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Budd-Chiari Syndrome

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Abstract: Budd-Chiari syndrome (BCS) is a rare disease with an incidence of 0.1 to 10 per million inhabitants a year caused by impaired venous outflow from the liver mostly at the level of hepatic veins and inferior vena cava. Etiological factors include hypercoagulable conditions, myeloprolipherative diseases, anatomical variability of the inferior vena cava, and environmental conditions. Survival rates in treated patients range from 42 to 100% depending on the etiology and the presence of risk factors including parameters of Child-Pugh score, sodium and creatinine plasma levels, and the choice of treatment. Without treatment, 90% of patients die within 3 years, mostly due to complications of liver cirrhosis. BCS can be classified according to etiology (primary, secondary), clinical course (acute, chronic, acute or chronic lesion), and morphology (truncal, radicular, and venooclusive type). The diagnosis is established by demonstrating obstruction of the venous outflow and structural changes of the liver, portal venous system, or a secondary pathology by ultrasound, computed tomography, or magnetic resonance. Laboratory and hematological tests are an integral part

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of the comprehensive workup and are invaluable in recognizing hematological and coagulation disorders that may be identified in up to 75% of patients with BCS. The recommended therapeutic approach to BCS is based on a stepwise algorithm beginning with medical treatment (a consensus of expert opinion recommends anticoagulation in all patients), endovascular treatment to restore vessel patency (angioplasty, stenting, and local thrombolysis), placement of transjugular portosystemic shunt (TIPS), and orthotopic liver transplantation as a last resort rescue treatment.

Introduction

Budd-Chiari syndrome (BCS) is a result of impaired hepatic venous outflow at any point from the efferent acinar vein up to the end of the inferior vena cava.

BCS is a rare entity in western countries, where it occurs predominantly in women between their third and fourth decade. In Asia, it is more prevalent in men around 45 years of age (Plessier and Valla, 2008). Whilst rare, BCS is accompanied by high mortality. If untreated, 70% of patients die within one year and 90% within three years, usually from complications of liver cirrhosis (Murad et al., 2004; Fu et al., 2011). Better insight into the nature of the disease may accelerate the diagnostic process and lead to timely treatment and improve outcome of the patient.

This article summarizes facts about epidemiology, classification, diagnosis, and treatment options in BCS.

History

The syndrome was first described in 1845 by an English physician named George Budd, who observed a triad of symptoms associated with this disease – abdominal pain, ascites, and hepatomegaly. But it was not until 1899 when Austrian pathologist Hans Chiari described its histopathology.

Epidemiology and etiology

The incidence of BCS is uncertain due to its rarity and figures from different locations vary greatly by as much as two orders of magnitude from 0.1 to 10 per million inhabitants a year (Valla, 2009; Renc et al., 2013; Ageno et al., 2017). In developed countries, thrombosis of the inferior vena cava (IVC) and hepatic veins (HV) in BCS is mostly a result of hypercoagulable conditions and myeloproliferative diseases (Valla, 2004; Plessier and Valla, 2008). Increased incidence of BCS in India, Nepal, South Africa, and in China along the Yellow river suggest the influence of environmental factors, living standard, and microbial infections (Zhang and Li, 2007). A membrane in the IVC above the confluence with HV can be retrospectively demonstrated in up to 70% of patients who died of complications of BCS. This membrane is thought to mechanically facilitate the development of BCS. Although these structures are inborn, the mechanism of their development remains unclear.

In Western countries, BCS is more common in females and occurs more commonly at the level of the hepatic veins. In Asia, occlusion of the IVC is more common and its incidence is comparable between the genders.

Prognosis

Five-year survival rates range from 42 to 100% depending on the etiology and the presence of risk factors including parameters of Child-Pugh score, sodium and creatinine plasma levels, and the choice of treatment (Valla, 2006). Murad et al. (2004) derived a prognostic formula that distinguished three classes based on the calculated score:

score = 1.27 × encephalopathy + 1.04 × ascites + 0.72 × INR + 0.004 × bilirubin

where ascites and encephalopathy are set to 1 when present, INR (International Normalized Ratio) higher than 2.3 also set to 1 and bilirubin used as a continuous variable in μmol/l. Class I represent scores between 0 and 1.1, class II between 1.1 and 1.5, and class III scores 1.5 and higher with 5-year survival rates of 89, 74, and 42% respectively.

As shown by Rautou and associates (2009), other prognostic scores including the Child-Pugh score, model for end-stage liver disease (MELD), Rotterdam index and New Clichy have their prognostic value too.

Best survival rates, irrespective of the prognostic score, have been reported in cohorts where venous drainage of the liver was restored by interventional methods (Eapen et al., 2006). The most common cause of death in patients treated for BCS is liver failure, postoperative multiorgan failure and sepsis (Murad et al., 2004).

Classification

There are several classifications of BCS (Senzolo et al., 2005). A classification suggested by prof. Wang included eight types based on anatomical location and the extent of the outflow obstruction (Wang and Jones, 1996). Less complex classifications used at present divide BCS according to the velocity of its development to fulminant, acute, subacute and chronic and by etiology as primary or secondary BCS (Plessier and Valla, 2008). Some authors do not consider cases with obstruction of small liver veins below 300 μm in diameter as BCS.

Etiology: primary and secondary BCS

Primary BCS occurs in patients with primary hematological disorders or hypercoagulable conditions. Up to half of patients with BCS are diagnosed with myeloproliferative disorders, polycythemia vera, essential thrombocytemia, or, in particular, primary myelofibrosis (Zhang and Li, 2007). The most common primary hypercoagulable conditions in these patients are mutations of factor V Leiden, factor II (prothrombin), JAK-2 tyrosine kinase genes and antiphospholipid syndrome (Colaizzo et al., 2008). The role of hyperhomocysteinemia and primary deficiency

of protein C or S or antithrombin III is difficult to establish due to the associated liver disease. BCS occurs in up to 30% of patients with primary nocturnal hemoglobinuria, which is 5% of all diagnosed patients in developed countries. Other risk factors include pregnancy, hypereosinophilic syndrome, ulcerative colitis, oral contraception, and several underlying conditions may act in synergy. Therefore, comprehensive hematological examination is indicated in every patient with excluded secondary etiology of BCS.

Secondary BCS describes any disease that causes BCS by invasion or compression of the IVC or HV with their consequent thrombosis. This includes focal liver lesions (hepatocellular carcinoma, abscess, cyst, …), renal or adrenal adenocarcinoma, blunt abdominal trauma, and rarely primary sarcomas of IVC or myxoma of the right atrium (Mukund and Gamanagatti, 2011).

Clinical: **type I** – acute, **type II** – chronic, **type III** – acute or chronic lesion with the worst prognosis (Langlet et al., 2003).

Morphology: according to the location of the obstruction – **truncal type (I)** with obstruction of the IVC (±HV), **radicular type (II)** with obstruction of HV, **venoocclusive type (III)** with obstruction of small centrilobular veins (Figure 1) (Lambert, 2016).

Pathophysiology

Between plates of hepatocytes and the sinusoids there is a narrow space of Disse. Microvillous projections from the hepatocytes into this space are responsible for metabolism and transport of molecules between blood and the hepatocyte. Impaired blood outflow results in increased intrasinusoidal pressure, dilation of sinusoids, and extravasation of erythrocytes that accumulate in the space of Disse. In early stages, congestion affects the perivenular zone but gradually extends towards the periportal zone (Valla, 2003). Persistent reduction of hepatic perfusion

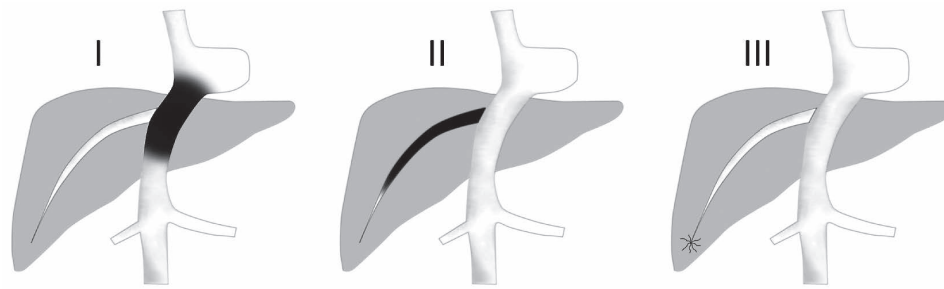

Figure 1 – Schematic drawing of the types of Budd-Chiari syndrome according to the location of the obstruction – truncal type (I) with obstruction of the IVC (±HV), radicular type (II) with obstruction of HV, venoocclusive type (III) with obstruction of small centrilobular veins.

Table 1 – The most common symptoms in patients with Budd-Chiari syndrome

	Okuda et al. (1998)	Hadengue et al. (1994)	Murad et al. (2004)	Singh et al. (2000)
Number of patients	157	88	237	81
Location of obstruction	IVC	HV	HV	HV (75%) IVC (25%)
Abdominal pain	23	72	–	32
Hepatomegaly	55	88	76	61
Ascites	31	83	84	81
Leg swelling	32	33	–	62
Jaundice	6	33	50	33

IVC – inferior vena cava; HV – hepatic veins

results in ischemic injury to the hepatocytes followed by fibrosis and nodular regenerative changes, resulting in liver cirrhosis. However, large regenerative nodules that develop to maintain perfusion of the liver may in turn lead to compression of adjacent intrahepatic veins (Cazals-Hatem et al., 2003).

Clinical presentation

BCS may cause a range of symptoms that present with various severities. Although some patients may be virtually asymptomatic, most of them develop some symptoms that are a result of liver failure (portal hypertension with variceal bleeding, encephalopathy, ascites, weakness, fatigue) or extension of secondary pathology and metastatic disease. Clinical presentation depends on the extent of hepatic venous outflow obstruction and velocity of its development. The most common symptoms are summarized in Table 1.

Diagnosis

Imaging is the mainstay of diagnosis of BCS, which is established by demonstrating obstruction of IVC, HV and structural changes of the liver, portal venous system, or a secondary pathology.

Ultrasound (US) is a first-line imaging method with high sensitivity and specificity of up to 85% (Bolondi et al., 1991). Venous outflow obstruction is demonstrated by absent flow and echogenic luminal content. Blood flow in a stenosis becomes accelerated with spectral broadening indicating turbulence. US can detect structural changes of the liver parenchyma (macronodular cirrhosis, focal liver lesions), hypertrophy of the caudate lobe (which is typical for BCS), ascites, collateral blood flow, or direct invasion and compression of the IVC by a tumoral mass.

Contrast-enhanced **computed tomography (CT)** is performed in portal venous phase in order to achieve good contrast filling in the portal, mesenteric,

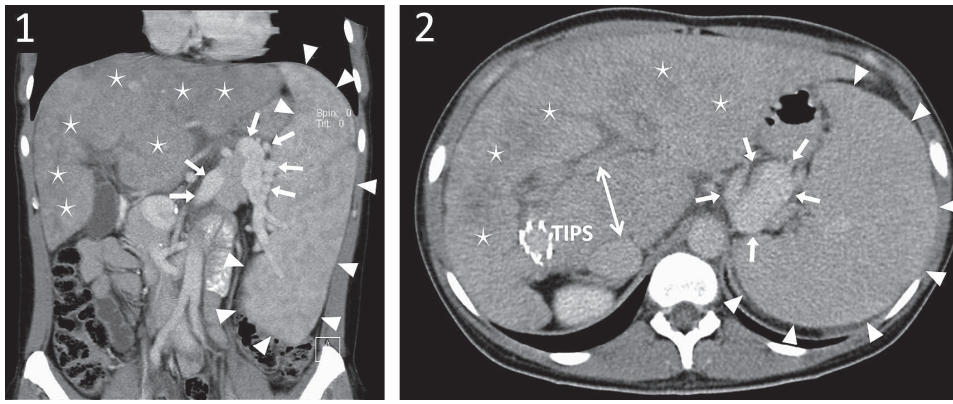

Figure 2 – Computed tomography (CT) in portal venous phase of a 32-years-old female patient with Budd-Chiari syndrome, who developed liver cirrhosis, portal hypertension, and splenomegaly showing macronodular regeneration in the liver parenchyma that has mottled appearance (asterisks), an enlarged caudate lobe (two-headed arrow), dilated splenic vein with conspicuous collateral veins (arrows), and enlarged spleen (arrowheads) in coronal (1) and axial (2) plane. This patient received a transjugular portosystemic shunt (TIPS) in order to reduce porto-systemic gradient and the risk of variceal bleeding.

and hepatic veins and in inferior vena cava for optimal detection of their pathology. Structural changes of the liver are similar to findings in US. Inhomogeneous enhancement of the liver parenchyma results in mottled appearance with relative sparing of the caudate lobe and perivenous parenchyma that have separate venous drainage (Figure 2). These changes are more obvious on **magnetic resonance (MR)**, which provides images with far better tissue contrast (Zhou et al., 2014).

Invasive imaging methods that can be used to depict obstruction of the venous outflow include **angiography** of the inferior vena cava and hepatic veins (**cavography**). They are commonly used together with interventional procedures to restore patency of the vessels.

Liver biopsy is indicated in patients with suspected venoocclusive type (III) of BCS of unknown etiology, when macroscopic obstruction of the venous outflow has been excluded by imaging.

Gastroscopy is warranted in patients with liver cirrhosis to exclude esophageal or fundal varices and perform their ligation in order to decrease the risk of variceal bleeding. Portal hypertensive gastropathy refers to macroscopic changes of the gastric mucosa with prominent vessels that result in mosaic appearance.

Laboratory and hematological tests are an integral part of comprehensive workup of patients with BCS and are invaluable in hematological and coagulation disorders that may be recognized in up to 75% of patients (Singh et al., 2000). In 25% of patients, at least two underlying conditions can be identified (Aydinli and Bayraktar, 2007). Primary BCS requires laboratory test for factor V Leiden and factor II (prothrombin) mutations, for the presence of antiphospholipid antibodies

and plasma levels of homocysteine, protein C, protein S, and antithrombin III (EASL, 2016). The deficiency of antithrombin III or protein C and S can also be the result of liver dysfunction. Myeloproliferative disorders are common in BCS and their diagnosis is based on bone marrow biopsy. Mutations of the JAK-2 kinase gene can be demonstrated in the majority of patients, including those with latent forms (Boissinot et al., 2006). Indeed, standard biochemical and haematological blood analysis including electrolytes, proteins (including serum electrophoresis), liver and kidney function tests, complete blood count, and coagulation tests are required as well.

Treatment

The recommended therapeutic approach to BCS is based on a stepwise algorithm beginning with medical treatment, endovascular treatment to restore vessel patency (angioplasty, stenting, and local thrombolysis), placement of transjugular portosystemic shunt (TIPS), and orthotopic liver transplantation as a rescue treatment.

Although no prospective randomized trials on **anticoagulation therapy** in BCS have been conducted so far, a consensus of expert opinions recommended anticoagulation in all patients (Janssen et al., 2003). The risk of bleeding complications in patients with BCS is comparable to patients with anticoagulation therapy for other indications (Plessier and Valla, 2008). Its main goal is to prevent progression of the thrombosis. As soon as the diagnosis of BCS has been established, anticoagulation with low molecular weight heparin (LMWH) with a target value of AntiXa between 0.5 and 0.8 IU/ml has to be initiated without delay (DeLeve et al., 2009). Before switching from LMWH to oral anticoagulants, contraindications such as liver cirrhosis, portal hypertension, and esophageal varices have to be excluded and the diagnostic workup (screening for coagulation disorders, or liver biopsy if indicated) has to be completed (DeLeve et al., 2009). The recommended targeted INR (International Normalized Ratio) for treatment of patients with BCS with vitamin K antagonists is between 2.5 and 3 (Senzolo et al., 2005; Mancuso, 2011). Regular INR testing is necessary to ensure that the target INR range is maintained. In patients with primary BCS, prolonged, possibly even lifelong anticoagulation therapy is recommended. There are limited data about the use of direct factor Xa inhibitors (e.g. rivaroxaban) in patients with BCS.

In general, **thrombolysis** in BCS, whether systemic or intraarterial into the hepatic artery, is ineffective, unless it is administered locally (HV, IVC, TIPS) very early in case of acute thrombosis of HV or TIPS followed by endovascular intervention. In such patients, no major complications have been reported (Sharma et al., 2004).

Treatment of consequent **portal hypertension** and its complications is symptomatic and treatment recommendations are based on their management in patients with liver cirrhosis: diuretic therapy (spironolactone, furosemide) or paracentesis for ascites, endoscopic ligation of esophageal varices, administration

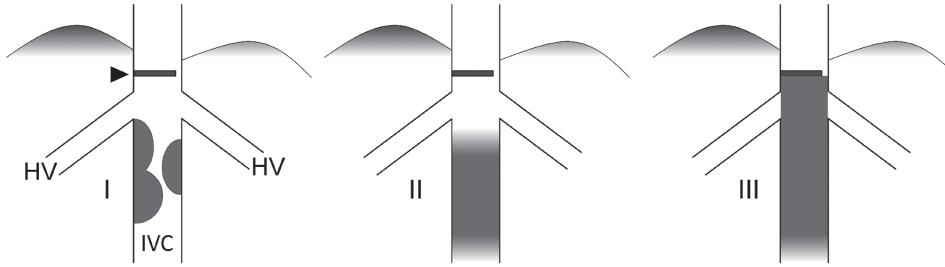

Figure 3 – Schematic drawing of types of occlusion of inferior vena cava (IVC) and their relationship to the hepatic veins (HV) with regard to planning of interventional procedures. Location of a membrane in the IVC, found in up to 70% of patients with Budd-Chiari syndrome, is denoted by an arrowhead. In type I (left) and II (middle), the thrombosis does not involve confluence with the hepatic veins.

of proton pump inhibitors and beta-blockers to reduce the risk of bleeding from esophageal varices in selected patients (EASL, 2016).

Medical therapy alone may not be sufficient in patients who have persistent abnormal liver function tests, intractable ascites, coagulopathy, encephalopathy, abdominal discomfort, hepatorenal syndrome, gastrointestinal bleeding or ongoing necrosis shown on liver biopsy (Menon et al., 2004). These patients require timely relief of hepatic obstruction, decompression of portal hypertension, or orthotopic liver transplantation as the last resort rescue treatment.

The increasing use of **interventional procedures** in the treatment of BCS is guided by the type of venous occlusion (Wang et al., 2005). It consists in aspiration thrombectomy followed by predilatation with a small diameter catheter, which decreases the risk of pulmonary embolism and allows introduction of a thrombolytic catheter with subsequent administration of thrombolytic agent.

In patients with **thrombosed IVC**, endovascular treatment is indicated in patients with subtypes I and II, and in patients with acute IVC thrombosis of subtype III (Figure 3). In patients with subtype III (i.e. that extends beyond HV) subacute to chronic thrombosis, conservative treatment or, ultimately, placement of TIPS are preferred (Pelage et al., 2003; Ruihua et al., 2013). Recanalization of the occluded IVC results in rapid clinical improvement, and has good mid-term results (Han et al., 2013).

Patients with **occluded HV and patent IVC** are suitable for angioplasty and stenting if the HV has a straight course and is of sufficient diameter (≥7 mm). The HV is usually accessed through the internal jugular vein. If this is not successful or feasible, percutaneous transhepatic approach or access through the femoral vein is another option. In acute thrombosis, thrombolytic therapy alone has good results as well (Mukund and Gamanagatti, 2011).

In short stenosis, interventional treatment with stenting warrants excellent long-term patency – 97% in IVC and 91% in HV, but angioplasty alone has a high rate

of restenosis. If restenosis occurs after stenting, it is usually due to inadequate anticoagulant therapy that has to be maintained for at least 6 months after the intervention (Mukund and Gamanagatti, 2011). In general, 5-year survival in patients treated by endovascular recanalization is 86% in patients with intermediate disease severity and 77% with severe BCS (Eapen et al., 2006).

In some patients, who developed liver cirrhosis, placement of **transjugular portosystemic shunt (TIPS)** is indicated to decrease porto-systemic gradient in order to reduce the risk of variceal bleeding. The procedure should be planned in collaboration with a surgeon who performs liver transplantations (Menon et al., 2004). Introduction of TIPS requires endovascular access using an internal jugular approach to the ostium of the hepatic veins. The portal vein is punctured through the remnant of the hepatic vein, if present, or directly from the inferior vena cava. Introduction of TIPS has a 5-year survival without the need for liver transplantation of 78% (Garcia-Pagán et al., 2008). The use of a TIPS stent covered with polytetrafluorethylene (ePTFE) or TIPS stentgraft improves long-term patency rates compared to bare stents and therefore became a standard (Hernández-Guerra et al., 2004; Šafka et al., 2005; Renc et al., 2013; Dulíček et al., 2016). Surgical construction of a portosystemic shunt is rarely required and some cases can be solved by combination of endovascular and surgical therapy (hybrid procedures).

Orthotopic liver transplantation is the last resort rescue treatment if conservative and interventional therapy does not prevent development of liver cirrhosis and progressive liver failure in chronic BCS. Its indication should be carefully evaluated. However, in patients presenting with fulminant hepatic failure, it is an urgent indication. Patients after liver transplantation require life-long immunosuppressive therapy. Although liver transplantation can cure a majority of the hereditary thrombophilias, in most of the patients with BCS, multiple etiologic factors may be present and long-term or life-long anticoagulation therapy may also be indicated. Five-year survival of BCS patients after orthotopic liver transplantation is around 90% (Hefaiedh et al., 2013).

Conclusion

Budd-Chiari syndrome is a result of impaired hepatic venous outflow at any point from the efferent acinar vein up to the end of the inferior vena cava. Congestion in the liver sinusoids results in portal hypertension, liver fibrosis or cirrhosis, and their complications. Imaging is the mainstay of diagnosis of BCS. Laboratory and haematological tests are invaluable in recognizing hematological and coagulation disorders that may be identified in up to 75% of the patients. The recommended therapeutic approach to BCS is based on a stepwise algorithm beginning with medical treatment. A consensus of expert opinion recommends anticoagulation in all patients. In selected patients, it can be combined with local thrombolysis, angioplasty and stenting. Restoring outflow vessel patency by endovascular

treatment results in improved survival. In patients with progressive liver failure and portal hypertension, placement of TIPS may decrease the risk of variceal bleeding and relieve symptoms. Orthotopic liver transplantation is the last resort with good long-term results.

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Interleukin-2, *Interferon-gamma* Gene Polymorphisms in Recurrent Aphthous Stomatitis

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Abstract: Recurrent aphthous stomatitis (RAS) is the most common oral ulcerative inflammatory disease with unknown etiology. *IL-2* and *IFN-γ* are secreted by Th1 cells and the elevated levels of them have been reported in RAS. Single nucleotide polymorphisms (SNPs) of *IL-2* and *IFN-γ* genes could alter the cytokine production. The aim of this study was to investigate frequencies of *IL-2* and *IFN-γ* alleles and genotypes in a group of patients with minor-RAS (MiRAS). PCR-SSP method used to type genomic DNA of 64 Iranian patients with MiRAS

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for *IL-2* gene (G –330 T) and (G +166 T) and *IFN-γ* gene at position UTR5644 (A/T). Frequency of each allele and genotype was compared with control group. *IL-2* +166 G allele was significantly lower among patients which was reflected in significantly decreased of GG genotype at this position, while *IL-2* +166 T allele was significantly higher among patients, *IL-2* GT genotype was also significantly higher in RAS patients. No significant differences were found regarding *IL-2* –330 G/T allele frequencies, while *IL-2* GT genotype at this position was significantly higher among patients and *IL-2* –330 TT genotype was significantly lower among RAS patients. Although no significant differences were found in *IFN-γ* allele frequencies at UTR5644 (A/T), AT genotype at this position was significantly overrepresented among patients compared with controls. Results of this study suggest that certain SNPs of *IL-2* and *IFN-γ* genes have association with predisposition of individuals to RAS. More studies in different ethnic groups are needed to confirm results of this study.

Introduction

Recurrent aphthous stomatitis (RAS) is the most common oral painful ulcerative inflammatory disease of unknown etiology (Preeti et al., 2011). Three clinical manifestations have been described for RAS: Minor (MiRAS), Major (MaRAS), Herpetiform Ulcers (HU). MiRAS is the most common manifestation which affects about 80% of RAS patients (Bazrafshani et al., 2002a). Although the precise etiology of RAS has not been understood yet, it has been designated that some immune dysfunction may underlie RAS pathogenesis (Chavan et al., 2012). Compelling evidences demonstrate that a dysregulated, local, cell-mediated immune response, which conduce to accumulation of T-cells and leads to tissue breakdown, might be responsible for formation of RAS ulcers (Akintoye and Greenberg, 2005).

An increased systematic production of Th1 cytokines and local transcription of Th1 genes have been reported in patients with RAS (Borra et al., 2004; Lewkowicz et al., 2005). Interleukin-2 (IL-2) and Interferon-gamma (IFN-γ) are two cytokines secreted by Th1 cells, which are considered as pro-inflammatory cytokines the elevated levels of both have been reported in RAS patients (Albanidou-Farmaki et al., 2007; Chavan et al., 2012). Treating CD4+ cells with IFN-γ may lead to Th2-to-Th1 exchange, and as a result of grow inhibitory of IFN-γ, the number of Th2 cells decreases while Th1 cells proliferate (Schroder et al., 2004).

IL-2 and IFN-γ might be strong candidates for involvement with RAS pathogenesis. Cytokine gene polymorphism plays an essential role in cytokine secretion. This study was designed to evaluate the possible single nucleotide polymorphisms (SNPs) in the gene of IL-2 at positions +166 (G/T), –330 (G/T) and also in the gene of IFN-γ at position UTR5644 (A/T) in a sample of Iranian patients with MiRAS. To our best knowledge, this is the first investigation of IL-2 and IFN-γ SNPs in individuals with MiRAS.

Material and Methods

Subjects

The Ethical Committee of Tehran University of Medical Sciences approved this project. Written informed consent was obtained from all subjects included to this study before sampling. Five ml of blood was obtained from sixty-four Iranian patients with MiRAS (24 men and 40 women) from the Department of Oral Medicine, School of Dentistry of Tehran University of Medical Sciences. 23 cases have less than three aphthous episodes per month, while 41 cases had 3 or more aphthous episodes per month. 140 age, sex and ethnicity matched healthy controls (101 men and 40 women), who were randomly selected to be enrolled in this study.

All patients were assessed by an oral medicine specialist and diagnosis of RAS was made based on accepted international clinical criteria (Ship et al., 2000). Neither patients nor controls had history of smoking or any systemic diseases such as Behcet's syndrome, diabetes mellitus, PFAPA syndrome, HIV infection. History of exposure to radiation, drugs consumption, and pregnancy at the time of study and any other periodontal diseases considered as exclusion criteria for both experimental and control groups.

Genotyping

DNA was isolated using phenol-chloroform method. IL-2 and IFN-γ gene typing was done by polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany), the method described in detail previously (Amirzargar et al., 2008). Briefly, amplification was carried out, using a thermal cycler Techne Flexigene apparatus, and the presence or absence of PCR product was visualized by 2% agarose gel electrophoresis. After electrophoresis, the gel was placed on a UV transilluminator and a picture for interpretation and documentation was taken. The allele and genotype frequencies of IL-2 (G –330 T), (G +166 T) and IFN-γ (UTR5644 A/T) were determined.

Statistics

Data analysis was performed using SPSS statistical software package (version 15.0). Chi-square test was used to compare frequencies of alleles, genotypes and haplotypes between patients and control groups. The odds ratio (OR) and 95% confidence intervals (CI) were calculated. Comparison of medians of quantitative variables was performed, using Mann-Whitney U-test. P-value (p) of <0.05 was considered significant. Allele frequencies were estimated by direct gene counting.

Results

Alleles, genotypes and haplotype frequencies

IL-2 and IFN-γ allelic and genotype frequencies in RAS patients and healthy controls are shown in Table 1. IL-2 haplotype frequency also is presented in Table 1.

Table 1 – Comparison of alleles, genotypes and haplotypes frequencies of *IL-2* and *IFN-γ* between patients with RAS and the control group

Cytokine position	Alleles/ genotypes/ haplotypes	RAS (n=60), n (%)	Controls (n=140), n (%)	P-value	Odds ratio (95% confidence interval)
IL-2 –330	G	58 (48.3)	110 (39.6)	0.1290	1.43 (0.91–2.25)
	T	62 (51.7)	168 (60.4)	0.1290	0.70 (0.44–1.10)
	GG	3 (5.0)	8 (5.8)	0.5650	0.86 (0.17–3.75)
	GT	52 (86.7)	94 (67.6)	0.0089	3.11 (1.29–7.76)
	TT	5 (8.3)	37 (26.6)	0.0066	0.25 (0.08–0.72)
IL-2 +166	G	80 (66.7)	219 (78.8)	0.0147	0.54 (0.33–0.89)
	T	40 (33.3)	59 (21.2)	0.0147	1.86 (1.12–3.07)
	GG	22 (36.7)	82 (59.0)	0.0061	0.40 (0.21–0.79)
	GT	36 (60.0)	55 (39.6)	0.0124	2.29 (1.18–4.46)
	TT	2 (3.3)	2 (1.4)	0.3500	2.36 (0.23–24.15)
–330, +166	GG	54 (29.8)	107 (38.8)	0.0635	0.67 (0.44–1.02)
	TG	55 (30.4)	112 (40.6)	0.0345	0.64 (0.42–0.97)
	TT	37 (20.5)	56 (20.3)	0.9360	1.01 (0.62–1.65)
	GT	35 (19.3)	1 (0.3)	0.0000	65.92 (9.57–1307.56)
IFN-γ UTR5644	A	62 (51.7)	140 (50.7)	0.9490	1.04 (0.66–1.63)
	T	58 (48.3)	136 (49.3)	0.9490	0.96 (0.61–1.51)
	AA	14 (23.3)	43 (31.2)	0.3430	0.67 (0.31–1.42)
	AT	34 (56.7)	54 (39.1)	0.0330	2.03 (1.05–3.94)
	TT	12 (20.0)	41 (29.7)	0.2130	0.59 (0.27–1.29)

RAS – recurrent aphthous stomatitis

IL-2 polymorphisms

IL-2 +166 G allele was significantly lower (OR=0.54, CI=0.33–0.89, p=0.014) in RAS patients compared to controls, which was reflected in significantly lower GG genotype (OR=0.40, CI=0.21–0.79, p=0.061) at this position. IL-2 +166 T allele was significantly higher among patients (OR=1.86, CI=1.21–3.07, p=0.014). TT genotype at this position was more common among patients but did not reach significance (p=0.3).

While IL-2 G +166 allele was significantly lower among patients and IL-2 T +166 allele was significantly higher among study group, IL-2 GT genotype at the same position was significantly higher (OR=2.29, CI=1.18–4.46, p=0.012) among RAS patients in comparison with healthy controls.

There was no significant differences in IL-2 (G –330 T) allele frequencies, while IL-2 GT genotype at position –330 was significantly higher (OR=3.11, CI=1.29–7.79, p=0.0089) among patients compared with controls. TT genotype at the same position was significantly lower (OR=0.25, CI=0.08–0.72, p=0.0066) among RAS patients compared with controls.

Comparison of IL-2 haplotype frequencies between the patients and controls indicated that TG haplotypes were significantly lower (OR=0.64, CI=0.42–0.97, p=0.03) in the patient group, while GT haplotype was significantly higher (OR=65.92, CI=9.57–1307.56, p=0.0000) among patients with RAS.

IFN-γ polymorphisms

There was no significant differences in IFN-γ allele frequencies at UTR5644 (A/T), while IFN-γ AT genotype at the same position was significantly overrepresented (OR=2.03, CI=1.05–3.94, p=0.033) among study group compared with healthy controls.

Discussion

Although association of a number of cytokines gene polymorphisms with RAS have been investigated (Najafi et al., 2014, 2015), to our best knowledge, this is the first time that association of IL-2 and IFN-γ gene polymorphisms with RAS is reported. Buno et al. (1998) demonstrated elevated levels of IL-2 and IFN-γ mRNA in RAS lesions, consistent with a cell-mediated immune response, thereby formation of aphthous ulcers is mainly dependent on the activation of the Th1-type immune response, which leads to elevated levels of Th1 cytokines such as IL-2 and IFN-γ (Buno et al., 1998; Lewkowicz et al., 2011).

To the best of our knowledge, we for the first time investigated IL-2 and IFN-γ SNPs in RAS. Some significant differences in alleles, genotype and haplotypes were found between study patients and controls. IL-2 SNPs were investigated at positions –330 (G/T) and +166 (G/T) in RAS patients and healthy controls. The results of our investigation indicated that G +166 allele was significantly lower in the patient group, while T +166 allele was significantly higher among patients. The +166 GG genotype and –330 TT genotype were significantly lower among the patient group. While IL-2 –330 GT genotype and +166 GT genotype were significantly higher among RAS patients, it seems that individuals who are heterogeneous in these positions are predisposed to RAS. The results of this study illustrates that the G to T exchange at position +166 of IL-2 gene might be responsible for increased IL-2 secretion.

The distribution of IFN-γ AT genotype at position UTR5644 was significantly higher among RAS patients compared with controls, indicating that UTR5644 AT genotype is likely to be responsible for the elevated level of IFN-γ in RAS patients (Buno et al., 1998; Lewkowicz et al., 2011). It seems that heterogeneity at this position of the IFN-γ gene predisposed individuals to RAS.

A Th1 type immune response has been shown in RAS pathogenesis (Bazrafshani et al., 2003), which eradicates pathogens and also cause immunopathology. Elevated levels of Th1 cytokines such as tumour necrosis factor (TNF), IL-2 and IFN-γ and on the other hand lower levels of IL-4, IL-10 and IL-5 which are secreted by Th2 cells in patients with RAS, support this hypothesis (Buno et al., 1998). Bazrafshani

et al. (2002b) have demonstrated no significant association between RAS and TNF polymorphisms. These findings support the notion that genetic factors related to Th1 type immune response more likely operating through the IFN-γ and IL-2 polymorphisms, and genetically predispose individuals to RAS. Indeed more studies in other ethnic groups and larger populations are needed to confirm these findings.

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Hereditary Multiple Exostoses: Clinical, Molecular and Radiologic Survey in 9 Families

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Abstract: Hereditary multiple exostoses (HME) represents a heterogeneous group of diseases often associated with progressive skeletal deformities. Most frequently, mutations in *EXT1* and *EXT2* genes with autosomal dominant inheritance are responsible for HME. In our group of 9 families with HME we evaluated the clinical course of the disease and analysed molecular background using Sanger sequencing and MLPA in *EXT1* and *EXT2* genes. The mean age in our group of patients, when the first exostosis was recognised was 4.5 years (range 2–10 years) and the number of exostoses per one patient documented on X-ray ranged from 2 to 54. Most of the exostoses developed before the growth was completed and they were dominantly localised in the distal femurs, proximal tibia, proximal humerus and distal radius. In all patients, at least one to 8 surgeries were necessary due to complaints and local complications, but neither patient

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developed malignant transformation. In half of the patients, the disease resulted in short stature. DNA analyses were positive in 7 families. In five probands, different *EXT1* gene mutations resulting in premature stop-codon (p.Gly124Argfs*65, p.Leu191*, p.Trp364Lysfs*11, p.Val371Glyfs*10, p.Leu490Profs*31) were found. In two probands, nonsense mutations were found in *EXT2* gene (p.Val187Profs*115, p.Cys319fs*46). Five mutations have been novel and two mutations have occurred *de novo* in probands. Although the risk for malignant transformation is usually low, especially in patients with low number of exostoses, early diagnostics and longitudinal follow up of patients is of a big importance, because early surgery can prevent progression of secondary bone deformities.

Introduction

Hereditary multiple exostoses (HME) manifests as remodelling deformities in bones due to disturbed chondrocyte proliferation and maturation that leads to abnormal bone growth and development of isolated or multiple exophytic masses near the joints of long bones (Karasick et al., 1997; Stieber and Dormans, 2005; Kok et al., 2013). HME belongs to the group of glycosaminoglycan biosynthesis deficiencies associated with skeletal and connective tissue disorders (Hennet and Cabalzar, 2015). The inheritance of HME is autosomal dominant and most patients have non-sense mutation in the *EXT1* gene on chromosome 8q24 or *EXT2* gene on chromosome 11p11-13 encoding exostosin glycosyltransferase 1 and 2 (EXT1; OMIM *608177 and EXT2; OMIM *608210) (Wuyts et al., 1998; Francannet et al., 2001).

Exostoses are localised most frequently in the metaphyses of long bones, but they may also develop on the diaphyses of long bones. They are usually painless, but secondary complaints may arise due local pressure of exostosis on neighbouring areas of soft tissues. Bone lesions on flat bones, vertebrae, and the ribs are less common and the skull is usually not involved (OMIM #133700).

We present the results of clinical, radiologic and molecular survey in 9 families with hereditary multiple exostoses.

Methods

Total genomic DNA was available from all patients and parents of patient 4 and patient 9. All exons of *EXT1* (ENSG00000182197; ENST00000378204) and *EXT2* (ENSG00000151348; ENST00000395673) genes were amplified by PCR from genomic DNA isolated from leukocytes and analyzed by direct sequencing using 3500xL genetic analyzer (Applied Biosystems, USA). PCR primers are available upon request. All samples were tested for large deletions/duplications using the SALSA MLPA P215-B2 EXT probe mix (MRC-Holland, Amsterdam, Netherlands) according to the manufacturer's instructions. Fragment analysis was performed on 3500xL genetic analyzer (Applied Biosystems, USA), and the MLPA data were analysed using Coffalyser.Net software (MRC-Holland, Netherlands).

Ethics

The study was approved by the Ethics Committee of the General University Hospital in Prague and was conducted in agreement with institutional guidelines. Written informed consent for molecular analyses was obtained from all patients and parents of affected children.

Results

Patients

Altogether, 9 probands and 3 other family members with multiple exostoses at the age between 6 and 76 years and two 18 months old girls from family 4 were recommended to our outpatient clinic for skeletal dysplasia for genetic testing and counselling. All but one proband are Caucasians from different regions of the Czech Republic, one proband is offspring of parents from the Czech Republic and Asia.

The average age, when the first exostosis was recognized clinically, was 4.5 years (range 2–10 years) and the average age, when the diagnosis of multiple exostoses was confirmed by radiographic examinations, was 8.6 years (range 3–15 years) (Table 1). Exostoses in our patients were dominantly localised in distal femur, proximal tibia, proximal humerus and distal radius (Figure 1). Most of the exostoses developed during pubertal growth spurt. Stabilisation of the disease occurred after

Table 1 – Clinical data and results of radiographic examination in 9 families with hereditary multiple exostoses

Family	Patient	Relationship to the proband	Age (years)	Growth (percentile)	Age at onset/age at diagnosis	Number of exostoses on X-rays	Number of surgeries
1	1	proband	16	<1	3/3 years	31	2
	2	mother	48	30	4.5/14 years	19	1
	3	grandmother	76	<1	6/15 years	30	?
2	4	proband	11	40	2/3 years	54	3
3	5	proband	19	2.6	0–3/3 years	36	7
4	6	proband	30	50	6/9 years	35	7
	7	daughter A	1.5	20	–	0	–
	8	daughter B	1.5	20	–	0	–
5	9	proband	16	2.7	5/5 years	44	1
6	10	proband	32	30	3/13 years	28	8
7	11	proband	9	13	4/4 years	16	2
8	12	proband	27	60	3/10 years	10	2
9	13	proband	10	0.7	9/10 years	39	–
	14	mother	40	<3	10/15 years	2	2

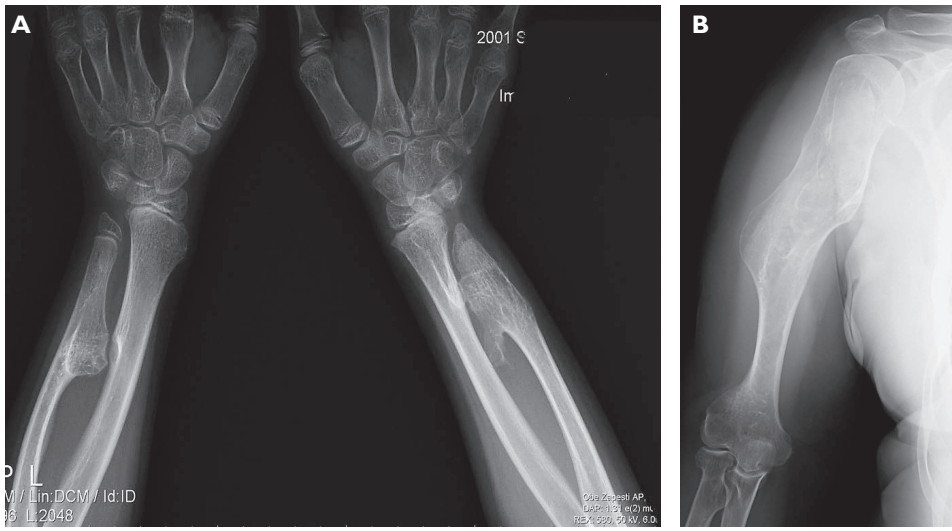

Figure 1 – Different size and localisation of exostoses in five patients with multiple hereditary exostoses due to heterozygous nonsense mutations in EXT1 and EXT2 genes.
A) Exostoses on distal forearms cause deformation of diametaphyses of long bones. Significant exostoses are located particularly on ulnae leading to their relative shortening, outward bowing of radii and formation of "bayonet hand" deformity of the forearm (P1 at the age of 13 years); B) Significant broad exostoses are apparent on the proximal metaphysis and on the upper half of proximal diaphysis of the right humerus with a significant deformity of the humeral diametaphysis (P3 at the age of 71 years).

the growth was completed, but the disease resulted in the short stature in 6 out of 13 patients (46%). The number of exostoses per one patient documented on X-ray ranged from 2 to 54 (Table 1). In practically all patients, at least one to 8 surgeries were necessary due to complaints and local complications of exostoses, but no patient developed malignant transformation, so far.

Mutation analysis of EXT1 and EXT2 genes
DNA analyses in 7 patients from 5 families revealed the presence of heterozygous mutation in *EXT1* gene and mutation in *EXT1* was also found in two asymptomatic young girls (P7, P8) from family 4. Mutations in *EXT2* gene were found in three patients P12, P13 and P14 (mother of P13). Five mutations have been novel and two mutations, one in each analysed gene, have occurred *de novo* in probands (Table 2). Large deletions/duplications affecting *EXT1* and *EXT2* genes were not found.

Discussion

Both enzymes, exostosin glycosyltransferase 1 and exostosin glycosyltransferase 2 (EXT1 and EXT2), are involved in the synthesis of heparan sulphate proteoglycans

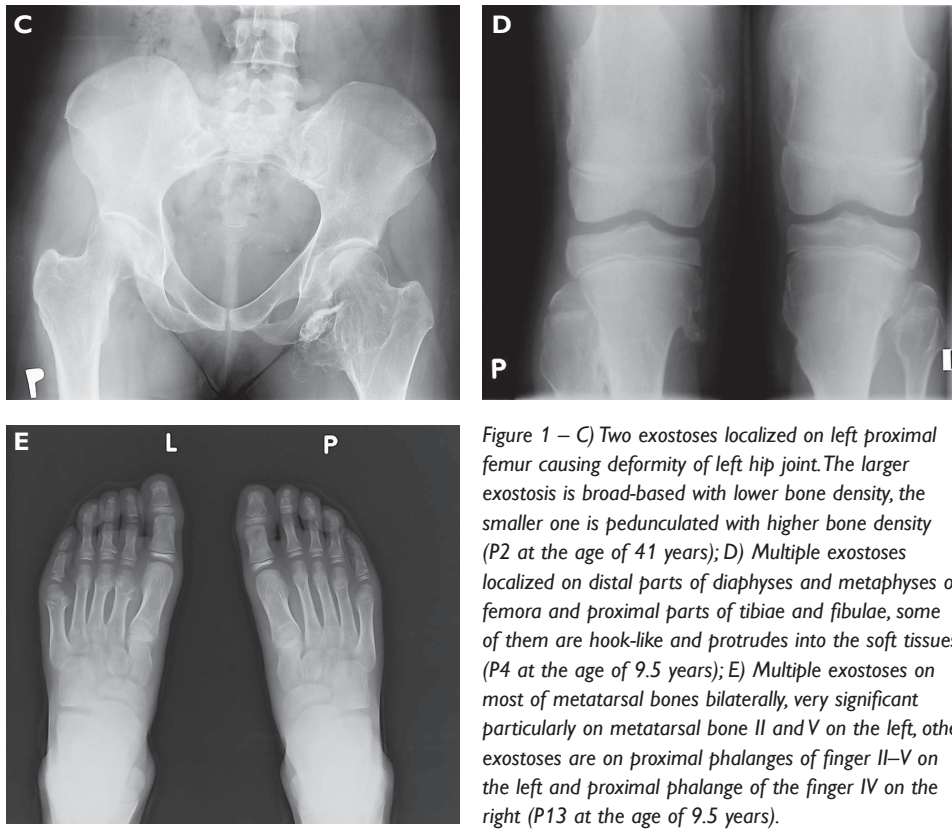

Figure 1 – C) Two exostoses localized on left proximal femur causing deformity of left hip joint. The larger exostosis is broad-based with lower bone density, the smaller one is pedunculated with higher bone density (P2 at the age of 41 years); D) Multiple exostoses localized on distal parts of diaphyses and metaphyses of femora and proximal parts of tibiae and fibulae, some of them are hook-like and protrudes into the soft tissues (P4 at the age of 9.5 years); E) Multiple exostoses on most of metatarsal bones bilaterally, very significant particularly on metatarsal bone II and V on the left, other exostoses are on proximal phalanges of finger II–V on the left and proximal phalange of the finger IV on the right (P13 at the age of 9.5 years).

on the cell surface and in the extracellular matrix (Wuyts et al., 1998; Francannet et al., 2001). *EXT1* and *EXT2* genes encoding these enzymes have tumour suppressor function and mutations in *EXT1* and *EXT2* genes may cause a loss of heterozygosity (LOH). It means a 2-hit tumour formation model, when a single germ-line mutation in *EXT1* or *EXT2* results in the predisposition for disease and the second local somatic mutation allows for aberrant growth of exostoses (Hecht et al., 1995; Raskind et al., 1995).

Growth of exostoses may result in short stature, limb-length discrepancies, valgus deformities of the knee and ankle, asymmetry of the pectoral and pelvic girdles, bowing of the radius with ulnar deviation of the wrist, and subluxation of the radiocapitellar joint (Stieber and Dormans, 2005). In both aspects, clinical and radiological, the group of our patients corresponds well with other studies describing the localisation of exostosis predominantly around the knee (Bovée, 2008).

The prevalence of HME is estimated at 1:50 000, and it seems to be higher in males (male-to-female ratio 1.5:1) (Bovée, 2008). In our study, only half of probands

Table 2 – *EXT1* and *EXT2* mutations found in 9 families with hereditary multiple exostoses

Patient	Gene	Mutation	Predicted protein change	*de novo* mutation	Reference
1	*EXT1*	c.572T>G	p.Leu191*		novel
2	*EXT1*	c.572T>G	p.Leu191*		novel
3	*EXT1*	c.572T>G	p.Leu191*		novel
4	*EXT1*	c.1112delT	p.Val371Glyfs*10	yes	novel
5	*EXT1*, *EXT2*	negative			
6	*EXT1*	c.369dupA	p.Gly124Argfs*65		novel
7	*EXT1*	c.369dupA	p.Gly124Argfs*65		novel
8	*EXT1*	c.369dupA	p.Gly124Argfs*65		novel
9	*EXT1*	c.1468dupC	p.Leu490Profs*31	yes	Seki et al. (2001)
10	*EXT1*	c.1092_1100delinsCTC AGAAATTGCTCAGCA	p.Trp364Cysfs*11	not tested	novel
11	*EXT1*, *EXT2*	negative			
12	*EXT2*	c.956delG	p.Cys319fs*46	not tested	novel
13	*EXT2*	c.459_462delTGTT	p.Val187Profs*115		Song et al. (1999)
14	*EXT2*	c.459_462delTGTT	p.Val187Profs*115		Song et al. (1999)

are males. The diagnosis of HME in childhood is mostly based on family history of the disease with autosomal dominant inheritance, clinical evaluation, radiographic examination and genetic testing.

The results of molecular analyses in our study are in agreement with the literature. Most mutations in *EXT1* and *EXT2* in families with HME are private. A more severe course of the disease was shown to be significantly associated with mutations in *EXT1* gene, whereas a moderate phenotype is more often associated with mutations in *EXT2* (Francannet et al., 2001; Heinritz et al., 2009). In our study, mutations in *EXT1* gene were found in 55% of probands. It is a much less than 85% found in 33 unrelated Polish patients (Jamsheer et al., 2014). Mutations in *EXT2* gene were found in two probands. All except two mutations identified in our study are novel. EXT1 mutation p.Leu490Profs*31 in proband 5 and ETX2 mutation p.Val187Profs*115 found in family 9 have been already described (Song et al., 1999; Seki et al., 2001). In our families with HME, the molecular basis of the disease was not recognized in 22% of probands. In recent study of significantly larger patient

group (112 patients) from Japan, 34% of patients did not exhibit mutations in *EXT1* or *EXT2* genes (Ishimaru et al., 2016).

Precise diagnostics in patients with multiple exostoses is not always easy, especially in patients without mutation in *EXT1* and *EXT2* genes, because exostosis may also develop in other clinical conditions including metachondromatosis (OMIM #156250) and the Langer-Giedion syndrome (LGS; OMIM #150230) also known as trichorhinophalangeal syndrome type II and in patients with fibrodysplasia ossificans progressiva (FOP; OMIM #135100), occipital horn syndrome (OMIM #304150), and the adult stage of hereditary hypophosphatemia (OMIM #307800); these exostoses are located at sites of tendon and muscle attachment. A relatively rare variant of the supracondylar process, on the anteromedial surface of the distal humerus, can be confused with an exostosis; the variant is said to be present in about 1% of persons of European descent (Silverman, 1985). Recently, exostosin-like glycosyltransferase 3 (EXTL3) that regulates the biosynthesis of heparan sulphate (HS) and is important for both skeletal development and haematopoiesis has been linked to distinct clinical phenotype by two groups. *EXTL3* mutations are responsible for "neuro-immuno-skeletal dysplasia syndrome" with variable skeletal abnormalities, neurodevelopmental defects and combined immunodeficiency (Oud et al., 2017; Volpi et al., 2017).

Conclusion

Early diagnostics and longitudinal follow up of patients with hereditary multiple exostoses are of great importance. In some patients, surgery can prevent progression of secondary deformities. Although the risk for malignant transformation of the cartilaginous portion of the exostosis in patients with isolated exostosis is usually low (0.5–1%), the risk increases in patients with multiple exostoses up to 5% (Vanhoenacker et al., 2001; Stieber and Dormans, 2005; Bovée, 2008). The onset of malignant transformation towards secondary peripheral chondrosarcoma is most frequent in the fourth decade, it seldom occurs in children below 10 years and adults after 50 years (Ochsner, 1978).

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IgG4-related Disease – A Patient with Multiple Organ Involvement

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Key words: IgG4 – Fibrosis – Sclerosis – Immunosuppressive therapy

Abstract: IgG4-related diseases represent a heterogeneous group of conditions characterised by elevated serum IgG4 levels and fibrotic or sclerosing changes in the affected organs or systems accompanied by IgG4-positive plasma cells. A disease associated with IgG4 may affect virtually any organ – salivary glands, periorbital tissue, kidneys, lungs, meninges, aorta, prostate, pericardium or skin. Histopathological findings are uniform, characterised by a major lymphoplasmocytic infiltrate and the presence of IgG4-producing plasma cells, irrespective of the affected site. It can be difficult to establish a correct diagnosis due to the lack of clinical symptoms. Treatment with immunosuppressive drugs provides good results and requires interdisciplinary cooperation.

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Introduction

IgG4-related disease represents a relatively newly defined condition comprised of a collection of disorders characterised by IgG4 hypergammaglobulinaemia and the presence of IgG4-positive plasma cells in affected organs with fibrotic or sclerotising changes (Kamisawa et al., 2015). IgG4-related disease may affect virtually any organ – salivary glands, periorbital tissue, kidneys, lungs, meninges, aorta, prostate, pericardium, and skin. However, its histopathology is uniform, showing a large lymphoplasmacytic infiltrate enriched in IgG4-positive plasma cells, irrespective of the localisation of involved organs (Mahajan et al., 2014). Mikulicz syndrome is a disease that was originally considered to be a variant of Sjögren's syndrome; at present, it is one of the examples of IgG4-related disease presentation. Inflammatory orbital pseudotumour, Küttner's tumour and idiopathic retroperitoneal fibrosis (Ormond's disease) are other disorders belonging to the group (Brito-Zerón et al., 2014). Being relatively rare, these disorders still have to be taken into consideration when examining patients.

Case report

A 74-year-old male patient was referred to our immunology outpatient department in order to exclude a systemic inflammatory disease. He had a history of the following diseases: epidemic typhus in 1945, infectious hepatitis A in 1953, myocardial infarction in 2003, and crural vein thrombosis on the left-hand side in 2010. A year and a half ago, the patient experienced orbital oedema and a burning sensation for the first time. Due to professional exposure – the patient grew African violets – allergy was considered; therefore, allergy assessment was performed which revealed nasal polyposis, with no findings of allergy to current inhalation allergens. Orbitopathy with bilateral exophthalmos followed shortly, with no findings supporting an endocrine aetiology. This was followed by the enlargement of salivary and lacrimal glands, with no pronounced xerophthalmia or xerostomia. This was followed by an asymmetrical submandibular gland swelling on the left. The patient had no fever; he reported no subjective complaints: absence of appetite, tiredness or pain upon admission. Objective assessment revealed a clear bilateral eye protrusion with lower eyelid oedema and an asymmetrical submandibular salivary gland swelling on the left. From a cardiopulmonary point of view, the patient was compensated, without any pathology found on the heart, lungs or abdomen. Laboratory findings: ESR 85/120, CRP < 5 mg/l, and biochemical screening showed no significant pathology. Immunology revealed polyclonal hypergammaglobulinaemia, with increased levels of all IgG1–4 subgroups (IgG1 18.1 g/l; IgG2 12.5 g/l; IgG3 5.83 g/l; IgG4 5.24 g/l), with no M gradient, with positive antinuclear antibodies (ANA) with a cytoplasm-type of immunofluorescence at a 1:40 titre, antibodies to extractable nuclear antigens (ENA), anti-neutrophil cytoplasmic antibodies (ANCA), and anti-phospholipid antibodies (ACLA, anti-beta GPIb) were negative. Head and neck

ultrasonography showed a symmetrical salivary gland swelling with reactively enlarged neck lymphatic glands. Head and neck MRI (magnetic resonance imaging) assessment revealed a bilateral symmetric retrobulbar mass outside of the extraocular muscles and parotic and submandibular gland swelling. PET (positron emission tomography) imaging showed locations with glucose hypermetabolism in parotic glands and the left submandibular gland, and in neck and mediastinal lymph nodes. A biopsy examination of the salivary gland was performed which showed significant reactive hyperplasia of the lymphadenoid tissue with lymphatic follicles corresponding to the diagnosis of Mikulicz syndrome. A CT (computed tomography) examination of the abdomen and lower pelvis revealed a non-functional left kidney which was slightly wrinkled and had a thick hollow system wall, without dilation, with several cortical cysts. The right kidney had a regular size and structures with normal excretion functions. An increased density was seen around large abdominal vessels, more to the left, which presented itself as infiltration, rather than fibrosis. The patient was diagnosed with an IgG4-associated disease – accompanied by inflammatory orbital pseudotumour, Küttner's tumour, Mikulicz syndrome and Ormond's disease – idiopathic retroperitoneal fibrosis. Corticosteroid therapy was indicated. Hypergammaglobulinaemia and ESR reached normal values in 4 weeks, and orbitopathy disappeared completely. Corticosteroids were administered for seven months and gradually discontinued. The patient had absolutely no subjective complaints and felt excellent. Follow-up examinations were performed every six months, with blood sample collection for immunological examination. Polyclonal hypergammaglobulinaemia (IgG 21 g/l) and increased IgG4 levels (4.6 g/l) were detected in the laboratory results two years later. Again, immunosuppressive therapy using methylprednisolone (Medrol) was administered to the patient – the initial dose of 32 mg was reduced to 2 mg once in two days six months later – which has kept the disease in remission.

Discussion

IgG4-associated diseases are a newly defined group of disorders, some of which were already known in the past (Table 1). They are characterised by elevated IgG4 serum levels and the presence of plasma cells in the inflammatory infiltrate producing these antibodies. There is a vast variety of clinical presentations and a range of involved organs and systems (Stone et al., 2015). The pathogenic role of IgG4 antibodies is poorly understood (Ebbo et al., 2012). We have relatively vast experience in the diagnosis and treatment of Ormond's disease – idiopathic retroperitoneal fibrosis – the treatment of which is provided in cooperation with an urologist and the vascular surgery department (Průcha et al., 2016). Our case report shows vast multiple organ involvement of various organs and systems. Mikulicz syndrome and inflammatory orbital pseudotumour were presented as clinically dominant entities. Idiopathic retroperitoneal fibrosis that was discovered

Table 1 – IgG4-associated diseases

• Mikulicz syndrome with the involvement of salivary and lacrimal glands
• Küttner's tumour affecting submandibular glands
• Riedel's thyroiditis
• Inflammatory pseudotumours affecting orbits, lungs, kidney
• Mediastinal fibrosis
• Multifocal fibrosclerosis affecting aorta, thyroid gland, retroperitoneal space
• Ormond's diseases – idiopathic retroperitoneal fibrosis
• Periarteritis and periaortitis
• Inflammatory aortic aneurysm
• Idiopathic hypocomplementemic tubulointerstitial nephritis

in a relatively inactive stage led to the loss of one kidney without the patient noticing. From the practical point of view, it is necessary to stress that patients with Ormond's disease present themselves with largely non-specific complaints – tiredness, sometimes subfebrile temperature, abdominal pain, and back pain. A common basic biochemical examination does not usually reveal any pathology, except for increased ESR and elevated C-reactive protein levels in half of the cases. In the absence of clear clinical symptomatology, diagnosis can be made based on ultrasound abdominal examination which can reveal the involvement of the kidney hollow system or abdominal aorta during an active disease. The gold standard of diagnosis is histopathological examination of the biopsy specimen. Differential diagnostics are required to exclude tumorous processes in the retroperitoneum (sarcomas, haemoblastosis, neuroblastomas, germ cell tumours, metastatic processes from other solid or carcinoid tumours). Similarly, secondary fibrosis from inflammatory processes (sarcoidosis, specific spondylitis or vasculitis, connective tissue disease) must also be excluded. We have diagnosed more than 20 patients with the disease during the last four years of cooperation. From the practical point of view, it is important to stress that the disease responds very well to systemic corticosteroid therapy (Van Bommel et al., 2007). Immunosuppressive therapy is the treatment of choice, and has no alternative. Urological or vascular surgical treatment of the affected renal hollow system or the abdominal aorta, respectively, should be provided as a matter of course. However, systemic corticosteroid monotherapy more often leads to exacerbation of the disease and, therefore, combined immunosuppressive treatment should be preferred, as appropriate.

Conclusion

IgG4-related diseases are a relatively rare group of conditions, at times with poor clinical symptomatology and a vast variety of involved organs or systems. Its diagnosis and treatment require interdisciplinary cooperation, which has the potential to be the most beneficial for the patient.

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Colonic Perforation: A Medical Complication

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Key words: Hypothyroidism – Sigmoid perforation – Constipation – Thyroid – Hartmann's procedure

Abstract: Hypothyroidism is a common comorbidity that on acute presentation is often overlooked. It can be an easily managed condition; however non-compliance can have severe consequences. In the presented case it was requirement for emergency surgery that resulted in stoma formation. This case is a first example of the need to include patient's decision making process with regards to medication adherence in the setting of chronic disease.

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Introduction

The Hartmann's procedure is the removal of the rectosigmoid colon with the formation of a rectal stump. This procedure is most commonly performed as emergency surgery for acute peritonitis, roughly 77% of cases (Pares et al., 2005). Other conditions that may require a Hartmann's procedure are intestinal obstruction (of which the most common cause is malignancy) and gastrointestinal bleeding. This procedure is reserved for critically unwell patients, as the gold standard operation would be anastomosis with diverting ileostomy (Liu et al., 2011).

Subclinical hypothyroidism occurs in roughly 3% of men and 8% of women (Chistiakov, 2005). Hypothyroidism is a condition where there is a lack of free thyroid hormones which are important biochemical modulators of metabolism. They have effects on the majority of body systems including vital actions like protein synthesis and regulating sympathetic activity. Clinical presentation of hypothyroidism includes bradycardia, drowsiness, hypothermia, congestive cardiac failure, immobile bowel and ileus (Canaris et al., 2000).

Hypothyroidism is known to cause gastrointestinal dysmotility however this is commonly unrecognised. Hypomotility, atony, pseudo-obstruction and in extreme cases even perforation has arisen as the result of hypothyroidism (Bergeron et al., 1997; Zachariah and Raja, 2010). The Association of Clinical Biochemistry, the British Thyroid Association and the British Thyroid Foundation state the incidence of hypothyroidism is around 1–2% demonstrating that although significant bowel complications are very rare there is a large proportion of the population at risk. We discuss a rare case of hypothyroidism causing a significant surgical emergency.

Case report

A 52-year-old lady with a background of acquired primary hypothyroidism secondary to thyroid malignancy, previous cholecystectomy and asthma who denied taking any regular medications presented with a fresh bleeding per rectum and lower abdominal pain. On arrival to the emergency department the patient was hypotensive, hypothermic and bradycardic. The patient lower abdominal tenderness and rectal examination revealed fresh blood. She was initially managed with analgesia, intravenous fluid resuscitation, intravenous antibiotics and tranexamic acid. Admission bloods were as follows: haemoglobin 117, white blood count 7.0, platelet 205, urea 6.9, estimated glomerular filtration rate 48, creatinine 105 (C-reactive protein not performed). She underwent an urgent CT (computed tomography) with intravenous contrast which identified moderate pneumoperitoneum extending from the pelvis closely associated to an inflamed sigmoid colon. No significant evidence of diverticulitis or malignancy was seen on the CT. Marked faecal loading was demonstrated throughout the colon and rectum. There were also changes congruent with sigmoid and rectal colitis with no obvious

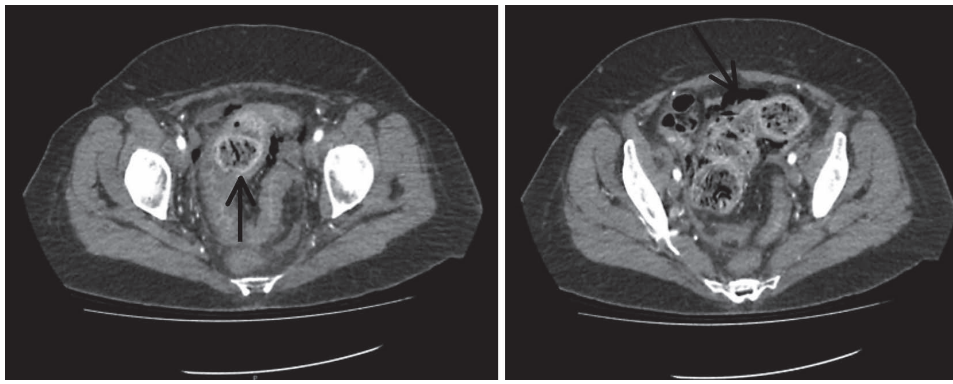

Figure 1 – Abdominal computed tomography scan shows pneumoperitoneum with enhancement of the distal sigmoid and rectum with faecal impaction.

site of mesenteric bleeding (Figure 1). The working diagnosis prior to theatre was of a stercoral perforation. The patient subsequently underwent an emergency laparotomy.

In keeping with the CT findings the patient had a sigmoid colon perforation with stercoral diffuse peritonitis with no other evident precipitating factors other than constipation. Therefore a Hartmann's procedure was performed which was uncomplicated. Two intra-abdominal surgical drains were placed and remained in-situ on transfer to the intensive therapy unit (ITU) for post-operative care. Post-operative bloods were performed in ITU which identified that the patient was markedly hypothyroid. Thyroid stimulating hormone (TSH) > 100 mU/l and free thyroxine (T4) < 0.3 pmol/l. Treatment with IV tri-iodothyronine (T3) and IV antibiotics as per local antibiotic guidelines for intra-abdominal sepsis was given.

Prior to admission the patient had been taking no regular medication including thyroxine. She had not opened her bowels for 14 days and had not sought any medical help until the point of admission. After three days the patient was discharged from ITU and the intra-abdominal drains were removed. With specialist input from the endocrine team the intravenous thyroxine replacement was converted to oral levothyroxine. Inflammatory markers improved and she was switched to oral antibiotics and learnt to manage her own stoma, which started to function well. Histological analysis of the resected sigmoid did not reveal any evidence of malignancy, diverticulosis or colitis. During admission, other contributing factors for her constipation were excluded and the only abnormality identified was her marked hypothyroidism. She was counselled appropriately about compliance and was seen as an outpatient by the endocrine team. The patient declined stoma reversal during her routine surgical follow up.

Discussion

Acquired primary hypothyroidism is a recognised consequence of thyroidectomy for malignancy that is easily managed by the consumption of oral thyroxine. In this case however, poor compliance to this treatment strategy resulted in severe hypothyroidism. It is clearly documented that decreased levels of thyroxine cause a proportional reduction in basal metabolic rate, which in turn reduces gut motility and causes constipation (Müller-Lissner et al., 2005; Ebert, 2010). Severe hypothyroidism and associated constipation has rarely been associated with surgical intervention (Bergeron et al., 1997).

Current literature has rarely demonstrated hypothyroidism causing significant surgical complications however this case adds to the cases as set forward by Bergeron et al. (1997), and Zachariah and Raja (2010). Bergeron et al. (1997) demonstrated 6 cases of hypothyroidism causing gastrointestinal complications including 1 case of gastrointestinal perforation (site unidentified at laparotomy), and Zachariah and Raja (2010) demonstrated a recto-sigmoid perforation in a patient with significant hypothyroidism. Perforation is a result of severe constipation is multi-factorial; however, the impact of hypothyroidism is poorly identified. Biochemical thyroid assessment is a simple test that can be incorporated into the assessment of the surgical patient. In modern practice it is important to consider the cost benefit ratio before performing additionally investigations on patients. Although significant surgical complications of hypothyroidism are rare it is difficult to discern what proportion of patients' symptoms may be affected by low levels of free T4.

Compliance of all medications, including thyroxine, is a well-known phenomenon throughout the world of medicine. Studies have shown states that only 50% of patients with chronic conditions comply with their treatment regime (Sabaté, 2003). Reasons for non-compliance with treatment are multifactorial, often including side effect profiles and lack of understanding of the benefits of taking the medication (Ngoh, 2009). In many cases these issues can be resolved by in depth discussions of advantages and disadvantages with the patient, and including them in the decision making process (Conn et al., 2016).

An aspect of long-term therapy that can be overlooked is managing the most commonly encountered side effects whilst on essential medications such thyroxine, anti-epileptics and analgesia. In the setting of chronic pain managed with opioids all clinicians are acute aware of the need for anti-emetics and laxatives in conjunction with the morphine derivative to obtain an effective balance of analgesia and unwanted symptoms (McNicol et al., 2003). However, constipation and sub-therapeutic thyroxine treatment is not as commonly encountered and is therefore not at the forefront of concerns for this subset of patients.

After a decade of taking her thyroxine this lady made an active decision to stop her thyroxine. The patient perceived that cessation of thyroxine treatment resulted in improved symptom control of her irritable bowel syndrome. Despite general

practitioners and consultants challenging this health belief the patient remained non-compliant. This lack of compliance was echoed biochemically with TSH levels > 100 recorded in 2012 and on admission to hospital in 2016, despite the patient's assertion that she was taking regular thyroxine.

Improving compliance is both time-intensive and multifaceted. Evidence based techniques include acceptance of the need for compliance, patient's understanding of the need for daily medication and regular contact with health professionals for on-going negotiations with regards to the treatment and its impact on the patient's lifestyle (Winnick et al., 2005).

In conclusion this clinical encounter stresses the vital importance of communication in the setting of compliance and adherence of chronic conditions and long-term medications. It highlights that surgeons must not ignore or overlook potential medical conditions that may contribute to the numbers and complexities of surgical admissions. A more open view of the medical complexities of the surgical patient may prevent life threatening surgical complications such as perforation.

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Meropenem-induced Valproic Acid Elimination: A Case Report of Clinically Relevant Drug Interaction

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Abstract: We present two case reports of drug interaction between valproic acid and meropenem. In comparison with expected population-kinetic based serum levels, we observed 90.8 and 93.5% decrease in valproic acid serum levels during concomitant administration with meropenem. If carbapenems need to be administered to valproic acid treated patient, other anticonvulsant addition seems to be the appropriate as most probably the valproic acid dose escalation would not be sufficient to achieve therapeutic serum concentration.

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Introduction

Valproic acid (2-propylpentanoic acid, VPA) was introduced for clinical use in France in 1967. Over the next half-century it has become a well-established first-line and widely used agent for convulsion treatment both for its broad spectrum of activity against generalized and partial seizures and for its acceptable safety profile (Peterson and Naunton, 2005). VPA serum therapeutic range is 50–100 mg/l (346–693 μmol/l) in seizure treatment (Bentué-Ferrer et al., 2010). Although VPA therapeutic drug monitoring is not routinely recommended in common neurological practice (Tomson et al., 2007) it can be useful in polypharmacy to reveal possible drug interactions or in the intensive care units when VPA is initiated for the onset of seizure treatment (Loh et al., 2010).

There are at least three metabolic pathways of VPA elimination in humans: glucuronidation, mitochondrial beta-oxidation (both considered major routes accounting for 50 and 40% of dose, respectively) and cytochrome P450-mediated oxidation (minor route responsible for elimination of approximately 10% of dose) (Ghodke-Puranik et al., 2013). Concurrently, VPA is regenerated from VPA-glucuronide by acylpeptide hydrolase-mediated hydrolysis. Carbapenems inhibit acylpeptide hydrolase and thus VPA regeneration from VPA-glucuronide, resulting in reduced VPA serum levels (Nakajima et al., 2004; Suzuki et al., 2010, 2011).

There are few case reports describing decreased VPA serum levels by carbapenems (Nacarkucuk et al., 2004; Clause et al., 2005; Fudio et al., 2006; Suntimaleeworakul et al., 2012). A recent study also documented the decreased VPA levels caused by concomitant use of meropenem using a large number of therapeutic drug monitoring (TDM) records (Wen et al., 2017). We present additional two cases of this significant drug interaction.

Case report 1

A 59-year-old polymorbid male (90 kg, 180 cm) admitted to the Department of Anesthesiology and Intensive Care after cardiopulmonary resuscitation for cardio-respiratory arrest. The sixth day of hospital stay VPA treatment was initiated for generalized myoclonic seizures probably related to brain ischemia. Patient was loaded with 1,600 mg VPA administered via 24 h-continuous intravenous infusion, followed by maintenance doses of 500 mg given every 8 hours as crushed slow-release tablets through the nasogastric tube. The tenth day of hospital stay meropenem therapy was initiated (2 g intravenous bolus followed by 4 g/day in continuous intravenous infusion) for pulmonary infection. The other concomitant medication included norepinephrine, unfractionated heparin (UFH), insulin, propofol, potassium supplementation, omeprazole, prednisone, diosmectite and probiotics. After five days of concomitant VPA and meropenem administration, blood sample was taken, showing a serum level of VPA of 8.0 mg/l (determined using a cloned enzyme donor immunoassay with quantification limit of 3 mg/l). In

spite of the low VPA levels no other seizures episode occurred. Subsequently VPA treatment was discontinued.

Case report 2

A 56-year-old man (92 kg, 178 cm) with chronic thrombotic pulmonary hypertension was admitted to the surgical ward for elective pulmonary vein endarterectomy. Patients' medical history included congestive hepatopathy, history of minor stroke, mild chronic kidney disease, dyslipidemia, hypertension and schizophrenia. On the second postoperative day after successful surgery intravenous meropenem 2 g three times a day was initiated because of pulmonary infection. As generalized myoclonic seizures occurred the day after, VPA treatment was initiated. Intravenous loading dose of 1,200 mg was followed by 100 mg/hour continuous infusion for 24 hours. Subsequently the patient was treated with maintenance doses of 300 mg every 8 hours administered as crushed slow release tablets through the nasogastric tube. On the postoperative day 7 the trough level of VPA was 3.4 mg/l (determined using cloned enzyme donor immunoassay). Concomitant medication included norepinephrine, UFH, insulin, propofol, sufentanyl, potassium supplementation, vancomycin, acetaminophen, dipyrone, omeprazole, furosemide, risperidone and treprostinil. In spite of the low VPA levels no other seizures episode occurred through the rest of hospital stay. Meropenem was withdrawn on postoperative day 8 and VPA two weeks later shortly before the hospital discharge.

Discussion

Food and Drug Administration Professional Drug Information (FDA-PDI) states following mean VPA population pharmacokinetic parameters: volume of

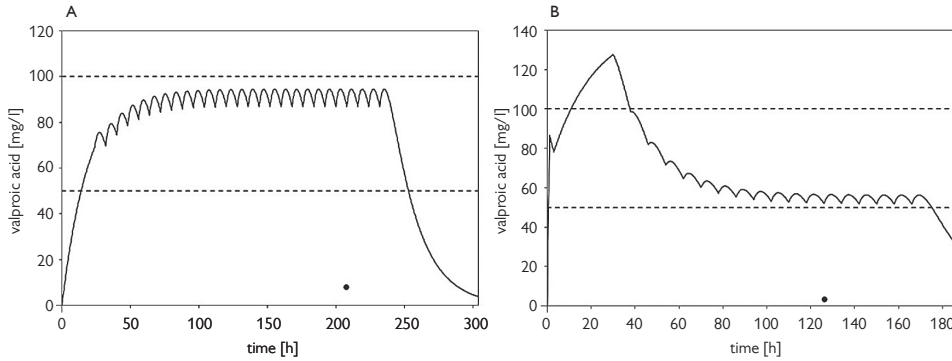

Figure 1 – A priori simulation of valproic acid plasma levels time profile based on population pharmacokinetics compared with measured valproic acid concentration. A) case report 1; B) case report 2. Dotted lines represent therapeutic range.

distribution 11 l/1.73 m^2, clearance 0.56 l/h/1.73 m^2 and terminal elimination half-life ($T_{1/2}$) 16 h. Simulation of VPA serum levels based on FDA-PDI population pharmacokinetics compared with measured VPA concentration is shown in Figure 1. Pharmacokinetic models show that measured VPA serum concentrations corresponded to only 9.2 and 6.5% of concentrations estimated based on population data. Fitting of the simulated pharmacokinetic profile curve with observed drug concentration points resulted in VPA $T_{1/2}$ of 1.6 and 1.4 h. Very short $T_{1/2}$ indicates that VPA dose escalation is meaningless in this case, while switch to another antiepileptic agent seems to be more relevant for effective anticonvulsive treatment.

It should be noted, however, that in theory the case 1 VPA levels could be also affected by reduced VPA absorption due to concomitant diosmectite administration. However, diosmectite was administered in at least 2 h distance from the other medication and no adsorbent was administered to the patient in case 2 where comparably low concentration of VPA was measured. In both cases slow release tablets were crushed to allow administration through nasogastric tube. Nevertheless nasogastric tube should have negligible impact. VPA bioavailability is approaching 100% when immediate release form is used and 8 hour dosing interval would not result in as low serum trough levels as were measured if clearance of the drug was not affected. Based on the literature data VPA-meropenem interaction is the most probable explanation for this observation. As absorption extent and volume of distribution is likely not to be affected by concomitant meropenem administration, significant increase in VPA clearance due to inhibition of VPA-glucuronide cleavage is most probable mechanism of this interaction.

Carbapenems are known for seizure threshold lowering properties in comparison with non-carbapenem antibiotics. Even though this adverse event was described mostly for imipenem/cilastatin, head to head comparison with meropenem showed similar risks for imipenem and meropenem (Cannon et al., 2014). In case 2, possible contribution of meropenem in triggering seizures cannot be excluded. Considering carbapenem-VPA interaction, appropriate treatment of suspected carbapenem-induced seizures should not include VPA.

Conclusion

We present two case reports of drug interaction between VPA and meropenem. In comparison with expected population-kinetic based serum levels, we observed 90.8 and 93.5% decrease in VPA serum levels during concomitant administration with meropenem. It is important for the physicians to recognize drug interaction between VPA and carbapenems, especially when treating carbapenem induced seizures. If carbapenems need to be administered to VPA treated patient other anticonvulsant addition seems to be appropriate as VPA dose escalation would most probably not be sufficient to achieve therapeutic serum concentration.

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