Časopis Medicinskog fakulteta Univerziteta u Beogradu

UNIVERZITET U BEOGRADU MEDICINSKI FAKULTET
UNIVERSITY OF BELGRADE SCHOOL OF MEDICINE

МЕДИЦИНСКИ ФАКУЛТЕТ УНИВЕРЗИТЕТ У БЕОГРАДУ

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INFLAMMATORY ABDOMINAL AORTIC ANEURYSM AND RETROPERITONEAL FIBROSIS

INFLAMATORNA ANEURIZMA ABDOMINALNE AORTE I RETROPERITONEALNA FIBROZA

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Summary

The inflammatory aneurysm of abdominal aorta (IAAA) accounts for 3–11% of all abdominal aortic aneurysms, and occurs predominantly in men. It has similar pathological mechanisms like idiopathic retroperitoneal fibrosis (IRF) and perianeurysmal retroperitoneal fibrosis (PRF), thus presenting the same non-specific systemic inflammatory disorder known as chronic periaortitis (CP).

Recognition of CP early in its course is important in order to reduce morbidity due to complications, such as renal failure and mortality from aortic rupture. However, the initial symptoms of CP are non-specific, which makes early clinical diagnosis extremely difficult.

Various studies have shown favorable outcomes following corticosteroid treatment in nearly 90% of patients. The positive effects of adding different immunosuppressants to corticoid therapy have established such "duo-therapy" as a durable treatment option. Also, cessation of smoking has a positive effect on disease course and it should be an integral part of every treatment regimen.

Operative repair of the abdominal aortic aneurysm is considered to be the definitive treatment for IAAA. The effects of both open and endovascular interventions on the inflammatory component however remain unclear. Resolution of periaortic fibrotic tissue after surgery for IAAA is still debated. Some investigators have reported a complete resolution while the others have noted partial regression and even persistence of the inflammatory cuff.

Current treatment strategies are based upon "the best available evidence", mostly derived from clinical experience obtained by case series involving a small number of patients. Studies of a higher evidence level are very much needed to adjust our approach to such patients and to establish guidelines for treating this complex and serious disorder.

Keywords: inflammatory abdominal aortic aneurysm, retroperitoneal fibrosis, chronic periaortitis, corticosteroids, immunosuppressants, abdominal aortic surgery, EVAR

Sažetak

Inflamatorne aneurizme čine 3-11% svih aneurizmi abdominalne aorte (AAA), javljajući se pretežno u muškaraca. Patofiziološki mehanizam nastanka ovih aneurizmi isti je kao i kod idiopatske i perianeurizmalne retroperitonealne fibroze, čineći tako nespecifični sistemski inflamatorni entitet poznat kao hronični periaortitis. Rano prepoznavanje ovog stanja važno je sa aspekta smanjenja morbiditeta i mortaliteta, pre svega otkazivanja bubrega i rupture aneurizme aorte.

Mnoge studije su pokazale značaj tretmana preparatima kortikosteorida, dovodeći do željenih efekata u skoro 90% bolesnika. Dodavanjem različitih imunosupresivnih lekova postavljeni su osnovi „dualne terapije" kao dugoročnog rešenja. Takođe, prekid pušenja pozitivno utiče na tok bolesti i predstavlja integralni deo svakog režima lečenja.

Iako se smatra da je operativno lečenje AAA definitivna terapijska opcija, efekti otvorene i endovaskularne hirurgije na inflamatornu komponentu AAA ipak ostaju nerazjašnjeni. Rezolucija periaortnog fibroznog tkiva nakon hirurškog lečenja i dalje ostaje predmet debate. Neki autori su prijavilni potpunu rezoluciju, dok su drugi primetili parcijalnu regresiju uz perzistirajući paraaortalni „cuff".

Trenutne strategije lečenja bazirane su na osnovu „najboljih dostupnih dokaza", uglavnom dobijenih iz kliničkog iskustva baziranog na individualnim slučajevima i istraživanjima sa malim brojem pacijenata. Studije od većeg kliničkog značaja su neophodne kako bismo prilagodili pristup takvim pacijentima i formirali vodiče za lečenje ovog kompleksnog i ozbiljnog poremećaja.

Ključne reči: inflamatorna aneurizma abdominalne aorte, retroperitonealna fibroza, hronični periaortitis, kortikosteroidi, imunosupresivi, hirurgija abdominalne aorte, EVAR.

Introduction

An inflammatory aneurysm of abdominal aorta (IAAA) is defined as an aneurysm with a > 1 cm thick periaortic inflammatory rind, as seen on computed tomography (CT) scan. The term inflammatory aneurysm was introduced by Walker in 1972, who described a group of abdominal aortic aneurysms (AAAs) intraoperatively characterized by a thickened wall, marked perianeurysmal and retroperitoneal fibrosis and adhesions to adjacent organs (1).

IAAA, idiopathic retroperitoneal fibrosis (IRF) and perianeurysmal retroperitoneal fibrosis (PRF) are actually various clinical presentations of the same non-specific systemic inflammatory disorder known as chronic periaortitis (CP) and do not represent separate entities. While IAAA is characterized by the triad of back/ab-

dominal pain, pulsatile abdominal aortic aneurysm and an elevated erythrocyte sedimentation rate (ESR), IRF and PRF cause even less specific symptoms and may lead to obstruction of the ureters and adjacent abdominal structures or even an end-stage renal disease (2-6). These varieties of RF can be idiopathic or secondary to other causes, the latter remaining beyond the scoop of this study.

Despite differences in their clinical presentation, all abovementioned varieties of CP are sharing identical histopathological characteristics: advanced atherosclerosis of the abdominal aorta, the development of fibroblast proliferation and chronic inflammatory infiltrates in the aortic adventitia, medial thinning in dilated or undilated aorta with a varying degree of periaortic fibrosis and deposits, and extension to involve adjacent structures (7). Apart from an increased aortic diameter in IAAA, there are no other differences in histology between IAAA and IRF. These conditions present a challenge for a surgeon and treatment strategies have not been clearly defined yet (8).

Incidence and epidemiology

The IAAA accounts for 3–11% of all AAAs. It occurs predominantly in men (male to female ratio from 6:1 to 30:1). The mean age at presentation is 62–68 year, which is 5–10 years younger than in patients with non-inflammatory AAAs (1, 9-13). The data on its annual incidence are incoherent due to overlap with IRF, varying from 0,1 to 1,3 per 100.000 persons per year (14, 15). Most studies indicate that IRF occurs earlier than IAAA, at the age of 40 to 60 years, and is twice more frequent in men (16-18).

The proportion of smokers in IAAA patients is significantly higher (77–100%). (11) There is evidence that a familial tendency to aneurysm formation is 10 times more frequent in patients with inflammatory aneurysm, leading to the assumption of a genetic predisposition (19).

Interestingly, the exposure to asbestos is a well–known risk factor for developing IRF (14, 20), but its relation to IAAA remains unclear.

Pathogenesis

CP and its spectrum of clinical disorders that include IAAA and IRF are characterized by chronic inflammation in the presence of advanced atherosclerosis (6). The etiology of the clearly antigen-triggered inflammatory response has not been fully understood so far and currently there are two leading theories on issue of the pathogenesis of CP.

The first theory is that RF is the expression of a vast local inflammatory response to aortic atherosclerosis and degradation of the aortic wall (7, 21-23).

Autoimmunity to a component of atherosclerotic plaque, oxidized low-density lipoprotein (LDL), and ceroid has been proposed as the antigenic stimulus in the initiation of the inflammatory process. Degradation products from lipids deposited in the wall, elastin degradation products, etc. may lead in the further course to the disintegration of the extracellular matrix via the release and activation of proteolytic enzymes from immune cells, e. g. metallomatrix proteases and can finally cause aneurysm genesis through inflammatory degradation of the aortic wall (24).

The second leading theory is that IAAA/RF is the expression of a systemic autoimmune disease (25). Vaglio et al reported vasculitis with fibrinoid necrosis involving the aortic vasa vasorum and the small and medium retroperitoneal vessels, suggesting that CP could be a systemic autoimmune disease, perhaps involving a vasculitic process of small and medium vessels (22). This supports previous studies, which reported a prevalence of small–and medium–vessel vasculitis in 10–80% (26, 27). The association of various autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, Wegener's granulomatosis, polyarteritis nodosa, and giant cell arteritis, with periaortitis and their response to steroids and other immunosuppressive agents further strengthens the concept of autoimmunity (27-32).

Clinical manifestations

Recognition of CP early in its course is important in order to reduce morbidity due to complications, such as renal failure and mortality from aortic rupture. However, the initial symptoms of CP are non-specific, which makes early clinical diagnosis extremely difficult. As a consequence, the diagnosis is often delayed until significant organ involvement, such as ureteral obstruction and/or renal failure, is present.

The onset of IAAA is virtually always associated with abdominal, back or flank pain (65–90 %), which are seen significantly less frequently in non-inflammatory aneurysms, namely 8% versus 18% (33). Lack of appetite and weight loss are seen in almost 50% of patients. Fever is seen in isolated cases but it does not represent typical element of the clinical picture (9).

The onset of IRF is characterized with even less specific clinical presentation. The most frequent clinical manifestation of IRF is a dull pain in the lower back, abdomen or flank. Additional symptoms include malaise, anorexia, vomiting, fever, weight loss, vomiting, lower extremity oedema and testicular pain (16).

At earlier stages of IAAA and IRF there are no specific abnormalities in laboratory findings, but inflammatory markers, such as erythrocyte sedimentation rate and C-reactive protein that are elevated in the majority of patients (34). Leukocytosis is seen in isolated cases. In patients with more advanced disease and depending upon the presence of ureteral obstruction impaired renal function is present in the majority of patients while urinary sediment tends to be normal (15).

Diagnosis

Due to unspecific clinical manifestations, in the first place it is of crucial importance to "think of it". Currently, the diagnosis of IAAA/IRF mostly depends upon imaging studies. Ultrasonography can detect the presence of AAA, urinary tract obstruction or hydronephrosis (35). It can even reveal a poorly emarginated hypoechoic retroperitoneal mass which, combined with coexisting hydronephrosis, might lead to correct diagnosis of IRF.

However, it is definitely not the most optimal imaging study for the diagnosis of IAAA or IRF. CT definitely provides more complete information on IAAA than ultrasound. At CT findings, the aortic wall thickening in IAAA is usually limited to the anterior and lateral aspect of the aorta while the posterior aortic wall remains spared (37, 38). (Figure 1)

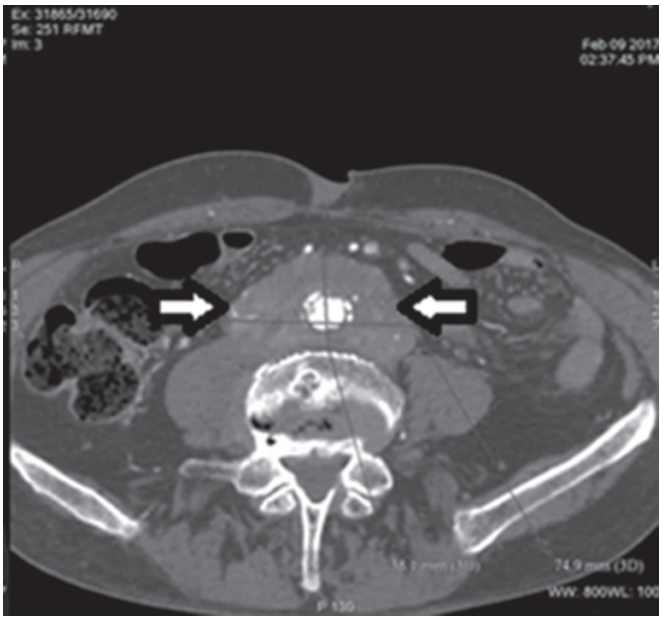

Figure 1. Computed tomography scan findings of periaortal retroperitoneal fibrosis

CT also plays a key role in diagnosis of IRF because it is characterized by certain pathognomonic findings (39). A confluent soft tissue density indicates a mass that develops and surrounds the aorta and often encircles the inferior vena cava. In 25% of the cases, a lymphadenopathy neighbouring the mass is seen (15). A contrast-enhanced CT can also visualize the degree of fibrosis and evaluate response to therapy, so it plays a very important role in elimination of other pathology (lymphadenopathy, tumors, etc).

CT-guided biopsy is highly desired in unclear cases of IRF but there is no consensus on its routine application so far. Current literature suggests taking a biopsy in subsequent settings, such as clinical, laboratory, and/or radiological findings that suggest an underlying infection or malignancy, an atypical location of the fibro-inflammatory mass, a limited experience and/or expertise regarding RF, and unresponsiveness to initial therapy.

Magnetic resonance imaging delivers findings similar to those of a CT while offering advantage of avoiding contrast (40). Several studies have proposed that a signal in T2-weighed scans is more efficient in indicating malignancies and that a better definition of the RF might be obtained (40, 41).

Positron emission tomography has a low specificity because it cannot distinguish between IRF and secondary forms of RF but it is a reliable technique to assess the metabolic or inflammatory activity of the disease and may lead to early diagnosis of relapses (42).

Differential diagnosis

The thickness of aortic wall and inflammatory rind surrounding the aorta on CT makes differential diagnosis between IAAA and non-inflammatory AAA relatively easy.

The differential diagnosis of IRF is more complex and it includes malignancies (lymphoma, sarcomas, etc.), infections such as tuberculosis, an inflammatory pseudotumor, retroperitoneal fibromatosis, Erdheim-Chester disease and virtually all other malignant and non-malignant retroperitoneal masses (43, 44). In patients who have a radiologic diagnosis of RF, causes of secondary RF should be sought: the history of radiation therapy, prior surgery such as lymphadenectomy, colectomy, or aneurysmectomy, the medication history, particularly searching for drugs associated with RF, including ergot-derivatives, methysergide, bromocriptine, beta blockers, methyldopa, hydralazine, and analgesics. Upon elimination of possible causes of secondary RF, diagnosis of primary or IRF can be established.

Whenever other pathology cannot be excluded by CT or magnetic resonance imaging (MRI), a biopsy should be performed to establish the diagnosis (45).

Treatment strategies

The main goals of treatment of CP are to stop the progression of the disease, to inhibit relapses, and to prevent

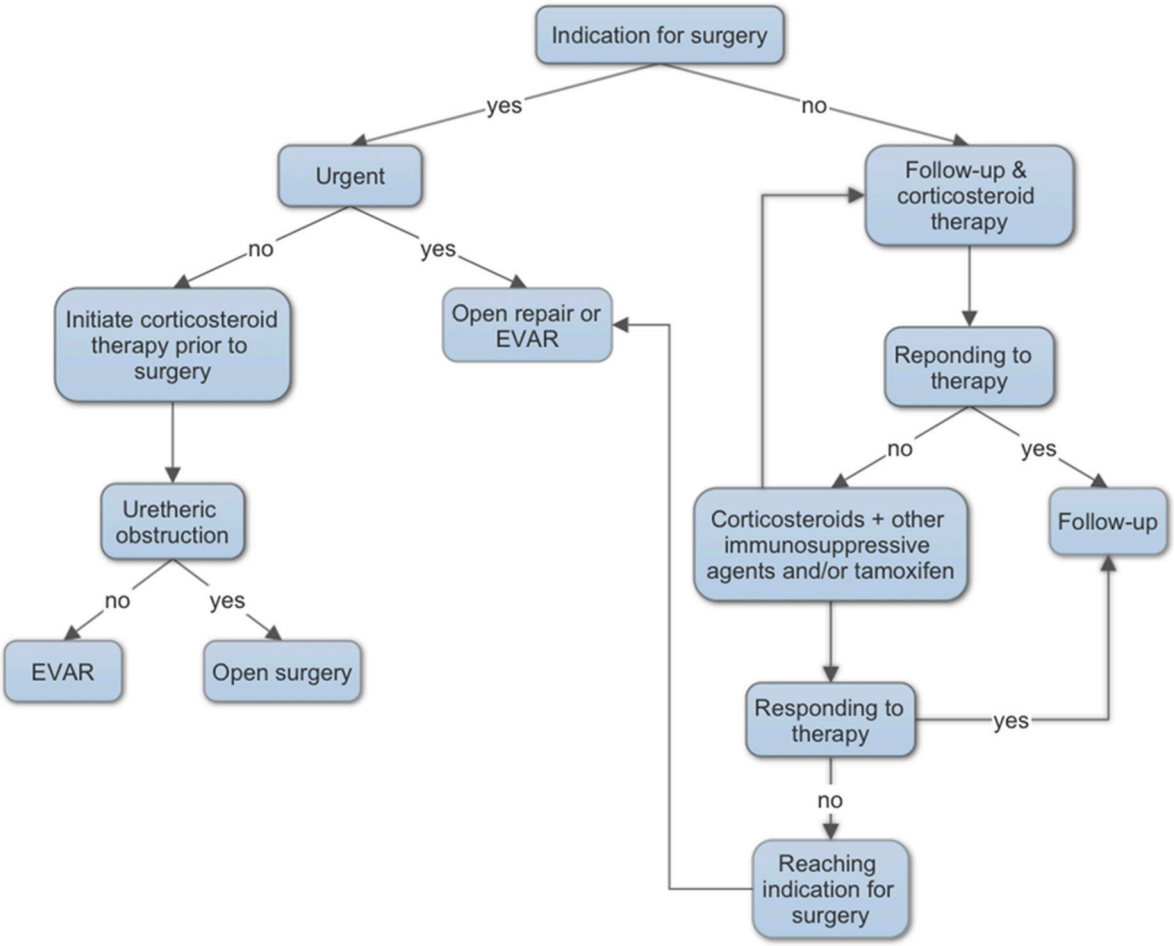

Figure 2. Treatment algorithm for patients with inflammatory abdominal aortic aneurysm and retroperitoneal fibrosis

development of complications such as urinary obstruction or aneurysm rupture. As soon as the diagnosis has been established, medical and/or surgical treatment is to be initiated. (Figure 2) Since there are no randomized trials to guide management of CP, treatment strategies are primarily based upon clinical experience obtained by case series. Most of these studies are retrospective and uncontrolled, and involve small number of patients (4, 5).

Corticosteroid treatment

Various studies have shown positive results following corticosteroid treatment in nearly 90% of patients, demonstrated by resolution of clinical symptoms, normalization of inflammatory markers, improvement of obstructive complications and significant reduction of the fibro-inflammatory mass (46-51). Patients responding to steroid treatment experience a mortality of less than 10%, which was measured over many years of follow-up and this excludes cases regarding malignancies (52, 53). These and other similar observations lead to a general agreement among clinicians that therapy with corticosteroids plays a fundamental role in treatment and represents the first-line in treatment.

In a recent review, Caspary et al also suggested that in patients with infectious condition, once the infection has been ruled out, immunosuppressant agents should be applied to all forms of inflammatory aortic disease and that corticosteroid treatment should be the primary choice (54). Although standards for the dose and duration of steroid treatment are still lacking, there is a general agreement that treatment should be initiated at a medium-high dose, which is then to be tapered under close monitoring.

IRF can be treated with prednisone 1 mg/kg per day (maximum dosage is 80 mg daily) for approximately four weeks. When improvement is detected, dosage is to be tapered over to about two or three months to 10 mg/day which is to be maintained for the following 6 to 18 months. Karder et al showed that treatment with prednisolone has led to disease remission in 9 of 11 patients with IRF (55). Initial dosage was 60 mg every second day for 8 weeks, which was tapered to 5 mg in the following 5 months.

Afterwards, Bommel et al reported satisfactory results in 75% of patients with applying prednisone 60 mg daily for 6 weeks, and then tapering it to 10 mg daily over the following two months (51). With regard to other regimens, lower or higher doses also presented positive results in curbing disease activity and preserving renal function (48, 56). While tapering the dosage, 25-50% patients are showing relapse of disease (48, 51). These relapsing patients show positive results after increasing corticosteroid therapy dosage (48).

Vaglio et al presented results in which the fibro-inflammatory mass reduces in mass to a greater extent during the first month of corticosteroid treatment, whereas in later phases it seems to stabilize (6). Serious comorbid conditions contraindicating surgery and persistence of symptoms and inflammation following surgery may be other indications for steroids (57).

The benefit from corticosteroid therapy differs among patients with CP, but predictors of responsiveness to this therapy are still lacking. Older patients and patients in whom the biopsy shows a great inflammatory component seem to experience greater benefit (58). Patients undergoing the treatment should be monitored closely by control of inflammatory markers, serum creatinine and imaging studies. Despite the lack of consensus, it seems reasonable to perform blood tests every 4-8 weeks and imaging studies every 2-4 months (59, 60). In addition, as the cessation of smoking has a positive effect on disease course, it should be an integral part of every treatment regimen (35).

Immunosuppressive therapy

An inadequate response to corticosteroids alone calls for the adding of another immunosuppressing agent. The positive effects of adding different immunosuppressants to corticoid therapy have been reported by Marcolongo et al (56). Following four to six months of no response to initial therapy with corticosteroids alone, it is preferred to add methotrexate, due to positive responses in other immunological mediated diseases that follow a chronic way of development (61). Starting dosage is 7,5 mg/week after which the dose is to be increased by 2,5 mg every month up to the moment when a significant effect is seen or the toxicity limits have been reached (maximum dose is 20 mg/week). If there is a lack of improvement within four to six weeks, a CT scan and biopsy are highly desired in IRF to verify diagnosis.

Azathioprine has been also used effectively as steroid-sparing agents/to lower the dose and in patients unresponsive to steroids alone (8, 27, 62). Cyclophosphamide may be useful in severe cases especially if vasculitis is present in the fibrotic mass (63). Scheel et al conducted a prospective study comprised of 28 patients receiving "duo-therapy" consisting of 40 mg prednisone tapered over 6 months and mycophenolate mofetil 1 g twice daily for a mean of 2 years (64). All patients showed remission of disease and normalization of creatinine and ESR levels, and 25/28 patients showed at least 25% shrinkage of the fibro-inflammatory mass.

As a last resort for corticosteroid-resistant cases, frequently relapsing disease or contraindication to steroids, tamoxifen, a selective estrogen receptor modulator, has been used in the treatment of IRF (53, 65-69). Tamoxifen has been found to be effective as a single agent and in combination with steroids. Van Bommel et al administered tamoxifen 20 mg twice daily in a group of 19 patients diagnosed with CP, of which 15 had satisfactory results defined by a decrease in CRP and ESR levels (53). Fourteen of these 15 patients showed decrease of the fibro-inflammatory mass on the CT. Vaglio et al performed an open-label randomized controlled trial comparing therapy with prednisone in comparison with tamoxifen therapy (16).

After a follow up of 18 months the relapse rates were 17% for prednisone and 50% for tamoxifen with the adverse effects more frequent in the prednisone group. However, the evidence on role of tamoxifen in treatment of IRF so far has been limited to a few positive case reports and small series of patients with observed resolution in fibrosis (63, 69-73). Further controlled studies are obviously needed to define more precisely the role of tamoxifen in treatment to CP-related disorders.

The interest in biologicals in treatment of CP related disorders has been growing over the last couple of years. Severel reports have shown positive responses resulting from the use of biologic agents. Couderc et al presented results suggesting that RF may occur alongside rheumatoid arthritis when patients are undergoing a treatment with anti-TNF-alpha, but the link between these agents and the developing disease remains unclear (74). Catanoso et al showed that infliximab led to improvement of refractory CP (75). Infliximab was given in a dosage of 5mg/kg at week 0, 2, 6, and 8 and after that weekly for the duration of 36 months. Vaglio et al presented the positive results of treatment with tocilizumab for a patient with refractory disease that was given 8 mg/kg once every four weeks for six months, which led to the improvement of symptoms and normalization of inflammatory markers (76).

Surgical treatment

Combined medical therapy along with surgery remains controversial, although scarce studies reported good results (77). When a severe ureteral obstruction is already present, immediate relief is demanded through either ureteral stenting or percutaneous nephrostomy simultaneously with application of corticosteroid therapy. Fry et al have shown that such initial conservative treatment is preferred over direct surgery (48). Wagenknecht et al, in their large retrospective series, reported a reduction of ureteral restenosis from 48% to 10% when steroids were used along with surgery in IRF (62).

The potential role of medical therapy in treatment of IAAA has been poorly analyzed (77). Even though a reduction in aneurysm diameter under steroid therapy has not been observed yet, treatment with corticosteroids

might be justified, while the aneurysm does not reach the operative criteria (4, 6, 33, 54). There are individual reports on the complete resolution of clinical symptoms and all inflammatory changes to the aortic wall and retroperitoneum under steroid therapy (33). Kardar et al. experienced regression of inflammation and fibrosis, therewith facilitating better surgical dissection (55).

As far as IAAA is concerned, the indications for surgical treatment are assessed in much the same way as in non-inflammatory aneurysms. Surgery is recommended when the aneurysm is greater than 5 cm in maximal transverse diameter, having in mind that the risk of rupture in an aneurysm measuring 5 cm is around 25% and postrupture mortality is more than 75% (78). The frequent onset of pain tends to prompt earlier surgery. Operative repair of the aneurysm is the definitive treatment for IAAA. Traditionally, open surgical repair (OSR) of IAAA was considered much more difficult than in non-inflammatory variety due to inflammatory adhesions involving adjacent organs, such as the duodenum, sigmoid colon, vena cava or ureters. Attempts to detach these structures from the aneurysm can cause damage to these organs and they initially contributed to significantly higher morbidity and mortality compared with non-inflammatory aneurysms (1, 11). Improvements in surgical techniques have led to a gradual reduction in the mortality rate, which was virtually comparable with non-IAAA in the 1990s, namely 6.8% versus 11% (35, 37, 79).

If anatomy permits, there seem to be no other obstacles to endovascular treatment of IAAA. A number of studies have shown that open and endovascular repair have similar results regarding terms of mortality and morbidity (35, 80). Puchner et al also concluded that endovascular repair of an IAAA excludes the aneurysms and leads to a decrease in size of the aneurysmal sac and the extent of periaortic fibrosis with acceptable interventional and long-term morbidity (81). In a systematic review, Paravastu et al collected data from 35 studies comprising 999 patients and 21 studies comprising 121 patients who underwent open and endovascular surgical repair (82). The authors concluded that endovascular repair is associated with a lower 1-year mortality when compared to open surgical repair. On the other hand, open surgical repair is preferred in patients diagnosed with hydronephrosis. Recently, Kakkos et al performed a retrospective study of 27 patients with an intact IAAA comparing outcome of the IAAA after OSR and endovascular aneurysm repair (EVAR) and concluded EVAR to be associated with decreased operating time, transfusion needs, hospitalization, and morbidity (83).

The effects of both open and endovascular interventions on the inflammatory component remain unclear. Resolution of periaortic fibrotic tissue after surgery for IAAA is still debated. Some investigators have reported a complete resolution while the others have noted partial regression and even persistence of the inflammatory cuff (84-86). Although Swartbol et al reported that the cytokine response seems to be lower during EVAR comparing to open surgical repair, a regression in fibrotic periaortitis is seen significantly less frequently following EVAR (37, 87).

Van Bommel et al concluded EVAR to be inferior in comparison with open repair in achieving regression of chronic periaortitis (79). It is possible that the endografts themselves are capable of triggering an inflammatory reaction in the aortic wall and directly adjacent structures thereby resulting in a new low-grade periaortic fibrosis as reported in a small number of patients (86). Thus, the indications EVAR, at least in IAAA with ureteral involvement, should be considered from a critical perspective.

Conclussion

Concomitant presence of inflammatory abdominal aortic aneurysm and retroperitoneal fibrosis generates several diagnostic and therapeutic dilemmas. Reaching an adequate diagnosis is often challenging, while the current treatment strategies for management of various presentations of chronic periaortitis are based upon "the best available evidence", mostly derived from clinical experience obtained by case series involving a small number of patients. Studies of a higher evidence level are very much needed to adjust our approach to such patients and to establish guidelines for treating this complex and serious disorder.

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TRACHEOBRONCHIAL ASPIRATION SYNDROME

SINDROM TRAHEOBRONHIJALNE ASPIRACIJE

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Summary

Aspiration–inhalation of solid or liquid materials into the airways–occurs when solids or liquids are introduced into the supraglottic area rapidly or in large volumes or when airway defences are reduced by structural abnormalities or neurological impairment. Tracheobronchial aspiration (TBA) syndrome is currently among leading causes of morbidity and mortality in the world when it comes to accidents, and it is the leading cause in one-year-old children. During the last several years, destructive effects of some food and drugs on respiratory system have been revealed. TBA may cause no complications at all or may result in a range of complications from paroxysmal cough to respiratory failure and lethal outcome. Rapid diagnosis is of utmost importance in correct interventions that should be done in a timely manner, and in prevention of complications, which are sometimes irreversible. This is a review of TBA syndrome etiopathogenesis with special reference to those caused by solid foreign bodies, including those of biological origin, hydrocarbons, lipids, and water – drowning. Special attention is given to TBA syndrome in children, and to complications such as aspiration pneumonia and pneumonitis. Current approach in treatment of the syndrome is explained in dependence of its etiology. Importance, indications and possibilities of interventional bronchoscopy are highlighted.

Keywords: Aspiration, tracheobronchial; foreign body, pneumonia; pneumonitis; bronchoscopy, interventional; criotherapy

Sažetak

Aspiracija – unošenje čvrstog ili tečnog sadržaja različitih (bio)hemijskih svojstava u disajne puteve udisanjem – događa se kad se čvrsto telo ili tečnost unesu u supraglotičnu regiju ili velike disajne puteve brzo ili u većoj količini ili u slučaju da je odbrana disajnih puteva smanjena strukturnim poremećajima ili/i neurološkim oštećenjem. Danas je sindrom traheobronhijalne aspiracije (TBA) među vodećim uzrocima morbiditeta i mortaliteta u svetu u odnosu na sve ostale zadesne slučajeve a vodeći kod dece u prvoj godini života. Poslednjih godina su rasvetljeni razorni efektni nekih životnih namirnica i lekova na respiratorni sistem. TBA ne mora da izazove ikakve komplikacija a može da dovede do čitavog spektra patoloških stanja, od paroksizmalnog kašlja do respiratorne insuficijencije i letalnog ishoda. Brza dijagnoza je od ključnog značaja u korektnom, pravovremenom zbrinjavanju i sprečavanju komplilkacija od kojih su neke ireverzibilne. U radu je dat pregled etiopagoneze sindroma TBA sa posebnom osvrtom na one izazvane aspiracijom čvrstih stranih tela, uključujući ona biljnog i životinjskog porekla, ugljovodonika, lipida i vode – utopljenja. Poseban osvrt je dat na TBA kod dece kao i na komplikacije kao što su aspiraciona pneumonija i pneumonitis. Izneseni su savremeni vidovi zbrinjavanja ovih bolesnika zavisno od etiologije sindroma. Naglašen je značaj, indikacije i mogućnosti interventne bronhoskopije u dijagnostičko-terapijskom pristupu.

Ključne reči: Aspiracija, traheobronhijalna; strano telo; pneumonija; pneumonitis; bronhoskopija, interventna; krioterapija.

Uvod

Aspiracija–unošenje čvrstog ili tečnog sadržaja različitih (bio)hemijskih svojstava u disajne puteve udisanjem–aspiracija–događa se kad se čvrsto telo ili tečnost brzo unesu u supraglotičnu regiju ili velike disajne puteve ili kad je odbrana disajnih puteva smanjena strukturnim poremećajima ili/i neurološkim oštećenjem (1-5).

Sindrom traheobronhijalne aspiracije (TBA) je danas među vodećim uzrocima morbiditeta i mortaliteta u svetu u odnosu na sve ostale zadesne slučajeve, a vodeći kod dece u prvoj godini života (2, 5). Većina od oko 80% prepoznatih slučajeva bila je kod mlađih od 15 godina. Brojne retrospektivne studije su pokazale da je ova učestalost u pedijatrijskoj praksi najveća kod mlađih od tri godine, posebno kod dečaka (2).

Etiologija i patogeneza aspiracije stranog sadržaja u disajne puteve

Sindrom TBA je najčešće izazvan aspiracijom neorganskog ili organskog stranog tela, uključujući i ona biljnog i životinjskogporekla, orofaringealnog ili gastičnog sadržaja, ugljovodonika, lipida i vode (1, 2, 6). Kad strano telo dospe u distalni deo bronhijalnog sistema a ne izazove akutnu opstrukciju, posledice ovakve aspiracije variraju u zavisnosti od prirode aspiriranog materijala. Zavisno od sastava aspiriranog sadržaja, sindrom TBA može da se javi pod slikom aspiracione pneumonije i pneumonitisa, lipoidne pneumonije, odnosno, utopljenja vodom (1).

Poslednjih godina su rasvetljeni i ranije malo poznati razorni efekti sastojaka pojedinih aspiriranih životnih namirnica i lekova na respiratorni sistem (7, 8). Lekovi u vidu tableta i pilula čine 7% svih aspiriranih stranih tela sa raznolikim posledicama u vidu opstrukcije, sistemskih i lokalnih efekata (9). Dijagnoza se postavlja

Tabela 1. Najčešće komplikacije traheobronhijalne aspiracije

AKUTNE	KASNIJE ILI HRONIČNE
• Hemoptizije • Asfiksija • Edem larinksa • Pneumotoraks • Pneumomedijastinum • Ruptura traheje/bronha • Cardiac arrestx	• Postoopstrukcijski emfizem • Atelektaza • Striktura bronha • Pneumonija • Uporni kašalj • Uporne hemoptizije • Lokalizovane bronhiektazije • Hronična postopstruktivna pneumonija • Apsces pluća • Bronho-pleuralna fistula • Traheo-ezofagusna fistula • Medijastinitis

na osnovu anamneze sa tačnim podatkom o vremenu uzimanja i vrsti leka, često uz normalan radiogram grudnog koša i u odsustvu stranog tela prilikom bronhoskopije. Organski materijali mogu da izazovu tešku lokalizovanu inflamaciju sluzokože, a granulaciono tkivo da se razvije već za nekoliko sati (5, 9).

Predmeti koji apsorbuju vodu, kao što su zrno pasulja, kukuruza i razne semenke, mogu da nabubre i od parcijalne opstrukcije dovedu do totalne. Tako, kod osoba koje su inhalirale male organske predmete, simptomi mogu potpuno da izostanu sve dok se ne desi totalna opstrukcija. Neki delovi biljaka kao što je popino prase mogu da migriraju distalno i dovedu do hronične inflamacije koja zahteva resekciju pluća. Semenke lubenice su bile najčešća aspirirana strana tela (66%) u velikoj desetogodišnjoj studiji Univerzitetske dečje klinike u Bagdadu a kiki riki u američkoj studiji u Memfisu (10, 11). Dvadesetogodišnja jordanska studija je našla da su u jednoj trećini slučajeva strana tela semenke, najviše od lubenice, orasi i orašasti plodovi pa biljke u jednoj četvrtini slučajeva (12). Prema istraživanju Klinike za otorinolaringologiju u Tuzli, tokom 50-godišnjeg perioda, u 87% slučajeva aspirirana strana tela kod dece od osam meseci do 14 godina bila su organskog porekla (13).

Poseban problem predstvljaju živa strana tela u bronhijalnom stablu (14, 15). Opisi aspiracije ribe datiraju iz 16.veka. Do nje dolazi usled navike ribara da ulov stave u usta. Pri naglom pregrizanju, riba lako sklizne u bronhijalno stablo, odakle se teško u komadićima eliminiše zbog brzo nastalog raspadanja. U savremenoj literaturi su opisi aspiracije živog stranog tela retki. Novi prikaz živih tela u bronhijalnom stablu baziran na literaturi objavljenoj na više jezika (engleski, latinski, španski, ruski, francuski, nemački, slovački i kineski) ukazuje da su to najčešće ribe, pijavice (*Hirudo medicinalis*) i crvi (*Ascaris*) (14)

Najčešći faktori rizika i stanja predispozicije za TBA su hronične teške bolesti, medicinske i stomatološke intervencije u orofaringealnoj i laringotrahealnoj zoni, izmenjeno psihičko stanje i poremećaj gutanja (1, 6, 16). Više ovih faktora može da bude prisutno istovremeno kod starih osoba.

Kako se najveći broj TBA događa bez prisustva svedoka, pravovremena dijagnoza se zasniva na visokom stepenu sumnje. Klinička slika i radiografski nalaz mogu veoma da variraju a zavise od prirode i količine aspirirarnog sadržaja ili aspiriranog predmeta. Aspiracija stranog tela ili relativno velika količina aspiriranog materijala može da izazove akutnu ili subakutnu sliku koja se kreće od zagrcnjavanja, akutne pneumonije, ugušenja zalogajem hrane i iznenadne smrti (17). Ponavljana aspiracija male količine želudačne kiseline može postupno da dovede do rekurentne pneumonije, bronhiektazija ili plućne fibroze. Vreme od aspiracije stranog tela do pojave tegoba varira. Simptomi, najčešće u vidu kašlja, mogu da se ispolje u prva 24 sata ili posle šest meseci od aspiracije (18).

Komplikacije TBA mogu da budu akutne ili hronične, odnosno, da se jave kasnije iza različito dugog perioda iza aspiracije (Tabela 1), (1, 16, 17). Sindrom TBA spada u ona stanja koja zahtevaju hitno prepoznavanje i lečenje, kako bi se ove komplikacije svele na minimum.

Aspiraciona pneumonija i pneumonitis

Aspiraciona pneumonija je zapaljenski proces na plućima nastao posle aspiracije orofaringealnog ili egzogenog sadržaja u traheobronhijalno stablo. Procenjuje se da čini oko 15% obolelih sa pneumonijom ali ova učestalost je veća kod poremećaja stanja svesti (cerebrovaskularni insult, alkoholizam, stanje posle opšte anestezije, trovanje lekovima i dr.), neuroloških oboljenja (parkinsonizam, miastenia gravis, epilepsija i dr.), disfagije, mehaničkog ometanja funkcije glotisa i ezofagealnih sfinktera (traheostoma, ishrana putem nazogastrične sonde, bronhoskopija) kao i starijih osoba sa hroničnim udruženim bolestim (16). Aspiraciona pneumonija predstavlja uzrok smrti u skoro 20 % osoba u staračkim domovima (19). Komplikacije mogu da se jave u vidu apscesa pluća, empijema pleure i bronhopleuralne fistule.

Aspiracioni pneumonitis nastaje aspiracijom sadržaja sa hemijskim dejstvom kao što su želudačni sadržaj,

ulja (mineralna ulja, petrolej, parafin, sredstava protiv opstipacije), lipidi (masnoće iz hrane). Može da dovede do teških hemijskih oštećenja traheobronhijalnog stabla. Prognoza bolesti zavisi od količine aspiriranog sadržaja. Akutni respiratorni distres sindrom (ARDS) nastaje kao teška komplikacijakoja u više od 50% dovodi do smrtnogishoda (20, 21). Veoma je važno da se posebna pažnja usmeri na preventivne mere nastanka ovih patoloških stanja, a posebno kod osoba sa navedenim visokim rizikom.

Lipoidna pneumonija i pneumonitis

Aspiracijom masnih supstanci nastaje egzogena lipoidna pneumonija, dok aspiracija ugljovodonika dovodi do akutnog hemijskog pnemonitisa, koji je vrlo sličan lipoidnoj pneumoniji. Akutna egzogena lipoidna pneumonija obično je povezana sa određenim zanimanjima ili načinom ponašanja („gutači vatre", osobe koje izvlače dizel gorivo iz rezervoara), dok je hronična posledica dugotrajnog korišćenja supstanci koje sadrže masnoće (laksativi, sjaj za usne, balzam za usne i drugo) (22-24).

Početak bolesti najčešće je neprimetan, a klinička slika nespecifična, pri čemu u simptomatologiji dominiraju kašalj i dispneja. Radiografske manifestacije su, takođe, potpuno nespecifične, tako da se nalazi kompjuterizovane tomografije grudnog koša kreću od parenhimskih infiltrata u plućima, perifernih nodula i zasenčenja po tipu mlečnog stakla, često kombinovanih sa zadebljanjem interlobarnih septi i trakastim intralobularnim zadebljanjima (eng. crazy-paving)(25). Ovakve radiografske manifestacije predstavljaju diferencijalno-dijagnostički problem, prvenstveno zbog sličnosti sa drugim bolestima pluća (pneumonija, karcinom, fibroza).

Za postavljanje dijagnoze najvažnija je dobro uzeta anamneza, posebno njen deo o izloženosti faktorima rizika. Definitivna dijagnoza se postavlja nalazom makrofaga ispunjenih mastima u sputumu, bronhoalveolarnom lavatu ili uzorku biopsije plućnog parenhima (1). Tok bolesti je u najvećem broju slučajeva povoljan sa retkim komplikacijama.

Aspiracija vode – utopljenje

Pod utopljenjem se podrazumevaju dva stanja: smrt zbog asfiksije posle potapanja u tečnostima (treći najčešći uzrok zadesne smrti u svetu) ali i stanje neposrednog preživljavanja asfiksije posle potapanja u tečnostima, sa svim eventualnim komplikacijama. Patofiziološki mehanizmi ove aspiracije nisu sasvim jasni, ali se smatra da, posle inicijalnog laringospazma, dolazi do relaksacije muskulature i prodora vode u donje disajne puteve, što je često praćeno, uz efekte dodatnih faktora (hladnoća), srčanim zastojem (1, 26-28).

Kod preživelih osoba, česte su komplikacije (hipoksijska encefalopatija, aspiraciona pneumonija, ARDS i drugo), čiji nastanak, tok i ishod zavise od trajanja i uslova potapanja, u koje spada i specifična težina vode (1, 28). Respiratorna insuficijencija je najznačajnija posledica kod preživelih utopljenika. Pa ipak, povrede drugih delova tela, posebno glave i cervikalne kičme, mogu da se očekuju i zahtevaju procenu stanja. Utopljenici mogu da se oporave bez ikakvih sekvela uz uslov da nije došlo do ireverzibilnog oštećenja usled moždane hipoksije.

Aspiracioni sindrom kod dece

Aspiracija stranog sadržaja u disajne puteve je relativno česta pojava čija ispoljavanja mogu da se kreću u spektru od potpuno neprimetnih, minimalnih respiratornih smetnji, pa do masivnih koje dovode do respiratorne insuficijencije i smrti. Deca mogu da aspiriraju različite sadržaje: u uzrastu novorođenčeta–mekonijumom kontaminiranu plodovu vodu, kasnije, u uzrastu do tri godine, sitne delove koštunjavih plodova, toksične tečnosti (organske rastvarače, insekticide), sekret iz nosa i usta, gastrički sadržaj i egzogeno kontaminirani materijal (2, 4, 5, 11-13).

Aspiracija klinički može da se ispolji znacima i simptomima opstrukcije velikih disajnih puteva (atelektaza, asfiksija, hiperinflacija, sviranje u grudima, stridor i apneja), akutnog hemijskog oštećenja (difuzni infiltrati, akutni respiratorni distres sindrom), infekcije (pneumonija, apsces pluća i empijem pleure) ili recidivnog hemijskog oštećenja (bronhitis, bronhiolitis, pneumonija, atelektaza, sviranje u grudima, hronični kašalj, apneja, laringospazam i gastroezofagusni refluks /GER/) (2, 29).

Stanja i poremećaji koji su udruženi sa aspiracionim sindromima mogu da se podele na strukturne (mikrognacija, makroglosija, rascep nepca, rascep larinksa i traheje, traheoezofagusna fistula, vaskulni prstenovi, GER, traheostoma i nazogastrička sonda), neuromišićne (izmenjena svest, poremećaj akta gutanja, cerebralna paraliza, povišen intrakranijalni pritisak, paraliza glasnica, mišićna distrofija, mijastenija gravis, Guillain-Barré sindrom, spinalna mišićna atrofija tip I) i ostale (trauma, opstrukcijska apneja u spavanju) (29).

Aspiracija stranog tela (AST) je važan uzrok morbiditeta i mortaliteta kod dece, za koji se vezuje 5% smrtnih ishoda kod dece mlađe od četiri godine. U zemljama bez razvijene politike prevencije, ovi pokazatelji su još lošiji (2, 5, 29). Aspiracija stranog tela je češća kod dečaka zbog nivoa aktivnosti i kod dece mlađe od tri godine zbog nerazvijenosti molara i neadekvatnog žvakanja.

Dijagnoza aspiracionog sindroma kod dece može da bude teška zbog toga što nedostaju dovoljno specifične analize kojima bi se uspostavila veza između aspiraci-

je i kliničkog ispoljavanja plućne bolesti. GER je često udružen sa respiratornim poremećajima, ali je aspiracija samo jedan od mogućih objašnjenja ove udruženosti. Gutanje je veoma složena funkcija i njegov poremećaj se smatra važnim uzrokom recidivnih pneumonija kod dece. I dijagnoza i lečenje recidivnih aspiracija treba da budu individualizovani za svakog bolesnika.

Sumnja na AST kod dece se postavlja na osnovu naglo nastalog zagrcnjavanja, kašlja praćenog pojavom stridora, sviranja u grudima ili oslabljenog disanja. Za odluku o bronhoskopiji presudna se sumnja postavljena posle uzimanja anamneze, a ne radiološki ili klinički nalaz koji često nemaju dovoljnu dijagnostičku specifičnost i senzitivnost (30).

Potrebno je da se rano posumnja, brzo dijagnostikuje i potpuno ukloni strano telo. Zakašnjenje u vađenju stranog tela može da ima za posledicu teške bronhopulmonalne sekvele–recidivne pneumonije i bronhiektazije. U iračkoj studiji je od 2710 dece kod kojih je u desetogodišnjem periodu postavljena sumnja na AST, to i dokazano u 84% slučajeva (10).

Terapijski pristup bolesniku sa sindromom TBA

Lečenje varira i zavisi od etiologije sindroma TBA (primer: aspirirano strano telo, želudačni sadržaj ili ugljovodonik) i kliničke slike (primer: hipoksemija ili znaci infekcije). Kod aspiracione pneumonije i pneumonitisa sa ili bez drugih komplikacija, terapiju čine antibiotici koji pokrivaju anaerobnu floru. Oksigenoterapija se primenjuje u stanjima hipoksemije. Inhalirani sadržaj treba da se odstrani posturalnom drenažom ili bronoskopijom. Posebno kod dece, lečenje recidivnih aspiracija treba da bude individualizovano.

U slučaju lipoidne pneumonije, terapijski pristup nije određen. Kortkosteroidi se primenjuju samo u pojedinim slučajevima sa progresivnim oštećenjem pluća, dok se, takođe u pojedinim slučajevima, primenjuju imunoglobulini ili bronhoalveolarna lavaža celih pluća. Ponavljana bronhoalveolarna lavaža se primenjuje i kod utopljenika iza aspiracije peska, gde radiografski pregled pomaže u dijagnozi. U slučaju potrebe, tokom bronhoalveolarne lavaže dodatno se primenjuje oksigenoterapija, koja može da bude izvedena i putem ekstrakorporalne membranske oksigenacije (31, 32).

Inicijalno lečenje utopljenika podrazumeva kardiopulmonalnu reanimaciju, parenteralnu nadoknadu tečnosti, intubaciju sa mehaničkom ventilacijom kod davljenika bez svesti, odnosno, primenu neinvazivne ventilacije kod svesnih osoba. Kod određenih davljenika primenjuje se terapijska hipotermija (33). Posebno hlađenje glave/mozga može da bude značajno u prevenciji neuroloških komplikacija. Prema dosadašnjim saznanjima, profilaktička primena antibiotika i kortikosteroida se retko preporučuju.

U pristupu bolesniku sa sindromom TBA posebno mesto zauzimaju interventne bronhoskopske procedure. Bronhoskopija ima najznačajniju ulogu i u dijagnostici i u lečenju aspiracije stranog tela. Pre intervencije, prvo je potrebno da se bronhoskopski odredi anatomska lokalizacija, vrsta i sastav aspiriranog materijala, odnos sa okolnim strukturama, prisustvo edema, fistule i/ili granulacija i na osnovu toga planira interventna proceduru, uz pažljivu procenu rizika. Koriste se i fleksibilna i rigidna bronhoskopija, nekada i kombinovano u istoj intervenciji. Rigidna bronhoskopija u opštoj anesteziji ima prednost u zbrinjavanju opstrukcije velikih disajnih puteva izazvane čvrstim ili polučvrstim stranim telima a fleksibilna u aspiraciji tečnog sadržaja ili za ekstrakciju stranih tela u segmentnim i subsegmentnim bronhima (1, 34).

Čvrsta strana tela koja ne sadrže vodu ekstrahuju se forcepsom ili posebnim instrumentima kao što je „korpica“ (eng, basket) a ako sadrže vodu, metoda izbora je krioekstrakcija (primena kriosonde sa tečnim azotom ili ugljen dioksidom, kojom se strano telo zamrzava i u celini uklanja) (35, 36). Ova metoda se često koristi za ekstrakciju koaguluma iz traheobronhijalnog stabla (37). Krioterapija granulacionog tkiva radi prevencije fibrostenoze bronha posle aspiracije je metoda koja se danas intenzivno ispituje (38). Krioekstrakcija se pokazala uspešnom i u ekstrakciji aspirirane žvakaće gume (39). Manje traheoezofagealne fistule mogu da se reše, izmedju ostalog, i postavljanjem trahealnog stenta. Poseban terapijski problem je atelektaza kod bolesnika u jedinicama intenzivne nege na mehaničkoj ventilaciji. Ako se ne sumnja na fistulu, aspiraciju stranog tela ili želudačnog sadržaja, terapijska bronhoskopija sa lavažom je u ovim slučajevima metoda drugog izbora i rezervisana je za bolesnike kod kojih nije postignut odgovor na intenzivnu fizikalnu terapiju.

Stanja iza aspiracije živih stranih tela zahtevaju dodatnu terapiju zavisno od nađenog uzročnika (antihelmintici itd) (15).

Zaključak

Traheobronhijalna aspiracija je stanje koje ne mora klinički da se ispolji a može da izazove komplikacije sa širokim spektrom patoloških poremećaja, od kojih su neki ireverzibilni a neki ugrožavaju život. Brza dijagnoza je od ključnog značaja u korektnom, pravovremenom zbrinjavanju i sprečavanju komplikacija. Interventna bronhoskopija ima značjno mesto u terapijskom pristupu TBA, uključujući i metodu krioekstrakcije. U prevenciji aspiracije stranog tela kod dece nephodno je

sprovođenje kontinuiranih programa edukacije, kao i označavanje pakovanja zrna koštunjavog voća sa upozorenjem da njihovo uzimanje može da bude opasno za decu mlađu od pet godina.

Napomena

Rad je usmeno izložen na mini simpozijumu Sindrom traheobronhijalne aspiracije, na 45. simpozijumu Stremljenja i novine u medicini, Medicinski fakultet u Beogradu, 7.12.2016. godine.

Rad je podržan od strane Ministarstva za nauku i tehnološki razvoj Republike Srbije ugovorom broj 175095.

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ANALYSIS OF THE ATTITUDE PATIENTS FROM RURAL AND PATIENTS FROM URBAN AREAS IN BOSNIA AND HERZEGOVINA HAVE TOWARDS SKIN TUMORS

ANALIZA ODNOSA PACIJENATA SEOSKE I URBANE SREDINE U BOSNI I HERCEGOVINI PREMA TUMORIMA KOŽE

Darko Lukić[1], Zoran Kričković[2], Vesna Gajanin[3], Spomenka Paurević[4], Sanja Sibinčić[5], Nenad Babić[3], Duško Ivić[6]

Summary

A prospective study, which lasted from September 2014 to October 2016, covered the subjects who were examined for various benign tumor changes on the skin. The examinees were divided into 2 groups. The first group A (92 examinees) was composed of the examinees who inhabited a rural area. The second group B (98 examinees) was consisted of examinees who lived in an urban environment. The analyzed examinees gravitate towards rural and urban areas of Banja Luka, Gradiska, Stanari, Prijedor and Teslic in Bosnia and Herzegovina.

The aim of this study is to analyze the attitude of examinees from rural and urban areas towards skin tumors. Parameters that were used for comparison of results are: personal attitude to skin tumors and previous skin examinations.

There was no statistically significant difference in terms of prejudices among the respondents: that there is no risk of skin cancer, if a person is not exposed to the sun, and/or if changes in the skin are innate, as well as in the number of examinees who said they were not afraid of a skin tumor. In group A the number of examinees that have no opinion about skin tumors is three times higher, while in group B the number of examinees who said that they had a phobia of skin cancer is three times higher.

It turned out that regular self-examination of the skin in group A is performed only by 7 (7.6%) examinees, while in group B it is done by 21 (21.5%) examinees, which proved to be statistically significantly different. Occasionally skin examination by a specialist family doctor (and/or a dermatologist) has been performed by 3 (3.3%) examinees of group A and 10 (10.3%) from group B. 4 (4.1%) examinees from group B and no one from group A perform dermoscopic examination occasionally.

It was found that regular dermoscopy inspections or skin examinations by a specialist family doctor and/or dermatologist have not been done by any examinees from both analyzed groups.

Attitude toward skin tumors of group A is more leisurely (less responsible), so for that group there is a possibility of higher risk degree for late diagnosis of malignant skin tumors.

Keywords: skin, tumors

Sažetak

Prospektivnom studijom, koja je trajala od septembra 2014. do oktobra 2016. godine, obuhvaćeni su ispitanici koji su pregledani radi različitih benignih tumorskih promjena na koži. Ispitanici su podijeljeni u 2 grupe. Prvu, grupu A (92 ispitanika), činili su ispitanici koji su nastanjeni u seoskoj sredini. Drugu, grupu B (98 ispitanika), činili su ispitanici koji su nastanjeni u gradskoj sredini. Analizirani ispitanici gravitiraju seoskim i urbanim sredinama Banja Luke, Gradiške, Stanara, Prijedora i Teslića u Bosni i Hercegovini.

Cilj rada je analiza odnosa pacijenata seoske i urbane sredine prema tumorima kože. Parametri za poređenje rezultata bili su: lični stav ispitanika prema tumorima kože i prethodni pregledi kože.

Nije pronađena statistički bitna razlika u pogledu predrasude kod ispitanika: da nema opasnosti od tumora kože, ukoliko se osoba ne izlaže suncu, i/ili ukoliko su promjene na koži prirođene, kao i u broju ispitanika koji su izjavili da se ne plaše tumora kože. U grupi A je trostruko više ispitanika koji nemaju nikakav stav o tumorima kože, dok je u grupi B tri puta više ispitanika koji su izjavili da imaju fobiju od kancera kože.

Ispostavilo se da redovan samopregled kože u grupi A izvodi svega 7 (7,6%) ispitanika, a u grupi B 21 (21,5%), što se pokazalo statistički značajno različito. Povremene preglede kože kod specijaliste porodične medicine (i/ili dermatologa) praktikuju 3(3,3%) ispitanika iz grupe A i 10(10,3%) iz grupe B. Povremeni dermoskopski pregled praktikuju 4(4,1%) ispitanika iz grupe B, a iz grupe A ni jedan.

Uočeno je da redovne dermoskopske preglede ili preglede kože kod specijaliste porodične medicine i/ili dermatologa, ne izvodi ni jedan ispitanik u obe analizirane grupe.

Odnos prema tumorima kože ispitanika grupe A je više ležeran (manje odgovoran), pa je za tu grupu moguć viši stepen rizika od kasne dijagnoze malignih tumora kože.

Ključne reči: koža, tumori

Introduction

According to their biological behavior skin tumors can be benign, precancerous and malignant. According to histogenetic origin they can be divided into keratinocyte, melanocyte, adnexal and mesenchymal (vascular, hematolymphoid, origin of muscle and connective tissue) (1).

Not all skin changes are tumors, especially not malignant ones, but it does not mean that all changes within the clinical picture should not be analyzed very subtly and seriously. The strategy of a good treatment of skin cancer has to take all possible changes in the skin into account, which is (or not) the tumor, and their repercussions on the treatment of skin diseases (2, 3).

Benign skin changes are more common than malignant, but they often worry patients and there should be serious professional considerations.

The importance of early diagnosis of skin cancer is invaluable. Since it is easy and simple to examine the skin by inspection, palpation, magnifying glass, dermoscopy etc. it is logical to assume that an early diagnosis will become dominant over time. However, despite the progress in knowledge about skin cancer the diagnosis is often delayed.

Improvements of diagnostic methods and additional efforts in the clinical, dermoscopic and PH verification of small, initial skin lesions contribute to the implementation of non-radical, limited surgical interventions. Today, most authors use optimally radical tumorectomy of non-melanocytic skin cancer (NMSC) of small diameter, when additional therapy is not necessary (4-8).

It is still an imperative to use more massive education of the population to suppress prejudices about skin tumors and favor professional and scientific truth.

The aim of the study

The aim of this study is to analyze the attitude of examinees from rural and urban areas towards tumor changes on the skin.

Materials and methods

A prospective study, which lasted from September 2014 to October 2016, covered the examinees that were examined for various benign tumor changes on the skin. The examinees were divided into 2 groups. The first group A (92 examinees) was composed of the examinees who inhabited a rural area. The second group B (98 examinees) contained the examinees who lived in the city.

Analyzed examinees gravitate towards rural and urban areas of Banja Luka, Gradiska, Stanari, Prijedor and Teslic.

The analyzed patients came to be examined by a family doctor, a dermatologist and/or plastic surgeon, or they were indirectly interviewed and referred by urologists and / or gynecologist.

The observed parameters are examinees' gender and relevant findings of cutaneous changes as a reason for coming to skin examination (Tables 1 and 2). Parameters for results comparison were personal attitude to skin tumors and previous skin examination (Tables 3 and 4).

Table 1. Gender

gender	Group A	Group B
Female	59(64,1%)	57(58,1%)
Male	33(35.9%)	41(41.9%)
Total	92(100%)	98(100%)

Table 2. Clinical finding

Clinical finding	Group A	Group B
Seborrheic keratosis	16(17,3%)	19(19,4%)
Dermatofibroma	3(3,3%)	4(4,1%)
Hemangioma	15(16,3%)	10(10,2%)
Papilloma (fibroepithelial polyp)	8(8,7%)	11(11, 2%)
Nevi	50(54,4%)	54(55,1%)
Total	92(100%)	98(100%)

Results

The research results are shown in Table 4 and Table 5. The parameters for result comparison were attitude to skin tumors and previous skin examination.

Table 3. Examinee's attitude to skin tumors

Attitude to skin tumors	Group A	Group B
There is no particular attitude to skin tumors	15(16,3%)	5(5,1%)
There is a phobia of skin cancer	9(9,8%)	28(28,5%)
Is not afraid of skin cancer	17(18,4%)	11(11.3%)
Does not expose the skin to the sun and there is no reason for concern	13(14,2%)	24(24,5%)
Changes are congenital and there is no reason for concern	24(26,1%)	26(26,5%)
Does not want to explore the skin	14(15,2%)	4(4,1%)
Total	92(100 %)	98(100%)

Table 4. Previous skin examinations

Previous skin examination	Group A	Group B
Self-Examination		
No	70(76,1%)	51(52,0%)
Occasionally	15(16,3%)	26(26,5%)
Regularly	7(7,6%)	21(21,5%)
Examination by a family doctor and/or dermatologist		
No	89(96,7%)	88(89,7 %)
Occasionally / if necessary	3(3,3%)	10(10,3%)
Regularly	0(0%)	0(0%)
Dermoscopy		
No	92(100%)	94(95,9%)
Occasionally / if necessary	0(0%)	4(4,1%)
Regularly	0(0%)	0(0%)
Total	92(100 %)	98(100 %)

Statistical analysis of the observed groups A and B is as follows: Statistical comparison analysis (Chi -Square Tests) of the parameters results of the examinee's personal opinion about skin tumors, showing that Chi-square = 24.764 df = 5 significance p = 00:00 <0.05, which is statistically significantly different.

At the same time, a statistically significant difference was observed in terms of the characteristics of "self-examination of the skin" where the Chi-square = 12.758 df = 2 significance p = 0.002 <0.01.

Discussion

The analysis included 190 examinees with benign skin tumors. Their attitude towards these tumors was analyzed. Examinees were divided according to their place of living to those who live in villages and those who live in urban areas.

Benign tumor lesions are numerous and any of them can cause concern. The reason of layman' concerns are the most often nevi and seborrheic keratosis.

Nevi are hamartomas, ie, benign formations that occur because of congenital developmental abnormalities with the appearance of malformations composed by ripe or nearly ripe structure (1). Nevi vary considerably in their clinical appearance: straight changes, mildly elevated changes (often with raised center and flat periphery), papillomatous changes, dome and stalk changes (2).

Seborrheic keratoses are benign papillomatous changes, originating from the epidermis. They are placed on the seborrheic areas of the face, chest and back. They are clearly limited, infiltrating the bases. They can be flat or slightly raised with light to dark brown velvet surface or they can be rough with keratoid caps. 1 Acanthotic keratosis is a common type of seborrheic keratoses where occasionally so-called bovenoid transformation, i.e. the creation of basal cell carcinoma (BCC) or squamous cell (PCC) carcinoma "in situ", inside of seborrheic keratosis (1, 2).

Our research has shown mainly equal distribution of benign skin changes in both groups. Nevi dominated in group A with 54.4% and in group B with 55.1% examinees. The other two skin changes are usually seborrheic keratoses (17.3% examinees from group A, and 19.4% from group B), and hemangiomas 16.3% examinees in the group A and 10.2% in the group B.

At the same time, our research has shown that the total of 20 (10.5%) examinees have no personal attitude to skin tumors. This approach to skin tumors is predominant in rural areas with 15 (16.3%) of examinees versus 5 (5.1%) of the respondents who live in the city.

Overall, in both groups there were 37 (19.4%) who had a phobia of malignant skin tumors. In the urban population there are hree times more examinees 28 (28.5%), stating that they have a phobia of skin cancer, compared to only 9 (9.8%) like-minded people who live in the village.

Overall, in both groups there were 28 (14.7%) examinees who said they were not afraid of skin cancer. By this characteristic, there are no statistically significant differences between the two groups.

Belief that there is no risk of skin cancer if the one is not exposed to the sun light, and/or that skin changes are not dangerous if they exist from birth, was found in 87 (45.7%) examinees in both groups. In Group A this prejudice is characteristic for 37 (19.4%) examinees, while in group B 50 (26.3%) examinees. In terms of these prejudices there is no significant difference in the attitude of the examinees.

The total number of examinees in both groups, which are benevolent towards skin changes and "do not want to examine their skin", is 18 (9.5%). This attitude is dominant among the examinees from rural area 14 (15.2%) versus 4 (4.1%) the examinees who live in the city. Thus, according to this characteristic it is found to be a significant difference.

The possibility of early diagnosis of suspicious skin lesions implies responsible and regular self-examination of the skin, and consulting a doctor of family medicine and if there is still any doubt, dermatologist and plastic surgeon are to be consulted as well (2-5).

Clinical diagnosis of pigmented and non-pigmented skin lesions is often not accurate (3-8). In cases where a suspicious, atypical tumor of the skin exists, it is wise to examine the suspicious lesion by computer dermoscopy. The sensibility of the digital computer dermoscopy (when it is performed by an experienced specialist) is from 84% to 96%, and specificity is from 98% to 100% (9, 10).

Dermoscopy is now widely used in the EU, USA and Australia. It is non-invasive, painless and bloodless, superficial contact microscopy, with "in vivo" structure visualization of the epidermis and dermis i.e., detection of changes that are not visible by the inspection examination or magnifying glass. This diagnosis does not imply any adverse effects, can be repeated indefinitely, regardless the age of patient (11-17).

Our testing has shown that 121 (63.6%) of examinees of both groups, generally do not perform self-examination of the skin. A regular skin self-examination in the rural population is carried out by 15 (16.3%) examinees and in the city's population by 26 (26.5%).

At the same time, the research has shown that regular self-examination of the skin in the rural population is performed only by 7 (7.6%) examinees, while in urban population it is done by 21 (21.4%), which proved to be statistically significantly different.

In the previous period, there were 3 (3.3%) of examinees from rural areas and 10 (10.3%) from the city that were examined by a doctor of family medicine and/or a dermatologist, because of skin changes, which was significantly different. Considering both groups 177 (93.1%) examinees had no earlier skin examinations on any issue.

4 (4.1%) examinees of the urban population had had a dermoscopic examination before, while examinees from rural area had not.

The observed groups are identical in terms of regular dermoscopic skin examination, or skin exam performed by a doctor of family medicine and/or dermatologist, since such no analyzed examinee had done that. Also, among the examined groups there is no statistically significant difference in terms of the number of those who do not have any kind of skin inspections of any scope or method.

By the way, the majority of both groups have never heard of computer dermoscopy. A small number of examinees learned about the procedure from media.

Conclusion

There was no statistically significant difference in terms of prejudices among the respondents: that there is no risk of skin cancer, if a person is not exposed to the sun, and / or if changes in the skin are innate, as well as in the number of examinees who said they were not afraid of skin cancer. In group A the number of examinees that have no opinion about skin tumors is three times higher, while in group B the number of examinees who said that they had a phobia of skin cancer is three times higher.

It turned out that regular self-examination of the skin in group A was performed only by 7 (7.6%) examinees, while in group B it was performed by 21 (21.5%) examinees, which is proved to be statistically significantly different. Occasionally skin examination by a specialist of family medicine (and/or a dermatologist) has been performed by 3 (3.3%) examinees from group A and 10 (10.3%) from group B. 4 (4.1%) examinees from group B and no one from group A occasionally perform dermoscopic examination.

It was found that regular dermoscopic inspections or skin examinations by a specialist of family medicine and/or dermatologist have not been performed by any examinees from both analyzed groups.

Attitude toward skin tumors of the group A is more leisurely (less responsible), so for that group there is a possibility of higher risk degree for late diagnosis of malignant skin tumors.

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DIABETIC VASCULAR DISEASE – CELLULAR AND MOLECULAR APPROACH

DIJABETSKA VASKULARNA BOLEST – ĆELIJSKI I MOLEKULSKI PRISTUP

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Summary

The term diabetes mellitus refers to a state of chronic hyperglycemia due to absolute or relative deficiency of insulin secretion with disordered metabolism of carbohydrates, lipids and proteins. More than 35 million people in Europe are diagnosed with diabetes. In 2030, it is expected that this figure will rise to 43 million. According to the International diabetes federation (IDF), more than 415 million people around the world are diagnosed with diabetes, and in 2040, IDF estimates that 642 million people will have diabetes. The metabolic syndrome (cluster of more or less related metabolic and cardiovascular derangements including visceral obesity, insulin resistance, dyslipidemia, hypertension and glucose intolerance) significantly contributes to development of diabetes mellitus type 2. This syndrome is characterized by a primary cellular defect in insulin action due to disorders in insulin signal transduction (insulin is unable to achieve its biological effects adequately). Under these conditions, insulin resistance in combination with hyperinsulinemia causes numerous metabolic and cardiovascular disorders that are a leading cause of morbidity and mortality worldwide. Thus, 65% of people with diabetes around the world die from cardiovascular disease. Besides, this serious condition is manifested by development of chronic angiopathic complications, such as microangiopathy and atherosclerosis. Atherosclerosis is a progressive, multifactorial, diffuse, multisystemic, chronic, inflammatory disease, which is manifested by disorders of vascular, immune and metabolic system. Pathogenesis of accelerated atherosclerosis in people with diabetes is not fully understood. Endothelial dysfunction is recognized as the crucial step in atherogenesis. A lot of studies have confirmed the role of dyslipidemia, hyperglycemia, oxidative stress and various mediators of inflammation in initial proatherogenic processes. After foam cell formation, mediators of inflammation initiate a series of intracellular events that include the induction of inflammatory cytokines. Thus, a vicious circle of inflammation, modification of lipoproteins and further inflammation can be maintained in the artery. Inflammatory process, matrix-degrading metalloproteinases activity, platelets aggregation and smooth muscle cells proliferation play a central role in development of fibrotic plaque. It has been shown that inflammation is closely related to the development of atherosclerotic plaque rupture. Having in mind an increase in diabetic vascular disease prevalence in future, it is necessary to take preventive actions to decrease the risk factors (inappropriate diet rich in carbohydrates and saturated fatty acids, smoking, sedentary lifestyle and physical inactivity). Apart from lifestyle changes, the usage of hypocaloric diet and increasing the level of physical activity, in patients with diabetic vascular disease, it is necessary to apply psychoeducation, as well as appropriate cognitive behavioral and medical therapy. However, although various studies related to this health problem have been carried out, scientists are still far from a complete understanding of the cellular and molecular basis of this problem.

Keywords: diabetic vascular disease, insulin resistance, atherosclerosis, inflammation

Sažetak

Šećerna bolest (dijabetes mellitus / *diabetes mellitus*) se definiše kao stanje hronične hiperglikemije nastale usled apsolutnog ili relativnog nedostatka insulina, koje dovodi do poremećaja metabolizma ugljenih hidrata, lipida i proteina. Preko 35 miliona ljudi u Evropi boluje od dijabetesa, a procenjuje se da će do 2030. godine 43 miliona ljudi oboleti od ove bolesti. Prema aktuelnim podacima Međunarodnog udruženja za dijabetes (*International diabetes federation* /*IDF*/), od dijabetesa boluje više od 415 miliona ljudi širom sveta, a očekuje se da taj broj do 2040. godine poraste na 642 miliona. Metabolički sindrom (udružena pojava visceralnog tipa gojaznosti, insulinske rezistencije, dislipidemije, hipertenzije i intolerancije glukoze) značajno doprinosi razvoju dijabetesa melitusa tip 2. Ovaj sindrom prevashodno karakteriše primarni ćelijski defekt dejstva insulina, tj. insulin usled defekta u signalnoj transdukciji nije u mogućnosti da ostvari svoje biološke efekte. U takvim uslovima, insulinska rezistencija u kombinaciji sa posledičnom hiperinsulinemijom izaziva brojne metaboličke i kardiovaskularne poremećaje koji predstavljaju vodeći uzrok morbiditeta i mortaliteta u svetu. Tako je ustanovljeno da 65% ljudi sa dijabetesom u svetu umire od kardiovaskularnih oboljenja. Osim toga, ovo teško stanje odlikuje razvoj hroničnih angiopatskih komplikacija, kao što su mikroangiopatija i ateroskleroza. Ateroskleroza je progresivna, višefaktorska, difuzna, multisistemska, hronična, zapaljenjska bolest kod koje postoji poremećaj vaskularnog, imunskog i metaboličkog sistema. Patogeneza ubrzane ateroskleroze u dijabetičara nije do kraja razjašnjena. Disfunkcija endotela ima bitnu ulogu u aterogenezi. Brojne studije su potvrdile ulogu dislipidemije, hiperglikemije, oksidacijskog stresa i različitih medijatora zapaljenja u početnim proaterogenim procesima. Nakon stvaranja penastih ćelija, medijatori zapaljenja iniciraju seriju intracelularnih događaja koji uključuju indukciju inflamacijskih citokina. Tako se može održavati začarani krug zapaljenja, modifikacije lipoproteina i daljeg zapaljenja u arteriji. Inflamacijski proces, aktivnost matriks metaloproteinaza, agregcija trombocita i proliferacija vaskularnih glatkih mišićnih ćelija imaju glavnu ulogu u razvoju fibroznog plaka. Dokazano je da je zapaljenje tesno povezano s razvojem rupture ateroskleroznog plaka. Imajući u vidu tendenciju sve većeg porasta prevalencije dijabetske vaskularne bolesti u narednim decenijama, neophodno je posebnu pažnju usmeriti na preduzimanje preventivnih mera za suzbijanje faktora rizika na koje se može uticati (neadekvatna ishrana bogata ugljenim hidratima i zasićenim masnim kiselinama, pušenje, sedentarni način života i fizička neaktivnost). Osim promene stila života, primene hipokalorijske dijete i povećanja fizičke aktivnosti, kod obolelih od dijabetske vaskularne bolesti važna je i psihoedukacija, kao i primena odgovarajuće kognitivno-bihejvioralne i medikamentne terapije. Međutim, uprkos raznim istraživanjima ovog zdravstvenog problema, naučnici su još uvek daleko od potpunog razumevanja njegove ćelijske i molekulske osnove.

Ključne reči: dijabetska vaskularna bolest, insulinska rezistencija, ateroskleroza, inflamacija

Uvod

Ateroskleroza predstavlja zaštitni, zapaljenjsko-proliferacijski odgovor usmeren prema različitim asgensima koji mogu da prouzrokuju bolest. Ako se iritacija (lezija) održava prekomerno dugo, ona postaje naglašena, odnosno postaje bolest sama po sebi.

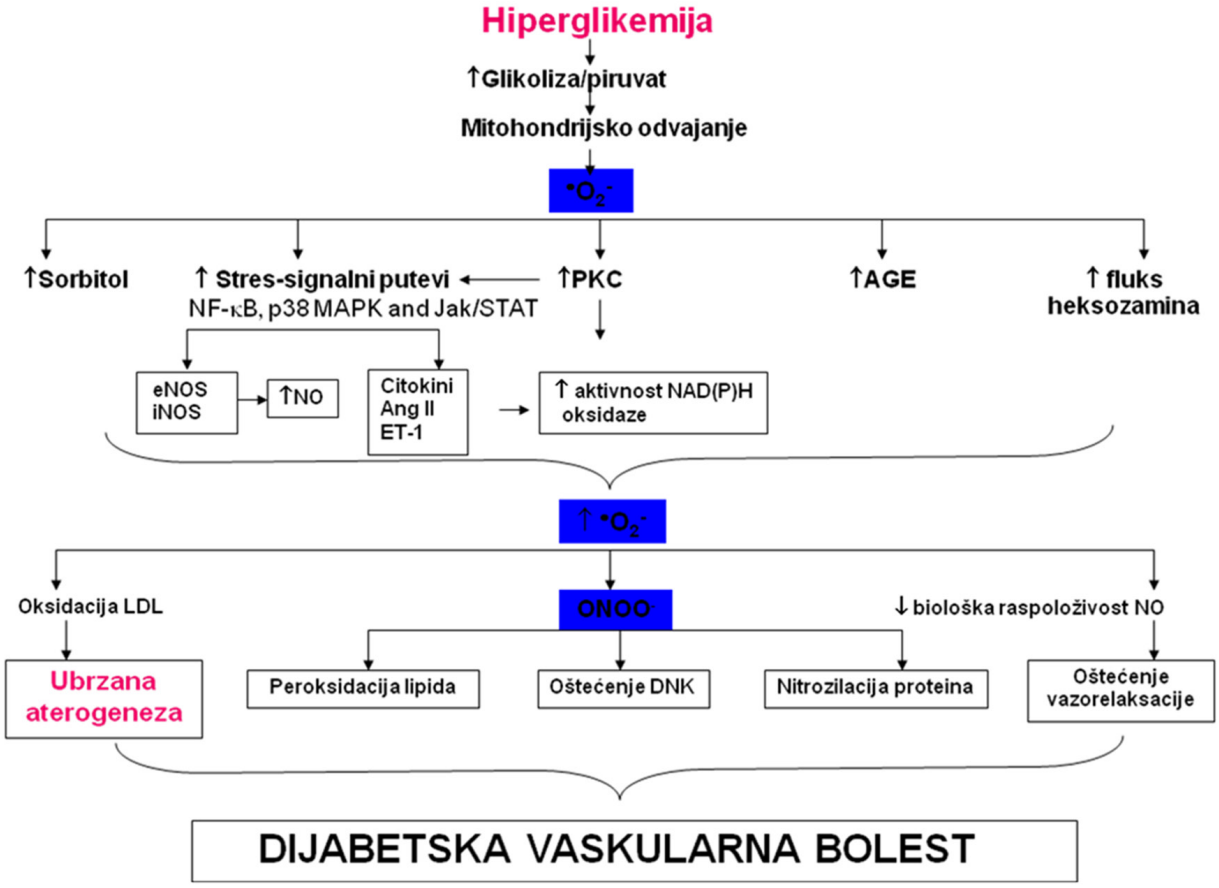
Rasel Ros (15)

Šećerna bolest (dijabetes melitus /*diabetes mellitus*/) svakih šest sekundi odnese jedan život, a dve osobe u svetu obole od ove bolesti (1). Dijabetes je oboljenje evolutivnog toka, velike učestalosti, zbog čega se u savremenoj literaturi označava kao pandemijski problem morbiditeta i mortaliteta (2). Definiše se kao stanje hronične hiperglikemije, nastale usled apsolutnog ili relativnog nedostatka insulina, koje dovodi do poremećaja metabolizma ugljenih hidrata, lipida i proteina, a kao jednu od dominantmih posledica ima razvoj hroničnih angiopatskih komplikacija u vidu mikroangiopatije i ateroskleroze (Shema 1) (3).

Dijabetes i kardiovaskularne bolesti su uzročnici više od 60% smrtnih slučajeva u svetu (4). Preko 35 miliona ljudi u Evropi boluje od dijabetesa, a procenjuje se da će do 2030. godine 43 miliona ljudi oboleti od ove bolesti (5). U Srbiji 710000 ljudi ima dijabetes, a to je ujedno i peti vodeći uzrok smrti u našoj zemlji. Čak 90% ovih pacijenata boluje od dijabetesa tip 2, a zbog blagih simptoma bolesti trećina njih i ne zna da su oboleli (6).

Prema aktuelnim podacima *Međunarodnog udruženja za dijabetes* (*International diabetes federation–IDF*), od dijabetesa boluje više od 415 miliona ljudi širom sveta, a očekuje se da taj broj do 2040. godine poraste na 642 miliona. Svake godine broj obolelih od dijabetesa tip 1 (prema prethodnoj klasifikaciji označen kao insulin zavisni dijabetes) poraste za tri procenta (7). Ovaj tip dijabetesa pogađa decu u najranijem uzrastu (8). Javlja se naglo, često i kod zdravih i vitkih ljudi. Iako još uvek nema prevencije i spada u grupu hroničnih autoimunskih bolesti, stručnjaci u samom startu savetuju insulinsku terapiju (3, 8).

Najčešća forma dijabetesa je tip 2 (prema prethodnoj klasifikaciji označen kao insulin nezavisni dijabetes), jer devetoro od deset ljudi ima upravo ovaj tip dijabetesa (9). Dijabetes tip 2 je progresivna bolest, koja sve više pogađa mlade i osobe između 35. i 64. godine. Karakteriše se dugim asimptomatskim predijabetesnim stanjem, koje može da traje godinama (3, 9). Za njegov nastanak su, pored starenja populacije, uglavnom odgovorni nepravilna ishrana, sedentarni način života i nasledna osnova (1, 3, 4, 7). Povećana prevalencija dijabetesa kod radno aktivnog stanovništva, zbog dugotrajnog toka bolesti i propratnih komplikacija višestruko povećava troškove lečenja (2, 6, 9).



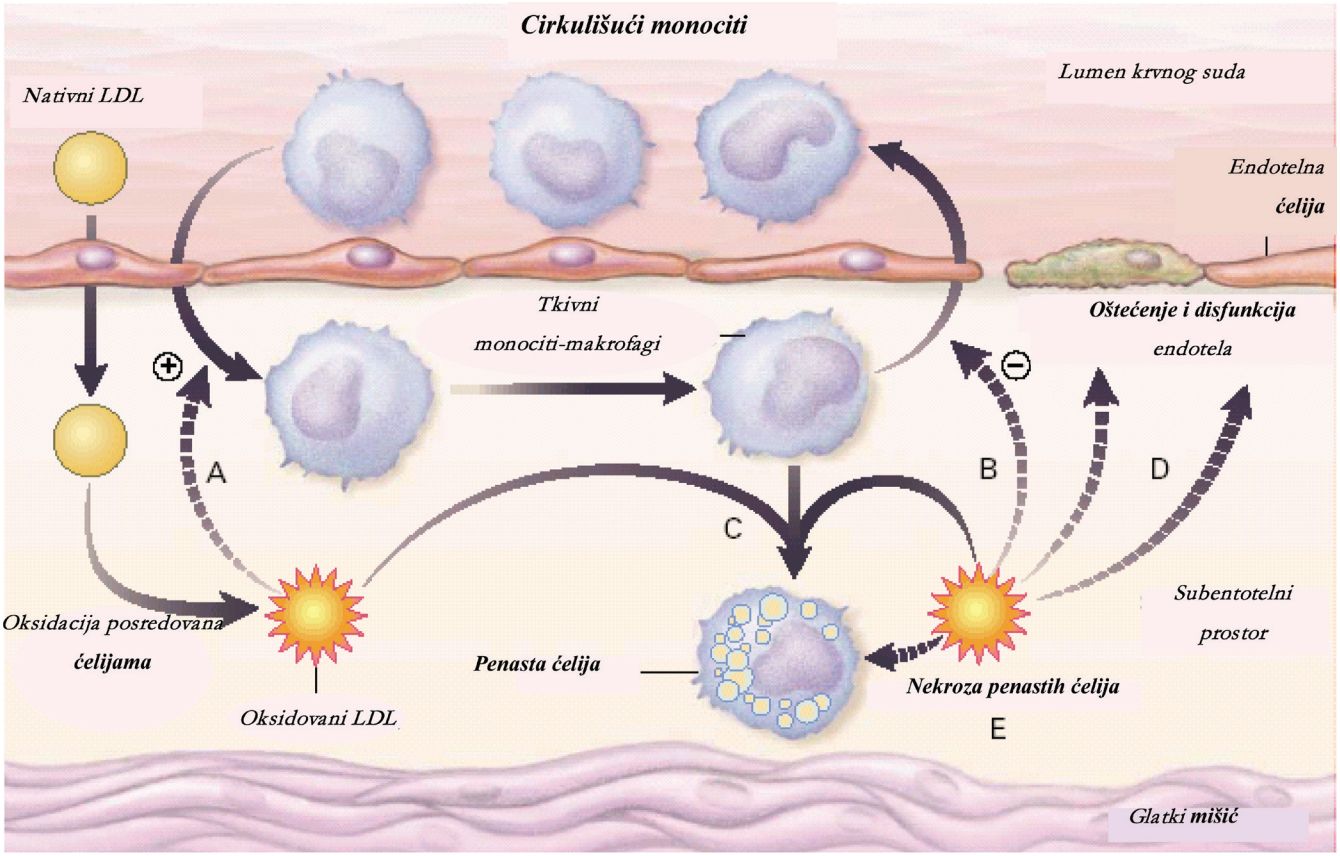
Shema 1. Dijabetska vaskularna bolest

Metabolički sindrom predstavlja udruženu pojavu intolerancije glukoze, arterijske hipertenzije, dislipidemije, centralnog (abdominalnog /visceralnog/) tipa gojaznosti, kao i postojanje drugih metaboličkih poremećaja u čijoj se osnovi nalazi insulinska rezistencija (10). Ovaj sindrom prevashodno odlikuje primarni ćelijski defekt dejstva insulina, tj. insulin usled neadekvatne signalne transdukcije nije u mogućnosti da ostvari svoju biološku ulogu (10, 11). U takvim uslovima insulinska rezistencija u kombinaciji sa posledičnom hiperinsulinemijom izaziva brojne hronične masovne nezarazne bolesti (dijabetes melitus tip 2, arterijska hipertenzija, ubrzana ateroskleroza sa svojim kardiovaskularnim i cerebrovaskularnim komplikacijama /hemodinamičke znatne stenoze krvnih sudova, fisure, rupture, hemoragije, tromboza, embolija, i dr./, nealkoholna masna bolest jetre /NAFLD, engl. *nonalcoholic fatty liver disease*/, sindrom policističnih ovarijuma i pojedine maligne bolesti /karcinom dojke, i dr./), koje imaju pandemijski karakter i predstavljaju vodeći uzrok morbiditeta i mortaliteta u svetu (11). Ovaj veoma čest skup patofizioloških poremećaja koji imaju metaboličko poreklo, označen još kao sindrom X i sindrom insulinske rezistencije, prisutan je kod približno svake četvrte odrasle osobe (12). On ujedno predstavlja faktor rizika za razvoj bolesti srca i krvnih sudova, pa se zbog toga naziva "smrtonosni kvartet" (11). Štaviše, metabolički sindrom je najviše odgovoran za razvoj kardiovaskularnih oboljenja u poslednjoj deceniji (12).

Osobe sa metaboličkim sindromom imaju dva puta veći rizik za nastanak mortaliteta usled kardiovaskularnog oboljenja i tri puta veći rizik da obole od infarkta miokarda ili moždanog udara u odnosu na zdrave osobe (11). Uz to, ove osobe imaju i višestruko veći rizik da obole od dijabetesa tip 2 (11, 13). Takođe, sve komponente metaboličkog sindroma su nezavisni uzročnici kardiovaskularnih događaja, kao što su moždani udar, kardiomiopatija, bolest koronarnih arterija, infarkt miokarda, srčana insuficijencija i iznenadna srčana smrt (11). Ustanovljeno je, takođe, da 65% ljudi sa dijabetesom u svetu umire od kardiovaskularnih oboljenja (7).

Insulinska rezistencija i ateroskleroza

Ateroskleroza je proces koji uzrokuje otvrdnuće i suženje arterija. U zavisnosti od lokalizacije, stepena suženja, anatomskih karakteristika vaskularne mreže i dužine trajanja okluzije, ateroskleroza i ishemija tkiva koju ona izaziva najčešće mogu prouzrokovati infarkt miokarda, moždani udar i perifernu vaskularnu bolest (14). Aterosklerozi se s razlogom pridaje veliki značaj na svim nivoima medicinskog istraživanja, s obzirom da njene posledice prouzrokuju smrt više od 19 miliona ljudi godišnje (4). Međutim, uprkos posebnoj pažnji koja se posvećuje proučavanju ateroskleroze, pokretač primarnog patološkog događaja još uvek je nepoznat. U tom smislu, posebno polje istraživanja predstavlja



Shema 2. Rani događaji u aterogenezi ((15) modifikivano)

inflamacija, imajući u vidu ulogu koju ona ima u svim stadijumima aterogeneze (endotelnoj disfunkciji, masnoj mrlji, tranzitornoj leziji, fibroznom plaku i komplikovanoj leziji) (14). S tim u vezi, prema savremenom poimanju, ateroskleroza se definiše kao progresivna, polifaktorska, difuzna, multiorganska, hronična zapaljenjska bolest kod koje postoji poremećaj vaskularnog, imunskog i metaboličkog sistema (14-16).

Moderan način ishrane (visok procenat šećera, masti i razni aditivi u hrani) i njen izmenjen hrononutritivan ritam čine da je ljudska populacija u zemljama u tranziciji i u ekonomski razvijenim zemljama u postprandijalnom stanju tokom celog dana (17). U postprandijalnom periodu dolazi do porasta nivoa inflamacijskih citokina, aktivacije imunskog sistema i oksidacijskog stresa (16, 17). Ova hronična aktivacija imunskog sistema, uzrokovana prekomernom ishranom, može se uočiti i pre kliničkih manifestacija gojaznosti. Pored toga, stanje hronične inflamacije predstavlja zajednički faktor u procesima ubrzane aterogeneze, insulinske rezistencije i gojaznosti. Ova inflamacija je niskog stepena i manifestuje se dvostrukim ili trostrukim porastom nivoa citokina, kao što su faktor tumorske nekroze alfa (TNFa), interleukin-6 (IL-6) i C reaktivni protein (CRP) (16). Povećan broj i kvalitativna izmenjenost ćelija masnog tkiva u gojaznosti može dovesti do hronične aktivacije imunskog sistema sa posledičnim razvojem insulinske rezistencije, metaboličkog sindroma i dijabetes melitusa tip 2 (16, 18).

Pokazano je da je gojaznost kod adolescenata i odraslih osoba povezana sa učestalom pojavom ranih aterosklerozних lezija (Shema 2) (11, 19).

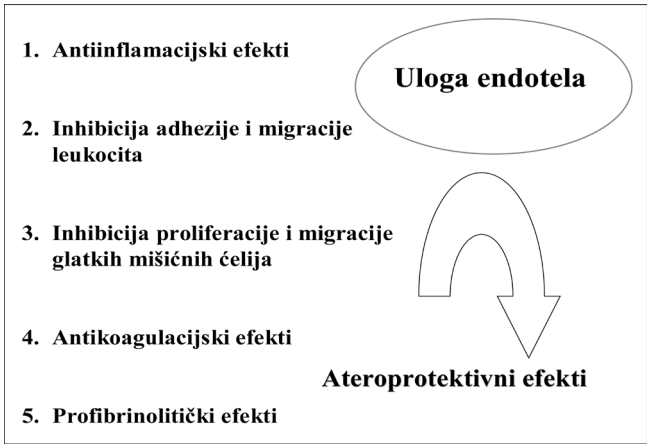
Tako je, na primer, ateroskleroza koronarnih krvnih sudova ubrzana različitim mehanizmima koji su karakteristični za stanja gojaznosti (pojačan tonus simpatičkog autonomnog nervnog sistema, naglašeno prisustvo slobodnih masnih kiselina u cirkulaciji, povećan intravaskularni volumen i napregnutost vaskularnog zida, inflamacija i promene lipoproteina koje povećavaju aterogeni potencijal, i dr.) (11). Pored toga, poznato je da povećane količine insulina kod obolelih od metaboličkog sindroma, trudnica sa gestacijskim dijabetesom i dijabetičara (bolesnika sa dijabetesom tip 2, kao i onih sa tipom 1 ove bolesti, naročito sa manifestnom mikroalbuminurijom) deluju direktno na zid krvnog suda, dovodeći do nakupljanja lipida i bubrenja medije arterijskog zida (10, 11, 18, 20). Istovremeno insulin uzrokuje proliferaciju vaskularnih glatkih mišićnih ćelija, pa na taj način deluje kao faktor rasta (11, 21). Takođe, pokazano je da se vazodilatacijsko dejstvo insulina može potpuno blokirati primenom inhibitora azot monoksid sintaze (NOS), što sugeriše da se vazodilatacijski efekat insulina dominantno ostvaruje preko azot monoksida (NO). Tako, u fiziološkim uslovima povećavanjem nivoa NO putem fosfatidil-inozitol-3 (PI3) kinazne aktivacije, insulin ublažava inflamacijsku realciju endotela, inhibirajući ekspresiju adhezivnih molekula i smanjujući aktivnost inflamacijskih citokina, što se interpretira kao njegovo antiaterogeno delovanje (11, 13). Međutim, u uslovima insulinske rezistencije stimulisan je *ras* mitogen aktivirani signalni put protein kinaze (MAP), tako da insulin ispoljava svoj aterogeni efekat (stimulacija ekstracelularne produkcije matriksa, ćelijski rast, mutacije u endotelu, i dr.). U takvim uslovima insulin stimuliše i produkciju inhibitora aktivatora plazminogena-1 (PAI-1) u vaskularnim glatkim mišićnim ćelijama, čime se inhibira fibrinoliza (13). Pokazano je, takođe, da kod osoba sa insulinskom rezistencijom postoji sinergizam između hiperinsulinemije i hiperlipidemije u odnosu na redukovanu elastičnost zida krvnog suda (11, 13, 18). Generalno posmatrano, progresija ateroskleroze kod osoba obolelih od metaboličkog sindroma i šećerne bolesti se objašnjava gubitkom insulinskih efekata koji se ostvaruju posredstvom PI3 kinaznog puta, pri čemu je očuvana stimulacija MAP kinaznog puta, koja dovodi do proliferacije vaskularnih glatkih mišićnih ćelija, povećane produkcije endotelina-1 (ET-1), kolagena, faktora rasta i proinflamacijskih citokina (10, 11, 13, 16).

Endotelna disfunkcija

Pobornici inflamacijske teorije aterogeneze ističu ulogu disfunkcije endotela u inflamaciji kao ključnog faktora u nastanku ateroskleroze (15). Prema ovom konceptu morfološki i funkcijski očuvan vaskularni endotel predstavlja dinamički autokrini i parakrini organ, koji putem svojih biološki aktivnih produkata (Tabela 1) učestvuje u modulaciji sudovnog tonusa i održavanju vaskularne homeostaze (Shema 3) (14).

Tabela 1. Najznačajnije biološki aktivne supstance koje oslobađa endotel

Vazodilatacijski i vazokonstrikcijski faktori	Azot monoksid (NO); Prostaciklin; Bradikinin; Endotelni hiperpolarišući faktor (EDRF); Angiotenzin; Endotelin; Tromboksan
Faktori rasta	Trombocitni faktor rasta (PDGF); Insulinu sličan faktor rasta (IGF); Transformišući faktor rasta (TGF); Bazni faktor rasta fibroblasta (bFGF)
Faktori koagulacije i fibrinolize	Trombomodulin; Heparin; Protein C; Protein S; Glikozaminoglikani; Tkivni aktivator plazminogena (tPA); Urokinaza; fon Vilebrandov faktor (vWF); Faktor V; Inhibitor aktivatora plazminogena (PAI); Tkivni faktor (TF)
Modulatori inflamacijskog i imunskog odgovora	Selektini; Intracelularni adhezivni molekuli (ICAMs); Vaskularni ćelijski adhezivni molekuli (VCAMs); Trombocitni adhezivni molekuli (PECAMs); Citokini; Faktor tumorske nekroze α (TNFα)



Shema 3. Uloga endotela

Nasuprot tome, u patološkim uslovima vaskularna homeostaza je narušena favorizovanjem vazokonstrikcije, tako da se arhitektonika krvnih sudova menja i omogućava nastanak morfoloških promena u njihovim zidovima, odnosno nastanak ateroskleroze (15). U tom smislu najnovija istraživanja ukazuju da morfološki normalne arterije sa funkcijski izmenjenim odgovorom endotela (endotelnom disfunkcijom), predstavljaju ciljno mesto rane ateroskleroze (Sheme 1, 2) (11, 13, 14, 16).

Primarno oštećenje endotela može biti izazvano mehaničkim (arterijska hipertenzija), metaboličkim (homocistein), endokrinim (kateholamini), imunskim, inflamacijskim i drugim činiocima (14). Na endotelu se tom prilikom povećava ekspresija specifičnih molekula, koji su odgovorni za adheziju, migraciju i akumulaciju monocita i T limfocita (14, 16).

Tako su u uslovima insulinske rezistencije prisutna početna mikrovaskularna i makrovaskularna oštećenja kojima prethodi endotelna disfunkcija. U takvim uslovima u endotelu se zapaža poremećaj produkcije NO, koji je udružen sa poremećajem ravnoteže vaskularnih faktora, naročito odnosa između NO i ET-1 (11). Zatim početno oštećenje endotela uzrokuje povećanu produkciju solubilnih vaskularnih ćelijskih adhezivnih molekula (sVCAM), intracelularnih adhezivnih molekula (ICAM, /engl. *intercellular adhesion molecules*/), ET-1, selektina, trombomodulina, fon Vilebrandovog (*von Wilebrand*) faktora, i dr., koju mnogi naučnici danas vide kao „markere endotelne aktivacije" (11, 13, 15, 16).

Adhezija mononuklearnih ćelija iz krvi (monocita i T limfocita) za vaskularne endotelne ćelije posredovana je ICAM-1 i VCAM-1 molekulima na endotelnim ćelijama i LFA-1 (leukocitni funkcijski antigen-1) i VLA-4 (engl. *very late antigen-4 molecule*) molekulima na mononuklearnim ćelijama (14, 15). S obzirom na to da se antitelima koja su usmerena protiv ICAM-1 i VCAM-1 ne sprečavaju u potpunosti adhezivni procesi, pretpostavlja se učešće i drugih molekula u ovim procesima (14).

Aktivacija monocita i T limfocita dovodi do ushodne regulacije receptora na njihovoj površini, kao što su molekuli nalik mucinu (engl. *mucin-like molecules*), koji se vezuju za selektine, integrini, koji se vezuju za adhezivne molekule iz superfamilije imunoglobulina i receptori koji se vezuju za hemotaksijske molekule. Ove ligand-receptor interakcije dalje aktiviraju mononuklearne ćelije, indukuju ćelijsku proliferaciju i pomažu u definisanju i lokalizaciji inflamacijskog odgovora na mestu lezije.

Poznato je, takođe, da adhezija monocita i T limfocita može nastati nakon povećanog ispoljavanja jednog ili više adhezivnih molekula, koji mogu delovati sinergistički sa hemotaksijskim molekulima (monocitnim proteinom hemotakse-1 /MCP-1, engl. *monocyte chemotactic protein-1*/, osteopontinom, interleukinom-8 /IL-8/ ili modifikovanim lipoproteinima male gustine /LDL, engl. *low density lipoproteins*/) (15).

Danas najveći broj naučnika smatra da je aktivacija endotela indukovana dislipidemijom inicijalni stadijum koji pokreće ateroskleroznu kaskadu (1, 11, 13-16, 18). Pokazano je da kada se površina endotelnih ćelija izloži povećanim nivoima holesterola, funkcijske promene se prvo javljaju u fokalnim područjima endotela (14). One su praćene povećanim preuzimanjem specifičnih peroksidaza i LDL molekula, čime se zatvara krug pozitivne povratne sprege i započinje aterogeneza (1, 13).

Pokazano je, takođe, da posle uvođenja dijete bogate holesterolom vrlo brzo dolazi do fokalne ekspresije VCAM-1 na predilekcionim mestima (13, 14). Pored toga, sastojak modifikovanih lipoproteina, lizofosfatidilholin, aktivira prepisivanje gena za VCAM-1 u endotelne ćelije. Lipoprotein(a), međutim, indukuje pojavu ICAM-1 na endotelnim ćelijama, dok ne utiče na ispoljavanje VCAM-1 i E-selektina. Uz to, i nativni LDL molekuli, vezujući se za LDL receptor, povećavaju koncentraciju VCAM-1 i E-selektina na humanim vaskularnim endotelnim ćelijama (14). S druge strane, ekspresiju ovih molekula izazivaju i pojačavaju TNFα i interleukin-1b (IL-1β), koji vode poreklo ili iz cirkulacije, ili iz samog vaskularnog zida (16). Ovi molekuli, koji predstavljaju deo postojeće lokalne ili sistemske inflamacijske reakcije, osim podsticanja ekspresije adhezivnih molekula, dovode do promena prokoagulacijskih i fibrinolitičkih karakteristika endotela, pri čemu površina endotela postaje trombogena (14).

Menjajući morfološke osobine endotela, ovi citokini utiču i na produkciju NO i prostaciklina i indukuju endotel na sintezu drugih citokina, čime se njihovo proinflamacijsko delovanje pojačava (16). Kao aktivatori endotelnih ćelija, IL-1 i TNFα indukuju sintezu specifičnih membranskih glikoproteina i dovode do preraspodele citoskeleta endotelnih ćelija. IL-1 i TNFα takođe stimulišu endotelne ćelije da stvaraju IL-8, koji snažno privlači i aktivira leukocite. Ulazak leukocita kroz morfološki intaktan endotel uključuje i direktnu migraciju kao odgovor na druge citokine, tzv. hemokine (14). U

ranoj fazi aterogeneze to su MCP-1, koji je lokalizovan u aterosklerozno leziji, IL-8, interleukin-16 (IL-16) i peptidni fragment komplementa (C5a), koji se oslobađa u hiperholesterolemiji (14, 16).

U novijoj literaturi se navodi mogućnost aktivacije endotela kao posledice prepoznavanja antigena na endotelnim ćelijama od strane senzibilisanih T limfocita (13, 14, 16). Od velikog značaja u mogućoj specifičnoj imunskoj reakciji je prisustvo molekula glavnog kompleksa tkivne podudarnosti (MHC–engl. *major histocompatibility complex*) klase II (molekula u okviru koga se prezentuju antigeni) i CD28 molekula (aktivacijskog kostimulacijskog molekula) na endotelnim ćelijama, zahvaljujući kojima one mogu da aktiviraju memorijske T limfocite. Poznato je, takođe, da humane endotelne ćelije podstiču T limfocite da oslobađaju interleukin-2 (IL-2) i interferon g (IFNγ), kao i da taj efekat nije posredovan preko CD28 molekula (14).

Hiperglikemija

Hiperglikemija je okidač kaskadnih mehanizama koji dovode do oštećenja endotela krvnih sudova. Ona dovodi do promene funkcije oksidacijskih sistema stvarajući u vaskulaturi milje skon oksidaciji, čime se inhibira proliferacija endotelnih ćelija, tj. ometa reparacija oštećenja endotela. Takođe, hiperglikemija povećava adhezivnost trombocita stimulišući sintezu tromboksana, a smanjujući sintezu prostaciklina (3). Osim toga, hiperglikemija povećava sintezu diacilglicerola (DAG), i na taj način intracelularno dovodi do aktivacije signalnog sistema PKC. Shodno tome, dolazi do povećane produkcije endotelnih faktora rasta, kao što su vaskularni endotelni faktor rasta (VEGF, engl. *vascular endothelial growth factor*), epidermni faktor rasta (EGF, engl. *epidermal growth factor*) i transformišući faktor rasta beta (TGFß, engl. *transforming growth factor β*), koji potom dovode do migracije i proliferacije VGMĆ (11). Uz to, prekomerna ekspresija faktora rasta, koja nastaje pod uplivom hiperglikemije, doprinosi nastanku i razvoju novih krvnih sudova (neovaskularizacija /angiogeneza/) (11, 22). Važna posledica hiperglikemije je i povećana neenzimska glikacija belančevina (proces u kojem se glukoza hemijski veže za proteine bez uticaja enzima). Pokazano je da glikacija belančevina plazme ubrzava razvoj ateroskleroze u bolesnika sa insulinskom rezistencijom i dijabetesom. Kod ovih bolesnika stabilno glicirane belančevine plazme vežu se na odgovarajuće receptore na membranama endotelnih ćelija i monocita, što zatim aktivira ove ćelije (Shema 1) (11).

Poremećaji lipida i lipoproteina

Dijabetes je često udružen s poremećajem metabolizma lipida, pri čemu mehanizam nastanka tih poremećaja zavisi od tipa dijabetesa. Kod obolelih od dijabetesa tip 1 lipidi u krvi su obično normalni kada je terapija insulinom odgovarajuća. Međutim, kada je terapija neadekvatna ili pre započinjanja terapije insulinom dolazi do pojave izrazite hipertrigliceridemije, uz relativno često istovremeno povećanje nivoa ukupnog holesterola i LDL (18). U takvim uslovima, u kojima dominira apsolutni i/ili relativni nedostatak insulina povećano je oslobađanje masnih kiselina iz adipocita, iz kojih se u jetri sintetišu trigliceridi (TG), tako da je zbog toga u hepatocitima povećano stvaranje lipoproteina vrlo male gustine (VLDL, engl. *very low density lipoproteins*) (3, 18).

S druge strane, u perifernim tkivima je zbog nedostatka insulina smanjena aktivnost lipoproteinske lipaze (LPL), a time i razgradnja VLDL, što za posledicu ima povećanje VLDL u krvi i nastanak izrazite hipertrigliceridemije (18). Pored toga, glikozilacija (ugradnja šećera u proteine) dovodi do smanjenja aktivnosti receptora za LDL (LDLR) i nagomilavanja LDL čestica u cirkulaciji (Sheme 1, 2) (3, 18). Osim toga, hiperglikemija je okidač kaskadnih mehanizama koji dovode do oslobađanja reaktivnih kiseoničkih vrsta (RKV), pa se na taj način dodatno ubrzava i olakšava aterogeneza.

Hipertrigliceridemija je takođe ispoljena u preko 50% bolesnika koji imaju dijabetes tip 2, s obzirom da rezistencija na insulin snažno stimuliše jetru na lučenje povećane količine TG i VLDL (18). Prisutna je i hiperholesterolemija, koja se dovodi u vezu sa smanjenom sposobnošću izmenjenih LDL čestica (najzastupljenija je izrazito aterogena subpopulacija LDL3) za vezivanje za specifične receptore (3, 18).

Aterogenom lipidnom profilu ovih bolesnika pridonosi i naglašeno sniženje lipoproteina velike gustine (HDL, engl. *high density lipoproteins*/) u krvi (18). Značajno je da lipoproteinski poremećaji mogu perzistirati i posle postizanja dobre metaboličke kontrole dijabetesa, tj. u fazama bolesti koje odlikuje dobra glikoregulacija. Osim toga, trajno visoki nivo TG viđa se u gojaznih dijabetičara i u onih koji imaju porodičnu formu hipertrigliceridemije (3, 18). U tim slučajevima, pored lečenja dijabetesa, potrebna je i medikamentna terapija hiperlipidemije (18).

Štetan efekat izmenjenog lipidnog i lipoproteinskog profila na endotelnu funkciju obolelih od metaboličkog sindroma i dijabetesa (hipertrigliceridemija, porast LDL i sniženje HDL dokumentovan je nizom istraživanja, koja su pokazala da je vazodilatacija krvnog suda u negativnoj korelaciji sa povišenim nivoom TG i LDL čestica, odnosno sniženim nivoom HDL partikula (3, 11). Ustanovljeno je, takođe, da hipertrigliceridemija dovodi do porasta koncentracije selektina i adhezivnih molekula (VCAM-1 i ICAM-1), kao i poremećaja permeabilnosti endotelnih ćelija (11). Uz to, LDL čestice, postaju podložne oksidacijskoj modifikaciji, inhibirajući stvaranje NO.

Istovremeno, oksidovane LDL čestice (oxLDL) aktivacijom citokina mogu da stimulišu proizvodnju solubilnih adhezivnih molekula (11, 15). Takođe, ove čestice moduliraju prostaglandin I2/tromboksan A2 (PGI2/TXA2) odnos, uzrokujući protrombogeno stanje (11). Osim toga, u stanjima hiperglikemije nastaje glikacija LDL čestica, koja zatim olakšava vezivanje ovih čestica za receptore čistače na membranama makrofaga i vaskularnih glatkih mišićnih ćelija, što doprinosi ubrzanoj aterogenezi (11, 18). Takođe, povećana koncentracija citokina koje produkuju makrofagi i vaskularne glatke mišićne ćelije potom podstiče proliferaciju endotela kapilara i time razvoj mikroangiopatije (11) (Shema 1).

Masne mrlje (žuto prebojene, ravne lezije veličine do nekoliko milimetara), koje se razvijaju na području lokalizovane endotelne disfunkcije, predstavljaju akumulacije penastih ćelija, uglavnom makrofaga bogatih lipidima, a u manjoj meri glatkih mišićnih i endotelnih ćelija (14). Inicijalni događaj u nastanku penastih ćelija, a time i masnih mrlja, izgleda da je vezan za interakciju monocita i makrofaga iz krvi i endotelnih ćelija (Shema 2) (15).

Hiperholesterolemija udružena sa povišenim nivoima aterogenih lipoproteina LDL i VLDL u krvi, vodi ka hroničnom prisustvu LDL čestica u arterijskom zidu (15, 18). Takvo stanje pogoduje stvaranju masnih mrlja, jer je ulazak lipida u subendotel veći od njihovog odstranjivanja iz arterijskog zida (14). Poznato je da nastanak penastih ćelija određuju lokalna sekrecija citokina i dostupnost modifikovanih lipoproteina, koji se vezuju za specifična mesta na površini makrofaga označena kao receptori "čistači" klase A (SRA, engl. *scavenger receptors class A*). Time se naglašava značaj lokalne inflamacijske reakcije u arterijskom zidu, jer RKV, koje se tom prilikom stvaraju, modifikuju kako lipoproteine, tako i produkciju citokina. Pokazano je da mononuklearne fagocitne ćelije još u krvi mogu fagocitovati u manjem obimu nativne, a u većoj meri izmenjene LDL čestice nakon njihovog vezivanja za SRA. Međutim, ove čestice mogu prodreti direktno u subendotel, gde podležu fagocitozi, pri čemu važnu ulogu imaju SRA. Ovi receptori, koji posreduju u preuzimanju i razgradnji modifikovanih LDL čestica, ne funkcionišu po principu negativne povratne sprege, tako da i kada se akumulira velika količina lipidnih partikula u ćeliji, ona i dalje nastavlja da ih unosi. Na taj način nastaju preko svake mere lipidima opterećene ćelije, koje su zbog svog izgleda nazvane penaste ćelije (14, 15). U ovoj fazi razvoja masnih mrlja veliki broj penastih ćelija migrira nazad u cirkulaciju.

Smatra se da taj proces ima zaštitnu ulogu u inflamacijskom odgovoru i da služi za "čišćenje" zidova krvnih sudova od naslaga lipida koji su iz cirkulacije prodrli u subendotel (15). Do progresije rasta masnih mrlja, a time i progresije procesa ateroskleroze, dolazi u uslovima kontinuirane inflamacije (14, 16). Tada LDL čestice, čija je koncentracija u krvi povećana, procesom pasivne difuzije prelaze iz cirkulacije u intimu krvnog suda, gde ih "zarobljavaju" glikozaminoglikani, a RKV izlažu svom dejstvu (14, 16). LDL čestice postaju vrlo osetljive na različite stimuluse i mogu biti modifikovane oksidacijom, glikozilacijom ili inkorporacijom u imunske komplekse (14).

U eksperimentalnom modelu ateroskleroze indukovane dijetom bogatom holesterolom monociti su prve ćelije koje se nalaze uz endotel (15). One, zatim, migriraju u subendotelni prostor, "gutaju" oksidovani holesterol i pretvaraju se u penaste ćelije (Shema 2) (14, 15).

Oksidacijska modifikacija LDL čestica, koja se odvija u dve faze, započinje konverzijom holesterol estara i fosfolipida u hidroperokside, izoprostane i aldehide kratkih lanaca. Ovakav LDL molekul se naziva minimalno modifikovan LDL, jer trpi minimalne promene na apolipoproteinu B i vezuje se za receptore koji internalizuju nativne LDL molekule. Osim toga, minimalno modifikovan LDL može stimulisati endotelne ćelije da sekretuju MCP-1 i faktor stimulacije kolonija makrofaga (M-CSF, engl. *macrophage colony stimulating factor*).

U drugoj fazi oksidacije LDL čestica dolazi do daljih strukturnih promena proteinskih komponenti molekula, uključujući modifikaciju apolipoproteina B i stvaranje lizofosfatidilholina (15). Eksperimentalno je dokazano da oksidovani lipoproteini male gustine (oxLDL) i lizofosfatidilholin mogu aktivirati PKC u endotelnim ćelijama, čime se inhibira stvaranje NO i pogoršava već postojeća endotelna disfunkcija (14, 16).

Sposobnost oxLDL molekula da indukuje akumulaciju holesterola u makrofagima bila je njegova prva opisana proaterogena osobina. Ostali proaterogeni efekti oxLDL čestica, koji se odnose na endotelne ćelije, uključuju ekspresiju faktora rasta koji deluju na vaskularne glatke mišićne ćelije, stvaranje superoksidnih anjona i apoptozu endotelnih ćelija (14).

Potvrđeno je da inflamacijski odgovor utiče na kretanje lipoproteina unutar arterijskog zida. Medijatori inflamacije (TNFα, IL-1 i M-CSF) povećavaju vezivanje LDL za endotel i vaskularne glatke mišićne ćelije. Posle vezivanja za SRA *in vitro*, modifikovana LDL čestica inicira seriju intracelularnih događaja, među kojima aktivacija urokinaze i inflamacijskih citokina ima značajnu ulogu. Na taj način, zahvaljujući prisustvu ovih lipida, u arteriji se može održavati začarani krug inflamacije, modifikacije lipoproteina i dalje inflamacije (14). S tim u vezi, LDL molekuli povećavaju ekspresiju SRA na makrofagima i tako u uslovima inflamacije olakšavaju nastanak penastih ćelija (14, 15) (Shema 2). Takođe, u nastanku penastih ćelija učestvuju i drugi receptori koji vezuju oxLDL (CD36, makrosijalin /CD68/) i HDL receptor, koji se označava kao SB-1 (11).

Pored makrofaga u stvaranju penastih ćelija učestvuju i vaskularne glatke mišićne ćelije, koje stiču osobine lipofaga. Naime, penaste ćelije luče trombocitni faktor rasta (PDGF, engl. *platelet derived growth factor*), osteopontin i druge faktore rasta i citokine koji stimulišu proliferaciju i migraciju vaskularnih glatkih mišićnih ćelija u intimu krvnog suda (14).

Oksidacijski stres

Oksidacijski stres se definiše kao povećano stvaranje oksidanasa i/ili slabljenje antioksidacijskih zaštitnih mehanizama (11). U stanjima insulinske rezistencije RKV nastaju redukcijom molekulskog kiseonika ili redukcijom vode, formirajući pri tom superoksidne anjone i hidroksilne radikale (Tabela 2) (3, 11). Takođe, NO doprinosi povećanom stvaranju RKV i formiranju reaktivnih azotnih intermedijernih jedinjenja (Tabela 3) (11).

Tabela 2. Reaktivne kiseoničke vrste

Vrsta	Ime	Poreklo
3O2	Triplet kiseonika	Stabilni atmosferski oblik
1O2	Singlet kiseonika	3O2, peroksidacija
RH	Matični molekul	RH
R*	Slobodni radikal	RH
ROO*	Peroksi radikal	R* + O2, ROOH
*O2H	Hidroperoksi radikal	*O2- + H+
* O2.-	Superoksid anjon radikal	O2 + e-
*OH.	Hidroksi radikal	H2O2, H2O
H2O2	Vodonik-peroksid	*O2-, biološko nastajanje
RO*	Alkoksi radikal	ROOH, ROOR
ROOH	Hidroperoksid	ROO, 1O2
ROOR	Peroksid	Peroksidacija
>c/O/c<	Epoksid	Nezasićen ROO* ili ROO*
ArO*	Peroksi i aroksi-radikali	Fenolski antioksidansi

Tabela 3. Biološki aktivni azotovi oksidi

Simbol	Oksidacijsko stanje	Ime	Dejstvo
NO-	+1	Nitroksil anjon	Miorelaksacijsko
N2O	+1	Azot suboksid (oksidul)	Anestetičko
NO	+2	Azot monoksid (azotoksid)	Vazodilatacijsko
NO+	+3	Nitrozil katjon (nitrozonijum)	Mutageno, RAV
NO2-	+3	Nitrit	Oksidans
N2O3	+3	Azot trioksid	Oksidans, RAV
NO2	+4	Azot dioksid	Oksidans
N2O4	+4	Azot tetroksid	RAV
ONOO-	+5	Peroksinitrit	Oksidans, antimikrobno, RAV
NO2+	+5	Nitril katjon (nitronijum)	Oksidans, RAV
NO3-	+5	Nitrat	Završni proizvod oksidacije

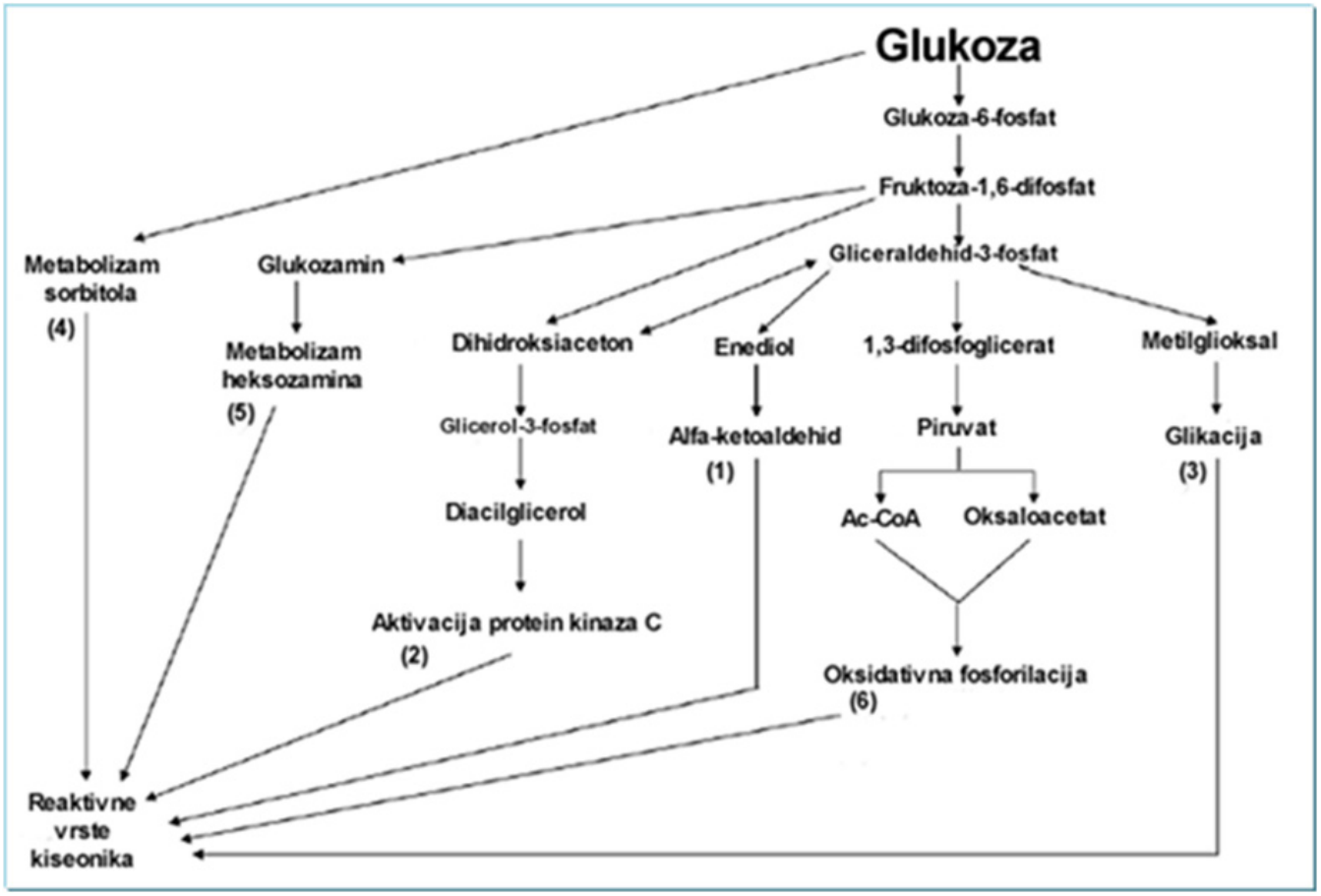
RAV-Reaktivna azotna vrsta

Osim toga, do olakšanog nastanka oksidacijskog stresa u bolesnika sa insulinskom rezistencijom dovode hiperglikemija, povišen unos slobodnih masnih kiselina, kao i izlaganje ultraviolentnom zračenju (3, 11). Štaviše, hiperglikemija predstavlja glavni faktor koji doprinosi nastanku oksidacijskog stresa u dijabetesu, jer je pokazano da stanje hiperglikemije utiče na stvaranje RKV i promenu oksidoredukcijske ravnoteže. Ove promene se događaju kroz nekoliko mehanizama (pojačan metabolizam glukoze preko poliolskog puta i povećanog stvaranja sorbitola, povećano intracelularno formiranje krajnjih produkata glikozilacije /AGE/, aktivacija PKC i povećana produkcija peroksidnih anjona u mitohondrijskom transportnom lancu elektrona) (11) (Shema 1).

U uslovima hiperglikemije postoji pojačano pretvaranje glukoze u sorbitol uz smanjivanje količine redukovanog oblika nikotinamid adenin dinukleotid fosfata (NADPH), koji je ključni kofaktor za stvaranje najvažnijeg intraćelijskog antioksidansa, a to je redukovani glutation. Aktivacijom poliolskog puta, tj. nagomilavanjem sorbitola i njegove konverzije u fruktozu, kao i povećanjem odnosa NADH/NAD, može doći do aktivacije PKC i inhibicije enzima gliceraldehid 3-fosfat-dehidrogenaze (GAPDH) (Shema 4) (11, 18).

Sledeći mehanizam doprinosa hiperglikemije oksidacijskom stresu predstavlja formiranje krajnjih produkata glikozilacije (AGE) (Shema 1). AGE nastaju kovalentnim vezivanjem aldehida i ketona redukovanih šećerom za slobodne aminogrupe proteina, formirajući Šifove (*Schiff*) baze, koje spontano prelaze u stabilnije ketoamine ("*Amadori product*"). Ova jedinjenja mogu biti direktno pretvorena u AGE ili podleći autooksidaciji i formirati karbonilna intermedijerna jedinjenja (kao i sami molekuli glukoze), koja podležu kompleksnim hemijskim reakcijama stvarajući ireverzibilne AGE strukture (11, 13). Utvrđeno je da AGE signalno pobuđuju ćelijske receptore za AGE (RAGE), koji su smešteni na površini ćelija. Ovi receptori su pored AGE zaduženi i za druge proinflamacijske molekule. RAGE se nalaze u velikom broju tkiva, a njihov broj i funkcija su naročito izraženi na mestima odvijanja ateroskleroznog procesa ili na mestima žarišta Alchajmerove bolesti u centralnom nervnom sistemu. Jedna od najvažnijih posledica vezivanja RAGE-ligand kompleksa je produkcija intracelularnih RKV preko aktivacije sistema NADPH oksidaze. Produkovane RKV potom aktiviraju put *ras*/MAPK, što dovodi do aktivacije NF-kB sa posledičnim transkripcijskim aktiviranjem RAGE i mnogih drugih genski uslovljenih produkata povezanih sa aterosklerozom (11).

Hiperglikemija dovodi do direktne ili indirektne produkcije RKV preko aktivacije puta DAG-PKC (16). Familija PKC se sastoji od više različitih izoformi, od kojih je najveći deo aktiviran lipidnim "drugim glasnikom" DAG. U uslovima hiperglikemije nastaje pojačano stvaranje dihidroksi-aceton-fosfata, koji se redukuje u glicerol-3 fosfat sa posledičnom pojačanom sintezom DAG (Shema 4). Aktivacija različitih PKC dovodi do niza



Shema 4. Stvaranje reaktivnih kiseoničkih vrsta u hiperglikemiji

promena u prenošenju ćelijskih signala. Tako je PKC-α moćni aktivator NADPH oksidaze, što posledično uzrokuje stvaranje RKV. Aktivacija PKC smanjuje produkciju NO inhibicijom endotelne NOS (eNOS) i indukuje ekspresiju VEGF, dovodeći do povećanja permeabilnosti krvnih sudova (11).

Hiperglikemija takođe može da dovede do inaktivacije antioksidacijskih proteina, kao što su bakar/cink superoksid dismutaze (Cu/Zn SOD, i dr.), smanjujući na taj način njihove antioksidacijske sposobnosti (11). Pored toga, bolesnici sa metaboličkim sindromom imaju izmenjen mitohondrijski metabolizam, skladištenje lipida, kao i povišene nivoe cirkulišućih slobodnih masnih kiselina, što sve favorizuje oksidacijsko-redukcijski disbalans i posledični razvoj oštećenja jetre, srca i krvnih sudova (13, 16). Slično tome, nedavna eksperimentalna istraživanja metaboličkog sindroma su takođe potvrdila da su srce (23) i jetra oštećeni organi i izvori poremećene oksidacijsko-redukcijske ravnoteže (24-26).

Progresija aterosklerozne lezije

Tranzitorne lezije objedinjuju karakteristike masnih mrlja i fibroznog plaka. U ovom stadijumu aterogeneze pojačava se fagocitoza oxLDL, koja dodatno aktivira monocitno-makrofagne ćelije, tako da one lučenjem mnogobrojnih citokina rasplamsavaju postojeći inflamacijski odgovor (14). U takvim uslovima dolazi do proliferacije vaskularnih glatkih mišićnih ćelija u intimi i mediji, kao i migracije ovih ćelija iz medije u intimu.

Vaskularne glatke mišićne ćelije počinju da luče PDGF i VEGF, koji stimulišu njihovo sopstveno umnožavanje, kao i sintezu velike količine proteina matriksa (glikozaminoglikana, elastina, kolagena izoforme 1 i 3), koji učestvuju u formiranju fibrozne kape ateroskleroznog plaka. Poznato je da su i bazni faktor rasta fibroblasta (bFGF, engl. *basic fibroblast growth factor*), kao i epitelni faktor rasta koji vezuje heparin (HB-EGF, engl. *heparin binding epithelial growth factor*) snažni aktivatori proliferacije i migracije vaskularnih glatkih mišićnih ćelija (14, 15). Takođe, migraciju vaskularnih glatkih mišićnih ćelija može indukovati lizofosfatidilholin, koji se akumulira u oxLDL česticama, najverovatnije u sadejstvu sa PDGF ili ET-1 (14). Na proliferaciju vaskularnih glatkih mišićnih ćelija u intimi i akumulaciju matriksa utiču i transformišući faktor rasta b (TGFβ, engl. *transforming growth factor b*), angiotenzin II i insulinu sličan faktor rasta-1 (IGF-1, engl. *insuline-like growth factor-1*). Angiotenzin II, verovatno posredstvom aktivirane protein kinaze, izaziva rast i migraciju vaskularnih glatkih mišićnih ćelija. IL-1β, interleukin-4 (IL-4) i IL-8 mogu takođe indukovati migraciju i proliferaciju vaskularnih glatkih mišićnih ćelija stimulacijom lipoksigenaznog puta metabolizma arahidonske kiseline (14, 16).

Nedostatak NO, heparan-sulfat proteoglikana i drugih inhibitora faktora rasta, koji nastaje usled oštećenja, takođe može doprineti migraciji i proliferaciji vaskularnih glatkih mišićnih ćelija. Nedavno je u kulturi vaskularnih glatkih mišićnih ćelija demonstrirana sposobnost serotonina da poveća sintezu IL-6. Ovaj multifunkcijski proinflamacijski citokin, osim što izaziva kontrakciju i proliferaciju vaskularnih glatkih mišićnih ćelija, utiče i na druge komponente tranzitorne lezije (monocite, makrofage i endotelne ćelije) indukcijom oslobađanja VEGF i MCP-1 (14). IL-6 stimuliše sintezu svih reaktanata akutne faze zapaljenja, koji imaju klinički značaj u postavljanju dijagnoze, proceni težine inflamacije i prognozi kardiovaskularnih bolesti (5, 13).

Prisustvo limfocita u ateroskleroznoj leziji pobuđuje veliko interesovanje, jer su oni najčešće pokazatelji antigenom pokrenute specifične inflamacije. S obzirom na to da su B limfociti retko zastupljeni (oko 1%), limfocite u aterosklerozним promenama praktično čine T limfociti. Faktori rasta koje produkuju T limfociti (HB-EGF i bFGF) podstiču proliferaciju vaskularnih glatkih mišićnih ćelija i olakšavaju prokoagulacijsku aktivnost makrofaga (14).

Aktivirani makrofagi eksprimiraju antigene klase II MHC, kao što je HLA-DR, koji im omogućavaju da prezentuju antigene T limfocitima. Vaskularne glatke mišićne ćelije iz aterosklerozne lezije, takođe, poseduju klasu II MHC molekula na svojoj površini, verovatno indukovanu dejstvom INFγ, i mogu prezentovati antigene T limfocitima (14). Jedan od potencijalnih antigena je oxLDL čestica, koju mogu produkovati makrofagi (11, 15). Kao indukujući antigeni spominju se, takođe, virusni antigeni i proteini toplotnog šoka (HSPs, engl. *heat shock proteins*) (14). Smatra se da kombinacija prisutnih potencijalnih antigena i medijatora zapaljenja u nastaloj mikrosredini uslovljava ćelijsku interakciju, što dalje određuje sudbinu tranzitorne lezije (11, 14, 15).

U uznapredovalim stadijumima ateroskleroze nastaje ***fibrozni plak***, koji predstavlja metabolički veoma dinamičnu tvorevinu sastavljenu iz centralnog lipidnog jezgra, fibroznog omotača i osnove sastavljene od vaskularnih glatkih mišićnih ćelija (14).

Makrofagi su stožerne ćelije u inflamacijskom procesu koji je udružen sa razvojem fibroznog plaka, pre svega zbog potenciranja inflamacijskog odgovora usled produkcije različitih inflamacijskih medijatora (15). Oni su "čistači" i antigen-prezentujuće ćelije, koje sekretuju hemokine, TNFα, IL-1 i druge citokine, faktore rasta (PDGF, TGFβ, bFGF i dr.), RKV i proteolitičke enzime (14-16). Humani makrofagi, takođe, sintetišu i sekretuju IGF-1, koji je unutar fibroznog plaka važan za monocitnu hemotaksu, aktivaciju i oslobađanje TNFα (14).

Prisustvo aktivnog imunskog odgovora u fibroznom plaku potvrđuju i aktivirani T limfociti koji sekretuju limfokine (IL-1, IL-2, TNFα, INFγ), doprinoseći, na taj način, njegovom razvoju i destabilizaciji (3, 14).

Imunohistohemijskim analizama u fibroznom plaku su identifikovane i vaskularne dendritične ćelije (VDC, engl. *vascular dendritic cells*), za koje se pretpostavlja da su odgovorne za lokalnu aktivaciju i preživljavanje limfocita. Najveći broj VDC, koje su u kontaktu sa T limfocitima, ima u zonama inflamacijskog infiltrata sa postojećom neovaskularizacijom (22). U ostalim zonama ove ćelije su u dodiru sa makrofagima. Ustanovljeno je, takođe, da se VDC nalaze u mediji ispod aterosklerozne lezije i u adventiciji oko *vasa vasorum* (14, 22). Pošto se VDC, koje su u kontaktu sa T limfocitima nalaze u dve zone, moguće je da iz intime VDC migriraju kroz mediju i adventiciju u drenirajući limfni čvor, gde prezentuju antigene nastale u plaku usled dugotrajne inflamacije, indukujući, na taj način, specifičnu reakciju (14).

Ćelije u fibroznom plaku (makrofagi, trombociti, ćelije endotela i vaskularne glatke mišićne ćelije) predstavljaju bogat izvor različitih oksidacijskih produkata metabolizma arahidonske kiseline (eikosanoida), koji kao moćni medijatori zapaljenja mogu imati značajnu ulogu u progresiji aterosklerozne lezije.

Ćelije endotela prvenstveno produkuju prostaciklin (PGI2), trombociti stvaraju TXA2 i 12-hidroksieikosatetraenoičnu kiselinu (12-HETE), dok makrofagi i vaskularne glatke mišićne ćelije sintetišu prostaglandine (PGE2 i PGI2) i monohidroksi masne kiseline (HETES) (14). Ubrzanom razvoju fibroznog plaka mogu doprineti i RKV čiji je izvor metabolička kaskada arahidonske kiseline (11). U uslovima progredirajuće hipoksije, ovi oksidansi su u stanju da na ćelijskom nivou prouzrokuju poremećaje u prenosu signala kao i poremećaje funkcije plazma membrane i membrana ćelijskih organela, čime se umanjuje sposobnost ćelijske samoregulacije (14, 22).

Vanćelijski matriks je zapreminski najznačajniji deo fibroznog plaka (14). Dominantnu ulogu u nastanku poremećaja njegove građe imaju monocitno-makrofagne ćelije, a u mnogo manjoj meri i vaskularne glatke mišićne ćelije, koje luče matriks metaloproteinaze (MMP, engl. *matrix-degrading metalloproteinases*), čime učestvuju u remodelovanju oštećene intime tokom rasta plaka (13, 14). U razgradnji matriksa ovi enzimi iz grupe endopeptidaza imaju najznačajniju ulogu. MMP (želatinaze, kolagenaze, stromelizini), koje imaju ulogu i u migraciji vaskularnih glatkih mišićnih ćelija iz medije u intimu, se obično luče u inaktivnom (zimogenom) obliku.

U uslovima hroničnog zapaljenja njihovu aktivaciju, koja se odvija izvan ćelija, stimulišu mnogobrojni agensi (fagocitoza lipida, oxLDL, oksidacijski stres, solubilni produkti aktiviranih T limfocita, IL-1, TNFα i drugi citokini) (14-16). Treba istaći da neravnoteža između faktora sinteze i razgradnje ekstraćelijskog matriksa ima

glavnu ulogu u rupturi ateroskleroznog plaka i njenim kasnijim posledicama (14).

Patofiziološku osnovu nastanka ***komplikovanih aterogenih plakova*** čini fisura ili ruptura fibroznog sloja koja nastaje zbog smanjene sinteze i povećane razgradnje matriksa (14, 15).

Najčešći vid rupture je pucanje fibrozne kape sa posledičnom trombozom na mestu kontakta krvi sa sadržajem plaka (15).

Plakovi koji su skloni rupturi imaju tanku fibroznu kapu u kojoj se nalazi veliki broj inflamacijskih ćelija i lipidni centralni deo, koji zauzima više od 50% zapremine plaka. Predilekciono mesto za rupturu plaka je rubni region, gde je fibrozna kapa najtanja, koncentracija penastih ćelija najviša i pritisak lipidne srži najveći (14).

Poznato je da je zapaljenje blisko povezano sa rupturom ateromatozne ploče. U humanom ateromu uočeno je prisustvo aktiviranih T limfocita, koji su prisutni u većem broju zajedno sa makrofagima na mestu rupture. Postoje dokazi da hronična imunska stimulacija unutar ateroma vodi produkciji INFγ iz T limfocita, koji inhibira sintezu kolagena u vulnerabilnom regionu fibrozne kape (14, 16). Produkcija INFγ se takođe povezuje sa aktivacijom makrofagnih funkcija, koje su odgovorne za vulnerabilnost plaka (15).

Pokazano je da vaskularne glatke mišićne ćelije u ateroskleroznom plaku mogu eksprimirati na svojoj površini fas receptor, koji je neophodan za pokretanje procesa apoptoze, vezivanjem za fas ligand smešten na membrani pojedinih T ćelija (14). U daljoj evoluciji plaka makrofagi i vaskularne glatke mišićne ćelije podležu apoptozi, naročito u prisustvu visokih koncentracija oxLDL molekula. Uz to, makrofagi su sposobni da razgrade ekstracelularni matriks fagocitozom ili sekrecijom aktivatora plazminogena i MMP, koji mogu oslabiti fibroznu kapu, predisponirajući je za rupturu (14, 15).

Aktivirani mastociti, koji su prisutni u malom broju u rubnom regionu plaka, mogu da sekretuju moćne proteolitičke enzime, kao što su triptaze i himaze, koje učestvuju u aktivaciji pro-MMP (14-16). Neutrofili su takođe sposobni da razaraju tkivo sekrecijom proteolitičkih enzima. Pored toga, dosadašnja saznanja jasno ukazuju da interakcije između neutrofila i T limfocita, koje se ostvaruju putem citokina ili njihovim međusobnim kontaktom doprinose olakšanom odvijanju inflamacije i slabljenju vezivne kape (Tabela 4) (14). Vezivna kapa takođe može da bude oslabljena pritiskom i istezanjem zbog naglog porasta ateromskog jezgra ili krvavljenja u ploču (15).

Terapijski pristup

Imajući u vidu tendenciju sve većeg porasta prevalencije dijabetske vaskularne bolesti u narednim decenijama, neophodno je posebnu pažnju usmeriti na preduzimanje preventivnih mera za suzbijanje faktora rizika na koje se može uticati (neadekvatna ishrana bogata ugljenim hidratima i zasićenim masnim kiselinama, pušenje, sedentarni način života i fizička neaktivnost) (1-4, 13, 27-29). Osim promene stila života, primene hipokalorijske dijete i povećanja fizičke aktivnosti (preporučuje se svakodnevno praktikovanje pola sata bržeg hodanja ili plivanja, odnosno vožnje bicikla), važna je i primena odgovarajuće medikamentne terapije (3, 5, 7, 13).

Tabela 4. Interakcije između neutrofila i T limfocita u hroničnom inflamacijskom odgovoru

Citokini koje stvaraju neutrofili	***Efekat na T limfocite***
Hemokini: CXCL1, CXCL5, CXCL6, CXCL7, CXCL8, IL-8	Hemoatraktanti T limfocita
IL-4	Th2 ćelijska diferencijacija
IL-12	Th1 ćelijska diferencijacija
TNFα	Kostimulator T ćelijske aktivacije
IL-10	Inhibicija T ćelijske proliferacije i aktivacije
IL-6	Doprinos Th2 i Th17 ćelijskoj diferencijaciji
TGFβ	Inhibicija T ćelijske proliferacije
Citokini koje stvaraju T limfociti	***Efekat na neutrofile***
IFNg	Produženje životnog veka
	De novo sinteza molekula MHC II klase
	De novo sinteza CD80 i CD86 molekula
	Pojačana aktivnost fagocita
IL-2	Aktivacija neutrofila
TNFα	Ushodna regulacija ekspresije CD83 molekula
	Produženje životnog veka
	Degranulacija
	De novo sinteza proteina
Kontakt između neutrofila i T limfocita	***Efekat na neutrofile***
	Stvaranje RKV
	Ushodna regulacija MHC II klase i CD69
	Efekat na T ćelije
	Indukcija sinteze IFN

Tabela 5. Glavne karakteristike procene distresa i depresije u dijabetičara

Procena vlastitih osećanja i iskustava u periodu dužem od dve nedelje
Disfunkcija vegetativnog nervnog sistema (poremećaji spavanja, apetita i energetskog bilansa)
Izmenjeno emocionalno reagovanje (manjak životne energije i smanjeno interesovanje za svakodnevne aktivnosti)
Poremećaji ponašanja (uznemirenost i hipodinamija)
Kognitivni simptomi (slabije pamćenje ili gubitak koncentracije); osim toga, mogu da se jave osećanje krivice i autodestruktivne misli

Tabela 6. Kognitivne komponente kognitivno-bihejvioralne terapije dijabetesa

Usvajanje ideje da napetost i potiskivanje negativnih emocija mogu da izazovu simptome bolesti
Razumevanje povezanosti misli i osećanja
Prepoznavanje i eliminisanje „grešaka u razmišljanju“ (npr., „razmišljanje po obrascu sve ili ništa“, personalizacija, preuveličavanje, minimalizovanje, itd.)
Analiza negativnih misli i njihova zamena svrsishodnijim mislima postavljenjem sebi sledećih pitanja: Šta ne mogu ili neću da progutam? Da li se jedem u sebi? Kako postupam sa svojim osećanjima? Zbog čega sam ljut (ljuta)? Kako postupam sa svojom agresivnošću? U kojoj meri izbegavam sukobe? Postoji li u meni potisnuta čežnja za rajem detinjstva lišenim sukoba, u kojem bih bio (bila) samo voljen (voljena), zbrinut (zbrinuta) i zaštićen (zaštićena), a ne bih morao (morala) da se zubima i noktima probijam kroz život?

Psihijatrijski poremećaji (depresija i anksiozna stanja) se češće javljaju u dijabetičara u odnosu na opštu populaciju. Pored toga, ustanovljeno je i da osobe koje pate od depresije, bipolarnih psihoza i shizofrenije imaju veći rizik da obole od dijabetesa u poređenju sa opštom populacijom. Stoga svi dijabetičari treba da ispitaju prisustvo simptoma depresije i anksioznih stanja (Tabela 5) (30). Poznato je da se emocije o kojima se nerado govori (veliki emocionalni lomovi), ili se ne razumeju na pravilan način, često izraze na telesnom planu (somatizacija). Zato je veoma važno da ljudi skloni somatizaciji nauče kako da neprijatne emocije prihvate, razumeju i izraze kroz motorno, verbalno ili socijalno ponašanje (30-32).

U Tabeli 6 navedene su kognitivne komponente kognitivno-bihejvioralne terapije koje se smatraju pogodnim za primenu kod dijabetičara (30, 31). Psihoterapijski rad se najviše bazira na povećanju kapaciteta dijabetičara da razume i obradi svoja osećanja, a ne da ih direktno prazni kroz telo (30). Kroz odnos koji se razvija s psihoterapeutom i povezivanje telesnih smetnji i psihičkog stanja, kao i kroz ispoljavanje i prihvatanje emocija u sigurnoj sredini, simptomi se smanjuju ili potpuno nestaju, i postiže se psihološki i telesni oporavak (30, 32).

Zaključak

Kod pacijenata sa dijabetesom tip 2 rizik za razvoj kardiovaskularnih bolesti je izuzetno veliki. Ateroskleroza, odnosno oštećenja velikih krvnih sudova srca i oboljenja koja nastaju zbog oštećenja samog srčanog mišića najčešće su kardiovaskularne bolesti kod dijabetičara. Zato bi pacijenta sa dijabetesom u startu trebalo lečiti kao kardiovaskularnog bolesnika. Uz to, potrebna su i dalja istraživanja molekulskih mehanizama dijabeteske vaskularne bolesti, kako bi se sprečio njen nastanak, odnosno pronašao efikasniji terapijski pristup.

Napomena

Ovaj rad je finansiran sredstvima iz projekta broj 175015 Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije.

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DISABILITY MILESTONES IN HUNTINGTON DISEASE
MILJOKAZI PROGRESIJE HUNTINGTONOVE BOLESTI

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Summary

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease which is characterized by the presence of involuntary choreatic movements, cognitive impoverishment and behavioral disorders.

The objectives of this research were: analysis of the clinical specifics of people with HD and definition of chronology in the occurrence of milestone disease progression.

56 patients with HD were interviewed by telephone about their age, family history, age at onset of the disease, first symptoms, current symptoms and their beginning. Also, the number of trinucleotide (CAG) repeats was determined by PCR method.

In group of 50 alive patients the average age was 54.5 years, age at the beginning of disease 46.7 years, disease duration 7.8 years and latency to diagnosis 2 years, approximately. Family history was positive for 22 patients. Most frequently first symptoms were involuntary movements as isolated symptom (24 patients). In a progression of the disease swallowing difficulty and choking were first problem in everyday life for patients and they occurred 4.5 years after the first symptoms. Talking difficulty, falls, dependence on the assistance of another person during bathing and eating and walking difficulty happened after 4.6, 5.2, 6.2, 7.5 and 7.5 years, respectively. The average number of trinucleotide repeats was 43.8, in range from 40 to 51 repeats.

HD in short term leads to significant disability with the emergence of problems with swallowing, speaking and walking, leading thus to complete depending on the physical assistance of another person.

Keywords: Huntington disease, progression, chorea, trinucleotide repeats

Sažetak

Huntingtonova bolest je autozomno dominantna neurodegenerativna bolest koja se karakteriše nevoljnim pokretima, kognitivnim osiromašenjem i poremećajem ponašanja.

Ciljevi istraživanja su analiza kliničke specifičnosti pacijenata sa Hantingtonovom bolesti i definisanje hronologije u razvoju miljokaza progresije bolesti.

Pedeset i šest pacijenata je intervjuisano telefonski i dobijeni su podaci o njihovoj starosti, porodičnoj anamnezi, starosti na početku bolesti, prvim simptomima i trenutnim simptomima kao i o trenutku njihovog nastanka**.** Takođe, broj trinukleotidnih CAG ponovaka je ispitan PCR metodom.

U našoj grupi ispitanika od 50 živih pacijenata prosečna starost je iznosila 54,5 godina, starost na početku bolesti 46,7 godina, trajanje bolesti 7,8 godina a latenca do postavljanja dijagnoze 2 godine. Porodična anamneza je bila pozitivna za 22 bolesnika. Najčešći prvi symptom bili su nevoljni pokreti kao izolovan symptom (24 bolesnika). U daljoj progresiji bolesti problem sa gutanjem i zagricnjavanje su bili prvi problem koji je otežavao bolesnicima svakodnevni život i pojavio se u proseku za 4,5 godina posle prvog simptoma. Problemi sa gutanjem, padanje, zavisnost od drugih osoba prilikom kupanja i jela, otežano hodanje desili su se posle 4,6; 5,2; 6,2; 7,5 i 7,5 godina, redom. Broj trinukleotidnih ponovaka se kretao od 40 do 51, u proseku 43.8.

Hantingtonova bolest u kratkom vremenskom period dovodi do značajne onesposobljenosti sa nastankom problema pri gutanju, pričanju i hodanju, dovodeći potom do potpune zavisnosti od fizičke pomoći druge osobe**.**

Ključne reči: Hantingtonova bolest, horea, pregoresija, trinukleotidni ponovci

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease which is characterized by the presence of involuntary choreatic movements, cognitive impoverishment and behavioral disorders (1).

The prevalence of HD is 3 -7 on 100 000 residents in Western Europe. It rarely appears in Japan, China, Finland and Africa, so the frequency of HD in Japan is from 0.1 to 0.38 per 100 000 inhabitants, while the prevalence in some populations of Western European origin exceeds 15 per 100 000 (2). Due to the isolation of the population frequency of HD on Lake Marakoibo in Venezuela is 700 per 100 000 (3).

Choreatic movements, by which it got its name, are typical for the disease but dystonia can also be recorded, later incoordination of movements with immobility, bradykinesia and rigidity in the final stages of the disease. These symptoms are associated with a damage of cognitive functions such as attention and memory, as well as psychiatric symptoms, including personality changes, depression, psychosis and dementia that often precede motor symptoms. Other symptoms, including weight loss, muscle atrophy, cardiac dysfunction, and testicular atrophy, as well as endocrine abnormalities that become evident with a progression of the disease (4).

The gene responsible for HD is located on 4p16.3 locus. In that gene multiple repetitions of the CAG triplet nu-

cleotides (cytosine, adenine, guanine) occur, encoding implantation of the amino acid glutamine in the protein called hantingtin (5). Forty and more repetitions are associated with the expression of the disease. If there are 36 to 39 repeats, penetrance of mutation is reduced, so some carriers will manifest the disease, and some will not (6).

The brain in the final stage of the disease is 400g lighter compared to the average weight of 1300-1400g (7,8). This atrophy is the result of an extensive atrophy in the caudate nucleus and in putamen, but it is also a result of a serious neuron loss in deeper layers of the cerebral cortex (9). The hippocampus and the thalamus are also affected, but cerebellum significantly less. White mass, including the corpus callosum is hypotrophic (10). The loss of cortical neurons leads to cognitive and behavioral problems (11).

In the treatment of HD tetrabenazine is used and it lowers vesicular depots of catecholamine, a drug that suppresses chorea and is associated with global clinical improvement (12). Neuroleptic drugs which block the postsynaptic dopamine receptors, fluphenazine (13), and haloperidol (14) can be effective in the suppression of chorea. It is considered that taking tricyclic antidepressants and serotonin reuptake inhibitors can improve the condition of HD patients (15).

Aim

The objectives of our research were:
1. Analysis of the clinical specifics of people with HD
2. Definition of chronology in the occurrence of milestone disease progression

Materials and methods

There were 56 patients who participated in the research all of whom were diagnosed with Huntington's disease through the neurological examination at the Neurology Clinic of the Clinical Center of Serbia. The diagnosis was based on clinical findings, positive family history, and confirmed with the analysis of trinucleotid repeats in IT 15 gene.

Patients were informed of the study over the telephone when they were asked if either them or the members of their family could answer the questions from an open questionnaire. The questionnaire collected data on the patient's age, family history related to HD, the age of the patient at the time of diagnosis and at the time of occurrence of the first symptoms. The patients were also questioned about the type of their first symptoms. Further, patients and their family members gave information about the current clinical picture and about the problems associated with speaking, swallowing and chewing, walking, falling, mood, sleeping, appetite and weight changes. It is also tested whether the patient can independently feed, bath, go out of the house, do the housework and if he/she uses diapers, catheter or wheelchairs. Apart from all these symptoms, data were also given about when the symptoms first appeared, in relation to the occurrence of the first symptoms.

After data collection, answers from the patients or their family members were grouped and analyzed statistically.

In addition to the questionnaire, in the research we have also covered data on the number of trinucleotide CAG repeats by PCR method. Blood samples were taken from all patients to determine the number of CAG repeats in a known manner (16). Briefly, genomic DNA was isolated from peripheral blood samples using standard procedures which including proteinase K and phenol chloroform insulation. Polymerase chain reaction (PCR) was performed with the primers: HC-1 (5'-ATG GCC TTC AAG TCC CTC AAG GAG TCC TCC3 ') and HC-2 (5'-CAG CAG CTG CGG TGCBCTG-3'). The amplification was performed in 25-ml reaction mixture which contained 1 X PCR buffer (Pharmacia), 200 mM each dNTPs, 1.5 mM Mg2Cl, 100 ng of each primer, 5% glycerol (final concentration), 1 U of Taq polymerase (Pharmacia) and 200 ng of genomic DNA. Terms of the cycle were: 3 min. Initial denaturation at 97 ° C, followed with 35 cycles of 1 min at 95 ° C, 1 min at 60 ° C and 2 min at 72 ° C, and the final 10 min at 72 ° C. Samples were first analyzed on 2% agarose gel. For determination of the precise number of CAG repeats part of each reaction (7 mL) was mixed with 3 ml of buffer (0.25% bromophenol blue, 0.25% xylene cianol in 100% formamide) and denatured with heat at 100 ° C for 10 min before adding to 6% poliakrilamid- urea denaturing gel. Samples were electrophoresis in 0.5 X TBE buffer. The product of amplification was detected by silver staining using the standard procedure. In our laboratory the genes which contain at least 40 repeats confirm the diagnosis of HD.

Results

The research included 56 patients with the diagnosis of Huntington's chorea confirmed also by genetic testing. Of the contacted patients ten have died, and for the six of the dead families were not willing to give any information. There were 19 men and 37 women. At the time of the telephone interviews, patients were aged 54.45 ± 9.61 (25-74) years, and at the time when the first symptoms appeared they were 46.69 ± 10.37 (23-65) years. We did not get the data on family history from 10 patients, whose families have not been informed of any relatives with the same disease. For 11 patients family history was allegedly negative with frequent data of early deaths of one or both parents, for 7 it was insecure

Table 1. Clinical and demographic specifics of patients with Huntington's disease

Male/Female	19:37
Age*	54.45±9.61 (25-74)
Alive∞	50/56 (82,1)
Trinucleotide repeats number*	43.87 (40-51)
Age at onset*	46.69±10.37 (23-65)
Latency to diagnosis*	1.90±2.87 (0-12)
Disease duration*	7.81±5.18 (0-28)
First symptoms∞	
Involutary movements	24/49 (42,9)
Involuntary movements, walking difficulty, instability, falls	5/49 (8.9)
Involuntary movements, aggressiveness, agitation, anxiety	4/49 (7.1)
Involuntary movements, depression	2/49 (3.6)
Other	14/49 (25)
Family history∞ ,	
No data	10/50 (20)
Negative	11/50 (22.5)
Uncertain	7/50 (14)
Positive	22/50 (44)

*average value with SD and range in a parenthesis; ∞ number of patients (percentage)

(there are relatives with similar symptoms who are not diagnosed and treated), and for 22 patients family history for HD was positive (in 9 cases mother was ill, and in 13 that was the case with the father).

The average age in the beginning of the disease for patients whose mother was ill was 42.55 ± 7.35 years, for those with diseased father 45.67 ± 6.62 years, and for patients with a negative family history of HD 50.1 ± 10.14 years. There is no statistically significant difference between the age at the begging of the disease for patients who have inherited disease from the mother and patients who inherited the disease from father. Also, although the disease occurred later in patients with a negative family history, compared to those with positive family history, this difference was not shown to be statistically significant. The number of trinucleotide repeats in our patients ranged from 40 to 51. Clinical demographic characteristics of the patients are shown in Table 1.

Involuntary movements were first, isolated symptoms for 24/49 patients, while the occurrence of walk disorder at the same time, with instability, falling and sticking was observed in 5 patients. In 4 patients aggressiveness, anxiety and nervousness were manifested along with the involuntary movements, and in 2 that was a case with depression. Other symptoms (absence, obliviousness, cognitive disorders, facial tics, swallowing disorders and /or speaking, dizziness, disturbance of sphincter control and psychiatric symptoms in the form of delusions) occurred in 14 patients alone or in combination.

In specified telephone interview patients' family members gave information that in the current clinical picture involuntary movements occurred in 45 patients. In five patients involuntary movements were not present. Walk which is either difficult and requires help or is impossible was characteristic of 23/50 patients, while 20 patients were falling. Nearly 80% (39/50) of patients choked, and slightly higher percentage of them spoke with difficulty and incomprehensibly (42/50). In 34 patients mood change was recorded, of which 12 patients are depressive, reticent and recluse, 10 are anxious or aggressive, 7 have a changeable mood, and 2 are oblivious. The combination of anxiety, aggressiveness and obliviousness occurred in another 3 patients. Sleeping problems were found in 28 patients, most commonly in the form of early morning awakening and getting up frequently during the night (11 patients). Three patients reported that their sleep quality reduced because of the great anxiety during sleeping. Seven patients put themselves to sleep only with the help of sleeping pills, while 5 patients say that their need for sleep increased. Appetite is reduced in nine patients, and increased in four, while 29 patients lost weight. 23/50 patients depend on feeding and require help; 32/50 do not conduct their own hygiene; 35/50 do not leave the house, while ten go out periodically, often with the help in the walks. There are 38 patients who cannot perform, seven use diapers, one patient uses catheter and six wheelchairs (Figure 1).

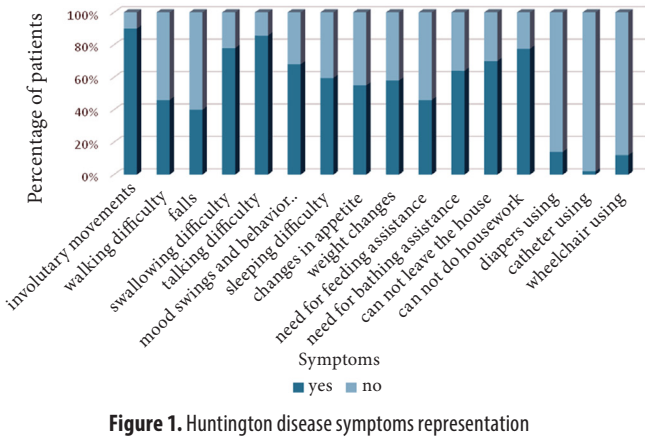

Figure 1. Huntington disease symptoms representation

Table 2. Milestones in progression of Huntington disease

Milestone	Years after first symptom (average value±SD)
Swallowing difficulty	4.45±4.02
Talking difficulty	4.58±3.01
Falls	5.25± 4.02
Need for bathing assistance	6.22±4.05
Need for feeding assistance	7.47±4.27
Walking difficulty	7.53± 4.63
Catheter using	9±0
Diapers using	9.11±3.68
Wheelchair using	10±6.54

Listed symptoms and status patients reached out in a short period of time (Table 2). The earliest evolved interferences with swallowing, chewing and speech, and the latest complete immobility with the attachment to the wheelchair (Figure 2).

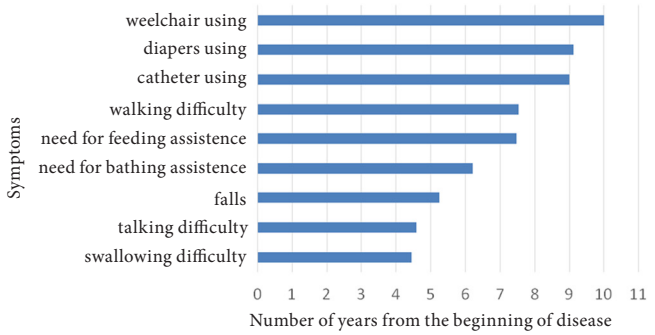

Figure 2. Milestones in progression of Huntington disease

Discussion

Although it is known that Huntington's disease equally affects men and women, the sex ratio of the patients included in our study was almost 2: 1 in favor of women (37:19). These results can be explained by the fact that women are major participants in studies on subjects under the risk for HD, as well as being more likely to require predictive genetic analysis for HD (17,18,19). Also, it is known that women with Huntington's disease live longer, which could be another explanation for why there are more women in random sample (20).

The first symptoms in our patients were reported on average of 46.7 years of age, which is consistent with many other studies that indicate that the first symptoms occur in the 4th and 5th decade of life (21). Our youngest patient was 23 years old at the beginning of the disease, and the oldest was 65 years old, which fits the range of 8 to 83 years in other studies (22).

Of the total of 50 interviewed patients, 22 had a positive family history, of which 9 patients inherited disease from the mother and 13 from the father. It is known that sex of the parents does not affect the heriditet because HD is an autosomal dominant disease, and it is transmitted on 50% of the offspring (23). Significant difference was not shown between the age at diagnosis in our patients who have inherited the disease from their mother and patients who have inherited the disease from their father, which is in accordance with the results of the study from Venezuela (24).

A quarter of our surveyed patients, more precisely 11 of them have a negative family history of HD. It is interesting that our results are consistent with research conducted in British Columbia, in which also a quarter of patients had a negative family history. In 7.8% of the total number of patients in the same research it is proved or probable de novo mutations due to the instability of CAG repeats (25).

The first symptom in our patients were involuntary movements (in 24 patients independently and in other 11 patients they were associated with other symptoms). These results were expected, due to the fact that other studies had shown that in the diagnostic stage chorea is the first and most evident sign of the disease (23). Changes in mood and behavior, including aggression, agitation, nervousness, depression, absence, obliviousness, cognitive disorders, as well as psychotic signs such as hallucinations and paranoia are reported in 15 of our patients as the symptoms with which the disease began. It is known that these symptoms often precede the motor signs of the disease, but are often ignored or attributed to other causes (5,26).

Latency of developing the first symptoms to diagnosis lasted for an average of about 2 years, although the longest latency was even 12 years long. This is common for patients with HD, especially if their involuntary movements are weakly expressed, the patient do not notice them or denies them, or they do not interfere with patients daily activities (on face, as in our patient).

With an average of 4.5 years after the appearance of first symptoms, patients have begun to swallow with difficulty, to chew and have spasms during meals, and shortly after that they started to speak difficult and incomprehensible. Our patients developed problems with speech much earlier than most patients in a similar research (27) where this symptom appeared between 6 and 10 years after the beginning of the disease. A year later, the patients would usually begin to fall, which would interfere with the independent walk and led to difficult and disabled walk for about 2 years, or 7.5 years after the beginning of the disease. Results of the research show that with the majority of patients problems with walking occurred in the period of 5-6 years from the beginning of the disease. Six to seven years since the beginning of the disease, patients are hampered in their daily activities such as eating and hygiene of the body and require help in performing them. Loss of the sphincter control our patients developed for an average of 9 years, which is a bit earlier than the majority of patients in the research mentioned above, in which urinary incontinence emerged after 10 years.

Conclusion

Huntington's chorea is a chronic progressive disease that in the short term it leads to significant disability with the emergence of problems with swallowing, speaking and walking, leading then to complete depending on the physical assistance of another person.

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MOLECULAR PREVENTION OF CHRONIC NON-COMMUNICABLE DISEASES: HOW CLOSE ARE WE?

MOLEKULARNA PREVENCIJA HRONIČNIH NEZARAZNIH BOLESTI: KOLIKO SMO BLIZU?

Tatjana Pekmezović

Summary

Chronic non-communicable diseases (CNDs) are a leading cause of death worldwide; preterm mortality in people younger than 70 years accounts for 40% of the total of 38 million deaths due to CNDs. Heterogeneity and complexity of CNDs cause shifting the prevention focus towards the molecular level in order to contribute to the global decrease of disease burden. Given that fact, the aims of molecular prevention are determination of crucial genetic, epigenetic and environmental factors that influence different responses to agents, as well as those that modify responses to agent exposure and tendency of development of chronic diseases. Also, it is important to mention the recognition of new pathways of pharmacologic modulation.

Although basic postulates of molecular preventions are still at their beginning, the fact that significant results in the field of clarifying CNDs etiology and early pathogenesis, risk assessment and modeling, as well as targeting of agents with high preventable efficacy have already been achieved, it is clear that there is a possibility to decrease CNDs burden in the earliest phases of its natural course. Accordingly, it is important to change the belief that a person without clinical symptoms and signs of disease is necessarily healthy. On the other hand, there is a need to balance the risks and health; molecular prevention has its own place in that interspace. Studies investigating the effects of potential preventive measures at molecular level have clearly determined high-risk cohorts, outcomes and other design elements, similarly to clinical studies. There is an intensive development of new research fields, like nutrigenomics which investigates the impact of diet on metabolic pathways and homeostasis, that is, their regulation in early stages of diseases associated with nutrition, as well as the level of susceptibility of person with certain genotype to those diseases.

The investigations in the fields of molecular prevention may contribute to new biomarkers development or help the setting of strategies for CNDs molecular prevention and nanotherapy. In other words, they represent the marker of genomics applying in population sciences.

Keywords: chronic non-communicable diseases, molecular prevention, gen-environment interaction, nutrigenomics, nutrigenetics

Sažetak

Hronične nezarazne bolesti (HNB) su vodeći uzrok umiranja na globalnom nivou; od 38 miliona smrtnih ishoda u svetu zbog HNB, više od 40% čini prevremeni mortalitet koji se odnosi na osobe mlađe od 70 godina. Heterogenost i kompleksnost HNB sve više uslovljavaju pomeranje fokusa prevencije ka molekularnom nivou, doprinoseći na taj način globalnom smanjenju opterećenja populacije bolestima. Stoga su ciljevi istraživanja u okviru molekularne prevencije utvrđivanje ključnih genetskih, epigenetskih i sredinskih faktora koji utiču na različite odgovore na agense, odnosno onih koji modifikuju odgovore na izloženost agensima i sklonost ka razvoju hroničnih bolesti, a prepoznaju se i novi putevi za farmakološku modulaciju.

Iako su bazične postavke molekularne prevencije još uvek u začetku, činjenica da su postignuti značajni rezultati na polju rasvetljavanja etiologije i rane patogeneze HNB, u proceni rizika i njegovom modelovanju, targetovanju agenasa sa širokom preventivnom efektivnošću, jasno ukazuju na mogućnost smanjenja bremena HNB u najranijim fazama njihovog prirodnog toka. U skladu s tim, značajna je i promena uverenja da osoba bez vidljivih simptoma i znakova bolesti ne mora nužno biti zdrava, odnosno da postoji potreba za balansiranjem između rizika i zdravlja i da u tom međuprostoru molekularna prevencija ima svoje mesto. Studije u kojima se testira efekat potencijalnih preventivnih sredstava na molekularnom nivou, slično kliničkim studijama, kao ključne determinante imaju jasno definisane visokorizične kohorte, ishode i druge elemente dizajna. Intenzivno se razvijaju nove oblasti, kao što je npr. nutrigenomika, koja proučava uticaj ishrane na metaboličke puteve i homeostazu, odnosno njihovu regulaciju u ranim stadijumima bolesti povezanih sa ishranom, kao i stepen do koga je osoba sa odgovarajućim genotipom podložna ovim bolestima.

Istraživanja u oblasti molekularne prevencije mogu da doprinesu otkrivanju novih biomarkera, pomažu u formulisanju strategija za molekularnu prevenciju HNB i nanoterapiju, odnosno predstavljaju putokaz za aplikaciju genomike u populacionim naukama.

Ključne reči: hronične nezarazne bolesti, molekularna prevencija, interakcije gen-sredina, nutrigenomika, nutrigenetika

Uvod

Heterogenost i kompleksnost mnogih hroničnih nezaraznih bolesti (HNB) uslovljavaju pomeranje fokusa njihove prevencije prema molekularnom nivou, doprinoseći na taj način globalnom smanjenju opterećenja populacije bolestima. S druge strane, molekularna prevencija je fokusirana na otkrivanje ključnih genetskih, epigenetskih, stohastičkih i sredinskih činilaca, koji modifikuju odgovore na izloženost agensima i sklonost ka razvoju hroničnih bolesti.

Hronične nezarazne bolesti u svetu i Srbiji

Hronične nezarazne bolesti (HNB) su vodeći uzrok umiranja na globalnom nivou, kako u nerazvijenim, tako i u zemljama u razvoju (1). Od 38 miliona smrtnih ishoda zbog HNB u svetu, na godišnjem nivou, više od 40% (16 miliona) čini prevremeni mortalitet koji se odnosi na osobe mlađe od 70 godina (1). Prevalencija HNB je takođe u porastu. Preko 70% svih smrtnih ishoda širom sveta posledica su različitih kardiovaskularnih oboljenja (17,5 miliona smrtnih slučajeva godi-

šnje), malignih bolesti (8,2 miliona smrtnih slučajeva godišnje), moždanog udara (6 miliona smrtnih slučajeva godišnje), dijabetesa (1,5 miliona smrtnih slučajeva godišnje) i hronične opstuktivne bolesti pluća (HOBP) (4 miliona smrtnih slučajeva godišnje) (1). U Republici Srbiji, najčešćih pet uzroka prevremene smrtnosti su ishemijska bolest srca, moždani udar, kardiomiopatije, karcinom pluća i samopovređivanje (2). S druge strane, ako se posmatra period od 1990. godine do 2010. godine u našoj zemlji, može se uočiti opadanje smrtnosti od zaraznih bolesti i porast u rangu prevremene smrtnosti, pre svega zbog malignih bolesti, dijabetesa, HOBP-a (3). Različite HNB se međusobno razlikuju u odnosu na broj godina korigovanih u odnosu na onesposobljenost, odnosno broj godina izgubljenog života zbog prevremene smrtnosti i onesposobljenosti. Većinu HNB karakteriše povećanje opterećenja u periodu između 1990. i 2010. godine. Za neke bolesti, broj godina izgubljenog života povećao se za 100% (na primer, Alchajmerova demencija) (3). Suočeni sa ovakvim trendovima, na globalnom nivou donete su različite strategije za borbu protiv HNB. Tako je Svetska zdravstvena organizacija (SZO) postavila 9 meta kada su u pitanju HNB do 2025. godine. Jedna od njih je smanjenje prevremene smrtnosti za 25%, šest se odnosi na redukciju faktora rizika, a dve na dostupnost lekova i medicinskih tehnologija i odgovarajuću pokrivenost populacije (4).

Pomeranje fokusa prevencije od tradicionalne ka molekularnoj

U tradicionalnoj epidemiologiji izloženost i bolest se posmatraju kao dve zavisne varijable. Molekularno-epidemiološki pristup širi vidik i omogućava precizniju kvantifikaciju izloženosti koja se dogodila u prošlosti, kao i rekonstrukciju stepena ekspozicije preko bioloških merenja, odnosno markera izloženosti. S druge strane, postoje klinički ishodi koji se statističkom metodologijom dovode u vezu sa markerima izloženosti. Međutim, ovakvim pristupom je moguće obuhvatiti i prekliničke događaje i sledstveno tome, njihovu prevenciju.

Poseban zamah molekularna epidemiologija dobija mapiranjem humanog genoma, čime se uspostavlja spona između genetske epidemiologije, kliničke medicine i javnog zdravlja. Projekat humanog genoma imao je kao primarni cilj određivanje tačne sekvence molekula DNK, kao i identifikaciju i mapiranje oko 20.000-25.000 gena ljudskog genoma. Suštinski cilj Projekta bio je uspostavljanje moderne biotehnologije, koja bi doprinela preciznijem postavljanju dijagnoze, lečenju i prevenciji bolesti (5).

Osim otkrivanja najvažnijih genetskih, epigenetskih, stohastičkih i sredinskih činilaca, koji modifikuju odgovore na izloženost agensima i sklonost ka razvoju hroničnih bolesti, istraživanja u oblasti molekularne prevencije doprinose prepoznavanju novih puteva za farmakološku modulaciju (6). Iako su bazične postavke molekularne prevencije još uvek u začetku, činjenice da su postignuti značajni rezultati na polju rasvetljavanja etiologije i rane patogeneze HNB u proceni rizika i njegovom modelovanju, targetovanju agenasa sa širokom preventivnom efektivnošću, jasno ukazuju na mogućnost smanjenja bremena HNB u najranijim fazama njihovog prirodnog toka. U skladu s tim, značajna je i promena uverenja da osoba bez kliničkih simptoma i znakova bolesti ne mora nužno biti zdrava, odnosno da postoji potreba za balansiranjem između rizika i zdravlja i da u tom međuprostoru molekularna prevencija ima svoje mesto.

Studije u kojima se testira efekat potencijalnih preventivnih sredstava na molekularnom nivou, slično epidemiološkim i kliničkim studijama, kao ključne determinante imaju jasno definisane visokorizične kohorte, ishode i druge elemente dizajna. Sprovođenje molekularno-genetičkih studija regulisano je jasnim smernicama sprovođenja prediktivne dijagnostike, odnosno genomskog skrininga kod zdravih osoba (7). Istraživačke linije idu u smeru populacionog preventivnog sekvenciranja koje ima za cilj da identifikuje faktore podložnosti kod odraslih osoba bez prisutnih poznatih faktora rizika. Drugim rečima, preventivno genomsko sekvenciranje sprovodi se s ciljem da doprinese otkrivanju onih osoba koje nose mutacije za koje ne znaju, a koje predstavljaju predispoziciju za neko potencijalno preventabilno oboljenje (8).

Najranije molekularno-epidemiološke studije potiču iz oblasti malignih tumora (9). Osnovna ideja je da se identifikacijom genetskih polimorfizama pokuša modifikacija asocijacije između izloženosti i bolesti, i na taj način identifikuju segmenti populacije koji su posebno osetljivi na određenu ekspoziciju. Ukoliko se posmatra put između izloženosti sredinskom faktoru rizika i nastanka bolesti, jasno se uočava da uobičajene genetske varijacije, odnosno polimorfizmi, mogu uticati na enzimsku aktivnost u različitim tačkama tog puta. Kao rezultat tih uticaja, može doći do formiranja DNK adukata, ali i njihovog izmicanja enzimima reparacije, što rezultuje mutacijama u kritičnim genima uključenim u kontrolu rasta (10).

Činjenica je da je za mnoge bolesti interakcija gen-sredina ključni događaj u patogenezi. To otvara mogućnost modifikovanja ove interakcije, što bi moglo da predstavlja ključ primarne prevencije (11-15). Na primer, karcinom cerviksa predstavlja, još uvek važan javnozdravstveni problem, uprkos činjenici da se radi o jednoj od najpreventabilnijih i najkurabilnijih malignih bolesti, s obzirom na postojanje vakcine protiv humanog papiloma virusa (HPV) u primarnoj, i efektivnog skrininga u sekundarnoj prevenciji (16). Međutim, treba imati u vidu činjenicu da se kod većine HPV pozitivnih žena ni-

kada ne razvije karcinom cerviksa, dok se kod određenog, mada neuporedivo manjeg broja žena koje nikada nisu bile inficirane HPV, ipak razvija cervikalni kancer (17). To nameće zaključak da i drugi prisutni faktori rizika imaju mogućnost indukovanja bolesti. Jedan od njih je pušenje, nezavisni faktor rizika za nastanak karcinoma cerviksa, bez obzira na seksualno ponašanje i socijalnoekonomski status. Dejstvo pušenja dodatno pojačava interakciju sa HPV (17).

Dobro je poznato da ekspresija virusnih proteina blokira tumor-supresorne gene, čime se kompromituje njihova funkcija u regulaciji ćelijske proliferacije i inicira apoptoza. Ispitivanja u tri populacije sa različitom etničkom strukturom pokazala su da je rizik za pojavu cervikalnog kancera kod žena inficiranih HPV veoma visok, ali i veoma različit, uprkos sličnoj stopi infekcije. Slična je situacija i sa rizikom žena koje puše da dobiju cervikalni kancer. To jasno govori da postoje i neki drugi, etnički, odnosno genetski specifični faktori i da njihov uticaj jasno modifikuje delovanje klasičnih sredinskih faktora rizika (18).

Nutrigenetika i nutrigenomika

Hrana, ishrana i nutritivni status se smatraju važnim determinantama i jednim od najodgovornijih faktora rizika za nastanak HNB. Prema podacima SZO, oko 16 miliona (1,0%) godina korigovanih u odnosu na onesposobljenost (engl. DALYs-disability adjusted life years, broj godina zdravog života izgubljenih zbog onesposobljenosti ili prevremene smrtnosti) i 1,7 miliona (2,8%) smrtnih ishoda na globalnom nivou posledica su nedovoljnog konzumiranja voća i povrća (19). Takođe, procenjuje se da bi smanjenje korišćenja soli u hrani sa 9-12 g/dan, koliko sada iznosi svetski prosek na preporučenih 5 g/dan, moglo da ima ogroman uticaj na krvni pritisak i kardiovaskularne bolesti (19).

U vezi sa navedenim činjenicama, intenzivno se radi na razvijanju novih oblasti, kao što su nutrigenomika i nutrigenetika. Nutrigenomika se bavi proučavanjem uticaja ishrane na metaboličke puteve i homeostazu, odnosno njihovu regulaciju u ranim stadijumima bolesti povezanih sa ishranom, kao i stepen do koga je osoba sa odgovarajućim genotipom podložna ovim bolestima (20). Drugim rečima, nutrigenomika je nauka o delovanju hranljivih materija (nutrijenata) na ekspresiju gena, ili preciznije, otvara se mogućnost za identifikaciju gena koji utiču na rizik od bolesti povezanih sa ishranom (21).

Nutrigenetika pokušava da pruži odgovor zašto hrana i načini ishrane imaju različiti efekat na svakog pojedinca (22). Izučavanje uloge genetskih varijacija u objašnjenju individualne različitosti u odgovoru na ishranu, predstavlja osnovu za proučavanje podložnosti za bolesti koje su povezane sa ishranom. Istorijski gledano, ishrana je uticala na ekspresiju gena, što je omogućilo formiranje fenotipskih karakteristika koje su uspešno mogle da odgovore na sredinske stimuluse i dozvole efikasniju eksploataciju resursa hrane. Ovakva adaptacija bila je ključna za humani rast i razvoj.

Danas se intenzivno razvijaju tzv. "omics" nauke (transkriptomika, proteomika, metabolomika), koje omogućavaju determinisanje interakcija između nutrijenata i drugih bioaktivnih komponenti hrane i gena, a takve relacije imaju značaja za uspešnije lečenje i personalizovanu ishranu (23). U tom smislu razmišlja se o epigenetskim mehanizmima koji leže u osnovi modifikacije fenotipa, a čija je modulacija moguća preko nutrijenata. Poslednjih godina raste interesovanje za epigenetske mehanizme čija disregulacija može imati značaja za razvoj bolesti u humanoj populaciji (24).

Ako se razmatra tzv. fetalna hipoteza o poreklu bolesti, onda su jasne relacije između ishrane majke, fetalnog epigenetskog programiranja i pojave bolesti u adultnom dobu, odnosno naglašava se da ishrana u najranijim periodima života ''programira'' neželjene ishode u adultnom periodu. Takvi efekti rane nutritivne izloženosti na rizik za adultnu gojaznost, hipertenziju i insulinsku rezistenciju, pokazani su na različitim animalnim modelima (24).

U oblasti nutrigenomike, aktivno se traga za tzv. ''genomic-based'' fenotipskim biomarkerima, koji bi bili validni i omogućili ranu detekciju, ili tačnije, otkrivanje bolesti u prekliničkoj fazi i efektivnu primenu strategija ishrane u prevenciji. Kao primer, može poslužiti metabolički sindrom, kompleksna, multifaktorska, poligenska bolest. Naglašava se komplementarnost dva pristupa, ishrane, koja je važna za najranije faze bolesti i očuvanje homeostaze, i lekova, neophodnih za tretman kasnijih faza oboljenja (20).

U istraživanjima u oblasti nutrigenomike primenjuju uglavnom dve strategije. Prva obezbeđuje detaljne podatke na molekularnom nivou o interakcijama između nutrijenata i genoma, dok se u drugoj težište stavlja na humanu ishranu (21). Primenom prvog pristupa identifikuju se transkripcioni faktori koji funkcionišu kao nutritivni senzori i geni koji su njihove mete, otkrivaju se signalni putevi i karakterišu glavni nutritivni signali, meri se genska ekspresija i metaboličke konsekvence specifičnih mikronutrijenata i makronutrijenata, otkrivaju se genotipovi koji su faktori rizika za nastanak bolesti povezanih sa ishranom, kao što su dijabetes, hipertenzija ili ateroskleroza i kvantifikuje njihov uticaj. Drugi pristup uključuje aplikaciju nutritivne biologije u otkrivanju biomarkera rane metaboličke disregulacije i podložnosti koje su pod uticajem ishrane (21, 25).

Inkorporiranje genomike u nutritivnu praksu nudi potencijal za personalizovanu ishranu i pomoć u pre-

venciji, targetovanjem molekularnih mehanizama koji prethode bolesti, a koji odgovaraju na nutriente (21). Najbolji primer za to je fenilketonurija, gde se odgovarajućom ishranom minimiziraju posledice bolesti (26). Kod ovih osoba sprovođenje lečenja ishranom se započinje u prvim danima života, a suština je u doživotnoj primeni specijalnih preparata koji ne sadrže fenilalanin.

***

Istraživanja u oblasti molekularne prevencije mogu da doprinesu otkrivanju novih biomarkera, preciznijem merenju podložnosti za bolest, odnosno personalizovanoj proceni rizika, novim saznanjima o efektima interakcija gen-sredina i posebno gen-hrana (27). Pored toga, primena genomike u populacionim naukama doprinosi formulisanju novih strategija za prevenciju i kontrolu HNB, koje bi trebalo da pomognu optimizovanju zdravlja, prevencije i lečenja bolesti.

Napomena

Mini simpozijum „Molekularna prevencija hroničnih nezaraznih bolesti: koliko smo blizu?“ održan je na 45. simpozijumu Stremljenja i novine u medicini, Medicinski fakultet u Beogradu, 06.12.2016. godine.

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ADVANCEMENTS IN MESENCHYMAL STEM CELL TREATMENT FOR BUERGER'S DISEASE

NAPREDAK U TERAPIJI BIRGEROVE BOLESTI MEZENHIMALNIM STEM ĆELIJAMA

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Summary

Buerger's disease or thromboangiitis obliterans, is a non-atherosclerotic inflammatory process which mostly involves medium and small sized arteries and veins in lower and upper extremities. It is categorized as vasculitis. The disease is known to be closely linked to smoking. Buerger's disease is a long-term debilitating condition because of the pain, the development of ulcers and gangrene, and the risk of amputation.

Drugs effective on erythrocyte flexibility, agents acting on platelets, non-steroidal anti-inflammatory drugs and vascular reconstruction are among several therapeutic methods for Buerger's disease. However, the applied therapies are insufficiently effective. Still, the base of treatment is smoking cessation. Lately, cell therapy has offered us entirely new possibilities.

Mesenchymal stem cell (MSC) treatment has been proposed as a novel approach for tissue engineering and regenerative medicine for various ischemic disorders, including Buerger's disease. In 2016, the first MSC- based therapy has received regulatory approval for the treatment of Buerger's disease in India. In March 2017, orphan designation was granted by the European Commission for autologous adipose tissue-derived MSC for the treatment of Buerger's disease. Novel therapeutic approach needs to be validated in the upcoming studies conducted in different clinical centers.

Key words: Buerger's disease, mesenchymal stem cells, orphan drug

Sažetak

Birgerova bolest (thromboangiitis obliterans) je neaterosklerotski inflamatorni proces koji uglavnom zahvata male i srednje arterije i vene u donjim i gornjim udovima. Okarakterisan je kao vrsta vaskulitisa. Nastanak, napredovanje i težina bolesti su povezani sa pušenjem. Birgerovu bolest karakterišu bol, pojava ishemičnih ulkusa, gangrena i rizik od amputacije, što značajno utiče na kvalitet života pacijenta.

Trombolitička terapija, primena nesteroidnih antiinflamatornih lekova i vaskularno-hirurški rekonstruktivni zahvati su neki od terapijskih pristupa u lečenju Birgerove bolesti. Međutim, ovi načini lečenja nisu dovoljno efikasni. Još uvek najbolji efekat na poboljšanje bolesti ima prekid pušenja. U poslednje vreme, ćelijska terapija je ponudila sasvim nove mogućnosti lečenju Birgerove bolesti.

Terapija mezenhimskim matičnim ćelijama (MMĆ) je prepoznata kao novi pristup u tkivnom inženjerstvu i regenerativnoj medicini, primenljiv u lečenju različitih ishemijski poremećaja, uključujući Birgerovu bolest. Prva terapija koja koristi MMĆ u lečenju Birgerove bolesti je odobrena u Indiji 2016. godine. U martu 2017. godine Evropska komisija je terapiju autolognim MMĆ iz adipoznog tkiva uvrstila u lekove „siročiće" za Birgerovu bolest. Ovaj novi terapijski pristup je neophodno validirati u narednim studijama u više kliničkih centara.

Ključne reči: Birgerova bolest, mezenhimske matične ćelije, lek „siročić"

Introduction

Thromboangiitis obliterans (TAO), also known as Buerger's disease, is a nonatherosclerotic process that most commonly affects the small and medium-sized arteries and veins in the upper and lower extremities. TAO was first described in 1879, when two Austrian surgeons, Felix von Winiwarter and Theodor Billroth, reported in the German Archives of Clinical Surgery a single case of what they described as "presenile spontaneous gangrene" (1). Almost half a century later, in 1924. Buerger described in detail the absence of large vessel involvement as opposed to what was thought by Winiwarter and Billroth, linking this disease with smoking, thus determining tobacco use as a predisposing factor and naming this condition thromboangiitis obliterans (2), (Figure 1, Figure 2).

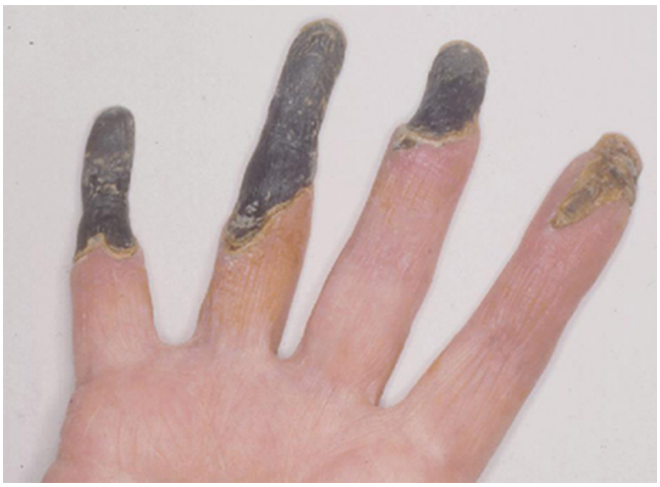

Figure 1. Trombangitis obliterans in progress

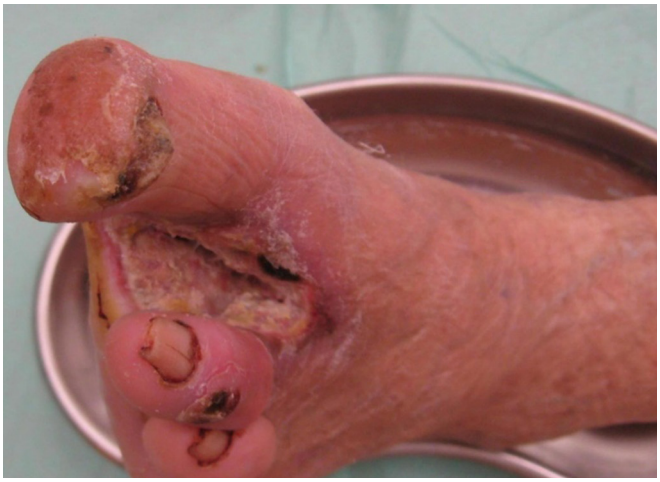

Figure 2. Thrombangitis obliteralis in progress

Epidemiology

TAO is a rare disease, since it affects approximately 1 in 10.000 people in the European Union (EU). This is equivalent to a total of around 52.000 people. Today it is proven that this disease is closely associated with smoking and that it mostly affects male patients aged 25 to 40. The ratio is 3 to 1 in favor of men. With a significant decrease in the number of smokers in the world, consequently the prevalence of this disease has been reduced as well. In Europe, it is from 0.5 up to 5.3 %, while high values of over 60% are still found in India and Pakistan. The greatest number of patients has been recorded among Jews of Ashkenazi ancestry living in Israel with prevalence of up to 80%. (3,4)

Etiology and Pathogenesis

The etiology of Buerger's disease is still unknown, despite a great progress in medicine. The fact is that although TAO is a type of vasculitis, it differs from other forms of vasculitis, due to much less intense cellular activity in the wall of the blood vessel. Immunologic markers, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), rheumatoid factor and antinuclear antibody levels, are usually normal or slightly elevated, which is not the case in other types of vasculitis (4).

Tobacco use or exposure plays a central role in the initiation and progression of the disease. It is considered that the patients have a sort of nicotine hypersensitivity or allergy. Namely, it is believed that tobacco glycoprotein (TGP) could be related to changes in blood vessels and some works have proven that the development of Buerger's disease can be directly monitored by measuring the urine level of nicotine metabolite cotinine (5).

As for genetic predisposition, it has not yet been proven, although some studies have mentioned higher prevalence of allele histocompatibility complex HLA A9 A5 and B5 (6).

Many have tried to prove the importance of hypercoagulability in connection to the development of this disease. Although it has not yet been definitively verified, some studies have shown that patients with TAO, who have elevated level of homocysteine, may also have a higher amputation rate than those with normal homocysteine levels (5 ,6).

Immunological aspects of this disease are highly important since one of the explanations for this disease is that it is an autoimmune disorder with antibodies directed towards vascular endothelium in response to antigens in tobacco. The presence of different antibodies, such as antinuclear, antielastin, anticollagens I and III, and antinicotine antibodies, as well as identification of deposits of immunoglobulin (Ig) G, IgC3, and IgC4 in the blood vessels of patients, provided evidence to the theory of the immune character of the disease (7, 8).

Histopathology

Disease progress can be divided into three phases, acute, intermediate and chronic phase. In all stages of the disease, the normal architecture of the vessel wall, subjacent to the occlusive thrombus and including the internal elastic lamina remains essentially intact, which distinguishes this disease from all other types of vasculitis?

Acute-phase lesion is characterized by acute inflammation involving all coats of the vessel wall, especially of the veins, in association with occlusive thrombosis around which polymorphonuclears accumulate on periphery, creating micro abscesses. Progressive organization of the occlusive thrombus in the arteries and veins with the possibility of recanalization is found in intermediate phase, while the thrombus formation with fibrotic changes is a clear sign that the disease is in its end stage.

Clinical Features

Buerger's disease is frequent among men younger than 45 and smokers. One of early symptoms are leg claudications, the necessity for frequent resting, mild swelling in extremities and ischemic ulcers on the fingers and toes. A significant number of patients have superficial thrombophlebitis and paresthesia, while more than 80% of patients have changes on 3 to 4 extremities (9,10,11).

Diagnosis

Since there are neither analyses nor special diagnostic methods for determining this disease, it is highly important to perform comprehensive laboratory analyses

in order to exclude other diseases (Table 1) and to take into consideration all differential diagnostic investigations in order to be positive that the patient is suffering from this disease (Table 2) (12,13, 14). Angiography is present with specific "corkscrew" sign. (Figure 3)

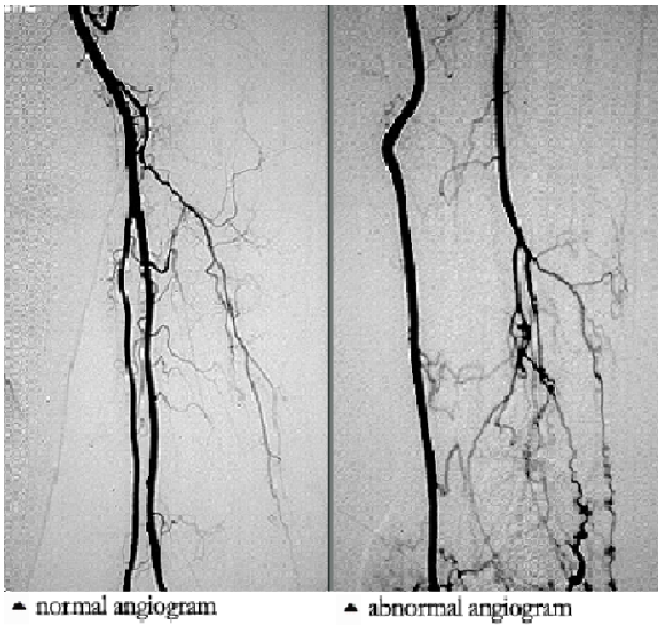

Figure 3. Normal and angiogram in TAO disease

Table 1. Diagnostic Investigations for Buerger's Disease

Diagnostic Investigations for Buerger's Disease
Blood Count
Liver function
Renal function
Fasting blood sugar
Erythrocyte sedimentation rate
C-reactive protein
Antinuclear antibodies
Rheumatoid factor
Complement Measurements
Anticentromere antibodies (for CREST)
Anti-Scl-70 antibodies (for Scleroderma)
Antiphospholipid antibodies
Lipid profile
Urinalysis
Toxicologic screen for cocaine and cannabis
Cryoproteins
Segmental Arterial Doppler Pressures
Arteriography
Echocardiography (to exclude source of emboli)
Computed tomography (to exclude potential source of emboli)
Biopsy (in proximal artedy involvement or unusual locations)
Complete thrombophilia screen: Protein C, S, Antithrombin III, Factor V Leiden, Prothrombin 20210, and Homocysteinemia

CREST- Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia. *Adapted from (12)

Table 2. Diagnostic criteria for Buerger's Disease

Diagnostic Criteria	
Shionoya criteria (13)	**Olin criteria** (14)
Onset before age 50	Onset before age 45
Smoking history	Current (or recent past) tobacco use
Infrapopliteal arterial occlusions upper limb involvement or phlebitis migraines	Distal extremity ischemia (infrapapliteal and/or intrabrachial), such as claudication, rest pain, ischemic ulcers, and gangrene documented with noninvasive testing
	Laboratory tests to exclude autoimmune or connective tissue diseases and diabetes mellitus
Absence of atherosclerotic risk factors other than smoking	Exclude proximal source of emboli with echocardiography and arteriography
	Demonstrate consistent arteriographic findings in the involved and clinically noninvolved limbs

Note: A biopsy is rarely needed to make the diagnosis, unless the patient presents with an unusual characteristic, such as large artery involvement or age greater than 45 years

Treatment

The most effective treatment for Buerger's disease is smoking cessation. In case the smoking continues, the chronic phase of this disease mostly ends with amputation. Nevertheless, the researches show that only 43 to 70 % of patients succeed in giving up smoking, regardless the fact that stopping smoking means avoiding amputation (15).

Since we do not know its etiology, Buerger's disease therapy has changed over time, starting from platelet activity blocking, to vasodilatation, changing metabolism of the cells and up to nowadays application of cell therapy.

Acetylsalicylic acid (ASA) and Clopidogrel are used for platelet inhibition and are both successful in disease prevention, giving advantage to Clopidogrel use, due to its more powerful inhibition of platelet activity. Both medicines represent basic therapy and have no effect on decreasing symptoms or preventing disease progress for longer periods of time (15).

Vasodilator drugs have been used in attempt to improve blood flow proximal to lesion or occlusion. However, vasodilators have the capacity to reduce overall systemic vascular resistance, leading to a reduction in perfusion pressure and subsequently causing the ischemic insult in the hypoperfused extremity. Therefore, vasodilators are not suitable for TAO therapy.

Calcium channel blockers, such as nifedipine, amlodipine or verapamil are believed to provide more efficient oxygen utilization in diseased extremity (17).

Pentoxifylline is a medicine that prevents red blood cell deformability, decreases blood viscosity, platelet ad-

hesiveness and secures reduction in fibrinogen levels. Though its usage may increase the pain-free walking distance in many, the long-term benefit and improvement in quality of life is limited (16).

Cilostazol is a phosphodiesterase type III inhibitor which inhibits cyclic adenosine monophosphate (cAMP) phosphodiesterase, thus decreasing platelet aggregation and providing muscle cell relaxation. Prolonged use of this medicine has numerous side effects such as headaches, of which patients need to be informed (18).

A few studies suggest surgical treatment that provides blood flow to a diseased extremity. The results have shown that this type of treatment is most successful in patients who have given up smoking (17,18). Sympathectomy, performed by laparoscopic or open surgery method in order to decrease arterial spasm in patients with Buerger's disease, has not provided satisfactory results (19,20, 21,22).

Iloprost, a synthetic analogue of prostaglandin 12, acts through vasodilatation and effects platelets, preventing their granule release. Many studies have addressed treatments with prostaglandins and endothelin as a cure and possible decrease of amputation cases in patients with Buerger's disease, but without expected results (23,24,25).

Seeking for alternative therapeutic approach, in the past decade cell therapy was introduced and it opened up entirely new chapter in Buerger's disease treatment.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are undifferentiated cells found in many organs and tissues of adult organisms, including humans. MSCs, like other stem cells, possess several features that make them special and different from other cell types in the body. The main feature of stem cells is their capacity for self-renewal, which enables that upon cell division at least one of daughter cells remains a stem cell. The decision of the cell fate, to move towards differentiation or to remain in the state of stemness, depends on the type of stem cell, microenvironment where it is situated and the needs of the tissue and the organism as a whole (26). MSCs have potency to differentiate into various cell types, meaning that they are able to become different cells of the specific tissue type. In addition, thanks to their plasticity, they are able, under specific in vitro conditions, to differentiate into cell types different from their origin. This means that stem cells of mesodermal origin are able to give cells of ectodermal or endodermal origin, and vice versa. Human MSCs (hMSCs) are the non-haematopoietic, multipotent stem cells with the capacity to differentiate into mesodermal lineage such as osteocytes, adipocytes and chondrocytes as well as ectodermal (neurocytes) and endodermal lineages (hepatocytes) (27). Furthermore, important characteristic of MSCs is homing, which is their ability to receive signals of specific tissue factors that are secreted in the place of injury or defect in the body? Attracted by these paracrine factors, they travel to that place and repair damaged tissue (28).

Mesenchymal stem cells possess all those features, and together with their low apoptotic potential and high proliferative rate, they have come in the focus of regenerative medicine. MSCs express immunoregulatory capacity, because they secrete large amounts of bioactive agents that inhibit T-cell recognition and expansion, as well as inhibition of maturation od B cells and other immune cell types (29). They also possess trophic effects, since they secrete a great number of cytokines and growth factors (30). Secretion of anti-inflammatory molecules makes MSCs an effective tool in the treatment of chronic conditions, like autoimmune, inflammatory and degenerative diseases (31, 32, 33).

What makes MSCs especially attractive for regenerative medicine is the fact that they can be isolated from various tissues and in enormous number, using minimal invasive procedures. hMSCs for the first time were discovered in the bone marrow (34). Until now, they have been isolated from various tissues, including adipose tissue (35), amniotic fluid (36), endometrium (37), dental tissues (38), umbilical cord (39), Wharton's jelly (40) and many other tissues. Many studies compared MSCs form different sources, and although there are slight differences between them, these are not of such importance that would influence their regenerative capacity (41, 42). In the last decade, adipose tissue became especially attractive as a source of MSCs in terms of cell and tissue regeneration, for several reasons. Adipose tissue can be easily obtained using non -invasive procedures. A large number of MSCs can be harvested from adipose tissue. It was estimated that there are one hundred times more MSCs in the same amount of adipose tissue compared to bone marrow (43). They express cell surface markers defined for MSCs, like cluster of differentiation (CD) 29, CD44, CD73, CD90, CD105 and lack the expression of CD14, CD34, CD45 and HLA (human leucocyte antigen)-DR. Moreover, adipose tissue is available during whole lifespan, and its usage has few, if any, ethical issues. Many preclinical and clinical studies showed their benefits and safeness (44, 45, 46).

MSCs-based therapy for Buerger's disease

MSC treatment has been proposed as a novel approach for tissue engineering and regenerative medicine for various ischemic disorders, such as stroke, coronary artery disease, peripheral arterial disease (PAD) and critical limb ischemia (CLI), the most advanced clinical stage of peripheral arterial disease (47).

The first reported human study using intramuscular administration of allogenic human umbilical cord-derived MSCs was conducted in four patients with Buerger's disease. Improved ulcer recovery time, enhanced limb perfusion, and relieved the symptoms of rest pain were observed (48). In the cohort of nine patients with Buerger's disease and three with diabetic foot ulcers, it has been shown that intramuscular administration of autologous bone marrow derived MSCs have accelerated ulcer healing and improved pain-free walking distance (49).

Lee et al (50) have demonstrated that autologous adipose tissue-derived MSCs treatment of patients with Buerger's disease and diabetic foot improved walking claudication distance, collateral vessel formation, wound healing, and clinical symptoms, especially pain relief.

Similar MSCs treatments have been successful in patients with systemic sclerosis (51) and diabetes mellitus (52,53).

Therapeutic perspectives for Buerger's disease

After completion of the phase 1/2 clinical trial, using intramuscular administration of off the-shelf allogeneic bone marrow-derived MSCs into patients with critical limb ischemia (CLI), Stempeutics Research Pvt. Ltd. (Bangalore, India) has reported that the MSCs were well tolerated with no adverse events or rejection. A reduction in the number of ulcers was also demonstrated. No significant increase in amputation rate was observed. The efficacy of allogeneic bone marrow-derived MSCs has been assessed in phase 2/3 clinical trials (54).

In 2016, the Stempeutics Research has received regulatory approval for 'limited marketing' of its product for the treatment of CLI due to Buerger's disease — the first stem cell based biologics to be approved by the Drugs Controller General of India (DCGI) (55). The product "stempeucel" — patented in the US, Japan, China and Malaysia — is derived from allogeneic pooled mesenchymal stromal cells, extracted from the bone marrow of adult voluntary donors. The goal of the Stempeutics Research is to complete the study on 200 patients and seek full marketing authorization by the end of 2017.

Two phase 1 trials using intramuscular administration of allogeneic placenta-derived MSCs have been conducted by Pluristem Therapeutics Inc. (Haifa, Israel) since 2010. The study has demonstrated that these cells were safe with no adverse effects in patients with CLI and Buerger's disease. No specific anti-MSC HLA class I or II antibodies were detected. Only one out of 27 patients (3.7%) had a major amputation within six months. This therapy significantly improved blood flow and quality of life and reduced pain score (56).

On 20 March 2017, orphan designation (EU/3/17/1854) was granted by the European Commission to SPC GmbH, Germany, for autologous adipose tissue-derived mesenchymal stem cells (also known as VascoStem) for the treatment of Buerger's disease (57). The medicine is composed of MSCs that are extracted from the patient's own fat tissue. Taking patient's adipose tissue is a simple procedure, which can be done under local anesthesia, requiring only about 20g of fat. After extraction, it is grown in laboratory to increase the number before being injected back into the patient. The effects of the MSCs have been evaluated in experimental models, and clinical trials in patients with Buerger's disease are ongoing. The number of people affected with Buerger's disease in EU is below the ceiling for orphan designation, which is 5 people in 10.000. Besides the rarity of the condition to be treated, orphan medicinal product designations are based on additional criteria, such as: the seriousness of the condition, lack of alternative methods of diagnosis, prevention or treatment, insufficient returns on investment.

Designated orphan medicinal products are products that are still under investigation and are considered for orphan designation on the basis of potential activity. An orphan designation is not a marketing authorization. As a consequence, demonstration of quality, safety and efficacy is necessary before a product can be granted a marketing authorization. It is expected that treatment with this medicine will reduce damage to the blood vessels and so improve the symptoms of the Buerger's disease. Novel therapeutic approach needs to be validated in the studies conducted in different clinical centers.

Conclusion

TAO treatment has so far proved to have no long-term results. A group of patients, despite chronic therapy, is still facing the risk of limbs loss. Using the methods of implantation of MSCs, there is a possibility for establishing a process of angiogenesis, aiming to achieve significant long-term therapeutic improvement.

Although early results are promising, there is still a long path to cross from establishing a suitable production processes to imminent application of stem cells in targeted muscle groups. Nevertheless, the success of such treatment would allow patients with other vascular diseases to have hope and possibility to get significant correction in their clinical picture.

Acknowledgements

This work is funded by the Ministry of Education, Science and Technological Development, Republic of Serbia (grant no. III 41004).

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Uredništvo i administracija: 11105 Beograd, Dr Subotića br. 8, soba 311

Realizacija: SPRINT, BEOGRAD

Tiraž: 300 primeraka