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APPLICATION OF LYMPHOEDEMA THERAPY FOLLOWING INJURIES TO ANKLE JOINT

ZASTOSOWANIE TERAPII PRZECIWOBRZĘKOWEJ PO URAZACH STAWU SKOKOWEGO

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S u m m a r y

Traumatic injuries of the ankle joint are one of the most frequent types of injuries to a motor organ. They usually occur on one side of the body and most often involve twisting. The mechanical injury of the ankle joint often leads to post-traumatic lymphoedema**,** which is very difficult to treat and appears not only at the site of the injury but also in the distal parts of the limb. Lymphoedema of the limbs is a result of the disorder to the structure and function of the lymphatic system; it occurs as a result of the lack of outflow of tissue fluid and lymph from the tissues. The condition leads to a disorder in the function of tissues and organs and replacement of their cell structure by connective tissue**.** An initial slight and spontaneously subsiding swelling may lead to an extreme expansion of the lower limb, causing deformation and disability. The aims of the treatment are to stabilize the edema, reduce swelling, make the patient more comfortable, as well as to prevent and treat any complications. The elements of complex lymphoedema therapy include: skin care, compression and support using multilayered low stretch bandages, pneumatic compression, manual lymphatic drainage and exercises. In the majority of cases manual lymphatic drainage is the best form of treatment. The aims of the drainage are to ease the outflow of remaining lymph fluid, prevent diseases caused by its retention and eliminate lymphoedema and inflammation caused by the retention of lymph fluid. Drainage of lymphatic fluid has been particularly effective in combating swellings following injury to the ankle joint. It is interesting to note that the development of swellings following injury to the ankle joint is often ignored both by the patient and by the medical staff. Untreated lymphoedema, besides hampering physiotherapy treatment in patients, often leads to severe dermatological and neurological complications.

S t r e s z c z e n i e

Urazowe uszkodzenia stawu skokowego są jednymi z najczęściej występujących obrażeń narządu ruchu. Zwykle występują jednostronnie i najczęściej mają charakter skręcenia. Uraz mechaniczny często prowadzi do powstawania przewlekłego, niezwykle trudnego do wyleczenia obrzęku pourazowego występującego w miejscu urazu oraz w dystalnych częściach kończyny. Obrzęk chłonny kończyn jest wynikiem zaburzeń struktury lub funkcji układu limfatycznego; powstaje z powodu braku odpływu płynu tkankowego i chłonki z tkanek. Stan ten prowadzi do zaburzeń funkcji tkanek i narządów oraz zastępowania ich struktur komórkowych tkanką łączną. Początkowo nieznaczne, samoistnie ustępujące obrzmienie, może doprowadzić do ogromnych rozmiarów kończyny dolnej, powodując deformację i niesprawność. Celem leczenia jest stabilizacja obrzęku, jego zmniejszenie, poprawienie komfortu życia pacjenta, a także zapobieganie i leczenie powikłań. Elementami kompleksowej terapii przeciwobrzękowej są: pielęgnacja skóry, ucisk i opatrywanie bandażami o niskim stopniu rozciągliwości, kompresja pneumatyczna, drenaż limfatyczny oraz ćwiczenia rehabilitacyjne. W większości przypadków najlepszą metodą leczenia jest manualny drenaż limfatyczny. Celem drenażu jest ułatwienie odpływu zalegającej chłonki, przeciwdziałanie powstawaniu chorób wywołanych jej zastojem, likwidacja obrzęków zastoinowych chłonnych i zapalnych. Drenaż

limfatyczny szczególne zastosowanie znalazł w zwalczaniu obrzęków towarzyszących urazom stawu skokowego.

Na uwagę zasługuje fakt, iż występowanie obrzęków pourazowych często zostaje zaniedbywane zarówno przez pacjentów, jak i personel medyczny. Nieleczone obrzęki chłonne prócz tego, że utrudniają usprawnianie fizjoterapeutyczne pacjentów, prowadzą często do groźnych powikłań dermatologicznych i neurologicznych.

Key words: injuries of the ankle joint, lymphoedema of the lower limb, complex physical therapy, manual lymphatic drainage

Słowa kluczowe: urazy stawu skokowego, obrzęk limfatyczny kończyn dolnych, kompleksowy charakter fizjoterapii, manualny drenaż limfatyczny

THE INJURIES TO THE TIBIA-ANKLE JOINT– CLINICAL SYMPTOMS

In addition to its complex structure and activities, the tibia-ankle joint is constantly susceptible to different types of overwork. In most cases they occur due to medial injuries i.e. resulting from the forces which cause the excessive extent of physiological movements in the joint. The extent of the injuries to the tibia-ankle joint depends on the size of the force which causes the injury and the endurance of the bone tissue and ligaments. The mechanisms of the injuries to the ankle joint are complex and the variety of patomorphological and radiology images causes a lot of difficulties in their classification. However, certain regularities and a typical character of the images have been observed for a long time [1].

The fractures of the tibia- ankle joint differ according to the seriousness of the injury- from the closed ones caused by an injury which has a mild impact and which is stable and applies just to one ankle, to the open ones- caused by an injury with a powerful impact which leads to the fractures with joint dislocation, as well as to a wide damage of the soft tissues. The classification of the ankle fractures is complex. The most common classification is the Lauge-Hansen classification scheme which differentiates four main types of tibia-ankle joint fractures: supination-eversion rotation; supination-adduction, pronation-external rotation; pronation-adduction. The first word in each of these types describes the position of the foot at the time of the injury and the second term- the direction of the force which causes the injury.

The most common injuries are the supination-adduction ones. They result from the excessive adduction of the back of the foot applied when the foot is in its supine position. The characteristic symptoms of the first stage of this fracture are the swelling and soreness caused by the pressure in the area of lateral malleolus in its upper part or in the ankle-tibia joint. The pain increases, particularly during the attempts of the foot pronation.

Additionally, in the second stage a swelling appears on the anterior-medial side of the ankle. The livid skin in the area might spread down towards the foot. The attempts of passive and active movements are conducive to a feeling of pain on the lateral and medial side of the joint [2, 3].

The fractures and dislocation of the talus are particularly dangerous due to the fact that most of its surface takes part in the joint formation.

That is why its anatomical recreation is so crucial in maintaining the proper efficacy of the gait mechanism. The fractures of the calcaneus are usually caused by the axial force applied to the heel, most commonly resulting from an automobile accident or falling from a height. They can be bilateral, often involving the fracture of the lumbar spine. The injuries in the area of the lower part of the ankle joint after the fractures of calcaneus may lead to the lowering of the longitudinal foot curve, the position of the talus in the dorsal curve in the ankle-tibia joint and the widening of the diaphysis of the heel bone, as a result of which wearing shoes might cause pain [4].

The sprains of the ankle joint are the injuries which sportsmen very often suffer from. The dislocation of the ligaments in lateral malleolus occur most often whereas the sprain of the medial collateral ligaments or the dislocation which includes the damage to the tibia – fibular syndesmosis occur rarely. The sprained joint appears as a result of surpassing the proper range of the movements in this joint. After the impact of the traumatic force stops, the surfaces of the joint return to their proper location. The consequences of the sprain are the distention and tearing of the ligaments and the articular capsule. The sprain involves the outflow of blood into the articular cavity and its surrounding tissues, as a result of which an inflammation or livid spots in the injured part appear.

The tension and what follows the tearing of the ligament may occur in each of its parts, however the

injury often occurs in the place where they are attached to the bone. Sometimes the ligament is torn together with a fragment of the bone. In spite of no apparent changes visible on the radiograph, the pain is characteristic of the sprain injuries in the ankle joint. This symptom impairs the activity of the whole limb. It is caused by the rich innervation of the articular capsule and ligament apparatus. According to Dziak, the estimation of the level of the injury to the ligament elements is extremely difficult. That is why in the sprains of the ankle joint a three-degree division has been accepted:

I degree- a little fracture of the articular capsule, a slight swelling in the injured area with sensitivity above the ligament, no apparent changes on the X- ray pictures;

II degree- the tearing of the articular capsule, stretching or tearing of the ligament fibres; a considerable swelling, pain and haematoma, possible slight changes on an X ray picture in forced positions;

III degree- involves the entire tearing of the ligaments, a considerable swelling and haematoma, in the X ray picture the dislocation of the talus up to 15% (forced position) [4, 5].

THE INJURIES TO THE LATERAL SECTION OF THE ANKLE-TIBIA JOINT CONSTITUTE 85% OF FOOT SPRAINS

The tearing of the lateral ligaments of the ankle-tibia joint is usually caused by the excessive supination of the foot. A complex of ligaments from the lateral section of the joint consists of the following elements: the anterior talofibular ligament, the posterior talofibular ligament, the calcaneofibular ligament. The additional elements which support the lateral section are: the lower strap of the fibular muscle and the lateral talocalcaneal ligament. The ligaments of the lateral malleolus limit the interior rotation of the foot, the anterior displacement of the talus and inversion. The most characteristic symptoms of the injuries to the lateral section involve the pain in the area where the anterior talofibular ligament is attached to the talus, particularly while bending with overpressure. A complex of ligaments of the medial malleolus is mainly built up of the deltoid ligament – its superficial layer and deep layer. The supportive stablizers include fibular muscles, tibial muscles and Achilles tendon. Deltoid ligament limits the foot inversion and lateral displacement of the talus. The injuries to the medial section of the ankle occur rarely and they do not have any major clinical importance. The causes for the injury are the excessive pronation and exterior dislocation. The most exterior fragment of deltoid ligament (the tibiocalcaneal ligament), less frequently the tibiotalar ligament and the tibionavicular ligament, are broken off or torn. The characteristic symptoms of the injured articular capsule which is usually torn off in its anterior part are haematoma, swelling and soreness of the anterior area of the ankle-tibia joint [6, 7].

The ankle sprain means a permanent displacement of the bone ends of the joint from its natural attachment borders. When the displacement is incomplete, we call it the ankle sprain. The incomplete dislocation involves the injuries of the articular capsule, ligaments and joint cartilage whose range depends on the size and type of the injury. The sprain of the talus is caused by forced inversion of the foot together with the tearing of the talocalcaneal ligaments, talofibular ligament ligaments and talonavicular ligament. There are also some non-characteristic symptoms which involve pain and swelling. The specific symptoms are: the increased warming-up of the skin above the joint and the deformation of the joint outline caused by the displacement of the ankle. By pressing the vessels the sprained ankle causes the formation of haematoma, what is more it has an impact on the appearance of the threat of tissue necrosis [8, 9].

THE LYMPHATIC SYSTEM AND ITS FUNCTIONS

The lymphatic system should be treated as a heterogeneous group of tissues and organs, which constitutes a certain functional whole. That is why the lymphatic system comprises: cells- lymphocytes, macrophages, organised lymphoid tissues, such as lymph nodes, spleen, bone marrow, lymphoid tissues of intestine, lungs and liver, dendritic skin cells; tissues- intercellular space; liquids- tissue liquid and lymph [10, 11].

The functions the lymphatic system is responsible for can be classified in a detailed way, into a four-group division:

1. Assuring the return of the protein to the circular system from the beyond-vascular space, which means the maintenance of the proper content of tissue liquid and the primary matter of the tissue.
2. Transport of water with mineral salts**;** physiological drainage of the tissues. What is more- transport and

processing of the chemical products (molecules) released by the cells (enzymes, cytokines and other) as well as their subcellular structures (receptors, DNA fragments and other).

3. The removal (filtering out) of foreign organic compounds (bacteria, viruses, fungi) and inorganic compounds (carbon, silicon dioxide) which penetrate into the tissue spaces from outside (immunological processes of the system).
4. The removal of dead and mutated cells.

The lymphatic system of the lower limb and the venous system consist of the superficial and deep layer. The superficial lymphatic vessels are more numerous than the deep ones. They run mostly along the tibial vein and fibular vein (peroneal vein). The lymph circulation in the area of the lower limb is shaped by the deep popliteal lymph nodes and inguinal lymph nodes which can be found in the deep layer as well as in the superficial layer. The superficial layer of inguinal lymph nodes is located right beneath the skin in the inguinal area. The lymph nodes are located vertically and horizontally. The leading vessels of the vertical path transport lymph from the superficial vessels of the lower limb, and on its way accompany the tibial vein. On the other hand, the horizontal path receives the inflow from the exterior sex organs, perineum, buttocks, as well as from the anterolateral wall of the stomach and the fundus of uterus. Most of the efferent vessels flow into the deep inguinal lymph nodes, few of them to the lymph nodes in the hip area. Deep inguinal lymph nodes are few and located medially from the thigh vein. Lymph from the deep vessels and superficial inguinal lymph nodes, as well as from the vessels in the penis or clitoris reaches them. Few popliteal lymph nodes are deeply rooted in the adipose tissue. There are three groups of the popliteal lymph nodes: the posterior group, located entirely superficially, the lateral one from the arch of the peroneal vein, the medial group- i.e. a few tiny lymph nodes located medially and laterally in relation to the popliteal vessels; the anterior group is constituted of one lymph node located deep between popliteal artery and knee articular capsule. The popliteal lymph nodes are connected by the lymph canal which collects lymph from the foot, shank and knee joint. The efferent lymph vessels make a few lymphatic collecting trunks which form two groups: superficial and deep. The superficial vessels accompany the thigh-popliteal vein and flow into the vertical path of the superficial inguinal lymph nodes. The most important way which takes lymph from the popliteal lymph nodes is a deep group of vessels which is made up of a few vessels going in the direction of the deep inguinal lymph nodes together with the thigh vein. The superficial lymph nodes of the foot are most developed on the plantar side. The vessels which emerge from this network run up accompanied by the tibial and peroneal veins, some of them flow into the popliteal lymph nodes, the others (the smaller part) into the superficial inguinal lymph nodes. They drain lymph from the lateral part of the foot, the area of lateral malleolus, the posterior area of the shank. The rest of the foot is supplied by numerous lymph nodes which begin with the network winding round toes and medial malleolus. These vessels run on the anterior and posterior side of the ankle along the tibial vein and become joined with the superficial vessels of the buttocks, then they flow into the vertical path of the superficial inguinal lymph nodes. The deep lymph vessels are connected by the junctions with the network of superficial vessels and they form the main and additional pathways. The main pathway is created by thigh vessels and it leads to the deep inguinal lymph nodes; whereas the additional one is formed by the obturatory lymph nodes and the lymph nodes in the buttocks, upper and lower. These vessels lead lymph to the internal lymph nodes in the hip [11, 12].

PATHOPHYSIOLOGY OF LYMPHOEDEMA

Lymphoedema results from the disturbance in the transport of lymph in the lymphatic system; i.e. the concentration of the substances overburdening the lymphatic system (water, protein, fat and cells) in the subcellular space exceeds the capability of the system to transport them into the blood circulation. The protein substances which overburden the lymphatic system are mainly the proteins of blood plasma, which continuously leave the blood vessels; the water burden is the water which is filtered on the arterial side of the capillary vessels and is not reabsorbed on their venous side. What is more, the migrating and settled lymph cells, metabolic products, the cells which undergo apoptosis, endothelial cells and other overburden the lymphatic system. The lack of proper treatment of the lymphoedema leads to its constant progression, and in the end the tissues overgrow and become fibred: proliferation of keratinocytes, fibroblasts and accumulation of collagen [13].

As far as the lower limbs are concerned, lymphoedema is caused by impairing the efficacy of the lym-

phatic system, which stems from its functional and structural disturbances. As a result, after the capabilities of the system are exhausted, the transport of the lymph becomes difficult. The accumulation of the liquid which is rich in protein in the beyond-vascular space is clinically apparent by the growth of swellings in the toe, the top of the foot, shank or thigh. The disturbance of the lymph drainage may lead to the colonization of the swollen tissues by the microorganisms which penetrate the skin and it also contributes to the development of the inflammation.

The most popular division of the lymphoedema has been based on the pathophysiological criteria.

1. primary lymphoedema:
   - congenital lymphoedema- is present from birth, caused by the lack or insufficient formation of lymph vessels, it has a genetic background, can be a hereditary illness;
   - lymphoedema praecox – appears at the age of puberty, most commonly caused by an infection in the limbs, an injury or a rapid growth which leads to the underdevelopment of lymph vessels;
   - lymphoedema tarta- develops due to the above mentioned reasons, as well as might be caused by pregnancy after the age of 30.
2. secondary lymphoedema:
   - caused by the closing of the lymph vessels by the external factors (pressure by the tuber, becoming fibred) or internal (bacteria or parasitic infections)
   - caused by the damage to the lymph pathways and lymph nodes resulting from an injury (fracture, dislocation, bruise), surgery (mechanical damage to the lymph vessels or surgical removal of lymph nodes), radiotherapy, the lack of limb function (paralysis, paresis, long lasting limb immobilization), insufficiencies of the venous system or surgical operations, reconstruction operations of the arterial system in the lower limbs, rheumatic illnesses [14].

Pathologic lymphoedema occurs when the growth in the volume of a particular area exceeds 30%. The reasons why the volume of the tissue liquid increases and the lymphoedema appears are: the increase of the filter pressure which involves the widening of arterioles, the narrowing of the veins, the increase of the vein pressure caused by, for example, the inefficiency of the circulation, valvular insufficiency, the influence of gravitation, the decrease in the osmotic pressure gradient caused by the decrease in the concentration of protein in blood plasma and the accumulation of active osmotic agents in the space filled by the extracellular liquid, the increase of penetrability of capillary vessels (histamine and supportive substances, kinines); the insufficient lymph flow.

The post-traumatic lymphoedema develops early after open fractures as well as closed bone fractures and the injuries of lower limb soft tissues, treated surgically or by means of preservative treatment- the external immobilization in a plaster dressing. In most of the cases a thrombosis of the deep veins occurs. It is characterized by the appearance of a characteristic swelling with the symptoms of the injury. The enlargement of regional lymph nodes can be observed. In advanced stages it can lead to the skin reverse flow and the closure of the section of the lymph vessels' trunks.

Brunner's classification of lymphoedema of lower limbs involves five degrees.

The first degree - a mild, soft edema which involves the foot and shank, occurs by the end of the day, disappears idiopathically after lifting the limb. Daily swelling which disappears idiopathically after the night with positive Stemmer's sign (the thickening of skin folds under the second toe, skin that is hard to lift) is characteristic for the second degree. The third degree always requires physiotherapic treatment - it is a permanent edema which does not disappear after lifting the foot. In the fourth degree pharmacological treatment must be applied (a permanent edema which deforms the limb, often complicated by different inflammatory skin changes: eczema, erysipelas, lymph shunting. In extreme situations the so-called Elephantiasis, or Lymphatic Filariasis – a gross enlargement which deforms the limb might appear. Stage fifth is characterized by the thickening of the skin (scleroderma) and muscle changes (dystrophy) which distorts the limb functions [15, 16].

METHODS OF DIAGNOSIS

The information about the beginning of the disorder, the precise reasons for the development of the disorder, as well as the types of discomfort, the progress of the disorder, the size and location of the tissue edema are extremely crucial in the proper diagnosis and classification of the disorder.

The diagnosis of the lymphoedema should always involve physical examination which is aimed at finding the edema, determining its advancement stage and the introductory differential diagnosis. We start by a precise examination of the lower limbs. The presence of lymphoedema depends on its asymmetric location, a characteristic deepening of the transverse skin fold, the edema of the skin surface. Lymphoedema is pale, painless, which differs from a bluish- red edema which appears during the venous insufficiency.

What is more, as far as lymphoedema is concerned, the skin temperature is not increased (venous edema involves an excessive skin warming), there is also no considerable pain involved. Warts, erysipelas, toe mycosis are also found in lymphoedema. Physical examination should be supplemented by the measurement of the lower limb periphery in the foot area, ankle, shank and thigh of both lower limbs. This measurement, conducted always in the same spots, is aimed at ascertaining the efficiency of applied treatment or the progress of the disorder. Additionally, a precise measurement of the lower limb assures choosing the proper size of compression materials (stockings, tights) [17].

The pictorial diagnostic examination (first throw examination) involves ultrasonography of soft tissues and Doppler ultrasonography. Doppler ultrasonography is particularly aimed at eliminating the venous insufficiency as the cause of the edema. What is more, by means of this examination the state of arterial vascularity before the application of the scheduled compression treatment is assessed.

Computer tomography and magnetic resonance imaging are rarely employed. They provide information about the location, size, the content of the liquid, muscular changes in an advanced lymphoedema of the lower limb.

Lymphography (second throw examination) is a contrastive examination of lymph nodes, aimed at exposing the anatomical structure and function of the lymphatic system. Direct lymphography is based on adding a contrastive agent to a lymph vessel. This examination is burdened by a large degree of complications, that is why it is rarely performed. Indirect lymphography involves putting the contrast into the foot skin. Nowadays it is also occasionally performed. Isotope lymphography (lymphoscintigraphy) involves injecting a colloid marked by technetium. Anatomical and functional state of lymphatic system is assessed. Due to the lack of complication risk it is performed most often [17].

THE TREATMENT OF POST TRAUMATIC EDEMA OF LOWER LIMBS

Treating post traumatic edema after the injuries to the ankle is not easy and that is why its broad view should be considered. A complex anti-edema therapy comprises: skin care, kinesitherapy, compressotherapy (stretchy sleeves, compression stockings, bandaging), pneumatic massage, lymphatic drainage, automassage, electrical stimulation (TENS), pharmacological treatment, educating the patient and the family. The objective of the physiotherapeutic treatment of lymphoedema is fighting against disorders associated with it. Reducing the volume of the lower limb, the improvement of the consistency of the injury-afflicted tissues, the restoration of joint flexibility, the decrease of the body weight are crucial.

As far as the patients with lymphoedema are concerned, looking after the skin and toe nails and observing the areas which might be the potential infection gateways should be applied. This procedure should involve: moisturizing dry skin with the cream which does not contain active and irritating agents, the inflammation of vellus hair necessitates washing the skin with a solution which acts as a disinfectant and dries the skin; lubricating the skin and using keratinolytic substances, skin protection (wearing gloves during household chores or other chores, wearing comfortable shoes which do not cause attrition and giving up cosmetic activities which might damage cell continuity).

Among the methods of pressotherapy of the first stage manual lymph drainage MLD and compression treatment can be mentioned; supportive methods involve lifting the limb and treating it by means of heat (local influence of temperature approximately 41 degrees decreases the temporary volume of lymph, warm compress of therapeutic mud, applying heat on the whole body- warm baths, sauna are contraindicated).

The importance of bandaging during the treatment of lower limb edema has already been known in the ancient times, which can be proved by the paintings on the Sahara walls made four thousands years ago. The research indicates that bandaging increases the absorption of protein and water. While applying this method of treatment the proper type of bandage, technique of putting it on and the clinical condition of the patient should be considered. The bandages which are characterized by low or average tensility are a source of the

proper pressure while resting or doing physical activities. A multi-layered bandage application is advised in lymphoedema treatment.

Compression stockings and tights have been widely used in treating lower limb lymphoedema. Made from nylon, cotton with the addition of rubber and depending on the compression category, these products are characterized by a precisely stated compression

on the lower limb defined in mmHg. The condition of the successful treatment is the size and the type of compression properly chosen for a particular patient. The research proves that the compression has a positive impact on the lymph flow, however it should be emphasized that these products only have a subsidiary character in lymphoedema treatment.

Compression therapy products are manufactured in four compression categories.

I (20-30 mmHg) applied in the first degree of lymphoedema, II (30-40 mmHg) used in the second and third degrees of lymphoedema, IV (50-60 mmHg) used in degrees IV and V of lymphoedema. The contraindications of its application are the chronic ischemia of the lower limbs and skin inflammation, lymph shunting and considerable limitation of the joint movability, particularly in elderly patients.

Another achievement in the treatment of the lower limb lymphoedema is the device for the compression massage. The device is equipped with a rubber cuff which consists of from 5 to 12 chambers, which is put on the swollen lower limb. Pumping performed by each of the chambers mechanically removes the edema. These interventions are particularly effective after the previous application of manual lymphatic drainage [18, 19, 20].

Kinesitherapy involves all types of exercises which facilitate the drainage (outflow) of lymph from the lower limb (isometric and respiratory), which support the muscular pump (active free and in alleviation) and relaxing. The exercises should be done in the positions which facilitate the gravitational lymph outflow (lying on the back with lifting lower limbs) a few times a day, in short sessions with few repetitions. A high intensity might be the cause of post traumatic lymhoedema.

Surgical treatment of lymphoedema is based on cutting out hypodermic tissue with fascia. Nowadays thanks to the development of micrurgy, ligament-vein junction, lymph node-vein junction and the vein insertions which contain a valve injected into the lymphatic vessel (i.e. lymphatic-venous-lymphatic anastomosis) are performed [19].

MANUAL LYMPHATIC DRAINAGE

The methods of lymphatic drainage used in Poland are based on the techniques suggested in the 30s of the last century by E Vodder- a Danish doctor- who dealt with the examination of lymphatic drainage, its influence on the organism and protective function which improves the circulatory system.

In order to apply drainage as one of the methods of anti-lymphoedema therapy a few rules of its use should be considered:

1. It is applied according to the recommendations and contraindications. It is particularly important in case of the patients who suffered from infectious illnesses, quite a severe inflammation with edema, after amputation and resection in the cancer treatment. More freedom can be applied in cases of post traumatic edema (twisting, fractures, post traumatic amputations). Lymphatic drainage is recommended for all the injuries which are associated with edema, exudation, and skin changes which stem from the disorders in lymph circulation. The contraindications include edema and exudation in severe inflammatory conditions, untreated infectious diseases and during the course of cancer illnesses (there is a risk of metastasis).
2. Drainage is conducted in the direction from the periphery to venous outlets. In order to start drainage we must first take into consideration the lymph nodes and lymph vessels which are located in the nearest position to the venous outlets. Then the next steps are taken by keeping the same direction. Such procedure allows the lymph to flow into the circulatory system and enables the mechanisms conditioning its flow to act. That is why lymphatic drainage of the lower limb should be started in the area of inguinal lymph nodes, then the lymph vessels in the thigh should be drained in the direction from the knee joint to the hip joint. First we focus on the lymph nodes in the knee joint, next lymph vessels in the shank in the direction from the ankle to the knee joint, the lymph nodes of the ankle, the lymph vessels of the metatarsus, toes (in the direction from toes to the ankle).
3. While applying lymphatic drainage the position of the patient, which facilitates the outflow of lymph is essential. In case of the drainage in lower limbs it is important that the lower limbs are located near the thorax and there should be a slight bend in the hip and knee joint (it will decrease the muscular tension and facilitate the lymph flow).

4. Drainage movements should be made in a slow manner (the lymph current necessitates it). All the techniques should be done with the frequency of about 10- 15 movements per minute. However, a larger number of repetitions is required (lymphatic drainage is the most efficient if each technique is repeated 3-5 times).
5. Grasps should be applied smoothly and softly, it loosens soft tissues of vessels and skeletal muscles.
6. The movements which are applied during the technique must be pushing movements. That is why we always perform stroking from distal to proximal parts. Rubbing is characterized by a gradual increase of pressure on the tissue by moving in the direction of the grasp and releasing the compression while going back or moving towards the distal areas.
7. The length of the session depends on the patient, however it should not exceed 20 minutes in case of partial lymphatic drainage massage and 60 minutes in case of a full lymphatic drainage massage. It can be performed every day and in order to strengthen the obtained results 2 or 3 massages weekly are recommended. The reaction of the patient is dependant on the patient's health condition.
8. During the massage the lymph nodes should be taken care of at least twice. As far as the lower limbs are concerned the same applies to the hip joint, the knee joint and the ankle.
9. The extent to which the lymphatic drainage massage should be applied on the body depends on the patient's condition and the type of illness, as well as the response of the patient's body to the massage.

Following the above mentioned rules meticulously allows to achieve the desired effects of the treatment [11, 21].

Manual lymphatic drainage is based on four basic types of grasps: stroking, circular rubbing, spiral-like rubbing and pressing. General stroking is aimed at preparing a large area for the massage (e.g. stoking the whole lower limb).

Local stroking is included in the proper massage, ideally adjusted to the shape of the area where the massage is applied. (e.g. stroking the shank).

Circular rubbing is similar to the segmentary technique in the massage (a triple rubbing in one spot, next moving with pressing forward and backward reducing the degree of massaging).

Spiral rubbing is performed similarly to the classical massage technique, the only difference is associated with the fact that in lymphatic drainage technique the rate of applying the grasp is much slower and the degree of pressing is increased while moving in proximal direction and reduced while returning in the distal direction. In lymphatic drainage only longitudinal pressing is performed, which is very slow and has a relaxing and draining impact. Pincer compression is a technique similar to the classical massage technique with an additional draining element. This compression is based on rolling the hand over the area the massage is applied to. It can be performed with the use of one or both hands (pumping in the popliteal fossa) [22, 11, 12].

The principles of lymphatic drainage should not be perceived as the fixed ones. An individual approach to each patient is crucial. The way of performing the manual lymphatic drainage and the intensity of the treatment should be defined by the physiotherapist. Most importantly, however, lymphatic drainage should be performed in such a way that it becomes an effective method which is not harmful to the patient. This technique should only help to obtain the best result.

CONCLUSION

Lymphoedema, regardless of its origin, is a serious problem for the patient and the physiotherapist. In most cases manual lymphatic drainage, combined with a compression therapy, is the best method. However, it requires a lot of patience from the patient. It is often very hard for a patient to accept the fact that lymphoedema is often a long-continued illness and the only possible method of treatment is a symptomatic treatment, which should be repeated. It should be emphasized that a complex anti-edema therapy is quite easy, it does not require a lot of expenditures and can be performed in most of the wards, even in out-patients' departments. Since so many patients suffering from post-traumatic edema still remain untreated, the therapy described above gives hope to those patients for the improvement of their heath and life.

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REVIEW / PRACA POGLĄDOWA

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CANCER STEM CELLS: CONCEPT AND MARKERS

NOWOTWOROWE KOMÓRKI MACIERZYSTE: KONCEPCJA I MARKERY

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S u m m a r y

Normal stem cells in the adult organism are responsible for tissue renewal and repair of aged or damaged tissue. The concept of cancer as a stem cell disease has the potential to dramatically change our view of the problem of its treatment. The existence of a cancer stem cells population is characterized by (a) an observation that only a minority of cancer cells within each tumor are usually responsible for oncogenic potential; (b) a distinctive profile of surface markers; (c) ability for recreating the full phenotypic heterogeneity of the parent tumor. Cancer stem cells can be identified by: side population, specific markers and self-renewal pathways. By separating the disease into a stem cell activation phase and a tumor progression phase, historical cancer studies can be reinterpreted with new understanding. Research efforts to understand the growth of tumor stem cells as well as to identify tumor stem cell antigens could lead to new targeted approaches.

S t r e s z c z e n i e

Normalne komórki macierzyste w dorosłym organizmie są odpowiedzialne za procesy odnowy i reparacji zużytych i zniszczonych tkanek. Koncepcja nowotworu jako choroby komórek macierzystych niesie potencjał dramatycznej zmiany punktu widzenia choroby nowotworowej i możliwości terapeutycznych. Istnienie nowotworowych komórek macierzystych jest określone przez (a) obserwację, że jedynie mniejszość komórek nowotworowych w obrębie nowotworu jest odpowiedzialna za potencjał onkogenny; (b) specyficzny profil markerów komórkowych; (c) zdolność do odtworzenia w hodowli właściwości fenotypowych pierwotnego guza. Nowotworowe komórki macierzyste mogą być identyfikowane przez: populację boczną, specyficzne markery oraz szlaki samoodnowy.

Dzięki wyodrębnieniu w rozwoju nowotworu fazy aktywacji komórkowej oraz fazy progresji guza, historyczne badania nad nowotworami, mogą być zreinterpretowane w nowym znaczeniu. Dalsze badania nad rozwojem nowotworowych komórek macierzystych oraz zidentyfikowanie kolejnych markerów komórek macierzystych może doprowadzić do rozwoju nowych możliwości terapeutycznych.

Key words: cancer stem cells, leukemic stem cells, side population, stem cell markers and immunophenotype
Słowa kluczowe: nowotworowe komórki macierzyste, białaczkowe komórki macierzyste, populacja boczna, markery, immunofenotyp

NORMAL AND MALIGNANT STEM CELLS

Normal stem cells in the adult organism are responsible for tissue renewal and repair of aged or damaged tissue. A substantial characteristic of stem cells is their ability for self-renewal without loss of proliferation capacity with each cell division. The stem cells are immortal, and rather resistant to action of drugs [1].

The existence of a cancer stem cells (CSC) population is characterized by (a) an observation that only a minority of cancer cells within each tumor are usually responsible for oncogenic potential; (b) a distinctive profile of surface markers; (c) ability for recreating the full phenotypic heterogeneity of the parent tumor

[2, 3]. Malignant stem cells have been described as the source of several types of human cancer. These unique cell types are typically rare and possess properties that are distinct from most other tumor cells.

DEFINITIONS

Stem cells (SC) – Cells which have three distinctive properties: self-renewal, the capability to develop into multiple lineages, and the potential to proliferate extensively.

Cancer stem cells (CSC) – The cancer stem cells constitute a small subset of cancer cells being a reservoir of self-sustaining cells with the exclusive ability to self-renew and maintain the tumor. These cancer stem cells have the capacity to both divide and expand the cancer stem cell pool and to differentiate into the heterogeneous non-tumorigenic cancer cell types that in most cases appear to constitute the bulk of the cancer cells within the tumor.

Leukemic stem cells (LCS) – Cancer stem cells originating acute or chronic leukemia.

Hematopoietic stem cells (HSC) – Stem cell responsible for repopulating of bone marrow.

Multidrug resistance (MDR) – Simultaneous resistance to several structurally unrelated drugs that do not necessarily have a common mechanism of action.

Side population (SP) – Population of cells gated in flow cytometry analysis, which do not accumulate the fluorescent dyes Hoechst 33342 and rhodamine 123. Most cells accumulate these dyes, while stem cells do not, as these compounds are effluxed by ABCG2 (BCRP) and ABCB1 (PGP) proteins.

Cluster of differentiation (CD) – Molecules on the cell surface, as recognized by specific sets of antibodies, used to identify the cell type, stage of differentiation and activity of a cell.

THE CANCER STEM CELL HYPOTHESIS AND ITS IMPLICATIONS

The concept of cancer as a stem cell disease has the potential to dramatically change our view of the problem of its treatment. By separating the disease into a stem cell activation phase and a tumor progression phase, previous cancer studies can be reinterpreted with new understanding. Research efforts to understand the growth of tumor stem cells as well as identify tumor stem cell antigens could lead to new targeted approaches. Normal stem cells are relatively drug resistant. Probably cancer stem cells are also refractory to therapies that have been developed to eradicate the rapidly dividing cells within the tumor that constitute the majority of the non-stem cell component of tumors, thus they are unlikely to be curative and relapses would be expected. The cancer stem cell hypothesis will require changes in the diagnosis and treatment of tumors.

Carcinogenesis possibly arises from neoplastic stem cells. Cancer stem cells can arise from a stem cell losing growth regulation could directly become a cancer stem cell, or a mature (i.e., differentiated or committed) cell could acquire the properties of self renewal and become a cancer stem cell [4-6]. A fraction of cells in a tumor are known to survive radiation treatment and cytotoxic drug exposure. Stem cells express drug transporters, DNA repair systems, and are refractory to programmed cell death, all properties that would serve to allow a cancer cell to resist our efforts to eliminate it [7-9]. Some form of normal stem or progenitor cell undergoes a mutation, giving rise to an entity that is functionally defined as a leukemic stem cell. The normal stem cells continue to differentiate into the hematopoietic lineage giving rise to erythrocytes, platelets, leukocytes, and granulocytes. The mutated stem cells have properties similar to the normal stem cells and can also differentiate into the hematopoietic lineage carrying the defect/s or can remain and accumulate as blast cells [10].

DEVELOPMENT OF A CONCEPT OF CANCER STEM CELLS

In 1977 Fialkow et al provided evidence of the stem cell origin of a human hematologic malignancy [11]. In 1977 Hamburger and Salmon, showed the relevance of stem cells in biology of tumors [12]. The first isolation of leukemia stem cells was done in human acute myeloid leukemia after transplantation into SCID mice [13]. The first isolation of cancer stem cells in a solid tumor was performed in breast cancer cells [14] (Table 1).

Table 1. *History of development of cancer stem cell conception*

Tabela 1. *Historia rozwoju koncepcji nowotworowych komórek macierzystych*

Year	Facts
1977	Fialkow et al: possible relationship between HSCs and human leukemias [11].
1977	Hamburger and Salmon: subpopulation of cells in a tumor will grow in soft agar, and termed these cells "tumor stem cells" [12].
1992	Reynolds and Weiss: isolation a small population of cells (<0,1% of total cells) from the adult striatum that could proliferate and generate multipotent clones of cells (neurospheres) [15, 16].
1994	Dick et al: cancerous stem cells were present in acute myelogenous leukemia by isolating such cells and documenting their self-renewing capacity, which is the critical property of all stem cells [13, 17].
1994-1997	Dick et al: [CD34+, CD38-] leukemic stem cells retained differentiative capacity, giving rise to CD38+ and Lin+ populations [13, 17].
2000	Miyamoto et al.: first study showing that CSC populations can change during disease progression in a human hematologic malignancy [18].
2003	Al-Hajj et al.: first isolation of cancer stem cells in a solid tumor [14].
2003	Hemmati et al.: description of the isolation of tumor stem cells from pediatric brain cancers [19].

STEM CELLS: SIDE POPULATION CELLS

Stem cells can be sorted by flow cytometry as cell population not accumulating fluorescent dyes, such as Hoechst 33342 and rhodamine 123, as these compounds are effluxed by drug resistance proteins ABCG2 (BCRP) and ABCB1 (PGP). These cells are referred to as "dull cells" or "side population" (SP) cells (Figure 1). A large fraction of hematopoietic stem cells are found in the SP fraction and when isolated from mice and transplanted into irradiated mice, small numbers of these SP cells can reconstitute the bone marrow [20]. SP cells can be isolated from many tissues including the brain, breast, lung, heart, pancreas, testes, skin and liver, and these cells might represent lineage-specific stem cells [7, 20-27].

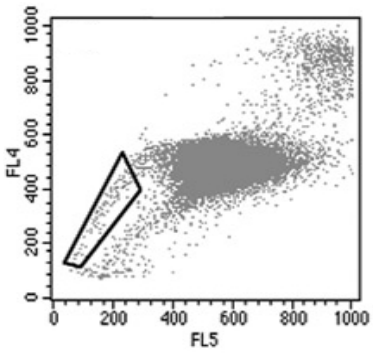

Fig. 1. *Side population detected by flow cytometry (fluorescences and stains: FL5 – red Hoechst, FL4 – blue Hoechst)*

Ryc. 1. *Populacja boczna w obrazie cytometrii przepływowej (fluorescencje i barwniki: FL5 – red Hoechst, FL4 – blue Hoechst)*

STEM CELL MARKERS

Some of the stem cell markers are distributed widely throughout different organs and tissues, other stem cell antigens may be restricted to certain organ systems (Table 2). Examples of broadly expressed stem cell markers are CD133, BMI, Mushi-1 antigen, and Oct-4. In several organs, gap junctional intercellular communication-linked antigens (GJICs) are expressed preferentially in mature cells, and loss of it may be indicative of malignant transformation. Although stem cells have been discovered in several organs, little is known regarding their phenotype. An exception is the HSC, which represents a well defined cell in terms of function and phenotype. HSC is characterized as Lin-/CD45+/CD34+/CD38- cells.

CANCER STEM CELL MARKERS

In the hematopoietic system as well as in other normal tissues, the normal stem cell must be both self-renewing and pluripotent. Although stem cells can self-renew, they are generally quiescent, spending most of their time in G0. This cell population is characterized by a specific surface marker phenotype that, remarkably, is negative for expression of all lineage-specific differentiation antigens (Lin neg) [28] (Table 2). Studies of acute myelogenous leukemia (AML) have shown that only 0,1-1% of all cells have leukemia-initiating activity [13]. Stem cell cannot be defined based solely on surface markers in the absence of linking marker expression to a self-renewal assay. None of the markers used to isolate stem cells in various normal and cancerous tissues is expressed exclusively by stem cells (Table 3). Cells of each type of AML have been isolated and divided into two subtypes with phenotypes CD34+CD38+ and CD34+CD38-. Antigen CD133 was used to successfully enrich for brain tumor stem cells, but it is also present on normal brain stem cells and on many non-stem cells in various tumors and tissues. The same is true for other commonly used markers, such as CD44, Sca1, and Thy1. The vast majority of cells that express these markers are not stem cells. Markers used to identify stem cells from one organ are frequently not useful for identifying stem cells in other tissues, such as Sca-1, which is useful for the identification of murine blood stem cells.

Table 2. *Surface antigens as stem cell markers*
Tabela 2. *Antygeny powierzchniowe o charakterze markerów komórek macierzystych*

Stem cell marker	Characteristics
CD34	A cell surface glycoprotein which functions as a cell-cell adhesion factor. It may also mediate the attachment of stem cells to bone marrow extracellular matrix or directly to stromal cells.
CD38	A surface glycoprotein present on the of many immune cells; marker of cell activation.
CD44	A cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration.
CD90 (Thy1)	Glycophosphatidylinositol (GPI) anchored conserved cell surface protein with a single V-like immunoglobulin domain, originally discovered as a thymocyte antigen.
CD105	Endoglin, a type I membrane glycoprotein located on cell surfaces and is part of the TGF beta receptor complex.
CD117	A cytokine receptor expressed on the surface of hematopoietic stem cells as well as other cell types. CD117 is the receptor for the cytokine stem cell factor (SCF), also known as "steel factor" or "c-kit ligand".
CD133	A glycoprotein also known in humans and rodents as Prominin 1 (PROM1). CD133 is expressed in hematopoietic stem cells, endothelial progenitor cells, glioblastomas, neuronal and glial stem cells and some other cell types.
CD135	A cytokine receptor expressed on the surface of hematopoietic progenitor cells.
CD150	A marker of various progenitor cells; belongs to SLAM (Signaling lymphocyte activation molecule) family.
Sca1	A marker of murine hematopoietic stem cells.

Table 3. *Phenotype of various stem cells*
Tabela 3. *Fenotyp różnych komórek macierzystych*

Specific stem cells (SC)	Markers
Human bone marrow SC population	CD34+, CD38-, Lin-, Thy-1+
Unknown SC population	CD34+, CD38-, Lin-, Thy-1-
CD34+, CD38+, Lin+	committed progenitors and mature elements
Acute myeloid leukemia	CD34+ CD38- CD44+ CD123+ (IL-3 receptor α chain), CD33+
Chronic myeloid leukemia	translocation t(9;22)(q34;q11)
Breast cancer	CD44+, CD24−/low, Lin–
Breast cancer	CD24+ CD44+ B38.1 ESA
Glioblastoma multiforme and medulloblastoma	CD133+
Glioblastoma	Lin-, CD133+, nestin
Prostate cancer	CD44+, SP cells (Hoechst dye excluding)
Lung	Bronchioalveolar stem cells (BASCs)
Multiple myeloma	subpopulation of CD138- cell
Squamous cell tumors	CD29+
Pancreatic cancer	CD44+, CD24+, and epithelial-specific antigen (ESA)
Head and neck squamous cell carcinoma	CD44+, BMI1+, cytokeratin

THE LEUKEMIC STEM CELL

Leukemic stem cell (LSCs) can be isolated based on their cell surface markers using the currently available cell-sorting technologies [10]. Tumor-associated properties in the LSC could include mutations in the kinase domains, transcription factors, and tumor suppressors, or alterations in the growth and survival mechanisms mediated through NF-kappa B or PI3 kinase, or changes in physiology, glucose metabolism, or responses to oxidative stress. LSCs in AML was a first population of CSC detected. Recently, LSCs for childhood acute lymphoblastic leukemia of B-lineage was detected among population with TEL-AML rearrangement [29].

CANCER STEM CELLS IN SOLID TUMORS

Evidence for the existence of cancer stem cells in solid tumors has been more difficult to obtain for several reasons. Cells within solid tumors are less accessible, and functional assays suitable for detecting and quantifying normal stem cells from many organs have not yet been developed. Non-stem cells can be converted into cancer stem cells. Activation could occur through hormonal stimulation; tissue damage caused by inflammation, radiation, chemicals, or infections; or inactivation of certain tumor suppressor genes [30]. Gene mutations can contribute to improper function of metabolic and signaling pathways. BRCA1 and BRCA2 mutations are thought to reduce the capacity for DNA repair and therefore raise the risk of breast cancer, by increasing the probability of downstream genetic events associated with tumor progression. The similar role have BCR-ABL and MLL rearrangements in leukemias. For renewal tissues such as the colon or skin, the stem cells divide at a very slow rate. Other childhood cancers such as Wilms tumor and leukemias arise from genetic events in kidney and hematopoietic stem cells, respectively. These types of cancer require the fewest genetic events, as the target cell is a fully activated stem cell. Stem cells are also be activated by hormones and/or growth factors in conditional growth tissues, as described by Knudson [31]. An example is the breast, which undergoes dramatic growth during puberty and is then stimulated by estrogen and other hormones monthly during the woman's reproductive life [32]. Activated stem cells in the breast would be the target cell for breast cancer. Inactivation of P53 and other genes would transform breast tissue stem cells into hyperplastic lesions, the target for further events

leading to the progression of pre-malignant cells to malignancy.

CONCLUSIONS

The concept of cancer as a stem cell disease has the potential to dramatically change our view of the problem. The discovery of cancer stem cells in solid tumors has changed our view of carcinogenesis and chemotherapy. There is now abundant evidence that stem-cell properties are highly relevant to the biology of several human cancers. By separating the disease into a stem cell activation phase and a tumor progression phase, historical cancer studies can be reinterpreted with new understanding. Research efforts to understand the growth of tumor stem cells as well as to identify tumor stem cell antigens could lead to new targeted approaches.

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REVIEW / PRACA POGLĄDOWA

Jan Styczyński

CANCER STEM CELLS: SELF-RENEWAL PATHWAYS

NOWOTWOROWE KOMÓRKI MACIERZYSTE: SZLAKI SAMOODNOWY

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S u m m a r y

Cancer stem cells have been described as a rare population of cancer cells exhibiting stem cell properties such as self-renewing, differentiation, tissue reconstitution, and multiple drug resistance. Cancer stem cells and "normal" (embryonic and adult) stem cells share several common features. At the molecular level, distinct sets of genes have emerged that can be associated with "stemness". In particular the Wnt (Wnt-3, β-catenin), Notch (Notch-1) and Hedgehog (Gli1 and 2, PTCH1, SMO) signaling pathways, the polycomb family transcriptional repressor Bmi1, octamer-binding transcription factor Oct-4, the drug efflux pump breast cancer resistance protein (BCRP), and human telomerase reverse transcriptase have been associated with stem cells. Research efforts to understand the growth of tumor stem cells as well as to identify tumor stem cell pathways of self-renewal could lead to new targeted approaches.

S t r e s z c z e n i e

Nowotworowe komórki macierzyste są populacją rzadkich komórek nowotworowych wykazujących właściwości komórek macierzystych obejmujących zdolności do: samoodnowy, różnicowania, odnowy tkankowej i oporności wielolekowej na cytostatyki. Nowotworowe i normalne (embrionalne i somatyczne) komórki macierzyste posiadają wiele cech wspólnych. Na poziomie molekularnym jest to związane z licznymi zestawami genów charakterystycznych dla komórek macierzystych. W szczególności obejmują one następujące białka i szlaki sygnałowe: Wnt (Wnt-3, β-catenin), Notch (Notch-1) i Hedgehog (Gli1 and 2, PTCH1, SMO), represor transkrypcyjny rodziny białek polycomb - Bmi1, czynnik transkrypcyjny Oct-4, białko oporności wielolekowej BCRP (breast cancer resistance protein), oraz odwrotną transkryptazę ludzkiej telomerazy. Dalsze poznanie mechanizmów decydujących o wzroście komórek macierzystych nowotworu oraz szlaków sygnałowych samoodnowy może przyczynić się do rozwoju nowych metod terapeutycznych.

Key words: cancer stem cells, leukemic stem cells, self-renewal, metabolic pathways, stem cell markers
Słowa kluczowe: nowotworowe komórki macierzyste, białaczkowe komórki macierzyste, samoodnowa, szlaki metaboliczne, markery komórek macierzystych

STEM CELL SELF-RENEWAL PATHWAYS

Cancer stem cells (CSC) have been described as a rare population of cancer cells exhibiting stem cell properties such as self-renewing, differentiation, tissue reconstitution, and multiple drug resistance. Cancer stem cells and "normal" (embryonic and adult) stem cells share several common features. At the molecular level, distinct sets of genes have emerged that can be associated with "stemness".

In particular the Wnt (Wnt-3, β-catenin), Notch (Notch-1) and Hedgehog (Gli1 and 2, PTCH1, SMO) signaling pathways, the polycomb family transcriptional repressor Bmi1, octamer-binding transcription factor Oct-4, the drug efflux pump breast cancer resistance protein (BCRP), and human telomerase reverse transcriptase (hTERT) have been associated with stem cells. Biochemical pathways that are active in the ma-

jority of tumor cells might be of little functional relevance for the biology of CSC, whereas biochemical pathways active only on a small minority of cancer cells might play key roles in CSC biology and thus in the overall long-term behavior of a tumor.

MOLECULES AND SIGNALING PATHWAYS OF STEM CELL

The Bmi1 oncogene, a member of the Polycomb group ring finger (PCGF) gene family, was shown to be expressed at high levels in hematopoietic stem cells (HSCs) and progressively down-regulated during hematopoietic differentiation [1, 2] (Table 1). A second molecule that is likely to play a key role in the molecular machinery of both HSC and LSC (leukemic stem cells) self-renewal is the protein phosphatase and tensin homologue (PTEN), a known tumor suppressor [3]. Genes required for self-renewal of normal HSCs can play opposite roles in the development of leukemia. In some cases they are necessary for long-term expansion of the transformed clone (Bmi1), but in others they act as tumor suppressors and prevent leukemic transformation (Pten).

Table 1. *Stem cell markers in normal and cancer stem cells*
Tabela 1. *Markery normalnych i nowotworowych komórek macierzystych*

Markers Markery	Description Charakterystyka
BMI-1	Oncogen present in HSC cells, responsible for preservation of gene silencing
Oct-4	Broadly expressed stem cell markers; stage-specific embryonic antigen and transcription factor
GJIC	Gap junctional intercellular communication-linked antigens
WNT	Signaling molecule
NF-kappaB	Tumor-specific transcription factor
PI3 kinase	Tumor-specific molecular factor
PTEN	Tumor suppressor; protein phosphatase and tensin homologue
hTERT	Human telomerase reverse transcriptase
HH	Hedgehod molecule; signaling molecule
Patched (PTCH)	Protein which has a tumor suppressor function
SMO	Smoothened G protein

It was found that the Bmi-1, Notch, Wnt and Sonic hedgehog pathways, tumor suppressor genes and oncogenes are involved in regulation of self-renewal of both normal and cancer stem cells [4-6]. Additional studies implicate the Wnt beta-catenin pathway in the maintenance of stem-cell self-renewal in other tissues as well [7]. Several studies suggest that epigenetic reprogramming is responsible for the loss of the tumor cells' capacity to form tumors [8]. The stem cell origin will dictate the tumor type, with contributions by the genetic background of the individual and microenvironmental influences.

Human telomerase reverse transcriptase (hTERT) the in vivo expression of hTERT has repeatedly proven to be extremely heterogeneous among cancer cells, especially in vivo [9, 10]. One of the genes involved in the control of stem cell self-renewal, Bmi1, is also able to upregulate hTERT expression in epithelial cells [2].

Directly targeting the growth of stem cells could be a fruitful avenue. Because of work in Drosophila and other developmental systems, a great deal about the growth regulatory pathways operative in embryonic cells is already known [11]. One such pathway, involving the Hedgehog (HH) and WNT signaling molecules, contains a large number of genes that can act as tumor suppressor genes or oncogenes in mammalian cells [12]. Patched (PTCH) codes for the receptor that binds HH molecules and is mutated in patients with nevoid basal cell carcinoma syndrome [13]. PTCH is also mutated in nearly all sporadic basal cell carcinomas and in some medulloblastomas [14]. The mammalian HH genes, ie. sonic hedgehog (SHH), Indian hedgehog (IHH), and desert hedgehog (DHH) are over-expressed in a wide variety of cancers, including small-cell lung, pancreas, gastric, breast, and prostate [15-18]. HH family over-expression and PTCH mutation both have the effect of constitutive action of smoothened (SMO), a G protein-coupled receptor that is a key signaling component of the pathway. Constitutive HH family expression could lead to stem cell activation and appears to be a common feature of many cancers.

Studies of the HH-PTCH pathway in tumors provide support for the importance of tumor stem cells in cancer, indicating that proliferation of normal stem cells is regulated by signals from surrounding normal cells. Transformation of these stem cells can lead to a pre-malignant stem cell with abnormal HH expression or deficient PTCH activity. Such cells can grow in an unrestrained manner, leading to local proliferation. Additional genetic events give rise to a tumor stem cell that can generate more tumor stem cells as well as mature tumor cells. This model leads to specific hypotheses that can be tested as well as new avenues for therapeutics [19].

The Notch protein is another protein important to the growth and differentiation of stem cells Notch is

processed by the enzyme γ-secretase, the same enzyme that processes the APP protein important in Alzheimer Disease.

MOLECULAR PATHWAYS REGULATING HSCs AND LSCs

It is generally accepted that self-renewal is the hallmark property of stem cells in both normal and neoplastic tissues. Recent research has delineated molecular pathways that regulate the self-renewal capacity of HSCs (Table 2). Over-expression and knockout experiments have identified several genes, transcription factors, and cell cycle regulators that modulate the self renewal and differentiation of HSCs [20]. Genes such as SCL, GATA-2, LMO-2, and AML-1 (also known as CBFA2 or RUNX1) regulae early hematopoiesis. The deregulation of these genes through chromosomal aberrations leads to the genesis of several hematopoietic malignancies. For example, transcriptional activation of the AML-1 gene is required for definitive hematopoiesis. As a result of translocation t(8;21), the fusion protein AML-ETO, one of the most frequent chromosomal abnormalities in AML, is generated [21]. Constitutive expression of AML-ETO has been shown to increase the frequency of self-renewal in stem cells [22]. Of interest, such increased self-renewal is of no apparent pathogenic consequence, presumably because secondary mutations are necessary for the expression of the leukemic phenotype [23].

Table 2. *Molecular pathways involved in maintenance of stem cell self-renewal capacity*
Tabela 2. *Szlaki molekularne związane z podtrzymywaniem zdolności samoodnowy komórek macierzystych*

Pathway Szlak sygnałowy	Description Charakterystyka
Bmi1	involved in regulation of self-renewal of both normal and cancer stem cells
Notch (Notch/Jagged)	regulating the integration of extracellular regulatory signals controlling HSC fate
Wnt beta-catenin	maintenance of stem-cell self-renewal in various tissues
Sonic hedgehog	regulation of self-renewal of both normal and cancer stem cells; overexpressed in a wide variety of cancers

Other transcription factors such as the Homeobox (Hox) genes, including HoxB4, and the Wnt signaling pathway have well-described roles in regulating the self-renewal and differentiation of HSCs [24]. HoxB4 promotes the expansion of HSCs whereas they retain their ability to differentiate into normal lymphoid and myeloid cells. It is abundantly expressed in HSCs but declines as terminal differentiation proceeds [25]. Of note, deregulated expression of Hox family members such as HoxA9 is commonly observed in AML. The Wnt signaling pathway has been shown to be critical to the development of several organs and recent studies have illustrated its important role in the regulation of hematopoietic stem and progenitor cell function [26]. Over-expression of beta-catenin, a downstream activator of the Wnt signaling pathway, expands the transplantable HSC pool in long-term cultures [27]. Furthermore, activation of Wnt signaling also increases the expression of other transcription factors and cell cycle regulators important in HSC renewal, such as HoxB4 and Notch-1 [28].

The Notch/Jagged pathway is important in regulating the integration of extracellular regulatory signals controlling HSC fate. Members of the Notch family have critical roles in keeping HSCs in an undifferentiated state and may act as a gatekeeper for factors governing self-renewal and lineage commitment. The gene encoding the Notch receptor was originally identified as the gene rearranged by recurrent chromosomal translocations in some patients with T-cell acute lymphoblastic leukemia [29].

Other transcription factors and cell cycle regulators associated with oncogenesis, such as Bmi-1 may play role in the regulation of proliferation of both HSCs and LSCs [5, 30]. Bmi-1 is a member of the Polycomb family of genes thought to be responsible for the preservation of gene silencing [1]. It is highly expressed in purified HSCs and its expression declines with differentiation. It seems to regulate stem cell renewal by modulating other genes that are important in cellular functions such as proliferation, survival, and lineage commitment. Bmi-1 has an essential role in regulating the proliferative potential of leukemic stem cells [31].

Differential expression of several transcription factors controls the fate of HSCs and plays a critical role in the determination of self-renewal, differentiation, and lineage commitment. These pathways are under the control of various intracellular stimuli as well as cytokines and stromal factors from adjacent cells in the bone marrow microenvironment.

CONCLUSIONS

Research efforts to understand the growth of tumor stem cells as well as to identify tumor stem cell pathways of self-renewal could lead to new targeted approaches. Cancer diagnostics, prevention, and therapeutics are likely to be greatly aided by this new insight. Normal stem cells are being intensively studied to develop approaches for replacing damaged cells and

tissues in the body. The insight from such work is likely to aid the understanding of cancer stem cells.

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REVIEW / PRACA POGLĄDOWA

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CANCER STEM CELLS: NEW CONCEPT OF DRUG RESISTANCE

NOWOTWOROWE KOMÓRKI MACIERZYSTE: NOWA KONCEPCJA OPORNOŚCI WIELOLEKOWEJ NA CYTOSTATYKI

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S u m m a r y

Cancer stem cell shares many properties of the normal stem cell. It follows that cancer stem cells might also possess these resistance mechanisms. Normal stem cells show properties that provide for a long lifespan such as relative quiescence, resistance to drugs and toxins through the expression of several ATP binding cassette (ABC) transporters, an active DNA repair capacity, and a resistance to apoptosis. Members of the ABC-transporter superfamily, have important roles in normal physiology in the transport of drugs across the placenta and the intestine, and are important components of the blood-brain and blood-testis barriers. Important role for these ABC transporters is protecting cells from toxins. The drug-transporting property of stem cells conferred by these ABC transporters is an important marker in the isolation and analysis of haematopoietic and cancer stem cells. By inhibiting the main transporters of chemotherapy drugs, it is regarded that drug resistance could be avoided and tumor cells eliminated. The properties of leukemic stem cells indicate that current chemotherapy drugs might not be effective. The use of current cytotoxic agents probably will not cure many cases of leukemia because the agents either target both the leukemic and normal stem cell populations or do not target any of these populations.

S t r e s z c z e n i e

Nowotworowe komórki macierzyste posiadają liczne cechy normalnych komórek macierzystych. Szczególną wspólna cechą jest posiadanie mechanizmów oporności wielolekowej. Normalne komórki macierzyste posiadają właściwości odpowiadające za długi czas przeżycia w fazie nieaktywnej G0, oporność na liczne leki i obce substancje, ekspresję białek oporności wielolekowej transporterów ATP-zależnych (rodzina ABC, ATP binding cassette), aktywne mechanizmy naprawy DNA i oporność na apoptozę. Białka transportujące należące do rodziny ABC, odgrywają ważną rolę fizjologiczną w przenoszeniu leków przez łożysko oraz ścianę jelit; są również ważnym elementem bariery krew-mózg oraz krew-jądra. Istotnym zadaniem białek ABC jest ochrona komórek przed działaniem różnych toksyn. Właściwość usuwania leków przez białka rodziny ABC jest ważną funkcją, obecną w hematopoetycznych i nowotworowych komórkach macierzystych. Istnieją potencjalne możliwości zahamowania transporterów błonowych, dzięki czemu oporność wielolekowa może być usunięta, a komórki nowotworowe wyeliminowane. Właściwości białaczkowych komórek macierzystych wskazują, że obecnie stosowane leki cytostatyczne mogą być nieefektywne u wielu pacjentów. Istnieje ryzyko, że aktualnie stosowana chemioterapia może być równie aktywna lub równie nieaktywna wobec białaczkowych i normalnych komórek macierzystych.

Key words: cancer stem cells, leukemic stem cells, drug resistance, ABC proteins
Słowa kluczowe: nowotworowe komórki macierzyste, białaczkowe komórki macierzyste, oporność na cytostatyki, białka ABC

MECHANISMS OF MULTIDRUG RESISTANCE IN CANCER STEM CELLS

It is generally accepted that normal stem cells show properties that provide for a long lifespan such as relative quiescence, resistance to drugs and toxins through the expression of several ATP binding cassette (ABC) transporters, an active DNA repair capacity, and a resistance to apoptosis. Cancer stem cell shares many properties of the normal stem cell. It follows that cancer stem cells might also possess these resistance mechanisms. The paradigm that drug resistance originates in the stem-cell phenotype might stimulate new strategies for the development of anticancer therapies. For example, non-stem-cell tumor cells often express multidrug resistance proteins ABCG2 and ABCB1. These genes are highly expressed in drug-resistant cells, and histopathological studies have reported increased expression of the ABCB1 transporter in more differentiated tumors [1]. In addition, in a range of cell lines, differentiating agents induce expression of ABCB1, inhibit cell growth, and increase the expression of markers of maturation. Normal HSCs possess several characteristics that protect them from potential insults. LSCs have similar properties, including quiescence, resistance to drugs and toxins through the expression of ATP-associated transporters, and resistance to apoptotic stimuli [2]. Cancer stem cells (with either inherent or acquired capabilities for self-renewal) give rise to cells that lack long-term self-renewal capability but retain a finite ability to divide [2]. Stem-cell populations have been identified in a range of hematopoietic and solid tumors, and might represent the cell of origin of these tumors. Normal and cancer stem cells express high levels of ATP-binding cassette (ABC) transporters, such as ABCB1,which encodes P-glycoprotein, and the half-transporter ABCG2, which was originally identified in mitoxantrone-resistant cells. The drug-transporting property of stem cells conferred by ABC transporters is the basis for the "side-population" phenotype that arises from the exclusion of the fluorescent dye Hoechst 33342 [2]. Cancer stem cells are likely to share many of the properties of normal stem cells that provide for a long lifespan, including relative quiescence, resistance to drugs and toxins through the expression of several ABC transporters, an active DNA-repair capacity and a resistance to apoptosis. Therefore, tumors might have a built-in population of drug-resistant pluripotent cells that can survive chemotherapy and repopulate the tumor.

THE ROLE OF LEUKEMIC STEM CELLS IN DRUG RESISTANCE AND RELAPSE

Data accumulated in recent years suggest that a small population of leukemic stem cells (LSCs), through their hematopoietic stem cells (HSC)-like properties, survive chemotherapy and sustain the disease. High levels of ATP-binding cassette transporters have been reported in both normal and cancer stem cells [3]. Indeed, HSCs, but not lineage-committed progenitors, express high levels of genes responsible for multidrug resistance-related transporters [4]. As mentioned earlier, the efflux of fluorescent dyes such as Hoechst 33342 has been used to isolate HSCs [5]. Similarly, LSCs either inherently possess drug resistance mechanisms or acquire them through mutations [2]. Other properties of LSCs are also likely to contribute to drug resistance and relapse. Stem cell progenies such as longterm culture-initiating cells and AML colony-forming units are in an active cell cycle, whereas several studies have clearly shown that LSCs remain quiescent. Guzman et al. [6] showed that as much as 96% of the LSC population, as defined by the phenotype CD34+CD38-CD123+, were in the G0 phase of the cell cycle. This resting status of the putative LSCs protects them from the commonly used cell cycle–specific chemotherapeutic agents. It is likely that secondary events, such as the development of mutations, further contribute to the intrinsic resistant properties of LSCs. In a multi-step pathogenic process, quiescent LSCs may carry the initial mutagenic event leading to genomic instability and the induction of secondary mutations that are responsible for a more resistant phenotype. Alternatively, random secondary mutations or mutations occurring as a result of selective pressure caused by therapy may contribute to disease progression or resistance (Table 1).

Table 1. *Methods of anticancer therapy, which might be insuffcient against cancer stem cells*

Tabela 1. *Metody terapii przeciwnowotworowej, nieskuteczne wobec komórek macierzystych nowotworu*

Metod Metoda	Reason of failure in therapy Powód niepowodzenia terapii
Imatinib	Gene mutations
Anti-CD33	Presence of CD33 on normal cells
Inhibitors of ABC proteins	Important physiological role
Flt3 inhibitor CEP701	Unknown

This has been seen in patients with chronic myelogenous leukemia (CML) treated with imatinib mesylate, in whom mutations of the ATP-binding site of BCR-ABL are well documented [7].

MECHANISMS OF MULTIDRUG RESISTANCE IN METASTASES

Cytotoxic chemotherapy kills most cells in a tumor, but cancer stem cells survive due to their relative high resistance to drugs and because of their silent replication. Despite the small number of such cells, their property of being immortal is expected to be sufficient to allow tumor recurrence. The relapse can occur many years after initial treatment by chemotherapy or radiotherapy. It is hypothesized that if tumors are derived from an early stem cell or its progenitor cells, the metastases are formed readily and are phenotypically more heterogeneous. Metastases derived from a later stem cell are more homogenous and have more restricted metastatic potential [8]. Cellular heterogeneity of metastases, their growth in distinct areas of body under different environment might be consequence of cancer stem cell differentiation and/or dedifferentiation.

The CSC model can also shed new light on the biology of metastases and explain why, despite extensive intra-tumor heterogeneity, comparison of paired samples of primary tumors and autologous lymph node and/or distant-site metastases usually reveals striking similarities over a wide range of parameters, including tissue morphology [9], repertoire of somatic genetic mutations [10], expression of tumor-suppressor and immunomodulatory proteins [11], expression of epigenetically controlled genes [12], and overall transcriptional profile as defined by gene expression arrays [13].

ROLE OF ABC PROTEINS IN DRUG RESISTANCE

The identification of potent, specific and non-toxic inhibitors of ABCB1, ABCG2 and ABCC1 is required before the full effects of blocking these transporters can be determined [14]. However, this might be difficult to accomplish in vivo without the destruction of normal stem cells, especially of hematopoietic stem cells, that depend on the expression of drug transporters to survive drug therapy. Stem-cell-driven tissue repopulation not only mediates regrowth of tumors, but also mediates regrowth of normal tissues in the adult, including the bone marrow, gastrointestinal tract and hair follicles. Whether a "therapeutic window" exists that would allow the destruction of cancer stem cells but not normal stem cells remains to be determined [2].

One of the innate resistance mechanisms of stem cells is the expression of one or more ATP-binding cassette (ABC) transporters (Table 2). These pumps play a role in protecting stem cells from xenobiotic toxins [3]. ABCG2 and ABCB1/MDR1 genes are expressed in the vast majority of stem cells and in most tumor stem cells [4, 15]. These transporters can efflux fluorescent dyes such as rhodamine and Hoechst 33342, and this property allows the stem cells to be separated from non-stem cells on a cell sorter [16]. The combined use of chemotherapy drugs and ABC transporter inhibitors could be used to specifically target cancer stem cells [17]. In fact, there are highly specific inhibitors of ABCB1 (PGP) in clinical use and ABCG2 (BCRP) inhibitors in development [18]. These therapies would be predicted to have toxic effects on the patient's normal stem cells, and both ABCG2 and ABCB1 play a role in the blood-brain barrier, which suggests that this approach would have to be carefully titrated to avoid excessive toxicity.

Table 2. *Most important multidrug resistance protein belonging to ABC family*

Tabela 2. *Najważniejsze białka oporności wielolekowej należące do rodziny ABC*

Gene Gen	Protein Białko	Drugs transported by the protein Leki transportowane przez białko	Other substrates Inne substraty
ABCB1	PGP/MDR	Doxorubicin, etoposide, vinblastine, paclitaxel	Digoxine
ABCC1	MRP1	Doxorubicin, daunorubicin, vincristine, etoposide, camptothecine, methotrexate	Rhodamine
ABCC2	MRP2	Vinblastine, cisplatine, doxorubicin, methotrexate	Sulfinpyrazone
ABCC3	MRP3	Methotrexate, etoposide	
ABCC4	MRP4	6-mercaptopurine, 6-thioguanine, Methotrexate and its metabolites	cAMP, cGMP
ABCC5	MRP5	6-merkaptopuryna, 6-tioguanina, methotrexate and its metabolites	cAMP, cGMP
ABCC6	MRP6	Etoposide	
ABCG2	MXR/BCRP	Mitoxantrone, topotecan, doxorubicin, daunorubicin, irinotecan, methotrexate, imatinib	Hoechst, Rhodamine

DRUG TRANSPORTERS IN STEM CELLS

Stem cells have many properties that separate them from mature, differentiated cells. In addition to their ability to self-renew and differentiate, they are quiescent, dividing infrequently. They also require specific environments comprising other cells, stroma and growth factors for their survival [19]. One particularly intriguing property of stem cells is that they express high levels of specific ABC drug transporters. For example, hematopoietic stem cells express high levels of ABCG2, but the gene is turned off in most committed progenitor and mature blood cells [4]. The two ABC transporter-encoding genes that have been studied most extensively in stem cells are ABCB1, which encodes P-glycoprotein, and ABCG2 [4, 15].

Along with ABCC1, they represent the three principal multidrug-resistance genes that have been identified in tumor cells. These genes, members of the ABC-transporter superfamily, are promiscuous transporters of both hydrophobic and hydrophilic compounds [3, 20]. These transporters also have important roles in normal physiology in the transport of drugs across the placenta and the intestine (more accurately, the retention of drugs in the intestinal lumen), and are important components of the blood-brain and blood-testis barriers. Important role for these ABC transporters is protecting cells from toxins. The drug-transporting property of stem cells conferred by these ABC transporters is an important marker in the isolation and analysis of haematopoietic stem cells.

OVERCOMING DRUG RESISTANCE

By inhibiting the main transporters of chemotherapy drugs, it was thought that drug resistance could be avoided and tumor cells eliminated. Therefore, much effort has been devoted to the development of inhibitors of ABC transporters. First-generation compounds included drugs identified as ABCB1 inhibitors, such as verapamil and cyclosporine (multi-protein, universal inhibitor), that were in clinical use to treat other diseases. These inhibitors were combined with a range of chemotherapy regimens for many cancers [3]. As the results were not convincing, subsequent clinical trials were attempted with second-generation inhibitors such as PSC 833 and VX-710. The results of these trials were largely negative, failing in some cases because of pharmacokinetic interaction between the chemotherapeutic agent and the ABCB1 inhibitor. These studies might also have failed because of the presence of additional transporters, such as ABCC1 and ABCG2, that were not targeted by the inhibitor. Although the results of these trials were negative, correlative studies did show that transport by ABCB1 could be inhibited. Efflux activity was assessed with a radionuclide-imaging agent (99mTc-Sestamibi), confirming that some human tumors have ABCB1 activity that can be suppressed with the ABCB1 inhibitors VX 710, PSC 833 and tariquidar (XR9576) [21, 22]. The increased 99mTc-Sestamibi retention in the entire tumor following treatment with tariquidar indicates that the transporter expressing phenotype of the cancer stem cell persists in the committed, abnormally developing progenitors that comprise the proliferative pool of cancer cells. Third generation inhibitors include zosuquidar.

As cancer stem cells express drug transporters that make them resistant to many chemotherapy agents, anticancer strategies should include efforts to target these cells with their special properties. Clinical studies have attempted to overcome drug resistance through combination therapies in which a cytotoxic drug was given along with an ABC-transporter inhibitor. In a new paradigm, transport inhibitors might be thought of as tumor stem cell sensitizing agents, that allow the most crucial and most drug-resistant cells in a tumor to be destroyed. If the stem cells are the main mediators of drug resistance, however, ABC inhibitors would not necessarily reduce tumor burden immediately, but efficacy could be observed using alternative end points, such as the frequency of relapse or the time to relapse. A skeptic would counter that these effects would surely have been reported in the trials conducted so far, if ABCB1 inhibitors did indeed destroy cancer stem cells.

However, it is possible that the cytotoxic drugs or ABC inhibitors tested were inefficient in killing cancer stem cells. An inhibitor of drug transport might be most beneficial when combined with an anticancer agent that specifically targets the stem cells, such as imatinib, which targets the leukemia stem cells that carry the BCR-ABL fusion protein. Another potential reason that clinical trials involving drug-transport inhibitors have not proven successful is that the wrong transporter was inhibited. Most of the studies evaluating cells with the SP phenotype have shown that stem cells overexpress ABCG2, rather than ABCB1, which has been the transporter targeted in most clinical studies [23]. To properly evaluate the latter possibility it will be important to develop an inhibitor for ABCG2.

The compound fumitremorgin C (FTC) is a natural product that specifically inhibits ABCG2 [24]. However this compound is toxic to cells, as well as to mice, and is not thought to be suitable for clinical studies. Chemically synthesized derivatives of FTC such as Ko143 have been developed, and several of these show high specificity and low toxicity [25]. In mice, these compounds have been shown to sensitize mouse tumor cells to drugs. Studies with Ko143 have also shown that inhibition of ABCG2 allows for a greater absorption of certain drugs across the intestine [25]. In addition, the compound GF120918 is an ABCB1 inhibitor that has been shown to inhibit ABCG2 in vitro and apparently also in vivo [26].

CIRCUMVENTION OF DRUG RESISTANCE

Because anthracyclines, alkylating agents, nucleoside analogs, and topoisomerase inhibitors currently used in the treatment of acute myeloid leukemia (AML) often fail, they may not be targeting LSCs very effectively [27]. For example, Ara-C, which is a cycle-active agent commonly used in treating leukemia, shows virtually no activity with isolated LSCs [28]. From a therapeutic perspective, the nature of the LSC may vary depending upon the stage during which it arose. Accordingly, drug resistance and various characteristics that are relevant to therapy may also differ, based on the origin of the diseased cell. The properties of leukemic stem cells indicate that current chemotherapy drugs will not be effective. The use of current cytotoxic agents is not effective in leukemia because the agents target both the leukemic and normal stem cell populations.

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REVIEW / PRACA POGLĄDOWA

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CANCER STEM CELLS: NEW THERAPEUTIC OPOORTUNITIES

NOWOTWOROWE KOMÓRKI MACIERZYSTE: NOWE MOŻLIWOŚCI TERAPEUTYCZNE

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S u m m a r y

Although stem cells can self-renew, they are generally quiescent, spending most of their time in G0. Because stem cells can repair their DNA as they self-renew, they have the potential to accumulate mutations acquired after exposure to carcinogens. Current investigations are attempting to combine molecular targeted therapy concepts with the concept of the cancer stem cells. Molecular targeted therapy can be divided into 6 categories that correspond to following types of targets, presented in this review: (1) surface molecules; (2) ABC proteins; (3) specific oncoproteins; (4) normal stem cell pathways; (5) survival factors, present in cancer stem cells, such as NF-κB pathway, PI3K, downstream signaling molecules and related signal-transduction pathways; (6) oxidative stress.

S t r e s z c z e n i e

Chociaż komórki macierzyste posiadają właściwości samoodnawiania, zasadniczo pozostają w uśpieniu, w fazie spoczynkowej G0. Komórki macierzyste posiadają aktywne mechanizmy naprawy DNA; posiadają jednak również możliwości akumulowania mutacji nabywanych pod wpływem działania czynników mutagennych. Aktualne badania w dziedzinie komórek macierzystych zmierzają do połączenia koncepcji terapii celowanej i koncepcji nowotworowych komórek macierzystych. Molekularna terapia celowana może być podzielona na 6 kategorii, które korespondują z następującymi celami, określonymi właściwościami komórek macierzystych: (1) markery powierzchniowe; (2) białka rodziny ABC; (3) specyficzne onkoproteiny; (4) szlaki sygnałowe samoodnowy; (5) czynniki przeżycia obecne w nowotworowych komórkach macierzystych, takich jak szlak czynnika transkrypcyjnego NF-κB/PI3K; (6) stres oksydacyjny.

Key words: cancer stem cells, leukemic stem cells, drug resistance, ABC proteins, therapy
Słowa kluczowe: nowotworowe komórki macierzyste, białaczkowe komórki macierzyste, oporność na cytostatyki, białka ABC, terapia

EVALUATION OF TREATMENT EFFICACY

Traditionally, antitumor treatments are screened based on their capacity to induce a clinical response (i.e., a dramatic regression, either complete or partial, of the tumor lesion). This approach, however, tends to select for treatments that are active on the bulk of tumor cell populations but not necessarily on cancer stem cells (CSCs). From a purely theoretical point of view, anti-tumor treatments that selectively target the CSC subset might actually be unable to induce rapid shrinkage of tumor masses but might eliminate their capacity for long-term growth and therefore cause their arrest or slowly reduce their size. It is therefore likely that, alongside new treatment strategies, new approaches for the preclinical evaluation of their efficacy will need to be devised [1]. Consequently, new strategies are required that specifically and preferentially target the malignant stem cell population, while sparing normal stem cells [2]. More and more data support the concept that anti-neoplastic therapies are curative only when all cancer stem cells in a given tumor are eliminated. This

new concept may soon change dramatically our view regarding the treatment of solid tumors. Significant effort is directed on evaluation of target expression profiles in cancer stem cells in various tumors and leukemias and on the potential of tumor stem cells to escape therapy through differential expression of targets, plasticity and quiescence [3].

THERAPEUTIC CSC TARGETING

According to the CSC model, therapeutic approaches that do not eradicate the CSC compartment are likely to achieve little success; they might kill the majority of tumor cells and induce temporary regression of gross tumor lesions but fail to prevent disease relapse and metastatic dissemination [1, 4]. In support of this hypothesis is the finding that, in the hematopoietic system, both normal stem cells and CSCs, ie. hematopoietic stem cells (HSCs) and leukemic stem cells (LSCs) from acute myeloid leukemia (AML) patients mainly appear to be in a quiescent, non-dividing, G0 state, and therefore inherently resistant to the toxic effect of traditional chemotherapeutic regimens [5-7].

Although stem cells can self-renew, they are generally quiescent, spending most of their time in G0. Because stem cells can repair their DNA as they self-renew, they have the potential to accumulate mutations acquired after exposure to carcinogens [8].

A further concern is that normal stem cells and progenitor cells may prove to be more sensitive than cancer stem cells to the effects of chemotherapy. Normal colon stem cells, for example, can inhibit DNA repair mechanisms and thereby undergo apoptosis in response to DNA damage; this mechanism avoids the accumulation of harmful mutations [9]. If, however, colon cancer cells evade this protective mechanism, then chemotherapy could preferentially spare them. Recent studies have demonstrated that normal hematopoietic stem cells undergo premature senescence (ie., cellular "aging") when exposed to ionizing radiation or busulfan [10, 11].

IMPLICATIONS FOR CANCER THERAPY: OPPORTUNITIES AND CHALLENGES

The cancer stem cell hypothesis posits that cancer stem cells are a minority population of self-renewing cancer cells that fuel tumor growth and remain in patients after conventional therapy has been completed. The hypothesis predicts that effective tumor eradication will require obtaining agents that can target cancer stem cells while sparing normal stem cells. Experimental evidence in human AML suggests that, compared with the bulk population of leukemic blasts, the leukemia stem cells are relatively resistant to conventional chemotherapeutic agents. Although it has been speculated in solid tumors that conventional agents kill the non-tumorigenic cancer cells while sparing the cancer stem cells, this has not been proven. There are other models of drug resistance consistent with the existence of cancer stem cells that could explain relapse, including the classic view of mutation and selection.

It is important that agents directed against cancer stem cells discriminate between cancer stem cells and normal stem cells. This will require identification of realistic drug targets unique to cancer stem cells. The identification of such targets and the development of anticancer agents will require a fuller understanding of normal stem cell biology as well as the genetics and epigenetics of tumor progression. There is some indication that such an approach can be successful. For example, stem cells isolated from AML patients display differences from normal hematopoietic stem cells [12].

STRATEGIES TO OVERCOME LSC RESISTANCE TO THERAPY

As implied by the multi-step theory of carcinogenesis, LSCs are likely to have the fewest number of molecular aberrations in the population of the malignant cells and, as such, biologically most similar to normal HSCs. The primary challenge in developing treatment strategies targeted toward LSCs is to identify pro-apoptotic stimuli that spare the normal HSCs while exerting the desired effect on LSCs. A primary concern in the development of tumor stem cell-specific drugs is to overcome the inherent drug efflux pumps that are highly expressed in LSCs. Several agents effective in inhibiting the ATP-binding cassette transporters have been studied and found to have limited clinical efficacy [13, 14]. The biggest obstacle to this approach is the similarly high expression of these transporters in normal HSCs, making them equally susceptible to the inhibitors [8]. As such, strategies directed at pathways that specifically regulate LSC survival would probably be more fruitful [15]. The search for identification of survival pathways that are preferentially overexpressed in LSCs is ongoing and several recent studies have described means of differential activation of apoptosis

mechanisms in LSCs [5, 16, 17]. The transcription factor NF-κB was found to be constitutively activated in LSCs, but not in normal HSCs [16]. Therefore, NF-κB inhibitors were added to antileukemic agents such as idarubicin in experimental models [5, 16]. Recently, single-agent parthenolide, a potent inhibitor of NF-κB, was found to induce apoptosis in AML and CML LSCs and progenitors while sparing normal HSCs [17]. Notably, parthenolide was much more selective in eliminating LSCs and sparing normal hematopoietic cells than was the standard chemotherapy agent cytarabine [17]. Constitutive activation of the phosphatidylinositide-3 kinase is also necessary for the survival of LSCs and its pharmacologic inhibition by LY294002 leads to a dose-dependent decrease in survival [18]. The fate of LSCs depends on the relative expression of transcription factors and their regulation, usually by aberrant signaling pathways [19, 20]. Although it has been proposed that recruitment of LSCs from the G0 to the S phase of the cell cycle might contribute to their eradication by cell cycle-specific cytotoxic agents, it is possible that prolonging the ''quiescent phase'' could be beneficial. One could envision a scenario in which LSCs are maintained in a state of hibernation, thereby prolonging relapse-free survival. The best evidence for this possibility is the existence of patients with clinically distinct indolent or proliferative forms of AML as well as the wide variation in the duration of relapse-free interval in individual patients.

NEW AGENTS TO TARGET THE LEUKEMIC STEM CELL FOR THERAPEUTIC PURPOSES

A major cellular target of cancer therapy is directed against neoplastic stem cells. Current investigations are attempting to combine molecular targeted therapy concepts with the concept of the cancer stem cells.

Molecular targeted therapy can be divided into 6 categories that correspond to following types of targets: 1) surface molecules; 2) ABC proteins; 3) specific oncoproteins; 4) normal stem cell pathways; 5) survival factors, present in cancer stem cells only (NF-κB pathway, PI3K, downstream signaling molecules and related signal-transduction pathways); 6) oxidative stress [2, 3, 8, 21] (Table 1).

Surface molecules. The ideal target would be expressed on all neoplastic stem cells but not on normal stem cells. It is regarded that IL-3 receptor α chain, and CD33 are not expressed on normal stem cells. However, only subpopulation of neoplastic stem cells express these antigens. CD44 is also expressed on normal stem cells. In such cases, targeted therapy has to be combined with stem cell transplantation. Anti-CD33 consists of calicheamicin and a humanized anti-CD33 antibody (CMA-676). CD44 is a monoclonal antibodies, acting through up-regulation of p27. Other possible agents include antibodies conjugated with diphteria toxin against GM-CSF (CD116) granulocyte-macrophage-colony stimulating factor, IL-3(CD123) and urokinase plasminogen-activator receptor (CD87).

Table 1. *Targets in leukemic stem cells*
Tabela 1. *Cele molekularne w białaczkowych komórkach macierzystych*

General target Cel ogólny	Specific target Cel szczegółowy	Agents Leki
Surface molecules	IL-3 receptor α chain, CD33 CD44	
ABC proteins	PGP1 BCRP	Zosuquidar Fumitremorgin
Specific oncoproteins	BCR-ABL PML-RARα HER-2/neu Mutated RAS Mutated Kit	Imatinib All-trans retinoid acid Trastuzumab
Stem cell pathways	Bmi-1, Notch, Wnt, Sonic Hedgehog pathways SMO protein	Cyclopamine, SMO inhibitors
Survival factors	NF-κB	Proteasome inhibitors, Parthenolide (or its analogs), TDZD-8, inhibitors of mTOR
Oxidative stress	DNA damage response	Idarubicin or other chemotherapeutics, Parthenolide (or its analogs), TDZD-8, Heat shock

ABC proteins. The most important targets include: PGP1 and BCRP, with best known inhibitors zosuquidar and fumitremorgin, respectively.

Specific oncoproteins. Probably, disease-related oncogenic proteins play an important role in the initiation and progression of leukemia and solid tumors. Such oncoproteins are expressed early in tumor development, and are expressed in neoplastic stem cells. The best known include BCR-ABL (inhibitor: imatinib), PML-RARα (inhibitor: all-trans retinoid acid), HER-2/neu (inhibitor: trastuzumab), mutated RAS, mutated Kit. However, this therapy is not associated with the elimination of the entire neoplastic clone, due to drug resistance, mutated forms, other mutations, and also BCR-ABL-negative clones.

Normal stem cell pathways. Genes that have demonstrated their involvement in the regulation of self-renewal in normal stem cells include the Bmi-1, Notch, Wnt, and Sonic Hedgehog (HH) pathways. These genes are potential targets for therapy. Cyclopamine is a compound originally discovered in the

Corn Lily (Veratrum californicum), a plant teratogenic to sheep [22]. Cyclopamine specifically inhibits the SMO protein and can suppress the growth of cells and tumors that contain activated HH [23]. Human prostate tumors grown as xenografts in mice were completely eliminated following twenty-one days of treatment with cyclopamine [24], and UV-induced basal cell carcinomas were suppressed in mice given low levels of cyclopamine in their drinking water [25]. Both cyclopamine and SMO inhibitors are in development as anticancer agents. Several companies have developed γ-secretase inhibitors and these drugs may have applications to cancer therapy. Rapamycin is a bacterially derived molecule that inhibits the target of rapamycin (TOR) family of kinases [26]. Rapamycin has immunosuppressive properties and is also used to treat certain leukemias [27]. Yilmaz et al. have shown that rapamycin can selectively inhibit leukemia initiating cells in leukemias generated in mice harboring a conditional deletion of PTEN (phosphatase and tensin homolog deleted on chromosome 10), a phosphatidylinositol phosphate (PIP) phosphatase often mutated in human tumors [28]. Rapamycin depletes the leukemia-initiating cells while restoring the normal hematopoieitc stem cells, suggesting therapies can be devised specific for cancer stem cells. Additional strategies to directly target the growth of tumor stem cells can now be developed and may prove superior in effectiveness.

Survival factors. These factors are present in cancer stem cells only, such as NF-κB pathway. Proteasome inhibitors down-regulate NF-κB expression. Inhibition of survival factors (NF-κB/PI3K) include proteasome inhibitors, parthenolide and its analogs, TDZD-8 and ET-18-OCH3 [2]. Another pathway is phosphoinositide-3 kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway. Whether mTOR is activated in stem cells and whether inhibitors of mTOR (rapamycin) can counteract the growth of LCS is under investigation. Other downstream signaling molecules and related signal-transduction pathways are possible to known.

Oxidative stress. DNA damage response might be influenced by idarubicin or other chemotherapeutics, parthenolide and its analog TDZD-8 [2].

CONCLUSIONS

Self-renewal capacity at early phases of cancer accumulates oncogenic transformations leads to eventual development of cancer metastasis, a multi-step process. The effective treatment of cancer involves the complete eradication of cancer stem cells. Identifying and characterizing the unique molecular profiles like cell surface antigen markers, mechanisms controlling cell survival and responses to injury of cancer stem cells discriminating them from normal stem cell phenotype may provide opportunities for therapeutic intervention that would spare normal stem cells. Drugs specifically targeting the growth and proliferation of cancer stem cells will undoubtedly help in its complete eradication.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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THE CHANGES OF SERUM SOLUBLE TRANSFERRING RECEPTORS (sTfR) CONCENTRATION IN ALCOHOL DEPENDENT WOMEN

OCENA STĘŻENIA ROZPUSZCZALNYCH RECEPTORÓW TRANSFERYNY (sTfR) U KOBIET UZALEŻNIONYCH OD ALKOHOLU

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Summary

Alcohol abuse is propably one of the factor of cellular iron homeostasis.

In recent years, the soluble transferrin receptor (sTfR) has been introduced as a sensitive, early and highly quantitative new marker of iron depletion, increasing in proportion to issue iron deficit. Measurement of sTfR in serum has recently emerged as a tool for detecting iron deficiency and for differentiating between anemia caused by iron deficiency and that caused by a chronic disease because an elevated concentration of sTfR in serum is a sensitive indicator of iron depletion.

The measurement of sTfR concentration is a reliable laboratory test for detecting iron deficiency.

The aim of the study was to assess changes of serum sTfR concentration in alcohol dependent women. The study was performed on a group of 34 women with clinical diagnosis of alcoholism (ICD-10).

Conclusions: These results suggest that prolonged alcohol abuse as a chronic disease can disrupt cellular iron homeostasis and lead to iron deficiency.

Streszczenie

Uzależnienie od alkoholu jest przewlekłym, nawrotowym schorzeniem, powodującym powstanie wielorakich szkód zdrowotnych i zaburzeń u osoby uzależnionej. Z uwagi na wyjątkową rolę, jaką spełnia żelazo w ogólnym metabolizmie i utrzymaniu homeostazy ustroju, ocena jego zasobów jest w ostatnich latach przedmiotem szczególnej uwagi badaczy. W osoczu krążą rozpuszczalne receptory transferyny, których stężenie jest ściśle powiązane z ilością komórkowych receptorów transferyny. Poziom sTfR jest proporcjonalny całkowitego stężenia sTfR w organizmie. Oznaczanie stężenia sTfR w surowicy okazało się użyteczne w różnicowaniu niedokrwistości z niedoboru żelaza od niedokrwistości w przebiegu chorób przewlekłych, bowiem ogólnie stosowane parametry, takie jak stężenie ferrytyny i transferyny okazują się wówczas niewystarczające. Ilość komórkowych receptorów transferyny zależy od stopnia dostępności żelaza (przy obniżeniu poziomu żelaza w magazynach tkankowych

rośnie ilość receptorów). Nadużywanie alkoholu jest chorobą przewlekłą prowadzącą do zmian w metabolizmie żelaza.

Badaniom poddano 34 kobiet z Zespołem Zależności Alkoholowej, u badanych oznaczono aktywność AST, ALT, GGT, stężenie sTfR, morfologię.

Zespołowi uzależnienia od alkoholu, który traktowany jest jako schorzenie przewlekłe, może towarzyszyć zaburzenie gospodarki żelazowej prowadzące do niedoboru żelaza.

Key words: chronic alkohol abuse, serum soluble transferring receptors sTfR
Słowa kluczowe: nadużywanie alkoholu, rozpuszczalne receptory transferyny sTfR

Chronic alcohol abuse may lead to changes in iron metabolism and may influence the concentration and structure of the proteins, which store and transport iron in human organisms. Iron deficiency as well as its accumulation have been observed in people abusing alcohol. The mechanism of accumulation has not thoroughly been investigated yet.

Measurement of sTfR in serum has recently emerged as a tool for detecting iron deficiency and for differentiating between anemia caused by iron deficiency and that caused by chronic disease. Because an elevated concentration of sTfR in serum is a sensitive indicator of iron depletion.

METHODS

34 women treated for 30 days in Short Term Therapy and Detoxification Ward for Women in Bydgoszcz E. Warmiński City Hospital were studied. The age of the subjects was 38 ± 8 years, with the time of alcohol dependency of 6 ± 3 years.

Inclusion criteria were: 1) fulfilling the alcohol dependency criteria according to ICD-10; 2) informed, written consent to participate in the study; 3) alcohol abstinence period not longer than 7 days.

Exclusion criteria were: 1) dependency other than of alcohol and/or nicotine; 2) active phase of extrahepatic disease; 3) liver impairment other than due to alcohol (serological features of hepathotropic infection and biochemical abnormalities showing metabolic disorders).

In every subject biochemical parameters of liver function were assessed using standard laboratory procedures (Roche Hitach 912 and Elecsys 1010). Following Roche tests for the assessment of liver enyzmes levels were used (reference values in brackets): AST (<31 U/l), ALT (< 32 U/l), GGTP (9-39 U/l)

Serum soluble transferring receptors concentration were indicated of the IdeA sTfR IEMA test (1,3 – 3,3µg/l) .

The study was approved by the Local Ethics Committee and written informed consent was obtained from all subjects; financed from grant UMK 57/2007.

RESULTS

The demographic and clinical data of the investgated women are shows in table 1.

Table 1. *Demograpfical and clinical data of subjects in the study*

Clinical data	x	SD	
Age	36.08	6	23 – 45
Age of onset of alcohol dependence	30.75	5.4	21 – 41
sTfR	3.12	1.60	1,1 – 10
Hg	13.52	1.43	8.4 – 16.5
AST	44.24	73.04	10 – 443
ALT	32.67	42.46	6.4 – 256
GGT	57.45	81.59	9.0 – 387

Women with serum sTfR concentrations significantly higher were significantly older (43.18±6.9 vs 37.09±8.3 p=0.043) and had more significantly icreased hemoglobin levels (12.18±86 vs 13.92; p=0.013) than women with normal serum sTfR concentrations (Table 2).

Table 2. *Comparison of date in women with high and normal concentration of sTfR*

Investigated parameter	sTfR>3.30 µg/l x ± SD	sTfR Normal x ± SD	p
Age	43.18±6.9	37.09±8.30	0.43
Hemoglobin	12.18±1.86	13.92±1.02	0.013

There were no differences of GGT, AST, ALT CDT activity in alcohol dependent women with high and normal levels of sTfR.

DISCUSSION

Chronic alcohol abuse may lead to changes in iron metabolism and may influence the concentration and structure of the proteins, which store and transport iron in human organisms. Iron deficiency as well as its accumulation have been observed in people abusing alcohol. The mechanism of accumulation has not thoroughly been investigated yet. Cellular iron uptake is facilitated by transferring receptor (TfR)-mediated

endocytosis. More insight has been obtained in TfR physiology and the regulation of cellular iron homeostasis. The synthesis of TfR and the iron storage protein ferritin is regulated reciprocally at the post-transcriptional level according to the cellular iron status. As a result of externalization of TfR during the endocyting cycle, a soluble form of TfR can be detected in serum.

Circulating concentrations of sTfR are proportional to cellular expression of the membrane-associated sTfR. The intact receptor is present on virtually all mammalian cell surfaces, as it mediates the flow of transferring-bound iron from the extracellular pool into the cytosol. Soluble TfR is truncated form of the initially dimeric functional receptor.

The transferring receptor is a disulphide-linked transmembrane glycoprotein with two identical polypeptide chains (190 kD), which shuttles iron from the extracellular space into the cytosol.

Measurement of soluble transferrin receptor in serum has recently emerged as a tool for detecting iron deficiency and for differentiating between anemia caused by iron deficiency and that caused by chronic disease. Because an elevated concentration of sTfR in serum is a sensitive indicator of iron depletion.

The correct diagnosis of iron deficiency is essential for successful patient management because it may be the presenting of a serious illness such as a gastrointestinal malignancy. In many cases, iron deficiency is relatively simple to diagnose and treat. However, in some patients, typically those with other medical problems, the diagnosis can be difficult.

The soluble transferrin receptor (sTfR), a truncated form of the membrane-associated transferring receptor, has been reported to be a sensitive indicator of iron deficiency and unlike conventional laboratory tests, the sTfR is not acute phase reactant.

Cellular iron homeostasis is accomplished by the coordinated regulated expression of the transferring receptor and ferritin, which mediate iron uptake and storage, respectively.

Chronic alcohol abuse may lead to changes in iron metabolism and may influence the concentration and structure of the proteins, which store and transport iron in human organisms. Iron deficiency as well as its accumulation have been observed in people abusing alcohol. The mechanism of accumulation has not thoroughly been investigated yet. Presumably, the increased secretion of gastric acid in the above mentioned people maintains the iron compounds in the soluble state until they reach duodenum and reduces the capability of erythrocytes to oppose the permeating of the access of iron into blood.

Roauch and co-workers revealed in their experiments on rats that excessive intake of alcohol changes the distribution of iron between circulation and tissues which in consequence, leads to the increase of iron concentration in brain and liver.

Other researchers also observed the increased tissue turnover of iron and the increase of its intestinal absorption. It is possible that irregular iron absorption from the digestive tract may cause the excessive accumulation of this element in a human organism.

On the contrary, Moirand is of the opinion that iron overload changes from individual to individual and does not occur in case of chronic alcohol abuse.

It has been revealed in the performed research in the performed research that the older investigated women had significantly higher concentrations of sTfR, which means that they have been characterized by a decreased level of iron in relation to the younger group of investigated women. Maybe, some other factors, like age or sex of the examined people, overlapped with the main factor influencing metabolic derangements (i.e. alcohol). Additionally, the persons with a lower level of iron have been noticed to have a lower hemoglobin level too.

It seems to the authors of this article that the multidimensional structure of iron metabolism and resulting from it – the multifactor influence on metabolism balance, should become the subject of further deep research.

CONCLUSION

These results suggest that prolonged alcohol abuse as chronic disease can disrupt cellular iron homeostasis and lead to iron deficiency.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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THE PARTITION OF THE FLUCTUATION STRENGTH OF THE HUMAN CENTER OF PRESSURE

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Summary

Introduction: In this study we have used the Langevin equation for the two-dimensional Ornstein-Uhlenbeck process to investigate the partition of the fluctuation strength of the short-term center of pressure (COP) sway under quiet-standing conditions.

Materials and Methods: In our method the state of the postural control system is described by the diffusion or the fluctuation strength matrix and the friction coefficient. Matrices of diffusion and fluctuation strength, in particular, give the coefficient of partition of the fluctuation strength. That partition is examined in coordinate system, in which those fluctuations are uncorrelated. This coordinate system in general is rotated by an angle with respect to the system defined by the anteroposterior and mediolateral directions. Seventy three young subjects (25 male and 48 female, age: 19-25 years) were tested under eyes-open and eyes-closed conditions.

Results: It was observed that this coefficient has values from almost all available range (0.5, 1). Furthermore, its mean and standard deviation were significantly increased under eyes-closed conditions.

Conclusion: COP fluctuation strength is not equally distributed in all directions. The partition of these fluctuations, which in physical models is constant for different angles, here accepts different values. The coefficient of that partition is different for each subject. It was shown here that this coefficient increases and its values become less homogenous under eyes-closed conditions.

Streszczenie

Wstęp: W pracy użyliśmy równania Langevina dla dwuwymiarowego procesu Ornsteina-Uhlenbecka do badania partycji mocy fluktuacji krótkoczasowego błądzenia centrum nacisku (ang. center of pressure, COP) podczas niezaburzonego stania.

Materiały i Metody: W naszej metodzie stan systemu kontroli postawy jest opisany przez macierz dyfuzji lub mocy fluktuacji i współczynnik tarcia. Macierze dyfuzji i mocy fluktuacji, między innymi, dają współczynnik partycji mocy fluktuacji. Ta partycja jest analizowana w układzie współrzędnych, w którym te fluktuacje są nieskorelowane. Ten układ współrzędnych w ogólności jest obrócony o pewien kąt względem układu zdefiniowanego przez kierunki przód-tył i boczny. Siedemdziesięciu trzech młodych ludzi (25 mężczyzn i 48 kobiet, w wieku od 19 do 25 lat) badano w warunkach oczu otwartych i oczu zamkniętych.

Rezultaty: Zaobserwowano, że współczynnik ten przyjmuje wartości z prawie całego dostępnego przedziału (0.5, 1). Ponadto, jego średnia i odchylenie standardowe istotnie wzrosło w warunkach oczu zamkniętych.

Konkluzja: Moc fluktuacji COP nie jest równo rozłożona we wszystkich kierunkach. Partycja tych fluktuacji, która w fizycznych modelach jest stała dla różnych kątów, tutaj przyjmuje różne wartości. Współczynnik tej partycji jest inny dla każdej osoby. Zostało tutaj pokazane, że ten współczynnik rośnie i jego wartości stają się bardziej niejednorodne w warunkach oczu zamkniętych.

Key words: visual input, postural control system, Ornstein-Uhlenbeck process, posturography, human stabilogram, statistical analysis

Słowa kluczowe: sygnał wizualny, system kontroli postawy, proces Ornsteina-Uhlenbecka, posturografia, ludzki stabilogram, analiza statystyczna

INTRODUCTION

The postural sway is a two-dimensional stochastic process. Several methods have been designed to analyze that sway under quiet-standing conditions. Most important among them are those which take into account its stochastic character. However, two-dimensional aspect of postural sway remains poorly investigated.

Collins and De Luca have shown that postural sway is a stochastic process [1]. Next, they have introduced the stabilogram-diffusion analysis [2-5]. Using that analysis they have shown that three different scaling regions, referred to as the short-term, long-term and saturation region, can be distinguished on the plot of the mean square displacement of the center of pressure (COP) versus time interval. For these regions the diffusion coefficients and the scaling exponents were obtained in anteroposterior (AP) and mediolateral (ML) directions independently. As a result Collins and De Luca have concluded that the postural control system uses over short-term and long-term intervals, the open-loop and the closed-loop control scheme, respectively.

The Collins and De Luca's studies have inspired many theoretical investigations of the phenomenon. Chow et al. have used a pinned polymer model to describe stabilogram-diffusion plot obtained by Collins and De Luca [6-8]. Next using that model they showed that the impulse response of the human body is similar to response on random fluctuation [7]. Alonso-Sanchez and Hochberg [9] improved that analysis adding a nonlinear term. To analyze this model they have used the techniques of a dynamical renormalization group. To describe the short-term regime in these approaches the colored noise must be used. Frank et al. [10], to model that region have used the theory of multivariate Ornstein-Uhlenbeck processes with mean-field dependent coefficients. Alternatively to Collins and De Luca, Newell et al. [11] and Peterka [12] have found that maintaining an upright stance is continuous process. Newell et al. have suggested that COP sway can be modeled by simple linear Ornstein-Uhlenbeck process. On the other hand, Peterka has used the model of an inverted pendulum randomly disturbed and adjusted dependently from COP position and its integral and derivative with time delay. Finally Chiari et al. [13,14] have proposed a simple improvement of Collins and De Luca two-process random walk model. They have found connection between the diffusion coefficients and the scaling exponents, and integrated the description of the stabilogram-diffusion plot in the short-term and the long-term region.

In our earlier studies, it was proposed that the quiet-standing postural sway for the short-term intervals can be modeled by the two-dimensional Ornstein-Uhlenbeck process [15,16]. The COP sway in AP and ML directions were treated as one two-dimensional process and were described by the diffusion matrix, the matrix of the fluctuation strength and the friction coefficient. These matrices describe the magnitude of activity of the short-term COP sway and the orientation on the plane of support of its uncorrelated components, whereas, the friction coefficient describes the compensation of that activity. The activity of the COP sway is the consequence of the activity of the postural muscles. It was shown that the activity of the short-term postural sway is increased, among others, by aging with and without parkinsonism or exclusion of vision [15,16]. Beside that common conclusion it was also shown that combination of that effect due to age and that due to exclusion of vision causes its compensation in healthy elderly subject, but not in parkinsonian elderly subjects [16].

In this study we have used the method proposed in our earlier studies to investigate the partition of the fluctuation strength of the COP sway under quiet-standing conditions. That partition is examined in coordinate system in which those fluctuations are uncorrelated.

MATERIALS AND METHODS

To describe the short-term dynamics of COP in ML (x) and AP (y) directions we have used Langevin equation for the two-dimensional Ornstein-Uhlenbeck process:

$$\frac{d^2}{dt^2}\mathbf{r} = -\gamma \frac{d}{dt}\mathbf{r} + \mathbf{f}(t), \qquad (1)$$

where $\mathbf{r} = (x, y)$, γ is the friction coefficient and $\mathbf{f}(t)$ is the stochastic force per mass, which is assumed to have properties of white noise: $\langle \mathbf{f}(t) \rangle = \mathbf{0}$ and $\langle \mathbf{f}(t)\mathbf{f}(t') \rangle = \mathbf{G}\delta(t-t')$, where $\mathbf{G}$ is the constant 2x2-dimensional matrix of the fluctuation strength of this force, δ is the delta distribution and the angled brackets denote the ensemble averages or the time averages. We have assumed that in case of center of pressure sway, both averages are interchangeable [2, 3, 7, 10]. For the Ornstein-Uhlenbeck process given by

Eq. (1), the matrix of the mean squared displacement as a function of time interval Δt takes the form [17,18],

$$\langle \Delta \mathbf{r} \Delta \mathbf{r} \rangle = 2\mathbf{D}\left(\Delta t + \gamma^{-1}\left(\mathrm{e}^{-\gamma \Delta t} - 1\right)\right), \qquad (2)$$

where $\mathbf{D} = \mathbf{G}/2\gamma^2$ is the diffusion matrix and $\Delta \mathbf{r} = \mathbf{r}(t + \Delta t) - \mathbf{r}(t)$.

The function given in Eq. (2) has two asymptotic behaviors. For time intervals much shorter than γ^{-1}, Eq. (2) yields $\langle \Delta \mathbf{r} \Delta \mathbf{r} \rangle = \mathbf{D}\gamma \Delta t^2$, what corresponds to ballistic motion. On the other hand, for time intervals much larger than γ^{-1}, Eq. (2) becomes $\langle \Delta \mathbf{r} \Delta \mathbf{r} \rangle = 2\mathbf{D}\Delta t$. That equation is the matrix form of the Einstein-Smoluchowski equation describing uncorrelated random walk. These asymptotic behaviors define the Fokker-Planck time scale and the diffusion time scale, for $<< \gamma^{-1}$ and $>> \gamma^{-1}$, respectively.

In our method the state of the postural control system is described by the diffusion matrix **D** and the friction coefficient γ. Alternatively, instead of the diffusion matrix, the matrix of the fluctuation strength **G** can be used. The diffusion matrix allows analysis of the COP sway in (uv) coordinate system, turned with respect to the xy coordinate system, in which there is no correlation between displacements along its axes. That rotation obtained through the diagonalization of matrix **D** gives the diffusion coefficients along u and v directions, D_u and D_v, respectively. That matrix in diagonal form is characterized by the planar diffusion coefficient $D_r = tr\mathbf{D} = D_u + D_v$ and the ratios $n_u = D_u/D_r$ and $n_v = D_v/D_r$, which describe the partition of D_r on D_u and D_v, where tr denotes trace of matrix. Thus the short-term postural sway is characterized by the friction coefficient γ, the planar diffusion coefficient D_r, the ratios n_u or n_v and the angle ξ between u and y as well as v and x directions. The planar diffusion coefficient can be replaced by the coefficient of the planar fluctuation strength of the stochastic force $G_r = tr\mathbf{G} = 2\gamma^2 D_r$.

Seventy three young subjects (25 male and 48 female, age: 19-25 years) were investigated. These subjects had the following body parameters: 65 ± 13 kg weight and 170 ± 16 cm height. Subjects included in this study had no history or evidence of any neurological disorders affecting movements control or posture.

Using the standard force platform, (produced by Pro-Med, Janusz Olton, Poland), the COP position were gathered within 32 s at a rate of 32 Hz. Investigated subject, during test, stood in an upright standardized posture on the force platform. Their arms were relaxed comfortably along the body, and feet without shoes were positioned heels together with an angle between them of 30°, which position were standardized for each subjects by lines marked on the platform. For each subject, one test was performed, with the eyes open and fixed on a wall in front of them, and one, with the eyes closed.

The ethical guideline for that experiment was given by the Bioethical Commission at the Ludwik Rydygier Collegium Medicum in Bydgoszcz of Nicolaus Copernicus University in Toruń.

The least square was utilized to adjust the theoretical functions to the experimental data. T-test for paired normal variables was used to compare the parameters obtained for investigated group in both experimental conditions and Shapiro-Wilk test was used to verify the normality of these parameters.

RESULTS

The plot of the elements of the matrix of the mean square displacement $\langle \Delta \mathbf{r} \Delta \mathbf{r} \rangle$ as a function of time interval, for representative subject was shown in Fig. 1. Those plots obtained for the COP random walk is well approximated by the theoretical functions given by Eq. (2), in the short-term region, i.e., for time intervals less than 1 s. Similar plots were obtained for all investigated subjects in both experimental conditions. It has allowed to find above mentioned parameters, describing the postural sway of these subjects. Further we have dealt with the coefficient n_u, which describes the partition of fluctuation strength in coordinate system uv.

The distributions of coefficient n_u for eyes-open and eyes-closed conditions are shown in Fig. 2. It was observed that this coefficient has values from almost all available range (0.5, 1). When the coefficient n_u is equal to 0.5, the stochastic activity of center of pressure in all directions is the same. When this coefficient increases, it appears preferable direction, in which displacements are the strongest. Simultaneously in perpendicular direction displacements are the smallest. In the limiting case, when n_u is equal to 1, not achieved practically, displacements appear only along one direction and in the direction perpendicular to it there are no displacements. It is further seen, that distribution of this coefficient widens after closing of subjects' eyes. Thus their mean and standard deviation were increased, i.e., assuming values 0.67 ± 0.08 and 0.71 ± 0.10, under eyes-open conditions and under

eyes-closed conditions, respectively, and these means are significantly different (P<0.001). It means that for these subjects the contribution of D_u and D_v to D_r, increased and decreased, respectively, their mean values.

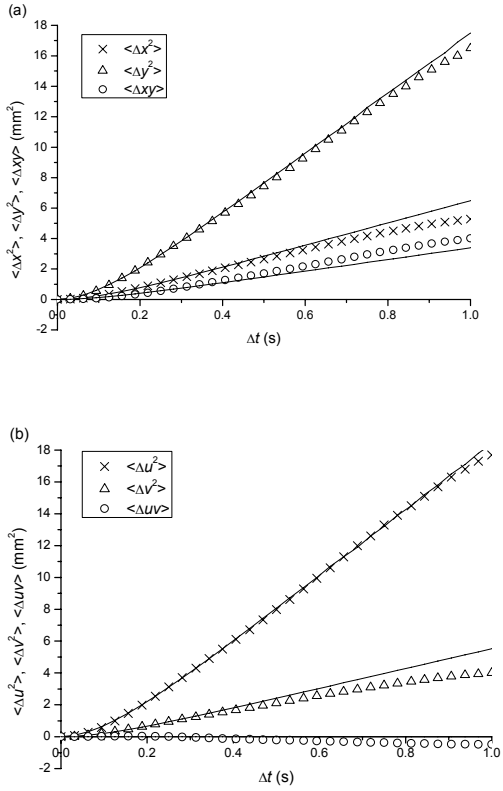

Fig. 1. *The plot of the diagonal and off-diagonal elements of the matrix of the mean squared displacement as a function of time interval Δt in xy (a) and uv (b) coordinate systems, for the representative subject under eyes-closed conditions and, approximated to them, theoretical ones given by Eq. (2) (solid lines), where theoretical <ΔuΔv> = 0*

Rys. 1. *Wykres diagonalnych i pozadiagonalnych elementów macierzy średniego kwadratowego przesunięcia jako funkcji interwału czasowego Δt w układzie współrzędnych xy (a) i uv (b), dla reprezentatywnego przypadku w warunkach oczu zamkniętych i, dopasowane do nich, funkcje teoretyczne dane przez równanie (2) (linie ciągłe), gdzie teoretyczne <ΔuΔv> = 0*

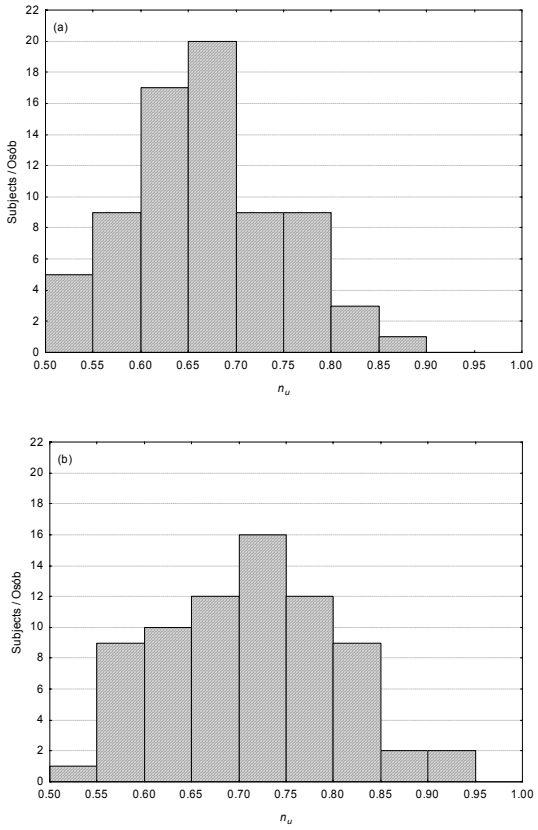

Fig. 2. *The histogram of the coefficient of the partition of the COP fluctuation strength n_u for young subjects under eyes-open (a) and eyes-closed (b) conditions*

Rys. 2. *Histogram współczynnika partycji mocy fluktuacji COP n_u dla młodych osób w warunkach oczu otwartych (a) i oczu zamkniętych (b)*

DISCUSSION AND CONCLUSIONS

We have investigated partition of the fluctuation strength of the short-term postural sway under quiet-standing conditions. It was examined, using method based on two-dimensional Ornstein-Uhlenbeck process. That process is described by two-dimensional Langevin equation of motion containing the friction force and the fluctuating force. The mean square displacement as a function of time interval for this process fits well this function for COP sway in the short-term region. Two-dimensional approach permits to find coordinate system *uv* in which COP displacements are uncorrelated. This coordinate system in general is rotated by an angle with respect to the system defined by the anatomy of the body. The status of the short-term postural sway under quiet-standing conditions is represented by the diffusion matrix, the matrix of the fluctuation strength and the friction coefficient. Matrices of diffusion and fluctuation strength can be characterized by following coefficients: the traces of these matrices, the angle defining the orientation of the *uv*

coordinate system with respect to the laboratory frame and the coefficient of the contribution of the diffusion coefficient and the coefficient of the fluctuation strength in u direction to traces of corresponding matrices. These parameters are well physiologically interpreted, since they describe the stochastic activity of the muscles of the lower limbs as well as mechanisms compensating that activity. In many cases, validity of our method was shown [15, 16, 19-22].

In this investigation it was shown that COP fluctuation strength is not equally distributed in all directions. Thus, partition of these fluctuations, which in physical models is constant for different angles, here accepts different values. Coefficient n_u, describing level of this partition, is different for each subject. It is also most dependent from direction in which the fluctuations are maximal for a given subject [19], and its increase accompanies an increase of COP fluctuations [16]. It was shown here that coefficient n_u increases and its values become less homogenous under eyes-closed conditions.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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EVALUATION OF RELATIONSHIP BETWEEN INTENSITY OF ANXIETY, PTSD SYMPTOMS AND DEPRESSION'S APPEARANCE IN VICTIMS OF DOMESTIC VIOLENCE

OCENA ZALEŻNOŚCI MIĘDZY WYMIARAMI LĘKU A OBECNOŚCIĄ OBJAWÓW PTSD I DEPRESJI U OFIAR PRZEMOCY DOMOWEJ

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Summary

P u r p o s e : The aim of work was to evaluate an intensity of anxiety as a trait and anxiety as a state as conditions of domestic violence victims. There was also the aim to evaluate a relation between those anxiety intensity and presence of posttraumatic stress disorder (PTSD) symptoms and depression.

M a t e r i a l : 39 women who had experienced trauma related to family violence formed the examined group.

M e t h o d s : The self-evaluation State Trait Anxiety Inventory (STAI) questionnaire, which had been compiled by Charles D. Spielberger, was used to evaluate intensity of anxiety as a trait and anxiety as a state. To evaluate presence of PTSD and symptoms' intensity there were 35-degree and 39-degree civilian versions of Mississippi PTSD Scale applied. To evaluate depression, Beck Depression Inventory and 17-degree version of Hamilton Depression Rating Scale were applied.

R e s u l t s : Victims of violence were characterized in most cases by the low anxiety intensity. Within the examined group of women in 36% of them PTSD and depression in 70% were ascertained. The intensity of both disorders correlated to each other. In women with PTSD diagnosis there were meaningfully higher average intensity of anxiety as a state and medium intensity of depression observed. The higher average level of PTSD symptoms was observed in the group of victims with the high intensity of anxiety.

C o n c l u s i o n s : Intensity of anxiety correlated to intensity of PTSD symptoms and depression. Patients with PTSD diagnosis experienced higher intensity of anxiety and depression.

Streszczenie

C e l : Celem pracy była ocena wymiaru lęku jako cechy i lęku jako stanu u ofiar przemocy domowej oraz ocena zależności między tymi wymiarami lęku a obecnością objawów PTSD i depresji.

M a t e r i a ł : Grupę badaną stanowiło 39 kobiet, które miały traumatyczne doświadczenia związane z przemocą domową.

M e t o d y : Oceny nasilenia lęku jako cechy i lęku jako stanu wykonano za pomocą kwestionariusza samooceny State Trait Anxiety Inventory (STAI), opracowanego przez Charlesa D. Spielebergera. Do oceny obecności PTSD i nasilenia objawów zastosowano 35 i 39 punktową cywilną wersję Mississippi PTSD Scale. Do oceny depresji posłużono się Inwentarzem Depresji Becka oraz 17 punktową wersją Skali Depresji Hamiltona.

W y n i k i : Ofiary przemocy w większości charakteryzowały się niskim poziomem lęku. W badanej grupie kobiet zanotowano u 36% PTSD oraz u ponad 70% depresję. Nasilenie obu zaburzeń korelowało ze sobą. U kobiet z diagnozą PTSD obserwowano istotnie wyższe średnie wartości nasilenia lęku jako stanu i średnie wartości głębokości depresji. Obserwowano większe średnie nasilenie objawów PTSD w grupie ofiar z wysokim lękiem.

W n i o s k i : Nasilenie lęku korelowało z nasileniem objawów PTSD i depresji. Osoby z diagnozą PTSD miały większe nasilenie lęku i depresji.

Key words: stress, trauma, domestic violence, anxiety, depression, PTSD
Słowa kluczowe: stres, trauma, przemoc domowa, lęk, depresja, PTSD

INTRODUCTION

Traumatic events may initiate or sharpen different mental disorders. One of the most common traumatic events which can be experienced by human being is trauma as a result of domestic violence. Domestic violence phenomenon means a deliberate activity taking advantage of force against any family member. It encroaches upon personal rights and goods, brings suffering and loses. According to the theory of evolution, phenomenon of violence within community is strictly bonded with a particular role of maternal child minding within mammals and with a quality of parental care within human beings [1]. Women who were physically or sexually abused achieve higher levels in tests measuring somatisation, depression, unrest, anxiety. They also commit suicide and are subjects of self-mutilation more often [2]. Serious trauma affects 1/3 of people, and 10-20% of them suffer continuously from PTSD [3]. Women more frequently than men react to stress by experiencing posttraumatic stress disorder [4]. Trauma which had been experienced in childhood, personality disorders, genetical susceptibility to psychical illness, inadequate supporting system, disadvantageous system of internal regulation, permanent psychical overloading, abuse of alcohol – all these factors have an impact on PTSD development [5]. As a result of the traumatic experience among people with PTSD, there were found functional brain changes as well as anatomical changes such as dying out of neurons in hyppocampus and reduction of hyppocampal volume in MRI. In women experiencing domestic violence the symptoms of depression and anxiety are diagnosed more often [6, 7, 8, 9, 10]. In the last few years a neuroanatomical, neurochemical basis of anxiety, stress disorders and posttraumatic stress were interpreted with a use of modern methods [11, 12, 13]. In anxiety disorders related to stress, some role is played by neural plasticity change, in conjunction with intracellular mechanism [14]. The basic factor causing anxiety is subjectively interpreted threat. The interpretation of threat is connected to individual experience. Anxiety meant as a state of emotions is conditioned on situation. However, a stronger negative evaluation of situation depends on anxiety as an element of personality. Intensity of such personality character as predisposition to more threatening reality evaluation depends, among others, on traumatic events, particularly when experienced in childhood. The victims of violence are submitted to traumatic situations regularly, sometimes for many years.

The low intensity of anxiety as a personality factor may have a preventive impact on situational anxiety stimulation, connected to violence or on further results of traumatic experience, which means development of posttraumatic stress disorder and depression.

The aim of this work was to evaluate intensity of anxiety as a trait and as a state as conditions of domestic violence victims as well as to evaluate relation between this intensity of anxiety and PTSD symptoms or depression presence.

MATERIAL

Selected aspects of psychical condition in 39 women aged between 17 and 55 were evaluated. The women experienced violence from a husband or a common-law husband, and two youngest victims from a father. The average age was 35.7 years, SD = 10.5 years. Inspected women were inhabitants of domestic violence victims' hostels in a voivodeship town. They experienced physical and psychological violence. Shapes of violence were different, like: beating, hitting with different objects, strangling, binding, financial limitation, limitation of mobility, demeaning, intimidating, using obscene expressions, compelling sexual intercourse, wheedling. The victims were forced to leave their dwellings to protect themselves and their children. The timing of continuous violence was different: from 6 months to 20 years. The period since the last traumatic event in most cases has been exceeding 6 months, in seldom cases not much over 1 month.

METHODS

I. Evaluation of intensity of anxiety as a trait and anxiety as a state.

The self-estimate questionnaire State Trait Anxiety Inventory (STAI), elaborated by Spielberger, Gorsuch and Lushene (1970) was used in the research. [15]. It consisted of two parts, each of them including 20 questions [16]. The Part One deals with evaluation of anxiety as a state, being experienced at the certain moment. The Part Two deals with anxiety as a trait, which means the permanent state [17]. The high results in intensity of anxiety/state give an evidence of difficult living situation impact on examined women. The high results in intensity of anxiety/trait give an evidence of permanent personality disposal to liability to respond

by anxiety to different life situations. In a research made by the State Trait Anxiety Inventory (STAI), raw results which had been achieved during examination, were exchanged for standardized results relating to sex and age – sten factor score [18]. Sten scale is a 10-degree scale. The scale between 1 and 4 is interpreted as low (it gives an evidence of low rate of anxiety as trait and state), the scale between 5 and 6 is medium (a medium intensity of anxiety as state and trait), and scale between 7 and 10 is interpreted as high level (they give evidence of high intensity of anxiety as state and trait).

II. Evaluation of PTSD presence and of symptoms intensity

A). The Mississippi PTSD Scale - 35 degrees version

The civilian version of Mississippi PTSD Scale is a result of adaptation of the basic version of scales used for PTSD evaluation in soldiers who have experienced military actions [19]. The above mentioned scale is used for evaluation of PTSD survey within the last month, with a help of 5-degree Likert scale and consists of 35 items. Low score which was achieved with a help of the scale, gives evidence of lacking PTSD symptoms. High score gives evidence of the disorder's presence. The possibly achieved scale is between 35 and 175 degrees. To recognise PTSD in war veterans with the use of Military M-PTSD scale, there was a requirement of 94 degrees at least [20]. In the civilian version the critical level to PTSD diagnosis was 96 degrees [21]. In the civilian version 11 item's word expressions were changed. The questionnaire includes 35 questions delimitating PTSD symptoms in conjunction with DSM III criterions. Symptoms belong to three different categories: 1. traumatic event's replay, 2. avoiding from trauma and emotional numbness, 3. continuous overexposed excitement. The other symptoms included in the scale relate, among others, to intensity of depression: melancholy, suicide tendencies and feeling of guilt. The Polish adaptation was described in Lis-Turlejska's and Łuszczyńska-Cieślak's work (2001) [22].

B). Mississippi PTSD Scale - 39 degrees version.

The scale consists of 39 items including PTSD symptoms description in accordance with DSM-IIIR criterions. The four items which were added later, evaluate repeated experiencing, psychogenic amnesia, excessive alertness and excitement rise [23].

III. Evaluation of depression's intensity

A). Beck Depression Inventory (BDI). In a research the last month's period was evaluated. [24].

B). HDRS- Hamilton Depression Rating Scale. In a research the 17-degree Hamilton Depression rating Scale was used [25].

STATISTICAL ANALYSIS

Results of research were measured with the tools of statistics. The file of statistic tests SPSS for Windows, version 13.0 was used. Independent-samples t test, Spearman correlation test, Mann-Whitney test were used.

RESULTS

In the inspected group of patients the anxiety as a trait and as a state was both low. The average intensity of anxiety/state raw results in the inspected group of women (victims of domestic violence) was 18.2 degrees, complying with SD = 8.8. The minimal intensity of anxiety/state in the inspected group was 0. whereas the maximum one was 41 degrees. The average intensity of anxiety/trait raw results among inspected women was 28.8 degrees, complying with SD = 11.1 and a range between 12 and 53. The meaningful correlation between the level of anxiety as a state and anxiety as a trait was noticed (Spearman correlation coefficient = 0.698; p = 0.000; correlation is significant at the 0.01 level).

After having assigned in subsequent age ranges the raw results that were achieved by use of STAI - sten scale, intensity of anxiety as a state was low in most of examined women. In more than 93% of inspected patients (93.9%) low intensity of anxiety was discovered. In 6.1% of women medium intensity of anxiety as a state was discovered and none of the examined women appeared to have high current intensity. The high intensity of anxiety as a trait was discovered in 3% women, medium in 12.5% and the most (84.4%) of women appeared to experience low intensity of anxiety as a trait. In the researched group of women who were victims of violence towards a partner, the average intensity of PTSD symptoms measured with Mississipi PTSD Scale, was 90.61, correlated with SD=25.5 (within 35-degree scale) and 101.58 correlated with SD= 28.27 (within 39-degree scale). PTSD was discovered in 36% of group members (over 96 degrees within the 35-item PTSD scale). Average intensity raw

results of anxiety/state in a group of PTSD diagnosed women (22.3; SD = 8.6) was meaningfully higher in comparison with the one in a group of victims who had not diagnosed posttraumatic stress disorder (15.3; SD = 8.1) (independent-samples t test; t = 2.3. df = 24.8; p = 0.029). The difference was not observed in average anxiety/trait intensity between a group of women with PTSD diagnosis (33.3 degrees; SD = 11.8) and those without diagnosed disorder (25.6; SD = 9.9) (independent-samples t test; p = 0.074)(tab. I).

Table I. *Anxiety and depression intensity as related to PTSD diagnosis*

	PTSD diagnosis (+) Average values (SD)	PTSD diagnosis (-) Average values (SD)	p
Anxiety / trait	33.3 (11.8)	25.6 (9.9)	0.074
Anxiety / state	22.3 (8.6)	15.3 (8.1)	0.029*
Depression (BDI)	32.9 (12)	20.5 (13.7)	0.015*
Depression (HDRS)	24.2 (8.5)	16 (8.4)	0.017*

* relevance; independent-samples t test

Intensity of anxiety/state in raw results within STAI scale correlated with intensity of PTSD symptoms in 35-degree Mississippi PTSD Scale (Spearman correlation coefficient = 0.504. p = 0.03; correlation is significant at the 0.01 level) as well as with PTSD symptoms intensity within 39-degree scale (Spearman correlation coefficient = 0.530. p = 0.002; correlation is significant at the 0.01 level). Intensity of anxiety/trait in raw results within STAI scale correlated with PTSD symptoms intensity within 35-degree Mississippi PTSD Scale (Spearman correlation coefficient = 0.592. p = 0.000; correlation is significant at the 0.01 level) and within 39-degree scale (Spearman correlation coefficient = 0.598. p = 0.000; correlation is significant at the 0.01 level). Intensity of anxiety within sten scale, after having attached it to proper age range, correlated meaningfully between PTSD symptoms intensity within 39-degree Mississippi PTSD Sale both in case of anxiety/trait (Spearman correlation coefficient = 0.483. p = 0.006; correlation is significant at the 0.01 level) and anxiety/state (Spearman correlation coefficient = 0.353. p = 0.047; correlation is significant at the 0.05 level) (tab. II).

Table II. *Correlation coefficient between anxiety intensity and PTSD symptoms intensity*

Anxiety (STAI)	35.Mississippi PTSD Scale	39. Mississippi PTSD Scale
Trait (raw)	0.592**	0.598**
Trait (stens)	0.480**	0.483**
State (raw)	0.504**	0.530**
State (stens)	0.346	0.353*

* p< 0.05 ** p< 0.01; Spearman correlation test

In a case of PTSD symptoms evaluation within 35-degree Mississippi PTSD Scale, the meaningful correlation applied to anxiety/trait intensity (Spearman correlation coefficient = 0.480. p = 0.006; correlation is significant at the 0.01 level), and yet correlation to anxiety/state intensity was not observed (Spearman correlation coefficient = 0.346. p = 0.053). In a group of women experiencing low anxiety/state intensity(<5 stens), the average PTSD symptoms intensity within 35-degree scale was 88.9 (SD = 22.6) , and in a group experiencing higher anxiety/state intensity (>= 5 stens) was higher (146 points, SD = 12.7) (independent-samples t test; t = 5.765. df = 1.465. p = 0.056). PTSD symptoms intensity was meaningfully higher (131 points, SD = 25) within 35-degree scale within the group of women experiencing higher anxiety intensity, than in women with lower sten range of anxiety (=> 5 stens) (84.62 points, SD = 19.5) (independent-samples t test; t = 3.923. df = 4.977. p = 0.011) (tab. III).

Table III. *PTSD symptoms and depression intensity in a group of women suffering from either high or low anxiety intensity*

Mean scores (SD)	Anxiety / state			Anxiety / trait		
	high	low	p	high	low	p
PTSD 35.	146 (12.7)	88.9 (22.6)	0.056	131 (25)	84.6 (19.5)	0.011
PTSD 39.	163 (11.3)	99.7 (25.1)	0.028*	144.6 (28)	95.1 (0.01)	0.014*

* relevance of difference; independent-samples t test

The meaningful difference concerning PTSD symptoms intensity (having been evaluated within 35-degree Mississippi PTSD Scale) was observed in a group experiencing low anxiety/state intensity (Mann-Whitney test, u =1.0. z = -2.258. p = 0.024). Similarly, yet more meaningful difference was in PTSD symptoms intensity in groups of women experiencing higher and lower anxiety/trait intensity (p = 0.006. u = 9. z = -2.738; Mann-Whitney test).

Meaningfully higher PTSD symptoms intensity (within 39-degree Mississippi PTSD Scale) was ob-

served between those women who had experienced higher anxiety intensity (=> 5 stens) as a state (independent-samples t test; t = 6.86. df = 1.75. p = 0.028) as well as a trait (independent-samples t test; t = 3.723. df = 5.008. p = 0.014). Average PTSD symptoms intensity in women with higher anxiety/state intensity was 163 points (SD = 11.3) and in women with lower anxiety 99.7 points (SD = 25.1). Average PTSD symptoms intensity in women experiencing higher anxiety/trait intensity was 144.6 points (SD = 28) and in ones with lower anxiety 95.19 points (SD = 22.2).

Depression of at least moderate intensity was found in 70% and 77% of inspected women (Beck Depression Inventory and Hamilton Depression Scale). Intensity of anxiety, both as a trait and a state, correlated to depression intensity having been self-evaluated by patients (applying to Beck Depression Inventory evaluation) and in depression evaluation by Hamilton Depression Scale. Anxiety as a state meaningfully correlated to depression intensity in a self-evaluation of examined women (Spearman correlation coefficient = 0.495. p = 0.007; correlation is significant at the 0.01 level) and to depression intensity within Hamilton Depression Scale (Spearman correlation coefficient = 0.544. p = 0.003; correlation is significant at the 0.01 level). Anxiety as trait meaningfully correlated to depression intensity in self-evaluation of examined women (Beck Depression Inventory) (Spearman correlation coefficient = 0.839. p = 0.000; correlation is significant at the 0.01 level) and to depression intensity within Hamilton Depression Scale (Spearman correlation coefficient = 0.637. p = 0.000; correlation is significant at the 0.01 level). PTSD symptoms intensity within the 35-degree Mississippi PTSD Scale correlated to depression intensity evaluated by the victims with a help of Beck Depression Inventory (Spearman correlation coefficient = 0.633. p = 0.000; correlation is significant at the 0.01 level) and to depression symptoms intensity examined by a physician with a help of Hamilton Depression Scale (Spearman correlation coefficient = 0.612. p = 0.000; correlation is significant at the 0.01 level)(tab. IV).

The average value of Beck Depression Inventory was meaningfully higher (32.9; SD = 12) in these women who were subject to posttraumatic stress disorders (=> 96 points within 35-degree Mississippi PTSD Scale) in comparison to women not suffering from PTSD (20.5; SD = 13.7 (independent-samples t test; t = 2.614; df = 25.838. p = 0.015). Similarly, in case of average depression values as evaluated within Hamilton Depression Scale, the average value of depression intensity was meaningfully higher (24.3 points; SD = 8.5) in women with diagnosed PTSD than in a part of examined victims not suffering from PTSD (16 points; SD = 8.4) (independent-samples t test; t = 2.596; df = 20.594. p = 0.017) (tab. 1). The average values of PTSD symptoms intensity within the 35-degree Mississippi PTSD Scale were meaningfully higher in a group of women suffering from at least moderate depression (101.2 points, SD = 24.7) in comparison to a part of women not suffering from depression or experiencing benignity (73.7 points, SD= 22.3)(p = 0.008. t = 2.87; df = 16.7; independent-samples t test).

Table IV. *Correlation coefficient between depression symptoms intensity and anxiety intensity or PTSD symptoms intensity*

	Depression (BDI)	Depression (HDRS)
Anxiety / state	0.839**	0.637**
Anxiety / trait	0.495**	0.544**
PTSD (35. degree scale)	0.633**	0.612**

* p< 0.05 ** p< 0.01; Spearman correlation test

DISCUSSION

In the examined group the victims of violence in most cases were characterised by low intensity of anxiety. In this part of women who had been diagnosed with PTSD on a basis of Mississippi PTSD Scale, meaningfully higher average values of anxiety/state and average values of depression intensity were observed. Moreover, the higher average PTSD symptoms intensity was observed in a part of victims suffering from high density anxiety. Within the examined group of women who had experienced domestic violence, PTSD was diagnosed in 36% and depression in 70%. Intensity of both disorders correlated with each other. Correlation between depression and anxiety disorders is general [26]. In the case of psychiatric effects after experiencing traumatic events, depression follows PTSD regularly.

The guard rail from posttraumatic stress disorder appearance may be presence of certain personality attributes, especially victims' intellectual values [27]. Termination of domestic violence and release from perpetrator's influence is related to many different factors, with intensity of anxiety among them [28]. Within the examined group majority of women experienced low intensity of anxiety as trait which could have impact on low intensity of anxiety as a state and

could lead to a decision to leave perpetrator. At the time of research women did not experience violence as they stayed at the single-mother hostels or at hotels for violence victims. Women experiencing violence need particular medical care and support [29].

CONCLUSIONS

1. Intensity of anxiety correlated to intensity of PTSD symptoms and depression.
2. Persons with PTSD diagnosis experienced higher intensity of anxiety and depression

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PITUITARY GLAND IMAGING WITH ^{99m}Tc-MIBI IN PATIENTS WITH CUSHING'S DISEASE

OBRAZOWANIE PRZYSADKI MÓZGU ^{99m}Tc-MIBI U CHORYCH NA CHOROBĘ CUSHINGA

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Summary

Introduction: ^{99m}Tc-MIBI (methoxyisobutylisonitrile) was created to examine myocardial perfusion. The subcellular site of the radiotracer retention in myocyties is mitochondrion. In diagnosis of pituitary adenomas nuclear medicine imaging methods are not used too often. This situation results from the fact that the use of magnetic resonance imaging with gadolinium enhancement enables to localize a tumor even in the case of microadenoma. However, in about 36-63% of cases the magnetic resonance examination does not reveal an adenoma in the pituitary gland despite the clinical symptoms of Cushing's disease.

The aim of the study was to evaluate the possibility of the pituitary adenoma imaging by means of Single Photon Emission Tomography (SPECT) using the ^{99m}Tc -MIBI in patients with Cushing's disease when MRI examination does not show microadenomas.

Material and methods: The research group consisted of 3 patients with Cushing's disease diagnosis performed on the basis of clinical and hormonal findings. All patients underwent pituitary SPECT with the use of ^{99m}Tc-MIBI following the magnetic resonance scanning of pituitary gland. The MRI did not reveal any changes. The control group consisted of 5 volunteers without hormonal dysfunction of pituitary gland who underwent SPECT examination because of coronary heart disease. The analysis of the SPECT images was both qualitative and semi - quantitative. The pituitary uptake was compared to the parietal bone uptake accepted as 100 %.

Results: The increased accumulation of ^{99m}Tc - MIBI in pituitary gland was noticed in patients with Cushing's disease. In pituitary glands of the control group the accumulation of the radiotracer was not observed. No accumulation was noticed in other parts of brain, except choroids plexus, in any of the examined persons. Accepting the radiotracer uptake in parietal bone as 100% we noted that accumulation in pituitary glands of persons with Cushing's disease was on the average 146%.

Conclusion: Single Photon Emission Computed Tomography using ^{99m}Tc-MIBI can be an useful and sensitive way of pituitary microadenoma detection in patients with Cushing's disease when microadenoma is invisible during the MRI scanning.

Streszczenie

Wstęp: ^{99m}Tc-MIBI (metoksyizobutyloizonitryl) stworzono do badania ukrwienia mięśnia sercowego. Miejscem gromadzenia znacznika w komórce są mitochondria.

W diagnostyce gruczolaków przysadki obrazowanie przy pomocy metod medycyny nuklearnej stosowane jest niezbyt często. Wynika to z faktu, że zastosowanie rezonansu magnetycznego ze wzmocnieniem gadoliny, umożliwia zwykle doskonałą lokalizację, również w przypadku mikrogruczolaków. Jednakże w 36-63% przypadków, mimo klinicznych cech choroby, badanie rezonansu magnetycznego nie wykazuje gruczolaka w przysadce.

Celem naszej pracy była ocena czy możliwe jest zobrazowanie gruczolaka przysadki za pomocą SPECT z użyciem ^{99m}Tc-MIBI u pacjentów z chorobą Cushinga, u których w badaniu MRI nie wykazano mikrogruczolaków.

M a t e r i a ł i m e t o d y : Grupę badaną stanowiło 3 pacjentów z chorobą Cushinga, u których rozpoznanie postawiono na podstawie badania klinicznego i badań hormonalnych. Wszystkim chorym wykonano scyntygrafię z użyciem ^{99m}Tc-MIBI po wcześniejszym wykonaniu MRI przysadki. Grupę kontrolną stanowiło 5 ochotników, bez klinicznych cech dysfunkcji hormonalnej, u których wykonano SPECT serca z powodu choroby wieńcowej.

Ocena wyniku badania była zarówno jakościowa, jak i półilościowa. Wychwyt znacznika w przysadce porównywano z wychwytem w rzucie kości ciemieniowej, który przyjmowano za 100%.

W y n i k i : Zwiększone gromadzenie znacznika ^{99m}Tc-MIBI w obrębie przysadki uwidoczniono u pacjentów z chorobą Cushinga. Nie stwierdzono natomiast wychwytu znacznika w przysadkach osób z grupy kontrolnej, ani w pozostałych obszarach mózgu (z wyjątkiem splotu naczyniówkowego). Przyjmując wychwyt w kości ciemieniowej jako 100%, stwierdzono, że gromadzenie w przysadkach osób z chorobą Cushinga wynosiło średnio 146%.

W n i o s e k : Tomografia emisyjna pojedynczego fotonu z użyciem ^{99m}Tc-MIBI jest przydatnym i czułym sposobem wykrywania mikrogruczolaków przysadki u pacjentów z chorobą Cushinga, u których są one niewidoczne w badaniu MRI.

Key words: Cushing's disease, pituitary microadenoma, SPECT, ^{99m}Tc-MIBI
Słowa kluczowe: choroba Cushinga, mikrogruczolaki przysadki, SPECT, ^{99m}Tc-MIBI

INTRODUCTION

Technetium ^{99m}Tc-MIBI (methoxyisobutylisonitrile) was created to examine myocardial perfusion. Accumulation of the radiotracer in myocardium is proportional to blood flow [1]. Retention of ^{99m}Tc-MIBI depends not only on blood flow but also on tissue viability [2]. The subcellular site of the radiotracer retention in myocyties is mitochondrion [3]. The mitochondrial retention of ^{99m}Tc-MIBI is not organ specific but it is a mechanism common to the majority of tissues. The investigation conducted by Piwnica - Worms shows that both uptake and retention of ^{99m}Tc-MIBI depend on metabolism and mitochondrial membrane potential [4].

The high level of the radiotracer accumulation should be observed in tumors. The uptake of the radiotracer in brain tumors was described by O'Tauma [5]; in normal conditions the radiotracer does not cross an undamaged blood – brain barrier. The ^{99m}Tc-MIBI is accumulated not only in malignant tumors but also in parathyroid adenomas [6-9]. According to Sandrock the adenoma of parathyroid have a great number of mitochondria [10]. Therefore, the uptake of ^{99m}Tc-MIBI is more intensive in adenoma cells than in the surrounding thyroid tissue, and it is washed out from adenoma at a slower rate.

In diagnosis of pituitary adenomas nuclear medicine imaging methods are not used too often. This situation results from the fact that the use of magnetic resonance imaging (MRI) with gadolinium enhancement enables to localize a tumor even in the case of microadenoma [11-13]. However, in about 36-63% of cases the magnetic resonance examination does not reveal an adenoma in the pituitary gland despite the clinical symptoms of Cushing's disease [14, 15].

The aim of our study was to evaluate the possibility of the pituitary adenoma imaging by means of Single Photon Emission Computed Tomography (SPECT) using the ^{99m}Tc -MIBI in patients with Cushing's disease when MRI examination does not show microadenomas.

MATERIAL AND METHODS

The research group consisted of 3 patients with Cushing's disease that had been diagnosed on the basis of clinical and hormonal findings. The blood cortisol level of the patients was elevated (the average level was about 12 ug/dl) and it showed no day and night rhythm. No abnormalities in pituitary glands were observed during MRI scanning in patients with Cushing's disease (Fig.1). All patients underwent pituitary scintigraphy using ^{99m}Tc- MIBI following the magnetic resonance scanning of their pituitary gland. The control group consisted of 5 volunteers without hormonal dysfunction of pituitary gland who underwent SPECT because of coronary heart disease.

All patients were examined in the Laboratory of Radioisotopes of the Medical University in Poznań. One-head gammacamera Diacam with low energy high resolution collimator (LEHR - Low Energy High Resolution) was used while measuring the emission from pituitary glands by computed tomography. The acquisition was started 2 hours after intravenous application of the 740 MBq of ^{99m}Tc-MIBI. The head turned round a patient's head by 360 degrees by elliptical course divided into 64 projections for 30 seconds.

The analysis of SPECT images was both qualitative and semi - quantitative. The regions of interest in each pituitary gland were drawn and then the radiotracer uptake was compared with the uptake in the region of interest in parietal bone, which was accepted as 100%.

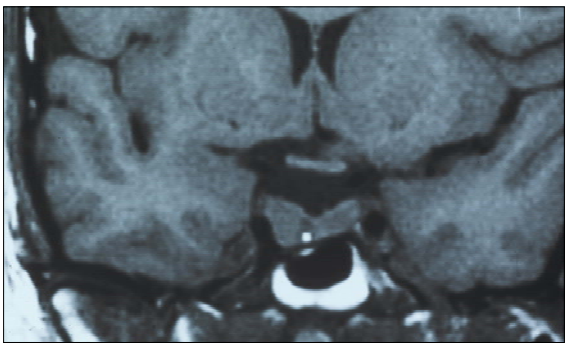

Fig. 1. *MRI scanning of the pituitary gland (no abnormalities)*
Fot. 1. *Rezonans magnetyczny przysadki (obraz bez zmian)*

RESULTS

The increased accumulation of ^{99m}Tc - MIBI in pituitary gland was noticed in patients with Cushing's disease. In the pituitary glands of patients of the control group the accumulation of the radiotracer was not observed. No accumulation was noticed in other parts of the brain, except chorioid plexus, in any of the examined persons. Accepting the radiotracer uptake in parietal bone as 100% we noted that accumulation in pituitary glands of persons with Cushing's disease achieved on average 146% (Fig. 2).

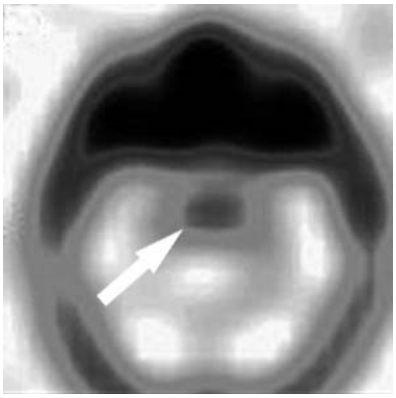

Fig. 2. *SPECT image of the pituitary gland. In the pituitary gland an area of an increased accumulation of ^{99m}Tc-MIBI is shown (arrow)*
Fot. 2. *Badanie SPECT przysadki mózgowej. Zwiększone gromadzenie radioizotopu ^{99m}Tc-MIBI uwidoczniono w obrębie przysadki mózgowej (strzałka)*

DISCUSSION

The Single Photon Emission Tomography (SPECT) is one of the tomography techniques imaging morphology and function of organs by displaying them in thin sections. Technetium ^{99m}Tc- MIBI has the ability to penetrate through damaged blood – brain barrier, but in normal conditions the radiotracer does not cross blood – brain barrier. [5]. The uptake of the radiotracer depends on both the number of mitochondria and the potentials of mitochondrial and cellular membranes [3]. Therefore, cells with high metabolic activity such as neoplastic tissues, can show high levels of the radiotracer uptake [5].

The sensitivity of the SPECT examination is high, but specificity of this method is rather low. The uptake of ^{99m}Tc-MIBI was observed in some intracranial tumors such as astrocytoma, glioblastoma and meningioma [4]. It seems that SPECT using ^{99m}Tc- MIBI could be particularly useful in case of tumor regrowth and also in the case of difficulties in estimation of hormonal activity of tumor. The postoperative or post radiotherapy tumor recurrence is often difficult to be seen in MRI. The surrounding tissue can be considered either as necrotic after treatment or as a regrowth of tumor.

We observed that no ^{99m}Tc-MIBI uptake is demonstrable in intracranial normal structures (except chorioid plexus), as it was noted in previous studies. In our research we observed that the accumulation of technetium is higher in pituitary gland adenomas than in parietal bone (which was accepted as 100%) and it achieved on average 146% in patients with Cushing's disease. No abnormalities in pituitary glands were observed during MRI scanning in the examined patients with Cushing's disease. Therefore, SPECT examination should be considered as a complementary diagnostic method when microadenoma of a pituitary gland is suspected.

CONCLUSION

Single Photon Emission Tomography using ^{99m}Tc-MIBI is a useful and sensitive way of pituitary gland microadenoma detection in patients with Cushing's disease when microadenoma is invisible during the MRI scanning.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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ASSOCIATION OF C-REACTIVE PROTEIN WITH APOLIPOPROTEINS OR TRADITIONAL LIPID MARKERS AS A MEASURE OF FUTURE CARDIOVASCULAR RISK IN APPARENTLY HEALTHY YOUNG NON-OBESE ADULTS

ASOCJACJA BIAŁKA C-REAKTYWNEGO Z APOLIPOPROTEINAMI ORAZ TRADYCYJNYMI MARKERAMI LIPIDOWYMI JAKO WSKAŹNIK RYZYKA CHORÓB SERCOWO-NACZYNIOWYCH U ZDROWYCH, MŁODYCH OSÓB

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Summary

Introduction: C-reactive protein has been shown an independent risk predictor of future coronary events in healthy subjects. Recent evidence suggests that apolipoproteins and apoB:apoAI ratio are strong predictors of cardiovascular disease risk. The levels of risk factors in young healthy individuals, albeit relatively low, may be more informative than generally recognized.

Aim of the study We assessed whether hsCRP and apolipoprotein measurement in young individuals could allow better detection of those at risk than traditional lipid markers.

Methods:: hsCRP, TC (total cholesterol), HDL-C, TG (triglycerides), apoAI, apoB100 were measured, LDL-C, non-HDL-C levels were calculated, waist circumference (Wc) was taken in 192 healthy, non-obese not smoking subjects aged 25-40 years with lipid concentrations within generally recommended values (104) and with moderate hyperlipidemia (88).

Results: Among individuals with normal lipids 33% had hsCRP>1mg/L (moderate relative cardiovascular risk), 14% hsCRP>3mg/L (high relative cardiovascular risk). In hyperlipidemia 48% had hsCRP>1 mg/L, 21% >3 mg/L. In females with normolipidemia CRP was significantly related to TG, apoB (R=0.4), apoB:apoAI (R=0.3) and HDL-C (R=-0.3). Apolipoproteins in all subjects were within the reference range but hsCRP increased with raising apoB and decreasing apoAI and was the most elevated in the highest apoB and the lowest apoAI concentrations in tertiles. No association of hsCRP with lipid markers was found. hsCRP>3 mg/L was related to higher frequency of increased apoB:apoAI ratio but not to TC:HDL-C.

Conclusions: hsCRP and apolipoprotein measurement in young non-obese individuals, instead of traditional lipids, could be more informative and allow better detection of those at risk of future cardiovascular disease.

Streszczenie

Wstęp: Białko C-reaktywne jest niezależnym wskaźnikiem ryzyka incydentów sercowo-naczyniowych u zdrowych osób. Wyniki ostatnich badań wskazują, że również apolipoproteiny i wskaźnik apoB:apoAI są dobrymi markerami w ocenie ryzyka chorób sercowo-naczyniowych. Stężenie tych wskaźników u zdrowych, młodych osób, aczkolwiek relatywnie niskie, może dostarczać wielu przydatnych informacji.

Celem pracy było określenie w jaki sposób stężenie hsCRP i apolipoprotein u młodych osób może pozwolić na lepszą ocenę ryzyka chorób sercowo-naczyniowych, w porównaniu z tradycyjnymi parametrami lipidowymi.

Materiał i metody: U 192 zdrowych, nieotyłych, niepalących osób w wieku 25-40 lat z prawidłowym stężeniem lipidów i nieznaczną hiperlipidemią oznaczono stężenie białka C-reaktywnego metodą o wysokiej czułości, cholesterolu całkowitego (TC), cholesterolu frakcji HDL (HDL-C), triglicerydów (TG), apolipoproteiny AI, apolipoproteiny B100. obliczono stężenie cholesterolu frakcji LDL i nie-HDL oraz dokonano pomiaru obwodu talii.

W y n i k i : W grupie zdrowych osób z prawidłowym poziomem lipidów u 33% wykazano podwyższone stężenie hsCRP>1mg/l (umiarkowane względne ryzyko chorób sercowo-naczyniowych), a u 14% wysokie stężenie hsCRP>3 mg/l (wysokie względne ryzyko chorób sercowo-naczyniowych). W grupie z hiperlipidemią 48% posiadało podwyższone stężenie hsCRP>1 mg/l, a 21% hsCRP>3 mg/l. W grupie kobiet z prawidłowym poziomem lipidów wykazano istotną korelację pomiędzy CRP a TG, apoB (R=0.4), apoB:apoAI (R=0.3) i HDL-C (R=-0.3). Stężenie apolipoprotein mieściło się w zakresie wartości referencyjnych, ale zaobserwowano tendencję do wzrostu stężenia CRP wraz ze wzrostem stężenia apoB i spadkiem stężenia apoAI. Stężenie hsCRP było najwyższe w najwyższym tercylu stężeń apoB i najniższym tercylu stężeń apoAI. Nie wykazano zależności pomiędzy hsCRP a tradycyjnymi parametrami lipidowymi. Ponadto, hsCRP> 3 mg/l było związane ze wzrostem wartości wskaźnika apoB:apoAI, czego nie zaobserwowano w przypadku wskaźnika TC:HDL-C.

W n i o s k i : Oznaczanie stężenia hsCRP i apolipoprotein u młodych, zdrowych osób, zamiast tradycyjnych parametrów lipidowych, może być bardziej przydatne w ocenie ryzyka wystąpienia chorób sercowo-naczyniowych w przyszłości.

Key words: C-reactive protein, apolipoproteins, cardiovascular risk

Słowa kluczowe: białko C-reaktywne, apolipoproteiny, ryzyko chorób sercowo-naczyniowych

INTRODUCTION

Since high sensitivity CRP assay has been developed, it was used as a predictive factor of cardiovascular risk [1]. Recently, it has been discovered that CRP possess proatherogenic properties, activates macrophages and enhances low density lipoprotein (LDL) uptake [2, 3]. There is increasing evidence that measurement of apolipoprotein B and apolipoprotein A may add valuable information in the clinical assessment of cardiovascular risk [4]. In healthy people, when LDL-C is within the normal range, higher apoB level may indicate an increased number of small dense atherogenic LDL particles that are easily oxidized and promote an inflammatory state and the development of plaques [5]. HDL particles possess anti-inflammatory properties, may modulate cell proliferation and migration and are cytoprotective against apoptosis induced by oxidized LDL [6]. ApoAI, as the major apolipoprotein in HDL particles manifests anti-inflammatory, antioxidant and anti-atherogenic properties [7].

The pathogenesis of atherosclerosis and cardiovascular disease often starts in childhood and measurement of sensitive markers may be especially valuable for identifying individuals at higher risk for disease [1]. It has been shown that hsCRP is a useful marker of low-grade inflammation in apparently, healthy subjects (8, 9). Apolipoproteins and apoB:apoAI ratio are also suggested strong predictors of cardiovascular risk [7] The levels of risk factors in young healthy individuals, albeit relatively low, may be more informative than generally recognized. We evaluated the association of CRP with novel and traditional lipid measures in apparently healthy adults with normal and moderately elevated lipid concentrations.

MATERIALS AND METHODS:

Study included 192 non-obese not smoking subjects, 112 women and 80 men aged 25- 40 years. The informed consent from each participant was obtained. Subjects with hyperglycemia or diabetes, hypertension, cardiovascular disease or any infection during a week preceding blood drawing were excluded. Among all, 104 participants had normal lipids and 88 had moderately elevated TC and/or TG. Fasting blood samples were collected and serum glucose, TC, HDL-C, TG, apoAI and apoB100 concentrations were measured (Abbott ARCHITECT ci8200). Reference ranges for apoB 53-182 mg/dL (women) and 49-173 mg/dL (men) and for apoA concentrations 101-223 mg/dL (women) and 95-186 mg/dL (men) were accepted according to the manufacturer data. LDL-C and non-HDL-C values were calculated. Remaining serum samples stored at -20˚C were assayed for CRP using high-sensitivity assay (BN II Dade Behring). The assay detection limit is 0.15 mg/L and CV is 5% for concentration of 0.35 and 0.5 mg/L. Three clinical cut points for hsCRP has been accepted <1.0 mg/L (low relative cardiovascular risk), 1-3 mg/L and >3.0 mg/L [10]. The following cut points were used : TC≤200 mg/dL (5.18 mmol/L), LDL-C≤130 mg/dL (3.36 mmol/L), HDL-C≥40 mg/dL (1.03 mmol/L) for men and ≥50 mg/dL (1.29 mmol/L) for women, TG≤150 mg/dL (1.71 mmol/L). All subjects were normoglycemic (≤100 mg/dL; 5.55 mmol/L) and non-obese : Wc≤ 80 cm for women and ≤ 94 cm for men [11]. As optimal TC:HDL-C ratio the values <3,4 (women) and <3.3 (men) were accepted. ApoB:apoAI ratio <0.6 (women) and <0.7 (men), reflected the low risk [12].

Data were presented as mean±standard deviation, except for CRP, where median, 25th and 75th percen-

tiles were given. Student t-test and U-Mann-Whitney test were used and Pearson's test for assessment of correlation between the variables after $\log_{10}$ transformation. Statistical analysis was performed using Statistica 6.0.

The study was approved by the Bioethics Committee at Collegium Medicum, Nicolaus Copernicus University.

RESULTS AND DISCUSSION

The median hsCRP concentration in subjects with normolipidemia was 0.59 mg/L (0.26-1.29) whereas in those with moderate hyperlipidemia was 0.96 mg/L (0.37-1.87), with slightly higher levels in females. Table I shows the characteristics of the study subjects.

Table I. *Characteristics of subjects with normal and elevated lipid values included in the study*

Variable	Normal lipid profile (n=104)		Moderate dyslipidemia (n=88)	
	Females (n=64)	Males (n=40)	Females (n=48)	Males (n=40)
Age (years)	27.5 ± 3.5	28.3 ± 3.8	30 ± 5[b]	30 ± 4
TC (mg/dL)	171 (151-189)	170 (154-186)	217 (206-232)[c]	218 (202-238)[c]
LDL-C (mg/dl)	86 (69-112)	103 (81-119)*	130 (116-151)	140 (115-161)[c]
HDL-C(mg/dL)	67 (57-77)	53 (45-58)**	68 (63-83)	53 (46-54)**
Non-HDL-C(mg/dL)	98 (83-122)	119 (98-132)**	145 (130-166)[c]	169 (149-180)[c]
TG (mg/dL)	64 (52-97)	80 (61-103)	85 (62-128)[b]	151 (94-187)**[c]
TC:HDL	2.5 ± 0.5	3.2 ± 0.6**	3.1 ± 0.6[c]	4.2 ± 0.7**[c]
hsCRP (mg/L)	0.65 (0.3-1.95)	0.52 (0.21-0.87)	1.02 (0.24-3.5)	0.9 (0.4-1.5)[a]
apoA (mg/dL)	134 ± 31	119 ± 33*	139 ± 44	108 ± 23**
apoB (mg/dL)	60 ± 19	64 ± 17	89 ± 30[c]	82 ± 20[c]
apoB:apoA	0.45 ± 0.12	0.55 ± 0.14**	0.61 ± 0.17	0.76 ± 0.15**[c]
Waist (cm)	72 (68-75)	86 (81-90)**	73 (69-77)	86 (82-94)**

Differences between females and males *p<0.05; **p<0.01
Differences between subjects with normal lipid profile and moderate hyperlipidemia: a p<0.05; b p<0.01; c p<0.0001

hsCRP concentration over 1 mg/L was found in 33% and hsCRP>3.0 mg/L in 14% of subjects with normal lipids. Among cases with hyperlipidemia 48% had CRP>1mg/L and 21% had CRP>3.0 mg/L.

The important determinant of CRP concentration in women was contraception use, however, in our study every fifth of non-users with normal lipids had hsCRP>1 mg/L. Oral contraceptives have been shown to increase the CRP level (3). In this study 28% were using oral or transdermal contraceptives which raised hsCRP (p<0.003) and apoAI values (p<0.04) and, only in women with normal lipids, raised TG concentration (p<0.0001). Similar findings were reported by Raitakari et al [13]. The mechanism by which oral contraceptives affect CRP concentration is not fully elucidated.

In females with normal lipids, CRP correlated with TG and apoB concentration (R=0.4; p<0.01, p<0.05), apoB:apoAI ratio and Wc (R=0.3; p<0.01, p<0.05) and inversely with HDL-C (R=-0.3; p<0.05). CRP was related only with TG and Wc in women with hyperlipidemia. No association of CRP with measured variables were found in men.

Of 192 subjects 36 (19%) had TG>150 mg/dL. Compared with TG<150 mg/dL they had lower HDL-C (52 vs 63 mg/dL; p<0.003), higher apoB (80 vs 67 mg/dL; p<0.03) and a tendency to higher CRP (1.70 vs 1.17 mg/L) concentrations.

Athough in all subjects apolipoprotein concentrations were within the reference range, a clear association of hsCRP with apolipoproteins was noticed. Concentration of hsCRP in all cases increased along with increasing apoB and decreasing apoAI concentration in tertiles. In subjects with normal lipids, hsCRP was the most elevated when apoB was in the highest tertile and apoAI in the lowest. There was no association of CRP neither with LDL-C and HDL-C nor with non-HDL-C and HDL-C.

Mean apoB:apoAI values were compared in different hsCRP categories, corresponding to relative CHD risk. In spite of the observed elevation of apoB:apoAI ratio in hsCRP categories, individuals with normal lipids were at low risk category (<0.6 for females; <0.7 for males) whereas those with hyperlipidemia were more likely to have moderate risk. Using TC:HDL-C ratio, especially in women, the risk may be underestimated since mean TC:HDL-C within hsCRP categories in most subjects, except males with hyperlipidemia, was low.

hsCRP>3 mg/L, among cases with elevated lipids, was related to higher frequency of moderate apoB:apoAI ratio (>0.6 or >0.7) but not TC:HDL-C.

To assess the relative cardiovascular risk three clinical cut points have been accepted. As we expected, most of the participants in our study with normal (67%) or moderately elevated lipids (52%) were at low risk (hsCRP<1.0 mg/L). Elevated level of hsCRP was recognized a stronger predictor of future cardiovascular events in healthy men than LDL-C (14). It has been shown that hsCRP is independent on lipid levels in both healthy and individuals with CVD, and that women with high CRP but normal LDL-C were four times more likely to have a coronary event as those with low CRP (15). In our study CRP testing was performed along with lipids and apolipoproteins to identify apparently healthy persons at risk of developing cardiovascular disease. The cut-off point of 130 mg/dL for LDL-C was used as it was shown that patients with LDL-C<130 mg/dL and hsCRP>1.0 mg/L are at higher

cardiovascular risk than those with LDL-C>130 mg/dL and hsCRP<1.0 mg/L (16) According to several studies hsCRP level can predict coronary risk even in the absence of hypercholesterolemia [17].

From our data we may conclude that every third, among healthy, non-obese young adults, with normal TC and LDL-C having hsCRP>1.0 mg/L may be at higher risk of CVD. Many epidemiological studies focused on conventional lipid parameters as risk markers for CVD, but recent studies suggested that apolipoproteins and their ratio may be a better prognostic indicator of risk. In our study apoB and apoB:apoAI values, but not LDL-C or TC:HDL-C, were related to CRP concentration in females with normal lipids suggesting their additional informative role in risk assessment.

The strongest evidence of the predictive value of apolipoproteins comes from AMORIS Study designed to compare levels of LDL-C and apoB as predictors of fatal acute myocardial infarction [18]. ApoB had higher sensitivity and specificity than LDL-C, especially in individuals with lower LDL-C levels. The superior ability of apoB could be due to the fact that it is a marker for the number of small dense LDL particles. Very recently, it has been confirmed that apoB:apoAI ratio is the strongest of all cardiovascular risk factors [12, 19].

Despite the evidence supporting predictive value of apolipoproteins, not all studies found them to be stronger predictors of risk than lipid parameters. The large population-based studies of middle-aged or older men and women either did not show that apoB was independently associated with risk in contrast to LDL-C, HDL-C and TG levels or have suggested that prediction of CVD by apoB:apoAI ratio was comparable with that of TC:HDL-C [7, 18, 20].

CONCLUSIONS

Nevertheless, in our opinion the prediction of risk, especially in young healthy individuals with normolipidemia, by measuring novel risk factors such as CRP or apolipoproteins seems to be more informative than generally recognized.

We are aware of the limitation of this study that include the relatively small group of young apparently healthy non-obese women and men, however we believe that our data could have several important clinical implications. We conclude that healthy, young, non-obese normolipidemic subjects are not free of cardiovascular risk having increased CRP concentration. Particularly, CRP and apolipoproteins seem to be the most useful to identify apparently healthy adults at risk of developing cardiovascular disease, who would benefit from targeted preventive interventions.

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SELF-ESTIMATED WORK LOAD IN PHYSIOTHERAPY

SAMOOCENA OBCIĄŻEŃ ZAWODOWYCH FIZJOTERAPEUTY

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S u m m a r y

The aim of the study was to evaluate the subjective work intensity by man and women working in physiotherapy departments of hospitals and in independent physiotherapy clinics of Bydgoszcz region in Poland. Additionally, frequency of application of different physiotherapy procedures in relation to their subjective strenuousness was calculated. Estimation of work loads and application of physiotherapeutic procedure during working days were performed with a questionnaire. It was found that both men and women are equally able to perform physiotherapy work. Lower physical capacity of women was probably compensated by their greater psychical capacity comparing to men. Fatiguing effects of physiotherapy procedures as well as the significant subjective work loads suggest that there is a need for proper physical education during university study preparing for physiotherapy profession.

S t r e s z c z e n i e

Celem pracy uczyniono próbę określenia wielkości obciążeń sfery fizycznej i psychicznej fizjoterapeuty na podstawie subiektywnej oceny osób wykonujących ten zawód – celem dalszym badań było także ustalenie częstości wykonywania podstawowych standardów rehabilitacyjnych.

Postawiono tezę o nierównym odbiorze uciążliwości pracy kobiet i mężczyzn w zawodzie fizjoterapeuty oraz niejednakowej częstości podejmowania w nim standardów różniących się stopniem swej uciążliwości.

Stosując metodę sondażu diagnostycznego badaniami objęto fizjoterapeutów zatrudnionych na oddziałach szpitalnych oraz terapeutów pracujących w salach kinezyterapeutycznych bydgoskich przychodni rehabilitacyjnych – uzyskano wyniki badań zespołu 90 osób, w którym przeważały kobiety.

W przypadku większości objętych oceną standardów, stwierdzono porównywalne dla mężczyzn i kobiet odczucia obciążeń fizycznych i psychicznych, co wskazuje, że fizjoterapia jest obszarem pracy dla osób obojga płci. W realizacji części standardów, kobiety w większym stopniu niż mężczyźni odczuwały obciążenia sfery fizycznej, a podobnej obserwacji dokonano w obszarze sfery psychicznej mężczyzn – standardy o znacznym stopniu uciążliwości były przez badany zespół wykonywane rzadziej niż charakteryzujące się małą uciążliwością.

Sformułowano wnioski dotyczące przydatności cech indywidualnych fizjoterapeuty i znaczenia jego psychofizycznych predyspozycji w pomyślnym wykonywaniu zawodu – także bezwzględnej konieczności zachowania zasad ergonomii pracy oraz profilaktyki przeciążeń.

Key words: physiotherapy, gender, therapeutic standards, wok loads
Słowa kluczowe: właściwości indywidualne, płeć, standardy terapeutyczne, obciążenia fizyczne, obciążenia psychiczne

INTRODUCTION

Professional, physiotherapeutic work needs significant physical and psychical effort [1, 2, 3, 4, 5, 6, 7].

Although, some studies indicate that average work loads for most physiotherapeutic procedures are rather

moderate in neurochirurgy the work loads are still classified as submaximal [8]. The problem of intensity of work load in physiotherapy may be especially important because of increasing feminization of this profession [1, 8, 9]. This can exert some limitation on possibility of work performance as well as on application of individual physiotherapy procedure. The aim of the study was, therefore, to evaluate the subjective estimation of work intensity by man and women working in physiotherapy departments of hospitals and physiotherapy clinics of Bydgoszcz region in Poland.

MATERIAL AND METHODS

The study was carried out in clinical departments of rehabilitation, neurology, orthopedics and paraplegic of hospitals and in kinezytherapy departments of rehabilitation clinics of Bydgoszcz region.

24 man (mean age 33 years) and 66 women (mean age 39 years), habitants of a big city, participated in the study. Over 60% of the women had been working in physiotherapy more than 10 years whereas only 50% of men continued the work in physiotherapy for maximum of 5 years. Over 60% of the subjects possessed university degree.

Estimation of work loads and application of physiotherapeutic procedure during working days were performed with questionnaire technics of Babie [10]. The subjects self-evaluated the physical and psychological intensity of work graphically on the maximum scale of 100 mm. They were also asked to inform about physiotherapy standards applied during the work, its frequency and strenuousness.

The data were analyzed by basic statistical methods with (χ^2) test and „Z" Guilford's test for independent groups [11].

RESULTS

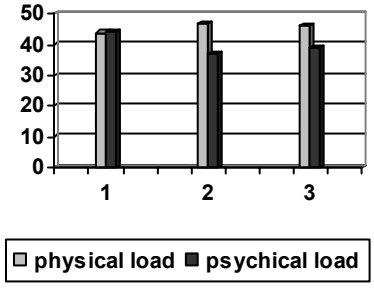
Almost all tested men (100%) and women (95%) presented opinion that gender does not influence on the functional capability in physiotherapy profession but they emphasized the importance of physical fitness necessary for realization of this profession. Physical endurance (48% of men and 53% of women) and movement co-ordination (52% and 30%, respectively) seems to be most important. This view was additionally supported by the opinion that the physiotherapy work is fatiguing and strenuous (75% of men and 83% of women) and thus they should be physically well prepared (74% and 76% of respondents, respectively). From that reason the physiotherapists prefer active rest (76% of men and 63% of women) by bicycling, walking, swimming, tennis during free time. Only 20% of respondents is of opinion that handicapped person would perform the job in physiotherapy.

Table I. *Self-estimated of subjective fatiguing of applied physiotherapy procedures and evaluated frequency of their application.*

Tabela I. *Liczbowa charakterystyka subiektywnego odczucia uciążliwości realizacji standardów terapeutycznych i częstości ich stosowania*

Standard	N	Frequency	Men	Women	D	Men	Women	D
			Physical load			Psychical Load		
Free exercise	1	6, 59	23, 57	22, 30	1, 27	33, 10	25, 48	7, 62
Free active exercise	2	7, 32	21, 19	32, 66	11, 47	25, 00	21, 56	3, 44
Individual exercise	3	7, 72	32, 50	38, 75	6, 25	42, 50	34, 22	8, 28
Passive exercise	4	7, 98	78, 33	82, 38	4, 05	48, 75	40, 85	7, 90
Isometric exercise	5	8, 14	19, 29	22, 81	3, 52	21, 67	22, 97	1, 30
Passive-active exercise	6	8, 15	64, 17	63, 64	0, 53	47, 08	36, 36	10, 72
Respiratory exercise	7	8, 51	18, 64	17, 66	0, 98	23, 64	19, 53	4, 11
Tests of movements	8	8, 60	30, 91	37, 03	6, 12	24, 55	23, 28	1, 27
Active exercise with resistance	9	9, 04	60, 91	72, 23	11, 32	43, 18	36, 23	6, 95
Data collection	10	9, 07	17, 50	17, 42	0, 08	29, 50	34, 19	4, 69
General exercises	11	9, 66	28, 18	35, 16	6, 98	37, 27	32, 03	5, 24
Locomotion tests	12	9, 84	24, 55	28, 81	4, 26	27, 73	23, 10	4, 63
Muscle strengths tests	13	11, 04	33, 75	38, 08	4, 33	28, 75	25, 62	3, 13
Learning of movements	14	13, 11	64, 17	69, 00	4, 83	56, 67	54, 38	2, 29
Physical measurements	15	13, 56	17, 73	27, 15	9, 42	18, 18	22, 23	4, 05
Verticalization	16	14, 09	68, 75	68, 79	0, 04	58, 75	52, 27	6, 48
Education therapy	17	14, 90	22, 73	26, 54	3, 81	62, 27	47, 31	14, 96*
After isometric relaxation	18	15, 51	44, 13	52, 54	8, 41	45, 00	43, 62	1, 38
Partial dry massage	19	15, 74	68, 91	66, 82	2, 09	49, 35	35, 30	14, 05*
Group exercise	20	16, 46	28, 04	32, 69	4, 65	51, 96	37, 77	14, 19*
Orthopedic learning.	21	16, 60	45, 00	46, 36	1, 36	48, 33	41, 67	6, 66
Total dry massage	22	18, 19	85, 83	86, 82	0, 99	53, 75	37, 88	15, 87*
Mobilization	23	18, 96	60, 00	63, 18	3, 18	62, 92	50, 00	12, 92
Profilaktic	24	19, 08	35, 43	31, 92	3, 51	36, 74	31, 31	5, 43
Redress exercise	25	19, 16	75, 43	75, 15	0, 28	61, 09	48, 64	12, 45
Family education	26	19, 46	19, 09	26, 27	7, 18	64, 55	53, 57	10, 98
Anticoagulant therapy	27	20, 69	51, 67	48, 85	2, 82	37, 50	33, 92	3, 58
Kinezotherapy	28	20, 83	72, 92	76, 90	3, 98	70, 36	63, 89	6, 47
Lymph drain	29	20, 86	63, 75	59, 62	4, 13	53, 75	38, 08	15, 67*
Redress extention	30	22, 60	40, 45	39, 00	1, 45	57, 27	41, 15	16, 12*

* statistically significant („Z"> 3, p = 0, 003)



1 – men; 2 – women; 3 – men and women

Fig. 1. *Physical and psychical work load of physiotherapy in men and women*

Ryc. 1. *Graficzna charakterystyka odczucia wielkości obciążenia fizycznego i psychicznego badanych mężczyzn i kobiet*

According to the questionnaired persons the most frequent physiotherapy procedures applied during the work were: free exercise, active in relieve, passive, passive-active and supplemented (Table 1). Both men and women estimated the performance of the standards as physically fatiguing but the men treated it also as a psychically fatiguing (Figure 1). Less frequently applied procedures (redress extension, lymph draining, total dry massage, partial dry massage, group exercise and patient education therapy) showed statistical differentiation in subjective estimation of fatiguing by men and women.

DISCUSSION

Results of the present study did not support the hypothesis that physiotherapy work load is subjective greater for women than for men. On the other hand, these subjective estimation of work load should not be analyzed on the basis of maximum oxygen consumption of the subject because of large discrepancy between the subjective sensation of the intensity of work and actual oxygen consumption [1, 12, 13]. According to Bilski [1] the relative intensity of aerobic component of the physiotherapy work should not be greater than 30%-35% VO_2max. Such level of intensity of the relative work makes the execution of the physiotherapy standards acceptable for both men and women without development of excessive fatigue. Realization of the work significantly depends on motivation and personality located in psychical sphere of human beings [14]. The results obtained revealed that although subjective physical work was greater for women they were psychologically less loaded than men. This could be a possible mechanism explaining why women are able to cope with physiotherapy work as well as men despite of their lower physical capacity. The results obtained have limited value since physiological measurements of physical activity during work should be applied for proper estimation of the physiotherapy work load [14, 15].

However, it might be conclude that:

1. Both men and women are equally able to perform physiotherapy work
2. Lower physical capacity of women was probably compensated by their greater psychical capacity comparing to men
3. Fatiguing effects of physiotherapy procedures as well as the significant subjective work loads suggest that there is a need for proper physical education during university study preparing for physiotherapy profession.

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BLOOD SEDIMENTATION. MODELING OF THE THIRD PHASE OF THE SEDIMENTATION CURVE

SEDYMENTACJA KRWI. MODELOWANIE TRZECIEJ FAZY KRZYWEJ SEDYMENTACJI

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S u m m a r y

Sedimentation curve for red blood cells (RBCs) suspended in native plasma was measured using an optical method. Blood was obtained from healthy donors. Samples of the RBCs suspension at haematocrit 45% were investigated. The third phase of the sedimentation curve was analysed. The time at which the third phase begins, the decay time for this part of the curve and the value of the sedimentation curve in the beginning of its third phase were determined. It is shown that the variability in the first two parameters in the population of healthy subjects is significant while the variability in the value of the sedimentation curve at the beginning of the third phase of this curve is relatively small.

S t r e s z c z e n i e

Krzywa sedymentacji dla czerwonych krwinek zawieszonych w osoczu została wyznaczona przy pomocy metody optycznej. Krew pobrano od zdrowych dawców. Badano próbki zawiesiny erytrocytów o hematokrycie 45%. Analizie poddana została trzecia faza krzywej sedymentacji. Wyznaczono czas rozpoczęcia trzeciej fazy, czas spadku trzeciej fazy krzywej i wartość krzywej sedymentacji dla chwili odpowiadającej rozpoczęciu jej trzeciej fazy. Pokazano, że zmienność dwóch pierwszych parametrów w populacji osób zdrowych jest znacząca statystycznie, podczas gdy zmienność wartości krzywej sedymentacji dla chwili wyznaczającej początek trzeciej fazy jest względnie mała.

Key words: erythrocyte sedimentation rate (ESR), sedimentation curve
Słowa kluczowe: szybkość opadu krwinek, krzywa sedymentacji

INTRODUCTION

Erythrocyte sedimentation rate (ESR) is one of the most commonly used parameters that characterize blood. The ESR test is nonspecific. This test is performed by a single reading of the height of the plasma that appears above the fraction containing RBCs after one hour of the sedimentation. The process of blood sedimentation is not fully recognized. Thus the studies of the properties of the sedimentation curve are still desirable to verify the utility of the parameters of the curve in clinical diagnosis.

Process of aggregation of red blood cells takes place in the presence of plasma proteins and other macromolecules and involves condensation and aggregation of RBCs [1]. The process of blood sedimentation consists of three characteristic phases. During the first phase one dimensional aggregates of RBCs called rouleaux are formed [2,3]. The behavior of one dimensional RBCs aggregates in sedimenting blood suspension was discussed in many studies [2-6] and the conclusions were contradictory. In the second phase, 3-D aggregates of the cells occur. The 3-D aggregates

sediment and when they reach the bottom of the container the deposit is formed. Packing of the deposit arises in the third phase of the process.

Sedimentation curve manifests the phases of the process by different slopes. The first relatively small slope of the curve reflects the rouleaux formation. Next the plasma-RBCs interface falls faster what manifests the sedimentation of the 3-D aggregates. Finally the asymptotic behavior of the curve reflects the packing of the cells [5, 6, 7, 9]. Up to now some mathematical models of this curve were presented. First this curve was mathematically modeled by a sigmoidal function [7,8]. Hung et al. [5] have found that the sedimentation curve is better modeled by three segments corresponding to the three phases of sedimentation process. Using this model they investigated an effect of fibrinogen, albumin and globulin concentration and haematocrit on the sedimentation kinetics. In these studies mainly the parameters of the first two phases of the sedimentation curve were analysed [5, 9]. There is an agreement between results obtained from the investigation of the sedimentation curve and results of other studies [4, 10-11].

In this study the third phase of the sedimentation curve was investigated. A simple mathematical model of the third segment of this curve was proposed. The variability of the parameters of the curve was analysed.

SAMPLE PREPARATION

Blood was obtained from 17 healthy donors. Blood was collected in sterile vacuum tubes containing anticoagulant (K_3EDTA). The blood samples were centrifuged at 3000 rpm for 5 min at 4^0C. The plasma was separated and the buffy coat (white blood cells WBCs) was discarded. Remaining RBCs or WBCs were eliminated by centrifuging for 15 minutes at 15000 rpm. RBCs were mixed with native plasma to adjust the haematocrit to 45%. The suspensions of RBCs were placed into a rectangular glass container of inner dimensions: height - 20 mm, width - 12 mm, depth - 0,5 mm.

The experiment was carried out according to the ethical guidelines laid down by the Bioethical Committeee at the Ludwik Rydygier Collegium Medicum of the Nicolaus Copernicus University in Bydgoszcz.

MEASUREMENT APPARATUS

The experimental setup is schematically shown in Fig. 1. As a light source a He-Ne laser (λ = 632,8 nm) is used. The laser beam is expanded, collimated and focused by lenses L_1, L_2 and L_3. The pinhole P_0 is used as a spatial filter. The beam is focused by lens L_3 on the sample (S). The beam width of the light at the beam waist position is 150 μm. The container is located so that its walls are normal to the beam axis. The light beam travels in the center of the container. The container is located on a motorized stage (MS). This stage provides vertical movement of the container with constant velocity 2 cm/min. The scattered light was collimated by the lens L_4 of the focal length f_4 = 11 cm and aperture of diameter d = 5 cm. Then the light focused by the lens L_5 of the focal length f_5 = 17 cm is detected by the photomultiplier Ph. The high intensity nonscattered component of light that appears when the beam passes through the highly transparent plasma is reduced by the circular nontransparent filter F of diameter d_f = 0,8 cm. Chopper CH permits to use lock-in technique for data recording. The continuous signals from the lock-in amplifier are measured every 0,6 s. It corresponds to the sampling interval 0,02 cm. The data were collected by personal computer. The measurements were performed at room temperature 22 ± 1^0C.

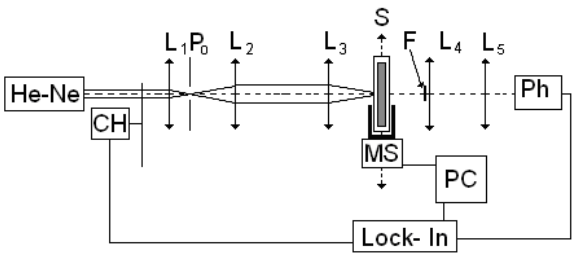

Fig. 1. *The scheme of experimental setup*
Ryc. 1. *Schemat układu pomiarowego*

RESULTS

Fig. 2 shows the scattered light intensity detected during sedimentation. High intensity values correspond to plasma, low fluctuating light intensity represents settling and aggregating RBCs. The boundary between plasma and RBC suspension is called sedimentation curve. The discontinuity in the intensity permits to find the position of the plasma-RBCs interface. In Fig. 3 sedimentation curves for two samples are depicted, where case (a) shows sedimentation curve for sample

from Fig. 2. From this figure one can recognize the three phases of the process. In the first phase there is a little (Fig. 3a) or no slope (Fig. 3b) in sedimentation curve. The duration of the first phase is 5 min in the case (a) and 18 min in the case (b). In the second phase the slope of this curve increases. Next in the third phase an asymptotic behavior of this function occurs. The boundary between the first and the second phase is quite sharp but this curve does not manifest exactly the boundary between the second and third phase.

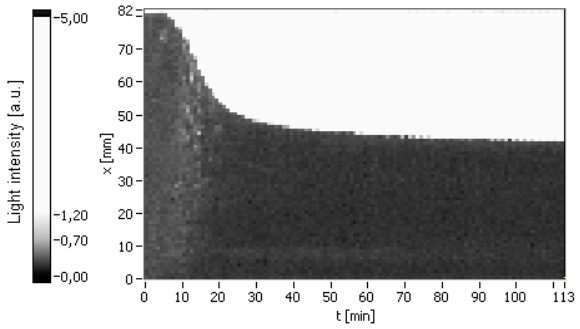

Fig. 2. *Light intensity changes along height x of the blood sample at haematocrit 45% during sedimentation*

Ryc. 2. *Zmiany natężenia światła wzdłuż wysokości próbki krwi o hematokrycie 45% w czasie trwania sedymentacji*

To model the sedimentation curve in its third phase $s_{III}(t)$ we propose the following formula

$$s_{III}(t) = s(0)\left[A + (1-A)\exp\left\{-\frac{t}{T_D}\right\}\right], \qquad (1)$$

where s(0) is the value of the curve at t = 0, T_D is the time of decay of the third phase of sedimentation curve and A is a parameter. Due to the fact that the boundary between the second and the third phase is not sharp we do not know the exact point in time when does the third phase start. The selection of the point on the curve since which we will fit formula (1) to experimental data is an individual case of each sample and in such selection we based on hitherto existing models of sedimentation curve. Fitting the formula (1) to the data in the third phase we have found the parameter A and T_D. The best-fit curves for two samples are drawn in Fig. 3. The modeled curves are drawn also in the first two phases. From Fig. 3 one can see that the function (1) fits the experimental data in the third phase very well. In the first two phases a departure of the function from the sedimentation curve takes place. The first point at which function expressed by equation (1) meets with the sedimentation curve s(t) defines the time of the beginning of the third phase T_3. In the case (a) it is 18 min and in case (b) it is 46 min. We can also find the value of sedimentation curve at the time T_3. From Fig. 3 we see that in the case (a) the value is 10,8 mm and in the case (b) it is 11,1 mm.

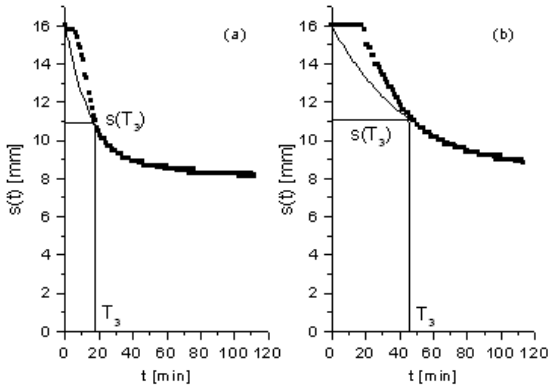

Fig. 3. *Sedimentation curves s(t) (square points) for samples at hematocrit 45% obtained from two different donors (a, b) and functions modeling third phase of these curves (solid curves)*

Ryc. 3. *Krzywe sedymentacji s(t) (punkty) dla próbek o hematokrycie 45% uzyskanych od dwóch różnych dawców i krzywe modelujące trzecią fazę tych krzywych (linia ciągła)*

Fig. 4 shows histograms of the parameters A, T_D, T_3 and $s(T_3)$ in the investigated population. The variables A, T_D and $s(T_3)$ are normal at 0,05 significance level according to Shapiro-Wilk's test while T_3 is not-normal at this same significance level. From Fig. 4 one can see a significant variability in T_D and T_3 while the variability in A and $s(T_3)$ is relatively small. Table I shows the mean values, standard deviations and coefficients of variation for the above mentioned variables. Especially the coefficient of variation manifests the very small variability in the parameter A as well as in the value of sedimentation curve at the beginning of its third phase.

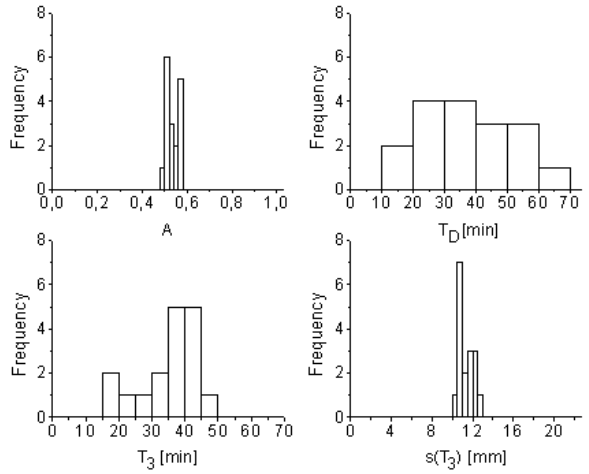

Fig. 4. *The histograms determined for: a) parameter A, b) the decay time of the third phase of sedimentation curve T_D, c) time of the beginning of the third phase T_3, d) the value of sedimentation curve at the time T_3*

Ryc. 4. *Histogramy rozkładu wartości dla: a) parametru A, b) czasu charakteryzującego spadek trzeciej fazy krzywej sedymentacji T_D, c) czasu rozpoczęcia trzeciej fazy T_3, d) wartości krzywej sedymentacji dla chwili T_3*

Table I. *Mean, standard deviation and coefficient of variation for parameters that characterize third phase of sedimentation curve*

Tabela I. *Wartość średnia, odchylenie standardowe i współczynnik zmienności dla parametrów charakteryzujących trzecią fazę krzywej sedymentacji*

Name of the variable	A	T_D [min]	T_3 [min]	s(T_3) [mm]
Mean value	0,533	36,97	34,76	11,35
Standard deviation	0,026	13,84	9,25	0,68
Coefficient of variation	0,048	0,374	0,266	0,060

DISCUSSION

Sedimentation curves show a significant variability in their shape for population of healthy donors. We have shown that variability of two parameters that describe the third phase of this curve is very small.

Although the blood sedimentation process is still not fully recognized it is commonly accepted that in its third phase the sediment is formed by settled 3-D aggregates of RBCs. During this phase an asymptotic packing of the cells occurs. This part of sediment can be called a forming deposit of the cells. The formation of the deposit is dependent on the interactions between the RBCs that create the structure of the deposit.

Up to now the most detailed study of the sedimentation curve were conducted by Holley et al. [9]. The study was performed for healthy donors. In the mathematical modeling of sedimentation curve a parameter responsible for the shape of the third phase was introduced. However in the analysis of the data this parameter was omitted. Also in the studies performed by Hung et al. [12] the first and the second phase of the process was investigated. In this study the analysis of the third phase of the sedimentation curve obtained for blood samples was taken into account giving an insight into the process of packing of RBCs.

We have shown that the third phase of the sedimentation curve can be described by a simple formula based on exponential function. With the use of the formula we have described the third phase using four parameters. It is shown that coefficients of variation of two parameters, the value of sedimentation curve at time T_3 and parameter A, are very small while the coefficients for the other parameters are relatively large. This suggests that the time of decay of the third part of sedimentation curve T_D and the time at which the third part of this curve starts T_3 can differentiate the subjects.

The ESR test asserted as a height of plasma after 60 minutes of sedimentation shows poor correlation with RBC aggregation [13]. As was mentioned earlier the behavior of the sedimentation curve in its third phase reflects the existence of the interactions between RBCs and in this way parameters of the sedimentation curve qualitatively reflect the interactions. Thus, further study is necessary to explain the meaning of the parameters T_3 and T_D in the description of the sedimentation process.

In conclusion, the results of our study show that the parameters of the third phase of the sedimentation curve differentiate the investigated subjects. It suggests that the investigation of packing of the RBCs can be useful in the study of aggregation and sedimentation process.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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BIOCHEMICAL MARKERS OF BONE CELLS ACTIVITY IN TYPE 1 DIABETIC CHILDREN AT ONSET AND IN THE FIRST YEAR OF FOLLOW-UP

WSKAŹNIKI PRZEBUDOWY TKANKI KOSTNEJ U DZIECI Z CUKRZYCĄ TYPU 1 NA POCZĄTKU CHOROBY I PODCZAS PIERWSZEGO ROKU LECZENIA

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S u m m a r y

Among many complications of type 1 diabetes in children, changes in bone metabolism leading to decreased bone mass may increase the risk of developing osteopenia/osteoporosis in adulthood. We evaluated bone turnover in pubertal children with insulin-dependent diabetes mellitus at onset and during one year follow-up by more-in-depth analysis of bone cells activity. 17 patients, with newly diagnosed type 1 diabetes and 22 healthy age-matched subjects were included in the study. Bone formation and resorption markers were determined by ELISA. Osteocalcin, in particular, and CTX were significantly lower in diabetics at disease onset but returned to levels similar as in controls after 3 months of insulin therapy. In diabetics HbA1c inversely correlated with OC at disease onset. Proper insulin therapy and good glycemic control have beneficial influence on bone turnover that may prevent against low bone mass in adult age.

S t r e s z c z e n i e

W cukrzycy typu 1 zmiany metabolizmu kostnego prowadzące do obniżenia masy kostnej mogą zwiększać ryzyko rozwoju osteopenii/osteoporozy w wieku dorosłym. Celem pracy była ocena obrotu kostnego u dzieci bezpośrednio po rozpoznaniu cukrzycy typu 1 i w ciągu pierwszego roku leczenia. Badaniem objęto 17 dzieci chorych oraz 22 zdrowych rówieśników. Wskaźniki przebudowy tkanki kostnej oznaczono metodą ELISA. U dzieci z cukrzycą typu 1 stwierdzono istotnie obniżony poziom markerów obrotu kostnego, szczególnie osteokalcyny, w odniesieniu do grupy kontrolnej. Po 3 miesiącach leczenia stężenia wskaźników obrotu kostnego osiągnęły wartości podobne do oznaczonych u dzieci zdrowych. Stężenie osteokalcyny było ujemnie skorelowane z poziomem hemoglobiny glikowanej bezpośrednio po rozpoznaniu choroby. Dobre wyrównanie glikemii powoduje wzrost poziomu markerów kościotworzenia i resorpcji, co zapobiega niskiej masie kostnej w późniejszym wieku.

Key words: bone turnover markers, osteocalcin (OC), crosslinked C-terminal telopeptides of type I collagen (CTX), tartrate-resistant acid phosphatase 5b (TRACP 5b), insulin-dependent diabetes mellitus (IDDM), children

Słowa kluczowe: markery obrotu kostnego, osteokalcyna (OC), C-końcowy usieciowany telopeptyd kolagenu typu 1 (CTX), winianooporna fosfataza kwaśna izoenzym 5b (TRACP 5b), cukrzyca insulinozależna (IDDM), dzieci

INTRODUCTION

Diabetes mellitus is a heterogeneous group of disorders characterized by raised blood glucose level. 50-60 % of patients with diabetes type 1 are younger than 16-18 years at onset [1, 2]. Among many complications of diabetes, changes in bone metabolism leading to decreased bone mass and the risk of developing osteopenia/osteoporosis in adult age have been reported. Bone is a tissue continuously remodeled, particularly in children and adolescents during growth. Measurement of bone markers, released during formation and degradation processes of bone provide insight into the patophysiology of bone turnover during growth and allows monitoring the response to treatment [3-7]. The processes of bone resorption and formation under physiological circumstances are tightly coupled. Recently, the possibility was discussed that osteoclasts not only resorb bone but also stimulate bone formation, that depends mainly on their number [8]**.** Using specific markers, such as TRACP 5b (tartrate-resistant acid phosphatase 5b) and CTX (crosslinked C-terminal telopeptides of type I collagen) osteoclast number and function may be separately assessed.

Osteocalcin (OC), TRACP 5b and CTX are regarded as specific and sensitive biochemical bone markers [3, 6]. OC, secreted by mature osteoblasts is a sensitive and highly

specific marker of their activity and bone mineralization. Osteocalcin is increased in childhood and during puberty [3, 5, 6].

Isoform 5b of TRACP released from mononuclear osteoclast precursors early during differentiation and from mature cells is regarded as an index of osteoclast number and bone resorption [3, 7, 10, 11]. CTX, a degradation product of collagen type I, is released when active bone resorption is started by matured osteoclasts and reflect their functionality [3, 4, 8, 9]. Bone resorption markers are very high in growing children and decrease gradually with age.

Recently, data on five biochemical bone markers in healthy children and adolescents have been published that may be of clinical utility as normal reference ranges in future studies on bone metabolism in different diseases [11, 12].

Until now few studies were undertaken to assess the influence of type 1 diabetes on bone remodeling in children and adolescents [13-21].

We evaluated bone turnover in children with insulin-dependent diabetes mellitus (IDDM) at onset and during one year follow-up, by analysis of bone resorption and formation process, measuring serum TRACP 5b released from osteoclasts, CTX a degradation product of collagen type I and osteocalcin, a marker of osteoblast's activity.

We assumed that metabolic effects of poor glycemic control at onset of type 1 diabetes mellitus lead to impaired bone turnover and therefore may affect bone mass later in life.

MATERIALS AND METHODS

17 pubertal children (12 boys aged 10-14 years, 5 girls aged 9-12 years) with recently diagnosed type 1 diabetes, recruited from Department of Pediatrics and Endocrinology, Children's Hospital in Bydgoszcz were included in the study. Children were classified as pubertal according to age ranges given by Institute of Mother and Child (Warsaw) [22]. We excluded those who suffered from chronic diseases other than IDDM and had taken medicines (other than insulin) known to affect bone metabolism. At onset of IDDM mean age of patients was 11.4 (1.4) yrs. They were treated with intravenous insulin infusion at starting dose of 0.05-0.1 U/kg/h of insulin. After metabolic equalization achievement they took insulin injections. Human regular insulin, human NPH insulin and rapid-acting insulin analogs were taken by the patients.

Diabetics regularly attended the follow-up control visits. Blood samples were collected three times: at onset of diabetes (5-11 days after diagnosis) and after 3 and 12 months.

In the control group 22 healthy pubertal children (15 boys aged 10-15, 7 girls aged 9-13), mean age 12.2 (1.7) yrs were included. Controls were recruited from the general population of the same region. The inclusion criteria were as follows: lack of evidence of systemic illnesses, endocrine or renal disorders, chronic diseases, obesity and not taking drugs known to affect bone metabolism. Diabetics and controls were asked to complete questionnaires pertinent to personal and family health, life style, dietary intake, physical activity, family history of fractures, current medication intake.

The study was approved by Ethical Committee of Collegium Medicum, N. Copernicus University. All parents and patients have been informed about the purpose of study. Participation was voluntary. Written informed consent was obtained from all the parents of the study participants.

Fasting blood samples in both IDDM children and controls were collected between 7 to 9 am. After centrifugation serum samples were kept frozen at -70^0C until assayed for bone marker concentrations. Serum calcium (Ca) and whole blood glycated hemoglobin (HbA1c) levels were measured after blood collection. Samples collected at three time-points were assayed in the same series for each of the bone markers.

Measurements of height (cm) and weight (kg) were taken at onset, after 3 and 12 months. Standing height (cm) and weight (kg) were measured using mechanical medical scales.

Calcium was measured with photometric assay on clinical chemistry analyzer (ARCHITECT® c8000 System, Abbott Laboratories, USA). HbA1c was measured with using the MULTIGENT™ Hemoglobin A_{1c} assay on the ARCHITECT® *c*8000™ System, Abbott Laboratories, USA. HbA1c was used as a marker of glycemic control.

Osteocalcin was measured in serum with N-MID® Osteocalcin ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark). Crosslaps were measured in serum with Serum CrossLaps® ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark). TRACP 5b was measured in serum with an immunocapture enzyme assay Metra® TRAP5b EIA Kit (Quidel Corporation, USA). As no reference values for bone markers were yet available in our Clinical Laboratory for children and adolescents, the obtained results in diabetic patients were compared to these found in the control group. However, our findings for osteoclacin and CTX in healthy pubertal girls and boys were very similar to those reported by others for Polish children [22, 23].

Data were expressed as means (SD) or medians (25th and 75th percentiles). Variables were tested for the normal distribution by Shapiro-Wilk test. Variables with nongaussian distribution were transformed logarithmically before analysis. Changes of variables during follow-up (longitudinal study) were compared by ANOVA for repeated measurements. We performed post hoc comparisons between values obtained at each time point by the Fisher LSD test. Pearson or Spearman rank correlation tests and Mann-Whitney *U*-test were used. P values equal to or less than 0.05 were considered statistically significant.

RESULTS

Mean age and proportion of boys and girls was similar in diabetic and control groups. Characteristics of the study participants was given in Table 1. There were no weight differences between diabetic children after 3 or 12 months of insulin therapy and controls, however the healthy ones were heavier than diabetics at onset (P=0.03). Children with type 1 diabetes increased slightly in height during 12 month's observation but at each time point the study group and controls did not differ significantly by height.

In diabetic children at onset of disease median HbA1c was significantly higher compared with values obtained after 3 and 12 months of insulin treatment and in controls (P<0.0001). At 3 and 12 months median HbA1c remained stable but was still significantly higher than in healthy children (P<0.0001).

Mean calcium concentration at onset of the disease, at 3 and 12 months did not change and was found to be within the reference range, however still significantly higher than in controls (P< 0.0005).

There were no significant gender differences found in OC, TRACP 5b and CTX levels at disease onset and during the follow-up in diabetic patients as well as in control group. However, at disease onset, the values of bone markers tended to be slightly lower in girls.

OC concentration at onset of type 1 diabetes was very low comparing with that of controls (P<0.0001); median OC level was as much as 40% lower in diabetics but increased significantly after 3 months (P<0.0001) following insulin treatment. After one year, osteocalcin values in patients returned toward the values similar as in control group (Table 1).

Table I. *Clinical and biochemical characteristics of the study participants*

	IDDM children			Controls
	Onset	3 months	12 months	
Weight,[1] kg	37 (28-44)	39 (36-46)	40 (36-56)	42 (37-49)
Height,[2] m	1.52 (0.09)	1.53 (0.09)	1.59 (0.01)	1.56 (0.01)
HbA1c,[1] %	11.5 (9.4-12.9)	6.6 (6.3-7.7)	7.4 (6.3-7.7)	5.6 (5.4-5.7)
Ca,[2] mmol/L	2.4 (0.1)	2.4 (0.1)	2.4 (0.1)	2.2 (0.1)
OC,[1] µg/L	61.6 (33.9-78.5)[a,b]	96.9(89.0-105.2)	96.1(70.5-144.2)	101.7(84.1-135.3)
CTX,[1] µg/L	1.6 (1.3-2.1)[c,d]	2.5 (1.9-3.3)	2.2 (1.4-2.6)	2.3 (2.2-2.5)
TRACP5b,[2] U/L	15.1 (4.5)[e,f]	17.4 (4.2)	18.7 (5.4)	18.9 (4.3)

[1] Median (25th–75th percentiles) [2] Mean (SD)
[a] P<0.0001 in comparison with controls; [b] P<0.0001 in comparison with values observed after 3 months of treatment; [c] P<0.01 in comparison with controls; [d] P<0.0005 in comparison with values observed after 3 months of treatment; [e] p<0.01 in comparison with controls; [f] P<0.03 in comparison with values observed after 12 months of treatment

Median CTX value at disease onset was 30% lower than in control group (P<0.01). CTX concentration increased after 3 months of treatment (P<0.005). After one year CTX values in patients and healthy children were similar (Table 1).

Baseline TRACP 5b activity was only 20% but significantly lower at disease onset comparing to healthy children (P<0.01) and after one year of follow-up (P<0.03). During follow-up TRACP 5b in diabetics increased gradually and at 12 months reached the control group values (Table 1).

Bone marker concentrations correlated directly with the age of onset of type 1 diabetes (R=0.55, P=0.03; R=0.68, P=0.004; R=0.59, P=0.02, respectively for OC, CTX and TRACP 5b).

OC positively correlated with CTX in diabetics at all three time points (R=0.73, P=0.001, R=0.57 and R=0.58, P=0.02) and with TRACP 5b after one year (R=0.64, P=0.01). Correlations of OC with CTX and

TRACP 5b were also found in control group *(R*=0.46, *P*=0.03; *R*=0.45, *P*=0.04).

HbA1c inversely and significantly correlated with OC (*R*=-0.55, *P*=0.03) in diabetics only at onset of disease.

DISCUSSION

Previous studies have shown that children and adolescents with IDDM seem to have increased risk of low bone mass and risk of osteopenia/osteoporosis in adulthood [14, 15, 24, 25]. However, there is no agreement about the effect of type 1 diabetes on bone turnover and the relationship between osteopenia and metabolic control [16, 19-21]. Osteopenia may result from impairment of bone formation and normal or increased bone resorption [13, 15]. Conflicting data on bone turnover in diabetes may be related to heterogeneity of patients included in the studies, duration of disease, type of insulin treatment and environmental factors. To investigate bone turnover in children and adolescents at onset of type 1 diabetes and during the first year follow-up we determined the serum level of sensitive and specific markers of bone cells activity.

It is well known that deficiency of insulin influences on bone forming cells and decreases bone matrix synthesis. Differentiated osteoblasts produce and secrete less osteocalcin that leads to diminished bone mineralization. In our study osteocalcin was found to be significantly decreased at onset of the disease that has also been observed in earlier findings [13, 17, 26, 27]. Inverse significant correlation of OC/TRACP 5b and osteocalcin with glycated hemoglobin level at disease onset and quick significant increase of OC after the start of insulin treatment to the level found in healthy children support the notion that poor glycemic control is related to insufficient bone formation.

Determination of resorption markers in the urine is confounded by marked circadian and intra-individual variation, and also by the necessity of expressing their concentration relative to creatinine, which itself changes with age and muscle mass [10]. Contrary to others [13, 19, 25], we measured sensitive and specific markers of osteoclast's activity in the serum.

Decreased resorption, as assessed by CTX, was found in our study at onset of type 1 diabetes. Similar data indicating low bone turnover in diabetic children were reported earlier by Gunczler et al [14, 15]. However, others have observed increased bone resorption and reduced bone formation [13, 25].

We have shown that CTX concentrations raised quickly within 3 months of treatment but after one year remained similar to these found in control group. Also, in adult patients the influence of type 1 diabetes on bone density and markers of bone remodeling was assessed. The results from this study suggested that although osteopenia in adulthood was quite common bone loss was attenuated [28]. It has been shown that among factors modulating bone metabolism a novel cytokine system RANKL/RANK/OPG (receptor activator of NF-κB ligand/receptor activator of nuclear factor NF-κB/osteoprotegerin), affecting the pathway of osteoclast formation and activity, play an important role. Insulin deficiency decreases IGF-1 (insulin-like growth factor 1) production that has been shown to impair osteoclastogenesis through regulation of RANKL and RANK expression in experimental animals using in vitro cultures [29]. Osteoprotegerin and RANKL are important regulators of terminal differentiation and fusion of mononuclear osteoclast precursors and resorptive function of multinucleated osteoclasts. OPG prevents activation of receptor RANK on osteoclast precursor cells by binding to RANK-ligand and determine osteoclast number and activity.

Indeed, increased concentration of osteoprotegerin (OPG) and positive correlation of OPG with HbA1c has been found in children with type 1 diabetes [30]. We did not measure serum OPG in this study but may assume that low CTX at disease onset could reflect down-regulation of osteoclast resorptive activity. On the other hand, at onset of diabetes TRACP 5b level was only slightly decreased. Moreover, minor changes during one year of insulin treatment indicate that TRACP 5b synthesis and release from osteoclast precursors may be less dependent on OPG/RANKL balance. However, lack of serum OPG measurements and respectively small number of study participants seem to be a certain limitation of our study.

CONCLUSIONS

Finally, our data support the presence of decreased bone metabolism mainly as the result of impaired bone formation at onset of diabetes type 1 in pubertal children. Bone turnover, in particular, bone formation is associated with improvement of glycemic control. Separate osteoclast activites seem to be affected in different ways.

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