker of ventilatory effectivity declined with age (31.0 vs. 28.8 vs. 26.6). VO_2 and $\%VO_2$ of VO_2max in AT remained stable across the childhood. WRpeak/kg (as hallmark of exercise tolerance) were growing steadily with age (4.15 W/kg vs. 4.50 W/kg vs. 4.87 W/kg) (Figure 1).

Tab. 2

Age group	8–10	11–14	15–18
Number of patients	n = 46	n = 48	n = 42
HR rest	71	65	61
±1.96 SD	(50–92)	(45–85)	(43–79)
O_2Pulse rest	2.22	2.68	3.99
±1.96 SD	(0.65–3.78)	(0.94–4.41)	(1.68–6.30)
VE rest	7.09	8.50	11.00
±1.96 SD	(1.65–12.52)	(3.63–13.37)	(3.61–18.39)
BF rest	19	19	18
±1.96 SD	(11–27)	(11–28)	(9 -27)
Vt rest	0.39	0.44	0.67
±1.96 SD	(0.08–0.70)	(0.16–0.72)	(0.11–1.23)
WR peak	134.9	193.9	320.6
±1.96 SD	(89.8–180)	(103.1–284.8)	(239–402.3)
WR peak/kg	4.15	4.50	4.87
±1.96 SD	(3.45–4.84)	(3.76–5.23)	(4.33–5.41)
VO_2max (abs.)	1.55	2.18	3.48
±1.96 SD	(0.90–2.21)	(1.18–3.19)	(2.43–4.53)
VO_2max/kg	48.29	51.00	52.81
±1.96 SD	(32.31–64.27)	(38.37–63.62)	(42.21–63.41)
RER	1.15	1.17	1.16
±1.96 SD	(0.98–1.33)	(0.96–1.37)	(0.97–1.35)
$\Delta VO_2/\Delta WR$	10.28	10.20	10.32
±1.96 SD	(6.76–13.80)	(7.25–13.15)	(7.66–12.98)
VE peak	61.6	81.6	122.5
±1.96 SD	(33.0–90.2)	(35.8–127.3)	(72.0–173.0)
BF peak	58	54	52
±1.96 SD	(41–76)	(42–66)	(34–70)
Vt peak	1.06	1.51	2.37
±1.96 SD	(0.55–1.56)	(0.72–2.31)	(1.48–3.27)
BR peak	19.65	22.63	25.93
±1.96 SD	(0–45.97)	(0–48.87)	(0–54.43)
HR peak	190	188	180
±1.96 SD	(166–213)	(169–206)	(156–204)
HRR1	39	31	21
±1.96 SD	(4–84)	(1 – 60)	(6 – 49)
HRR2	66	62	47
±1.96 SD	(37–85)	(27 – 98)	(15 – 79)
O_2Pulse peak	8.27	11.62	19.41
±1.96 SD	(5.18–11.37)	(6.02–17.22)	(13.37–25.45)

Age group	8–10	11–14	15–18
Sys BP peak	134	137	156
±1.96 SD	(107–161)	(104–170)	(127–185)
Dia BP peak	71	75	74
±1.96 SD	(53–89)	(56–93)	(56–92)
VE/VCO_2 slope	31.0	28.8	26.6
±1.96 SD	(24.9–37.0)	(23.5–34.1)	(22.3–30.9)
PET CO_2 peak	34	36	40
±1.96 SD	(29–39)	(30–41)	(33–46)
Vd/Vt peak	14.2	14.9	12.0
±1.96 SD	(7.5–20.9)	(10.6–19.1)	(7.1–16.8)
VO_2 at AT abs.	0.94	1.34	2.05
±1.96 SD	(0.40–1.47)	(0.58–2.11)	(1.20–2.90)
VO_2 at AT /kg	29.07	31.33	31.13
±1.96 SD	(13.06–44.07)	(17.24–45.42)	(20.84–42.42)
%VO_2 at AT	61	62	59
±1.96 SD	(35–62)	(37–88)	(39–79)
RER at AT	0.86	0.87	0.82
±1.96 SD	(0.68–1.04)	(0.72–1.01)	(0.65–0.99)
HR at AT	145	144	136
±1.96 SD	(109–182)	(105–183)	(106–165)
O_2Pulse at AT	6.49	9.34	15.18
±1.96 SD	(3.18–9.81)	(4.56–14.11)	(9.68–20.68)
VE at AT	26.5	36.4	48.4
±1.96 SD	(9.2–43.9)	(12.3–60.5)	(24.3–72.5)
BF at AT	38	34	30
±1.96 SD	(20–57)	(21–47)	(15–45)
BR at AT	64	66	70
±1.96 SD	(40–89)	(45–87)	(54–86)
VE/VCO_2 at AT	27.1	27.7	26.3
±1.96 SD	(22.5–31.7)	(23.4–31.9)	(22.3–30.2)
WR at AT	69.4	102.8	181.3
±1.96 SD	(28.6–110.2)	(31.9–173.6)	(91.9–270.7)
Vd/Vt at AT	10.09	13.14	12.32
±1.96 SD	(0.08–20.11)	(5.03–21.26)	(5.24–19.40)

HR rest, resting heart rate (beats/min); O_2Pulse rest (ml/beat); VE rest, resting minute ventilation (l/min); BF rest, breathing rate (1/min); Vt rest, resting tidal volume (l); WR peak, peak work rate (W); WR peak/kg, peak work rate per kg (W/kg); VO_2max abs., absolute maximal oxygen uptake (l/min); VO_2max/kg, oxygen uptake per kg (ml/min/kg); RER, respiratory exchange ratio (1); $\Delta VO_2/\Delta WR$, slope of work rate (W) to oxygen uptake (ml/min); VE peak, peak minute ventilation (l); BF peak, peak breathing rate (1); Vt peak, peak tidal volume (l); BR peak, peak breathing reserve (%); HR peak, peak heart rate (1/min); HRR1 – hear rate recovery in 1 minute (1/min); HRR2 – heart rate recovery in 2 minute (1/min); O2Pulse peak (ml/beat); Sys BP peak, peak systolic pressure (mmHg); Dia BP peak, peak diastolic pressure (mmHg); VE/VCO_2 slope, slope of respiratory minute ventilation to CO_2 production; PET CO_2 peak (mmHg); Vd/Vt peak, peak dead space ventilation (%); VO_2 at AT abs., absolute oxygen uptake at anaerobic threshold (l/min); VO_2 at AT/kg, oxygen uptake at anaerobic threshold per kg (ml/min/kg); %VO_2 at AT, percentage of maximal oxygen uptake in anaerobic threshold (%); RER at AT, respiratory exchange ratio at anaerobic threshold; HR at AT (1), heart rate at anaerobic threshold (1/min), O_2Pulse at AT (ml/beat); VE at AT, minute ventilation at anaerobic threshold (l/min); BF at AT, breathing rate at anaerobic threshold (1/min); BR at AT, breathing reserve at anaerobic threshold (%); VE/VCO_2 at at, slope of respiratory minute ventilation to CO_2 production; WR at AT, work rate at anaerobic threshold (W); Vd/Vt at AT, dead space ventilation at anaerobic threshold (%).

Tab. 3

Parameter	Regression equation
WRpeak	y = 0.1024 × (age) + 3.1829
VE/VCO_2	y = −0.6604 × (age) + 37.27
HR rest	y = −1.4176 × (age) + 204.11
HR peak	y = −1.5485 × (age) + 85.57
O_2Pulse	y = 1.5403 × (age) – 6.9403
VO_2max/kg	y = 0.596 × (age) + 43.009

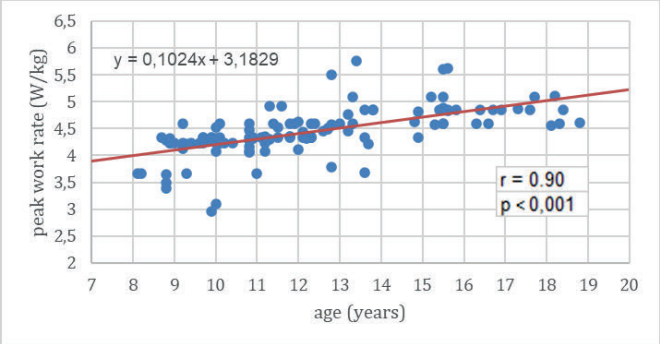

Fig. 1 The relation between age and the maximal work rate. The trendline represents linear regression of all data.

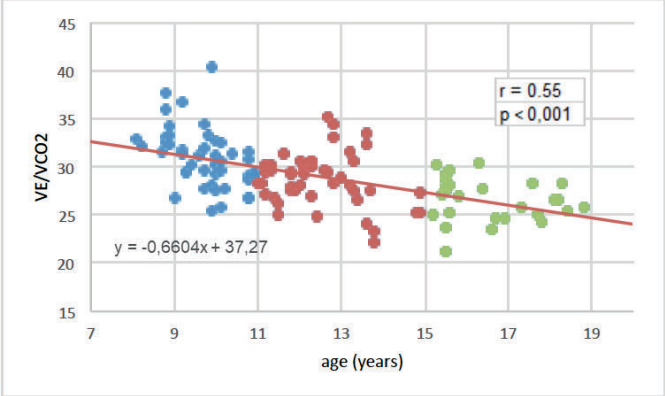

Fig. 2 The relation between age and the ventilation to carbon dioxide exhalation (VE/VCO_2) slope. The trendline represents linear regression of all data.

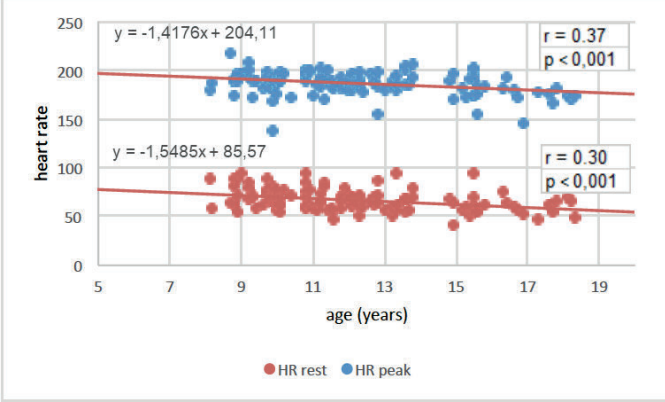

Fig. 3 The relation between age and resting heart rate (HR rest). The trendline represents linear regression of all data. The relation between age and peak heart rate (HR peak). The trendline represents linear regression of all data.

DISCUSSION

The primary aim of this study was to provide reference values for cardiopulmonary exercise testing in the cohort of healthy athletic children between 8 and 18 years of age. Data yielded are presented in Table 2. Upper and lower limit for age dependent variables are given for all 3 age groups which makes it possible to use these values as reference data.

VE/VCO_2 slope was decreasing steadily with age (Figure 2). Decrease in VE/VCO_2 slope with advancing age has been explained by a slightly lower pressure of CO_2 set point during exercise in the younger children and higher breathing efficiency in older children (larger tidal volumes and a relatively lower breathing frequency) (9, 16).

VO_2/WR remains unchanged with age. Calculation of the steepness of this slope is a valid measurement of O_2 flow or utilization in the exercising tissues (5). Our findings correlate with Harkel et al. (15). but our value was approximately 10.2 ml O_2/min per W in contrast of theirs 9.5 ml O_2/min per W in cohort very similar in account of age distribution. On the other hand, in our cohort we examined athletes where athlete's body adaptation might lead to processes which higher muscle efficiency in O_2 utilisation. Lower values are present in patients with impaired O_2 delivery to the exercising muscles such as patients with cardiac defects or malnourished patients.

During a progressive exercise test, the anaerobic threshold occurs when aerobic metabolism is insufficient to meet energy requirements. The AT indicates the highest oxygen uptake that can be sustained during exercise without developing lactic acidosis. The ability to sustain a high fractional utilization of athlete's maximal oxygen uptake (%VO_2max) in AT is considered crucial in order to maximize exercise effect. Anaerobic threshold is highly correlated to the distance running performance as compared to maximum aerobic capacity or VO_2max, because sustaining a high fractional utilization of the VO_2max for a long time delays the metabolic acidosis (4). It is not affected by patient effort or motivation and may be determined on a submaximal exercise test (2). VO_2 at AT is useful submaximal parameter in children. It is a good indicator of exercise capacity in children who are unable to perform to maximal exhaustion (12). The VO_2 at AT is a highly reproducible measure that provides insight into aerobic exercise capacity of children (13). Published data on normal values of VO_2 at AT are abundant but ranging from 45% to 75% of VO_2max (10, 18). Most recent study by Harkel et al. (16). reported 66% of VO_2max in children of age 8 and 9 and 60% of VO_2max in older children which is consistent with our findings (59–62%).

Peak VO_2 (VO_2max) kept rising throughout childhood with only very small inclination (Figure 5). Aerobic fitness is one of the most important components of physical fitness (15). The measurement of maximal VO_2max or VO_2peak during a progressive cardiopulmonary exercise test up to maximal exertion is widely considered the gold standard for assessing aerobic fitness (1). Comparing reference values for age in boys (16). with young athletes, boys of same age that are not athletes have lower VO_2max than athletes of same age. This states as a proof that regular exercise lead to increase in one's aerobic fitness even in children.

The maximum or peak HR achieved declined with age in all studies. Although in paediatric patients peak HR seems to remain constant throughout the paediatric years (11). We observed slight decline in peak HR in adolescents which is explainable with regular exercise that leads to athletic HR adaptation (Figure 3).

O_2Pulse (VO_2/HR) can be used as an indirect indicator of cardiac stroke volume (17). A plateau in the O_2Pulse at a low value implies limited cardiac output, either because of heart disease or disorders of the pulmonary circulation (7). The measurement of O_2Pulse during exercise can provide insight into the change in stroke volume during progressive exercise by assessing pattern of O_2Pulse changes in exercise and by estimating the value at peak exercise. (13). Recent study showed good correlation between O_2Pulse and stroke volume in adult patients undergoing CPET (8). In our cohort O_2Pulse was rising during examination from rest, throughout whole exercise until it reached plateau and was rising with age (Figure 4).

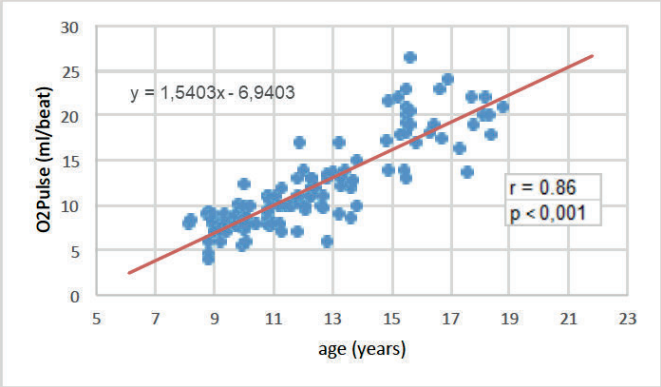

Fig. 4 The relation between age and peak O_2Pulse. The trendline represents linear regression of all data.

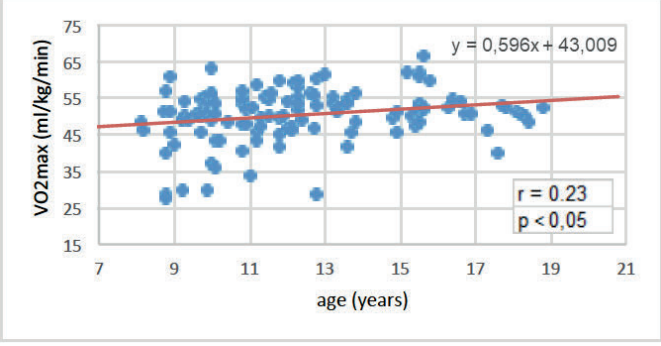

Fig. 5 The relation between age and VO_2max. The trendline represents linear regression of all data.

LIMITATIONS

Our dataset presents only male athletes reference values and is set to specific population of athletes competing in single sport. Reference values might be suitable for other types of athletes (as we found changes in CPET parameters

that are expected in highly active individuals) but confirmation from specific population locally is missing.

CONCLUSION

This work comprehensively provides a reference set of data for the most important cardiopulmonary variables that can be obtained during exercise testing in young athletes in Slovakia. Our work was set in specific population as these reference values are lacking. We found that many obtained other than performance parameters are not altered by regular exercise as they are showing physiological functions of body systems and are comparable with healthy inactive children. Performance results (VO_2max, WRpeak) were growing with age and were higher than in children of same age that are not athletes.

CONFLICT OF INTEREST

No conflict of interest to declare.

FUNDING

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The Difficult Path to Correct Diagnosis of Hepatolithiasis: A Case Report

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ABSTRACT

Hepatolithiasis is a benign disease, where stones are localized proximal to the confluence of hepatic ducts. The clinical picture may differ depending on whether the stones cause complete, partial, or intermittent biliary obstruction. The course can vary from asymptomatic to fatal, thus, early diagnosis and treatment is critical for a good prognosis. The gold standard in imaging is magnetic resonance cholangiopancreatography (MRCP). However, correct diagnosis can be challenging due to atypical clinical picture and laboratory findings. We present a case where hepatolithiasis was misdiagnosed initially due to incomplete reporting and documentation of MRCP. Choledocholithiasis was diagnosed based on initial MRCP, and endoscopic stone extraction was indicated. However, an unusual post-interventional course and signs of obstructive cholangitis led to an endoscopic re-intervention, which confirmed absence of pathology in extrahepatic biliary ducts. The cholangitis recurrence required intensive antibiotic treatment, and CT examination revealed intrahepatic S3 bile duct dilatation. Thus, a re-evaluation of initial MRCP and repeated MRCP confirmed hepatolithiasis. Further, laparoscopic bisegmentectomy was chosen as the definitive treatment due to the location of the lesion. The patient recovered and remained symptom free upon a 12 month follow up.

KEYWORDS

hepatolithiasis; intrahepatic stones; choledocholithiasis; laparoscopic liver resection; laparoscopic bisegmentectomy

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INTRODUCTION

Hepatolithiasis is the presence of stones proximal to the confluence of right and left hepatic duct. The symptomatology depends on the stage and severity of the obstruction and can vary from the asymptomatic course to cholangitis with fulminant sepsis. Typical clinical manifestations are cholangitis of varying intensity and jaundice (1). The treatment options are not uniform and despite treatment, hepatolithiasis often recurs. Recurrent inflammation of the bile ducts results in their fibrosis and stenosis, abscesses formation, cirrhosis, or liver fibrosis. In the long term, there is also an increased risk of developing cholangiocellular carcinoma, which occurs in 3.3% to 10% of patients with hepatolithiasis (2). Genetic predisposition, bile stasis, recurrent cholangitis, and biliary parasites are considered risk factors for hepatolithiasis (3, 4).

CASE REPORT

A 44-year-old male patient was referred to our hospital in August 2019 with a diagnosis of choledocholithiasis, which had been verified by magnetic resonance cholangiopancreatography (MRCP). Only a description, and no image documentation, of the procedure was available. Apart from choledocholithiasis, no other pathology was described. The patient had undergone cholecystectomy due to biliary colic 11 years ago. However, during the recent visit, he reported radiating colic abdominal pain below the right rib arch and anorexia associated with weight loss. There were no signs of jaundice or fever. Laboratory findings indicated a slight increase in gamma glutamyl transferase (GGT; 4.77 μkat/l, normal range 0.03–0.92 μkat/l) and alanine aminotransferase (ALT; 1.47 μkat/l, normal range 0.1–0.85 μkat/l) serum levels. Both total and conjugated bilirubin, alkaline phosphatase (ALP), and aspartate aminotransferase (AST) levels were all within normal ranges.

The patient was admitted for endoscopic retrograde cholangiopancreatography (ERCP). During the examination, a short stump of the ductus cysticus was observed, and the hepatocholedochus was reported to be 7 mm wide. Above the papilla of Vater, the defect in filling of contrast revealed a choledocholite, 7 mm in diameter (Fig. 1). Other findings did not indicate apparent pathology. Extraction of choledocholite and lavage of bile ducts was performed. Postoperatively, there was an elevation in AST (2.85 μkat/l, normal range 0.1–0.85 μkat/l), ALT (4.47 μkat/l), and GGT (5, 99 μkat/l) levels; however, serum amylase levels did not increase. This observation was interpreted incorrectly and the patient was discharged.

Two weeks after initial ERCP, the patient returned with complaints of severe abdominal pain in the epigastrium, fever, and chills. Elevation of inflammatory (leukocytosis 13.3 × 10^9/l, normal range 3.9–10 × 10^9/l; CRP 27.7 mg/l, normal range 0–5 mg/l) and hepatic parameters (total bilirubin 31.5 μmol/l, normal range 5–21 μmol/l; ALT 2.42 μkat/l; AST 1.74 μkat/l; GGT 2.89 μkat/l) were detected in the laboratory findings. Serum amylase and ALP levels were normal. The patient was admitted and treated empirically

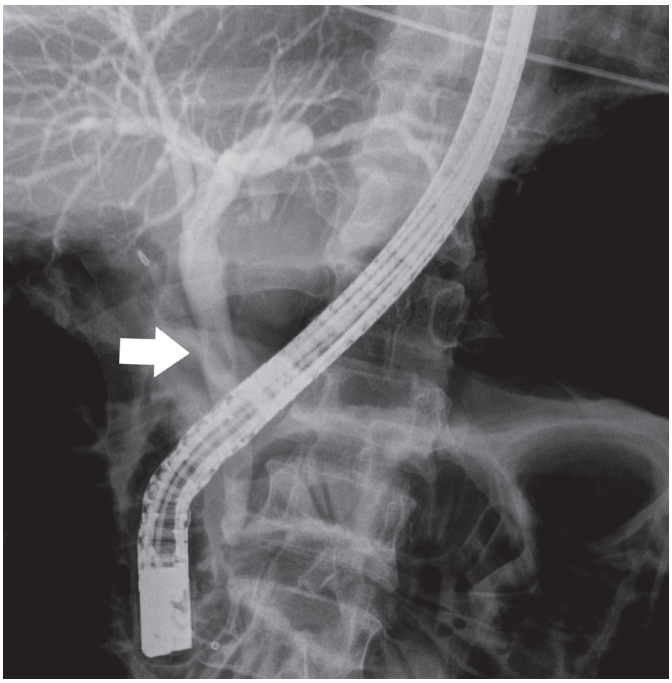
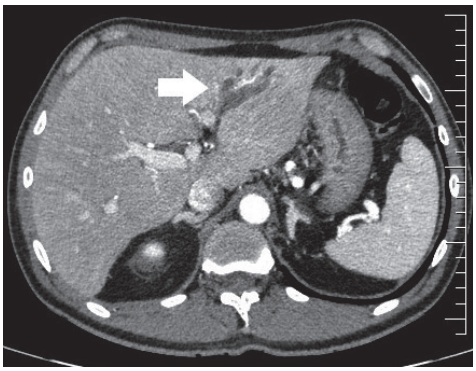


Fig. 1 Initial endoscopic retrograde cholangiopancreatography findings. Defect in the filling of contrast in the suprapapillar region – choledocholite (arrow). Other findings were without apparent pathology.

with antibiotics. Contrast-enhanced computed tomography (CT) showed dilation of bile ducts in the segment 3 (S3) of the liver to up to 10 mm (Fig. 2A). The patient was indicated for re-ERCP, where no pathology was found in the extrahepatic bile ducts, or in the right and left hepatic ducts. However, amputation of the subsegmental branch of the bile duct for S3 was observed (Fig. 2B). Cholangitis was managed conservatively; the patient was discharged and indicated for control MRCP one month apart.

On control MRCP, dilation of bile ducts was observed in segment 3 of the liver, without unambiguous presence of tumor (Fig. 3A). Subsequently, the finding of the initial MRCP was revisited. Image documentation, which was not available at the first examination, was requested. The finding of pathology in the S3 was already evident on the initial MRCP but was overlooked and not described in the written report (Fig. 3B). Due to the fact that the patient had a recurrence of biliary symptoms and it was not possible to rule out malignancy with certainty (despite the normal levels of tumor markers CA 19.9 and AFP), laparoscopic revision was finally preferred before further diagnostics. During the procedure, macroscopic changes were visible in the S3 of the liver (Fig. 4A) and perioperative ultrasonography confirmed the finding of dilation of the bile ducts with bile stones (Fig. 4B). Subsequently, the laparoscopic bi-segmentectomy S2–S3 was performed. The operative and postoperative course were without complications and the patient was discharged on the 4th postoperative day. Histological examination confirmed hepatolithiasis and ruled out malignancy (Fig. 4C). No residual hepatolithiasis was present in the control MRCP performed 3 months post-surgery. The patient had recovered, with no recurrence of biliary symptoms when followed-up for more than 12 months after the operation.

A



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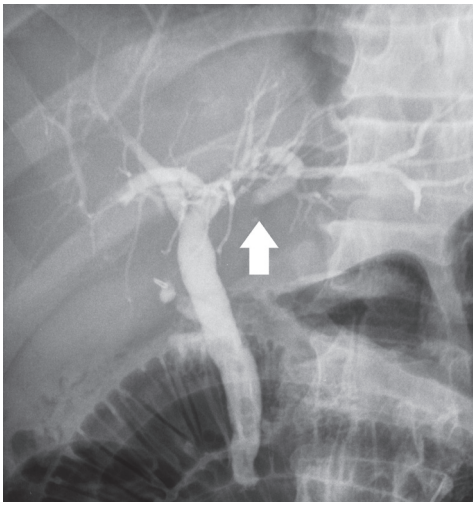
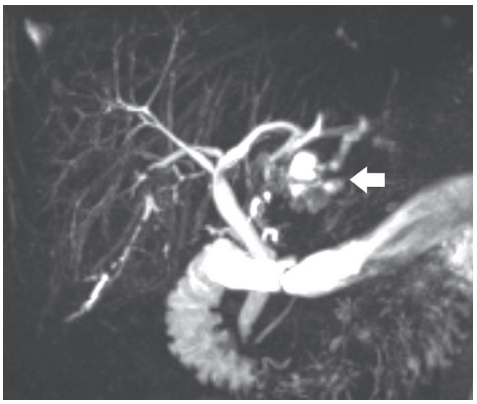


Fig. 2 Findings of imaging examinations during the first rehospitalisation. (A) Contrast-enhanced computed tomography (CT) scan shows dilatation of the bile ducts in segment 3 of the liver up to 10 mm, there is also higher post-contrast enhancement of the bile ducts walls and the surrounding liver parenchyma in this area (arrow) – CT signs of cholangitis; (B) re-endoscopic retrograde cholangiopancreatography examination without finding pathology on the extrahepatic bile ducts and on the right and left hepatic duct. There is visible amputation of the subsegmental branch of the bile duct in segment 3 of the liver (arrow).

A



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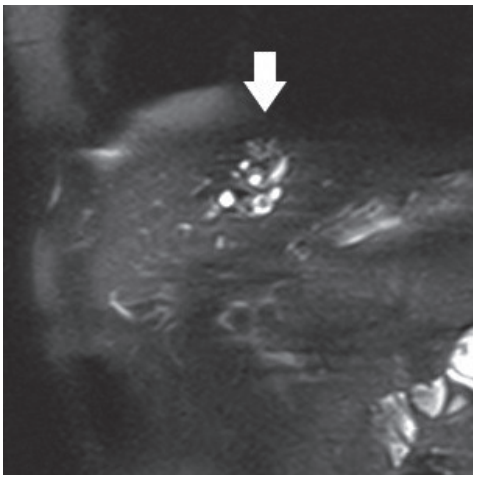
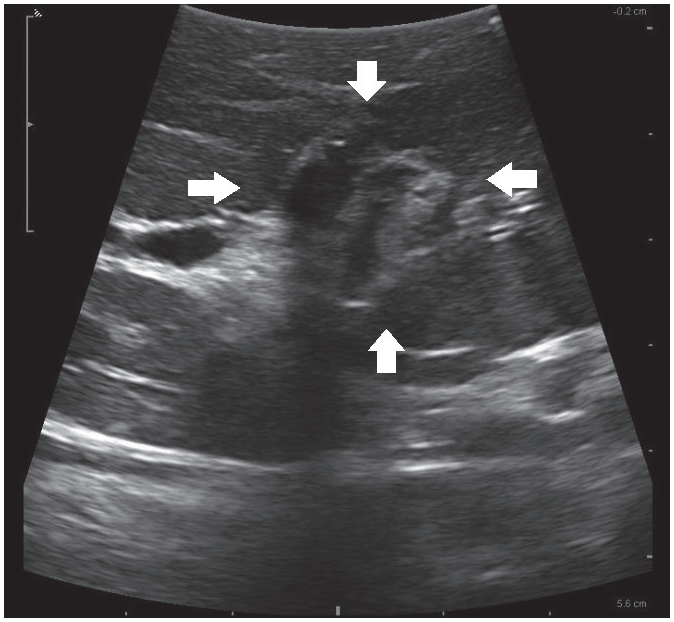


Fig. 3 Comparison of magnetic resonance cholangiopancreatography (MRCP) findings. (A) Control MRCP shows bile duct dilatation in the segment 3 of the liver (arrow); (B) Initial MRCP reveals apparent dilatation of bile ducts in the segment 3 of the liver at time of initial diagnosis (arrow).



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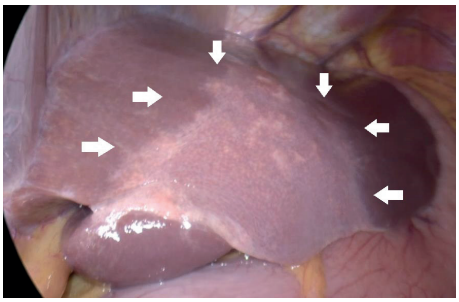

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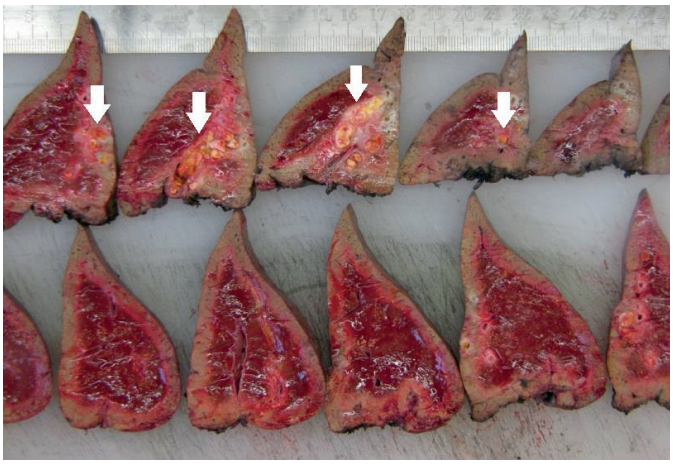

Fig. 4 Intraoperative and histopathological findings. (A) Macroscopic changes in the segment 3 of the liver during laparoscopic revision (arrows); (B) Intraoperative sonography revealed the dilatation of ducts and bile stones (arrows); (C) The specimen of the segments 2 and 3 of the liver, a large ductus filled with yellow stones is visible in serial sections (arrows).

DISCUSSION

Hepatolithiasis is a benign disease, but its symptoms of biliary obstruction and cholangitis can be fatal within 24 hours due to fulminant sepsis (1, 4). Early diagnosis and aggressive treatment are key to a good prognosis. In the case of extensive hepatolithiasis and recurrent cholangitis, cirrhosis, fibrosis, and eventually liver failure may occur. Another risk is development of cholangiocellular carcinoma (2, 4). The incidence of hepatolithiasis in the indigenous people of the Western countries remains very low; therefore, this diagnosis is not immediately considered in patients with biliary symptoms (5).

Clinical symptomatology depends mainly on the severity and location of biliary tract obstruction (1, 6). In our patient, especially during the primary examination, the typical clinical symptomatology was not fully plotted and the biliary symptoms were attributed to choledocholithiasis. Even during further course of the disease development to cholangitis, although abdominal pain was associated with chills and fever, jaundice was not clinically present.

In the case of obstruction and cholangitis, typically the markers of bile duct obstruction, hepatocyte damage, and inflammation are elevated. However, in case of incomplete obstruction, early after the onset of symptoms and when the hepatolithiasis is located in more peripheral ducts, laboratory finding may not be typical (6). In our patient, only some markers of obstruction and hepatocyte damage were found slightly elevated in the laboratory findings during the primary examination. This was probably due to incomplete obstruction without cholangitis. After the ERCP extraction of choledocholite, elevation of hepatic parameters occurred, but due to the description of initial MRCP and safe removal of choledocholite, this observation was misinterpreted and the patient was discharged. During rehospitalisation, the laboratory findings were almost typical of cholangitis.

Sonography and contrast-enhanced CT may contribute to the diagnosis of hepatolithiasis. However, the gold standard in the imaging of hepatolithiasis is MRCP (7). ERCP is usually indicated either as a predominantly therapeutic method, only after the bile duct obstruction is diagnosed, or in case of diagnostic doubts. In the case of our patient, the initial MRCP would have, upon careful evaluation, revealed hepatolithiasis in addition to choledocholithiasis. Relying on the description of MRCP without looking at the image documentation, the rarity of hepatolithiasis in our region, and the coincidence with choledocholithiasis were factors that influenced our decision-making and led to a delay in making a definitive diagnosis of hepatolithiasis. As in this case, ERCP is the method of choice for the treatment of choledocholithiasis. Acute contrast-enhanced CT revealed dilatation of the bile ducts in segment 3 of the liver and signs of cholangitis in this area. Subsequent re-ERCP revealed amputation of the subsegmental branch of the bile duct for S3, which could not be treated endoscopically due to its unavailability. Control MRCP led to a definitive diagnosis of hepatolithiasis. This case highlights the fact that the currently available laboratory and imaging techniques are able to establish a definitive diagnosis of hepatolithiasis, if properly examined and interpreted.

The biggest challenge, therefore, remains the adequate and effective treatment that allows for restoration of bile drainage by removing the bile stones or strictures and, if the parenchyma is damaged, its resection (3). Choice of treatment depends on the extent of hepatolithiasis (5, 8). Liver resection is considered as the optimal treatment because it removes damaged bile ducts and parenchyma, thereby minimizing the risk of recurrence of hepatolithiasis and cholangiocellular carcinoma formation (3, 9). Vetrone reports that up to 4.5% of liver specimens resected for hepatolithiasis have been shown by histological examination to have previously undiagnosed cholangiocellular carcinoma (9). However, resection cannot be performed in patients with extensive hepatolithiasis (3, 9). In the case of our patient, hepatolithiasis was localized only in segment 3 of the liver. Therefore, resection was chosen as the optimal therapy in this case as well. The operation and postoperative course were without complications and the patients biliary symptoms did not recur. Histological examination definitively ruled out the presence of cholangiocellular carcinoma. Our experience confirms the suitability of liver resection as the optimal and most effective solution in cases of localized hepatolithiasis.

In conclusion, the course of hepatolithiasis can vary from asymptomatic to fatal and also laboratory finding may not be typical. MRCP is the gold standard in imaging of hepatolithiasis. Nowadays, if currently available laboratory and imaging techniques are properly examined and interpreted, there should not be problem to establish a definitive diagnosis of hepatolithiasis. Also it is very important, that early diagnosis and treatment is crucial for a patient´s prognosis. Choice of treatment mainly depends on the extent of hepatolithiasis and in cases of localized hepatolithiasis, liver resection is considered as the optimal treatment.

DISCLOSURES

Drs. Peter Mikolajčík, Alexander Ferko, Michal Demeter, Martin Vorčák and Ľudovít Laca have no conflicts of interest or financial ties to disclose.

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Association of Tortuous Common Carotid Artery with Abnormal Distribution of the Ansa Cervicalis: A Case Report

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ABSTRACT

In the current study, we display a rare association of an aberrant innervation of the sternocleidomastoid muscle by the ansa cervicalis (AC) with a tortuous common carotid artery (TCCA). In specific, in a male cadaver we observed on the right side of the cervical region, a nerval branch of remarkable size originating from the most distal part of the AC's superior root and after piercing the superior belly of the omohyoid muscle innervated the distal portion of the sternocleidomastoid muscle. Furthermore, we noticed a tortuous course of the initial part of the right common carotid artery. We discuss the surgical significance of the awareness of AC's variations during neurotisation of the recurrent laryngeal nerve in cases of its damage, as well as the importance of aberrant innervation of the sternocleidomastoid muscle by AC for the preservation of muscle's functionality after accessory nerve's damage. Furthermore, we highlight the fact, that the knowledge of the relatively uncommon variant, such as TCCA is crucial for the physician in order to proceed more effectively in differential diagnosis of a palpable mass of the anterior cervical region or deal with symptoms such as dyspnea, dysphagia or symptoms of cerebrovascular insufficiency.

KEYWORDS

ansa cervicalis; common carotid artery; tortuosity; sternocleidomastoid muscle; variations

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INTRODUCTION

The ansa cervicalis (AC) or ansa hypoglossi as it is known is a cervical loop usually formed by first to third cervical nerves and innervating the infrahyoid muscles (1). There is a variability in regards to the cervical nerves which participate in its formation, the length of the loop with respect to the omohyoid muscle, the variable morphology of the AC's inferior root, the AC's relationship to the internal jugular vein or the likely participation of the vagus nerve in AC formation (2). However, the presence of additional AC's branches distributed to the adjacent muscles, such as the sternocleidomastoid muscle is rarely detected in the literature (3–4). As regards the tortuous common carotid artery (TCCA) is an usually asymptomatic elicosis of the common carotid artery of unclear origin which rarely may be palpated as a mass of the anterior cervical region or very rarely may be associated with symptoms of cerebrovascular insufficiency (5–6).

In the current study, we display a rare combination of an aberrant innervation of the sternocleidomastoid muscle by the AC, along with a TCCA, which is an interesting opportunity to search through the literature for similar anatomical variations.

CASE REPORT

During the routine dissection of the neck in our Department of Anatomy in a 82-year-old male cadaver whose cause of death was unrelated to the current case study, an infrequent combination of an aberrant innervation of the sternocleidomastoid muscle by the AC along with TCCA was detected. In particular, after meticulous dissection of the anterior, as well, lateral cervical region we observed on the right side, a nerval branch of remarkable size originating from the most distal part of the AC's superior root and after piercing the superior belly of the omohyoid muscle innervated the distal portion of the sternocleidomastoid muscle. Furthermore, we noticed a tortuous course of the initial part of the right common carotid artery (Figure 1). No other anatomical variations were present, or evidence of previous surgical procedures undertaken on the neck. The detected nerval and vascular variations, along with their relationship to the adjacent anatomical elements were documented by photographs.

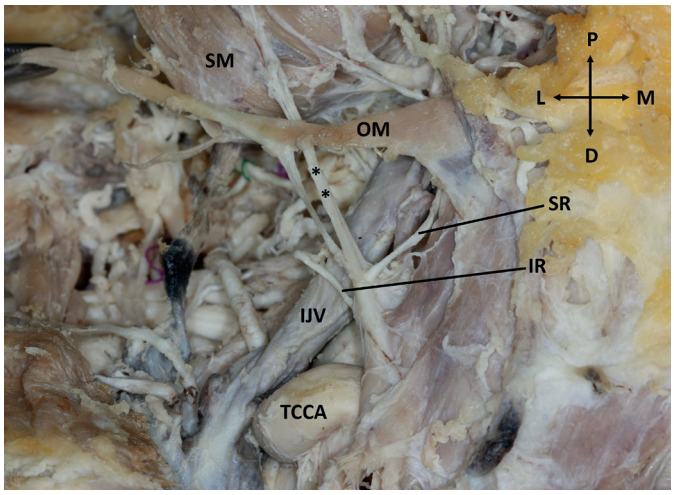

Fig. 1 On the right hemi-neck an aberrant branch (**) of the ansa cervicalis' superior root (SR) is seen piercing the superior belly of the omo-hyoid muscle (OM) and distributing to the inferior segment of the sterno-cleido-mastoid muscle (SM). The initial portion of the right common carotid artery displays a tortuous course (TCCA) (IR: inferior root of ansa cervicalis, IJV: internal jugular vein, P: proximal, D: distal, L: lateral, M: medial).

DISCUSSION

The AC as it is widely known is a neural loop formed by the union of a superior root originating from the ventral ramus of first cervical nerve (joining the hypoglossal nerve) and the inferior root being formed by the union of the ventral rami of the second and third cervical nerve. Ultimately, that nerval loop provides innervation to the infrahyoid muscles (1). There is a plethora of AC's variation concerning the pattern of cervical roots participating in its formation, the AC's length with respect to omohyoid muscle (proximal or distal to it), the various forms of AC in regard to the inferior root, the AC's relationship to the internal jugular vein, or the possible neural communications between cervical component (C1–C2 fibers), vagal component (C1–C2 fibers with the vagus nerve) and hypoglossal component (C1–C2 fibers with the hypoglossal nerve) (2). However, we consider that the presentation of classification of such morphological AC's patterns is beyond the aim of the current study.

Apart from the AC's formation from cervical fibers derived from the hypoglossal and vagus nerves, there are cases in which the cervical fibers of the AC are derived from the accessory nerve (7). Furthermore, a case of AC's formation by a cervical branch to the sternocleidomastoid muscle has been previously prescribed (8). It should be emphasized that the AC might be differentiated from the so-called "pseudo-ansa cervicalis", in which the superior root arises from the vagus nerve, whereas the inferior root from the superior cervical sympathetic ganglion (9).

Beyond the classic AC's branches to the infrahyoid muscles, there are reports in which the AC provides rami to the cardiac or phrenic nerves (3). The case in which the AC provides branch or branches to the adjacent sternocleidomastoid muscle, as occurs in the present study, has been very rarely detected in the literature. A first mention of such a branch has been brought by Piersol in 1911 (10). Later, Koizumi et al noticed a case in which the AC's superior root, provided a branch innervating the sternocleidomastoid muscle (3). Paraskevas et al presented a case of a nerve innervating the sternocleidomastoid muscle; that nerve was formed after the union of two rami of the AC's branch to the sternothyroid muscle (4). Blythe at al observed a nerve, similar in diameter to the accessory nerve that innervated the lower third of the sternocleidomastoid muscle (11). That nerve arised from the hypoglossal nerve proximal to the origin of the hypoglossal branch. In the current study a nerve trunk of remarkable size was seen arising from the most distal portion of the AC's superior root and after piercing the superior belly of the omohyoid muscle was distributed to the distal segment of the sternocleidomastoid muscle.

Due to close location of AC to recurrent laryngeal nerve, the AC may be utilized for neurotisation of that nerve, since the recurrent laryngeal nerve is active during phonation. The recurrent laryngeal nerve may be damaged during various surgical procedures undertaken in the region, such as oesophageal cancer surgery (12). Furthermore, AC can be used also in preventing the morbidity associated with tongue hemiatrophy after facial-hypoglossal anastomosis (13, 14) or as a donor, during neurotisation of brachial plexus (descending and ascending loop of AC) (15). Although AC's sacrifications does not induce serious functional consequences, it should be noted that the infrahyoid muscles which are innervated by the AC, play a role in laryngeal steadiness during phonation and deglutition (16).

The TCCA is an anatomical variant of unclear origin, usually accidentally detected during a surgical procedure or imaging of the neck or rarely observed as a mass of the cervical region. As regards the various types of spiral course of carotid arteries, three types of tortuosity have been described by Weibel et al, the tortuosity type, as an s- or c-shaped elongation, the kinking type as an acute angulation and the looping type as an exaggerated s-shaped curve resembling to a circular course (17). The TCCA is usually asymptomatic and accidentally detected in imaging of the region. In some instances individuals may feel a pulsating mass in the anterior cervical region (18). Rarely, patients should have a painful sensation, progressive dysphagia or dyspnea or symptoms of cerebrovascular insufficiency (5), or TCCA may be masquerading as a thyroid mass (6) or as a submandibular mass (19).

It must be emphasized that TCCA usually does not result in cerebrovascular disease; most commonly a TCCA aneurysm, usually of traumatic origin can lead to such pathologic condition. Usually, TCCAs do not display atherosclerotic lesions, however, the hypertension and the atherosclerotic lesions result in aortic arch elevation and a subsequent formation of a common carotid artery looping (5, 18). Predisposing factors associated with TCCA are old age, female sex, hypertension and other cardiovascular risk factors, whereas associated diseases usually genetic syndromes are among others, Marfan syndrome, arterial tortuosity syndrome, fibromuscular dysplasia and other inherited arteriopathies (20).

The diagnosis of a TCCA requires physical examination and imaging, such as ultrasonography with Doppler color flow image, computed tomography, magnetic resonance imaging, angiography and especially computed tomographic angiography (6). As regards the treatment options of TCCA, it is generally accepted that there is no need for a specific therapy. It has been shown that treatment of the likely associated hypertension could potentially reduce the dimensions of the TCCA's mass. Uncommonly, and especially in cases of existence of symptoms of cerebrovascular disease, a graft should be inserted after TCCA resection (5).

CONCLUSION

The precise awareness of AC's morphology and topography is crucial for the surgeon of the region in order not to damage it, since AC is utilized as a graft for larynx's re-innervation following recurrent laryngeal nerve paralysis. Moreover, the knowledge of partial innervation of the sternocleidomastoid muscle from the AC is essential for the surgeon of the region, since it can explain almost the total functionality of that muscle in potential laceration of the accessory nerve during lymph nodes' dissection at the cervical region. Furthermore, the possible presence of TCCA should be kept in mind of physician dealing with palpable masses of the anterior cervical region or dealing with differential diagnosis of dysphagia or dyspnea. Neurologists and neurosurgeons should be also aware of such anatomic variant, as a possible cause of cerebrovascular insufficiency or during carotid endarterectomies.

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Failure of Warfarin Anticoagulation Therapy after Administration of Oral Terbinafine

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ABSTRACT
Warfarin is widely used anticoagulant drug for a variety of diseases (thromboembolic disease, atrial fibrillation, etc.). It has three most important parallel metabolic pathways, CYP1A2, CYP3A4 and CYP2C9. Terbinafine is a potent CYP2D6 inhibitor. A possible drug interaction could lead to an increased pharmacological effect of the above drugs. Enzyme induction with CYP3A4, CYP2C9, CYP1A2 inducers may have occurred.
Case report: We present a case report of an 88-year-old male patient who has been successfully anticoagulated with warfarin due to atrial fibrillation. He was orally administered terbinafine to treat onychomycosis. Two weeks after initiation of this drug the patient experienced dizziness and feelings of instability, for which he was admitted to the neurology department. A low-efficient INR level was found at the baseline, presumably due to warfarin interaction with terbinafine. The induction of liver enzymes lasts 10–14 days, which matches the introduction of the antifungal agent.
Conclusion: Combined therapy with warfarin and oral terbinafine is actually rarely prescribed but, if used, their interaction can have serious consequences in many clinical situations for which anticoagulation therapy with warfarin is indicated.

KEYWORDS
warfarin; terbinafine; anticoagulation; atrial fibrillation; liver inductor

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INTRODUCTION

A large number of patients are currently treated with anticoagulants for a variety of diseases (thromboembolic disease, atrial fibrillation, mechanical heart valve, etc.) (1) to prevent ischemic stroke (2). One of the widely used drugs is warfarin, although it is today frequently replaced with direct oral anticoagulants (DOACs) with fewer adverse effects/drug interactions for example in atrial fibrillation (3).

We present a case report of a patient who has been successfully anticoagulated with warfarin due to atrial fibrillation. He was orally administered terbinafine to treat onychomycosis. Two weeks after initiation of this drug the patient experienced dizziness and feelings of instability, for which he was admitted to the neurology department. A low-efficient INR level was found at the baseline, presumably due to warfarin interaction with terbinafine.

CASE REPORT

This 88-year-old male patient was treated for coronary artery disease with paroxysmal atrial fibrillation, which was managed with warfarin anticoagulation. He also had a history of anterolateral myocardial infarction and a left anterior descending artery (LAD) stent. He had been treated for hyperlipidaemia, hyperuricaemia, impaired glucose tolerance and prostate hypertrophy. He had a history of cholecystectomy and inguinal herniotomy. He used the following oral medication which is summarized in Table 1.

Tab. 1 Patient medication at admission

Tramadol/paracetamol	75 mg/650 mg bid
Ezetimib	10 mg tbl daily
Loperamide hydrochloride	2 mg daily
Lansoprasole	30 mg daily
Allopurinol	100 mg bid
Nebivolol	2.5 mg daily
Alprazolam	0.5 mg daily
Trimetazidine	35 mg bid
Propafenone	15 mg daily
Terbinafine	250 mg daily
Warfarin	5 mg daily

The patient arrived at the emergency neurological department due to sudden onset of rotational vertigo which developed during the night. When he sat up in order to go to the toilet, everything began spinning around him. The problems have since continued; he spent the night in bed. The patient perceives no weakness of limbs. He complains of arduous articulation.

In the objective neurological status, the patient was oriented, without aphasia, with mild dysarthria. The blood pressure was 170/93. No nystagmus was observed, only the patient's left mouth corner was mildly lower. Taxia was mildly hypermetric in the right upper extremity. In the sedentary position, there was instability drawn to the back, standing positions II and III were appreciably unstable. Gait was basophobic and unstable with 2 forearm crutches.

Clinically this was a central vestibular syndrome with suspect of mild ischaemic stroke in the vertebrobasilar circulation. Brain CT revealed no focal changes (Figure 1). Ultrasonography detected insignificant atherosclerotic changes in the carotid artery bifurcations and hypoplasia of the distal part of the right vertebral artery. Baseline laboratory findings were satisfactory. Electromyography was performed during hospitalisation, detecting rather severe, mainly axonal sensory-motor polyneuropathy of the lower extremities. Neurological problems (vertigo and slight dysarthria) normalized within 24 hours and patient did not receive any further medication. One explanation of this transient ischemic attack in vertebrobasilar region was microembolization while reducing the effectiveness of anticoagulant therapy in atrial fibrillation.

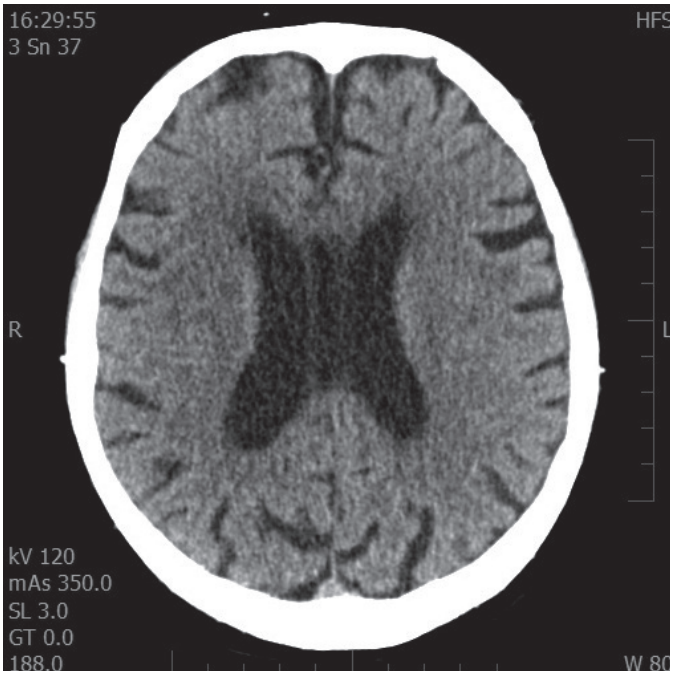

Fig. 1 Brain CT with very small isolated older hypodense foci in the bilateral periventricular area.

The patient had a low-efficient INR level on admission (see Table 2). He uses warfarin anticoagulation, dose 5 mg/day, with good long-term stability. He brought recently measured INR records with him, all of which were in the effective range of 2–3. This way he refuted the suspected non-compliance. No dietary error could be detected either. The only major recent change was the use of the antifungal agent terbinafine against onychomycosis (about 14 days before).

After consulting the attending physician, terbinafine was discontinued. The current warfarin dosage was preserved and LMWH at an anticoagulant dose was initiated. Repeated INR measurement was recommended.

The patient reported he was a non-smoker and never had used any food supplements with St. John's wort, tea or other food supplements.

The next INR measurement showed additional INR level decrease, despite the discontinuation of terbinafine and continued warfarin dose, which had provided a stable long-term condition (see Table 2).

Tab. 2 Patient medication and INR values in temporal sequence.

	Day 1 (on admission)	Day 3	Day 4	Day 7 (discharge)
Warfarin	5 mg	5 mg	7.5 mg	7.5 mg
Terbinafine	250 mg	0	0	0
Nadroparin	0	0,6 ml bid	0,6 ml bid	0
INR	1.91	1.53	1.48	2.4

After consulting the attending physician, the warfarin dose was increased to 7.5 mg/day and additional INR monitoring was recommended. Only the last INR lay in the therapeutic range. LMWH was discontinued and the patient was discharged from the hospital with a warfarin dose of 7.5 mg/day. The attending general practitioner was instructed on the probable need to reduce the warfarin dose back to the initial 5 mg/day after the liver enzyme induction effect completely disappears. In future, we recommend switching warfarin to some of direct anticoagulants.

DISCUSSION

The patient with paroxysmal atrial fibrillation using warfarin had an ineffective INR level caused by drug interaction with terbinafine (medication for onychomycosis); however, several medication problems were found in the patient during the initial medication review.

Few cases of warfarin-terbinafine interactions have been reported in literature (4, 5). Terbinafine is a potent CYP2D6 inhibitor metabolising several other drugs from among the patient's medication – propafenone, nebivolol, tramadol. Warfarin exploits three most important metabolic pathways, CYP1A2, CYP3A4 and CYP2C9. A drug interaction could lead to an increased effect of the above drugs. Enzyme induction with CYP3A4, CYP2C9, CYP1A2 inducers may have occurred. The time sequence after eliminating all the possible causes pointed to terbinafine. The induction of liver enzymes lasts 10–14 days, which matches the introduction of the antifungal agent. Furthermore, it is well-known that this induction persists after drug discontinuation, as is clear from the measured INR values.

Warfarin acts as an antagonist, antivitamin K. It prevents the synthesis of coagulation factors in the liver. The efficacy and safety of warfarin therapy is closely related to its biotransformation and high interaction potential. Warfarin is among drugs with a narrow therapeutic index and a poorly predictable effect due to genetic polymorphism – CYP2C9 oxidase, which provides its biotransformation. The effect of warfarin should therefore be monitored based on periodic INR measurement. Its mechanism of action is based on the effect on vitamin K epoxide reductase and vitamin K reductase, two key enzymes of vitamin K metabolism. Warfarin is a racemic mixture of two enantiomers (R, S) with different pharmacokinetic and pharmacodynamic properties. The most important drug interactions take place at the level of hepatic metabolism, i.e., inhibition or induction. Warfarin is metabolised via three forms of cytochrome P-450: R-form CYP1A2 and CYP3A4, S-form: CYP2C9 (6). Drugs that inhibit these enzymes can cause reduced warfarin conversion, with consequent accumulation of the active substance in plasma and subsequently a higher anticoagulation effect. Conversely, a number of active substances induce the production of cytochromes P450 involved in warfarin metabolism, with a consequent decrease in metabolised isomer levels and possible failure of the anticoagulation therapy.

Terbinafine is a fungicidal allylamine antifungal agent. It inhibits squalene epoxidase, the enzyme responsible for ergosterol synthesis, which leads to impaired cell wall function. It is not used in systemic mycoses because its bonding to plasma proteins is very strong and effective concentrations are only attained in the skin and its adnexes. Thus, terbinafine is normally primarily used against onychomycoses. It is well absorbed on oral administration, binds strongly to plasma proteins, and is biotransformed in the liver by CYP2C8, CYP2C9, CYP2C19 and CYP3A4 oxidases to inactive metabolites. It is a potent inhibitor of CYP2D6, its half-life is 22–26 hours and even as long as 200–400 hours in skin adnexa. However, terbinafine may be considered as a weak liver enzyme inducer as well but this interaction will only be clinically apparent in susceptible patients (the role of genetic polymorphism) (7).

CONCLUSION

Combined therapy with warfarin and terbinafine is actually rarely prescribed but, if used, their interaction can have serious consequences in many clinical situations for which anticoagulation therapy with warfarin is indicated.

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