The Connection between MicroRNAs from Visceral Adipose Tissue and Non-Alcoholic Fatty Liver Disease

Veronika Zubáňová[1,*], Zuzana Červinková[2], Otto Kučera[2], Vladimír Palička[1]

ABSTRACT

Non-Alcoholic Fatty Liver Disease (NAFLD) is one of the most important causes of liver disease worldwide leading the foreground cause of liver transplantation. Recently miRNAs, small non-coding molecules were identified as an important player in the negative translational regulation of many protein-coding genes involved in hepatic metabolism. Visceral adipose tissue was found to take part in lipid and glucose metabolism and to release many inflammatory mediators that may contribute to progression of NAFLD from simple steatosis to Non-Alcoholic SteatoHepatitis. Since visceral adipose tissue enlargement and dysregulated levels of miRNAs were observed in patients with NAFLD, the aim of this paper is to reflect the current knowledge of the role of miRNAs released from visceral adipose tissue and NAFLD.

KEYWORDS

non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; NAFLD; NASH; microRNA; miRNA; adipose tissue; inflammation; lipid metabolism

AUTHOR AFFILIATIONS

[1] Department of Clinical Biochemistry and Diagnostics, Charles University, Faculty of Medicine in Hradec Králové and University Hospital Hradec Králové, Czech Republic
[2] Department of Physiology, Charles University, Faculty of Medicine in Hradec Králové, Czech Republic
* Corresponding author: Department of Clinical Biochemistry and Diagnostics, Faculty Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic; e-mail: zubanovv@lfhk.cuni.cz

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INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) also known as Metabolic Associated Fatty Liver Disease (MAFLD) (1) is the most common chronic liver disease, which afflicts approximately a quarter of the worldwide population (2, 3). However, there also exist some variances in prevalence according to interindividual differences, such as ethnicity and gender. The highest prevalence has been reported in industrialized countries (Western Europe, USA and Middle East) and the lowest number of affected people is in Africa (3, 4). NAFLD is defined by liver fat accumulation in the absence of any other known cause of hepatic steatosis (extensive alcohol intake, chronic viral hepatitis, drug intake or genetic disorders etc.) (5). After hepatitis C, NAFLD is the second leading cause for liver transplantation in developed countries such as the USA. This is the result of asymptomatic follow-up in most of NAFLD patients, which may lead to late detection of the disease at an advanced stage (3).

MicroRNAs (miRNAs) are approximately 20 nucleotides long naturally occurring non-coding RNAs, which at post transcriptional level negatively regulate a large number of genes playing the role in many physiological and pathophysiological processes in the liver and in the human body in general (6–9). Until now, few thousand miRNAs were identified in human genome. miRNAs can be classified according to their characteristics and well defined role in organ development, and may be used as a very powerful, specific and sensitive biomarker in NAFLD diagnosis and follow-up as it was previously seen in many human diseases (7, 10). It has been suggested that microRNAs may influence almost 60% of human protein-coding genes (11). To regulate gene expression, they bind to 3′ untranslated region (UTR) of complementary mRNA (messenger RNA) target and inhibit its translation to protein or cause mRNA degradation. According to this effect, they are involved in the regulation of many biological processes, such as glucose, cholesterol or iron homeostasis, immune response or cellular proliferation, differentiation and cell death as it was reviewed in many articles (8, 9, 12–14). Since one miRNA can target multiple genes (miRNA multi-functionality) and multiple miRNAs may influence a single gene (redundancy), it is difficult to identify direct connection between miRNA and the target gene (9).

NAFLD PATHOGENESIS AND PROGRESSION

The pathogenesis of NAFLD is multifactorial and is triggered by environmental factors such as hypernutrition (excessive caloric intake, high intake of saturated fats, cholesterol, fructose, etc.) and physical inactivity in the context of genetic predisposition (15). Elevated incidence of NAFLD is related to the worldwide rise in metabolic syndrome. NAFLD is associated with increased risk of cardiovascular disease, metabolic syndrome and cancer development (16, 17). Metabolic syndrome (MS) is defined as a complex disorder including many interconnected factors (dyslipidemia, abdominal obesity, dysregulated glucose metabolism, etc.) that together contribute to higher risk of cardiovascular disease and type 2 diabetes mellitus (18). Above 90% of patients with NAFLD have more than one component of MS and about 60% of patients with liver disease have the complete diagnosis of MS (minimal 3 of 5 signs) (19). Insulin resistance (IR) was identified as a central point in pathogenesis of NAFLD, thus considering NAFLD to be the liver manifestation of metabolic syndrome. IR leads to the retention of triacylglycerols (TAG) by the hepatocyte together with impaired carbohydrate metabolism (20). In general, insulin decreases the production of VLDL (very low-density lipoprotein) by inhibition of adipose tissue lipolysis and hepatic VLDL production. Nevertheless, in patients with NAFLD (or MS) insulin fails to inhibit lipolysis and hepatic production, leading to an increase in serum triacylglycerols and their accumulation in the liver (21). In this case, the global prevalence of NAFLD among patients with type 2 diabetes mellitus (T2DM) was estimated to be almost 60% (22).

Several dysfunctions may participate in the pathogenesis of NAFLD such as alterations in metabolic pathways, imbalance in lipid import, *de novo* formation, oxidation and export, and thus leading to lipid accumulation in the liver (steatosis is defined as hepatic TAG content > 5% of liver weight) and to further progression – inflammation state (steatohepatitis) complicated with fibrosis, cirrhosis and carcinoma formation (Figure 1) (23). Non-Alcoholic SteatoHepatitis (NASH), an advanced stage of fatty liver disease, develops in 10–30% of patients with simple steatosis and in 40% of T2DM patients where is associated with a worse long-term prognosis (22). NASH is accompanied by hepatocellular damage characterized by hepatocellular ballooning, apoptosis, lobular inflammation and with or without hepatic fibrosis (23). Contrary to simple steatosis, NASH may progress to cirrhosis and, in some number of patients to hepatocellular carcinoma (23, 24). Unique miRNA profiles were found to be changed in obesity and different stages of NAFLD. Sharma et al. also found correlation of differentially expressed miRNA with various variables such as body weight, body mass index, fasting glycemia, TAG concentrations, adipocyte differentiation or adipose tissue inflammation (25). In addition to previously mentioned study, Klöting et al. found higher miR-17-5p, miR-132 and miR-134 expression and opposite pattern of miR-181a in omental fat comparing the group of patients with T2DM and normal glucose tolerance (26). Some miRNAs were identified to be dysregulated in omental and subcutaneous fat (subcutaneous adipose tissue – SAT) that significantly correlate with glucose and lipid metabolism parameters, adipose tissue morphology (macrophage infiltration, adipocyte volume) and visceral fat area, suggesting the role of miRNAs in obesity associated disorders (26). As an example, serum free fatty acid levels negatively correlate with adipose tissue level of miR-99a in human. Additionally, an expression of miR-155 was significantly related to macrophage infiltration and the strongest correlation was found between the mean omental adipocyte diameter and miR-198. These findings point out a role of miRNAs in immune cells attraction to adipose tissue and thus its proinflammatory role in NAFLD development (26). Furthermore, it has been demonstrated that visceral adipose tissue (VAT) and SAT exhibit specific gene expression patterns and in case of

that regulate different physiological functions (27). Up to now, an exact role of miRNAs is difficult to estimate, and *in silico* analyses are widely used. For instance, Capobianco et al. studied miRNA and protein expression profiles of visceral adipose tissue. Based on the transcriptomic and proteomic profiles, they found a functional association between dysregulated miRNA and corresponding protein. Two pairs were identified as contributors to altered gene expression in obesity, namely, miR-141/YWHAG (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide) and miR-520e/RAB11A (Ras-related protein). Decreased expression of miRNA was associated with up-regulation of their pairing proteins and this hypothesis was also validated by functional analysis with luciferase assay confirming binding site of these miRNAs on 3'UTR region of corresponding transcripts (28). However, further detailed research on miRNA functional studies is needed.

THE ROLE OF VISCERAL ADIPOSE TISSUE IN NAFLD

Adipose tissue plays a central role in health and disease. Its primary function is to store energy in the form of triacylglycerols and release them in the usable form during fasting and starvation, when energy is needed (29). In general, obesity is characterised by an excess of white adipose tissue. Central obesity, i.e., fat accumulated in VAT, is stated to be the major risk factor for metabolic-syndrome related disorders. Although, there is considerable physiological difference in genders, whereas in men VAT represents almost 20% of total fat and in women 8% (16, 29). The abdominal adipose tissue has been characterised as an endocrine tissue, releasing many hormonal signals that may affect the hypothalamic-pituitary-gonadal axis (30, 31). However, few epidemiological studies revealed the fact, that NAFLD occurred also in group of non-obese patients without metabolic syndrome (32, 33). In the group of non-obese Chinese adults, hepatic fat accumulation showed the relation to abdominal fat examined by CT (34).

A) VAT inflammation

In obesity, adipocytes enlarge their size due to higher accumulation of TAGs. However, it is hypothesised that this expansion may be limited and fat overload of adipocytes may create a hypoxic environment (35), the condition leading to adipocyte death (36). Monocytes are then attracted and infiltrate adipose tissue partially due to monocyte chemoattractant protein-1 (MCP-1), chemokine released from damaged adipocytes (37). Knockout or pharmacological blockage of MCP-1 receptor gene reduced macrophage infiltration of adipose tissue and insulin resistance in mouse on high-fat diet (38). In addition, the anatomy of the liver enables the portal and arterial circulation interaction together with hepatocytes and liver immune-system cells. In comparison to subcutaneous adipose tissue, VAT has a greater lipolytic potential and free fatty acids (FFAs) released from visceral fat are directly transported to the liver via portal circulation. These FFAs, together with exogenous fat supply and *de novo* lipogenesis are the main source of hepatic TAGs in NAFLD (39).

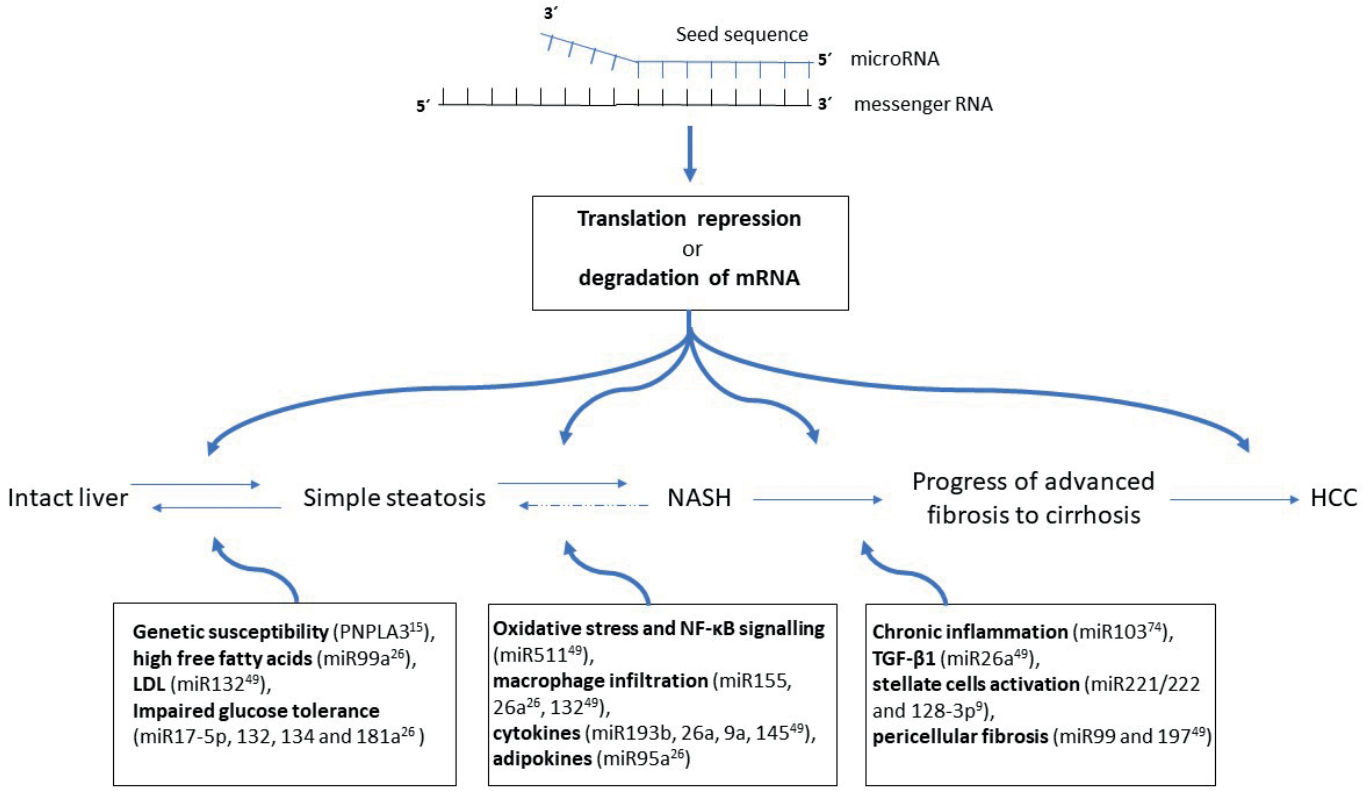

Fig. 1 Schematic diagram of possible effect of miRNAs on pathogenesis of NAFLD. HCC: Hepatocellular Carcinoma; NASH: Non-Alcoholic SteatoHepatitis; PNPLA3: Patatin-like Phospholipase Domain-containing Protein 3; TGF-β1: Transforming growth factor β1.

B) VAT mediators

The excessive expansion of VAT results in low-grade inflammation due to the presence of macrophages secreting several bioactive mediators. Dysregulation in soluble signalling molecules such as cytokines and adipokines (leptin, resistin, visfatin, interleukin 6 (IL-6) and 8 (IL-8), tumour necrosis factor alpha (TNF-α), adiponectin) secreted by visceral adipose tissue can partially contribute to proinflammatory state participating on NAFLD progression to NASH, NASH-associated fibrosis and insulin resistance (40, 41). Since the role of adiponectin is to protect the liver from inflammation via direct antagonism of TNF-α, dysregulation of adipocytokines may represent and important link between fat mass in obesity (metabolic syndrome) and NAFLD development (42). Adiponectin exhibits insulin-sensitising effects in the insulin-dependent tissues, such as the liver, skeletal muscle and adipose tissue. Concentrations of adiponectin were found to be downregulated in obesity and T2DM, in which inversely correlated with fat amount (43). Adipose tissue shows selective gene expression patterns, to which miRNAs contribute (26, 29, 44, 45). Enlargement of adipose tissue is associated with a transcriptional alteration of coding and also non-coding RNAs. In that case, miRNA expression from VAT may contribute to the pathogenesis of NAFLD, as it was detected in previous studies revealing their association with NAFLD (41, 46).

In comparison to subcutaneous adipose tissue, visceral adipose tissue contains larger adipocytes resistant to insulin and macrophages releasing pro-inflammatory cytokines such as TNF-α, IL-6 and C-reactive protein (47). Many miRNAs, such as miR-193b, miR-26a, miR-9a, miR-145, were found to regulate the production of MCP-1, TNF-α and adiponectin during visceral adipose tissue inflammatory process (48). Several adipokines have been reported to be related to changes in miRNA profile, and differential expression of miRNAs was found in the liver and also in VAT of patients with NAFLD (49). Estep et al. studied expression of miRNAs in visceral adipose tissue from NASH patients in which they found overall down-regulation of miRNA expression in comparison to non-NASH NAFLD. In addition, the decreased levels of miR-99 and miR-197 were significantly associated with pericellular fibrosis in NASH. Other miRNAs were confirmed to be associated with insulin receptor pathway (IGF1, IGFR13), cytokines (CCL3, IL6), genes connected to inflammation response (NFκB1, RELB, FAS) or ghrelin/obestatin gene. Moreover, four of differentially expressed miRNAs are positioned inside genes connected to metabolic syndrome, liver disease, obesity or T2DM, confirming their possible effect on target genes (49). This down-regulation of miRNAs was also found in the serum of NASH patients, but without any relationship to the stage of fibrosis (50). The study of Cheung et al. also supported these findings when they found eighteen miRNA species to be differentially expressed in the adipose tissue of NASH patient and confirmed their dysregulation in the liver of NASH patients (51).

In comparison, some miRNAs were found to be upregulated in adipose tissue. Chartoumpekis et al. found increased levels of miR-146a and miR-146b in obese mice fed by long-term high-fat diet (52). On the other hand, Latorre et al. investigated the decrease of few miRNAs in obese subject with NAFLD. In concordance with the results of human studies, palmitic acid used as an inductor of FA accumulation in hepatocytes *in vitro*, inhibited expression of many miRNAs in cell cultures, increased fatty acid transport, and fat formation, but reduces glucose metabolism and fatty acid oxidation (53). Down-regulation of miRNA miR-146a was also examined in peripheral blood mononuclear cells from T2DM patients when compared to healthy subjects suggesting the role of miR-146a in insulin resistance (54).

THE ROLE OF MICRORNA IN NAFLD DIAGNOSTICS

Until now, it has not been possible to predict NAFLD outcome through the routinely used blood and tissue markers due to their limited sensitivity and tissue specificity. The ultrasonography is a gold standard in NAFLD non-invasive diagnostics, but it has low sensitivity (detects fatty liver when approximately 30% of area is affected by liver steatosis) (55). The best tool for revealing the fine details of histopathological severity is liver biopsy. Nevertheless, this technique is invasive and could be accompanied by some severe complications, thus limiting its indication. Liver biopsy is usually performed at progressed stage of the disease and is not routinely used for mass screening or disease follow-up (56). Due to the limitations of liver biopsy, the use of non-invasive markers has emerged in recent years.

A) Circulating miRNAs

The biggest advantage of miRNAs is their use in non-invasive diagnostic due to their presence in the body fluids such as serum, plasma or urine and correlation between free circulating and tissue levels and thus reflecting the physiological/pathological state of tissue they are released from. Circulating miRNAs can result from a passive release in case of cell death or an active secretion to extracellular space in the form of microvesicles. In that case, they may indicate hepatocellular damage during liver injury or inflammation as described previously (56–58). Due to their presence in microvesicles and exosomes, they show high stability (contrary to long RNAs) in the environment containing RNases (RNA-degrading enzymes) (56, 58). The presence of specific surface proteins on exosomes reflects tissue or cell origin and function in cell-to-cell communications (56, 59). During NASH ballooning degeneration, cell death is enhanced, and many miRNAs are released into the circulation. This feature is thought to mirror molecular event in NAFLD (57). Some miRNAs also exist without cover or are incorporated in and protected by Argonaute 2 and surrounded proteins in high-density lipoproteins (HDL) (56, 58).

RESEARCH MODELS ON THE ROLE OF MICRORNAS IN NAFLD

Murine models play a key role in the deeper understanding of therapeutic effects and provide an opportunity for research using genetic manipulation. Popular models of obesity are genetically and diet-induced animals (mouse)

or, on *in vitro* level, preadipocyte cell-culture differentiated into adipocytes (60).

Adipocyte differentiation as a cellular process is regulated by several important regulatory factors, nevertheless the whole mechanism is not fully understood. miRNAs together with insulin secretion have been shown to be involved significantly in stem cell modulation and adipocyte differentiation (61, 62). These results were supported by *in vitro* functional analysis of Esau et al. The reduction of miR-143 by transfecting antisense oligonucleotides inhibited adipocyte differentiation, suggesting that miRNAs may regulate adipose tissue processes (63). Furthermore, the study of Bergenstrate revealed almost 70 miRNAs to be differentially expressed in mesenchymal stem cell-derived adipocytes (terminally differentiated adipocytes) compared to progenitor cells. They also validated the results of *in vivo* studies in mouse and *in vitro* studies in mutant mouse line (64). Moreover, miR-21, miR-27a, miR-103a, and miR-320 have been reported to be involved in adipocyte proliferation and differentiation (65–68). The functional study demonstrated insulin-induced improvement of glucose uptake in insulin-resistant adipocytes after anti-miR-320 oligo addition. Prior to these manipulations, miR-320 expression in insulin-resistant cell line (3T3-L1) was approximately 50 times higher compared to normal cell expression. These results suggest the role of miR-320 in targeting phosphoinositide-3-kinase, regulatory subunit 1 (68). The findings are in the accordance with study of Lin et al., who demonstrated influence of miR-27 overexpression in inhibition of adipocyte formation. Also, adipogenic regulators PPAR-γ (peroxisome proliferator-activated receptor γ) and C/EBPα (CCAAT-enhancer binding protein α) represent targets for this miRNA, which contributes to their close link (69). PPARs are ligand-activated transcription factors from nuclear receptor subfamily that function as lipid and glucose metabolism, energetic, inflammation and atherosclerotic regulators. Few isoforms can be found in different tissues: PPAR-α, PPAR-β/δ and PPAR-γ that is highly expressed in adipose tissue (70, 71). Additionally, data from PPAR-α null mice supported this hypothesis. Activation of this protein was reported to control the metabolic response of adipose tissue to diet income and induce cleavage of intracellular lipids through fatty acid oxidation (72). In adipose tissue, activation of PPAR-γ regulates the change of the macrophages to M2 subtype, that exerts in contrast to M1 type, anti-inflammatory action and may be preventive in NAFLD development (73). In another study, few miRNAs were found to be pro-adipogenic (miR-143, miR-103, miR-146b, miR-148, miR-33b) and serve in the process of preadipocytes accelerated adipogenesis. This process was accompanied by increased expression of transcription factors (PPARγ2), cell cycle regulators, glucose transporters and adipokines (74). However, miR-143 and miR-103 were found to be significantly decreased in adipocytes from obese mice. On the contrary, miR-222 and miR-221 were downregulated during adipogenesis and conversely upregulated in adipocytes from obese subjects. In general, miRNAs that were induced during adipogenesis, were underexpressed in adipocytes from obese mice and *vice versa*, suggesting that these changes are related to chronic local inflammatory environment in adipose tissue (74). Intronic miRNA – miR-33b and its target gene SREBP-1 (sterol regulatory element-binding protein 1) were also highly upregulated during preadipocyte differentiation and miR-33b inhibition decreases adipose cell differentiation and lipid droplet accretion (75). Moreover, miR-146b expression was increased in high fat (HF) diet-induced obese mice, db/db mice and ob/ob mice its and expression of its target gene SIRT1 (sirtuin 1) was found to be decreased in white adipose tissue representing induced adipogenesis based on SIRT1 pathway. In addition, *in vivo* neutralization of miR-146a revealed increased SIRT1 expression relieving the impact of diet-induced obesity (76).

Similarity of rodent model with human NAFLD or NASH is assessed by liver histology, where degree, phenotype of inflammation and fibrosis are rated (17). For example, HF diet leads to the development of the steatotic phenotype that most characterizes human NAFLD/NASH at early stage. Teufel et al. analysed systemic gene expression profile of liver tissues from patients at different stages of NAFLD compared to various rodent models of NAFLD. Mice model based on high-fat diet seems to be the most similar to human liver affected by NAFLD as documented by resemblance to expression patterns analysed at pathway level (17). Even though the nutritional manipulation-based mouse model is considered the best in terms of correlation to human pathology, no single model is able to reflects the whole process of human disease. However, currently, there are only few models that can mimic the individual aspects of NAFLD (high-fat/high-fructose diet mouse model) (17, 77, 78).

CONCLUSIONS

Genetics, environment and dietetic habits play a key role in NAFLD development and progression. Epigenetic factors influence genetic base of the disease and increase individual susceptibility to NAFLD and may stand for great phenotype variability.

Since visceral adipose tissue secretes significant amount of cytokines and adipokines, adipocytes play an important role in fatty liver development. Genes encoding these inflammatory molecules were found to be regulated by epigenetic factors, thus indicating that understanding of differential expression of miRNAs in the adipose tissue is useful for better understanding of mechanisms participating in the development of NAFLD and its progression to advanced stages. Hopefully, circulating miRNAs secreted from tissues, may become promising tool for non-invasive diagnostic differentiation between NASH and NAFLD.

AUTHOR CONTRIBUTIONS

All authors took part in writing the manuscript. All authors read and approved the final draft.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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The Effects of Ranitidine Treatment on the Risk of Necrotizing Enterocolitis in Preterm Infants: A Case-Control Study

Zlatan Zvizdic[1], Emir Milisic[1], Asmir Jonuzi[1], Sabina Terzic[2], Denisa Zvizdic[3], Semir Vranic[4, 5,*]

ABSTRACT

Introduction: Gastric acidity plays an important role in the protection of infants against various pathogens from the environment. The histamine-2 receptor blockers (H2-blockers) are off-labeled drugs that are frequently prescribed in preterm neonates to prevent stress ulcers. The impact of the H2-blockers on the development of the necrotizing enterocolitis (NEC) in preterm infants is still controversial, particularly in the developing world.

Materials and Methods: One hundred twenty-two preterm infants were enrolled in the study. The multivariate logistic regression model was used to identify potential postnatal risk factors associated with NEC.

Results: Preterm infants (n = 51) with total NEC, medical NEC, and surgical NEC had the highest rate of receiving ranitidine compared with controls (n = 71) (39.2%, 19.6%, and 47.6%, $p < 0.05$). Logistic regression analysis revealed that ranitidine use and nosocomial infections were significantly associated with NEC development (odds ratios 1.55 and 3.3).

Conclusions: We confirm that ranitidine administration was associated with an increased risk of NEC in preterm infants. H2-blockers use should be only administered in very strictly selected cases after careful consideration of the risk-benefit ratio.

KEYWORDS

ranitidine; necrotizing enterocolitis; preterm infants; developing countries

AUTHOR AFFILIATIONS

[1] Clinic of Pediatric Surgery, University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina
[2] Pediatric Clinic, Neonatal Intensive Care Unit, University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina
[3] Eye Clinic, Department of Pediatric Ophthalmology, University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina
[4] College of Medicine, QU Health, Qatar University, Doha, Qatar
[5] Biomedical and Pharmaceutical Research Unit, QU Health, Qatar University, Doha, Qatar
* Corresponding author: College of Medicine, QU Health, Qatar University, PO Box 2713, Doha, Qatar, e-mail: semir.vranic@gmail.com or svranic@qu.edu.qa

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INTRODUCTION

Despite many advances in the treatment of vulnerable preterm infants, necrotizing enterocolitis (NEC) remains the most common gastrointestinal tract emergency in the neonatal period with a substantial morbidity and a mortality rate (1–3). These rates may be substantially higher in developing countries (4–7). The well-established risk factor for NEC is prematurity while bowel ischemia, enteral feeding, and bacterial colonization pose an additional risk (8).

Prescription of inhibitors of gastric acid secretion (IGAS)/histamine-2 receptor blockers (H2-blockers) in preterm infants has been a widespread practice in the neonatal intensive care units (NICUs) because H2-blockers may prevent the gastric bleeding. The bleeding is usually caused by septic shock, hypotension, ischemia, and drugs such as indomethacin and corticosteroids (9). However, an increasing evidence suggests that some external factors including those that reduce gastric pH may affect the gastrointestinal bacterial milieu and thus predispose premature infants to inflammatory bowel disorder (10). Gastric acidity is considered a major non-immune defense barrier against infections in neonates through the reduction of the number of ingested bacteria. It consequently reduces the possibility of bacterial dissemination into the bloodstream via an immature and leaky gut barrier (11). Hence, the pathophysiological mechanism responsible for gastrointestinal bacterial overgrowth prompting infections may be triggered by blockade of gastric acid secretion by administration the H2-blockers (e.g., ranitidine, cimetidine) and proton pump inhibitors (PPIs; e.g., omeprazole). Bacterial overgrowth and infections due to an inefficient elimination of ingested pathogens are more common if gastric acidity is suppressed and the gastric pH rises above 4.0 (12–15). Since bacterial colonization and translocation are some of the important mechanisms in the pathogenesis of NEC, it is understandable the finding of some studies that the use of H2-blockers may predispose the development of NEC (16–18). Notably, Singh et al. could not confirm an association between H2-blockers administration and an increased risk of infection and NEC in preterm infants (19).

Several other risk factors for the NEC development in the neonatal intensive care units (NICU) have been reported including the use of invasive respiratory support (20), administration of morphine sulfate (21), the presence of circulation impairment requiring inotropic drug administration (22), erythrocyte transfusions (23), and nosocomial infections (24).

The data related to NEC are limited from resource-limited countries where the burden of infection is often the highest (25). In this context, we investigated the relationship between H2-blockers use and the development of NEC in a pediatric population originating from a middle-income population (Bosnia and Herzegovina). In addition, we explored other potential postnatal risk factors associated with NEC using multivariate logistic regression.

PATIENTS AND METHODS

PATIENTS AND SELECTION CRITERIA

We conducted a case-control study at the level III neonatal intensive care unit (NICU) of the University Clinical Center Sarajevo (Bosnia and Herzegovina), on 122 preterm infants classified into NEC group and control group. All patients were treated in the period 2008–2012. Sixty-seven (55%) of infants were born at our obstetrics department while the remaining 55 patients (45%) were transferred from other hospitals, typically (98%) within 24 hours following the birth.

NEC group included 51 consecutive preterm infants treated for NEC ≥ stage II. Based on the severity of the disease, the NEC group was further divided into medical NEC and surgical NEC groups. A randomly selected control group (N = 71) *was* formed from preterm infants without NEC whose clinical features were similar to those in the exposed group. The control group was treated in the same period as the NEC group (2008–2012).

The eligibility criteria were gestational age at delivery < 37 weeks of gestation and diagnosis of NEC ≥ stage II, defined according to modified Bell's staging criteria (26). Gestational age was determined by an early ultrasound and recorded as completed weeks. A preterm birth was defined as any birth before 37 weeks of gestation or fewer than 259 days since the first day of the woman's last menstrual period. Medical NEC was defined as NEC that met Bell stage IIA, IIB, or IIIA criteria (26). Surgical NEC was defined as NEC requiring a surgical treatment and met Bell stage IIIB criteria (Supplemental Table 1). The infants were ineligible if they had major congenital anomalies, congenital heart disease, and gastrointestinal disorders that required surgery. The preterm infants who developed infection or NEC within 48 hours after receiving ranitidine were also excluded from the study.

The ranitidine was administered intravenously to all patients using our in house protocol: for term infants: 1.5 mg/kg/dose every 8 hours and for preterm infants at 0.5 mg/kg/dose every 12 hours. All patients were treated with ranitidine 5–7 days (mean: 6.15 days). The treatment with ranitidine was immediately terminated if the risk of complications (infections, NEC) would appear (mean: 56.2 hours following the initiation with ranitidine, range: 28–84 hours). A neonatologist made the decision for ranitidine use. The indications for ranitidine use included prevention and treatment of potential digestive tract bleeding, primarily gastric bleeding confirmed by blood-stained aspirate from nasogastric tube.

Nosocomial infection was defined as an infection that occurs after 48 hours following birth, resulting in a positive blood, cerebrosinal fluid, or urine culture with clinical manifestation such as hospital-aquired bloodstream infection, nosocomial pneumonia, sepsis, urinary tract infection, and meningitis.

The infants' medical records were reviewed daily for medical course information until hospital discharge or death of an infant. A standardized form was used for data collection. All medical records were anonymized for the study purposes.

Institutional Review Board (IRB) of University Clinical Center Sarajevo approved the study (Number of approval: 0305-11118/2010) and waived informed consent request given the non-interventional nature of the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the IRB and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

STATISTICAL ANALYSIS

Descriptive statistics were used to characterize the patients' cohort. The association between H2-blockers use and NEC was evaluated using Chi-square test. Preterm infants with NEC were compared on their relative odds by using multivariate logistic regression. A multiple logistic regression analysis was then used to identify potential postnatal risk factors associated with the development of NEC. The results were expressed as odds ratios (ORs) and 95% confidence intervals (CIs). Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) IBM Version 26 (SPSS) (UNICOM Systems, Inc.). *P*-values < 0.05 were considered significant.

RESULTS

Demographic data and clinical characteristics of the cohort are summarized in Table 1.

The mean gestational age of the total NEC group, medical and surgical NEC group and control group were similar. There were also no statistically significant differences between the groups concerning sex and mode of delivery (p > 0.05). The Apgar score reported at 1 minute in infants of the study and control groups was 5.3 ± 2.3 and 6.1 ± 1.8, respectively, and the difference was of the borderline significance (p = 0.05). The five-minute Apgar score in infants in the surgical NEC group was also significantly higher compared with the control group (p = 0.03). We also observed differences in the birth weight between the two groups (p = 0.02), particularly between the medical NEC subgroup and control infants (p = 0.001).

Among infants with NEC, the medical NEC was diagnosed in 30 patients (58.8%), while 21 patients were in the group of surgical NEC (41.2%). Postnatal age at a time of appearance of NEC was 18.2 days (SD 12.8 days, range 2–57 days) while 49% of infants were older than 2 weeks. The patients were breastfed in a majority of cases (40/51).

Tab. 1 Demographic and clinical characteristics of the cohort.

	Total NEC n = 51	**Control group n = 71**	**Medical NEC n = 30**	**Surgical NEC n = 21**	***p*1 Total NEC vs. Control group**	***p*2 Medical NEC vs. Control group**	***p*3 Surgical NEC vs Control group**	***p*4 Medical NEC vs. Surgical NEC group**
Mean GA age at birth/weeks (Mean ± SD)	30.2 ± 3.7	30.9 ± 2.7	29.8 ± 3.2	30.6 ± 4.3	NS	NS	NS	NS
Mean birth weight/g (g) Mean ± SD	1502.7 ± 781.5	1609.8 ± 459.3	1316.7 ± 574.3	1768.6 ± 960.4	p = 0.02*	p = 0.001*	NS	NS
Gender (M:F) (n)	30/21	39/32	14/16	16/5	NS	NS	NS	NS
Type of delivery Vaginal / Caesarian (n)	33/18	36/35	17/13	16/5	NS	NS	NS	NS
APGAR 1 Mean ± SD	5.3 ± 2.3	6.1 ± 1.8	5.3 ± 2.2	5.1 ± 2.4	p = 0.05*	NS	NS	NS
APGAR 5 Mean ± SD	6.3 ± 1.8	6.8 ± 1.4	6.5 ± 1.9	5.9 ± 1.8	NS	NS	p = 0.03*	NS
Antenatal steroids (yes/no)	13/38	21/50	11/19	2/19	p = 0.6209 NS	p = 0.2904 NS	p = 0.1321 NS	p = 0.0302*
IUGR (yes/no)	7/44	4/67	5/25	2/19	p = 0.1253 NS	p = 0.0768 NS	p = 0.5282 NS	p = 0.4701 NS
PDA (yes/no)	14/37	3/68	9/21	5/16	p = 0.0003*	p = 0.8071 NS	p = 0.7517 NS	p = 0.6293 NS
Length of hospital stay ± SD	49.6 ± 36.3	35.6 ± 20.5	48.5 ± 32.2	51.2 ± 42.2	p = 0.0180*	p = 0.0310*	p = 0.0379*	NS
Mortality rate (Death/Total)	0.333 (17/51)	0.0986 (7/71)	0.1667 (5/30)	0.5714 (12/21)	p = 0.0039*	p = 0.3644	p < 0.0001*	p = 0.013*

* Significant p-values (*p* < 0.05)
NS = non-significant *p*-value; SD = standard deviation; NEC = necrotizing enterocolitis.

Tab. 2 Potential postnatal risk factors associated with the development of necrotizing enterocolitis in preterm infants.

Variable	OR	95% CI	*P*-value*
H2-blockers	1.5503	1.0514–2.2861	**0.03**
Nosocomial infections	3.3169	1.0993–10.0086	**0.03**
Intubation and ventilation	1.1848	0.9671–1.4515	0.10
Nasal continuous positive airway pressure	0.9117	0.7618–1.0911	0.31
Morphine sulfate	1.8500	0.9242–3.7031	0.08
Inotropes	0.9621	0.6542–1.4148	0.84
Red blood cell transfusions	0.6474	0.3022–1.3870	0.26

* Only significant variables were bolded.
OR = odds ratio; CI = confidence interval; H2-blockers = histamine-2 receptor blockers.

No baby was fed by a donor breast milk. Consequently, we have not observed any differences between study groups related to the type of enteral feed. In one patient, NEC developed before starting of enteral feeding. The most common gastrointestinal symptoms were abdominal distension (89%), gross or microscopic blood in the stool (56.9%) and increasing gastric residuum (46%).

During the study period, 20/51 (39.2%) preterm infants with NEC received H2-blockers, whereas only 6/71 (8.5%) preterm infants in the control group received H2-blockers ($p < 0.001$). Among NEC cases, 10/30 (33.3%) preterm infants with medical NEC and 10/21 (47.6%) infants with surgical NEC received H2-blockers (Figure 1). We also found a statistically significant association between H2-blockers use and NEC in both medical and surgical NEC groups in comparison with the control group ($p = 0.005$, $p = 0.0001$, respectively). In contrast, no significant difference was found between medical and surgical NEC subgroups regarding the H2-blockers use and the development of NEC ($p = 0.41$).

The results of the conditional logistic regression model using seven independent variables (H2-blockers, nosocomial infections, intubation and ventilation, nasal continuous positive airway pressure, morphine sulfate, inotropes, and red blood cell transfusions) showed that the model was statistically significant ($p < 0.001$). This model could reliably predict the patients with a higher likelihood of developing NEC. H2-blockers use was associated with an increased incidence of NEC (Table 2). In addition, nosocomial infections were significantly associated with the development of NEC (Table 2).

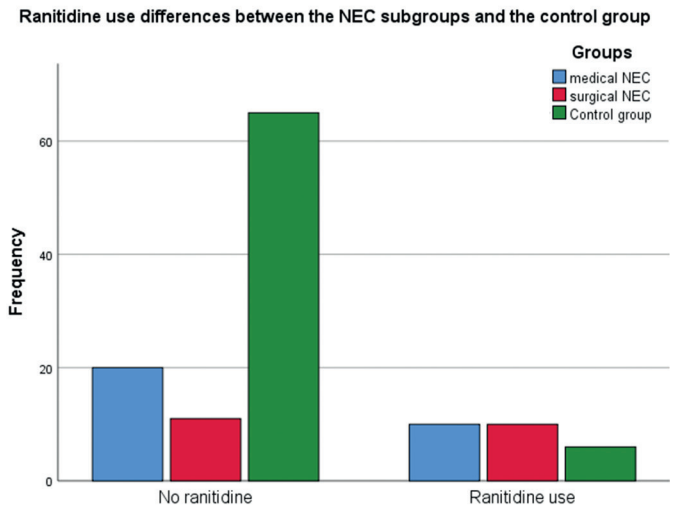

Fig. 1 Differences in the frequency of preterm infants receiving H2-blockers therapy.

DISCUSSION

NEC remains the most common gastrointestinal tract emergency in the neonatal period with a significant morbidity and mortality (2, 3, 27). Our study confirms the association between the use of H2-blockers use and NEC. Previous studies have also provided similar conclusions regarding the use of H2-blockers and NEC development (17, 18, 28–30). In a large cohort of > 11,000 very low birth weight (VLBW) infants, Guillet et al. suggested an association between the use of H2-blockers and NEC revealing that treatment with H2-blockers was associated with a higher incidence of NEC (28). Similarly, Terrin et al. in prospective study of 309 VLBW infants reported that ranitidine use was associated with increased risk of infection, NEC, and overall mortality (17). Bilali et al. in a case-control study that included 116 preterm infants with NEC reported that H2-blocker therapy was significantly associated with an increased incidence of NEC (30). Another evidence-based survey of Chandrasekaran and Fleming further confirmed that the use of ranitidine/H2 blockers is associated with an increased incidence of NEC (level 2b = individual cohort studies and low-quality randomized control trials) (31).

A recent systematic review with meta-analysis exploring the impact of H2-blockers use in neonates in NICUs on infections, NEC and mortality revealed that these drugs might increase the risk of infections and NEC in neonates. Consequently, the use of H2-blockers in neonates must be stringently considered only when necessary (32). Another systematic review by Green et al., based on 11 studies involving > 800 preterm and term neonates confirmed the efficacy of H2-blockers in preventing gastrointestinal bleeding. Given that no single study reported the incidence of NEC, the authors concluded that the safety of H2-blockers in this regard remained unclear (33).

In contrast to the numerous studies that have reported an association between H2-blockers use and NEC,

a retrospective study of Singh et al. reported that the use of ranitidine/omeprazole was not associated with adverse outcomes of late sepsis, NEC, and mortality in VLBW neonates (< 1500 grams) (19). The authors also emphasized the importance of the age at which ranitidine was commenced, suggesting that the administration of ranitidine and omeprazole in the later postnatal age of premature infants born < 1500 grams (37 days and 72 days after birth, respectively) allows for development and maturation of the immune system, thus reducing susceptibility to late onset sepsis or NEC (19). Interestingly, a systematic review on risk factors for NEC conducted by Samuels et al. did not reveal commonly assumed clinical neonatal risk factors for NEC such as central lines, red cell transfusions, H2-blockers, and a high osmolar formula feeding as important prognostic factors (34). A possible explanation for such findings may lie in the fact that the analyzed studies were not prognostic.

One of the relevant findings in our study is the impact of nosocomial infections on the NEC development among the preterm infants (35). A four-fold increase in the risk of bloodstream infection among the NICU infants who received H2-blockers was noted in the prospective study conducted by Beck-Sague et al. (36). Another study that included 91 children undergoing gastroesophageal reflux disease (GERD) treatment with gastric acidity inhibitors found an increased risk of intestinal and respiratory infectious diseases compared with 95 non-treated patients (14). Similarly, Graham et al. showed that H2-blockers use might increase the risk of infection in low birth weight (LBW) infants (37). The mechanisms by which H2-blockers use may contribute to the increase risk of NEC have not been clarified yet. It is well known that a significant function of gastric acidic juice is the inactivation of ingested microorganisms within a few minutes at pH < 3 in addition to protein denaturation, activation of pepsinogen and augmentation of intestinal absorption of iron, calcium, and vitamin B12 (38). The proposed mechanism of association between H2-blockers use and NEC in preterm infants is a loss of gastric acidity that causes an increased bacterial colonization of GI tract and consequently leads to the increased risk of preterm infants to gram-negative and opportunistic infections (17). It should be noted that the reasons for effects of ranitidine administration are not limited only to the reduction of gastric pH but also to an increase the production of pro-inflammatory cytokines and reduce immunological responses to infection (37). It is worth mentioning that the time for bacterial/substrate interaction is also increased due to reduced intestinal motility in preterm infants thus facilitating bacterial overgrowth (39).

Considering all the above concerns, it is understandable that H2-blockers induced hypochlorhydria predisposes infants to the enteric infections including NEC. An additional risk factor associated with H2-blockers use is the finding that H2-blockers strike down several functions of leukocytes, thereby leading to insufficient control of the production of inflammatory cytokines in the intestinal tract (40, 41). Since NEC is a multifactorial disease, it is unlikely that one factor (e.g. H2-blockers use) would independently increase the overall incidence of NEC, but its use increases the risk of this devastating disease.

Our study had some limitations including the inherent errors and bias of retrospective nature of the study (conducted in the period 2008–2012), small sample size and restriction to a single pediatric surgical center, which may limit the generalizability of our conclusions. A further limitation of our study is related to the lack of accuracy in indications for the ranitidine use. It may be possible that infants with early NEC symptoms were treated with ranitidine before an accurate diagnosis of NEC was made. Therefore, larger prospective studies are required to confirm the effect of H2-blockers on susceptibility to infections and NEC in preterm infants. In all pediatric indications ranitidine should be very cautiously used given the recent recommendation from the regulatory bodies from Europe, US and Canada to withdraw ranitidine from the market. Namely, it has been found that N-nitrosodimethylamine (NDMA) levels in ranitidine products might exhibit potentially carcinogenic effects.

CONCLUSIONS

Our study from the middle-income country provides further evidence for a possible association and role of H2-blockers use and NEC development in preterm infants, suggesting that H2-blockers use should be only administered in strictly selected cases after careful consideration of the risk-benefit ratio.

ABBREVIATIONS

CI	Confidence interval
FDA	Food and Drug Administration
GERD	Gastroesophageal reflux disease
GI	Gastrointestinal
H2-blockers	Histamine-2 receptor blockers
IGAS	Inhibitors of gastric acid secretion
IUGR	Intrauterine growth restriction
LBW	Low birth weight
NDMA	N-nitrosodimethylamine
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
OR	Odds ratio
PDA	Patent ductus arteriosus
PPI	Proton pump inhibitors
SD	Standard deviation
VLBW	Very low birth weight

AUTHOR CONTRIBUTIONS

Conceptualization and methodology, Z.Z. and S.V.; Formal analysis, Z.Z., E.M., A.J., S.T., D.Z..; Data curation, Z.Z., S.T., D.Z.; Writing – original draft preparation, Z.Z. and S.V..; Writing – review and editing, S.V. and Z.V..; Supervision, S.V. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Supplemental Table 1 Modified Bell's staging criteria for necrotizing enterocolitis.

Stage	Systemic signs	Abdominal signs	Radiographic signs
IA suspected	Temperature instability, apnea, bradycardia, lethargy	Gastric retention, abdominal distention, emesis, heme-positive stool	Normal or intestinal dilation, mild ileus
IB suspected	Same as above	Grossly bloody stool	Same as above
IIA definite, mildly ill	Same as above	Same as above, plus absent bowel sounds with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis
IIB definite, moderately ill	Same as above, plus mild metabolic acidosis and thrombocytopenia	Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as IIA, plus ascites
IIIA advanced, severely ill, intact bowel	Same as IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, DIC,and neutropenia	Same as above, plus signs of peritonitis, marked tenderness, and abdominal distention	Same as IIA, plus ascites
IIIB advanced, severely ill, perforated bowel	Same as IIIA	Same as IIIA	Same as above, plus pneumoperitoneum

Urban or Rural GP? In the Czech Republic It Is not just Distances That Matter

Jan Bělobrádek[1, 3,*], Luděk Šídlo[2], Kateřina Javorská[1, 3], David Halata[3]

ABSTRACT

This article proposes a combined mixed methods approach to categorising GP practices. It looks not only at location but also at differences in the nature of the work that rural GPs perform.

A data analysis was conducted of the largest health insurance company in the Czech Republic (5.9 million patients, 60% of the population, 100% coverage within the Czech Republic). We performed two data analyses, one for 2014–2015 and one for 2016, and divided GP practices into urban, intermediate, and rural groups (taking into account the OECD methodology). We compared groups in terms of the total annual cost in CZK per adult registered insurance holders. The total volume of data indicated the financial costs of €1.52 billion and €2.57 billion respectively.

Both analysis showed differences between all groups of practises which confirmed the assumption that the work of the GP is influenced by regionality. A multidisciplinary hospital is the main factor that fundamentally affects the way a GP's work in that area.

The proposed principle of categorising general practices combines geographical and cost characteristics. This requires knowledge of the cost data of healthcare payer and on the basic demographic knowledge of the area. We suggest this principe may be transferrable and particularly suitable for categorising general practice.

KEYWORDS

general practice; rural health; regional typology; workforce; efficiency

AUTHOR AFFILIATIONS

[1] Department of Preventive Medicine, Charles University, Faculty of Medicine in Hradec Králové and University Hospital Hradec Králové, Czech Republic

[2] Department of Demography and Geodemography, Charles University, Faculty of Science, Prague, Czech Republic

[3] Working Group on Rural Practise of the Czech GP Society, Czech Republic

[*] Corresponding author: Charles University, Faculty of Medicine in Hradec Králové, Department of Preventive Medicine, Hradec Králové, Czech Republic; mudrjanbelobradek@gmail.com

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INTRODUCTION

The issue of the division of territory into urban and rural one is a typical multidisciplinary topic, discussed in many disciplines – geography, demography, sociology, economics, etc. In many regions it is also a key social and political topic, especially in connection with urbanization trends that can be observed worldwide, regardless of the size, political or economic characteristics of the continent or state.

Difficulty of distinguishing rural GP practices is also a global topic that has attracted much research in all parts of the world (1–4). This is true regardless of the distribution in the given country, its economic level or used healthcare system. The key issues are how a given territory should be divided (definition of rural areas) (5–7), the equity in healthcare access (8–10), the specific nature of rural general practice (11–13), and long-term sustainability in the face of increasing urbanisation (14–16).

It might seem that a smaller area is an advantage, especially if it is a geographically homogeneous state in a stable socio-economic environment of the EU. Nevertheless, even a small Central European country such as the Czech Republic does not avoid the typical problems of rural medicine. Like elsewhere, doctors in the Czech Republic tend to prefer working in towns or cities (17). The increasing difficulty of finding medical staff for rural practices is an important local policy issue that is gradually becoming a strategic national issue (as part of the ongoing primary healthcare reforms instituted by the Czech Ministry of Health) (18).

Support for rural practices at many levels is being considered. Whether in the form of a direct financial incentive for doctors themselves (central or organized by the regional government), subsidies for the establishment, equipment and operation of practices, bonus mechanisms paid by health insurance companies, housing support (renting a municipal apartment or offering land to build a house) or supporting families of doctors (job opportunities for partners and children's education).

However, if similar support for rural practices was to exist, those practices must be properly defined (19). This is the only way how the transparency of the system, its sustainability and the minimization of clientelistic and corrupt influences are possible. In a country with a small area and a high number of healthcare providers, such as the Czech Republic is, it is not possible to rely only on geographical criteria. Compared to large countries, the distances here are short, nevertheless there is an outflow of workforce from rural areas. The question is, how it is possible to help with more precise determination of the borders between rural and urban areas? Other rural GP characteristics must also be taken into account (20).

Therefore, we decided on a combination of criteria: location and practice characteristics data, expressed in costs. In terms of regionality, we have created our own model of division of practices, which meets the conditions of the Czech Republic and takes into account the local health care system. We obtained not only cost data from the payer of the care, but also structure information of services that the payer reimburses for individual practices. We examined the extent to which the structure of GP activities is related to regionality and whether cost effectiveness or some of its components could be a suitable determinant for rurality in primary care.

The Czech Republic has currently the system of funding based on the general health care insurance (21, 22). Its health insurance companies not only pay for the care but are legally responsible for creating and maintaining the network of providers. The largest healthcare insurance company is the General Health Insurance Company (GHIC), which ensures 5.9 million patients (4.5 million adults) receive care. That is roughly 60% of the population. GHIC contracts cover almost 100% of the country and all healthcare service providers who are reimbursed through the public health insurance system (23).

The data held by the GHIC has been gradually made available for our research since 2016. The original intention was to monitor the differences between individual GP practices according to two criteria: 1. the size of the practice, ie. according to the number of registered patients, 2. the location, ie. location in the city or in the countryside. As the first results already showed that the differences according to the first criterion (size of practice) are insignificant, we continued to focus only on the second criterion (location of practice).

The Czech Republic has a capitation performance-based model of payments. Capitation performance-based model of payments – a lump sum payment for a registered insured person, the amount of which increases with the patient's age, is used in the Czech Republic. The basic performance package is reimbursed through capitation, while technical procedures and prevention are reimbursed separately (insurance company payments are made based on the actual procedures performed according to a catalogue of prices). Depending on the fulfilment of predetermined criteria (e.g. number of preventive examinations, tests for occult bleeding, influenza and other vaccinations, etc.), bonus supplements are also paid.

MATERIAL AND METHODS

The first research was conducted using data from 2014–2015. We investigated the differences in relation to the size of the pacient's list (total number of registered patients) and location of practice (urban or rural). The variables were a production of data (financial costs of GP activities – their procedures, ancillary services, specialist care, home care, and prescriptions). We compared the total annual costs in CZK per adult, that is, per "unit insurance holder" (registered insurance holders calculated using age indexation). This measure is comprehensive, enshrined in care reimbursement agreements and is generally accepted. Its aim is to prevent the disadvantage of age at registration (for example, a patient in the age of 20, GP will receive 0.9 times the capitation payment, for a patient in the age of 80 2.9 times the capitation payment). With regard to the age structure of the population in the Czech Republic, the number of unit insurance holders exceeds the number of registered insurance holders.

The basic criterion for defining location of the general practice was the presence of a multispecialty hospital.

According to the OECD method for defining territorial units we placed practices into three groups. We defined urban practices located in a town/city with a large hospital or near a town/city with a large hospital, where the catchment area was 5 km from the centre of the town/city (Type I). Usually these were GP practices in towns/cities with over 30,000 inhabitants and the remainder were all district towns (LAU 1 administrative centres). The intermediate group (Type II) comprised municipalities with extended state administration powers, where there are often smaller hospitals, and a catchment area of 10 km. The remaining areas were categorised as rural (Type III). The total number of practices (FIDs – facility ID number) does not take into account the number of hours the doctor works nor the corresponding practice hours.

The results of the above described analysis has not only generated interest in further research but led to the founding of the Working Group on Rural Practise of the Czech GP Society (24) in 2016. Nonetheless, the investigators recognised that the research methodology could be improved. A further analysis was therefore conducted, using data from 2016. The aim of this research was to investigate regional differenties between GP. The focus was on two areas: 1. Improving the accuracy of the selection of the categories, bearing in mind the OECD regional typology criteria (Table 1) (25, 26); and 2. using as wide a range of data as possible. The data was structured in the same way, updated for 2016, and included data on bonus programme payments (training, programme quality). Particular attention was paid to procedures performed directly within GP practices.

We also changed the way to establish the total number of providers because most of the data was provided by the GHIC relating to GP practices as whole providers (PIDs – provider ID number). There are many practices in the Czech Republic in which a doctor is only available for a limited time. These are, for example, situations where the GP from the city arrives some afternoon to work from the adjacent village. This separate unit cannot be considered as a full-fledged practice, especially with regard to its costs. When determining the location of a given PID number, we relied on a longer working time for the doctor.

It was found that there are acute care hospitals in many of the Type II towns, which was the reason why the Type II was divided further into two groups – Type IIa – is a town with an acute care hospital in at least one of the basic specialisation (internal, surgery, paediatrics, gynaecology), and Type IIb refers to a town with no such hospital. We also added Type IV – a corporate, where the nature of the practice is different (a larger company generally consisting of a number of GP practices working closely with doctors from various specialties and a large laboratory, operating either at the local or the national level and a large number of commercial activities). Type V refers to special general practices – military, prisons (Table 2).

RESULTS

DATA ANALYSIS

The initial analysis of the 2014–2015 data indicated that performance did not substantially differ in the size of the list of registered patients. In the Czech Republic the number of patients registered with a GP is determined by the capacity of the practice to provide the prerequisite care. However, practice location did have a marked effect on performance.

The costs of rural practices were lower (type III, total costs in the monitored variables lower by 7.3%) in comparison with urban practices (type I). The same was true for intermediate practices (type II, total costs in monitored variables lower by 5.9%). For some variables the difference was almost 30% (Table 3, Table 4).

Although the GHIC data sources were slightly different and types II and III changed parametrically, the conclusions regarding the 2016 data analysis were similar. Rural practices still had lower costs (Type III, total costs were 4.5% lower for the variables observed), the difference was more marked in relation to the intermediate practices (Type II, total costs were 7.2% lower for the variables observed) than urban ones (Type I). The largest differences in the variables were slightly over 30% (Table 3, Table 4).

Tab. 1 Distribution of Czech population in 2016 according to OECD regional typology (in %).

OECD 2016	Predominantly urban (PU)	Intermediate (IN)	Predominantly rural (PR)
Czech Republic	24.2	42.9	32.9

Note: based on data on number of inhabitants in the Czech Republic in 2014
Source: 29, 30

Tab. 2 Distribution of number of practices (FIDs in 2014–2015), and number of GP providers (PIDs in 2016) by type of region.

Period (level)		PU	IN			PR	Other		Total
		Type I	Type II	Type IIa	Type IIb	Type III	Type IV	Type V	
2014–2015 (FIDs)	abs.	3021	1907	–	–	477	–	–	5405
	%	**55.9**	**35.3**	–	–	**8.8**	–	–	100
2016 (PIDs)	abs.	2349	758	246	512	1389	49	41	4586
	%	**51.2**	**16.5**	5.4	11.2	**30.3**	1.1	0.9	100

Source: authors' calculations

Tab. 3 Basic GHIC input data (analyses for 2014–2015 and 2016).

	2014–2015	2016
FIDs (practices)	5405	
PIDs (providers)		4586
Number of registered insurance holders (in thousands)	4462.7	4689.8
Number of unit insurance holders (age indexed) (in thousands)	6480.7	6864.6
Total financial resources analysed (million EUR/year)	1516.9	2567.2
Analysed costs per registered insurance holder (EUR/year)	339.9	547.4
Analysed costs per unit insurance holder (EUR/year)	234.1	374.0

Source: GHIC

Tab. 4 Basic results of both analyses – differences in costs between types of practice by location (in %).

Selected data cycles	Type II/Type I	Type III/Type I	Type II/Type I	Type III/Type I
	2014–15 (difference in %)		2016 (difference in %)	
Capitation	-0.1	-2.9	0.0	0.1
Procedures	13.0	23.6	6.5	18.3
Specialists – in total	-11.8	-13.4	-13.5	-10.5
Ancillary services – in total	-19.1	-23.9	-10.4	-8.3
Prescriptions – own	24.4	29.8	17.7	32.2
Prescriptions – others	-7.4	-9.8	-5.1	-6.3
Total (complete analyses)	**-5.9**	**-7.3**	**-7.2**	**-4.5**

Source: GHIC, authors' calculations

DISCUSSION

The results of the two analyses show that the differences mainly concern the nature of the activities and skill set of rural GP practices. These are influenced by conditions within the local healthcare system, as is the case at the global level too (rural general practices all over the world are forced to adapt to their environments). Information of this kind, frequently published, based on data provided by the GP practices themselves (in questionnaires or interviews) (11–13, 20). However, there is still very little published in the literature on the verification of costs to the payer in relation to primary care (27, 28). Similar characteristics are shown by doctors from smaller towns, although there are usually a larger number of GP's offices in them. Therefore, the crucial factor is the distance from the regional centre with the hospital, which has a decisive influence on the equipment of practices and the range of services performed.

The Czech Republic has an unusually high number of patient contacts with the healthcare system (29). This is reflected in the large number of hospital beds and associated longer than average hospital stays compared with other OECD countries (30), as well as in the larger number of specialists (31). The distribution of GP practices in the Czech Republic is also one of the most unequal, with most specialists providing care in larger towns and cities (32). This of course has had an effect on GP practices as well. While the care for patients registered with urban GP practices is also provided by hospitals and specialists (patients frequently seek out these services themselves, as gatekeeping is minimal), these options are limited in rural practices and so they provide more of the care themselves.

It is obvious that the determined absolute amounts of funds are influenced by the setting of the local health care system. The setting of the amount of capitation and the amount for individual services affect the total costs that are specific to the Czech Republic in a given period. Relative differences between different groups of practices are more important for further research or research in other regions.

In terms of spectrum of activity, rural GPs around the world are forced to adapt to the environment in which they operate. Globally, GPs cannot ignore performances for which they receive remuneration and are rewarded. Although it can be assumed that the amount of reimbursement affects the range of services, the advantage in our analysis is the inclusion of total costs of practices. This minimizes the possibility of distorting the results, because

of the preference of a certain activity, which the payer placed more emphasis in the given period.

The costs of the GP segment used here (calculated as capitation per insurance holder) are not the same as the overall costs per head when calculated using the SHA methodology (A System of Health Accounts 2011) (33). In that system, all the costs of the healthcare system are calculated and the figure for the Czech Republic is €1,850 PPP (22). Our analyses were based only on the controllable GP costs relating to healthcare activities, paid for out of health insurance.

It is also necessary, to mention the similarity in the determination of the rural area in the case of results from the 2016 analysis and data published by the OECD. Our analysis considers 30.3% of GP practices to be rural; the OECD reports 32.9% of the population living in a predominantly rural area. We paid high attention to determining the degree of agreement between our methodology and the OECD methodology. This will be the topic of a separate publication.

In categorising the GP practices in the 2016 analysis, several variants were produced that were then compared with the OECD regional typology method. It uses the NUTS 3 level. We opted for a much more detailed image (municipalities with extended powers, a regional category used in the Czech Republic that is somewhere between LAU 1 and LAU 2), as it is better suited to distinguish between GP practices. However, differences in choice of method had no effect on categorising non-urban practices as intermediate and rural. What is important, though, is accurately defining urban practices (over 90% of which corresponded in all variants). These are the urban GP practices where we can best define the differences in the nature of the activities and thereby cost efficiency.

The practice included in the intermediate group seems to be interesting and promising for further research. This is a relatively heterogeneous group, for which other methods of division can be proposed. These practices operate in smaller towns, where there was usually a local hospital in the past. This created a network of related specialists. In some of these settlements, the hospital is still in operation, elsewhere its activities have been reduced to varying degrees, but often the hospital has been completely closed down (or transformed from acute hospital care to follow-up or social care). Cost data shows the highest efficiency of GP practices in the intermediate group. It can be explained by the optimal setting of the system, where more GPs and an adequate network of outpatient specialists operate in the given headquarters, but the easy path of patients to the necessary examination in a multidisciplinary hospital is hindered by a certain distance.

Multispecialty hospitals are more commonly used as a research variable in relation to access to care, than in relation to the patient, in rural medicine (19). But when considering the assessment criteria used in Central Europe, distance from the hospital or acute care unit is not as important (it is above the global standards) as the range of healthcare services available. This is because general practices operating in areas with a multispecialty hospital have a significant advantage in that the patient load can be shared more easily than is the case for their colleagues in small towns or rural areas. In the Czech Republic, the percentage of typical urban practices accounts for more than 50%.

The involvement of the evaluation of GP cost effectiveness as a determinant of the division of practices is possible only with the knowledge of the data of the health care payer. Data from health insurance companies can be used in the Bismarck system. However, in any health care system based on the dominant role of the state in the organization and reimbursement of health care, there are payers who collect performance data. These are therefore available, which makes our model transferable and applicable in other regions, regardless of the type of health system. It is very suitable for the GP segment, which provides a wide range of care for the entire population, not just a selective sample of patients.

Our principle of territorial division is a combined principle, because it helps to divide practices into urban and rural by means of two different, complementary criteria. In a situation, where we are working shorter distances in Central Europe, we are solving the same problem with the lack of medical staff on the periphery as other countries. Only by distance, or another simple geographical or geomorphological criterion, our problems cannot be sufficiently explained. It is a functional principle, because there are no problems with the exact definition of rural areas. Explaining this problem will be more difficult for researchers than answering the questions they originally wanted to answer. Finally, our principle can be described as dynamic, because it can respond flexibly to changes in the structure of the network of providers. For example, if the operation of a peripheral hospital is reduced or abolished, this will quickly affect the structure of local GP activities and the attractiveness of the region for healthcare professionals, while the geographical criteria will remain unchanged. Similar analyses can be performed repeatedly at any intervals, the results can be used in planning and local policy making.

CONCLUSIONS

The combined way of dividing GP practices into urban and rural practices is based on the use of two complementary factors. The location of the practice remains the first and basic one. Equally important are the characteristics of the activity, which can be defined and compared. In our research, we focused on the performance of the practice, quantified by the total cost of the health insurance company to its policyholders. These are payments for various items, directly related to the activities of the GP practice. So they provide comprehensive information about the spectrum of its work and at the same time about the cost effectiveness of this complex activity. It seems that in the Czech Republic there is a "rural way of working" of GPs, which could be more precisely defined and used as a determinant of rural areas, especially in areas that are not clearly urban or clearly rural.

In the Czech Republic practices in rural areas and small towns provide patient care using their own resources and that is reflected in their practice equipment and staff skill

set. They perform more procedures, make greater use of ancillary services, and issue more prescriptions, but they rely less on specialists. Results in less indicated care, saves the payer money and reduces the burden on the healthcare system. We have therefore shown that even in the small Czech Republic, regionality plays an important role in the provision of primary care.

In categorising the GP practices, we tried to follow the OECD criteria used to distinguish between three types of area (predominantly urban, intermediate, predominantly rural). We worked with different variants and ultimately obtained similar results. The key criterion is the presence of a multispecialty hospital in use with a network of related services (especially specialists) in that location. We considered the GP practices located in these areas to be typical urban ones. GP practices in the remaining areas operated efficiently (more own activities with lower total costs) regardless of whether they were categorised as intermediate or rural.

We believe that this principle of defining urban and rural GP practices is transferable and universally applicable. Although it requires knowledge of payers' cost data and a certain demographic framework, on the other hand, it can respond to changes in the network of providers (secondary and tertiary care) with a minimum of time, especially in peripheral regions. If the conditions in the provision of health services change (for example, the reduction of the activities of a peripheral hospital), it will be reflected very quickly in the activities of local GPs. It will be precisely they who will immediately be transferred to the higher demands on the range of services provided and the organization of health care.

ABBREVIATIONS

GP	General Practice
GHIC	General Health Insurance Company
OECD	Organisation for Economic Co-operation and Development
LAU	Local administrative unit
NUTS	Nomenclature of Units for Territorial Statistics
PU	Predominantly Urban
IN	Intermediate
PR	Predominantly Rural

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Diagnostic Value of Narrow Band Imaging in Visualization of Pathological Lesions in Larynx and Hypopharynx

Jana Šatanková[1, 2,*], Lucia Staníková[3], Anna Švejdová[1], Michal Černý[1, 2], Jan Laco[2, 4], Viktor Chrobok[1, 2]

ABSTRACT

Introduction: Narrow Band Imaging (NBI) is an endoscopic optical imaging enhancement technology that improves the contrast of mucosal surface texture and enhances visualization of mucosal and submucosal vasculature. Due to its properties, it can visualize suspected malignant or precancerous lesions earlier than conventional white light endoscopy. The aim of this study was to analyze the benefit of NBI in visualization of precancerous and malignant lesions in preoperative and intraoperative diagnostics and correlation with histopathologic results.

Methods: A total of 589 patients with suspicious laryngeal or hypopharyngeal lesion were investigated using conventional white light endoscopy (WLE) and NBI endoscopy with high-definition TV (HDTV NBI) from 10/2013 to 12/2019. Patients were divided into two groups based on pre-operative NBI examination (group A, 345 patients) and intraoperative NBI examination (group B, 244 patients). All suspicious lesions were graded to 5 types of Ni classification and correlated with histopathologic results. The SPSS version 8.0.4 statistical software package was used for statistical analysis. In diagnosing premalignant and malignant lesions sensitivity, specificity, positive predictive value, and negative predictive value were calculated.

Results: The agreement between NBI endoscopy and histopathological analysis in group A was statistically significant ($\kappa = 0.76$, $p < 0.001$), with a sensitivity of 86.2% (95% IS: 65.4–95.2) and specificity of 90.9% (95% IS: 70.6–94.1). Moreover, in group B was proven almost perfect agreement between NBI and histopathological analysis ($\kappa = 0.8461$, $p < 0.001$), with a sensitivity of 84.0% (95% IS: 60.2–92.4) and specificity of 96.0% (95% IS: 87.0–99.2).

Conclusions: Based on our results, NBI using the Ni classification has great potential in improving diagnosis of precancerous and malignant lesions and correlates strongly with histopathologic results. It serves as a useful adjunct to white light endoscopy in the diagnosis of laryngeal and hypopharyngeal lesions, especially using HDTV NBI.

KEYWORDS

narrow band imaging; white-light endoscopy; Ni classification; precancerous lesion; squamous cell carcinoma; larynx; hypopharynx

AUTHOR AFFILIATIONS

[1] Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Hradec Králové, Czech Republic
[2] Charles University, Faculty of Medicine in Hradec Králové, Czech Republic
[3] Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Ostrava, Czech Republic
[4] The Fingerland Department of Pathology, University Hospital Hradec Králové, Czech Republic
* Corresponding author: Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Hradec Králové, Czech Republic; e-mail: jana.satankova@fnhk.cz

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INTRODUCTION

Squamous cell carcinoma (SCC) is one of the most common types of head and neck cancer. Early detection, histopathological diagnosis and treatment significantly improve prognosis and reduce patient morbidity and mortality (1). The most important limiting factor of the early diagnosis is the fact, that precancerous and early malignant changes in the larynx and hypopharynx do not have specific clinical and macroscopical features different from symptoms and mucosal changes trigerred by common inflammatory disorders (2). Standard examination methods include rigid or flexible laryngoscopy with white light that lacks sensitivity to precancerous (high-grade dysplasia include carcinoma in situ) or early superficial lesions (T1 or T2 carcinoma). Radiologic examinations such as CT and MRI play a complementary role, as they are the only diagnostic tools that are capable of correctly evaluating deep structures, regional and distant lymph node metastases. Growth and progression of SCC relies on neoangiogenesis, a process where new blood vessels are formed from the surrounding pre-existing blood vessels (1). These new vessels lack the histological architecture and structural anatomy of pre-existing vessels that can be detected by conventional white light endoscopy in later stages of cancer. Earlier detection of these changes is very important for better prognosis of the patients (3). Development of new optical endoscopic methods allowed to detect discrete mucosal changes with pathological vascularization and started rapid progress in the diagnostic process of head and neck tumors. One of endoscopic methods that can visualize these changes and improve the diagnosis is NBI, especially HDTV NBI (2).

NBI as a special endoscopic optical imaging enhancement technology improves the contrast of mucosal surface texture and enhances visualization of mucosal and submucosal vasculature by using specially filtered light (blue and green). The blue light (wavelength 415 nm) corresponds to hemoglobin absorption spectrum and visualizes IPCL (intraepithelial papillary capillary loops). The green light (wavelength 540 nm) penetrates deeper and highlights submucosal vessels. In the definitive image, the mucosal microvascularization appears brown coloured and submucosal vessels are blue coloured (4). Finally, there is a substantial increase of colour contrast between blood vessels and surrounding tissue and a few millimeter changes can be identified. The optimal image is achieved by using HDTV NBI – NBI with high resolution (more than one million pixels) or with ultra high resolution 4K (more than 8 million pixels) (2).

Due to its properties, NBI can visualize suspicious malignant or precancerous lesions earlier than conventional WLE. NBI shows great potential by improving detection of tumours in upper aerodigestive tract (UADT) and currently is the most progressive technological tool in diagnosis of head and neck cancer (5).

Ni et al. proposed a classification system for description of changes in morfology and architecture of IPCL to distinguish between benign and malignant laryngeal lesions (6). This system describes five patterns of morphological changes in IPCL architecture (Table 1). Type I–III are usually associated with benign changes (Figure 1), type IV represents preneoplastic changes and type V neoplastic changes (Figure 2). Type V is further subdivided into Va, Vb a Vc (Table 1) (5). A new classification system as the result of consensus of European laryngological society was published by Arens et al. in 2016 (7). This system is a simplified version of the Ni classification using a descriptive evaluation of microvascular changes. This classification distinguishes only two types of vascular changes – longitudinal and perpendicular (7).

The aim of this study was 1) to evaluate the prehistological diagnostic value of NBI focusing on laryngeal and hypopharyngeal suspicious lesions and 2) to compare NBI with conventional WLE in this setting.

Tab. 1 Classification of intraepithelial papillary capillary loops (Ni et al. 2011).

Classification	Description
Type I	Thin, oblique and arborescent vessels are interconnected IPCLs are almost invisible
Type II	Diameter of oblique and arborescent vessels is enlarged IPCLs are almost invisible
Type III	IPCLs are obscured by white mucosa
Type IV	IPCLs can be recognized as small dots
Type Va	IPCLs appear as solid or hollow, with a brownish, speckled pattern and various shapes
Type Vb	IPCLs appear as irregular, tortuous, line – like shapes
Type Vc	IPCLs appear as brownish speckles or tortuous, line – like shapes with irregular distribution, scattered on the tumour surface

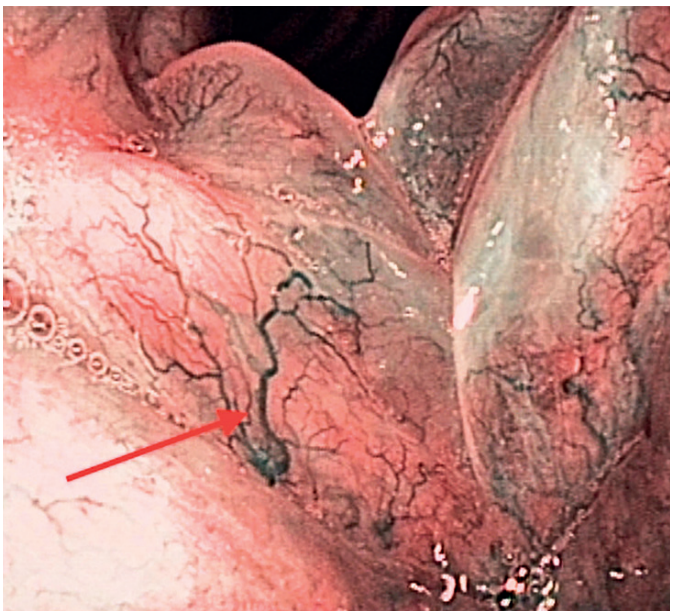


Fig. 1 Reinke oedema of both vocal cords – benign type of vascularization in NBI (the red arrow is showing oblique and arborescent vessels IPCL type II according to Ni classification).

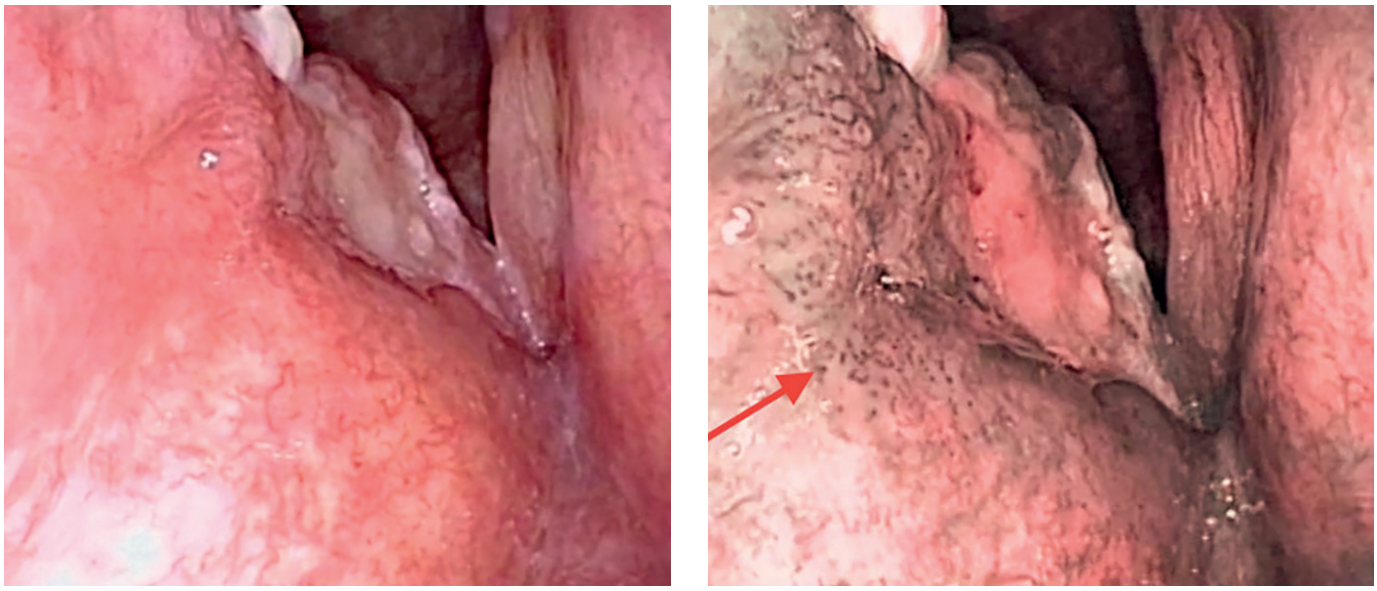


Fig. 2 Squamous cell carcinoma of right vocal cord and vestibular fold: (A) in conventional WLE, (B) in NBI malignant type of vascularization (the red arrow is pointing to the affected area with IPCL type Va).

MATERIAL AND METHODS

The presented study was conceived as a prospective study. Patients with clinically suspicious laryngeal or hypopharyngeal lesion that presented to the Department of Otorhinolaryngology and Head and Neck Surgery in Hradec Králové (Czech Republic) from October 2013 to December 2019 were recruited. A total of 589 patients were investigated using conventional WLE and NBI endoscopy with high-definition TV (HDTV NBI) from 10/2013 to 12/2019. Patients were divided into two groups based on preoperative NBI examination (group A = 345 patients) and intraoperative NBI examination (group B = 244 patients). All suspected lesions were graded to 5 types of Ni classification and correlated with histopathologic analysis.

The main reasons for referral were patients with chronic laryngitis, hoarseness or voice changes of unknown etiology lasting more than 3 weeks, benign – appearing lesions (polyps, cysts, vocal cord granulomas), leukoplakias, erythroplakias, macroscopic tumorous lesions of vocal cords or laryngeal papillomatosis. During the consultation in out-patient department (group A), flexible laryngoscopy was performed. After application of mesocain gel (trimecainhydrochloridum carbethopendeciniibromidum 10 mg/g), flexible videolaryngoscope was inserted through the nasal cavity. For local anesthesia of larynx we used lidocaine spray (10% lidocainum) with specially curved applicator that was applied transorally. Flexible endoscopy was performed with an Olympus ENF-VH or ENF-VQ 3.9 mm flexible endoscope connected to an Elvis Exera III CV 170 light source (Olympus Medical System, Tokyo, Japan) while patients were awake and seated. Each patient was examined by conventional WLE followed by NBI mode where the lesions were assessed in real time and captured. In conventional WLE we evaluated macroscopic characteristics of the lesion (chronic laryngitis, cyst, polyp, granuloma, Reinke oedema, leukoplakia, erythroplakia, papilloma, exophytic lesion) and the extent and spreading of the lesion (Figure 2). In NBI mode we described changes in microvascularization graded according to Ni classification. Any lesions considered being precancerous were graded IV, lesions suspected from malignancy were given grade V (Va, Vb or Vc). Endoscopic examination was performed only by three physicians. A clinically indicated biopsy was taken under general anaesthesia in the operating room. The biopsy specimens were fixed in 10% formalin and sent for histopathological examination. The tissue was routinely processed, stained with hematoxylin and eosin and examined by an experienced head and neck pathologist. Histopathological diagnoses were then compared to their respective Ni classification (Table 1). Based on this, sensitivity and specificity of NBI using the Ni classification for detection of laryngeal and hypopharyngeal cancer were calculated. Exclusion criteria were allergy to local anaesthetics, previous laryngeal or hypopharyngeal cancer, or inability to undergo flexible endoscopy due to increased gag reflex.

In group B patients underwent examination in general anaesthesia in the operating theatre (direct laryngoscopy or hypopharyngoscopy). After the endotracheal intubation, all anatomical sites were endoscopically evaluated by WLE and NBI using rigid 0° and 30° angled telescopes (Olympus Visera Pro system CV-170 with Olympus OTV S 7 camera head). Attention was paid to the superficial extension of the primary lesion with special emphasis to the additional suspected areas in the entire larynx and hypopharynx. The principles of classification of NBI findings and correlation with histopathological results were identical to the group A.

The SPSS version 8.0.4 statistical software package was used for statistical analysis. Sensitivity, specificity, positive predictive value, and negative predictive value for detection of laryngeal and hypopharyngeal cancer were calculated.

RESULTS

From October 2013 to December 2019, a total of 345 patients were included in group A. There were 248 (71.9%) males and 97 (28.1%) females (male to female ratio 2.5 : 1) aged 34 to 79 years (median 53; mean 58.4 ± 16.5).

In group B total of 244 patients were included. There were 169 (69.3%) males and 75 (30.7%) females (male to female ratio 2.3 : 1) aged 27 to 81 years (median 59; mean 56.2 ± 14.5). Detailed personal data are summarized in Table 2.

Tab. 2 Age, sex and comorbidities in two groups of patients.

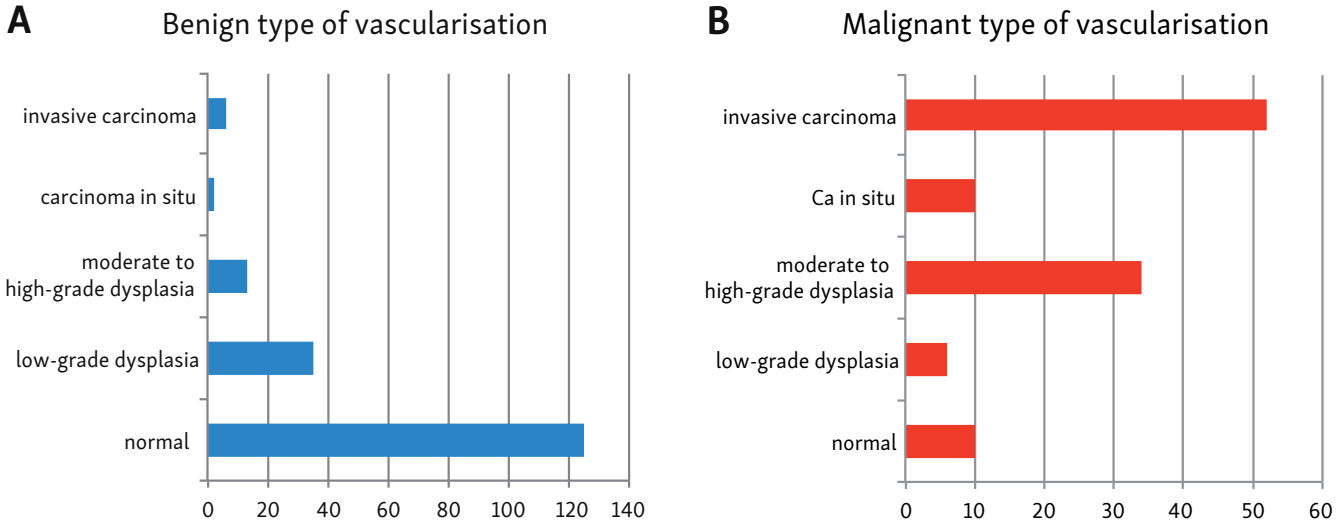
Characteristics	Group No. I	Group No. II
men	72.4%	69.3%
women	27.6%	30.7%
smokers	65.5%	71.7%
non-smokers	24.1%	19.7%
stop-smokers	10.4%	8.6%
age	64.3 y (43–82)	56.3 y (27–81)
hoarseness	79.3%	79.1%
chronic laryngitis	23.3%	29.1%
1 comorbidity < 60 y	74.1%	65.8%
≥ 2 comorbidities > 60 y	77.6%	72.2%
Extraesophageal reflux	17.2%	23.4%

In group A, we detected pathological findings in 324/345 (94%) patients when using the conventional WLE, especially chronic laryngitis (16%), leukoplakia (22%), erythroplakia (4%), exophytic tumour (35%), papilloma (15%) and Reinke oedema (8%). Using NBI endoscopy, larger extent of the lesion was visualized in 36% and new lesions not detected in white light in 6% cases (Table 3). Histopathological examination was made in 85% (293 patients). If we assumed benign mucosal vascularization in NBI, normal finding was reported by pathologist in 69.1% lesions, dysplastic changes in 26.4%, carcinoma in situ in 1.2% and invasive spinocellular carcinoma in 3.3% (Graph 1A). If we assumed malignant mucosal vascularization in NBI, an invasive spinocellular carcinoma was proven in 46.4% cases, carcinoma in situ in 8.9%, moderate to high-grade dysplasia in 30.4%, low-grade dysplasia in 5.4%, and chronic inflammatory changes without dysplasia in 8.9% cases (Graph 1B).

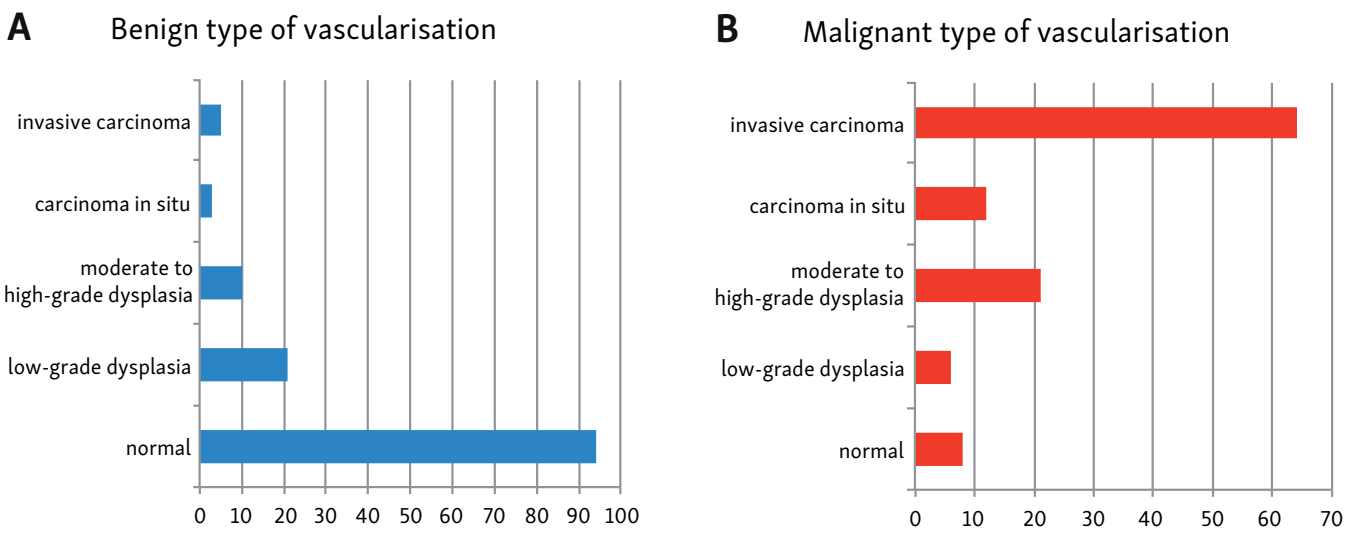
Tab. 3 Overview of own results (Statistics: software SPSS version 8.0.4).

NBI examination	Group No. I	Group No. II
Larger extent of lesion in NBI	36.0%	22.1%
New lesions in NBI	6.0%	17.6%
Sensitivity	86.2%	90.8%
Specificity	90.9%	94.7%
Positive predictive value	75.8%	93.6%
Negative predictive value	95.2%	86.5%
Overall agreement 95% IS	86.84% (76.4–93.4%)	89.64% (79.4–94.6%)
Ķ index (p < 0.001) 95% IS	0,76 (0.54–0.96)	0.8461 (0.8274–0.9654)

Character of mucosal vascularization (IPCL) in NBI was correlated with histopathological examination. Sensitivity of NBI in distinction of benign hyper- and parakeratosis, low-grade dysplasia, moderate to high-grade dysplasia from advanced precancerous stages (carcinoma in situ) and invasive spinocellular carcinoma (SCC) was 86.2% (95% IS: 65.4–95.2), while specificity was 90.9% (95% IS: 70.6–94.1). Overall agreement was 86.84% (95% IS: 76.4–93.4). According to Kappa index Ķ = 0.76 (95% IS: 0.54–0.96) (p < 0.001), we proved statistically significant agreement between NBI and histopathological examination (Table 3).



Graph 1 Comparison of vascular pattern of intraepithelial papillary capillary loops in NBI (according to Ni et al. 2011) and the results of histopathological analysis in group A. **(A)** benign type of vascularization in NBI, **(B)** malignant type of vascularization in NBI.



Graph 2 Comparison of vascular pattern of intraepithelial papillary capillary loops in NBI (according to Ni et al. 2011) and the results of histopathological analysis in group B. **(A)** benign type of vascularization in NBI, **(B)** malignant type of vascularization in NBI.

In group B, larger extent of the lesion was visualized in 22.1% and new lesions not detected in conventional white light in 17.6% cases (Table 3). Histopathological examination verified benign findings (hyperkeratosis, parakeratosis, chronic inflammatory changes, polyps, cysts and granulomas) in 37.3% of cases. Recurrent respiratory papillomatosis was present in 14.3% and low-grade dysplastic changes in 8.6% of cases (Graph 2A). As regards precancerous and malignant changes, we proved high-grade dysplasia in 13.9%, carcinoma in situ in 3.3% and invasive SCC in 22.5% of patients (Graph 2B). Statistical analysis confirmed the sensitivity of 90.8% (95% IS: 82–96) and specificity of 94.7% (95% IS: 79.4–94.6). Overall agreement was 89.64% (95% IS: 79.4–94.6). According to Kappa index $\kappa = 0.8461$ (95% IS: 0.8274–0.9654) ($p < 0.001$) we found statistically significant agreement between NBI and histopathological examination (Table 3).

DISCUSSION

Patients with UADT tumors are routinelly examined by conventional white light endoscopy. It is the most commonly used method to evaluate superficial spreading of SCC. In early stages of cancerogenesis, the tumors of UADT are hardly distinguished from normal tissue and usually not identified by conventional WLE (5). The growth of epithelial malignant tumors leads to escalated neoangiogenesis, which may be observed in endoscopic practice as changes in the IPCL arrangement, diameter, shape and as a loss of regularity (1). Technological improvement in endoscopic methods is the key for early detection of these changes. Alteration of microvascular architecture becomes more visible in high-grade dysplastic changes in comparison to low-grade dysplastic changes (1).

NBI is easily applicable and well tolerated in local anesthetics in out-patient department. Great practical significance is identification of new suspicious lesions. We found out larger extent of suspicious lesions in 36% of patients, and in 6% of cases we identified new lesions that were not be visible by conventional white light endoscopy. Piazza et al. (2010) proved larger extent of lesions in 26% and new lesions not visible in WLE in 9% (8).

Piazza et al. (2012) also proved the benefit of pre-operative using of NBI with detection up to 20 lesions not visible by white light endoscopy. The diagnostic benefit of NBI in better definition of neoplastic superficial spreading is substantially increased with using HDTV NBI (from 20.8% to 42.7%) (9).

Clinical application of NBI endoscopy requires a special practice and defined "learning curve". Correct recognition and diagnostics of lesions is long-lasting process. Villaseca et al. (2017) demonstrated, that accuracy of detection of malignant lesions increased with longer-lasting practical experience of the examiner and he recommends at least 200 examinations for correct identification of vascular changes (10). Piazza et al. (2010) demonstrated that 13.7% from 58 positive findings in NBI were false positive, if 70% of the findings have been diagnosed during the first three months of using NBI. The advantage of NBI examination in out-patient department is possibility of data recording and fast video analysis in a short time (8).

Piazza et al. (2010) assessed 279 patients with laryngeal cancer and reported a sensitivity of only 61% using flexible NBI alone, but the sensitivity increased substantially to 98% and specificity to 90% when NBI was coupled with HDTV (8). Shoeffel-Havakuk et al. (2017) compared NBI with WLE for the diagnosis of laryngeal cancer and reported a sensitivity of 58.6% when using NBI. Sensitivities reported in this study were based on three expert assessments of NBI and WLE images, not histopathological diagnosis like in other studies (11). Chang et al. (2016) consider NBI as an effective technique with high diagnostic accuracy (98.9%), sensitivity (97.2%) and specificity (100%), and therefore recommend using flexible endoscopy with NBI and taking biopsy in out-patient department. Diagnostic accuracy was not affected by size, localization, "learning" or history of head and neck cancer (12). In recent

publication by Hosono et al. (2019), sensitivity using HDTV NBI in laryngeal lesions was 84.4% (13). Others recent studies have demonstrated higher sensitivities of > 90% when using HDTV NBI in laryngeal and hypopharyngeal cancer (14).

Young and colleagues (2015) reported a sensitivity of 91.2% in 23 malignant lesions (15). Sakthivel et al. (2018) confirmed sensitivity up to 100% if WLE and NBI were combined (16). In study by Rzepakowska et al. (2018) the sensitivity was up to 98.8% with the HDTV NBI in diagnostics of precancerous and malignant laryngeal lesions (17). In our study we achieved sensitivity 86.2% and specificity 90.6% and according to Kappa index there was substantial agreement between NBI and histopathological results ($K = 0.76$; $p < 0.001$).

NBI has also limitations in preoperative diagnosis. To obtain a valid interpretation it is necessary to keep minimal distance between distal part of endoscope to the examined mucosa (3 mm and less) which can be difficult in patients with strong gag reflex even after using local anaesthetics. Another disadvantage is the impossibility to assess vertical extent of lesion. The cause of false negative findings might be due to hyperkeratosis making the assessment of pathological vascularization impossible (3, 18). In our study there were two patients with verrucous SCC where it was not possible to assess IPCL. In one patient with adenoid cystic carcinoma superficial pathological vascularization was not identified due to submucosal spreading of the tumour. Assessment of leukoplakia in NBI is controversial as well, described by a well-known phenomenon – the "umbrella effect", which reflects the submucosal vascular pattern being hidden under the hyperkeratotic plaque (Klimza et al. 2017) (19). To overcome the "umbrella effect", the NBI was used to categorise submucosal vascular pattern surrounding the plaque, as illustrated in Figure 3B (Klimza et al. 2017, Stanikova et al. 2017) (18, 19). Laryngeal recurrent respiratory papillomatosis (RRP) is another problematic lesion that can be confused with SCC (Figure 4). In our study we found

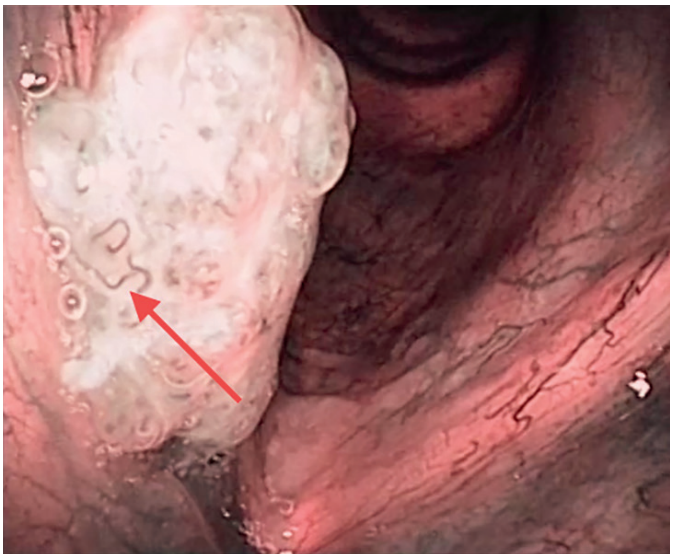


Fig. 3 Squamous cell carcinoma of right vocal cord (the red arrow is showing pathological vascularization – irregular, tortuous, line-like shapes – IPCL type Vb).

27 patients with RRP (8.3%), 3 cases were initially suspected from malignancy and proved to be false positive. However, if RRP is suspected other synchronous papillomatous lesions (Jackowska et al. 2018) can be detected in NBI. In our study NBI revealed other lesions not visible in WLE in 14/27 (51.8%) (20).

In peroperative using of HDTV NBI we demonstrated significant difference in comparison with conventional WLE. We identified larger extent of lesions in 22.1% and new suspicious lesions not visible by WLE in 17.6%. The sensitivity was 90.8%, specificity 94.7% and according to Kappa index ($K = 0.8461$; $p < 0.001$) almost perfect agreement between NBI and histopathological examination has been proven. We had low amount of false positive results and high number of true negative results, and therefore the specificity is so remarkable.

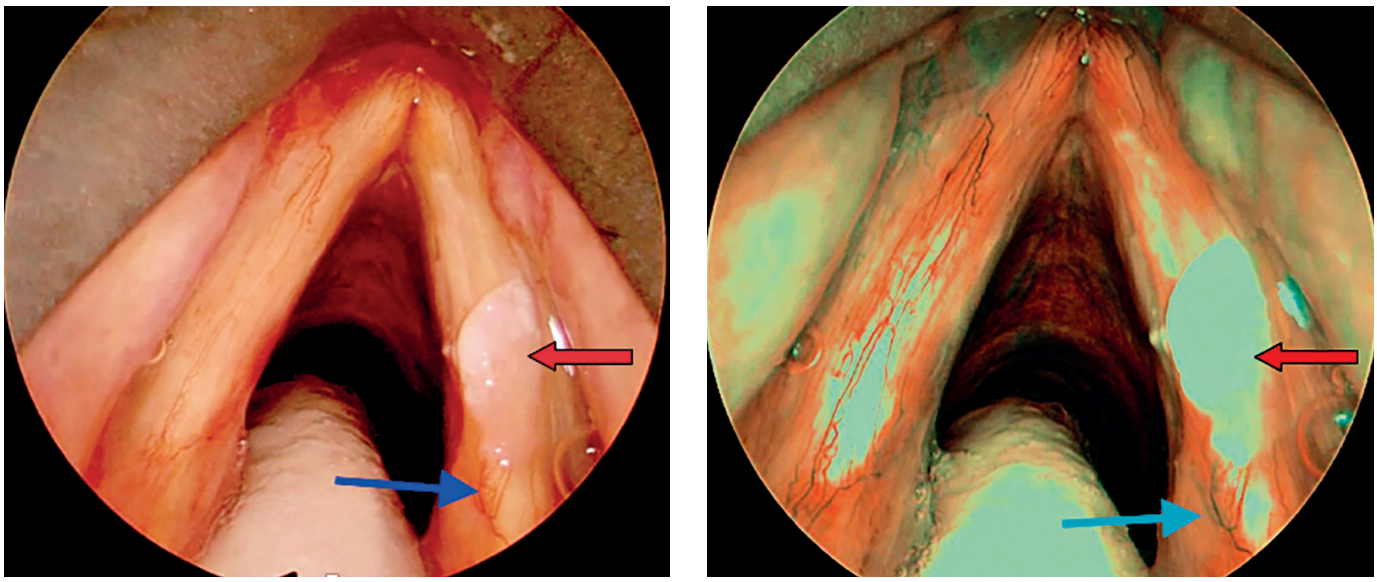


Fig. 4 Leukoplakia on the right vocal cord – intraoperative finding in direct laryngoscopy: (A) in conventional WLE, (B) in NBI (the red arrow is showing the lesion, the blue arrow is pointing to the surrounding IPCL type II).

It is difficult to determine margins between physiological and pathological findings when taking "ideal" biopsy in conventional WLE. There are many studies that evaluate positive intraoperative effect of NBI to reduce the number of positive resection margins (21, 22). They proved benefit of NBI in intraoperative diagnostics for better visualization of margins in superficial spreading lesions what is important in endoscopic surgery. Garofollo et al. (2015) proved significant reduction of positive resection margine using NBI (from 23.7% to 3.6%) in patients with early glottis carcinoma (cordectomy type I and II) (21). Piazza et al. (2010) confirmed the significance of NBI in 26 laryngeal tumors where HDTV NBI used intraoperatively led to enlargement of resection margins (23). In a recent study performed by Klimza et al. (2019) pathological lesions not visible in WLE were identified in 13.6% and histopathologically confirmed in 88.8% (33.3% invasive SCC, 66.6% high-grade dysplasia and carcinoma in situ) (22). Orita et al. (2008) confirmed intraoperative benefit of NBI during the endoscopic hypopharyngeal surgery (24).

CONCLUSION

The results in all above mentioned published studies as well as our study confirmed that NBI is a reliable method in early detection of precancerous and malignant changes, for discriminating between benign and malignant patterns in prehistological diagnosis.

Great practical significance is identification of new suspicious lesions not visible by conventional white light endoscopy that we proved preoperatively in 6% and intraoperatively in 17.6% of cases. We confirmed low amount of false positive results and high number of true negative results, and therefore the specificity was so remarkable. Especially in peroperative using of HDTV NBI we demonstrated almost perfect agreement between NBI and histopathological examination.

NBI has been proved to be a "gold standard" and routine diagnostic tool for assessment of laryngeal and hypopharyngeal pathology.

CONFLICT OF INTEREST

The authors declare that there is no actual or potential conflict of interest in relation to this article.

FINANCIAL DISCLOSURE

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Endoscopic Third Ventriculostomy for Obstructive Hydrocephalus and Ventriculocystostomy for Intraventricular Arachnoid Cysts

Bekir Akgun*, Sait Ozturk, Omer Batu Hergunsel, Fatih Serhat Erol, Fatih Demir

ABSTRACT

Objective: To evaluate and discuss the outcomes of a combination of ventriculocystostomy (VC) and endoscopic third ventriculostomy (ETV) for obstructive hydrocephalus (HCP) due to ventricular/cisternal arachnoid cysts, and only ETV for obstructive HCP due to different etiologies.

Methods: We retrospectively reviewed all 40 symptomatic patients (aged 4 months – 61 years) of obstructive HCP treated by ETV or VC+ETV during October 2014 – April 2019. VC+ETV was performed in 7 patients with intraventricular/cisternal arachnoid cyst and obstructive HCP. Only ETV was performed in 33 patients with obstructive HCP due to other etiologies.

Results: Successful ETV or VC+ETV surgery was performed in 35 patients. The procedure failed in 5 patients aged < 1 year; all these 5 patients had a head circumference (HC) of > 90 percentile at the time of surgery. Another 5 patients aged < 1 year showed successful ETV, with a HC of 75–90 percentiles.

Conclusion: ETV is a successful alternative treatment for obstructive HCP. The ventricular size may not demonstrate a remarkable reduction post-ETV than post-shunting. Accordingly, increased intracranial pressure may not effectively decrease during the early period post-ETV than post-shunting. Therefore, the success rates of VC and/or ETV are low in very young patients with very high HCs (> 90 percentile).

KEYWORDS

endoscope; third ventriculostomy; ventriculocystostomy

AUTHOR AFFILIATIONS

Firat University, School of Medicine, Department of Neurosurgery, Elazig, Turkey

* Corresponding author: Firat University Hospital, Department of Neurosurgery, Elazig, Turkey; e-mail: bekirakgun@yahoo.com

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Tab. 1 Demographic features of the patients and details of the surgeries.

Case	Age	Etiology of Hydrocephalus	Operation	Shunt History before ETV	Shunt Necessity after ETV
1	4 m	MMC + Chiari Type 2	ETV	–	+
2	6 m	MMC + Chiari Type 2	ETV	–	–
3	7 m	MMC + Chiari Type 2	ETV	–	+
4	8 m	IV arachnoid cyst (posterior of the 3rd ventricle)	VC + ETV	–	+
5	9 m	MMC + Chiari Type 2	ETV	–	+
6	9 m	Aqueductal stenosis	ETV	–	–
7	10 m	Aqueductal stenosis	ETV	–	+
8	11 m	IV arachnoid cyst (trigone of the right lateral ventricle)	VC + ETV	–	–
9	11 m	MMC + Chiari Type 2	ETV	–	–
10	12 m	Suprasellar arachnoid cyst	VC + ETV	–	–
11	2 y	MMC + Chiari Type 2	ETV	+	–
12	4 y	Suprasellar arachnoid cyst	VC + ETV	–	–
13	5 y	Aqueductal stenosis	ETV	–	–
14	5 y	Aqueductal stenosis	ETV	–	–
15	7 y	Pineal tumor	ETV	–	
16	8 y	IV arachnoid cyst (body of the left lateral ventricle)	VC + ETV	–	–
17	9 y	IV arachnoid cyst (posterior of the 3rd ventricle)	VC + ETV	–	–
18	9 y	Aqueductal stenosis	ETV	–	–
19	13 y	Quadrigeminal arachnoid cyst	VC + ETV	–	–
20	13 y	Aqueductal stenosis	ETV	+	–
21	14 y	Aqueductal stenosis	ETV	–	–
22	15 y	Pineal tumor	ETV	–	–
23	16 y	Pineal cyst	ETV	–	–
24	18 y	Aqueductal stenosis	ETV	–	–
25	20 y	Pineal cyst	ETV	–	–
26	21 y	Chiari Type 1	ETV	–	–
27	24 y	Aqueductal stenosis	ETV	–	–
28	30 y	Aqueductal stenosis	ETV	–	–
29	32 y	Aqueductal stenosis	ETV	+	–
30	34 y	Chiari Type 1	ETV	–	–
31	36 y	Chiari Type 1	ETV	–	–
32	40 y	Aqueductal stenosis	ETV	–	–
33	45 y	Chiari Type 1	ETV	–	–
34	47 y	Chiari Type 1	ETV	–	–
35	48 y	Chiari Type 1	ETV	–	–
36	51 y	Chiari Type 1	ETV	–	–
37	53 y	Aqueductal stenosis	ETV	–	–
38	57 y	Aqueductal stenosis	ETV	–	–
39	60 y	IV hemorrhage	ETV	–	–
40	61 y	Aqueductal stenosis	ETV	–	–

INTRODUCTION

Hydrocephalus (HCP) is one of the most common conditions encountered in neurosurgical practice. It is a spectrum of conditions involving an imbalance of cerebrospinal fluid (CSF) production and absorption, with resultant enlarged ventricles that are usually associated with the clinical sequelae of increased intracranial pressure (ICP) (1, 2). In 1923, Mixter first performed endoscopic third ventriculostomy (ETV) after successfully using a urethroscope to perform intervention in a pediatric patient with obstructive HCP (3). Shunt techniques became hugely popular in the 1950s considering the technical limitations of the endoscope, which resulted in high mortality and morbidity rates due to insufficient illumination and low-quality lenses (1, 4). However, there exists no ideal shunt system, and the complications encountered remain a great concern in the long-term management of HCP, with numerous drawbacks related to shunt malfunction and infection (1). In addition, neuroendoscopy gathered attention since the 1990s due to the production of more powerful light sources, small-sized cameras and the achievement of better quality in the optic systems with the advancements in the technology (5–7). Particularly, ETV has gained popularity in the recent years, with evident advantages over the ventriculoperitoneal shunt (VPS) as patients remain device-free (1, 2, 5). This technique provides direct communication between the third ventricle and interpeduncular and prepontine subarachnoid spaces so as to re-establish the normal CSF flow by perforating the floor of the third ventricle (2, 5, 7). Hence, it is the treatment of choice for obstructive HCP (stenosis of the aqueduct of Sylvius, Dandy–Walker malformation and Chiari malformations types I and II) (8). Recently, some preliminary reports of successful ETV employment in patients with communicating HCP have been reported, including idiopathic normal pressure HCP (9). However, past studies have shown that ETV is associated with more favorable outcomes in cases with obstructive HCP. The highest success rates have been reported in patients with obstructive HCP secondary to aqueductal stenosis (10).

Arachnoid cysts refer to a collection of CSF within a cyst wall lined with arachnoid cells and collagen. The pathophysiology of arachnoid cysts remains unclear. Although they are located mostly in the temporal fossa, several intracranial and intraspinal localizations have been reported. In addition, they can be found in the ventricles and cisterns (11, 12). Several arachnoid cysts are asymptomatic, while symptomatic arachnoid cysts can present with signs and/or symptoms resulting from increased ICP or local mass effect on the adjacent structures. Such cases often require surgical decompression of the cyst, which can be achieved by using craniotomy or shunt systems. Presently, we have techniques to treat intraventricular and/or intracisternal cysts that causes obstructive HCP with fenestration into the ventricle [ventriculocystostomy (VC)] and ETV performed simultaneously as a result of advancements in neuroendoscopy (11–14).

In this study, we discussed and analyzed our experience of the management and results of VC and ETV performed in patients with obstructive HCP due to ventricular or cisternal arachnoid cysts; and only ETV was performed for the treatment of obstructive HCP due to different etiologies.

MATERIAL AND METHODS

We retrospectively reviewed all cases of obstructive HCP treated with ETV or VC+ETV between October 2014 and April 2019 at our hospital. In total, 40 patients with symptomatic HCP of age 4 months – 61 years were evaluated, of which 18 were women and 22 were men. Seven patients showed obstructive HCP due to intraventricular or cisternal arachnoid cyst, and 33 showed obstructive HCP due to other etiologies such as Aquaductal stenosis, Chiari malformations, myelomeningocele and pineal cyst/tumor. Table 1 displays the enrolled patients' demographic features, etiologies of HCP, the applied surgical techniques, shunt history and the necessity of performing the shunt procedure after endoscopic procedures. The patients with colloid cysts and intraventricular tumors were excluded.

Tab. 2 Correlation between the head circumference and the success in patients aged <1 year.

Case	Age	Operation	Head circumference	Success of the procedure
1	4 m	ETV	> 90 percentile	–
2	6 m	ETV	75–90 percentile	+
3	7 m	ETV	> 90 percentile	–
4	8 m	VC + ETV	> 90 percentile	–
5	9 m	ETV	> 90 percentile	–
6	9 m	ETV	75–90 percentile	+
7	10 m	ETV	> 90 percentile	–
8	11 m	VC + ETV	75–90 percentile	+
9	11 m	ETV	75–90 percentile	+
10	12 m	VC + ETV	75–90 percentile	+

m: months, VC: ventriculocystostomy, ETV: endoscopic third ventriculostomy

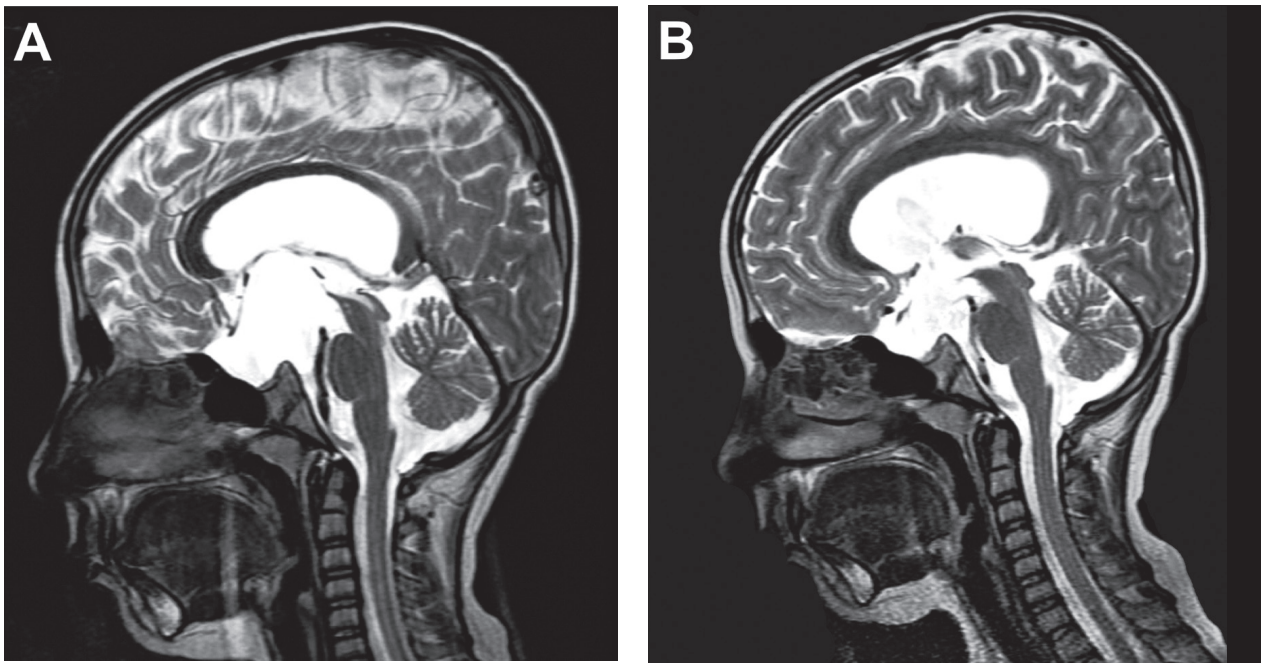

Fig. 1 T2W-sagittal MRI of a 12-month-old boy included in this study. (A) Preoperative MRI showing a suprasellar arachnoid cyst extending to the third ventricle and the foramen of Monro, which caused the dilation of the lateral ventricles. (B) Reduction in the sizes of the cyst and the third ventricle after VC+ETV treatment in the postoperative first-month MRI. In addition, the CSF flow artifacts were observed at the site of VC (from the foramen of Monroe to the third ventricle) and ETV (from the third ventricle to the prepontine cistern).

Table 2 displays the correlation between the head circumference (HC) and the success in patients aged < 1 year. The success rates of the procedures were primarily defined using clinical resolutions accompanied with radiological confirmations (Figure 1).

RESULTS

Seven patients were operated for intraventricular or cisternal arachnoid cysts by a combination treatment of VC and ETV. The surgical intervention was unsuccessful in only one 8-month-old patient from these 7 patients. In addition, this patient had a HC of > 90 percentile at the time of the surgical intervention.

Only ETV was performed in 33 patients with obstructive HCP due to other etiologies. Of these, 14 patients had aqueductal stenosis. The applied procedure failed in a 10-month-old boy who had a HC of > 90 percentile at the time of the surgery. In addition, 6 patients had meningomyelocele. ETV failed in 3 of these 6 pediatric patients aged 4, 7 and 9 months, who had a HC of > 90 percentile. These 3 patients had VPS history and were admitted because of shunt dysfunction. We performed ETV after shunt removal and achieved successful results; the treated patients remained shunt-free.

Successful ETV or VC+ETV (Figure 2) were performed in 35 of the 40 patients. VPS was applied in case of unsuccessful procedures. The procedures were unsuccessful in 5 patients aged < 1 year. In addition, they had a HC of > 90 percentile at the time of the surgery. ETV was successful in other 5 patients aged < 1 year who had HC of 75–90 percentiles. In our series, the procedure failure was not observed in adult patients and in those of age > 1 year.

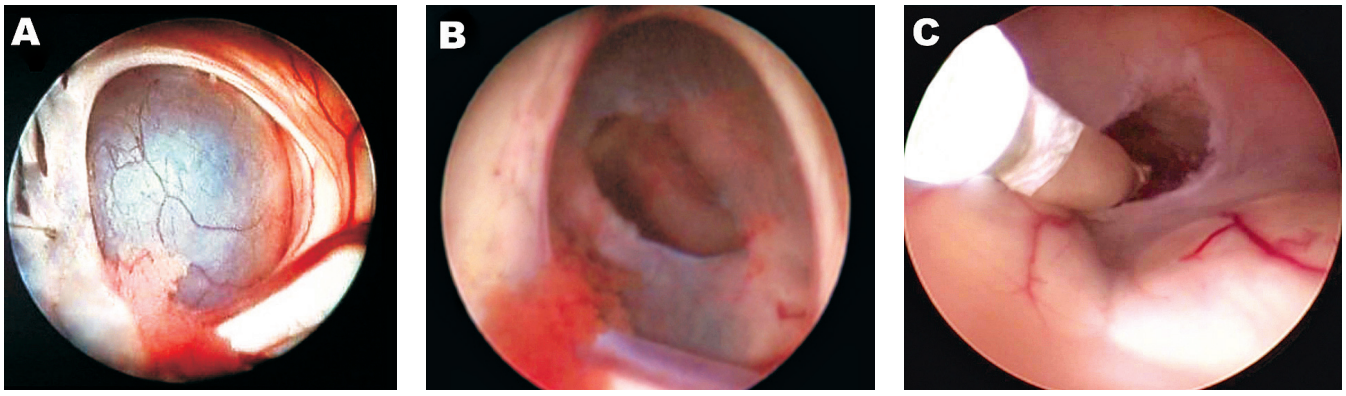

Fig. 2 Neuroendoscopic surgical photographs of a 12-month-old boy included in this study. (A) The view of the foramen of Monro and the third ventricle arachnoid cyst. (B) Fenestrated and dilated cyst wall. (C) After entering the cyst, we proceeded to the third ventricle floor and performed ETV. Fenestrated third ventricle floor can be seen.

Various complications arise in these kinds of neuroendoscopic procedures. For instance, CSF leakage can occur because of the failure of surgical closure. However, this condition can be observed in procedure failure. In 2 of the 5 patients in whom ETV failure was recorded, CSF fistula developed from the incision site after ETV. The first patient could be treated through the conservative method and the second one through surgical intervention (i.e. dural repair with synthetic dura material and fibrin sealant). Bradycardia occurred in 1 patient immediately after the fenestration of the third ventricular floor, and the patient was ameliorated after removing the endoscope from the third ventricle. Intraventricular hemorrhages were recorded in 2 patients, and they were controlled within a few minutes of performing irrigation.

DISCUSSION

VPS remains the most common treatment approach for HCP across the world. In the treatment of HCP and arachnoid cysts, significant reductions in the ventricular and/or cyst sizes could be noted after shunt surgeries in the early postoperative period (2, 5, 6, 11). Despite the sophisticated

Tab. 3 Case series of intraventricular arachnoid cysts managed with endoscopic surgery

Authors (ref)	Study period	n	Cyst localization	Primary clinical features at presentation	Complications	Success (n)
Ozek and Urgun (13)	1994–2010	34	suprasellar-prepontine	hydrocephalus and macrocrania (n = 32), developmental delay (n = 6), visual field defect (n = 9), endocrine dysfunction (n = 9), central tremor (n = 3), loss of visual acuity (n = 2), seizure (n = 2)	transient abducens nerve palsy (n = 1), growth hormone deficiency (n = 1), salt wasting syndrome (n = 1)	34
Cinalli G et al. (5)	1995–2008	14	quadrigeminal cistern	hydrocephalus (n = 10), intracranial hypertension (n = 7), enlarging cyst (n = 6), macrocrania (n = 4)	subdural collection (n = 1), postoperative CSF leak (n = 1), intraoperative bleeding (n = 2)	11 of 14
Tamburrini et al. (27)	2000–2006	26	quadrigeminal cistern, paraventricular, suprasellar, and choroid plexus	hydrocephalus (n = 15), headache (n = 8), increasing head circumference (n = 7), asymptomatic (n = 5), bulging fontanelle (n = 3), hemiparesis (n = 3), strabismus (n = 3), seizures (n = 2)	none	26
Ersahin and Kesikci (19)	2000–2007	17	quadrigeminal cistern	hydrocephalus (n = 17), macrocephaly (n = 12), bulging fontanelle (n = 7), headache (n = 3), sun-setting eyes (n = 2), nausea/vomiting (n = 2), psychomotor retardation (n = 2)	subdural collection (n = 3), CSF leak/meningitis (n = 1)	10 of 17
El-Ghandour (28)	2001–2009	25	Suprasellar	macrocrania (n = 12), intracranial hypertension (n = 12), developmental delay (n = 5), precocious puberty (n = 1), seizures (n = 1)	arterial bleeding (n = 1), CSF leak (n = 1), subdural collection (n = 1)	22 of 25
Knie et al. (12)	2002–2015	10	velum interpositum, quadrigeminal cistern	enlarging cyst (n = 5), seizures (n = 2), hydrocephalus (n = 2), hemiparesis (n = 1), optic atrophy (n = 1), mass effect (n = 1)	seizures (n = 2)	10
Copley P et al. (11)	2005-2016	29	suprasellar, velum interpositum, lateral ventricles, quadrigeminal cistern, third ventricle (posterior)	hydrocephalus (n = 29) increasing head circum./macrocephaly (n = 12), vomiting (n = 6) seizures (n = 5) bulging fontanelle (n = 4) developmental delay (n = 4) headaches (n = 3)	asemptomatic subdural CSF collection (n = 2), seizure (n = 1), wound leak (n = 1), diabetes insipidus (n = 1), intraoperative subdural hematoma (n = 1)	25 of 29

n: number of patients

advancements in the shunt systems, they are usually associated with several complications and numerous revisions due to either malfunction or infection. Thus, the provision of a long-term, quality-of-life to children with HCP seems extremely urgent. ETV could be an attractive alternative to the commonly utilized VPS. If it is effective, ETV can prevent long-term morbidity and mortality inherent to shunt-related complications (5, 15, 16).

Intraventricular or intracisternal arachnoid cysts near the ventricles are difficult to treat because of their deep locations. Craniotomy and cyst excision or fenestration therefore carries the risk of significant potential complications, such as neurological deficits (hemiparesis or cranial nerve injury), subdural hematomas, seizures, CSF leakages and meningitis. Moreover, open surgeries involve complication risks due to the long-duration of anesthesia. The significant potential morbidity of microsurgical fenestration and the high incidence of shunting complications have prompted neurosurgeons to search for an alternative procedure. Cumulatively, endoscopic fenestration has become the first choice of treatment because of its minimally invasive nature and the high success rates (11, 13, 14). Table 3 shows a literature review of the largest previously published case series of intraventricular arachnoid cysts managed with endoscopic surgery.

Although ETV, VC and other types of intraventricular neuroendoscopic interventions are considered safe, various complications resulting from them have been reported, including cardiac arrhythmias, injury to the hypothalamic–pituitary axis and/or structures near the floor of the third ventricle, vascular injuries, CSF fistulas, meningitis and sepsis (1, 8, 16, 17). Transient bradycardia is the most frequent hemodynamic alteration resultant from the impression of the third ventricular floor; therefore, on diagnosis, surgeons should pause fluid irrigation and ballooning and then remove the endoscope from the third ventricle. Intraoperative cardiac arrests have been reported very rarely in this condition. Bradycardia has been reported in only 1 patient in our series, immediately after the fenestration of the third ventricular floor. The patient was ameliorated after removing the endoscope from the third ventricle (6, 16, 18). Moreover, injuries to the fornix, hypothalamus and cranial nerves could be observed because of the anatomical proximity. Although it has rarely been reported, increased ICP due to closed and obstructed outflow channels or excessive irrigation can delay awakening and cause persistent confusion (2, 18). Intraventricular hemorrhage can occur because of choroid plexus, vein, or artery injury during the third ventricle floor perforation. Injury to the basilar artery is the most feared intraoperative complication. The fenestration point in the third ventricle floor should always be in front of the basilar tip. Before surgery, detailed evaluation of the radiological investigations must be performed to assess the possible variations, most of which can be controlled through continuous irrigation. In this study, intraventricular hemorrhages were recorded in 2 patients and controlled within a few minutes through irrigation (16–18). CSF leakage can occur because of the failure of surgical closure. Tight suturing of the layers and occlusion of the cortical and calvarial holes with hemostatic agents can prevent this complication (16, 17). In 2 patients, CSF fistula was detected at the incision site after ETV. The first patient could be treated conservatively (by resuturing the skin incision) and second one surgically (by dural repair with synthetic dura material and fibrin sealant). These 2 patients suffered from ICP symptoms after the treatment of the CSF fistulas; next, they were treated using VPS. Thus, CSF fistula was caused not only due to the surgical closure failure but also due to the application of the unsuccessful ETV procedure because of the high intraventricular volume and/or pressure. In other words, CSF leakage could indicate treatment failure.

The success of ETV can be determined by assessing clinical improvements and the absence of the need for further surgical procedure. Moreover, the simplest definition of successful ETV is 'freedom from a shunt'. Criteria as per the radiological investigations include decreased or stabilized ventricular size, especially that of the third ventricle, CSF flow through the fenestration point and straightened third ventricle floor that sprang downward (1, 16, 17). However, the ventricle size may not show a remarkable reduction on imaging post-ETV than post-shunting. However, this aspect does not necessarily reflect increased ICP (17). Hence, the main criterion for successful surgery should be the improvement of the clinical symptoms.

The correlation between ETV success and the patient's age is one of the most controversial issues. In a study by Erşahin and Kesikçi (19), almost all patients aged < 6 months who underwent both cyst fenestration and ETV required VPS postoperatively. In contrast, only a few studies indicated that there was no difference in the success rates in very young patients compared with older patients or that the rate was only slightly lower (20,21). Sufianov et al. (22) observed that ETV was successful in 71.4% of children aged 1–2 years and in 75.0% of children aged < 1 year. Recent studies have reported that the success of ETV mainly depended on the etiology of HCP and not on the patient's age alone. Several authorities consider patients with communicating HCP, especially due to the history of CSF infection or subarachnoid hemorrhage, to be more prone to ETV failure than those with obstructive HCP (2, 23). However, even in obstructive HCP, ETV failure can occasionally be observed in extremely young patients, especially in newborn and premature babies. Past studies have advocated different contributory factors to the worse outcomes of ETV in younger children. For instance, lesser efficacy for CSF reabsorption due to immaturity of the brain pathways in the arachnoid spaces/Pacchionian granulations, the development of new arachnoid membranes that obstruct the stoma, and low-gradient CSF reabsorption in newborns with open sutures are some of the contributing factors (15, 24).

Despite several investigations on the correlation between ETV success and the patient's age, no studies have yet reported the association between ETV success and HC or the ventricular volume. In our study, procedures were unsuccessful in 5 of 10 patients aged < 1 year. A common characteristic of these patients was a HC of > 90 percentile. HCP causes an increase in the intraventricular volume and pressure, which subsequently increase the fetal head size. Various investigations have shown that, in children with HCP, severe ventriculomegaly has been associated

with less favorable outcomes when compared with mild ventriculomegaly because of the high ventricular pressure or brain parenchyma compression (25). Furthermore, an increase in the fetal head size is associated with increased intraventricular pressure and volume (26). Shunts are mechanical drainage systems, with the features of rapid and high effectiveness. ETV re-establishes the physiological route of CSF dynamics, although the ventricular size may not result in a remarkable reduction post-ETV when compared with that post-shunting. Accordingly, increased ICP may not effectively decrease during the early period by ETV when compared with that by shunting. Therefore, the low success rates of VC and/or ETV may be observed in very young patients with a high HC.

CONCLUSIONS

ETV is currently considered as a successful alternative to the shunt systems in the treatment of HCP. However, most studies have reported that ETV has low success rates in patients aged < 1 year. In contrast, only a few studies have indicated the absence of any difference in the success rates in extremely young patients when compared with older patients. As per the past reports, in infants and very young patients, the underdeveloped subarachnoidal space plays a role in ETV failure. In this study, no failure was observed in children aged > 1 year and in adult patients. ETV or VC+ETV failure was recorded in half of the patients aged < 1 year; this report was consistent with those of the previous literature. Moreover, these patients had a HC of > 90 percentile at the time of the procedure, which makes it the focal point of our study. Furthermore, ETV failure was not observed in patients with a HC of < 90 percentile. Our results indicate that remarkable decrease in ICP may not be achieved by ETV in patients with enlarged ventricular volume and HC. Thus, the results of the present study contributes to the literature by addressing this important issue.

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A Case Series of Malignant Otitis Externa Mimicking Malignancy

Vengathajalam Selvamalar[1], Nik Adilah Nik Othman[1, 2,*], Mohd Khairi Md Daud[1, 2]

ABSTRACT

Malignant otitis externa is an inflammation of the external auditory canal with preceding osteomyelitis of the temporal bone and the adjacent structures that could be potentially lethal. Malignant otitis externa may present with cranial nerve involvements and massive spread of disease mimicking nasopharyngeal carcinoma or any other malignancies on imaging. Two elderly patients who presented with severe otalgia and significant facial nerve palsy and lower cranial nerve palsies showing extensive spread of disease are reported in this case series. They both had resolution of disease after a prolonged course of antibiotics and cortical mastoidectomy for disease clearance in one of them.

KEYWORDS

malignant otitis externa; Pseudomonas aeruginosa; osteomyelitis; cranial nerves palsies

AUTHOR AFFILIATIONS

[1] Department of Otorhinolaryngology-Head and Neck Surgery, School of Medical Sciences, Health Campus, University Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
[2] Hospital University Sains Malaysia, Health Campus, Kubang Kerian, 16150, Kelantan, Malaysia
* Corresponding author: School of Medical Sciences, Health Campus, University Sains Malaysia, Kubang Kerian, 16150, Kelantan, Malaysia; e-mail: adilahkk@usm.my

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INTRODUCTION

Malignant otitis externa (MOE) is an aggressive infection of the external ear canal and temporal bone. MOE was first reported by Toulmouche in 1838 (1). Another name for MOE is necrotizing otitis externa or osteomyelitis of the temporal bone. Patients with MOE usually presents with painful ear discharge with hearing loss, tinnitus and throbbing headaches that is not responding to medical treatment. The ear canal is edematous with granulation tissue at the osteocartilaginous junction on otoscopic examination. The usual causative organism is Pseudomonas aeruginosa and the infection can be lethal with extension of the infection to the skull base and central nervous system causing cranial nerve palsies, meningitis and brain abscesses. The commonly involved cranial nerve is the facial nerve as it exits the stylomastoid foramen, subsequently the glossopharyngeal, vagus and accessory nerves as they exit the jugular foramen and the hypoglossal nerve as it exits the hypoglossal canal. In petrous apex involvement, the trigeminal and abducens nerve can be affected and in cavernous sinus and clivus involvement, the olfactory, troclear and abducens nerve may be affected as well. We report two cases of malignant otitis externa in two elderly female patients presented with lower cranial nerve palsies and extensive spread of disease described on computed tomography (CT).

CASE REPORT 1

This patient is a 50-year-old lady who presented with dysphagia, lethargy and hoarseness for 2 weeks. She was having intermittent episodes of left ear discharge and pain for 3 months with throbbing headache and vomiting episodes. She was previously treated for acute otitis externa.

She appeared comfortable under room air with stable vital signs. On otoscopic examination, the left ear canal was filled with pus, oedematous and there was a mass occupying the canal arising from the posterosuperior wall. The mass was smooth surfaced and didn't bleed on probing while the left tympanic membrane couldn't be visualised. The right ear was normal. Her intraoral examination revealed the curtain sign with left palatopharyngeal weakness. However, there was no trismus. There was a firm fullness felt on the left preauricular region measuring 7 × 6 cm but there was no signs of inflammation. She also had left facial nerve palsy House-Brackmann Grade 2. Flexible nasopharyngolaryngoscopy showed pooling of saliva and the left vocal cord was immobile in paramedian position with a phonation gap consistent with left recurrent laryngeal nerve palsy. Her pure tone audiometry showed left moderate to profound mixed hearing loss and right mild to severe sensorineural hearing loss.

The total white blood cell count was raised up to 16.5 × 10^9/L. Both ESR and C-reactive protein were also raised with a reading of 123 mm/hr and 40 mg/L respectively. She had electrolytes imbalance with hyponatraemia and hyperkalemia secondary to her poor oral intake.

She was admitted for initiation of intravenous antibiotics, corrections of electrolytes and dietary optimization. She was started on ryles' tube feeding and was referred to the Endocrine team to manage her diabetic status.

This lady had underlying diabetis mellitus, on insulin regime with a diabetic foot ulcer and hypertension. Her foot ulcer was fairly clean and was dressed daily. Her sugar control in the ward was below the range of 10 mmol/L but her HbA1c showed poor sugar control with reading of 14.5%.

Swab was obtained from the left ear for culture and sensitivity and it grew Pseudomonas Aeruginosa. Biopsy was taken from the left external ear canal mass and was later reported as inflammatory granulation tissue by the pathologist.

She was started on intravenous ceftriaxone but once the culture grew Pseudomonas Aeruginosa, her antibiotics were upgraded to meropenem for better blood brain barrier penetration and was given for 2-weeks. She was also given gentamicin with betamethasone ear drops.

She underwent high resolution computed tomography (HRCT) to see the extent of the disease. As shown on Figure 1, there was a soft tissue density in the left middle ear, part of the external ear canal and the left mastoid air cells. The ossicles and incudomalleolar joint were intact. Even though the first genu and the tympanic segment of the facial nerve were not well visualized, the rest of the facial nerve course were normal.

The HRCT also showed features of skull base osteomyelitis with abscesses in the left parapharyngeal extending to left fossa of rossenmuller, left torus tubarius, retropharyngeal, masticator space and left temporomandibular joint as shown in Figure 2.

There was an intracranial extension with meningeal enhancement in the left temporal region with bony erosions at the petrous apex, left anterior occipital condyle, squamous part of temporal bone, sphenoid bone, tegmen

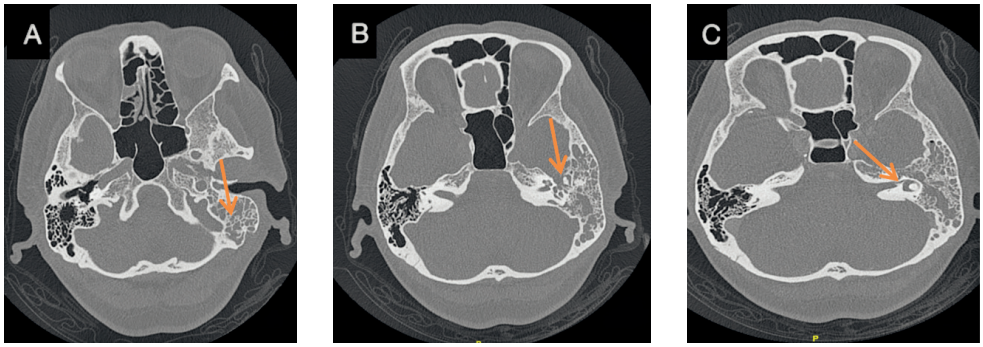

Fig. 1 HRCT temporal, axial cuts showing soft tissue density in the left mastoid air cells (A). The ossicles were intact (B). The left facial nerve course was normal (C).

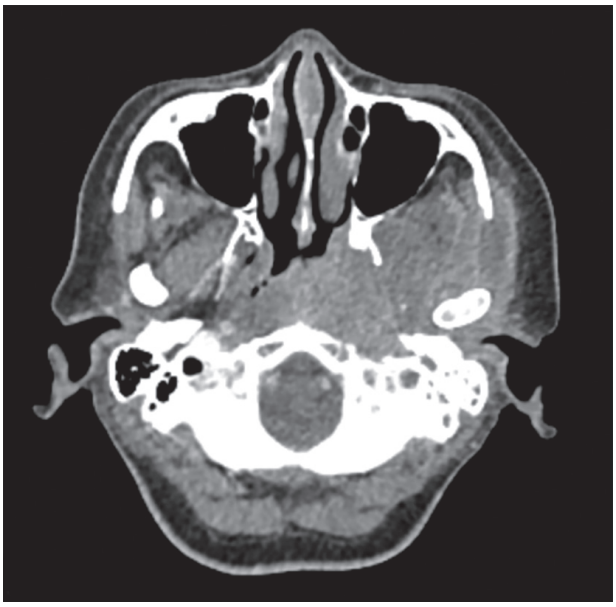

Fig. 2 HRCT temporal, axial cuts showing abscesses in the left parapharyngeal extending to left fossa of rossenmuller, left masticator space and left temporomandibular joint.

tympani, carotid canal, clivus and anterior part of foramen spinosum as shown in Figure 3.

After 2 weeks of intravenous meropenem, she was given oral ciprofloxacin for 4 weeks. Her symptoms improved as her otalgia and otorrhea resolved. Although her hoarseness persisted, she was well and was discharged home with oral ciprofloxacin.

She was seen again in clinic 2 weeks after and she was still pain free. Her ear symptoms such as otalgia and otorrhea improved. She was feeding via ryles tube with evident aspiration. However, there was a new finding of left hypoglossal nerve palsy showing significant muscle wasting of the left lateral tongue deviating to the left. The previously seen left facial nerve palsy reverted back to normal during examination. The other pre-existing left glossopharyngeal and left vagus nerve palsies showed no improvement. She was referred to the speech therapist for swallowing exercise. She was planned for a bone scan to monitor her disease progression and treatment response but patient refused for any further intervention.

CASE REPORT 2

The second case reported is a 53-year-old lady who presented with severe right ear otalgia for almost 5 months, throbbing in nature. She described it as a constant pain associated with right sided headache affecting her daily routine. The pain was relieved by oral analgesia but it only lasted a while. She also complaint of right ear fullness with tinnitus and reduced hearing. Otherwise she denied of any fever and there was no giddiness nor vertigo. The symptoms were not preceded by any respiratory infection or trauma.

She visited the local clinics multiple times and was given tropical ear drops and oral antibiotic but her symptoms were not relieved. She had underlying diabetic and hypertension, well controlled on oral medications and insulin injections.

She appeared comfortable under room air with no acute respiratory distress. There was right facial nerve palsy House-Brackmann Grade 2. On otoscopic examination, the right external ear canal was filled with a mass and the tympanic membrane could not be visualized; however the left ear canal was clear with intact tympanic membrane. She also had right glossopharyngeal and right vagus nerve palsies with curtain sign elicited intraorally and uvula deviated to the left. Her gag reflex was absent. The other cranial nerves examinations were unremarkable. There was a right level II lymph node measuring 2 × 1 cm, firm in consistency with well demarcated borders. Her pure tone audiometry showed right mild to severe mixed hearing loss and left mild to profound sloping sensorineural hearing loss.

She was admitted for intravenous ciprofloxacin given for 2 weeks, ofloxacin topical ear drops and pain control. During admission, her ESR was reported as 127 mm/hour and C-reactive protein was positive with a reading of 48 mg/L. Her total white blood cell count was 12.04 × 10^9/L. A biopsy was obtained from the right ear canal mass reported as compatible with malignant otitis externa. Nasal endoscopy showed an enlarged right torus tubarius obliterating the right fossa of Rossenmuller. Biopsy of the right torus tubarius revealed no malignancy. Other findings were normal. Swab for culture and sensitivity from the right ear canal was reported as negative for any growth of microorganism.

Magnetic resonance imaging (MRI) was done towards the end of the 2 weeks of admission to look for any evidence of cholesteatoma or malignant lesions. As shown in Figure 4, there was a diffuse enhancing soft tissue lesion inferior to the right temporal lobe involving the right masticator space, right carotid space, right parapharyngeal space, pharyngeal mucosal space and retropharyngeal space. The lesion extended till the dural layer superiorly, inferiorly until the upper oropharynx and medially to the nasopharynx.

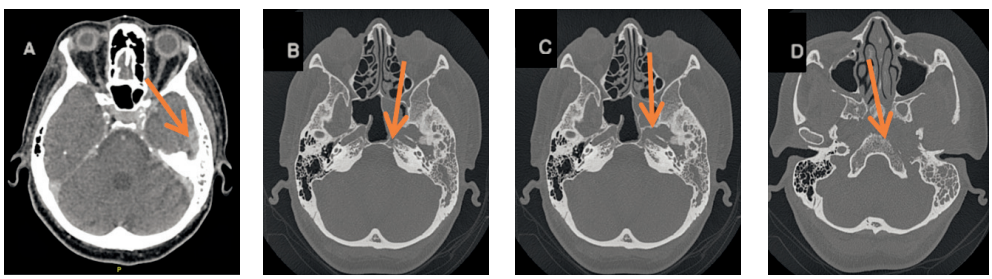

Fig. 3 HRCT temporal, axial cuts showing intracranial extension with meningeal enhancement in the left temporal region (A). Bony erosion were seen at left petrous apex (B), carotid canal (C) and clivus (D).

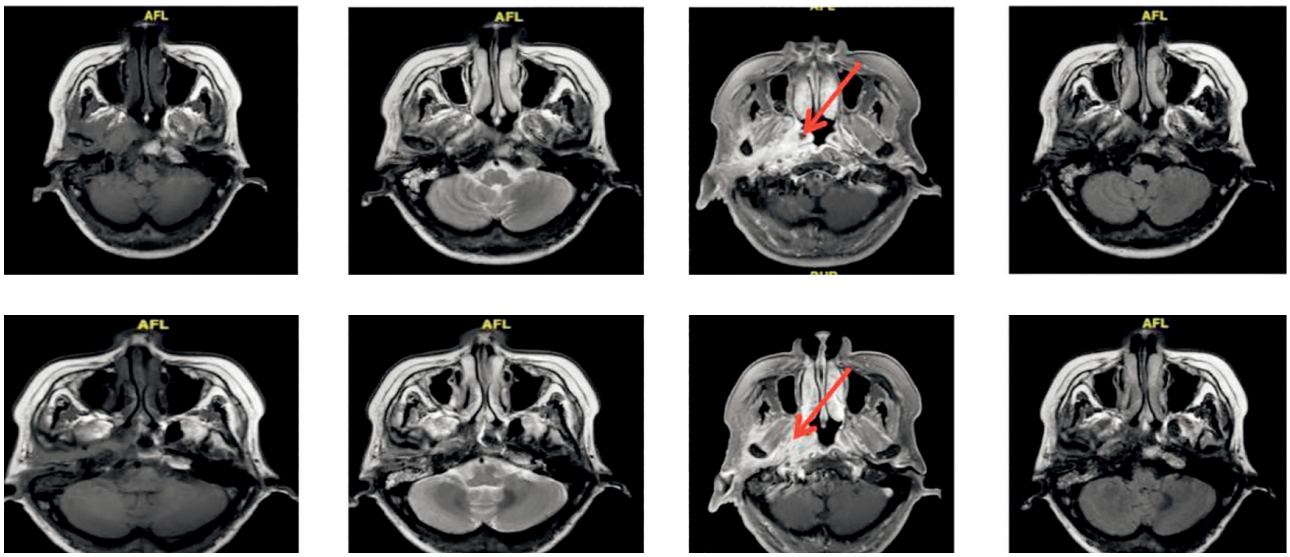

Fig. 4 MRI images, axial cuts showing diffuse enhancing soft tissue lesion involving the right masticator, right parapharyngeal and retropharyngeal space.

She was given another 6 weeks of oral ciprofloxacin to complete 8 weeks all together. Her symptoms improved clinically with reducing pain and resolved ear discharge but she underwent right cortical mastoidectomy to obtain a tissue biopsy as we were still uncertain of her diagnosis. Intra-operatively there was sagging of the supero-posterior wall with intact tympanic membrane. When the tympanomeatal flap was raised, there was a soft tissue mass seen within the right mastoid antrum and right middle ear which was later reported as chronic inflammation. The middle ear mucosa appeared thickened with mucoid discharge.

Post operatively, she was doing well. Her pain was well controlled and there was no more ear discharge. There was improvement in the cranial nerve functions with right facial nerve House-Brackmann Grade 1 and uvula was central during intraoral examination. Her total white blood cell count dropped to 9.8 × 10^9/L and her ESR reading was decreasing to 111 mm/hour with a negative reading of C-reactive protein. Her pure tone audiometry also showed improvement with right moderate mixed hearing loss and left mild to profound sensorineural hearing loss.

DISCUSSION

Malignant otitis externa is a fulminant osteomyelitis involving the external auditory canal and the skull base following an episode of external ear infection. Being termed as a misnomer, it behaves aggresively like a malignancy. Patient in old age or patients with diabetis mellitus and debilitating condition are at higher risk for MOE (1).

Patients with MOE presents with severe otalgia and throbbing headaches just like the cases reported in this series. The intensity of pain is usually measured with the visual analogue scale (VAS). Both our patients had significant pain score according to the VAS. They usually show no improvement to local treatment and due to the ongoing infection, they may also have a discharging ear. The most commonly grown organism in MOE is Pseudomonas aeruginosa (95%) (2) as reported in our first case report.

The otoscopic examination will reveal an edematous ear canal with granulation tissue at the osteocartilaginous junction. Besides the granulation tissue at the osteocartilaginous junction, there can also be a mass in the external ear canal. Biopsy of this mass is important to help to exclude other pathologies such as malignancies or cholesteatoma (3, 4) but most frequently, these biopsies obtained may not be significant (5) delaying the establishment of diagnosis.

MRI and CT scans are used to determine the anatomical extent of the disease and intracranial complications if any. Features of MOE on CT is reduce skull base density and bony erosions. Bony erosions of the skull base and petrous apex as seen in the first case report raises the suspicion of malignancy. In MOE, bony erosions on CT is only evident when at least 30% demineralisation of bone has happened which is usually in the later stage of the disease (6). MRI on the other hand determines the soft tissue changes, exact location and extent of the disease. MRI is highly sensitive but not so specific for MOE. The Technetium Tc 99 methylene diphosphonate bone scanning is the most useful imaging tool to evaluate the positive findings based on binding of the osteoblasts (3) but they may be positive in cases of malignancy as well (7).

As the disease progresses, patients can rarely present with cranial nerve involvement. Intracranial involvement can potentially alter the patient's mental status and even cause mortality. Cranial nerve involvement indicates a poor prognosis of the disease leading to death due to the complications (8, 9). The spread of infection from the external ear is through the fissures of Santorini and the osteocartilaginous junction. The lower cranial nerves are commonly affected in the skull base osteomyelitis as reported in both our cases. The other cause of lower cranial nerve palsy in these areas is the nasopharyngeal

carcinoma (NPC) that is related to the posterolateral spread of the disease as well as the jugular foramen infiltration (10).

The facial nerve is the first and most common to be affected in MOE as it exits the stylomastoid foramen (7) and both our cases had facial nerve palsy on presentation. This is followed in order by the glossopharyngeal, vagus and accessory nerves as they exit the jugular foramen and the hypoglossal nerve as it exits the hypoglossal canal (11). The trigeminal and abducens nerve can be affected as well if there is petrous apex involvement. The spread of infection to the cavernous sinus and clivus may cause the olfactory, troclear and abducens nerve palsy (12). If the treatment is initiated early, the cranial nerve palsies in MOE can be reverted back to normal. Both our cases had facial nerve and lower cranial nerves involvement and they showed improvement after treatment suggesting MOE rather than malignancy.

Navin reported 43% of cranial neuropathies in a retrospective analysis of 23 MOE cases where he mentioned that 60% cases were with facial nerve palsy, 30% with lower cranial nerves palsy in combinations of glossopharyngeal, vagus, accessory and hypoglossal nerve and 10% had an extended cranial nerve palsies involving the facial nerve, lower cranial nerves and other cranial nerves. Out of the 10 cases reported, 4 died due to unrelated causes, 5 had resolution of disease but no cranial nerve palsy improvement and 1 required prolonged treatment of oral ciprofloxacin. And it was speculated that facial nerve palsy does not resolve after treatment because of its longer course of involvement compared to the others. This case series reported a recovery rate of 87% with 0% of mortality (13). In another journal, Ethan et al. reported that involvement of facial nerve palsy showed a significant extension of disease to the parapharyngeal and nasopharyngeal region in addition to the mastoid on computer tomography (4) just like the two cases reported in this series.

The choice of antibiotics for treatment is from the fluoroquinolone group namely ciprofloxacin that can attain high level of bone and soft tissue penentration, and third generation cephalosporins such as ceftazidime (2) and aminoglycosides such as gentamicin may be used in ciprofloxacin-resistant patients.

Surgical options are kept in view for local debridement, abscess drainage and bony sequestrum removal. Even though our patient in the first case was given intravenous meropenem for 2 weeks, she was then given a long 4 weeks course of oral ciprofloxacin and for our patient in the second case, she was treated with 8 weeks course of ciprofloxacin and they both responded well to the treatment.

There may be many instances where MOE was mistaken for NPC because both these diseases invades the bony structures aggressively. Both the cases in this series had imagings with involvement of fossa of rossenmuller mimicking NPC. Goh et al reported 14 cases with skull base osteomyelitis where the imagings showed a nasopharyngeal bulge involving the fossa of rossenmuller (14). He also mentioned that in MOE, the soft tissue enhancement is greater or equal to the mucosa and the spread of disease is along the fascial plane preserving the architecture whereas it was vice versa in NPC. The presence of abscesses in imagings should also lead to infection rather than malignancy.

Another significant distinguishing factor of MOE is the lateral structure involvements such as TMJ and parotid gland involvement. NPC usually does not extend laterally except in late cases due to perineural invasion or contiguous spread along the auriculotemporal nerve (15).

Clival involvement on MRI is shown for both MOE and malignancy (16). But for MOE specifically, the clival involvement is shown enhanced with marrow space hypointensity on T1 weighted images but hyperintense on T2 weighted images with enhancement of dura, parapharyngeal fat plane effacement and soft tissue mass in skull base (17). Ozgen et al reported the important function of diffusion-weighted sequence on MRI in differentiating MOE and malignancy (18). He said that malignant tumours such as NPC and lymphomas have very low apparent diffusion coefficients (ADC), while benign lesions such as MOE have higher ADC.

The raise of inflammatory markers such total white blood cell count, ESR and CRP should also lead the suspicion of infection rather than malignancy. MOE patients may have a reactive node but not in typical fashion like NPC. NPC patients commonly present with nodal metastasis starting from the retropharyngeal region extending to level II, III, IV, V and supraclavicular.

MOE may spread causing further complications such as lateral sinus thrombosis, internal jugular vein thrombosis, opthalmoplegia and blindness which can mimick malignancy. It is also important to correlate with the clinical history and examination where in MOE, patients commonly with underlying diabetes mellitus presents with ear pain and ear discharge with ear swabs growing Pseudomonas Aeruginosa.

CONCLUSION

Although MOE with cranial nerve involvement indicates extensive progression of the disease, it doesn't worsen the prognosis of the disease itself. In view of aggressive features of disease in imaging, it is important to differentiate MOE from malignancy as early intervention with long-term high dose antibiotics may lead to resolution of disease although the cranial nerve functions might not be reverted back to normal in all cases.

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Ovotesticular Disorder of Sexual Development and Non-Palpable Testis

Radek Štichhauer[1,*], Antonín Šafus[1], David Neumann[2], Ivo Novák[3], Vladana Skutilová[4], Jan Laco[5]

ABSTRACT

Disorders of sexual development (DSD) refers to a group of diseases that links the mismatch between an individual's genetic and gonadal development and its phenotype. Ovotesticular DSD (true hermaphroditism) is one such disease, in which both male and female gonads are present.

A 15-year-old boy with a history of surgery for non-palpable testis was examined due to bilateral gynecomastia and known gonosomal mosaic of Klinefelter syndrome. The external genital was matured as male and, in the left half of the scrotum, there was a testicle of normal size. Despite uncertain resistance on the right side, however, the right testis was not palpable. Revision of the right groin revealed a surprising finding in the form of an ovary with a dilated fallopian tube, both of which were completely removed. Surgical revision of the left testis with biopsy was performed. The surgery was completed with a bilateral mastectomy. The postoperative course was uncomplicated, and the boy is content and fully integrated into his peer group. True hermaphroditism is a rare type of DSD. In the case described, DSD was not exhibited until puberty, after an examination for gynecomastia. The case also confirms the necessity of clarification and long-term follow-up of patients with unclear findings during surgery for non-palpable testis. Diagnostic laparoscopy is clearly indicated in these situations.

KEYWORDS

true hermaphroditism; ovotesticular DSD; gynecomastia; non-palpable testis; laparoscopy

AUTHOR AFFILIATIONS

[1] Department of Pediatric Surgery, University Hospital Hradec Králové, Faculty of Medicine Hradec Králové, Charles University, Czech Republic
[2] Department of Pediatrics, University Hospital Hradec Králové, Faculty of Medicine Hradec Králové, Charles University, Czech Republic
[3] Department of Urology, University Hospital Hradec Králové, Faculty of Medicine Hradec Králové, Charles University, Czech Republic
[4] Department of Medical Genetics, University Hospital Hradec Králové, Faculty of Medicine Hradec Králové, Charles University, Czech Republic
[5] Fingerland Department of Pathology, University Hospital Hradec Králové, Faculty of Medicine Hradec Králové, Charles University, Czech Republic
* Corresponding author: Department of Pediatric Surgery, University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic; e-mail: radek.stichhauer@fnhk.cz

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INTRODUCTION

Disorders of sexual development (DSD), previously termed "intersex", refers to a heterogeneous group of diseases that links the mismatch between an individual's genetic and gonadal development and their phenotype. A basic genetic status of "XX" primarily determines female genital organs and phenotype. A system of genes, namely, *TDF* (testis determining factor) and *SRY* (sex-determining region on Y-chromosome) located on the short arm of the Y chromosome, is required for male development. The presence of these genes leads to differentiation of originally undifferentiated gonads. The hormones produced by the fetal testicle then modify phenotypic sex (1, 2). However, there are numerous deviations and pathologies that can completely disrupt this process. Ovotesticular DSD (previously termed "true hermaphroditism") is such an example of a DSD in which both male and female gonads are present in an individual. Gonadal or phenotypic sexual disorder may occur in an individual immediately after birth; however, there are also variants that manifest later in adolescence, as in the case described in the present article, or during adulthood. Treatment of DSD involves an interdisciplinary approach, including pediatric, endocrine, surgical, urological and, finally, psychosocial care. The timing of surgery depends on the type of disorder, age at diagnosis, and psychosocial considerations (3). Given the hormonal production and the increased risk for malignant germ cell tumors in abnormally localized gonads, surgical treatment should not be postponed. At this point, DSD treatment is intertwined with treatment of non-palpable or pathologically undescended testes in otherwise healthy boys.

CASE REPORT

A 15-year-old boy was referred to the pediatric surgeon for bilateral gynecomastia and confirmed rare gonosomal mosaic of Klinefelter syndrome 46XX[84]/47XXY[16], previously examined by an endocrinologist. The patient was otherwise healthy, with normal height and body proportions. He had an unremarkable medical history, except for surgery for the presumed undescended right testis at 15 months of age. Symmetrical bilateral gynecomastia grade II was present and pubic hair within the context of pubertal development was evident (Fig. 1). The external genitalia were fully matured in the male line, Tanner G5, P4, A3. The left half of the scrotum contained a normal size testicle. The right side, however, exhibited elastic resistance in the groin extending into the scrotum, measuring approximately 2 × 2 cm in area; nevertheless, the right testis was not palpable. Laboratory investigations revealed a hypergonadotropic status testosterone level, although other biochemical examinations were within reference limits. Human chorionic gonadotropin and alpha-fetoprotein levels were unremarkable. With these results, the boy was referred to an outpatient department of Pediatric Surgery. According to the currently defunct operative protocol used to revise the right groin at the age of 15 months, it was found that tissue that was supposed to be in the right testicle rudiment was removed. Diagnostic laparoscopy was not performed at that time. However, microscopic examination of the removed tissue did not confirm the presence of testicular tissue. Subsequently, the boy was lost to surveillance and follow-up. At 15 years of age, the boy underwent ultrasonography after physical examination. Normal homogeneous testis on the left and a structure mimicking a small testicle on the right, with possible incipient hydrocele, were evident. After multidisciplinary board consultation, surgery was scheduled. The actual surgery was made two months after the first examination. The resistance in the right groin was already twice as large and visible.

First, diagnostic laparoscopy was performed with the patient under general anesthesia to assess the type of internal genital organs in the abdominal and pelvic cavities. Entrances to both inguinal canals were closed. The vas deferens and spermatic vessels entered the left inguinal canal, whereas vas deferens was absent on the right side,

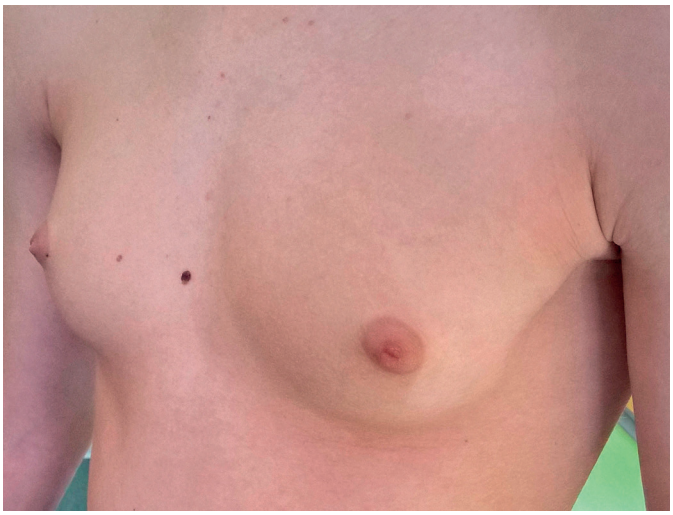

Fig. 1 Gynecomastia grade II on both sides.

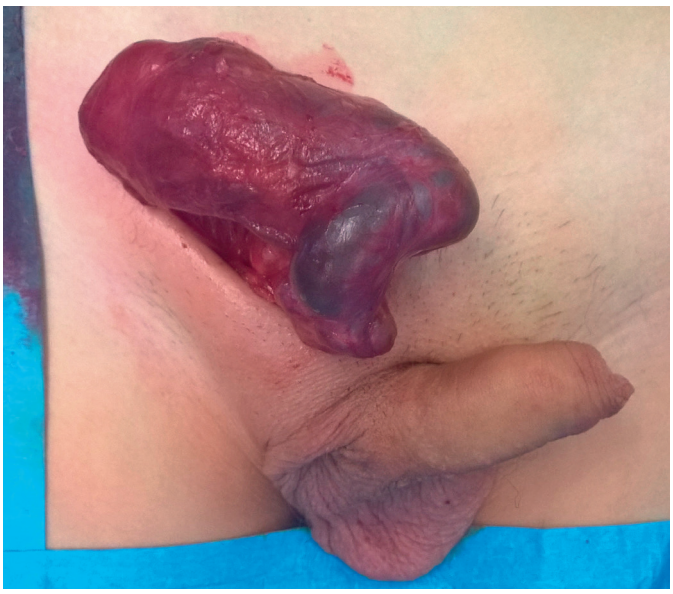

Fig. 2 Peroperative finding in the inguinal region – ovary and fallopian tube on the right.

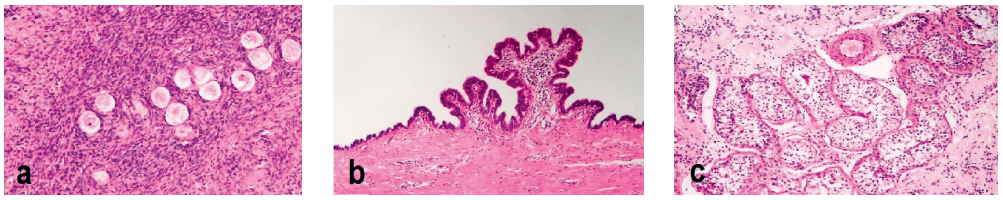

Fig. 3 Histological findings: (a) ovary, (b) Fallopian tube, (c) testis.

where only spermatic vessels entered the inguinal canal. Revision of the right inguinal region revealed a surprising finding of an ovary with a dilated Fallopian tube filled with serous fluid (Fig. 2); both of these organs were completely removed. Subsequently, surgical revision of the left, macroscopically normal, testis with a punch biopsy was performed. The testis was then fixed into the scrotum and has been left in situ. The surgery was completed with bilateral mastectomy, with a subareolar incision at the end of the procedure.

Microscopic examination of the tissue from the right groin revealed a fully developed but dilated fallopian tube and ovarian tissue with primordial follicles. Testicular tissue was absent. Examination of the left-side testis revealed complete sclerosis in approximately one-half of the tubules and absence of spermatogenesis in the remaining tubules. This finding was consistent with the diagnosis of germ cell aplasia. Within the interstitium, there were a few scattered cells with eosinophilic cytoplasm, most likely representing immature Leydig cells (Fig. 3).

The postoperative course was uncomplicated, and all surgical wounds healed per primam. The cosmetic outcomes in all regions were favorable. The boy is under continual care of a pediatric endocrinologist and a pediatric surgeon. His psychological well-being is favorable, and he does not need the specialized care of a mental health professional. He was fully satisfied with the postoperative outcomes and has fully integrated into his peer group.

DISCUSSION

DSD is a group of diseases manifesting as various abnormalities of genetic, gonadal, and phenotypic sex. The most common example of DSD is congenital adrenal hyperplasia, accounting for approximately 70–80% of cases. As surgical treatment, feminizing genitoplasty is usually performed. Ovotesticular disorders account for approximately 3–10% of DSDs, the most common of which include karyotype 46 XX (53%), and chromosomal mosaicisms (40%) and 46 XY (7%). According to Matsui (4), the most common gonadal combination is ovotestis with ovary (33.9%), followed by ovary and testis (24.2%), bilateral ovotestis (20.6%), and ovotestis and testis (16.4%). The true hermaphrodite, an individual with a simultaneously developed ovary and testicle and karyotype 46XX, 47 XXY, as in our case, is a very rare finding. Comprehensive studies are rare, and very few have described a large number of patients. Thus, extensive experience with treatment and long-term follow-up is lacking (3). There is a 2.6–4.6% increased risk for malignant transformation of ovotesticular gonads in boys with karyotype 46 XY (5). In girls with ovotesticular disorders, the presence of testis leads to early virilization; therefore, early surgical and endocrinological treatments are recommended (2). Other factors, such as age at diagnosis and psychosocial aspects, also play an important role. The majority of DSD defects are detected early after birth or in the first years of a child's life. Experience with the boy in our case and some other publications demonstrate that ovotesticular disorder may only become evident also later, in older children or even in adults. In a previous study, Caputo reported a case involving a 53-year-old man with hypergonadotropic hypogonadism that was revealed incidentally when the patient was treated for urolithiasis. Clinically, the man had micropenis, undifferentiated external organs, and normally formed secondary sexual characteristics. After genetic and magnetic resonance examination, he underwent laparoscopy with removal of a uterus with adnexa, ovary, and testis from the abdominal cavity (6, 7). The timing of surgery also involves psychosocial considerations. Gonadal organs are diverse in newborns or young children with ovotesticular disorders, and assessment of further development can be difficult. Previous publications have recommended postponement of definitive surgery until the pre-pubertal or adolescent period (1). In girls with karyotype 46 XX, however, there is agreement regarding the elimination of male or atypical gonads (testis, ovotestis) at an early age (8, 9).

The boy described in the present case, points out one more aspect, which is the suitability of diagnostic laparoscopy in the treatment of undescended and non-palpable testes. Cryptorchidism (i.e., impaired testicular descent and position in boys), is one of the most common congenital malformations in general. It affects approximately 1–4.6% of newborns but up to 30–45% of preterm newborns. At 1 year of age, the percentage of persistent cryptorchidism varies from 0.8–1.8% (1, 2, 10). Neonatal germ cells are converted in spermatogonia between months 3 and 9, and there is clear evidence of a decrease in germ cells after this period and fibrous rebuilding in pre-school age. The incidence of malignant germ cell tumors in undescended testes has been reported to be approximately 1.7% (10). For these reasons, cryptorchidism should be diagnosed before 6 months of age and surgery should be performed within the first year of life. Non-palpable testes (i.e., testes that are not palpable in the scrotum or inguinal region), have been described in 10–15% of cryptorchidism cases (10, 11). Clinical examination is usually followed by ultrasonography (USG). While the specificity of USG examination is 91% for testes located in the inguinal canal, the sensitivity is only 78%, and the yield of USG examination decreases significantly to approximately 30–40% in cases involving intra-abdominal retention (non-palpable testes) (10). In Europe, USG is used to diagnose non-palpable testes in approximately

49% of cases; in the United States, however, this figure is only 12% (12). Independently, USG examination is recommended as the next step in examining the patient under general anesthesia at the beginning of the operation. Currently, diagnostic laparoscopy is considered to be the gold standard modality in the treatment of non-palpable testis (12). Both, monocentric and multicentric studies describe ovotesticular disorders as an indication for gonadal exploratory diagnostic laparoscopy in cases of non-palpable testis (11, 13). In our case, diagnostic laparoscopy for non-palpable testis was not performed at 15 months of age, and the result of histological examination of the removed tissue from the right groin, where the testis was absent, was not correctly interpreted by the clinicians. At present, diagnostic laparoscopy is currently performed at our department in every patient with non-palpable testis or non-specific resistance in the scrotum or groin. This structure could be mistaken for atrophic testicular rudiment and the testicle itself could be located intra-abdominally. The preoperative ultrasound examination is not considered decisive. The final diagnosis is always ensured by histology. In case of unclear peroperative findings, histological examination can be performed peroperatively. Patients after these operations are observed annually until puberty.

CONCLUSION

True hermaphroditism is a rare type of ovotesticular DSD. In our case, it was not evident until puberty, following an examination for gynecomastia. After multidisciplinary examinations, surgery was performed and the ovary and fallopian tube were removed, with a histologically confirmed second testis fixed and mastectomy performed. The postoperative course was uncomplicated, and the boy is healthy, content, and fully integrated into his peer group. This case confirms the necessity of clarification and long-term follow-up of patients with unclear findings during surgery for non-palpable testis. Diagnostic laparoscopy is clearly indicated in all of these situations.

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ABBREVIATIONS

DSD	disorders of sexual development
USG	ultrasonography

AUTHOR CONTRIBUTIONS

SR designed the study and drafted the manuscript, and SR, SA, and NI performed the surgical procedures. ND performed pediatric and endocrinological examination of the patient and leads his follow-up; SV performed the biogenetics examination, and LJ performed histological examination. All authors revised and edited the draft, and are in agreement with the content of the manuscript submitted for publication.

AVAILABILITY OF DATA AND MATERIALS

All data are available upon reasonable request from the Department of Pediatric Surgery, University Hospital Hradec Králové and Charles University, Faculty of Medicine Hradec Králové, Czech Republic, Sokolska 581, 500 05 Hradec Králové, Czech Republic.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest regarding publication of this article.

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Rare Complication of Necrotizing Pancreatitis: Extension of Retroperitoneal Abscess into Femoral Region

Maja Karin[1], Ante Bogut[1], Ivan Romic[2,*], Hrvoje Silovski[2], Josip Figl[2], Danijel Pravdic[1], Mile Volaric[1], Emil Babic[1], Branko Bakula[3], Renata Romic[4]

ABSTRACT

Distant abscesses are uncommon during the episode of acute pancreatitis (AP). However, these are possible sequalae of necrotizing pancreatitis and should be treated appropriately to prevent serious septic complications. We demonstrate a case of a 56-year-old male patient who presented with severe necrotizing pancreatitis and distant retroperitoneal abscess that reached femoral region and was detected on diagnostic imaging scans. Combination of surgical and supportive therapy was employed, and the patient recovered well with no permanent consequences. Our article highlights the importance of quick and accurate diagnosis and timely intervention in this rare type of pancreatitis complication.

KEYWORDS

pancreatitis; retroperitoneal; drainage

AUTHOR AFFILIATIONS

[1] Internal Medicine Clinic with Dialysis Center, University Clinical Hospital Mostar, Bosnia and Herzegovina
[2] Surgery Department, University Clinical Hospital Zagreb, Croatia
[3] Surgery Department, University Clinical Hospital Sveti Duh, Zagreb, Croatia
[4] Family Medicine Department, Health centre Zagreb, Zagreb, Croatia
* Corresponding author: Department of Surgery, University Hospital Centre Zagreb, Kispaticeva 12, 10 000 Zagreb; e-mail: i.romic@gmail.com

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INTRODUCTION

Acute necrotizing pancreatitis (ANP) is a severe form of acute pancreatitis, characterized by necrosis of the pancreas and surrounding tissues with development of pancreatic collections and abscesses. It is associated with high rates of morbidity and mortality and long-term complications. Wide clinical picture, atypical presentation, local and systemic complications make treatment of acute necrotizing pancreatitis very challenging for both surgeons and gastroenterologists (1). Most severe complications of acute necrotizing pancreatitis are result of peripancreatic collections rich in digestive enzymes. It leads to necrosis of peripancreatic tissue and spreading of inflammation process along the retroperitoneum. The descent of necrotic retroperitoneal collections along the retroperitoneal space to the femoral region is a rare but possible manifestation of ANP that all clinicians should have on mind when evaluating femoral and inguinal swelling.

CASE REPORT

A 56-year-old male patient was admitted to the gastroenterology department because he had abdominal pain for the last 4 days, ascites and microcytic anemia. The patient had a history of heavy alcohol consumption. On physical examination, diffuse abdominal tenderness, ascites and ventral hernia were noted. C-reactive protein (CRP) was elevated to 88.9 mg/L (range 0–5), alkaline phosphatase 213 U/L, gamma glutamyl transferase (GGT) to 93 U/L (range 9–35) and serum amylase to 345 U/L (range 28–100). Abdominal ultrasound showed ascites and inhomogeneous calcification of pancreas suspicious of acute pancreatitis. Upper endoscopy showed esophageal and gastric varices. Conservative therapy was initiated and included broad spectrum antibiotics (Meropenem 3 × 1 g daily + Metronidazole 3 × 500 mg), parenteral nutrition, analgesics, intravenous fluids and electrolyte corrections. However, seventh day of the hospitalization, laboratory tests showed dramatic elevation of inflammatory markers: leukocytosis (25 × 10^9/L) and CRP of 290 mg/L along with increase in serum and urine amylase, 3299 U/L and 4340 U/L, respectively. The patient became febrile (40 °C) and developed large, painful bulge over the proximal part of right inguinal area and thigh.

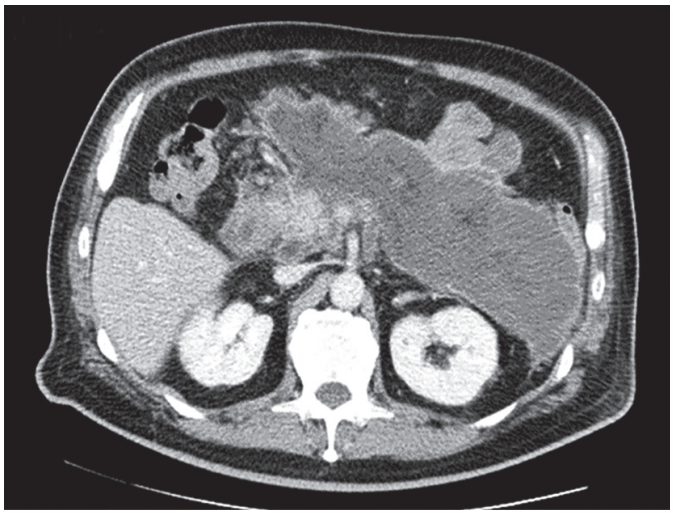

Fig. 1 MSCT showing a large retroperitoneal collection with abscess that occupied omental bursa, left pararenal and left iliacal retroperitoneal space with ascites intraperitoneally.

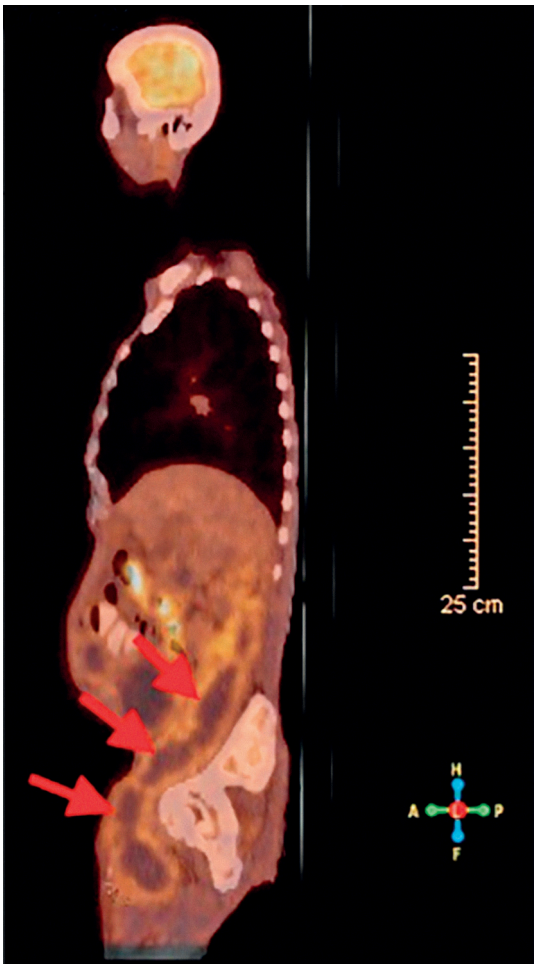

Fig. 2 PET/CT showing inflammatory process that represents communication between the retroperitoneal and left femoral region.

Multi-slice computed tomography (MSCT) showed a large retroperitoneal collection with abscess that occupied omental bursa, left pararenal and left iliac retroperitoneal space with ascites intraperitoneally (Figure 1). Due to a suspicion of systemic septic foci, a positron emission tomography (PET/CT) was indicated and it showed continuous abscess formation between upper retroperitoneal space, femoral triangle and proximal part of the right thigh with no accumulation of F-fluorodeoxyglucose (FDG) in other parts of the body (Figure 2).

Abdominal paracentesis and right femoral region aspiration performed simultaneously showed 8374 U/L amylase in the ascites and 36,450 U/L amylase in the thigh collection. Microbiological analysis confirmed polymicrobial infection (E. Coli, Enterococcus spp., Klebsiella pneumoniae and Bacteroides spp.). All bacterial species were sensitive to initial antibiotic combination, so it was continued for the next 14 days.

Consultation with surgeon was done and it was decided to perform laparotomy, necrosectomy and surgical

drainage of femoral collections because we have proven extensive infected pancreatic necrosis, femoral abscess and progression of septic complications. Minimally invasive approaches were not performed since interventional radiologist considered that it was not possible to adequately drain all collections percutaneously while laparoscopic techniques in our institutions were not at such high level to allow safe and sufficient laparoscopic pancreatic necrosectomy.

The patient was further treated with total parenteral nutrition, octreotide injections, transfusions of red blood cell concentrate, antibiotics and additional percutaneous intraabdominal abscess drainage on 6th postoperative day (POD). Patient's clinical condition improved, octreotide and parenteral nutrition were discontinued on POD 8, he started oral feeding on POD 9 and he was discharged on 24th POD with completely healed femoral and abdominal incisions. At the 1-year follow-up, the patient was asymptomatic and there were no repercussions on pancreatic endocrine or exocrine function.

DISCUSSION

Based on the revised Atlanta classification, there is a clinical spectrum of AP presentations which can be categorized as mild, moderately severe, or severe (2). This division depends on presence of organ failure and systemic complications. Acute pancreatitis is most (in 80% of cases) often a mild disease and treatment consists of supportive care with fluid resuscitation, pain control and nothing by mouth, while the role of antibiotics therapy and parenteral nutrition in mild cases is still widely debated (3, 4). On the other side, less common, but more severe form of AP when more than 30% of the gland is affected by necrosis, is called necrotizing pancreatitis and it may lead to septic complications, multi-organ failure and death. Necrotizing pancreatitis may result in acute or subacute development of sterile or infected pancreatic necroses, abscesses or pancreatic fistulas while late complications include pancreatic pseudocyst and pancreatic failure (5).

American Gastroenterological Association (AGA) and American College of Gastroenterology (ACG) guidelines suggest that AP can usually be diagnosed based on clinical symptoms and biochemical evidence of pancreatitis while imaging tests should be performed only if there is clinical deterioration or diagnostic dilemma (6, 7). Treatment depends on the severity of the disease and different scoring systems have been used to assess it and predict outcome (e.g., Ranson criteria, Glasgow score, Imrie score), but still, the course of AP is unpredictable in some cases so the adequate monitoring of clinical and laboratory signs are mandatory. When systemic inflammatory response syndrome and/or organ failure is detected, the patient should be admitted to an intensive care unit.

Regarding recent WSES (World society of emergency surgery) guidelines, the surgery is indicated in abdominal compartment syndrome, acute ongoing bleeding when an endovascular approach is unsuccessful, bowel ischemia or acute necrotizing cholecystitis during acute pancreatitis, bowel fistula extending into a peripancreatic collection and as a continuum in a step-up approach after percutaneous/endoscopic procedure (8).

Previous guidelines included some other surgery indications which changed over time mainly due to development of minimally invasive methods. Thus, the presence of infected pancreatic necrosis should be managed by Percutaneous drainage as the first-line treatment (step-up approach), alternatively, transgastric endoscopic necrosectomy and video-assisted retroperitoneal debridement may be considered. Surgery is therefore reserved when such methods are not available or these do not produce the desired result (9, 10).

Other indications that are less well defined, but many surgeons still consider these when deciding on surgery timing are: persistence of pancreatitis in spite of maximal medical therapy, deterioration of one or more organ systems or sterile pancreatic necrosis involving 50% or more of pancreatic tissue (11).

Abscesses are usually located retroperitoneally around the pancreas, but may spread along retroperitoneal planes into abdominal wall, and less commonly into intraperitoneal space after erosion of peripancreatic structures. Because of such pathogenesis of pancreatic necrotizing processes, secretions may extend to unusual anatomical locations, presenting with clinical features which may mimic various other disorders (12, 13).

There are reports of pancreatic abscesses reaching psoas muscle (14), hepato-gastric ligament (15) or scrotum (16) but this is the first report of an abscess as consequence of pancreatitis that was located in femoral region and upper thigh.

In addition, we found reports on extra-pancreatic location of post-pancreatitis pseudocyst complications location (mediastinum, pleura, spleen, stomach wall), but these were late (> 4 weeks) complications of AP with proved pseudocyst morphology so pathogenesis and disease course of clinical scenario reported in our study was different and it occurred in early phase of AP (17, 18).

We included octreotide in conservative therapy as some authors have suggested an indication for somatostatin and octreotide in the treatment of pancreatic fistulae. This is explained by its direct anti-inflammatory and cytoprotective effects as well as potent inhibition of exocrine secretion of the pancreas, which has an important role in the pathogenesis of acute pancreatitis (19).

Despite the rarity of distant pancreatic abscesses, gastroenterologists and surgeons should be aware of this clinical entity during the management of AP especially when unexplained septic complications or unusual abdominal, inguinal or femoral swellings develop.

CONCLUSION

The presented case highlights the need to consider important clinical challenges in diagnosis and therapy of acute necrotizing pancreatitis and its complications.

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Hirata's Disease: Rare Cause of Hypoglycaemia in Caucasian Male

Kateřina Žibřidová*, Barbora Havlínová, Eliška Svobodová, Pavel Žák, Jan Čáp, Filip Gabalec

ABSTRACT
Insulin autoimmune syndrome or Hirata's disease is an extremely rare condition leading to hypoglycaemia of variable severity due to autoantibodies against insulin. We present the first case documented in the Czech Republic.

KEYWORDS
hyperinsulinemic hypoglycaemia; insulin autoantibodies; Hirata's disease; insulin autoimmune syndrome

AUTHOR AFFILIATIONS
4th Department of Internal Medicine, University Hospital and Faculty of Medicine Hradec Králové, Charles University, Hradec Králové, Czech Republic
* Corresponding author: University Hospital Hradec Králové, 4th Department of Internal Medicine, Sokolská 581, 500 05 Hradec Králové, Czech Republic; e-mail: katerina.zibridova@fnhk.cz

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BACKGROUND

Insulin autoimmune syndrome or Hirata's disease is a rare disorder manifested by episodes of hypoglycaemia in the patient without previous treatment by insulin or its analogues. This condition is the third most prevalent cause of hyperinsulinemic hypoglycaemia in Asians, whereas it is extremely rare in the Caucasian population. Insulin autoimmune syndrome (IAS) is an infrequent condition characterised by episodes of postprandial and/or fasting hypoglycaemia and the presence of autoantibodies against endogenous insulin in patients with no prior exposure to exogenous insulin (1). It's named after Y. Hirata, who described this disease for the first time in 1970 (2). It affects mainly adults between 40–60 years of age with no difference in sexes, although some cases in children were also described (3–5). The exact prevalence is difficult to assume because of the rarity of the disease. The most cases were described in Eastern Asia, especially in Japan, where it is considered to be the third leading cause of hyperinsulinaemic hypoglycaemia right after insulinoma and extrapancreatic neoplasias (6). This condition seems to be significantly less frequent in other countries. However, the number of cases is generally rising in the last decade, probably due to higher awareness of the disease. In this paper, we would like to present, to our knowledge, the first case of IAS documented in the Czech Republic to spread awareness across the clinicians and to prevent a misdiagnosis of this rare condition.

CASE PRESENTATION

A 40-year-old Caucasian male was referred to our department with episodes of dizziness, hunger, sweating and blurry vision lasting ten days. These symptoms usually occurred 2–3 hours after the meal and during the night and got more severe and frequent in the past few days. He claimed there was a significant relieve after food administration. Before the admission to our department, the lowest measured glycaemia value was 2.6 mmol/l (normal range 3.9–5.6 mmol/l) and considerable elevation of C-peptide (6620 pmol/l, normal range 260–1730 pmol/l) was found. However, no abnormality on abdominal ultrasound was found; he was referred to our clinic with a high suspicion for insulinoma for further diagnostic evaluation.

His past medical history was significant for pollinosis, gastroesophageal reflux disease and low back pain. He used antihistaminics (levocetirizine) and topic corticosteroid (fluticasone) occasionally during the pollen season. He confessed taking Revitanerv (OTC preparation containing vitamins and alpha-lipoic acid) for leg and back pain for one month with the last administration eighteen days before the admission. There was no autoimmune or endocrine disease in family history, besides the fact that his father died of pancreatic cancer. He denied smoking and drinking alcohol or any drug abuse. His vital functions and physical examination at the admission were completely normal, except for being overweight with a BMI 27.7 kg/m^2 (80 kg/170 cm). Blood count was normal. Biochemistry revealed a slight elevation of liver enzymes and hyperuricaemia.

INVESTIGATION

Because of symptomatic hypoglycaemia (3.3 mmol/l) at admission and insufficient peroral food intake due to nausea we started an intravenous 10% glucose infusion to maintain the serum glucose level within the physiologic range. We performed the fasting test. Our patient developed symptomatic hypoglycaemia 2.3 mmol/L in 160 min with serum Insulin level 31910 mU/l (normal range 2.5–24 mU/l) and C-peptide 6304 pmol/l (normal range 260–1730 pmol/l). These findings did not support the diagnosis of an insulinoma, neither did imaging studies – CT of the abdomen showed only small stone in the gallbladder. Incipient chronic pancreatitis with no focal lesion was visible on endoscopic ultrasound examination.

We considered the possibility of an autoimmune form of hypoglycaemia in the differential diagnosis because of the inadequate elevation of serum level of Insulin, C-peptide and the insulin to C-peptide molar ratio equal to 5 (normal ratio < 1). The presence of autoantibodies against the endogenous insulin was confirmed by precipitation in 25% PEG – result of insulin after the process was 326.2 mU/l which is equal to 1,26% (compared with insulin level before the precipitation – 25860 mU/l). The same test with C-peptid showed the result of 17% (level before – 6204 pmol/l, after – 1056 pmol/l). ELISA was performed (Commercial kit Anti-Insulin, DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H., Austria) to determine the exact level of autoantibodies against human insulin, with the result of IAA 395.4 U/ml (reference range 0–10 U/ml). Further laboratory studies showed no abnormalities in serum cortisol level, CGA, thyroid gland function, serum protein electrophoresis and screening for rheumatologic autoantibodies (Table 1). Since the signs of possible type B insulin resistance (acanthosis nigricans, hyperandrogenism, hyperglycaemia and elevated HbA1c) were absent, we did not evaluate insulin receptor autoantibodies.

TREATMENT AND FOLLOW-UP

Peroral corticosteroids in a high dosage were started (Prednisone 1 mg/kg per day) and dietary modification with the elimination of high glycaemic index carbohydrates was implemented. Because of the persistent severe hypoglycaemia every night, therapeutic plasmapheresis was performed four times in total within the hospitalisation. After 43 days in total, with a significant decrease of serum insulin serum (1020.1 mU/l) and IAA titters (1.9 U/ml), our patient was discharged with a home medication of Prednisone 0.5 mg/kg per day.

Corticosteroids were slowly tapered down during regular follow-up. Nevertheless, the IAA titters gradually increased, and episodes of symptomatic hypoglycaemia occurred again. As a side effect of the long-lasting corticosteroids therapy, a deep vein thrombosis was diagnosed and treated. Another plasmapheresis was performed to

Tab. 1 Laboratory findings at the time of diagnosis, February 2020.

Analyte	Patient's value	Reference range
Sodium (mmol/l)	142	136–145
Potassium (mmol/l)	4.2	3.5–5.1
Chloride (mmol/l)	106	98–107
Urea (mmol/l)	2.2	2.8–8.1
Creatinine (umol/l)	77	45–84
Uric acid (umol/l)	427	143–339
Alcalic phosphatase (ukat/l)	1.35	0.58–1.75
Aspartate aminotransferase (ukat/l)	1.52	0.17–0.6
Alanine aminotransferase (ukat/l)	2.54	0.17–0.58
Gamma-glutamyl transferase (ukat/l)	0.82	0–0.67
Bilirubin (umol/l)	3	0–15
C-reactive protein (mg/l)	0.6	0–5
Total protein (g/l)	71	64–83
Albumin (g/l)	44.8	35–52
Glucose (mmol/l)	3.3	3.9–5.6
Insulin (mU/l)	31910	2.5–24
C-peptide (pmol/l)	6304	260–1730
HbA1c (mmol/mol)	38	20–42
TSH (mU/l)	1.6	0.27–4.2
fT4 (pmol/l)	14.6	12 – 22
TRAK (kU/l)	1.1	0–1.8
ATPO (kU/l)	29.3	0–34
ATG (kU/l)	12.7	0–115
Chromogranine A (ug/l)	53.19	0–101.9
Rheumatoid factor (latex fixation)	negative	
ANA immunofluorescence	negative	
ANCA immunofluorescence	negative	
ENA screening profil	negative	
anti-dsDNA	negative	
Anti-CCP (U/ml)	0.10	0–25
anti-insulin antibodies (U/ml)	337	0–10
Cortisol (nmol/l)	258	138–690
Red blood count (× 106)	5.17	4–5.8
Hemoglobin (g/l)	153	135–175
White blood count (× 109/l)	7.8	4–10
Platelet count (× 103/l)	204	150–400
PT (ratio)	0.82	0.8–1.2
APTT (ratio)	0.84	0.8–1.2

TSH – thyroid stimulating hormon, fT4 – free thyroxin, TRAK – anti-TSH receptor antibodies, ATPO – anti-thyreoperoxidase antibodies, ATG – anti-thyreoglobulin antibodies, ANA – anti-nuclear antibodies, ANCA – anti-neutrophil cytoplasm antibodies, ENA – extractable nuclear antigen antibodies, anti-dsDNA – anti-double stranded DNA antibodies, anti-CCP – anti-cyclic citrullinated peptid antibodies, PT – protrombin time, APTT – activated parcial tromboplastin time

Tab. 2

Drugs	Infection
Methimazole	Mumps
Propylthiouracil	Rubella
Carbimazole	Coxsackie B influenza
Alpha-lipoic acid	Hepatitis C
Prytinol	Varicella zoster virus
Glutathione	Measles
Methionine	
α-mercaptopropionil glycine	
Captopril	
Hydralazine	**Other conditions**
Torasemide	MGUS
Diltiazem	Multiple myeloma
Procainamide	Rheumatoid arthritis
Loxoprofen-sodium	SLE
Steroids	
Penicillamine	
Penicilin G	
Imipenem	
Isoniazid	
Alpha-interferon	
Acegratone	
Pantoprazole	
Clopidrogrel	
Albumin	
Gliclazide	
Garlic	

Assumed and revised by Censi et al. Ann Transl Med 2018; Sep; 6(17): 335.
MGUS – monoclonal gammopathy of undetermined significance,
SLE – systemic lupus erythematodes

reduce IAA level, and then immunotherapy with Rituximab (anti CD20 chimeric monoclonal antibody) in two doses of 1 g was applied with a significant decrease of IAA and complete elimination of symptomatic hypoglycaemic episodes. At follow up six months after diagnosis, our patient remained asymptomatic on Prednisone 0.25 mg/kg per day only (Figure 1).

DISCUSSION

We present the first case of IAS described in the Czech Republic. Etiopathological background of IAS is based on the presence of a combination of genetic predisposition and exogenous trigger. Mechanism of onset has been classified as a type VII hypersensitivity – a presence of autoantibodies against molecules circulating in the blood (7). This condition was associated more frequently with particular alleles of HLA antigens – specifically with HLA DRB1*0406 and less frequently with DRB1*0403(8). The

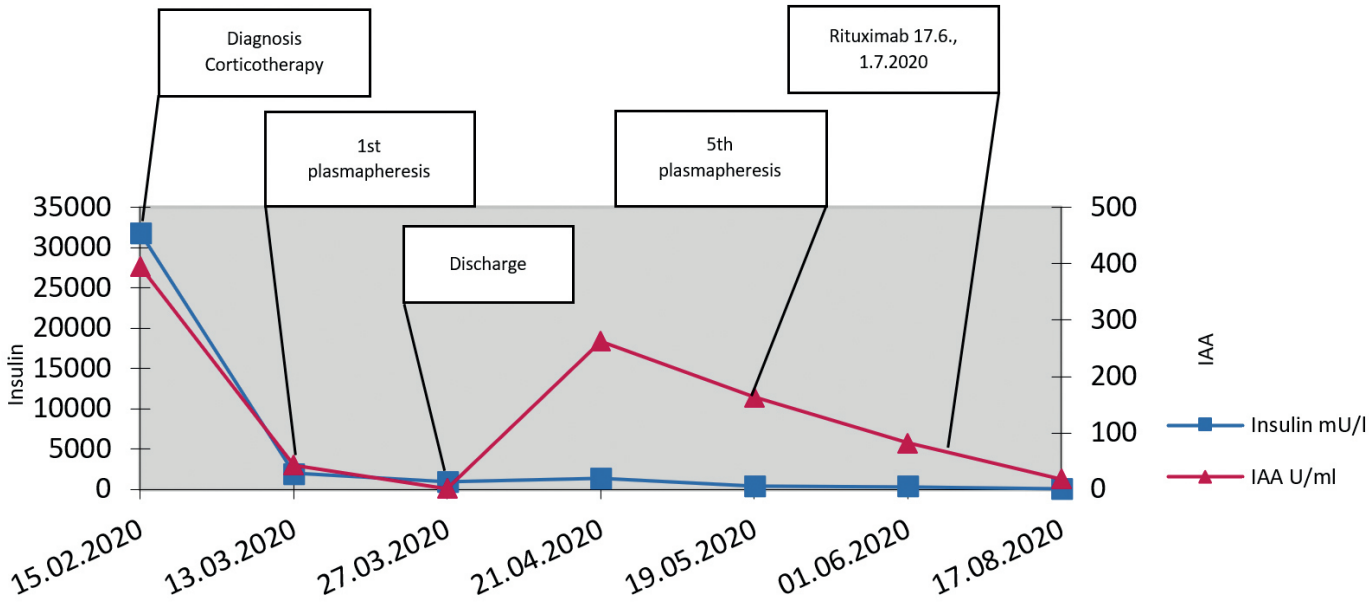

Fig. 1 Serum levels of insulin and anti-insulin autoantibodies during the treatment and follow-up.

first mentioned has a high prevalence in the Asian population, especially in Japan, which may explain a geographic distribution of IAS. The second cause taking part in the process of induction of the immune system starting the production of autoantibodies can be a viral infection or the exposition to drugs – especially the ones containing a sulfhydryl compound (Table 2) (9). Drug-induced onset was also present in our case. In our patient, the trigger was revealed later, after careful history taking. The patient has admitted administration of preparation containing alpha-lipoic acid. Drug-induced etiology is documented in more than 50% IAS cases. The presumed mechanism of development is deemed to be an interaction between the reductive part of the drug and the disulphide bonds in the insulin molecule which makes its structure more immunogenic due to molecular mimicry phenomenon (1, 10). Association between administration of alpha-lipoic acid, often used for a peripheral polyneuropathy, was firstly published in 2006 by Furukawa (11). Since then, others reported cases in both Asian and Caucasian population as the frequency of its use is rising (12–14). Besides the drugs and infection trigger, IAS was also documented together with another autoimmune inflammatory disease such as rheumatologic (rheumatoid arthritis, systemic lupus erythematodes), endocrine (Graves' disease) (1, 5) and also with the hematologic disease (monoclonal gammopathy of undetermined significance, multiple myeloma) (3). Spontaneous onset was also described in minor cases (3).

Refractory of the disease is also unique in our case. IAS is mostly a self-limited disorder, in some cases treated even with dietary modification and corticosteroids. In our case, several plasmapheresis and immunotherapy with Rituximab had to be applied to maintain blood glucose at a normal level. The therapeutic approach varies from the discontinuation of certain drug and diet modification to aggressive immunosuppressive therapy in cases with persistent severe hypoglycaemia. In most patients (over 80%) disease spontaneously resolved after drug withdrawal or with a restriction of high glycaemic carbohydrates food intake and increased frequency of meals (16). High dose corticosteroids are commonly used. Alpha-glucosidase inhibitors can also be used to lower postprandial blood glucose level as a stimulus for further secretion of insulin, but its intake is accompanied by frequent gastrointestinal discomfort. Nevertheless, in the minority of cases refractory to corticosteroids and regimen adjustment, it requires aggressive immunosuppressive therapy, as in our case. In the literature, the application of Cyclophosphamide, cyclosporin A, mycophenolate mofetil (3), azathioprine (16) and Rituximab (17) was documented, however, no randomised clinical trial was performed, or clinical effect of each regimen was analysed, so far to our best knowledge. Plasmapheresis also can be performed with the aim of faster decreasing serum IAA levels (17). In contrast with most other cases of ALA induced IAS the disease was not self-limiting, our patient required aggressive therapeutic approach – a combination of corticosteroids, plasmapheresis, and Rituximab with a significant clinical and laboratory effect. Pathogenesis of the IAS consists of interaction between the IAA and insulin and/or in the same cases proinsulin molecules by creating a macromolecule hindering its biologic effect. During a postprandial hyperglycaemic episode, insulin is secreted in pancreatic beta-cells to maintain blood glucose level within normal range and ensure proper glucose metabolism. When the significant titter of IAA with accurate affinity and avidity is present, insulin is bound to a macromolecule complex. Further secretion is then stimulated to exceed the capacity of antibodies. Blood glucose level slowly decreases, and insulin is slowly released due to the dissociation of immunocomplexes. Attributes of IAA are documented to be responsible for the duration and severity of hypoglycaemia. Association and dissociation rate constant, antibodies capacity and serum concentration are the most important characteristics, a high binding capacity and low

binding affinity for insulin allow the release of insulin and development of a hypoglycaemia (1, 10). Symptoms usually occur 30–120 days after the initiation of therapy with a certain drug (12) and present themselves as a Whipple's triad – symptomatic and by the laboratory test confirmed hypoglycaemia (blood glucose level < 4 mmol/L) with relief after glucose administration.

Pathognomonic laboratory findings are elevation of serum Insulin (> 24 mU/l, usually over 100–1000 mU/l, a much higher concentration than in insulinoma), elevated or normal serum C-peptide (260–1730 pmol/l) and detection of IAA (3). Insulin to C-peptide molar ratio can also be used. Usually, it should be less than one, whereas, in patients with IAS, it is usually > 1 (3). This diagnostic tool is not specific enough in view of a possibility of the presence of autoantibodies against both insulin and C-peptide in the same patient. In case of production of C-peptide binding autoantibodies the ratio can be false normal because of prolonged t1/2 due to its binding to IAA (10). Macrocomplexes of IAA and insulin can be assessed by precipitation in polyethene-glycol (15). Exact titter is later determined by ELISA (10). Administration of exogenous insulin or other hypoglycaemic medication, e.g. sulphonylurea should be considered in the differential diagnosis. In our patient symptoms typically started four weeks after administration of a preparation, presenting both adrenergic and neuroglycopenic symptoms 2–6 hours after the meal.

An extreme elevation of serum insulin and no abnormality on imaging studies brought us to a diagnosis of IAS, the first published case in the Czech Republic.

CONCLUSION

IAS should be considered a possible diagnosis in all cases of hyperinsulinaemic hypoglycaemia in non-acutely ill patients to prevent unnecessary expensive imaging studies and potentially harmful surgical procedures. In cases refractory to dietary regimen and corticosteroids, or cases when corticosteroids have considerable side effects, Rituximab and plasmapheresis should be considered as an alternative treatment.

PATIENTS CONSENT

Informed consent has been obtained from the patient.

DECLARATION OF INTEREST AND FUNDING

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Peritoneal Pseudocyst Causing Acute Abdomen as a Complication of Cronh's Disease

Christos Damaskos[1,*], Nikolaos Garmpis[2], Anna Garmpi[3], Aliki Liakea[4], Dimitrios Mantas[2]

ABSTRACT
Peritoneal pseudocysts (PPs) in patients who are diagnosed with Crohn's disease (CD), is a rarely diagnosed entity with unknown epidemiology, etiology and pathogenesis. We present the case of a 30-year old male with known CD who presented with an acute abdomen because of a PP. PPs are developed as a complication caused in patients, suffering from mainly thee conditions. Firstly, PPs appear in patients with continuous ambulatory peritoneal dialysis (CAPD), they are also developed in patients with peritoneal trauma and finally in CD patients. Our case belongs to these three reported cases in our literature review, since it refers to a CD patient that developed PPs. He underwent emergency laparotomy and excision of the cyst, with good postoperative results. A literature review of 22 publications show that PPs often represent a diagnostic and therapeutic problem as it has a variable presentation and there are no data on what the best treatment option is – surgical excision or aspiration.

KEYWORDS
peritoneal; pseudocyst; crohn; acute; abdomen

AUTHOR AFFILIATIONS
[1] Renal Transplantation Unit, Laiko General Hospital, Athens, Greece
[2] Second Department of Propedeutic Surgery, Laiko General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[3] First Department of Propedeutic Internal Medicine, Laiko General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[4] First Department of Pathology, Medical School, National and Kapodistrian University of Athens, Athens, Greece
* Corresponding author: Renal Transplantation Unit, Laiko General Hospital, Athens, Greece; 17 Agiou Thoma Street, 11527, Athens, Greece; e-mail: x_damaskos@yahoo.gr

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INTRODUCTION

Peritoneal pseudocysts (PPs) in patients who are diagnosed with Crohn's disease (CD), is a rarely diagnosed entity with unknown epidemiology, etiology and pathogenesis (1). These patients represent a diagnostic and therapeutic problem waiting to be solved. In various publications, PPs are also named as multilocular peritoneal inclusion cyst and inflammatory peritoneal cyst. PPs are reactive, fluid-filled cysts of the peritoneal serosa, often with septa in their cavity.

In this manuscript, we report on a case of a male patient with CD who presented with a PP.

CASE PRESENTATION

A 30 year-old patient with a known history of CD presented with abdominal pain and fever at the emergency department. The patient was diagnosed with CD ten years earlier affecting mainly his distal ileum for approximately 1 m. At the time of the diagnosis his Montreal classification was A2 L1 B1. He had been under treatment with azathioprine and mesalazine in a number of occasions. No past surgery was reported.

The abdominal pain has progressively worsened during the last three weeks. The patient reported diarrhea. A week earlier, he had visited his gastroenterologist and had had an abdominal computed tomography (CT) scan. CT reported signs of inflammation of the cecum and distal ileum. It also described a pelvic cystic formation approximately 6cm in diameter (Figure 1A, B).

In the emergency department he underwent an abdominal ultrasonography which indicated thickening of the distal ileum wall, presence of turbid fluid in the area of the appendix and a non-homogenous pelvic cystic mass of 11 cm in diameter with septa.

Due to the excruciating pain that could not be controlled with analgesics, the deteriorating general condition of the patient and the non-conclusive findings of the CT and ultrasound scans, it was decided the patient to undergo an emergency laparotomy.

Intraoperative findings consisted of cecal wall inflammation and a pelvic cyst. The cyst was drained of its clear fluid content. The abdomen presented with a diffuse inflammation. As the symptoms were totally attributed only to the cyst, it was decided not to explore the abdomen further although a rapid inspection of the abdominal cavity was undertaken to exclude other pathology. Sample of the fluid was sent for microbiology and histopathology examinations. A drain was place in the peritoneal cavity and the patient recovered uneventfully from anesthesia.

On the 5th postoperative day the patient was discharged from the hospital after an uneventful postoperative period. To date, the patient remains fit and healthy.

Histopathology reports were negative for malignancy and confirmed that the cyst wall sample was characteristic for a PP (Figure 2A, B, C). Microbiology reports were negative for bacteria or fungi growth.

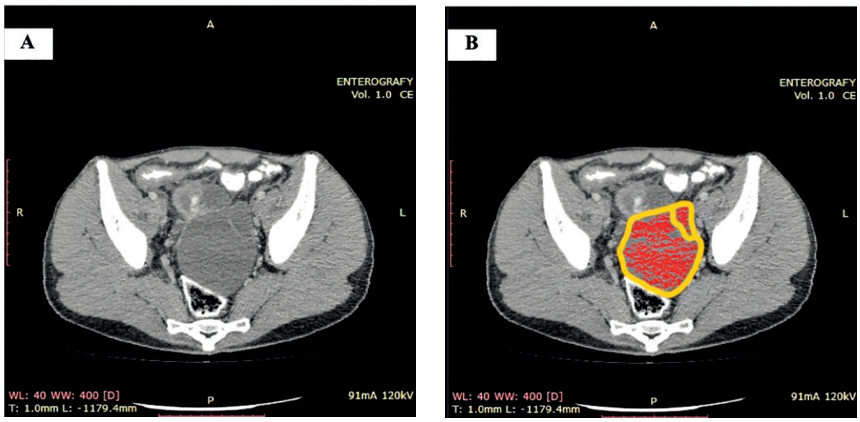

Fig. 1 A, B: Abdominal computed tomography (CT) reported a pelvic cystic formation.

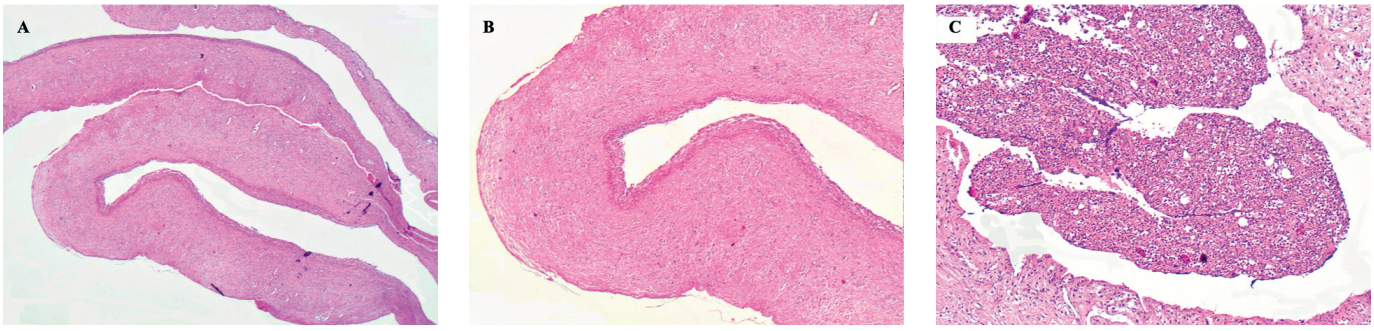

Fig. 2 A, B: Part of the thick cystic wall consisting of dense fibrous collagenous bundles, with mild vascular congestion and mild chronic inflammatory cell infiltration. No endothelial or epithelial lining is seen (Hematoxylin – Eosin, original magnification ×10 and ×20 respectively); C: Fragment of granulation tissue that covers the inner surface of the cyst, constisting of newly formed capillaries, mixed inflammatory cells (plasma cells, lymphocytes, neutrophils and monocytes) and cellular debris (Hematoxylin – Eosin, original magnification ×40).

DISCUSSION

Patients with CD who present with a PP are rarely described in the published case reports and series; only three cases are described in the 18 manuscripts that our literature search has yielded (Table 1). To date, scientific literature has produced contradictory reports regarding the epidemiology of PPs (1). The presence of PPs in patients with either ventriculo-peritoneal shunt for hydrocephalus (2–9, 21) or undergoing continuous ambulatory peritoneal dialysis (CAPD) (10–13, 22) is well described in the literature (Table 1). In the largest published case series by Veldhuis et al. (14) it is suggested that previous irritation of the peritoneum is present in over 70% of the cases, therefore PPs etiopathology might be linked to peritoneal trauma or disease. Peritoneal inflammation caused by CD or inflammatory bowel diseases in general are other ways of peritoneal irritation. Chronic pancreatitis or CD link to PPs may be explained by this hypothesis. Females tend to be affected more frequently, but no significant gender preponderance can be seen in Table 1. All ages seem to be equally affected although some authors report that 20–40 years of age are the peak period for PPs (15).

Tab. 1 Publications on peritoneal pseudocysts.

Authors	Year of publication	Number of cases	Males (%)	Median age (range)	Cause	Presentation	Location	Treatment
Latchaw et al. (5)	1981	NA	NA	NA	NA	NA	NA	NA
Piercy et al. (7)	1984	1	100	NA	Ventriculo-peritoneal shunt	Ureteropelvic junction obstruction and an enlarging right upper quadrant mass	Distal portion of the shunt	NA
Joffe & Friedberf (11)	1985	NA	NA	NA	CAPD	NA	NA	NA
Singh & Wadhwa (13)	1987	NA	NA	NA	CAPD	NA	NA	NA
Grunebaum et al. (4)	1988	6	NA	NA	Ventriculo-peritoneal shunt	NA	NA	NA
Namasivayam (12)	1991	NA	NA	NA	NA	NA	NA	NA
Urbanski et al. (16)	1991	1	0	25	Previously undiagnosed CD	10-day history of lower abdominal pain, diarrhea, and increasing abdominal pain	Pelvis	Laparotomy and excision
Besson et al. (2)	1995	22	NA	NA	Ventriculo-peritoneal shunt	Abdominal symptoms	NA	Laparotomy and excision (21), aspiration (7)
Ha et al. (10)	1995	NA	NA	NA	CAPD	NA	NA	NA
Oh et al. (6)	2001	11	0	NA	Ventriculo-peritoneal shunt	NA	NA	Laparoscopic cyst fenestration
Rovlias & Kotsou (8)	2001	NA	NA	NA	Ventriculo-peritoneal shunt	NA	NA	NA
Ying et al. (17)	2007	10	0	42.7 (35–62)	History of gynecological surgery	NA	Pelvis	Laparotomy and excision
Tamura et al. (9)	2013	1	100	22	Ventriculo-peritoneal shunt	Diffuse abdominal distention	Distal portion of the shunt	Laparotomy and excision
Veldhuis et al. (14)	2013	228	17.5	55 (18–89)	2.6% of patients had a known history of inflammatory bowel disease and/or pelvic inflammatory disease, 13.8% of women had a history of endometriosis and 67.1% of patients had a history of abdominal surgery, radiotherapy or trauma involving the abdomen	NA	Various	NA

Authors	Year of publication	Number of cases	Males (%)	Median age (range)	Cause	Presentation	Location	Treatment
Ercan et al. (3)	2014	1	0	22	Ventriculo-peritoneal shunt, CAPD	Nausea, vomiting and abdominal distension	NA	Aspiration
Mazziotti et al. (1)	2016	3	0	41.7 (35–49)	CD	Abdominal pain	Left ovary, small bowel & urinary bladder	Cyst aspiration
Afroza et al. (22)	2018	1	100	68	Peritoneal dialysis	Abdominal distension, abdominal pain, and vomiting	Pelvis	Medical management
Cusack et al. (21)	2019	1	100	37	A peritoneal pseudocyst around a ventriculoperitoneal shunt	Distended abdomen and fatigue	Pelvis	Paracentesis
Damaskos et al. (This study)	2021	1	100	30	CD	Abdominal pain	Pelvis	Emergency laparotomy & excision

NA: Not available; CD: Crohn's disease; CAPD: Continuous ambulatory peritoneal dialysis.

Symptomatic PPs present with pelvic or abdominal pain and a pelvic or abdominal mass. Fever is an infrequent finding and other symptoms may occur depending on the localization of the cyst: diarrhea, vomiting, urinary problems (1). PPs are considered as a rare cause when acute abdominal pain occurs. In most cases, ascites, abdominal distention, and peritoneal signs may be present. Iinflammation or a pseudocyst is developed in 5–20% of the cases. PPs cases reported in the literature are rather heterogeneous. Most publications are single case reports while only a small number of them reporting on a larger number of patients (14, 16).

The macroscopic characteristics of PPs include a thin wall of less than 3mm thickness, septa, no solid components, no cyst wall or septa enhancement in imaging. Apart from the lack of enhancement in imaging and the lack of solid components all other characteristics may be or not be present (14).

Some authors suggest that CA-125 is elevated in patients with pelvic PPs, but no firm data exist to support this conclusion (15). Presenting various imaging appearances, diagnosis is quite challenging. Ultrasound often set the diagnosis. Abdominal CT scan can also be used to detect PPs. However, at present, the pathological condition is monitored with the widespread use of magnetic resonance imaging (MRI) for diagnosis and follow-up examination of inflammatory bowel diseases. A technique also known with the term magnetic resonance enterography (MRE) has eased the diagnosis of both abdominal pathological findings and PPs as well (15). According to the majority of the cases presented, this method enables researchers to estimate all the useful findings such as the bowel wall thickening. In all cases, MRE allows to detect not only the presence, but also the progression of the PPs according to the treatment approach. However, biopsy is also recommended because PPs may result in squamous cell carcinoma.

To date, there is no treatment of choice. Patients that present with acute abdomen often undergo emergency laparoscopy or laparotomy. Patients with subtle clinical presentation often undergo selective excision or aspiration of the cyst fluid. The presented studies indicate that aspiration is a safe and effective nonsurgical alternative treatment for PPs in order to relieve the adverse symptoms. However, it is associated with a higher recurrence rate. Aspiration is considered as a suitable option for patients that do not want to undergo surgery, as no major complications occur. On the other hand, selective excision, being a surgical procedure, results in decreased postoperative pain and faster recovery. The complication rate is lower. However, the poor attachment of skin grafts could result in an unsuccessful outcome. There are no long-term results on the treatment of PPs, but most authors report good postoperative results and the recurrence is rare.

In the case of CD, treatment depends on disease severity. The first-choice treatment option is surgery, and many patients will require more than one surgery over their lifetime. However, numerous drugs have been reported to be used to treat PPs in CD. The application of medication, such as metronidazole, can manage the suppurative or perianal complications of CD. Conservative treatment of the PPs in CD can be effective in improving symptoms, but often fails to achieve complete treatment. To conclude, according to the presented cases, successful treatment of PPS in CD patients depends on the severity of the case and the clinical appearance of the patient. Conservative treatment is a safe and feasible alternative option to manage PPS. However, in severe cases, patients require emergent hospitalization and a surgical approach.

Another cause of intraabdominal cystic formation is the peritoneal cyst, developed as a complication of chronic pancreatitis. In these cases, dangerous acute hemorrhage can take place because of a pseudocyst of the pancreas. Comparable to the case of PPs, in chronic pancreatitis a pseudocyst appears following sudden abdominal pain. The main treatment option is surgery. Meconium pseudocyst is also considered as an intraabdominal cystic

formation in fetal bowel obstruction. The cases of prenatally diagnosed meconium peritonitis that have been reported in the literature are quite limited. Therefore, diagnosis of meconium peritonitis in the presence of a fetal intra-abdominal hyperechoic mass should be investigated (18–20).

The authors of this manuscript aim to inform physicians – especially general surgeons and gynecologists – on this underreported pathology that could often lead to troublesome diagnosis and treatment. In our patient, CD is considered the cause of the PP. The clinical presentation of a larger PP varies significantly depending on the organs affected and therefore physicians should have high suspicion of such pathology.

CONCLUSION

PPs represent a diagnostic and therapeutic problem as it has a variable presentation. Despite there are no data on what the best treatment option is – surgical excision or aspiration; postoperative results are good, and the recurrence is rare.

CONSENT

The patient's images have been anonymized to maintain privacy.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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Horseshoe Kidney Complicated by Xanthogranulomatous Pyelonephritis in a Young Girl: A Case Report and Review of the Literature

Jamal Musayev[1,*], Rashad Sholan[2], Adalat Hasanov[1], Rizvan Rustamov[3]

ABSTRACT

The cases of horseshoe kidney presented by xanthogranulomatous pyelonephritis are very rare. In this study, the case of XGP developing in HSK in a young female patient was presented due to its rare incidence and the previously reported cases were reviewed, as well. The patient, who has end-stage renal disease and was under treatment, admitted to the clinic for preemptive kidney transplantation. Bilateral open en bloc nephrectomy was performed before the kidney transplantation. The histopathological examination of the specimen was reported as XGP. Eight months later, living-donor organ transplantation was performed to the patient with the kidney obtained from her father. XGP can present as a complication of HSK. Moreover, HSK may rarely be manifested by end-stage renal disease in young patients. In such cases, who would undergo kidney transplantation, it is important to examine the HSK in detail and perform bilateral nephrectomy to prevent complications after transplantation.

KEYWORDS

horseshoe kidney; xanthogranulomatous pyelonephritis; bilateral nephrectomy; end-stage renal disease

AUTHOR AFFILIATIONS

[1] Department of Pathology, Azerbaijan Medical University, Baku, Azerbaijan
[2] Department of Kidney Diseases and Transplantology, Republican Treatment and Diagnostic Center, Baku, Azerbaijan
[3] Institut of Pathology, SRH Wald Klinikum, Gera, Germany
* Corresponding author: Sherifzade 212, Patoloji Anatomiya Burosu, AZ1012, Baku, Azerbaijan; e-mail: patolog.jamalmusaev@gmail.com

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INTRODUCTION

Horseshoe kidney (HSK) is the most common renal fusion anomaly with a prevalence of 0.25%; and is twice more common in the male compared to the female (1, 2). Approximately 1/3 of the cases with such anomalies are asymptomatic and they are detected randomly during autopsies or through the imaging methods (1, 2). The remaining cases occur as complications such as obstruction, infection, urolithiasis in the ureteropelvic junction, and rarely through secondary findings due to tumor and trauma (2).

Xanthogranulomatous pyelonephritis (XGP) is a rare disease causing renal parenchymal destruction; and it constitutes 0.6 to 1.0% of the cases of chronic pyelonephritis. It can be seen at all ages; however, it is more frequent in middle-aged women (3, 4).

The cases of HSK presented by XGP are very rare. In this study, the case of XGP developing in HSK in a young female patient was presented due to its rare incidence and the previously reported cases were reviewed, as well.

CASE REPORT

A 17-year old female patient, who has end-stage renal disease and was under treatment, admitted to the clinic for preemptive kidney transplantation with complaint of chronic fatigue. The partially palpable abdominal mass was determined on physical examination. The daily amount of urine was around 1 liter; and the protein amount in the 24-hour urine was 4000 mg. According to the laboratory tests, WBC was 10.3, creatinine was 4.5 mg/dL, GFR was 18 ml/min/1.73 m^2, hemoglobin was 8.5 gr/dL, and hematocrit was 27%. There were signs of pyuria in the urine and *E. Coli* reproduction in the urine culture. Native computed tomography (CT) revealed enlargement in both renal pelvis and calyces, numerous formations compatible with stone in their cavity, signs of atrophy in the renal parenchyma, and isthmus combining both kidneys in their lower poles (Figure 1). Considering the findings mentioned above, the patient was recommended bilateral open en bloc nephrectomy to prevent complications after the kidney transplantation.

For the nephrectomy, the abdominal cavity was opened with a section starting with supraumbilical incision and extending laterally with a Mercedes-type incision. The colon was medialized by incising the parietal peritoneum from the white line of Toldt, and the retroperitoneum was reached. Surrounding tissues were removed from both kidneys, and the arteries and veins were ligated. Both kidneys were removed as en bloc, together with the isthmus connecting the two kidneys. The amount of blood loss during surgery was 200 mg. The creatinine level was 7–9 mg/dL in the postoperative period. The patient received hemodialysis 3 times a week following the bilateral nephrectomy. Eight months later, living-donor organ transplantation was performed to the patient with the kidney obtained from her father. No complications and rejection have been observed in the patient within the seven years of follow-up.

In the macroscopic examination of the specimen, fusion was detected in the lower pole of the horseshoe kidney (Figure 2). There was dilatation in the renal pelvis and calyces. Signs of atrophy were observed in the renal parenchyma. Abundant purulent exudate and stones were detected in the renal pelvis. In the microscopic examination, renal tissue was atrophic. There were neutrophil infiltration areas and microabscess in the background of diffuse lymphocyte and plasma cell infiltration. Lipid-laden macrophage accumulations were observed in certain areas (Figure 3). The histopathological examination of the specimen was reported as XGP.

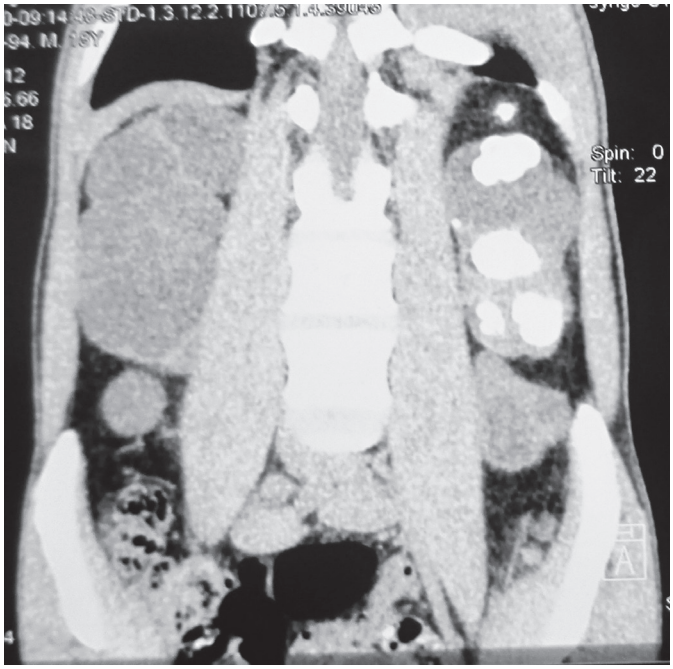

Fig. 1 CT scan of the abdomen revealed features of the bilateral hydronephrosis and pelvic urolithiasis.

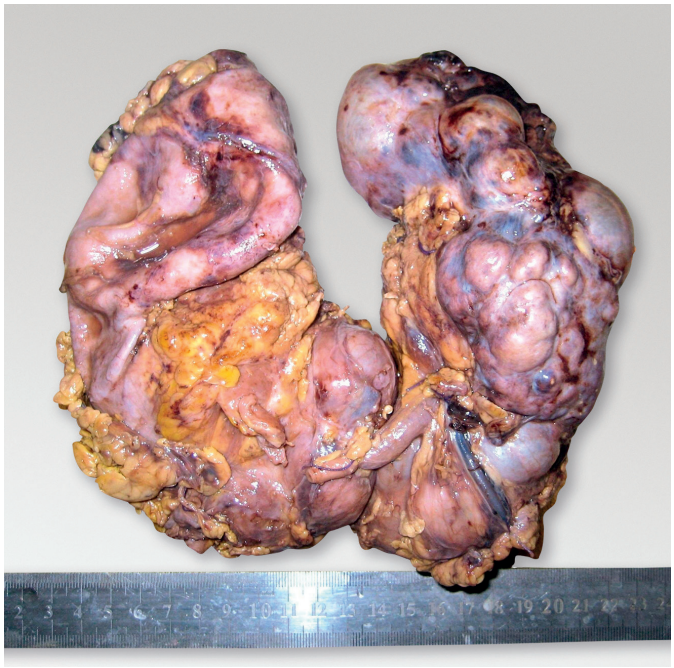

Fig. 2 Macroscopic view of the nephrectomy specimen.

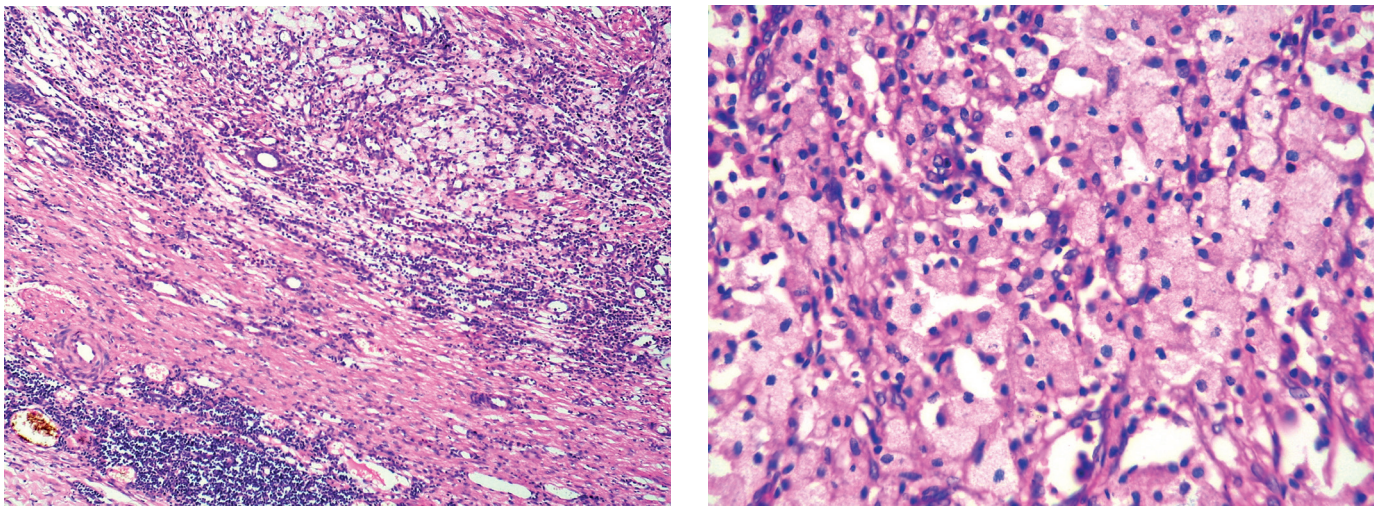

Fig. 3 Diffuse infiltration of atrophic renal parenchyma by lymphocytes, plasma cells and neutrophils (a), and aggregates of foamy macrophages (b) (×100, ×200; HE).

DISCUSSION

The combination of HSK and XGP is extremely rare and the first case was reported in 1994 by Hammadeh et al. (5). There have been only 7 cases reported in the English literature so far (5–11). The detailed characterization of all cases is presented in Table 1. What makes our case different from the previously reported cases is the concomitant end-stage renal disease, implementation of bilateral nephrectomy and subsequent kidney transplantation.

Congenital anomalies of the kidney and the urinary tract have been detected more frequently and earlier, particularly with the widespread of imaging methods within the last 30 years. Some of these anomalies, which form a wide spectrum, can be identified within the first years of life and in the antenatal period; however, asymptomatic anomalies such as unilateral renal agenesis, ectopia, malrotation, and fusion anomalies manifest themselves in older ages and adults (1, 12).

HSK is the most common fusion anomaly. It has been reported in the literature that the incidence rate in newborns is 1/400–1600 (1, 2). In the vast majority of the cases, fusion is present in the lower pole of the kidneys; therefore, U-shaped HSK is more common. Reverse U-shaped HSK, which results from the fusion of the upper pole, and L-shaped HSK, which is shaped due to lateral fusion, are rarely observed (2). In our case, fusion was in the lower pole and U-shaped HSC was present.

Approximately half of the complications related to HSK consists of obstructions and kidney stones (1, 2, 13).

Tab. 1 The general characteristics of horseshoe kidney cases complicated by xanthogranulomatous pyelonephritis.

Authors	Age (years)	Gender	Symptoms	Imaging method	Presence of stones	Involvement	Treatment
Hammadeh et al. (5), 1994	7	Male	Right flank mass	X-ray	Yes	Right kidney	Right heminephrectomy
Samuel et al. (6), 2001	6≥	NK	Pain, fever, hematuria, renal mass	X-ray, US, CT	Yes	Left kidney	Left heminephrectomy
Sausville et al. (7), 2009	48	Female	Fever	CT	Yes	Left kidney	Laparoscopic left hemine-phrectomy
Mongha et al. (8), 2010	62	Male	Pain, fever	US, CT	Yes	Left kidney	Left heminephrectomy
Basson and Witt (9), 2013	18≤	NK	NK	X-ray, CT	Yes	Right kidney	NK
Sawazaki et al. (10), 2017	75	Male	General fatigue, breathlessness	CT	No	Right kidney	Right heminephrectomy
Fernandez et al. (11), 2018	64	Female	Pain, decreased appetite, abdominal discomfort, chronic fatigue	CT	Yes	Bilateral	Laparoscopic left hemine-phrectomy
Musayev et al. Present case	17	Female	Chronic fatigue	CT	Yes	Bilateral	Bilateral nephrectomy

NK: Not known, US: Ultrasonography, CT: Computed tomography

Among the cases, 4% can manifest with malignancies and the most common malignancy accompanying HSK is the renal cell carcinoma (2, 13). Urinary tract infections are present in 19% of the cases; however, the cases of HSK complicated with XGP are very rare (13).

XGP was defined for the first time by Schlagenhaufer in 1916 (3, 4). The reasons for its occurrence are not definite; however, it is common in cases with long-term obstruction and infection. The presence of stones in the pelvis was reported in 47–100% of the cases (4). Obstruction findings and stones were present in both moiety of the kidney in our case.

The preoperative diagnosis process of XGP is quite complicated. In some of the cases, specific diagnosis can be determined by CT. However, the fact that there are not stable clinical and radiological findings of XGP as well as it can resemble tuberculosis, pyelonephritis, perinephric abscesses, and malignant tumors in the imaging methods, are important factors making the specific diagnosis difficult (3, 4). In such cases, determining the specific diagnosis and ruling out the above mentioned entities requiring different management is possible only with histopathological examination.

As can be seen from the table 1, the majority of cases of XGP developing in HSK are adults; only 2 pediatric cases have been reported (5, 6). XGP is more common in the female, while HSK is more common in the male. Our case was a 17-year-old female.

The most frequent imaging method used in the diagnostic process of the cases was CT, which was also used in our case. CT is valuable in these cases in terms of displaying the kidney stones, the involvement of perinephric adipose tissue characterizing XGP, and the anatomic findings specific to HSK (4, 9).

The incidence of urinary stones is high in the cases of both XGP and HSK. Urinary stone has been reported in the vast majority of cases with HSK manifesting with XGP, including our case. Only one case, who did not have a urinary stone, had a history of nephrolithotomy that was performed 34 years ago (10).

The risk of end-stage renal disease in HSK is higher than that of the general population. However, it has not been reported in the cases with the combination of HSK and XGP before our case (13). Six of the cases had unilateral and one case had bilateral involvement. Unilateral heminephrectomy was performed in the 6 cases where the treatment method was specified (5–8, 10, 11). Mortality was reported in only one case (10). Our case is the first case of HSK bilaterally complicated with XGP in the literature that underwent bilateral nephrectomy and was performed kidney transplantation subsequently.

CONCLUSION

Although rare, XGP can present as a complication of HSK. Moreover, HSK may rarely be manifested by end-stage renal disease in young patients. In such cases, who would undergo kidney transplantation, it is important to examine the HSK in detail and perform bilateral nephrectomy to prevent complications after transplantation.

ABBREVIATIONS

HSK	Horseshoe kidney
XGP	Xanthogranulomatous pyelonephritis
US	Ultrasonography
CT	Computed tomography
WBC	White blood cells

PRIOR PUBLICATION

This study was presented as an poster presentation at the 1st International Uroanatomy Congress, Izmir-Turkey, on June 14–16, 2013.

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A Case of Severe Thyroid Eye Disease Treated with Tocilizumab

Aysel Mehmet[1,*], Eirini Kanella Panagiotopoulou[1], Aristeidis Konstantinidis[1], Charalampos Papagoras[2], Panagiotis Skendros[2], Doukas Dardabounis[1], Athanasia Maria Mikropoulou[1], Georgios Labiris[1]

ABSTRACT
This is a case report describing a patient with severe thyroid eye disease complicated with dysthyroid optic neuropathy that was unresponsive to intravenous steroids and orbital radiotherapy but responded well to intravenous tocilizumab.

KEYWORDS
thyroid eye disease; tocilizumab

AUTHOR AFFILIATIONS
[1] University Eye Clinic, University Hospital of Alexandroupolis, Greece
[2] University Department of Internal Medicine, University Hospital of Alexandroupolis, Greece
* Corresponding author: University Eye Clinic, University Hospital of Alexandroupolis, 68132, Alexandroupolis, Greece; ayselmehmet83@hotmail.com

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INTRODUCTION

Thyroid eye disease (TED) is an inflammatory disorder of the orbit secondary to Graves' disease. Although the inflammatory phase is self-limiting it can severely affect the vision. Sight threatening TED is rare. The overall incidence of TED is 0.42/10,000/year with women and the 40–60 age group being mostly targeted by the disease (1). Only about 2% of patients with TED have complications that threaten the vision primarily due to optic neuropathy and/or corneal exposure.

Cessation of smoking is the most important lifestyle factor that positively influences TED; while systemic steroids is the first line medication in the case of moderate-to-severe and sight threatening TED (1). However poor or no response to steroids is occasionally encountered (2). In these cases, steroid administration could be attempted again; or proceed to cyclosporine or rituximab administration and/or orbital radiotherapy. Tocilizumab is a recombinant antihuman monoclonal antibody of the immunoglobulin G1 subclass directed against the interleukin-6 receptor (IL-6R) that has been used in TED patients with variable efficacy. Several theories have been proposed regarding tocilizumab's potential beneficial impact in TED. Among them, an overall modification of the immunomodulatory function, remodeling of the extracellular matrix, and reduction of the inflammatory response (3).

Within this context, we present a case with sight-threatening TED with minimal response to steroids and orbital radiotherapy, which responded well to tocilizumab treatment.

CASE DESCRIPTION

A 60-year-old man, heavy smoker (40 cigarettes/day, 80 pack/years), was referred to our outpatients' service by his doctor in February 2019 due to sight-threatening TED, which manifested with gradual reduction of his visual acuity. He was diagnosed with Graves' hyperthyroidism 14 years earlier, and ever since he was under regular systemic treatment with carbimazole. The patient had also had I^{131} treatment in July 2018.

On the initial examination his best corrected visual acuities (BCVA) were 0.4 logMAR in the right eye (OD) and 0.3 logMAR in the left eye (OS) and the Clinical Activity Score (CAS) was 5/7. The exophthalmometry measurements were 32 mm OD and 30 mm OS. The intraocular pressures (measured with Goldmann Applanation Tonometry-GAT IOPs) were 30 mmHg in both eyes in the primary gaze and 35 mmHg in the upgaze. The right upper eyelid was retracted 2 mm above the limbus and the left was 0.5 mm above the limbus. Both lower lids were retracted 1.5 mm below the limbus (Fig. 1 above). The patient was hypermetropic (+3 sph dpt spherical equivalent) and the angles were moderately closed in the superior quadrants and moderately open in the inferior quadrants and gave a plateau iris configuration on indentation gonioscopy. Fundoscopy showed early disc swelling in the OD and increased retinal nerve fiber thickness bilaterally (Fig. 2). Patient was advised to quit smoking and a fixed combination of dorzolamide-timolol (FCDT) bid was immediately started. Automated perimetry showed defects bilaterally (Fig. 3a).

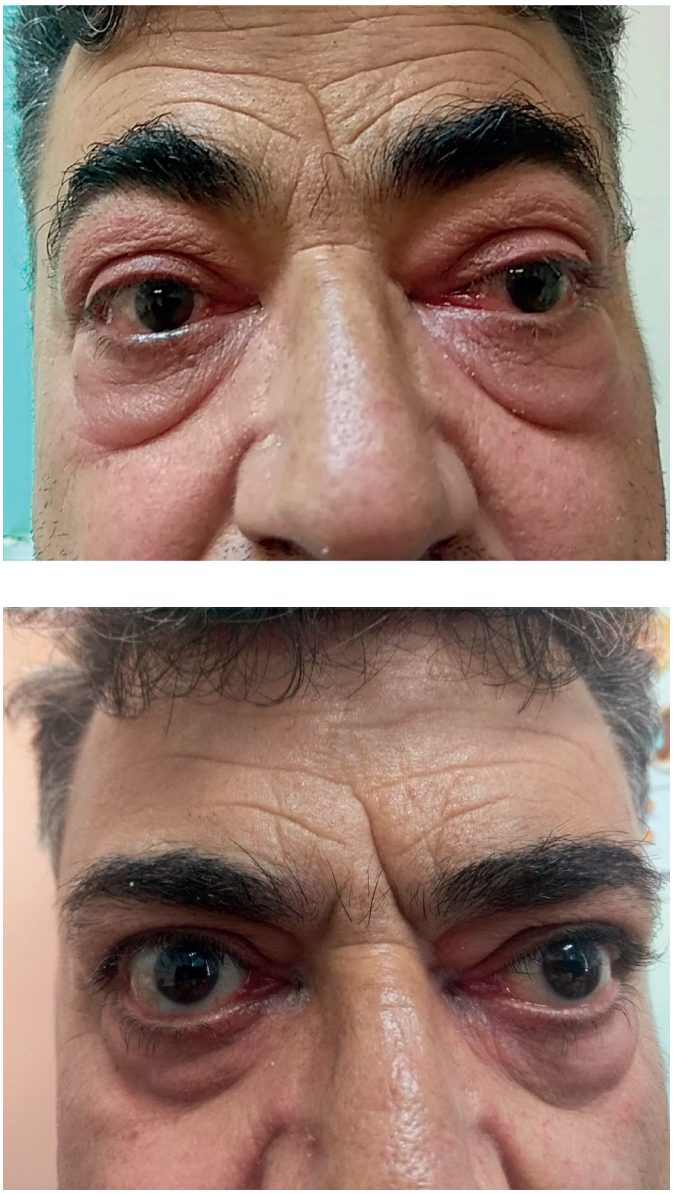

Fig. 1 Top: Baseline clinical picture. Upper and lower lid erythema and swelling of both eyes. Below: clinical picture after the 4-week course of tocilizumab. Marked reduction of erythema and swelling of all eyelids are noted.

In order to address the increased intraorbital pressure, the patient was scheduled immediately for a 3-day course of intravenous (iv) methylprednisolone 1000 mg/day. However, neither BCVA nor exophthalmos was improved. Orbital decompression was suggested but was rejected by the patient who was against any surgical intervention. As a second option, a 12-week course of intravenous methylprednisolone was proposed (500 mg/week for the first six weeks and then 250 mg/week for the following six weeks) as per EUGOGO recommendations (1) aiming to achieve a prolonged anti-inflammatory effect. At the end of the course the CAS score was 8/10 with no clinical improvement. The patient complained that his eyesight was worse and his BCVA was 0.5 logMAR OD and 0.4 logMAR OS.

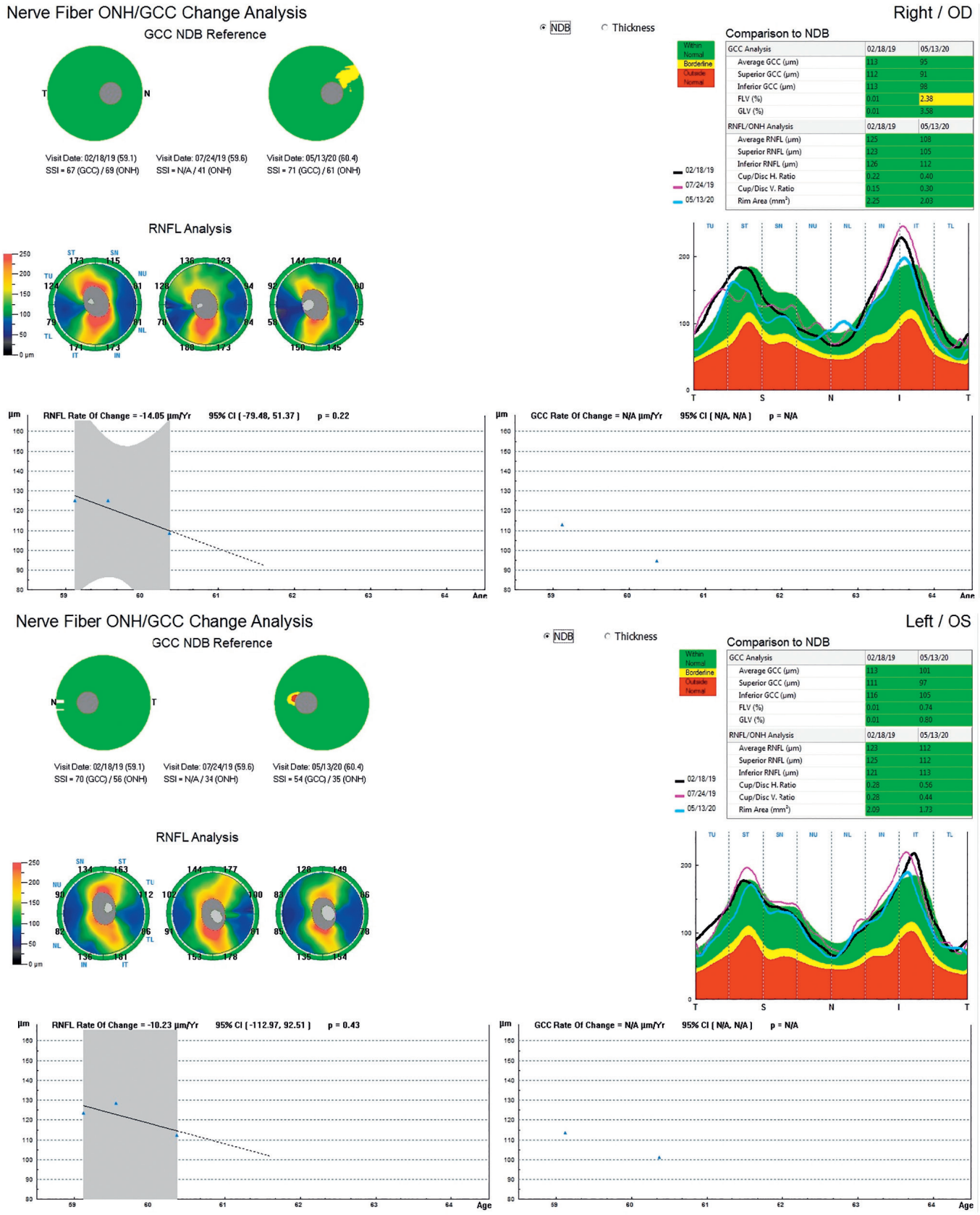

Fig. 2 Sequential measurements of the retinal nerve fiber layer of the right and left eye. 01/18/19: baseline OCT (black line), 7/24/19 OCT taken after the 12-week course of iv methylprednisolone (purple line), 5/13/20 OCT taken after the 4-week course of tocilizumab (light blue line). A reduction of the thickness of the retinal nerve fiber layer is shown (when comparing the height of the black line to the height of the light blue line).

GAT-IOPs were 27mmHg in both eyes despite the FCDT, with deterioration of his visual fields (Fig. 3b). The patient consented to orbital radiotherapy and had 10 sessions with a cumulative dose of 20Gy. Despite the treatment there was no improvement in BCVA, GAT-IOPs or visual fields (Fig. 3c). One month after the orbital radiotherapy he underwent thyroidectomy.

As the postoperative period was uneventful and in order to address the persisting sight-threatening situation we decided to start the patient on intravenous tocilizumab (RoACTEMRA, Roche International Ltd, Walwyn Garden City, UK), initially one injection every 4 weeks at a dose of 8 mg/ml. After the fourth infusion (one month after the first tocilizumab infusion) the patient had a marked improvement of his BCVA (0.2 logMAR OD, 0.0 logMAR OS), reduction of the IOP (20 mmHg bilaterally in primary position, 23 mmHg in upgaze), slight improvement of the lid retraction (the right upper eyelid was 1 mm above the limbus, the left upper eyelid 0.5 mm below the limbus but both lower lids remained 1.5 mm below the limbus), the range of ocular movements was extended and the visual fields were also improved (Fig. 3d). The exophthalmometry measurements remained the same (Fig. 1 below). The retinal nerve fiber thickness was also reduced bilaterally (Fig. 2). The patient had only managed to reduce smoking to 20 cigarettes/day. He was on a dietary supplement of selenium, topical artificial tears and the FCDT. Three months the course of tocilizumab all patient's indices remain stable.

DISCUSSION

Thyroid eye disease can potentially cause blindness in a small minority of patients. The mainstay of treatment for the moderate-to-severe disease is a 12-week course of intravenous steroids and for the sight threatening form a 3-day course of high-dose intravenous steroids and/or orbital decompression (1).

Intravenous steroids have been associated with recurrences and poor response as well as 6.5% morbidity and 0.5% mortality rate (4). For this reason other modalities have been tried such as cyclosporine, azathioprine,

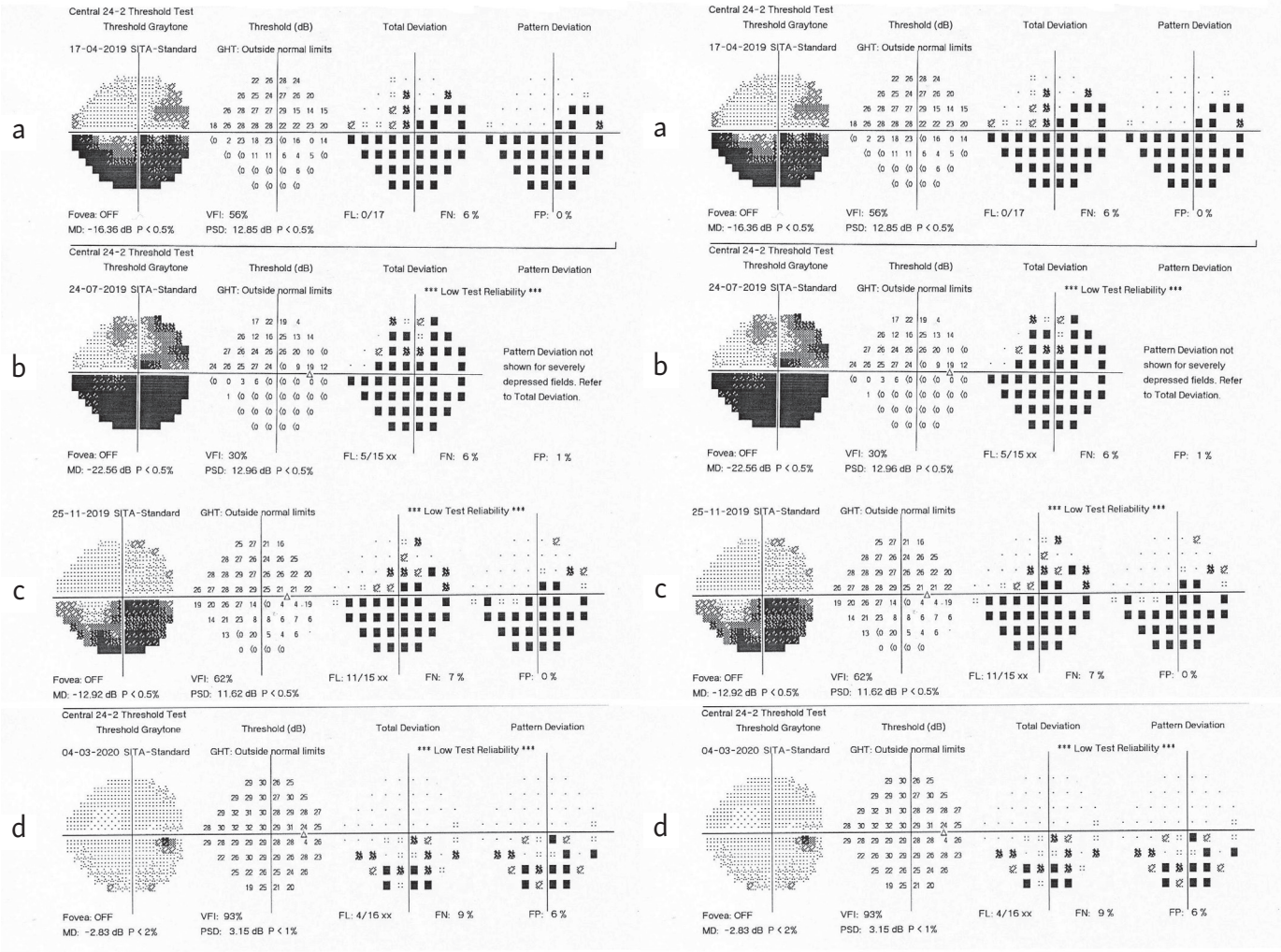

Fig. 3 Serial visual fields of the right eye (left) and left eye (right). total deviation and pattern deviation plots are only shown. (a) Baseline VF. Right eye: superior paracentral scotoma with dense inferior altitudinal scotoma. Left eye: Dense inferior altitudinal scotoma; (b) VFs after the 12-week course of intravenous methylprednisolone. There is no change in the visual field defects; (c) VFs after radiotherapy. There is no change in the visual fields defects; (d) VFs after the 4-week course of tocilizumab. Right eye: the superior paracentral scotoma has disappeared and the inferior altitudinal defect has decreased in depth (sensitivity has increased) and size. Left eye: The inferior altitudinal scotoma has decreased in depth (sensitivity has increased) and size.

mycophenolate mofetil, methotrexate along with specific immunosuppresants (rituximab, adalimumab, etanercept, infliximab) (5). These treatments are reserved for the moderate-to-severe TED. In the case of sight threatening TED more radical measures are required.

Radiotherapy has been used in the treatment of TED for many decades. It mainly acts on the orbital soft tissues and the extraocular muscles (6). Its effects are more potent and last longer when combined with steroids (oral or iv) (6, 7). The effects of radiotherapy may take days to a few weeks to develop (1). Given this time frame and bearing in mind that the patient received tocilizumab 10 weeks after the last session of radiotherapy and the clinical improvement started to take place already after the second tocilizumab infusion, we feel that it would be an unlikely coincidence that the improvement of the TED was due to the late effect of the combined effect of iv steroids/radiotherapy rather than the beneficial effect of tocilizumab.

In this case, sight-threatening TED was manifested with reduction of visual acuity, uncontrolled intraocular pressure and deterioration of visual fields in a man who responded poorly to both a 3-day high dosage treatment with intravenous methylprednisolone and to a 12-week course of the same steroid as suggested by EUGOGO. This 12-week course of iv steroids is recommended for moderate-to-severe TED by EUGOGO but still we elected to administer the treatment to the patient in order to achieve a prolonged anti-inflammatory effect with the aim to reduce the volume of the extraocular muscles that compressed the optic nerves as confirmed on the orbital CT and the visual fields. We decided to proceed with tocilizumab as orbital decompression was rejected by the patient and gradual deterioration of the visual fields and high GAT-IOPs were recorded. Literature suggests that tocilizumab in moderate-to-severe TED reduces the soft tissue swelling, Clinical Activity Score and exophthalmos but this improvement does not translate into significant clinical improvement from the patients' point of view (8, 9). The decision to favor tocilizumab over other immunoregulatory modalities was based on the safety profile of tocilizumab, the supporting evidence from the international literature (8, 10) and the lack of evidence that tocilizumab is inferior to the other medical treatments.

Our patient had very congested orbits with the soft tissues impinging on the optic nerves. We also felt that the high IOPs were due to poor aqueous outflow secondary to episcleral venous congestion. The patient's response was impressive which reflected on the improvement of the eyelid and conjunctival swelling and redness, the improvement of the ocular motility, the reduction of the IOP (presumably due to the decrease of the episcleral venous pressure) and the ameliorated perimetric findings secondary to the reduction of the soft tissues bulk. Exophthalmometry readings did not change although the upper and lower lid retraction was minimally improved compared to the baseline measurements.

We believe that tocilizumab can have a positive impact on the aforementioned aspects of TED. However it is very unlikely that it could be used in the case of exposure keratopathy as it does not reduce the eyelid apertures (8). In the same manner dysthyroid optic neuropathy which can cause quick and profound loss of vision should not be treated with tocilizumab as its effects take days to weeks to develop. It should, though, be used in cases were the first line medication fails to offer adequate alleviation of signs and symptoms of moderate-to-severe TED.

Sy et al. (10) reported two cases of sight-threatening TED who received iv tocilizumab after orbital decompression. Tocilizumab improved the signs of TED although the previous decompressions did not have the desired effects. The anti-inflammatory effect of tocilizumab was also confirmed with orbital fat biopsies.

Research has shown that thyroidectomy may improve the signs of TED but this process takes months to develop (11, 12). In the case of our patient we did not have the time to wait for the possible beneficial effects of thyroidectomy. For this reason we started the patient on the tocilizumab treatment 6 weeks after the thyroidectomy. We believe that the improvement of symptoms was due to the tocilizumab treatment as it is was already observed from the second intravenous infusion and the time course by that time was too short for the effects of the thyroidectomy to manifest. The patient was euthyroid on tablets of levothyroxine after the removal of the thyroid gland.

As far as it concerns side effects, tocilizumab can cause hepatitis, opportunistic infections or flare ups of latent infections as it interferes with the immune response and development or exacerbation of cancer (8). Our patient developed a moderate thrombocytopenia which recovered after 3 weeks without intervention.

To the best of our knowledge this is the first case reported where tocilizumab was used in a patient with severe TED. Although these cases require treatment that evoke a quick response we felt the rate of the development of the optic neuropathy gave us the luxury of time to use a medication that takes longer to act and indeed the clinical response supported our decision. We would like though to stress that tocilizumab (as other immunoregulatory medication) is not a substitute for the recommended management of severe TED and its main indication is moderate-to-severe disease.

CONCLUSIONS

In the present article we describe a case of sight threatening thyroid eye disease which was treated with tocilizumab. This IL-6 inhibitor plays a key role in reducing the swelling of the soft tissues in moderate-to-severe TED. Our patient benefited from this treatment as the extraocular muscles compressed the optic nerve causing symptoms and signs of dysthyroid optic neuropathy. The reduction of the soft tissue swelling led to an improvement of the optic nerve function that was maintained at the last follow up eight months after the last intravenous dose of tocilizumab.

AUTHORS' STATEMENT OF CONSENT

The authors have obtained written consent by the patient stating that the patient agrees to have the pictures attached to this paper published.

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