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Novel Approaches in Pilonidal Sinus Treatment

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Abstract: Pilonidal sinus is a very common inflammatory disease of the gluteal region. The ideal method of pilonidal sinus treatment should have a low recurrence rate with minimum excision. Moreover, the treatment method should have a short hospitalization time, should let the patient return to his normal life rapidly, should cause minimum loss of labour and should result a small scar only. In the presented review, modalities in pilonidal sinus treatment in the light of current information in the literature are evaluated.

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Introduction

Pilonidal sinus is a very common inflammatory disease of gluteal region (McCallum et al., 2008). Its incidence is 26/100,000 and it usually occurs in working males of age 15 to 30 (Søndenaa et al., 1995a; McCallum et al., 2008; Farrell and Murphy, 2011). In the literature, a wide range of factors is defined in its etiology. Keratin plugs, foreign substance reaction related to hairs and occurrence of dermopathy and debris in the hair follicles in the natal cleft are the factors most frequently held responsible (Page, 1969; von Laffert et al., 2011; Meinero et al., 2014). Pilonidal sinus disease and its treatment cause discomfort, limitation of mobility and deterioration of the quality of life of the patient (Marsh, 2008; Stewart et al., 2008). Despite the presence of several procedures defined in the treatment of chronic and recurrent pilonidal sinus disease, there isn't a standard view on which method is the best one. Recurrence rates of complex pilonidal sinus following surgical treatment vary between 0–46% (Shafik, 1996). The ideal method of pilonidal sinus treatment should have a low recurrence rate with minimum excision. Moreover, the exemplary treatment method should have short hospitalization time, should let the patient to return to his normal life rapidly, should cause minimum loss of labour and should result in a minimal scar (Bayhan et al., 2016). In this review, we aimed to evaluate the modalities in pilonidal sinus treatment in light of the current information in the literature.

Treatment options

Although pilonidal sinus abscess treatment is usually applied under local anaesthesia, excision often requires loco-regional or general anaesthesia (Isgör, 2011). The patients often lay in the prone position and sacrococcygeal protrusion up and the hips are separated from each other by the help of plasters. Drainage and antibiotic therapy usage are required after local anaesthesia in patients with abscess development in acute period. It is reported that prophylactic antibiotic usage in post-excision period doesn't reduce complications and recurrence; it doesn't accelerate healing and doesn't avoid lesion infections (Søndenaa et al., 1995b).

Conservative techniques

There are several methods published in the literature about surgical treatment of pilonidal sinus. The most frequently used are: primary closure, lay open, and the flap plasty techniques.

Lay open

Lay open method can include the resection of the sinus whether by a wide range excision of cavity and sinus or only resection of the sinus with a minimal incision, also the removal of the hairs and curettage of the cavity. The cavity occurring during a wide excision is formed so as to deepen toward the central position.

In the minimal lay open method, the healing is much faster, and hospitalization duration is shorter than wide excision (Gupta, 2005; Mohamed et al., 2005; Hosseini et al., 2006; Rao et al., 2010; Lorant et al., 2011). In a review analysing the studies performed with laying open and curettage techniques, a meta-analysis of 13 studies and 1,445 patients was made. In this study, the total recurrence rate was detected as 4.47%, complication rate as 1.44%, the length of operation time as 34.59 minutes and time of return to work as 8.4 days (Garg et al., 2016).

No matter the type of the excision, wide or minimal, the wound is left for secondary healing at the end. In such circumstances, the wound should be dressed daily, and infection development should be avoided (de Parades et al., 2013). With the marsupialization technique in which the edges of the wound are sutured, the injury is minimized, and this fastens the healing process (Mentes et al., 2004a). In recent years, various dressings and negative pressure wound care products are preferred (de Parades et al., 2013).

In a review published by McCallum et al. (2008), it is reported that the patients who are left for secondary healing following pilonidal sinus excision have a shorter hospitalization period and less recurrence development rate concerning the patients who undergo primary closure. However, there isn't a significant difference regarding wound infection. Moreover, wound healing time is much longer in patients left for secondary healing, and this negatively affects the quality of life.

Primary suture

While it is easy to close the wound in the middle line after the pilonidal sinus cavity and tracks are extracted, it also brings the problems such as the tension of the sutures and increase of this tension with movements. In the literature, recurrence rates following primary closure vary in a wide range of 0–42%. While recurrence rate following primary closure is found as 18.4% in the study of Can et al. (2009), this rate is reported as 4.3% by Toccaceli et al. (2008) and 4% by Muzi et al. (2009) (Iesalnieks et al., 2003).

Bascom technique

In Bascom technique, hair follicles in midline gluteal region are excised with incisions of 2–4 mm and then an incision is made in lateral a few centimetres parallel to the midline and a subcutaneous tunnel is opened towards the midline and the hair and granulation tissue is cleaned away. The wound isn't sutured. Bascom (1983) has reported that the average healing time of the patients in his series followed-up during 3.5 years is three weeks, and the recurrence rate is 16%.

Karydakis technique

Karydakis has indicated that suture line formed in midline will produce a serious tension following strong gluteal muscle spasms, and for this reason, the midline should be shifted. With the method he developed, after excision of the pilonidal

sinus tissue with a unilateral elliptical incision, he forms a deep flap in the contralateral side including subcutaneous tissue and then sutures this flap to the sacral fascia, and makes the primary closure of the skin on the lateral of the midline. In the 2–20 years of follow-up of 7,471 patients that he has operated with this technique, the recurrence rate is reported as 0–1% (Karydakis, 1992; de Parades et al., 2013).

Plasties

These methods have a broad range of variety and they are developed with indications related to plastic surgery and are used for the purpose of wound closure following pilonidal sinus surgery. Today the most common plasty methods are Limberg flap, V-Y plasty, and Z plasty. In the literature, wound infection, wound separation and seroma rates after V-Y plasty are reported respectively as: 0–10.2%, 0–10.2% and 0–4.6% (Schoeller et al., 1997; Milito et al., 1998; Nursal et al., 2010). In a study including 353 cases operated with Limberg flap, the recurrence rate is reported as 3.1%, and infection rate as 6.5% (Mentes et al., 2008). In another study made with modified Limberg flap the recurrence rate is indicated as 1.2% (Mentes et al., 2004b). Lee et al. (2008) defend the idea that primary closure can be used in the primary disease, but flap must be used in recurrent disease. Also, Lieto et al. (2010) have used the flap reconstruction technique in 55 recurrent pilonidal patient series and only one patient (1.8%) presented recurrence. In a retrospective study of 55 patients comparing fasciocutaneous V-Y advancement flap (VYF) and Limberg transposition flap (LTF) techniques for recurrent pilonidal sinus disease, it is shown that for the patients who underwent Limberg transposition flap, the duration of the operation is shorter. And also the volume extracted is smaller, the length of hospital stay is shorter and patients return to work earlier in LTF group compared to VYF group (Öz et al., 2015). In the case of repair of the defect with a flap in pilonidal sinus patients, instead of a V-Y flap, the first choice should be Limberg flap due to recurrence and shorter hospitalization time.

Minimal invasive therapies

Due to the advantages such as earlier discharge from the hospital, quicker return to work, minimally invasive techniques are being used more frequently by the patients and surgeons instead of wide excisions.

Endoscopic pilonidal sinus surgery is a new invasive treatment procedure in pilonidal sinus treatment. In this technique, pilonidal sinus, pilonidal fistula tract are extracted endoscopically, hair follicles and keratin debris are destructed (Meinero et al., 2016). In a study made by Meinero and Mori (2011), 11 pilonidal sinus patients are treated by video-endoscopic technique. The patients are discharged on the same day, and no recurrence is detected during six months follow-up. In another prospective endoscopic pilonidal sinus study of 250 patients operation was performed in two phases as diagnosis and surgery phase, while total wound healing

time is reported as 26.7 ± 10.4 days, total wound healing rate is 94.8%. In 5.2% of patients without wound healing, the external exotoxin quantity was statistically higher. No significant difference was found between the failure rates following the usage of endoscopic treatment as the primary treatment and the failure rates following the usage of endoscopic treatment after another treatment that failed. The average time of return to work was reported as 2 ± 0.5 days after the operation and recurrence developed in 12 (5%) patients during the follow-up period of 12 months (Meinero et al., 2016). In a study comparing the modified Limberg flap reconstruction (n=44) and crystallized phenol application (n=37), no significant difference is seen regarding recurrence and hematoma and wound infection development rates during average 16.5 months follow-up (Bayhan et al., 2016).

Fibrin glue which is another minimally invasive treatment method in pilonidal sinus surgery is also used in different treatment techniques. Filling of the sinus tract with fibrin glue is a new method that can be used instead of surgery. Moreover, fibrin glue can be used as a concealer on the wound surface left open after the surgery or to fill the dead space that occurs after primary closure. In four studies in the literature, fibrin glue was used in 113 patients after pilonidal sinus tracts were curetted and cleaned away (Lund and Leveson, 2005; Elsey and Lund, 2013; Isik et al., 2014; Smith et al., 2015). The success rate of these studies is reported as 80% in their meta-analysis. However, there isn't any information about the re-usage of fibrin glue in the patients who failed in these studies. Although similar studies were made for phenol and their success rates were found as 70%, this rate increased to 86.7% in repeating implementations (Kayaalp et al., 2010; Olmez et al., 2013). The high cost of the fibrin glue on phenol is another disadvantage.

Negative pressure wound therapy

In recent years, negative pressure wound care products are one of the adjuvant therapy options that are thought to be useful both for open and closed wounds. Negative pressure wound care products absorb the liquid and exudate accumulated in the wound; reduce the number of bacteria and tissue edema. Moreover, negative pressure-induced tissue deformity activates some intracellular pathways that accelerate wound healing (Farrell and Murphy, 2011). Negative pressure wound care products are also associated with an increase in factors accelerating wound healing such as: wound oxygenation, angiogenesis, and blood flow, granulation tissue formation (EWMA, 2008). Relative contraindications of the negative pressure wound care products are necrotic tissue formation with eschar on in the wound, priority of debridement requirement, presence of an untreated osteomyelitis around the wound, cancer in the wound or development of a fistula towards an organ or body cavity (Cooper and Young, 2000; Eryilmaz et al., 2015). When VAC (vacuum-assisted closure) treatment used as negative pressure wound care product is compared with standard gauze dressings, it is reported that VAC treatment further reduces the depth of chronic wounds, provides a faster recovery, causes fewer infections

and allows shorter hospitalization (Joseph et al., 2000; Vuerstaek et al., 2006; Mouës et al., 2007). But despite these advantages, each dressing change is subject to high costs.

Conclusion

Although pilonidal sinus disease is frequently observed, no standard treatment approach has been determined. In determining of the treatment method, the preferency of the surgeon, patient's request and extend of the disease are effective factors. Each method has a certain recurrence rate, and none of them is used as the gold standard. Although minimal treatment approaches are used more frequently in recent years, much more experience is needed.

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Severe Snakebite Envenoming in Intensive Care

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Abstract: Snakebites by exotic venomous snakes can cause serious or even life-threatening envenoming. In Europe and North America most victims are breeders, with a few snakebites from wild native American rattlesnakes. The envenomed victims may present in organ and/or system failure with muscle paralysis, respiratory failure, circulatory instability, acute kidney injury, severe coagulation disorder, and local disability – compartment syndrome and necrosis. Best managed by close collaboration between clinical toxicology and intensive care, most severe envenomings are managed primarily by intensive care physicians. Due to the low incidence of severe envenoming, the clinical course and correct management of these cases are not intrinsically familiar to most physicians. This review article summarizes the clinical syndromes caused by severe envenoming and the therapeutic options available in the intensive care setting.

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Introduction

Snakebites caused by dangerous exotic venomous snakes, which can lead to life-threatening envenoming, are rare in Europe and North America, where most victims are breeders. Due to the very low incidence of these injuries the literature consists mostly of case reports or summaries of case reports, often dating back 40 years or more, and most healthcare providers are not experienced with the management of these cases. The detailed clinical course including the early recognition of life-threatening complications and appropriate therapeutic interventions are therefore not commonly known by most physicians treating the envenomed patient.

Statistical reports on snakebites by dangerous non-native species and clinical courses of envenoming are relatively rare. In the UK there were 32 snakebites by 14 exotic species registered from 1970–1977, with two life-threatening clinical courses (Reid, 1978). Subsequently, a further 43 cases of snakebite envenomation caused by 26 species in the UK was published (Warrell, 2009). Eleven exotic snakebites treated in the ICU (intensive care unit) were recorded in Berlin from 1980–1991, three of them for severe envenoming (Köppel and Martens, 1992). In north-eastern Germany and south-eastern France 157 bites by exotic snakes were registered between 1996 and 2006 (Schaper et al., 2009). In Hungary from 1970–2006 80 cases caused by 19 species of exotic snakes were described, with one death (Malina et al., 2008). The Toxic Exposure Surveillance of the American Association of Poison Control Centres presented a yearly average of 39.9 envenomings by 77 species of exotic snakes with a total of three fatal courses in the period 1995–2004 (Seifert et al., 2007). A total of 258 snakebites involving at least 61 exotic venomous species were recorded in the US between 2005 and 2011 (Warrick et al., 2014). In the Czech Republic we registered 87 victims of exotic snakebites from 1999–2013. Twenty nine cases with systemic envenoming were treated in the ICU with ten of them considered potentially life-threatening (Valenta et al., 2014). All of these articles, however, summarized only reported snakebites. The real incidence is unknown but probably higher.

In most serious cases the envenomed victim may present in organ and/or system failure, with any or all of muscle paralysis, respiratory failure, haemodynamic instability, acute kidney injury, coagulation disorder (venom-induced consumption coagulopathy, VICC) and severe local injury due to necrosis and compartment syndrome. Severe envenomings and its serious complications requiring organ and systems support should be treated in the intensive care setting (Warrell, 2009).

Clinical course of severe envenoming

Signs of snakebite and envenoming

The bite is commonly visible as "double fang" marks but may be solitary from one fang or even multiple from several fangs in a row (e.g. the viper, *Bitis* sp.).

Conversely, the bite site may be minimally visible (e.g. *Bungarus*) and so the lack of an obvious bite site does not exclude envenomation. Not all bites, however, develop into envenoming. In approximately 10–30% of bites the snake does not inject venom (a "dry bite") (Spano et al., 2013; Valenta et al., 2014).

In most cases the first sign of envenoming is local pain and swelling, which can progress cranially on the effected limb. Subsequent lymphadenitis of the draining lymph-nodes may also occur. These signs and symptoms of localized envenoming do not always progress to systemic envenoming. With some species (e.g. *Bungarus* sp., *Micrurus* sp.) envenoming is indicated by the presence of prodromal or specific signs of systemic envenoming, with no or very few discrete local signs.

Prodromal symptoms of systemic envenoming commonly arise from autonomic dysfunction and include nausea, vomiting, headache, diarrhoea, sense of coldness or hotness, blurred vision, faintness and other non-specific signs.

The actual course of systemic envenoming is specific for each snake genera and may be complicated by allergic reactions including anaphylaxis. Severe anaphylaxis to the injected venom can develop rapidly, often much earlier than the signs and symptoms of envenoming. This may lead clinicians to miss the early signs of envenoming after the anaphylaxis has been successfully treated. Administration of horse antivenom is also associated with a risk of anaphylaxis including anaphylactic shock. Intravenous administration of hydrocortisone and antihistamines together with the dilution and slow i.v. infusion of antivenom has been recommended, although no adequately powered study has demonstrated significant benefit from the use of antihistamine premedication (Caron et al., 2009). Antivenom administration is definitive therapy in most cases of severe envenoming. Timely administration and adequate dosage of the appropriate antivenom can prevent the development of systemic symptoms of envenoming. Before administration of antivenom, due to its temporary or total unavailability, and during antivenom treatment, patients have to be treated symptomatically, sometimes with organs and systems support.

All snakebite victims should receive tetanus prophylaxis if their vaccination status is lapsed or unknown.

Muscle paralysis

Paralysis of striated muscle is a characteristic of envenomation caused by the elapid snakes (*Elapidae*). Curare-like neurotoxins (NTX-α) with postsynaptic effects, sometimes combined with highly potent presynaptically acting enzymes, are capable of causing paralysis of respiratory muscles and fatal respiratory failure (Warrell, 1995). This paralysis typically proceeds in a cranio-caudal direction. Its initial symptoms are ptosis, ophtalmoplegia, diplopia, dysarthria, dysphagia, salivation and weakening of facial muscles. Subsequently general weakness develops with weakness of neck muscles, loss of deep tendon reflexes and eventual weakness and paralysis of respiratory muscles. Death due to asphyxia will occur unless

respiratory support is initiated (White, 1995). Snakebites caused by some species of mambas may be further complicated by generalized muscle fasciculations (Rodríguez-Ithurralde et al., 1983).

Envenomation with a substantial quantity of NTX can cause fatal paralysis just in minutes, although it usually takes several dozen minutes, but it can rarely manifest in the interval up to 10–24 hours after snakebite (Warrell, 1995; Hung et al., 2009).

Life threatening muscle paralyzing NTX are contained mainly in the venom of elapids (*Elapidae*): cobras (*Naja* sp.) – especially Asian species, king cobra (*Ophiophagus hannah*), African mambas, Asian kraits (*Bungarus* sp.), American coral snakes (*Micrurus* sp.) and some Austropapuan terrestrial snakes, for example the taipan (*Oxyuranus* sp.). NTX are also contained in the venoms of the sea snakes. Much less toxic NTX-β (presynaptically acting phospholipases A2, PLA2) are contained in the snake venom of other families, some subfamilies of vipers (*Viperinae*) and rattlesnakes (*Crotalinae*). Clinically apparent effects from NTX-β may be present in envenoming from Russell's viper (*Daboia russelli*), South American rattlesnake (*Crotalus durissus*) and others. Neurotoxicity from these species is however restricted to facial muscle and general weakness. Severe respiratory muscle paralysis does not occur.

Treatment

In cases of envenoming by snakes with potent NTX, the victim should be transferred immediately to medical facilities where mechanical ventilatory support is available. If required, tracheal intubation should occur early to prevent aspiration.

The effect of the postsynaptic curare-like NTX-α can be decreased or eliminated by acetylcholinesterase inhibitors. To test the inhibitor's efficiency, 10 mg of edrophonium can be administered i.v. to an adult patient, atropine 0.5 mg i.v. should be used to block the drugs' muscarinic effects. The test is considered positive if the patient significantly improves (with a peak effect within 5 minutes) before gradually deteriorating as the drugs' effect wanes. Treatment can continue with long-acting cholinesterase inhibitors, typically neostigmine at a dose of 0.5 mg i.v. or SC every 20 minutes (Banerjee et al., 1972). This procedure has been shown to successfully treat envenoming by the curare-like NTX-α of cobras, including the king cobra. However it is not effective in envenomings caused by NTX combined with presynaptically acting enzymes, such as those found in the venom of the mambas (*Dendroaspis* sp.), kraits (*Bungarus* sp.), Australian tiger snakes (*Notechis* sp.), taipans (*Oxyuranus* sp.), and some other snakes.

In mamba bite envenoming, where respiratory failure is complicated by muscle fasciculation, non-depolarizing neuromuscular blocking agents and mechanical ventilation is first line treatment.

Although paralysis due to NTX-α may self-reverse with time on mechanical ventilation, symptomatic treatment should not be considered an alternative

to antivenom. Antivenom should be administered in all cases of severe envenoming.

Haemodynamic instability

Venom components affect the cardiovascular system by direct and indirect effects on the vasculature and myocardium. Toxins usually decrease vessel resistance, may cause tachy- or brady-dysrhythmias and impaired myocardial contractility (Hafeez and Majeed, 2004).

Vasodilation is caused by both direct action on the vascular system and by release of kinin-based hypotensive substances. It results in hypotension and in severe cases circulatory failure and distributive type of shock. Vasodilatory hypotension may be potentiated by fluid leakage and volume loss due to damage of endothelium and/or by haemorrhage. Hypotensive compounds are contained in the venoms of most viperids, vipers and rattlesnakes, but cannot be excluded in elapids, e.g. cobras and Austropapuan snakes (Meier and White, 1995).

Conversely, vasoconstriction resulting in systemic hypertension, coronary artery spasm and myocardial ischemia is caused by sarafotoxin present in the venom of the burrowing asps *Atractaspididae* (Warrell, 1995).

Specific compounds directly affecting the myocardium (cardiotoxins) can be found in the venoms of the cobras *Naja* sp., African vipers *Bitis* sp., some Austropapuan snakes and other species. These toxins can cause arrhythmias and contractility disturbances (Meier and White, 1995; Ismail et al., 2012).

Treatment

Intravenous administration of crystalloids increasing the intravascular volume is indicated in systemic hypotension, whereas the use of colloids or albumin remains controversial (Lira and Pinsky, 2014). Application of fresh frozen plasma (FFP) would provide not only volume replacement, but also a certain option to normalize potential coagulopathy. Therefore an administration of FFP can be helpful in the patients with hypotension in the presence of a haemostatic dysfunction. Blood pressure and organ perfusion can be stabilized in some cases with volume replacement only. Moreover, increased diuresis induced by volume replacement, could prevent imminent renal injury.

If hypotension persists despite volume replacement therapy, application of vasopressor support such as noradrenalin is justified. Treatment then requires invasive blood pressure (IBP) monitoring and controlled infusion rate according to blood pressure changes. Ongoing cardiac output monitoring as well as monitoring of myocardial contractility and filling by ultrasonography may be used to guide therapy more precisely. If envenoming is associated with disturbed cardiac rate, rhythm or contractility other therapeutic interventions may also be required (e.g. antidysrhythmics and inotropes).

Early administration of the correct antivenom is desirable.

Haemocoagulation disorder – VICC

Snake venoms contain a number of toxins and toxic enzymes affecting the haemocoagulation system. These substances may interfere with vast majority of the processes in plasma coagulation system, the function of platelets (PLT) and the endothelium. The haemocoagulation active components of snake venoms cause primarily generalization of thrombin activity by thrombin-like isomers or thrombin itself, with the manifestation of consumption coagulopathy. Toxic components also create direct or indirect fibrino(geno)lysis, affect the platelets and endothelial functions. These processes result in a non-characteristic haemocoagulation disorder, VICC, ranging from clinically silent changes in laboratory parameters to significant haemorrhage, (micro)thrombosis or embolism (Lu et al., 2005; Berling and Isbister, 2015).

Serious or life-threatening conditions include organ haemorrhage (brain, lungs, retroperitoneum, and gastrointestinal tract), generalized unmanageable bleeding and intravascular formation of thrombi (e.g. coronary and mesenteric vessels, brain) (Gawarammana et al., 2009). Damage to the endothelium and formation of micro-thrombi, among others, also contribute to organ disability or failure, affecting also kidney or lungs (White, 2005).

Manifestation of VICC can start relatively late. Apparent haemocoagulation laboratory abnormities can develop as far as several hours, with no detectable clinical symptomatology in that time. When suspecting coagulation disorder, thrombo-elastography (TEG) may be useful aid to early bed-side diagnosis.

Initial laboratory findings mostly involve prolonged prothrombin and activated partial thromboplastin times (PT, aPTT), decreased fibrinogen (FBG) levels and elevated levels of fibrin degradation products (FDP), including D-dimer. Antithrombin (AT) activity and PLT count are not necessarily affected, mainly in the initial phase. Subsequent laboratory tests may show immeasurable or significantly prolonged clotting times, reduced FBG up to zero levels and a massive rise in FDPs, mostly including D-dimers. These tests and their trends are valuable for estimating the severity of envenoming. Mild changes in laboratory coagulation parameters early after a bite do not exclude the later development of a serious coagulopathy.

Many snake venoms contain components that may cause a coagulopathy, including the vipers (*Atheris* sp., *Bitis* sp., *Cerastes* sp., *Daboia russelli*, *Echis* sp., *Macrovipera* sp.), *Crotalinae* subfamily (*Bothrops* sp., *Lachesis* sp., *Agkistrodon* sp., *Crotalus* sp., *Trimeresurus* sp.). They are also inherent in the venoms of Austropapuan elapids (*Acanthophis* sp., *Oxyuranus* sp., *Notechis* sp.), some *Naja* cobras, boigas (*Dispholidus typus*, *Thelotornis* sp.) and sea snakes (White, 2005).

Treatment

Targeted early antivenom treatment plays the key role in this type of envenoming. Repeated doses may be required because the antigenicity of some haemocoagulation effecting compounds in venom is low.

Substitution of the clotting factors (FFP and/or FBG) may be employed whilst awaiting antivenom treatment or if the antivenom is contraindicated, but the efficacy of this therapy is controversial.

If fibrinogen levels are decreasing (indicating a consumptive coagulopathy) and the venom is not neutralized by antivenom therapy, fibrinogen replacement is unlikely to restore plasma levels but may instead "feed the fire" of the consumptive coagulopathy leading to increased (micro)thrombi formation and the generation of more fibrin degradation products, worsening the coagulopathy and bleeding. Conversely, persisting afibrinogenaemia obviously carries an increased risk of serious haemorrhage, including organ haematomas. An alternative treatment for coagulopathy may be fresh frozen plasma (FFP) (White, 2005, 2009; Maduwage and Isbister, 2014). FFP contains, in addition to fibrinogen, other coagulation factors and inhibitors and the simultaneous replacement of all these proteins may temporarily improve coagulation status. Venom components either inhibit or activate platelets. Therefore in some cases thrombocytopenia does not occur or it is only mild. Platelet infusions to correct severe thrombocytopenia may simply worsen (micro) thrombi formation, similar to FBG administration. If active bleeding with severe thrombocytopenia occurs, guidelines for PLT substitution in DIC (disseminated intravascular coagulation) should be followed (Wada et al., 2013).

Heparin administration could be considered in cases of VICC, where the predominant symptom is thrombosis or embolic complication (Wada et al., 2013). Paul et al. (2003) found that a heparin treatment regime with an initial dose of 5,000 units and further doses of 2,500 units every 8 hours, in parallel with antivenom administration, reduced mortality in patients envenomed by the saw-scaled viper (*Echis carinatus*) and the Russell's viper (*Daboia russelli*) from 26% to 19%, but these results are not highly significant. However, because heparin administration will not terminate ongoing VICC and may worsen bleeding its use must be carefully considered in each case and its administration cannot be generally recommended (White, 2005).

Administration of tranexamic acid may also be efficacious in the treatment of VICC if bleeding has been worsened by fibrinolysis, particularly by increasing of plasminogen activation (Wada et al., 2013).

Treatment of last resort may be the use of plasma exchange therapy (Zengin et al., 2013). The outcome, however, is not always fully satisfying (Valenta et al., 2011).

The effectiveness of treatment should be repeatedly monitored by laboratory tests.

Organ dysfunction and failure

Acute kidney injury (AKI) frequently accompanies snakebite envenoming even if specific nephrotoxic components were not found in snake venoms. Renal insufficiency arises from myoglobinuria or haemoglobinuria secondary to rhabdomyolysis or haemolysis, the influence of destructive toxic enzymes

on renal tissue, endothelial dysfunction and obstruction of renal capillaries by microthrombi or combination of these factors. AKI is potentiated by hypovolemia due to intravascular volume loss from redistribution or bleeding (Top et al., 2006). Oligoanuria may be the first manifestation of systemic envenoming (Valenta et al., 2008), but it may also develop later in the clinical course, sometimes even due to inappropriate treatment.

AKI cannot be excluded in envenoming by most snakes, but commonly occurs after envenomation by vipers, pit vipers (and their relatives) and rattlesnakes.

Treatment

Blood pressure support by volume replacement therapy and vasopressors to maintain increased diuresis, alkalisation of urine and timely antivenom administration may prevent the development of AKI. Early antivenom treatment could act preventively in suspicion on AKI (e.g. myo-haemoglobinuria, consumptive coagulopathy, extensive swelling). Pharmacologically resistant oligoanuria requires the use of renal replacement therapy (RRT), as in other ICU patients. The presence of ongoing VICC can cause thrombotic occlusions of the device tubing set, even together with increased tendency for bleeding. If RRT is required and heparin is contraindicated, regional anticoagulation using citrate or prostacyclin may be employed (Valenta et al., 2011).

AKI is mostly reversible if envenoming is treated adequately.

Respiratory failure (acute respiratory distress syndrome, ARDS) may occur due to toxic endothelial injury and microthrombotic obstruction of the lung capillaries. Cannot be excluded even destructive effect of toxic enzymes on lung tissue. It may also rarely occur in cases of late or ineffective antivenom treatment in severe envenomings from the viper snakes (Valenta et al., 2010, 2011).

This type of respiratory failure requires an effective, early antivenom treatment, oxygen administration and respiratory support even mechanical ventilation (Bouziri et al., 2011).

Pancreatitis as a consequence of snakebite envenoming has been reported three times (Kjellström, 1989; Valenta et al., 2010; Sagheb et al., 2011).

Liver disability can be seen either as a simple rise in liver enzymes or, rarely, as hepatocellular necrosis. The pathophysiology is likely similar to that already described in respiratory failure (Barraviera et al., 1989).

Severe local disability

Compartment syndrome with intracompartmental pressure above 30–40 mm Hg limiting perfusion is a potentially limb threatening complication of severe

inflammation and swelling at the snake bit site (Warrell, 1995). This syndrome may develop from two main reasons. The first is the oppression caused by swelling of the affected area, especially in tight spaces of small joints. The second reason is the intrafascial expansion of osmotic active myonecrosis caused by myotoxins injected to close intrafascial space, if large fangs penetrate subfascially (e.g. rattlesnakes, puff adder, Russell's viper).

If there is a risk of compartment syndrome, the compartment pressure should be measured and the patient monitored for the development of the syndrome. If its value reaches above 30 mm Hg, there is a possibility to attempt reducing the pressure by the limb elevation, by intravenous administration of mannitol and by repeated antivenom administration in multiple doses. This procedure may preserve the perfusion and thus need for fasciotomy. Obviously, if the compartment pressure further rises and clinical manifestation (severe pain on minimal passive extension of muscles) is present, the fasciotomy have to be performed in time. Preventive use of fasciotomy is not recommended (Gold et al., 2003).

Necroses of the skin and subcutaneous tissue are caused by venom components of some vipers, rattlesnakes and cobras, predominantly by African species. Even if the antivenom treatment does not have essential influence on the local tissue disability, its administration may reduce the extent of necrosis. Early necrectomy and debridement are indicated (Warrell, 1995, 2009).

Phlegmon or abscess can also occur in the bitten area, but primary antibiotic prophylaxis after snakebite is not recommended (LoVecchio et al., 2002). If infectious complications develop, surgical and/or antibiotic treatment is required. The infectious organisms may be gram-positive, gram-negative, anaerobes or a mixture of different species. If the bacterial agents are not known, the pervasively acting, wide spectrum antibiotics with anaerobic cover should be initiated after collection of laboratory specimens for subsequent targeted therapy (Garg et al., 2009; Chen et al., 2011).

Conclusion

All victims bitten by dangerous exotic venomous snakes should be admitted, examined and observed in appropriate healthcare facilities. Because of the possibility of life-threatening organ failure all patients with systemic envenoming should be treated in an intensive care unit with availability of antivenoms, ideally by physicians with experience in clinical toxinology.

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The i-gel Supraglottic Airway as a Conduit for Fibreoptic Tracheal Intubation – A Randomized Comparison with the Single-use Intubating Laryngeal Mask Airway and CTrach Laryngeal Mask in Patients with Predicted Difficult Laryngoscopy

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Abstract: Fibreoptic intubation through a supraglottic airway is an alternative plan for airway management in difficult or failed laryngoscopy. The aim of this study was to compare three supraglottic airways as conduits in patients with at least one predictor for difficult laryngoscopy. The i-gel was compared with the single-use intubating laryngeal mask airway (sILMA) and CTrach laryngeal mask in 120 adult patients scheduled for elective surgeries under general anaesthesia using a prospective, randomized and single-blinded design. Primary outcome was success

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rate of tracheal intubation through the device, while secondary outcomes were times required for device insertion and tracheal tube placement, fibreoptic scores and the incidence of perioperative complications and postoperative complaints. The success rates showed no statistical difference between devices (i-gel 100%, CTrach 97.5%, ILMA 95%). Insertion time was shortest for the i-gel (12.4 s) compared with ILMA (19.3 s) and CTrach (24.4 s). Intubation time was shorter in the i-gel group (29.4 s) in comparison with the CTrach (39.8 s, $p<0.05$) and sILMA (51.9 s, $p<0.001$) groups. Best fibreoptic scores were observed also in the i-gel group. In total, 24 patients (20%) presented with difficult laryngoscopy. The i-gel showed significantly shorter times for insertion and fibreoptic intubation than the other two devices in this group. No difference was observed in the incidence of postoperative complaints. The i-gel is a suitable alternative to the sILMA and CTrach for fibrescope-guided tracheal intubation. Shorter insertion and intubation times with the i-gel may provide advantage in case of difficult oxygenation.

Introduction

Supraglottic airway devices (SADs) play an important role in modern anaesthetic practice (Michálek and Miller, 2014). Apart from airway maintenance during selected elective procedures, they may be used in difficult airway management situations. The SADs in these situations allow both spontaneous and controlled ventilation. SADs also can allow planned blind or fibreoptic intubation in expected and unexpected difficult laryngoscopies (Timmermann, 2011). The use of SADs (intubating laryngeal mask airway, ILMA®, Intavent Direct; classic laryngeal mask airway, LMA Classic®, Intavent Direct) for both ventilation and fibrescope-

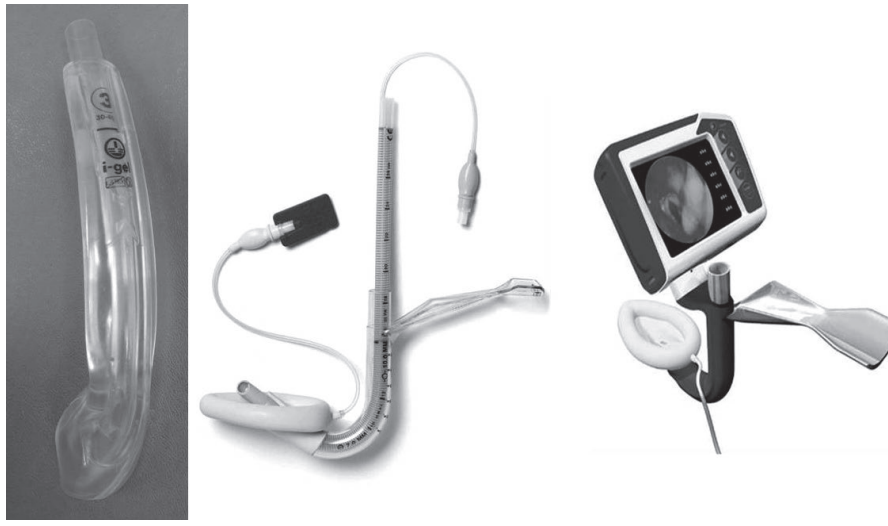

Figure 1 – Supraglottic airway devices used in the study – i-gel, sILMA, CTrach.

guided tracheal intubation is recommended by the Difficult Airway Society as an acceptable back-up plan during unexpected difficult intubation (Frerk et al., 2015).

Based on our previous experience, evidenced by case reports (Michalek et al., 2008; Campbell et al., 2009) and a manikin study (Michalek et al., 2010), we aimed to compare the i-gel as a conduit for fibrescope-guided tracheal intubation with the single-use intubating laryngeal mask airway (sILMA^{TM}, Laryngeal Mask Company, Mahé, Seychelles) and with the CTrach laryngeal mask (The Laryngeal Mask Company, Singapore) (Figure 1). The null hypothesis for this study was that each of these three devices would perform without a statistical difference in terms of success rate and time needed for their insertion and tracheal intubation.

Material and Methods

The study protocol was approved by both a local (Northern HSC Trust) and regional Ethical Committee (Office for Research and Ethical Committees Northern Ireland, 09/NIR03/44). The study was then registered with a public trial database (www.clinicaltrials.gov, NCT00983229). All participants received a Study Information Pack in advance and signed their written consent. In total, 120 participants were included in the study (Figure 2). Inclusion criteria were: ASA I-III patients, both genders, age 18–89 years, all elective procedures requiring tracheal intubation and at least one predictor of difficult laryngoscopy – Mallampati score II or higher, thyromental distance less than 6.5 cm, limited

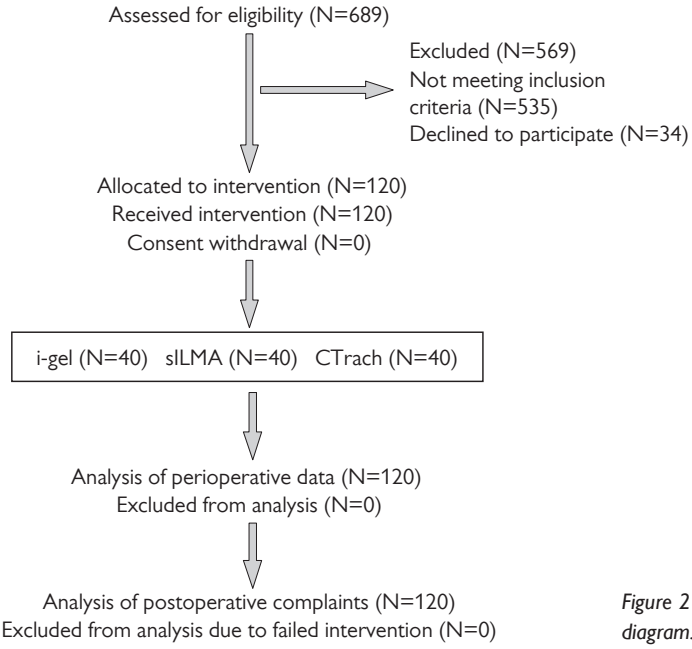

Figure 2 – CONSORT 2010 study flow diagram.

mouth opening and previous history of difficult laryngoscopy. Exclusion criteria were: ASA status IV or V patients, emergency surgical procedures and patients at increased risk for aspiration of gastric contents. Randomization was performed using a randomization software and sealed envelopes immediately prior to patient admission to the anaesthetic room. The operators were trained anaesthetists with previous experience in fibreoptic intubation. Induction of general anaesthesia was standardized and included fentanyl, propofol at a dose of 2–3 mg/kg (until loss of verbal contact) and non-depolarizing muscle relaxant. Non-depolarizing relaxant was given upon confirmation of feasible bag-mask ventilation. Following successful induction and confirmation of adequate muscle relaxation (TOF 0), an independent operator performed direct laryngoscopy using a standard Macintosh laryngoscopic blade and recorded the view according to the Cormack and Lehane classification (1–4) (Cormack and Lehane, 1984). Different operators then inserted the supraglottic airway devices and intubated through them. Procedure was divided into several steps:

Step 1: Insertion of a supraglottic airway device;

Step 2: Assessment of fibreoptic view through the device;

Step 3: Tracheal intubation through the supraglottic airway device;

Step 4: Removal of the SAD.

Following insertion of the SAD and confirmation of its satisfactory function – effective ventilation, oxygenation and no audible leak around the device – the operator inserted a flexible fibrescope with an external diameter of 5.2 mm (Olympus Medical Systems, Tokyo, Japan) with a loaded soft tracheal tube (Fastrach^{TM} silicone tube, Laryngeal Mask Co., Mahé, Seychelles) through a device in case of i-gel and sILMA, or switched on the light of the CTrach LMA. Endotracheal tubes size 7.0 were used for size 4 SADs, and sizes 7.5 tubes for size 5, respectively. Endotracheal tubes were railroaded over the fibreoptic scope in the i-gel and sILMA groups, while in the CTrach group, they were inserted into the trachea under the direct vision of the CTrach camera.

The primary outcome of this study was the success rate of tracheal intubation through each device. Secondary outcomes included: insertion time of supraglottic

Table 1 – Fibreoptic view scoring system (Kapila et al., 1997), and percentage of glottis opening (POGO) score (Levitan et al., 1998)

Fibreoptic view scoring system	POGO score
1 – Full view of vocal cords	0 – Vocal cords not visible
2 – Partial view of vocal cords, including arytenoids	100 – Full view of vocal cords
3 – Epiglottis only visible	Values between 0 and 100 are calculated according to proportion of the cords visible with the scope
4 – Other structures visible only (pharynx, LMA cuff)	

device, intubation time through the device, fibreoptic scores and incidence of postoperative complaints. Insertion time was defined as the time interval from when the device was handed to an anaesthetist until the first successful breath as visible with capnography. Intubation time was defined as the interval from the circuit disconnection until the first successful breath. We evaluated two different fibreoptic scores – "fibreoptic view scoring system" (Kapila et al., 1997) and "percentage of glottis opening" (POGO) score (Levitan et al., 1998) (Table 1). Serious complications such as massive intraoral bleed or aspiration of gastric contents were recorded. The following postoperative complaints were evaluated at 24 hours: sore throat, hoarseness, swallowing difficulties, tongue numbness and cough.

Statistics

Sample size was determined to be 120 patients in total – allowing an alpha-error of 0.05 and power of 80% (beta-error of 0.05). A 90% success rate was determined for the sILMA and CTrach based on the results of previous studies and a lowest meaningful success rate of the i-gel was set up as 65% (25% difference). All data were tested for normal distribution prior to final statistical analysis using the Shapiro-Wilk test. According to data distribution, either parametric (Fischer's exact test, chi-square test) or non-parametric (Kruskal-Wallis) tests were employed. InStat software (GraphPad Software Inc., La Jolla, USA) was used for all comparisons.

Results

Demographic data is shown in Table 2. There were no significant differences between the groups in terms of age, duration of surgery or weight. Total success rate of tracheal intubation through the device, as a primary outcome, did not differ significantly (i-gel 100%, CTrach 97.5%, sILMA 95%) (Table 3). Regarding secondary outcomes of the study, the i-gel showed significantly shorter insertion (Figure 3)

Table 2 – Patient demographic data, preoperative airway evaluation and perioperative data

	i-gel (n=40)	sILMA (n=40)	CTrach (n=40)
Gender (M/F)	10/30	16/24	21/19
Age (years, range)	48 (18–74)	47 (18–83)	49 (21–77)
ASA (I/II/III/IV)	9/30/1/0	17/20/3/0	10/26/4/0
Weight (kg)	78 [72–84]	76 [71–81]	83 [79–87]
Mallampati (I/II/III/IV)	8/21/9/2	11/17/12/0	3/32/5/0
Limited mouth opening	21 (52%)	22 (55%)	18 (45%)
Limited jaw protrusion	32 (80%)	31 (78%)	27 (68%)
Thyromental distance ≤ 6.5 cm	8 (20%)	5 (12%)	1 (2%)
Duration of surgery (min)	79 [71–87]	84 [76–92]	100 [88–112]

Data presented as mean [95% CI], mean (range) or number

and intubation times (Figure 4) than the other two devices tested (Table 4). The i-gel device also demonstrated the best coverage of the laryngeal inlet as evidenced by the highest concentration of fibreoptic scores of 1 and 2 in the i-gel group and by the highest concentration of POGO scores over 75% in the i-gel group (Figure 5).

Additional sub-group analysis for patients who experienced difficult laryngoscopy

Table 3 – Primary outcome of the study – Differences in success rates of intubation through the supraglottic airway devices

	Device		
Outcome (%)	i-gel	sILMA	CTrach
Success	40 (100%)	38 (90%)	39 (97.5%)
Failure	0	2 (10%)	1 (2.5%)

Statistical significance as confirmed with the Fisher's exact test; i-gel vs. sILMA – p=0.494, i-gel vs. CTrach – p=1.00, sILMA vs. CTrach – p=1.00

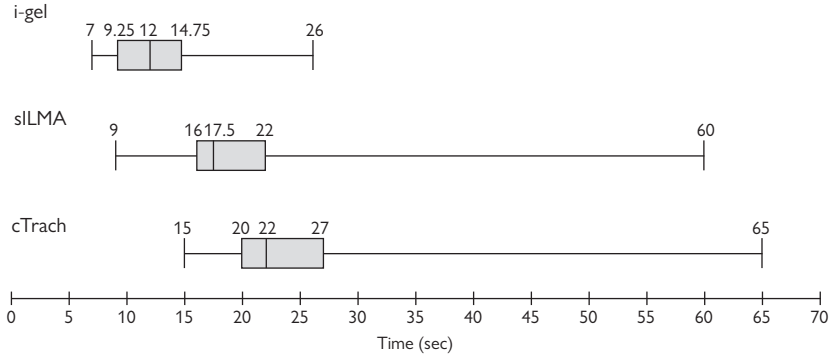

Figure 3 – Box-plot of insertion times of supraglottic airway devices.

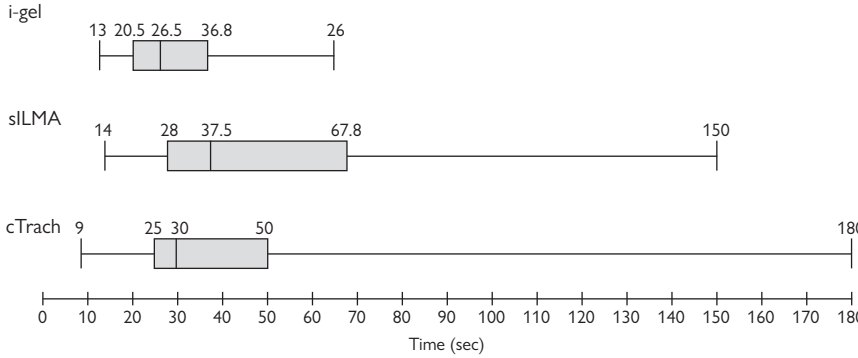

Figure 4 – Box-plot of intubation times through supraglottic airway devices.

Table 4 – Secondary outcomes of the study – Insertion time, intubation time, coverage of laryngeal inlet

	Device		
	i-gel (n=40)	sILMA (n=40)	CTrach (n=40)
Time insertion (s)	12.4 [11–13.8]	19.3 [16.3–22.3]	24.4 [21.4–27.4]
Difference		6.9 [4.4–10.6]***	12.0 [9.5–15.7]***
Time intubation (s)	29.4 [26–32.8]	51.9 [40.7–60.1]	39.8 [29.7–49.9]
Difference		22.5 [12.3–34.8]*	10.3 [2.7–23.6]***
Fibreoptic view score (1/2/3/4)	29/9/2/0	16/17/4/3[a]	11/22/6/1[b]
POGO score (0/10/25/50/75/100%)	1/1/0/4/13/21	7/2/5/3/15/8[c]	1/4/2/8/17/8[d]

Data presented as mean (s) [95% CI] for times, numbers (%) for fibreoptic view scoring system and percentage of glottic opening (POGO); *p<0.05, ***p<0.001 – Kruskal-Wallis test; [a]p=0.006 for scores 1, p=0.15 for scores 1+2, [b]p=0.0001 for scores 1, p=0.15 for scores 1+2 – Fisher's exact test; [c]p=0.01 for scores 75 – 100%, [d]p=0.04 – Fisher's exact test

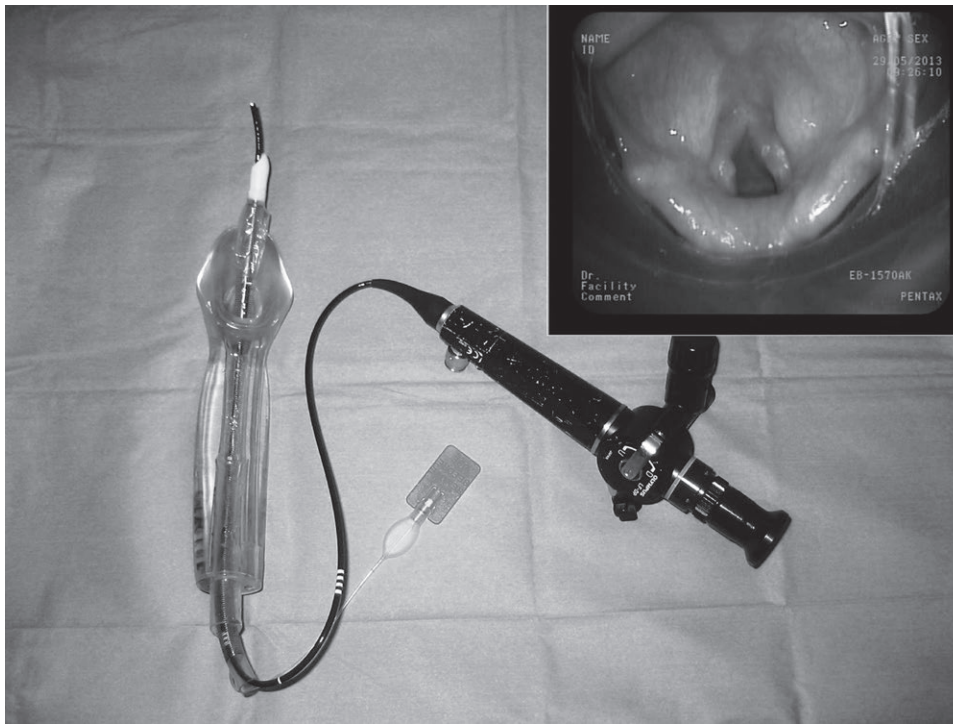

Figure 5 – Fibreoptic intubation through the i-gel and a view to the laryngeal inlet through the device.

(Cormack-Lehane scores 3 and 4) was carried out. In total 24 patients presented with difficult laryngoscopic views, 7 in the i-gel group, 12 in the sILMA group and 5 in the CTrach group, respectively. The i-gel was also inserted significantly faster than the other two devices in this sub-group and intubation times were also shorter (Table 5).

Post-hoc power analysis for primary and secondary outcomes was performed using final results of the study due to a significantly higher success rate in the i-gel group than expected. Based on the results of this study, a 95% success rate was considered as standard and 5% difference as significant. To detect this 5% difference at 95% significance and 80% power, 277 observations in each group and in total 834 patients would need to be recruited. Post-hoc analysis for secondary

Table 5 – Insertion time, intubation time, coverage of laryngeal inlet in the patients with difficult laryngoscopy (Cormack-Lehane grades 3, 4)

	Device		
	i-gel (n=7)	sILMA (n=12)	CTrach (n=5)
Time insertion (s)	12.3 [11.6–13]	24.8 [20.9–28.7]	24.8 [23.8–25.8]
Difference		12.5 [7.2–24.1]*	12.5 [8.8–15]***
Time intubation (s)	35.3 [24.6–46]	75.6 [48.4–102.8]	74.4 [20.3–128.5]
Difference		40.3 [14.4–68.5]*	39.1 [1–137.6]*

Data presented as mean (s) [95% CI] for times; *p<0.05, ***p<0.001 – Kruskal-Wallis test

Table 6 – Incidence of postoperative complaints at 6 and 24 hours after anaesthesia

Symptom	i-gel (%)	sILMA (%)	CTrach (%)	Fisher's exact test (P-value)
		6 hours		
Sore throat	67.50	65.00	65.00	1.000
Hoarse	27.50	25.00	47.50	**0.076**
Difficulty swallowing	12.50	25.00	25.00	0.325
Numb tongue	0.00	2.50	5.00	0.772
Cough	10.00	32.50	17.50	**0.054**
Nausea	30.00	17.50	20.00	0.474
		24 hours		
Sore throat	32.50	22.50	37.50	0.385
Hoarse	2.50	5.00	15.00	0.144
Difficulty swallowing	2.50	2.50	5.00	1.000
Numb tongue	0.00	2.50	0.00	1.000
Cough	2.50	17.50	10.00	**0.092**
Nausea	2.50	10.00	10.00	0.389

outcomes showed that given mean insertion time for the i-gel of 10 s (SD 4 s, powered for 3 s difference between the groups) a minimum of 28 observations would be needed in each group. Similarly, for intubation time (SD 30 s, difference 20 s) at least 36 patients would be required in each group of patients.

Analysis of the incidence of postoperative complaints is reported in Table 6. There was no difference found at the level of significance of 5% between the groups at 24 h. Some differences were observed at the 10% level of significance ($p<0.1$). The incidence of hoarseness was higher in the CTrach group while patients with sILMA reported more cough than in the other two groups.

Serious perioperative complications such as significant intraoral bleeding, vomiting or aspiration were not seen in this cohort. One female patient in the CTrach group experienced decreased oxygen saturation (spO_2 91%) after intubation associated with harsh wheezes over the right hemithorax. During intubation, no gastric fluid was seen in the oral cavity. Postoperative X-ray showed consolidation within the right lower lobe. This patient required additional oxygen on the ward for the following two days.

Discussion

The results of this study suggest that all three devices are reasonably good conduits for fibrescope-guided tracheal intubation in patients with at least one predictor of difficult laryngoscopy. The i-gel was associated with shorter time of insertion, better coverage of glottic opening and reduced intubation time than both the sILMA and CTrach.

The ILMA (Intavent Orthofix Ltd., Wokingham, UK) has been designed for either blind or fibrescope-guided tracheal intubation, in patients with expected and unexpected difficult airway (Ferson et al., 2001). Since its development in 1997, it has been used for both blind and fibrescope-guided tracheal intubations in patients with a difficult airway. The ILMA is still considered as a "gold standard" among supraglottic airways used for tracheal intubation. The original ILMA was a reusable device, a single-use ILMA has been available since 2007 (Teoh and Lim, 2007).

The i-gel (Intersurgical Ltd., Wokingham, UK) is a supraglottic airway device, with an integrated channel for derivation of gastric fluid, with a wide breathing channel allowing direct passage of a tracheal tube (Donaldson et al., 2011; Michalek et al., 2013).

The CTrach (The Laryngeal Mask Company, Singapore) is a special device for difficult airway management (Liu et al., 2006). It has special optical fibres built-in inside its bowl and a liquid crystal display which allows views of the larynx while the endotracheal tube is being placed (Liu et al., 2009).

Standard laryngeal mask airway (cLMA, LMA Classic) may be also used as a rescue device in the cases of difficult intubation, however its design with a narrow lumen makes subsequent placement of endotracheal tube technically difficult.

With a reference to these supraglottic airway devices, only a few randomized

studies and a small number of case reports detail tracheal intubation through the i-gel in patients with normal and potentially difficult airways. Initial manikin studies (de Lloyd et al., 2010; Michalek et al., 2010) compared i-gel with classical (cLMA) or intubating LMAs. De Lloyd et al. (2010) found fibrescope-guided intubation through the i-gel to be more successful and easier than through the cLMA. The i-gel was as successful as the ILMA for fibrescope-guided intubation while blind techniques with gum-elastic bougie or direct insertion of endotracheal tube through the device were significantly higher in the ILMA group (Michalek et al., 2010). Various authors have described fibrescope-guided tracheal intubation through the i-gel in patients with unpredicted difficult laryngoscopy (Sharma et al., 2007), craniofacial abnormities (Michalek et al., 2008), in the intensive care setting (Campbell et al., 2009) or in a "cannot intubate, cannot oxygenate" scenario (Corso et al., 2010). A few studies have focused on tracheal intubation through the i-gel in the operating room and its comparison with the ILMA. Both devices allowed high success rate of tracheal intubation using a fibrescope with similar intubation times (Kleine-Brueggeney et al., 2011). Limitations of this study may be that it used different tracheal tubes in each group and that predicted difficult laryngoscopy was not confirmed in the operating room. Another study showed significantly shorter intubation times and better visualisation of the glottis with the i-gel while total success rate was similar in both groups (Moore et al., 2015). This study did not specifically seek to recruit patients with predicted difficult intubation and used soft endotracheal tubes in both groups. Blind technique of endotracheal tube insertion was evaluated in two clinical trials. The ILMA was found to be superior to the i-gel as a conduit for blind insertion in the study by Theiler et al. (2011).

In the second study, the authors reported similar success rate of tracheal intubation through the devices on the first attempt, while total success rate was significantly higher with the ILMA (Halwagi et al., 2012).

Our study has several limitations. The sample size was not big enough to prove a difference in the success rate but sufficient for confirmation of difference in insertion and intubation times. Another limitation may be that the operators were more experienced in insertion of the i-gel for routine procedures under general anaesthesia in comparison with the other two devices. However, all operators had similar experience with fibreoptic intubation through each device studied. Operators performing the intubations were all relatively experienced in airway management and the results might be different with inexperienced trainees or novices in anaesthesia.

On the other hand, our study used identical soft endotracheal tubes in all patients and thus created comparable intubation conditions between the groups. Predicted difficulties were verified using direct laryngoscopy in order to validate the use of these SADs in real difficult intubation scenarios which was not performed in other published studies.

Conclusion

The i-gel, sILMA and LMA CTrach allow fibrescope-guided tracheal intubation with a very high first attempt, total success rate and overall low incidence of complications (Michalek et al., 2015). The i-gel device showed best coverage of the laryngeal inlet, as well as shortest insertion and intubation time. Based on the results of our study and other trials this device may become a first option SAD in difficult or failed laryngoscopy.

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Osteoarthrosis of Temporomandibular Joint Related to the Defects of Posterior Dentition: A Retrospective Study

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Key words: Temporomandibular joint – Osteoarthrosis/osteoarthritis – Posterior dentition – Supporting zones – OA etiologic factors

Abstract: Osteoarthrosis (OA) of temporomandibular joint (TMJ) is a progressive degenerative disease, gradually affecting cartilage, synovial membrane and bone structures. OA of TMJ clinically manifests with joint noises, pain and restricted mouth opening. In late stages, it results in severe damage of TMJ structures and development of ankylosis. Osteoarthrosis is a multifactorial disease; the occurrence is associated with TMJ overloading. The cohort included 619 patients [538 women (87%) and 81 men (13%), with average age 40.6 years (age range 8–89 years)] with TMJ disorder, who were examined in the year 2014 in Department of Dental Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic. The aim of this retrospective study was to analyse, if the lack of posterior teeth (supporting teeth zones) is the main etiologic factor of osteoarthrosis of TMJ. Diagnosis of OA was established on the clinical signs and the panoramic X-ray signs. Simultaneously other etiologic factors of OA were assessed. The presence of OA changes on X-ray had 171 patients (i.e. 27.6% of the total number of 619). 17.5% from these patients with OA had defect in posterior dentition. Other aetiological factors (stress, skeletal or vertebrogenous disorders) showed higher incidence of OA changes on X-ray. Defect of posterior dentition is not negligible, but it is not the main aetiological factor for osteoarthrosis of TMJ.

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Introduction

Temporomandibular joint (TMJ) osteoarthrosis (OA) is a chronic disease, which manifests with progressive degenerative changes of joint structures. OA develops because of imbalance between reparative and degenerative processes of the joint (Mercuri, 2008; Adebajo, 2009; Machoň and Hirjak, 2014).

TMJ OA might not be clinically manifested and might be accidentally found on panoramic radiograph during the initial phase (Figure 1). The first clinical symptoms include sound phenomena associated with jaw movements (crepitus or clicking in case of articular disc derangement or perforation). With the progression of degenerative changes, pain and restriction of mouth opening usually occur. In the

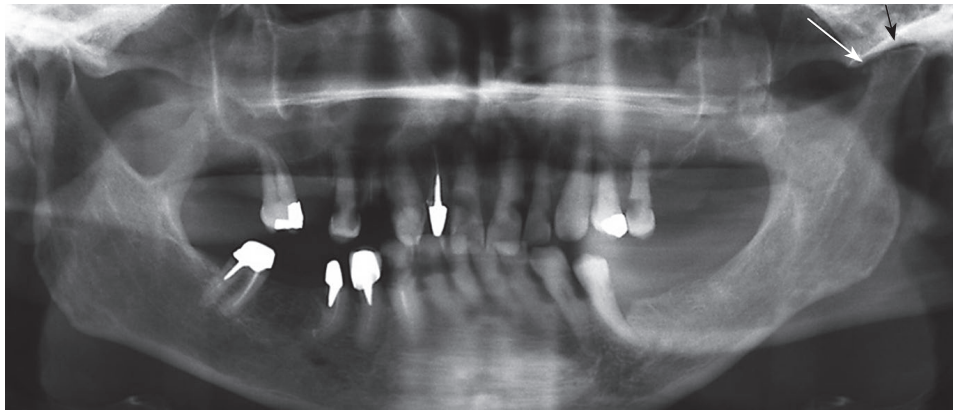

Figure 1 – Panoramic radiograph shows flattening of left condylar head of mandible, narrowing of joint space; incongruence of head surface and loose body in anterior aspect: degenerative changes.

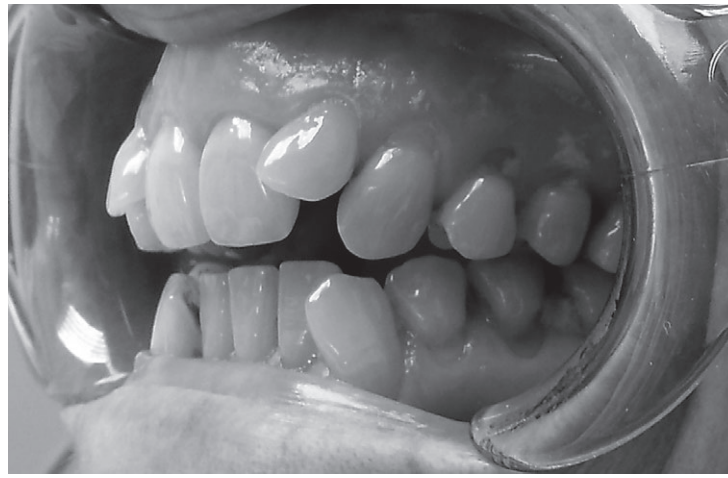

Figure 2 – Angle Class II malocclusion and anterior open bite, which developed as a result of severe bilateral osteoarthrosis of temporomandibular joint.

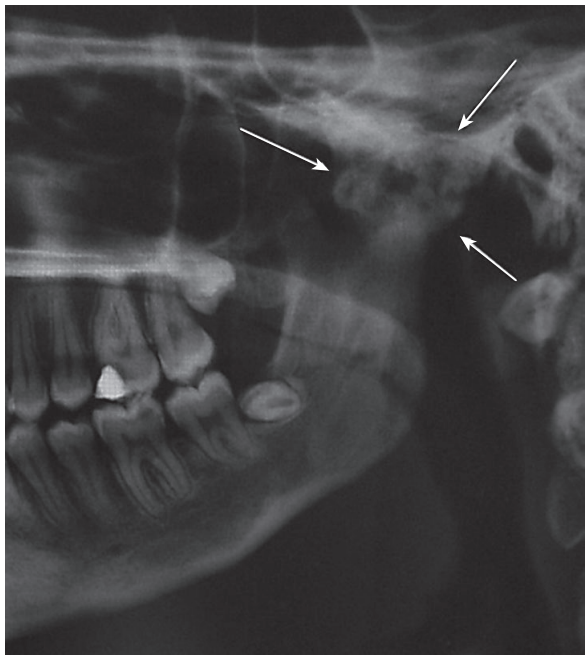

Figure 3 – Bone mass representing ankylosis of left temporomandibular joint in young patient: result of a trauma.

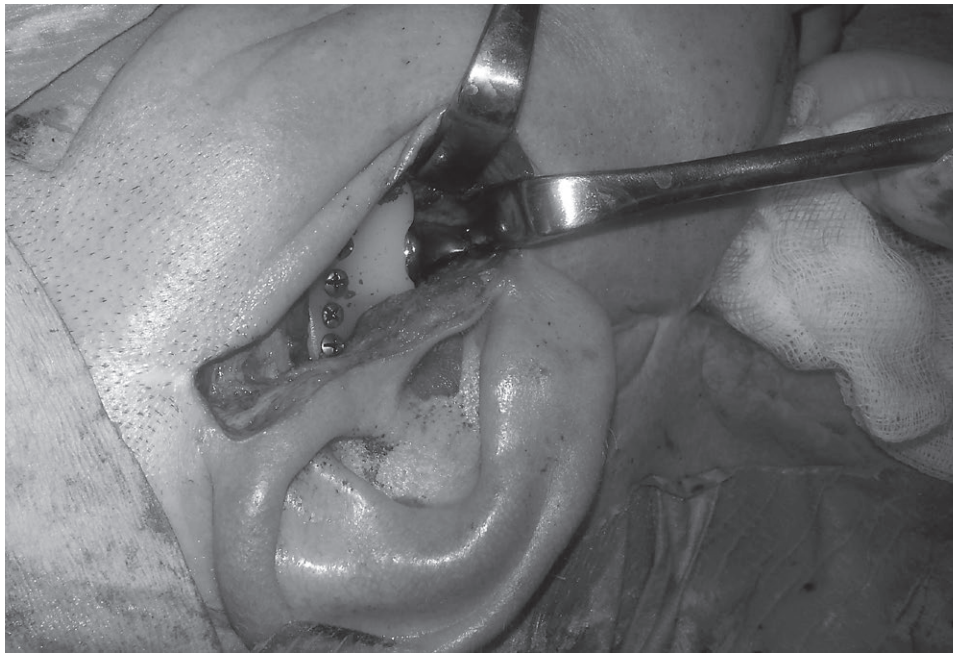

Figure 4 – Intraoperative photo of temporomandibular joint total alloplastic prosthesis (TMJ Concepts, Ventura, California, USA).

late stages of the disease, the height of the condylar process may be shortened. This condition leads to anterior open bite and development of Angle Class II (Figure 2). Degenerative changes of joint surfaces and disc perforation are risk factors for the development of ankylosis (pathological fusion between mandibular condylar head and glenoid fossa) (Figure 3). The most extensive arthritic changes and ankylosis are an indication for total joint replacement (Figure 4); other treatment modalities for OA are conservative, miniinvasive (arthrocenthesis, arthroscopy) or other types of open surgery, based on the severity of degenerative changes (Mercuri, 2008; Adebajo, 2009; Machoň and Hirjak, 2014).

Pathogenesis of OA lays in increased stress to joint structures that leads to an increase in intra-articular pressure and continuous disturbance of blood supply of the retrodiscal tissue. Cycle of transient hypoxia followed by reoxygenation of the retrodiscal tissue provokes the release of free oxygen radicals, which inhibit the synthesis of hyaluronic acid (HA) in the synovial joint. Interleukins and prostaglandins are released into joint spaces and induce activation of proteolytic enzymes (matrix metalloproteinases), which take part on chondromalacia development. Simultaneously occurring changes in composition of synovial fluid, including the lack of HA, lead to the loss of nutritional efficiency and lubricating function of the fluid. This condition results in increased friction and subsequently arthritic changes of subchondral bone (Blake et al., 1989; Grootveld et al., 1991; Nitzan, 1994, 2001; Laskin et al., 2006; Okeson, 2007; Mercuri, 2008; Machoň and Hirjak, 2014).

Increased stress may be caused by extensive load (macrotrauma or chronic microtraumatization as bruxism or functional overload). When this occurs in normal joint structures, the disease is called primary OA. Abnormal or even normal load acting against compromised joint structures (congenital collagen defects; metabolic, rheumatoid, infectious diseases) plays a role in the development of secondary OA (Laskin et al., 2006; Okeson, 2007; Mercuri, 2008; Machoň and Hirjak, 2014).

Unstable occlusion (bite/skeletal disorders, premature teeth contact) is one of the aetiological factors. Influence of orthopaedic instability (breakdown in relations between maximum intercuspidation and musculoskeletal stable position) on joint structures is a theoretical matter (Pullinger et al., 1993; Pullinger and Seligman, 2000). In the real situation, unstable occlusion increases the activity of masticatory muscles and joint surfaces are loaded during the mandibular movements. Relationship to the lateral part of the teeth is as following: when the maximum intercuspation of premolars and molars on both sides is achieved, there is a distribution of masticatory forces on all these teeth and load of the joint structures is minimized. If one/more teeth from this relationship are detached, this increases the force acting on the remaining teeth involved in that intercuspation and, the force transferred to the structure of the TMJ increases. Analogously, when only teeth on one side are in contact, then the masticatory forces transfer to

contralateral side. When there is a complete lack of posterior teeth (from the first premolar to the second or third molar), contact of the anterior teeth (in range between canines) is achieved, then it is not a stable occlusion (Okeson, 2007).

The authors focused on a group of patients with the lack of support zones (distal teeth) to confirm or reject the hypothesis, that a one- or two-sided lack of support zones causes overload of TMJ and thus contributes to the formation of degenerative changes in the structures of the temporomandibular joint (Pullinger et al., 1993; Pullinger and Seligman, 2000; Okeson, 2007).

Material and Methods

The study includes patients, who were examined in the Department of Dental Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic in the year 2014. From a total of 1,024 patients with temporomandibular joint disorders, patients with osteological, autoimmune, metabolic and rheumatoid diseases had been excluded.

After exclusion, the study consisted of 619 individuals. Patients were aged 8–89 years; the average age was 40.6 years. Women accounted for 87% of all patients (538 patients); the remaining 13% were men (81 patients). Osteoarthrosis was evaluated based on the presence of degenerative changes on the panoramic radiograph; therefore, the study included both symptomatic and non-symptomatic patents with OA. To evaluate the defects of the support zones, panoramic radiographs and clinical examination were used. A defect of the support zone was defined as one- or two-sided shortening of the upper or lower or both dental arches (from first premolar to second or third molar). Patients were evaluated for other possible etiologic factors: significant psychical stress load, skeletal defects, vertebral disorders (pain in the cervical spine and neck or neck muscles), bruxism, trauma (fractures of the lower jaw in the personal history).

Results

The examined group consisted of 619 patients (TMJ disorder, without further osteological, autoimmune, rheumatoid, metabolic diseases).
538 women (87%) and 81 men (13%).
The average age was 40.6 years (age range 8–89 years).

The presence of OA changes on X-ray:
171 patients (i.e. 27.6% of the total number of 619).
These were 162 women (94.8%) and 9 men (5.2%).
The average age was 46.3 years (age range 33–89 years).

Defect of supporting zones in patients with OA changes on X-ray:
Defect of supporting zones was found in 30 patients (17.5%) out of 171 patients with OA.

These were 9 men (30%) and 21 women (70%).
The average age was 57.9 years (age range 35–83 years).

When men only were evaluated with OA (9 patients), then the absence of the support zones was in all patients (100%).
If only women were evaluated with OA (162 patients), then the absence of the support zones was only 21 patients (12.9%).

Other etiological factors in patients with OA changes on X-ray (171 patients) (Figure 5):
31% (51 patients) – significant stress load
30% (50 patients) – skeletal malformation (hypoplastic mandible, Angle Class II)
28% (46 patients) – vertebrogenous disorders
12% (20 patients) – bruxism
3% (5 patients) – trauma (fractures of the mandible)

Discussion

Prevalence of osteoarthrosis, as well as overall temporomandibular joint diseases, is higher in women (70–72% of cases) (Pullinger et al., 1993; Pullinger and Seligman, 2000). The same result was found in this study (females 87%, males 13% with TMJ disorders; resp. 94.8% females and 5.2% males with OA). The aetiology of this disease is now considered as multifactorial. Increased psychological stress accompanied by anxiety and depression is reported as a vital factor (Dworkin and Massoth, 1994; Suvinen and Reade, 1995; Ferrando et al., 2004). This fact was confirmed in this study: a total of 31% of patients in this study diagnosed with OA admitted increased psychological burden. Patients often compensate increased psychological stress by conscious or unconscious parafunctional activities, which cause chronic microtraumatization of the joint and subsequent development of inflammatory degenerative changes. The mechanism of chronic TMJ overload operates in bruxism, skeletal defects (especially Angle Class II, open

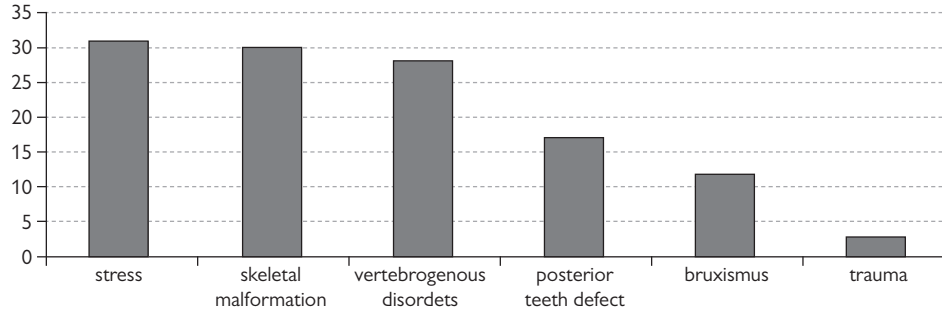

Figure 5 – Aetiological factors of osteoarthrosis.

bite) and vertebrogenous problems (Israel and Scrivani, 2000; Güler et al., 2003). Macrotrauma as the cause of developing OA of TMJ was only a marginal factor (3% of the patients had a history of trauma to the mandible; a total of 5 patients with a fracture of the lower jaw in all cases). Extensive studies, Probert et al. (1994) comprising 20,763 patients after car accident, showed the presence of TMJ disorder even only in 28 patients (i.e. 0.13%).

Pullinger et al. (1993), Pullinger and Seligman (2000) and Okeson (2007) evaluate one or both sides shortened dental arch as an important factor for overloading of ipsilateral/bilateral joint structures. In this study, degenerative changes developed only in 17.5% of patients with defect of supporting zones. The study shows, that the lack of posterior teeth is not the main aetiological factor of TMJ OA. Okeson (2007) refers to 57 other authors who diverge in opinion on relation between failure of occlusion and TMJ disorders. A total of 22 of these authors reject the relation, and other authors consider the defects of occlusion as the most important factor in the TMJ disorders development (Tsolka et al., 1994; Ciancaglini et al., 1999).

When only men with OA were evaluated (9 patients), then the defect in posterior dentition was seen in all these patients (100%). When considering the same condition in women: 21 patients with OA had the defect of posterior dentition (i.e. 12.9% out of 162 patients with OA). This result correlates with the work of Magnusson et al. (2010), which presents the lack of support zones as s significant risk factor of OA for men, not for women (per his work, increasing age is a risk factor for women is a risk factor). Data can be distorted by different gender representation in our compared to Magnusson's study (Magnusson investigates 170 men, 89 women, our study includes 81 men and 538 women). In studies of several different authors dealing with the masticatory forces (and their association with TMJ disorders), it was found, that men use larger chewing power than women (Shinogaya et al., 2001; Ferrario et al., 2004; Pizolato et al., 2007; Koc et al., 2010), with a difference of up to 300 N (that is 30 $kg \times m/s^2$!) (Waltimo and Könönen, 1993). Conversely, a higher value of the intra-articular pressure was measured in females than males, both at closed mouth position at rest and during clenching of the teeth, i.e. showing increased activity of the masticatory muscles (Nitzan, 2001).

Conclusion

Surprisingly, only a small percentage of patients examined for TMJ disorders in this study, had unsatisfactory dental situation in terms of lack of posterior teeth. The study shows, that lack of posterior dentition is not the essential factor in the development of osteoarthrosis. On the other hand, the percentage of patients, who have arthritic changes of TMJ and shortened dental arch at the same time, is not negligible. It indicates that these factors are involved in the development of osteoarthrosis, as well as in the development of other TMJ disorders.

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Can Anxiety Tested in the Elevated Plus-maze Be Related to Nociception Sensitivity in Adult Male Rats?

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Abstract: Methamphetamine (MA) is one of the most addictive psychostimulant drugs with a high potential for abuse. Our previous studies demonstrated that MA administered to pregnant rats increases pain sensitivity and anxiety in their adult offspring and makes them more sensitive to acute administration of the same drug in adulthood. Because individuals can differ considerably in terms of behaviour and physiology, such as rats that do not belong in some characteristics (e.g. anxiety) to average, can be described as low-responders or high-responders, are then more or less sensitive to pain. Therefore, prenatally MA-exposed adult male rats treated in adulthood with a single dose of MA (1 mg/ml/kg) or saline (1 ml/kg) were tested in the present study. We examined the effect of acute MA treatment on: (1) the anxiety in the Elevated plus-maze (EPM) test and memory in EPM re-test; (2) nociception sensitivity in the Plantar test; (3) the correlation between the anxiety, memory and the nociception. Our results demonstrate that: (1) MA has an anxiogenic effect on animals prenatally exposed to the same drug in the EPM; (2) all the differences induced by acute MA treatment disappeared within the time of 48 hours; (3) there was no effect of MA on nociception per se, but MA induced

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higher anxiety in individuals less sensitive to pain than in animals more sensitive to pain. In conclusion, the present study demonstrates unique data showing association between anxiety and nociceptive sensitivity of prenatally MA-exposed rats that is induced by acute drug administration.

Introduction

Methamphetamine (MA) is one of the most addictive psychostimulant drug with a high potential for abuse. It is also one of the most frequently used "hard" drug in the Czech Republic (Vavřínková et al., 2001) and due to its anorectic effects, it is one of the most commonly abused drug among women, even during pregnancy (Marwick, 2000). Our previous studies demonstrated that MA administered to pregnant rats increases pain sensitivity in their offspring in adulthood (Yamamotová et al., 2011). In addition, our recent study (Šlamberová et al., 2015) demonstrated that prenatal MA exposure increases anxiety of adult male rats and makes them more sensitive to acute administration of the same drug in adulthood.

In addition, there are studies showing that administration of MA in adulthood improves cognitive processes (Kornetsky et al., 1959), does not have any effects on cognition (Fialová et al., 2015), or impairs the learning abilities of adult male rats (Meredith et al., 2005; Schutová et al., 2009). This inconsistency seems to be caused by different dose of the drug, duration of the drug exposure and the type of cognition test used in the studies (Simoes et al., 2007; Belcher et al., 2008). Our previous study demonstrated that the effect of MA administration in adulthood on cognition is influenced by prenatal MA exposure, which seems to correlate with NMDA (N-methyl-D-aspartate) receptors in the hippocampus (Šlamberová et al., 2014).

As show in the study of Borta and Schwarting (2005) rats, although identical in strain, sex, age and housing conditions, can differ considerably in terms of behaviour and physiology. Such individual differences can be rather stable and may be detected by behavioural screening tests. There are studies showing that animals that do not belong in some characteristics (e.g. anxiety) to average (the most often incidence), but can be described as low-responders or high-responders, are then more or less sensitive to pain (Ho et al., 2002).

Studies of Rodgers and Dalvi (1997) and Rodgers et al. (1999) found that exposure to the Elevated plus-maze (EPM) test induces behavioural and physiological effects in rodents consistent with fear/anxiety. Maze-naive animals displayed high levels of risk assessment towards the open arms, and explore these areas less extensively than other parts of the maze. Immediately following the EPM test, pain latencies (Rodgers and Cole, 1994), skin conductance levels (Suer et al., 1998), and plasma corticosterone titers were shown to be significantly elevated (Rodgers et al., 1999). Also clinical studies (Lautenbacher and Krieg, 1994; Asmundson and Katz, 2009; Bras et al., 2010) repeatedly showed that anxiety decreases threshold of nociception, thereby increases pain sensitivity. Therefore,

the present study was set to examine possible correlations between the effects of MA on pain sensitivity and anxiety. To test anxiety EPM test was chosen as the most appropriate test of anxiety (Pometlová et al., 2009). For pain sensitivity the Plantar test was used as in our previous studies (Yamamotová et al., 2011).

Moreover, the experiment was expanded for the influence on learning and memory in accordance with the protocol "test/re-test" in the EPM (Carobrez and Bertoglio, 2005). After exploring the maze during the first testing the rat saves the information that the area is not dangerous, in that part of memory that relates to exploration of potentially dangerous areas, and the rat changes its behaviour during the retest. If the drug disrupts learning process, the pattern of behaviour is changed in comparison with the first test (File, 1993; Bertoglio and Carobrez, 2002; Bertoglio et al., 2005). Based on our previous results (Schutová et al., 2009) we assume that the influence on the consolidation of the memory trace will be not significant.

Methods

The procedures for animal experimentation in this study were reviewed and approved by the Institutional Animal Care and Use Committee and were in agreement with the Czech Government Requirements under the Policy of Human Care of Laboratory Animals (No. 246/1992) and with subsequent regulations from the Ministry of Agriculture of the Czech Republic.

Animals and prenatal care

Adult female (275–300 g) and male (375–400 g) albino Wistar rats provided by Charles River Laboratories International, Inc., were delivered by AnLab (Prague, the Czech Republic). Animals were housed four per cage by sex and left undisturbed for a week in a temperature-controlled (22–24 °C) colony room with free access to food and water on a 12 h (light):12 h (dark) cycle with lights on at 06:00 h. Eight females were impregnated as described in our previous study (Šlamberová et al., 2005). On gestational day (GD) 1 the daily injections of MA started and continued until the day of delivery, which usually occurred on GD 22. D-methamphetamine HCl (Sigma Aldrich, the Czech Republic) was diluted in distilled water in concentration of 5 mg/ml and injected subcutaneously (s.c.) in a volume of 1 ml/kg. The dose 5 mg/kg of MA was chosen because it induces similar fetal brain drug concentrations and similar behavioural changes to those found in humans (Acuff-Smith et al., 1996; Rambousek et al., 2014).

The day of the delivery was indexed as postnatal day (PD) 0. On PD 1, pups were weighed and the number of pups was adjusted to 12 per litter. Whenever possible, the number of male and female pups raised by a dam was equal. On PD 21, pups were weaned and group-housed by sex. Animals were left undisturbed until adulthood. In order to avoid litter effects, only two male rat from each litter was used in the present study (one for each adult injection). The rest of the animals were used in other experiments.

Schedule of the experiment

In total 16 adult male rats (PD 80–90) prenatally exposed to MA were tested in this experiment (n=8 rats/group). All of the animals were handled according to the protocol described in Behavioral Science Protocols (Geyer and Swerdlow, 2007) during three days prior to the EPM tests.

After the three days of handling, animals were tested in the EPM. Forty-five minutes prior to testing, animals were injected with either a challenge dose of MA (1 mg/ml/kg) or saline (1 ml/kg). The dose of MA was chosen based on our previous studies (Šlamberová et al., 2010; Schutová et al., 2013) showing that this dose does not induce stereotypical behaviours. The timing of the drug application was chosen based on the study of Rambousek et al. (2014) demonstrating that the peak of MA level in the brain (not in the blood) occurred between the 45th and 60th minute after administration.

To see the effect of MA on learning, the EPM experiment was further expanded for the protocol of "test/re-test" as shown in the study of Carobrez and Bertoglio (2005). In details, forty-eight hours after the EPM test, animals retook the test on the EPM (re-test) but without any drug administration. Directly after the re-test on the EPM animals were examined for nociception sensitivity in the Plantar test (see time-line at Figure 1).

Elevated plus-maze (EPM)

The same method was used as in our previous study (Pometlová et al., 2012), which was a modified protocol of Fernández Espejo (1997). All animals were habituated to the laboratory environment and the experimenter during the 3 days prior to the experiment (Geyer and Swerdlow, 2007). At the beginning of the test an animal was positioned on the center square of plus maze with the animal's nose pointing toward one of the closed arms (CA). Animal behaviour in the EPM was video-recorded for five minutes. Acquired video records were evaluated using the ODLog program (Macropod Software^{TM}). Animal behaviour was divided into four categories as follows.

The first category included behaviour related to anxiety. The anxiolytic effect on animal behaviour was described as activities in open arms (OA): the number

Prenatal MA exposure	Postnatal development (no disturbance)	3 days of habituation	MA or saline s.c. application	EPM test 45 min after injection	48-h break period	EPM re-test	Plantar test
ED 1–22	PD 1–60	Test day 1–3	Test day 4		Test day 5	Test day 6	

Figure 1 – Time-line of the experiment.

of entries to the OA and total time spent there. Anxiogenic effect on animal behaviour involved activities in the CA and the center: the number of entries to the CA and total time spent there, the number of protected head-dipping (DIP) and protected stretched attend postures (SAP) (Fernández Espejo, 1997). DIP was defined as head-dipping below surface of the maze that occurred on the center square, while the body of an animal remained in the CA or central platform. SAP was defined as forward elongation of head and shoulders followed by retraction to initial position. In this study it was recorded when it occurred on the central platform, while the body and all paws of an animal remained in the CA.

The second category described approach-avoidance conflict and also depicted types of behaviour with respect to OA and CA, i.e., positioning of an animal with all four paws in the center of the maze followed by retrieval (meaning moving backwards) to the CA.

The third behavioural category in the EPM was used to describe motor activity. Evaluated activities included the total number of entrances into all arms and sniffing in the center of the EPM (mobile or quiet olfactory exploration of the environment).

The last category included displacement behaviour as a measure of anxiety, i.e., self-grooming, etc. This category was not evaluated because at a dose of 1 mg/kg it did not occur.

Frequency of specific behavioural patterns as well as time spent engaged in specific behaviours were used for statistical analyses.

Plantar test

The same method was used as in our previous study (Yamamotová et al., 2011). Plantar test (Plantar test; Ugo Basile, Comerio, Italy) was used to measure nociceptive sensitivity. A beam generator, which is controlled by the experimenter under the floor of the Plexiglas box (size 27×17×14 cm) allows to stimulate the sole (planta) of the paw in a freely moving rat. The latency of paw withdrawal from painful heat stimulus was measured for each of the four paws. Latency to withdrawal of the tail was measured as a modified method of the tail-flick test. The stimulation was repeated four times. Each repetition progressed from more distal to more proximal parts of the tail. The inter-stimulus intervals ranged from 1/2 to 1 min in order to let the animals calm down from the preceding stimulus. The maximal intensity was set to 90 and cut-off time was 22 s to prevent tissue damage.

Statistical analyses

A one-way ANOVA (MA vs. saline) with repeated measure (test vs. re-test) was used for statistical analyses of the results of the EPM test and re-test. Bonferroni post-hoc test was used when appropriate. In the Plantar test, the factor treatment (MA vs. saline) comprised the between-subject variable. The within-subject variables

(factor pain) consisted of three measured body sites (average withdrawal latency of forelimb, hind limb and the tail).

An association between pain sensitivity and anxiety was analysed using correlation technique. As the data from EPM were not normally distributed Spearman rank order correlation was applied.

Differences were considered significant if $p<0.05$.

All statistical data in this report are presented as [F(N-1, n-N) = xx.xx; p<0.0x], where F = test criterion of ANOVA, N-1 = degrees of freedom of groups, n-N = degrees of freedom of individual subjects, p = probability level.

Results

Elevated plus-maze test

Concerning the anxiogenic/anxiolytic effect, there were no significant differences in the measure of entries and time spent in the OA (anxiolytic effect), nor in the CA (anxiogenic effect) of the EPM apparatus (Table 1). However, when more detailed analysis was conducted, animals injected with MA displayed decreased DIP [$F(1, 14) = 5.58$; $p<0.01$] and increased SAP [$F(1, 14) = 6.40$; $p<0.01$] during the EPM test relative to saline controls (Figure 2).

The approach-avoidance conflict was significantly changed by the MA administration in the EPM test [$F(1, 14) = 8.46$; $p<0.01$], thereby that MA administration increase the number of returns to the CA when compared to saline-treated animals in the EPM (Figure 2).

The motor activity that was evaluated as number of entries to all arms and frequency as well as time spent with sniffing was not significantly affected by MA administration (Table 1).

Table 1 – Nonsignificant effects on behaviour in the EPM

	Test		Re-test	
	Saline	MA	Saline	MA
OA entries	1.88 ± 1.05	1.50 ± 1.04	0.88 ± 0.45	0.50 ± 0.48
OA time (s)	24.50 ± 12.92	19.38 ± 12.91	13.88 ± 6.30	5.13 ± 6.25
CA entries	14.13 ± 1.83	20.00 ± 1.84	9.25 ± 1.93	10.75 ± 1.94
CA time (s)	132.63 ± 17.15	143.88 ± 17.15	162.00 ± 20.90	202.50 ± 20.91
Locomotion (arms entries)	8.88 ± 1.97	10.00 ± 1.97	4.13 ± 1.46	3.25 ± 1.45
Sniffing frequency	15.75 ± 2.05	19.13 ± 2.06	11.00 ± 2.29	9.75 ± 2.28
Sniffing time (s)	76.38 ± 9.99	77.00 ± 10.00	89.88 ± 13.13	53.88 ± 13.12

Numbers are means ± SEM (n=8); EPM – elevated plus-maze; MA – methamphetamine; OA – open arms; CA – closed arms

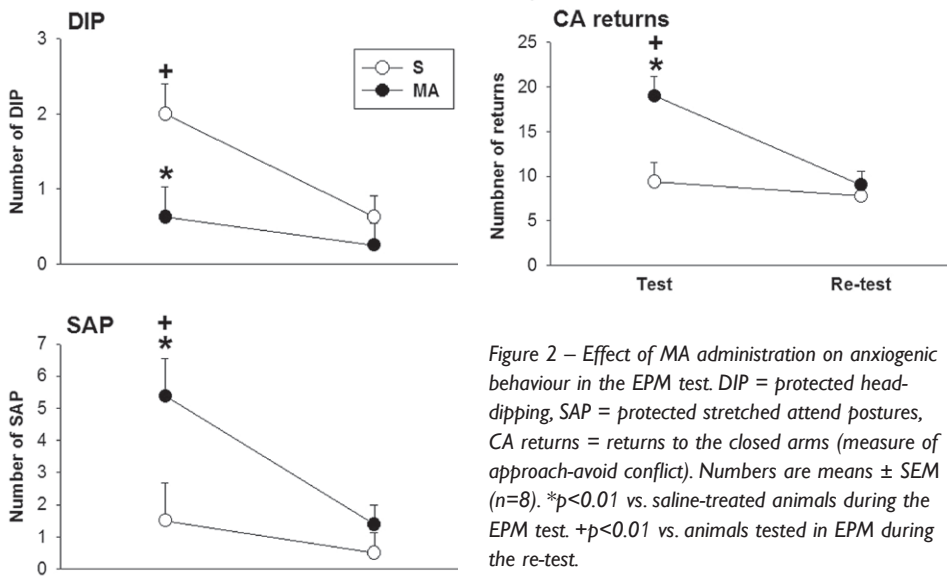

*Figure 2 – Effect of MA administration on anxiogenic behaviour in the EPM test. DIP = protected head-dipping, SAP = protected stretched attend postures, CA returns = returns to the closed arms (measure of approach-avoid conflict). Numbers are means ± SEM (n=8). *p<0.01 vs. saline-treated animals during the EPM test. +p<0.01 vs. animals tested in EPM during the re-test.*

Elevated plus-maze re-test

As shown in Figure 2, there were no differences induced by acute MA administration on DIP, SAP and returns to the CA as shown in the EPM test.

Plantar test

The withdrawal latencies on thermal stimulation were measured after the re-test in the EPM. Neither MA nor saline application influenced nociceptive sensitivity (main effect of treatment [$F(1, 14) = 2.13$; $p=0.17$]). Pain sensitivity measured on different

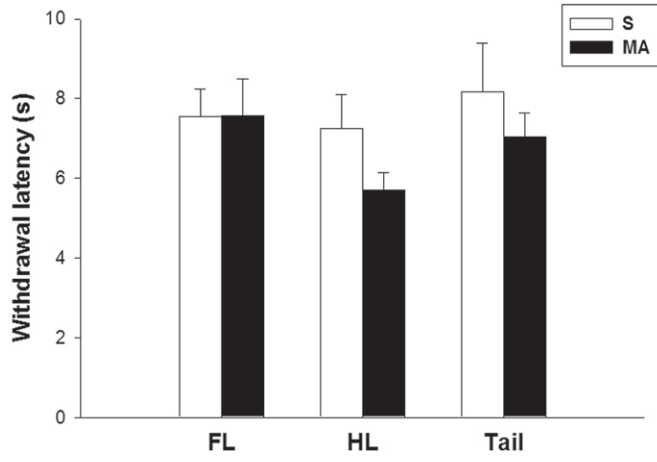

Figure 3 – Thermal nociceptive sensitivity after the re-test in the elevated plus-maze. No significant differences were found between groups in either forelimbs (FL), hind limbs (HL) or the tail. Data are expressed as means ± SEM.

body parts was also similar (main effect of factor pain [F(2, 28) = 1.09; p=0.35]). Interaction of factors treatment and pain was also nonsignificant [F(2, 28) = 0.46; p=0.64] (Figure 3).

Correlations

From correlation matrices evaluating pain sensitivity in relation to measured parameters from EPM follows that significant results were obtained only for those animals that were formerly treated with MA. More specifically, after application of MA, animals with lower pain threshold on the tail spent more time in OA [r=–0.71; p=0.048], more frequently visited OA [r=–0.74; p=0.036], generally spent more time in OA in relation to CA [r=–0.71; p=0.048], and average single visit in CA was shorter [r=0.86; p=0.006] (Figure 4). No significant correlations were found in animals previously treated with saline.

Discussion

Our results can be presented in three steps. First, the anxiety data from the EPM test and memory in re-test. Second, the results showing changes in nociception tested in the Plantar test. Third, the correlation between the anxiety, memory and the nociception.

First, our data from the EPM test did not show any robust changes induced by application of MA (no effect on entries and time spend in the open/closed

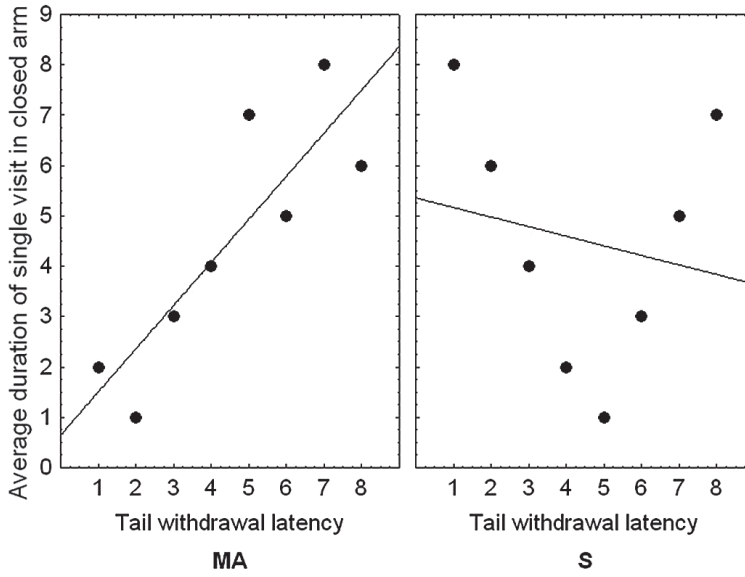

Figure 4 – Rank-order correlation between the tail withdrawal latency on thermal stimulus measured after re-test in EPM and the average duration of single visit in closed arms of the EPM after injection of MA or saline. Anxiogenic effect of MA was observed in animals with longer withdrawal latencies (r=0.8571; p=0.0065).

arms). However, our detailed analysis based on studies of Fernández Espejo (1997) and Pometlová et al. (2009) suggests rather anxiogenic effect of acute MA administration, such as increased number of SAP as well as number of CA returns. This is in agreement with our recent study showing anxiogenic effect of prenatal and acute MA exposure in different models of anxiety (Šlamberová et al., 2015). The only disputatious might be our finding showing decreased number of DIP after acute MA administration. Decreased of DIP is by Fernández Espejo (1997) interpreted as anxiolytic behaviour. Based on our results, however, one may offer different interpretation of these data. Note that DIP is defined as head-dipping below surface of the maze that occurred on the center square, while the body of an animal remained in the closed arm or central platform. It is possible that increase of such a behaviour means that these animals are more adventurous and are not afraid of heights so much. This is however only our speculation.

In addition to these findings, re-test in the EPM was conducted 48 hours after the first anxiety testing to test learning ability. Shortly we can say that all the differences between acute MA- and saline-treated groups disappeared within the time of 48 hours. Specifically, the effect of acute MA administration decreased with the time between test and re-test in the number of SAP and number of CA returns. In contrast, there was significant decrease of the number of DIP in saline-treated group, but no change in MA-treated group. This confirms ambiguous effect of acute MA, because after 48 hours the animals are both the same. It should be noted the EPM is a model of "state" anxiety that tests unconditioned responses (Belzung and Griebel, 2001). There are many factors that may affect the performance of the EPM test, such as strain, sex, age and housing conditions (Borta and Schwarting, 2005). Influence of acute administration of MA was not manifested, which is in agreement with our previous Morris water maze study (Schutová et al., 2009).

Second, our data show no differences in nociceptive sensitivity between MA- and saline-treated groups. Our previous studies (Yamamotová et al., 2004, 2011; Hrubá et al., 2010b; Yamamotová and Šlamberová, 2012) consistently showed that prenatal treatment of pregnant mothers with MA has long-term impact on nociception in their offspring. Even in adulthood these animals have decreased pain thresholds contrary to control animals. Although the mechanism underlying the increased pain perception induced by prenatal exposure to MA is not clear at present, during early stages of development prenatal MA may sensitize nociceptive system via up-regulation of the NMDA receptors activity which then cause an increase in the pain perception due to changes in central excitability (Šlamberová et al., 2009, 2014; Chen et al., 2010; Hrubá et al., 2010a; Vrajová et al., 2014).

After EPM re-test, the groups did not differ in nociceptive sensitivity. Given that both groups were exposed during the prenatal period to MA, there was no reason to assume that they will differ in this respect. From this point of view, we

can assume that the observed reaction to pain is specific characteristic of the individual.

Third, it was shown that chronic pain can lead to anxiety and anxiety can enhance pain (Koga et al., 2015). However, anxiety can exacerbate pain perception when the source of anxiety is related to the pain experience. When the source is related to something else, anxiety may even reduce the reaction to pain (Weisenberg et al., 1984). Classification of Wistar rats into high and low anxious animals according their behaviour in the EPM showed that despite their different basal levels of trait anxiety, rats did not exhibit different levels of mechanical hypersensitivity (Wilson et al., 2007). Contrary to these results, an increase of heat pain threshold was observed in high anxious rats compared to normal Wistar rats (Jochum et al., 2007).

In addition, our results demonstrated that in the re-test, the groups did not differ in the length of stay and frequency of visits in open and closed arms of the EPM. No relationships were further found between measured characteristics in the EPM and nociception. On the other hand, significant correlations were found between anxiety after application of MA and nociceptive sensitivity measured two days later. It can be interpreted as follows; assuming relatively stable patter in nociception, acute dose of MA had a greater anxiolytic effect in individuals more sensitive to pain than in animals less sensitive to pain and the other way around. A similar relationship between nociception and anxiety was observed in rats after administration of N-feruloylserotonin (extracted from *Leuzea carthamoides*) which has been demonstrated anxiolytic effect (Yamamotová et al., 2007).

There are several studies showing that anxiety in the EPM induces analgesia of non-opioid type. In mice, 5-min exposure to the EPM induced a mild, though enduring, elevation in tail-flick latencies and pre-treatments with the opiate antagonist naltrexone failed to block EPM-induced antinociception (Lee and Rodgers, 1990). In rats, the results shown that open EPM-induced antinociception did not display cross-tolerance to morphine and failed to be reversed by naltrexone (Cornelio and Nunes-de-Souza, 2009). Therefore, our animals with higher pain thresholds can be considered more anxious/stressed and the effect of MA might be therefore more anxiogenic, unlike less stressed animals, in which MA might be more anxiolytic.

We can summarize the present data the following way: first, MA has an anxiogenic effect on animals prenatally exposed to the same drug in the EPM; second, all the differences induced by acute MA treatment disappeared within the time of 48 hours; third, there was no effect of MA on nociception per se, but MA induced higher anxiety in individuals less sensitive to pain than in animals more sensitive to pain. To conclude, the present study demonstrates unique data showing association between anxiety and nociceptive sensitivity of prenatally MA-exposed rats that is induced by acute drug administration.

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Percutaneous Nephrolithotomy in a Patient with Mainz Pouch II Urinary Diversion: A Case Report

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Abstract: Mainz pouch II is a reliable and viable technique of continent urinary diversion. Patients are at increased risk of long-term complications including urolithiasis of the upper urinary tract and reservoir. We report the case of a 67-year-old male with prior Mainz pouch II due to invasive bladder cancer treated for a large renal calculus. Percutaneous nephrolithotomy (PCNL) was successfully performed. Stone management in these type of patients is of increased interest due to existed "anatomical challenges" concerning the access and safety during the procedure. To our knowledge this is the first case of PCNL in a patient with Mainz pouch II that has been reported in the literature.

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Introduction

Mainz pouch II or sigma-rectum pouch represents a continent urinary diversion for patients undergoing a radical cystectomy (Fisch et al., 1993). Sigmoid colon is utilized to create a low pressure and high capacity reservoir. Patients with urinary diversions are at increased risk for upper tract stones formation as well as calculi within the diversion segment (Okhunov et al., 2011). There are several treatment options for managing renal calculi. Following the establishment of minimal invasion techniques, percutaneous nephrolithotomy (PCNL) is considered the preferred option for treating renal stones in patients with urinary diversion.

Although PCNL is an efficient and safe technique, it may be a demanding procedure in case of urinary diversion. Gaining percutaneous access is a challenging step since identification of the neo-ureteral orifice and retrograde opacification of the collecting system is commonly difficult. There are several reports in the literature presenting the outcomes of PCNL in a variety of diversion types (El-Nahas et al., 2006; Hertzig et al., 2013). However, data regarding the outcomes of PCNL in patients with Mainz pouch II is missing. In the present report, we describe the management of a large renal stone in a patient with Mainz pouch II urinary diversion by PCNL.

Case report

A 67-year-old Caucasian male was referred to our Department of Urology for definitive treatment of a large renal calculus. According to his past medical history, he underwent radical cystectomy and Mainz pouch II urinary diversion due to invasive bladder cancer 8 years ago. He also underwent an open ureterolithotomy for stone removal 2 years ago. The patient was admitted to another institution due to acute pyelonephritis 2 months ago. Obstruction of the left kidney was identified by ultrasound and a nephrostomy tube was inserted. A computed

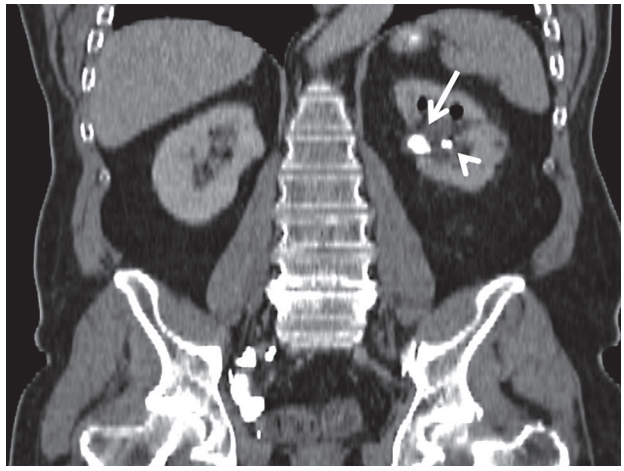

Figure 1 – Computed tomography scan revealing the stone (arrow – renal stone; arrowhead – nephrostomy tube).

tomography showed a 21×13×15 mm stone within the left renal pelvis (Figure 1). Surgical options were discussed and PCNL was considered the optimal option. Preoperative urine culture revealed multi-drug resistant *Pseudomonas aeruginosa* and Meropenem was initiated 24 h prior the procedure.

After inducing general anesthesia, the patient was placed in prone position. Nephrostomy tube was utilized and antegrade pyelography was performed for

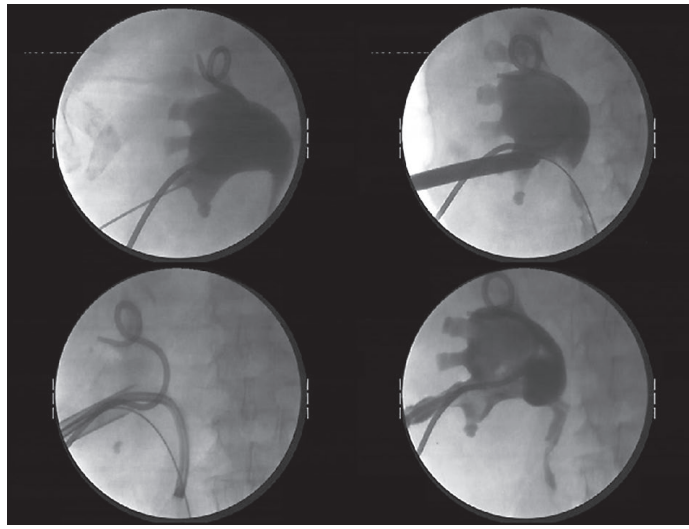

Figure 2 – Figure 2a (upper left) – an 18G needle inserted in lower calyx; Figure 2b (upper right) – obtaining the tract with 30F balloon dilation; Figure 2c (lower left) – inspection of the ureter with flexible nephroscope; Figure 2d (lower right) – insertion of Council catheter and antegrade pyelography.

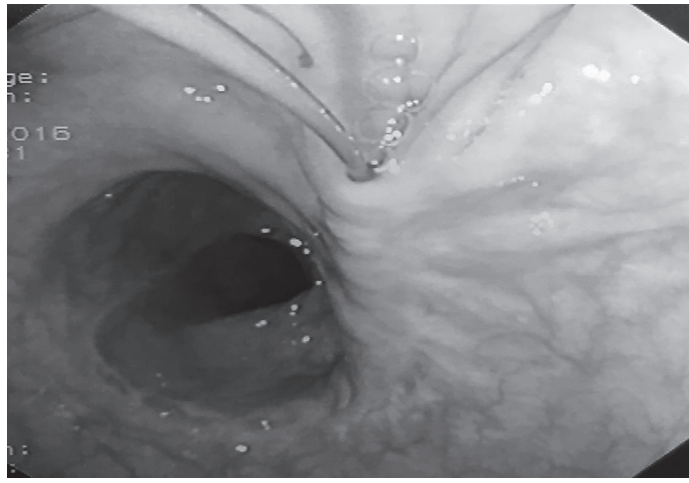

Figure 3 – Neo-ureteral orifice and safety wire.

the identification of optimal calyx. Puncture of posterior lower calyx was chosen using the "bull's eye" technique. After confirming the site of the needle within the collecting system (Figure 2a), a 0.035-inch hydrophilic-coated angled-tip guidewire was inserted. A 5F "hockey stick" catheter was then advanced guiding the wire down the ureter into the Mainz pouch. At this point, the guidewire was grasped and pulled out through the rectum by flexible colonoscope (Figure 3), obtaining a "through and through" wire. Guidewire was replaced with Amplatz 0.038-inch extra-stiff wire. A gas tube was placed into the pouch through the rectum. The

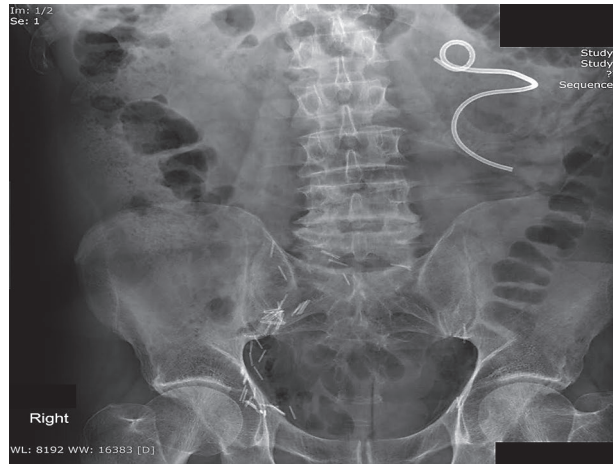

Figure 4 – Postoperative KUB (kidney-ureter-bladder).

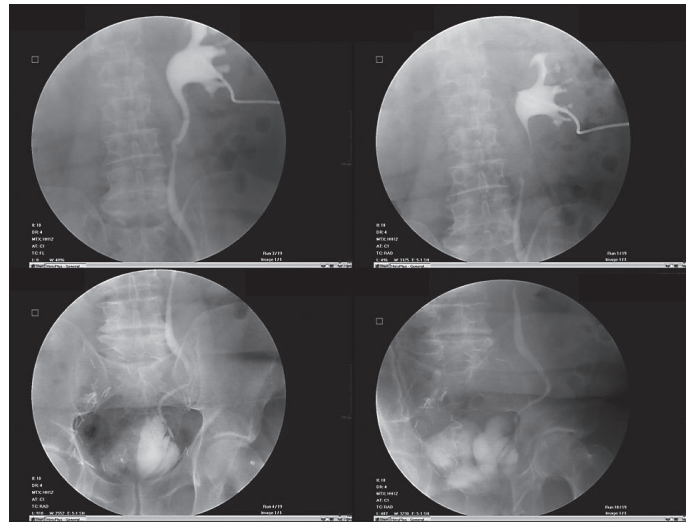

Figure 5 – Postoperative nephrostomography.

percutaneous tract was established by 30F balloon dilation (Figure 2b). The calculus was identified by rigid nephroscope, and fragmentation performed by ultrasonic lithotripter. Significant fragments were retrieved by duckbill stone grasper. Following stone removal, inspection of the upper calyces and ureter was performed by flexible nephroscope (Figure 2c). Spotted remaining fragments were removed by tipless nitinol basket while a significant fragment, identified within proximal ureter, was fragmented by Ho:YAG laser. Intra-operative stone-free status was confirmed by fluoroscopy and antegrade pyelography. An 18F council catheter was placed into the renal pelvis (Figure 2d). Sequential exchange and withdrawal of wires and catheters were performed antegradely to avoid retrograde microbial migration from the pouch to the collecting system.

Postoperative course was uneventful. Gas tube was removed the 1st post-operative day. Patient was discharged the 2nd post-operative day after removal of council catheter. Nephrostomy tube remained closed but not removed. Stone-free status was confirmed by KUB (kidney-ureter-bladder) the 2nd post-operative day (Figure 4). One month after the procedure, a nephrostomography was performed to confirm stone-free status and ureteral patency (Figure 5). Nephrostomy tube was safely removed. Stone analysis revealed a mixed type stone, composed by struvite and apatite.

Discussion

Mainz pouch II technique was first described in 1993 (Fisch et al., 1993). The recto-sigmoid reservoir is a low pressure pouch providing continence, protection of the upper urinary tract and minimal morbidity (Bastian et al., 2004). Compared to other types of continent diversions, Mainz II results to notable decrease in mean and peak bowel contraction pressures, as confirmed in postoperative recto-dynamic studies (Okhunov et al., 2011).

It is well-established that patients undergoing urinary diversion are at amplified risk of calculi formation. Reported prevalence varies between 3% and 43%. Risk factors include postoperative anatomical changes resulting to urinary stasis and mucus reflux into the upper tract, bacterial colonization and diversion-associated metabolic disorders (Okhunov et al., 2011).

As it has been previously reported, PCNL represents the preferred treatment option for large renal stones in patients with urinary diversion (Okhunov et al., 2011). Identification of the neo-ureteral orifices and retrograde pyelography are challenging steps in these cases. Ultrasound-guided or blind puncture is usually needed in order to obtain primary access and opacify the collecting system. In our case, ultrasound-guided nephrostomy insertion had preceded the operation. The nephrostomy tube was utilized for antegrade pyelography and lower calyx puncture under fluoroscopy guidance.

Under the contribution and assistance of gastroenterologists, we obtain a "through and through" wire. This step represents a significant part of the

procedure since it offers maximal safety by providing a retrograde pathway.

Stone analysis revealed a mixed type stone, consisting by struvite and calcium phosphate. As it has been reported before, the above stone types represent the most common types of diversion stones (Okhunov et al., 2011).

Surgical management of renal stone disease in patients with urinary diversion requires detailed evaluation and individualized consideration depending on stone location and burden, diversion type and surgeon's experience. To the best of our knowledge, this is the first case of percutaneous nephrolithotomy in a patient with Mainz pouch II urinary diversion that has been reported in the English literature. Although the experience regarding renal stone management in patients with Mainz pouch II is minimal, we may report that PCNL can be performed with safety and maximal efficacy. The combination of rigid and flexible scopes is mostly needed to obtain postoperative stone-free status. A "through and through" wire is strongly advised since it may provide retrograde and antegrade access to the collecting system.

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