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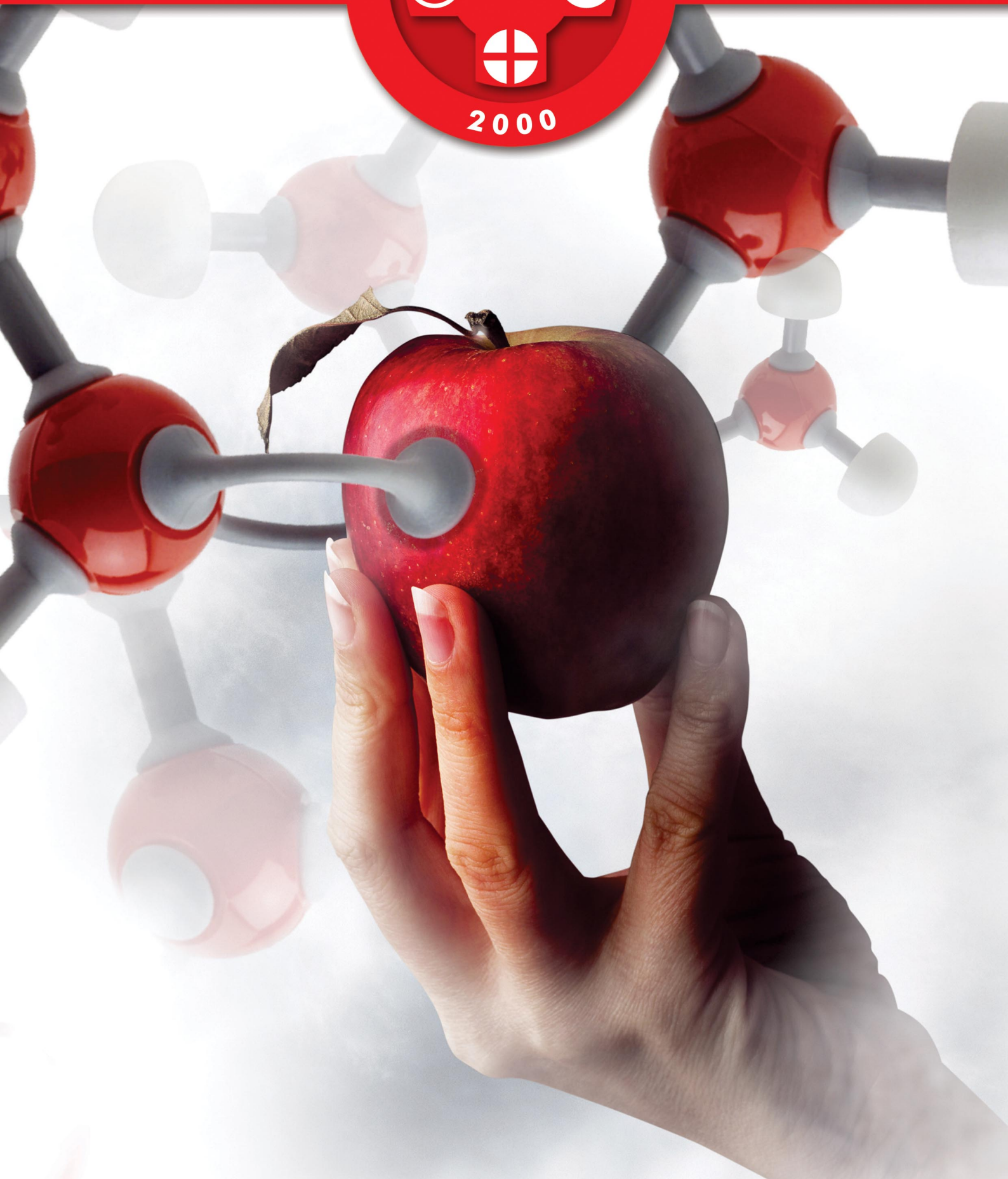

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TOWARDS ENDURANCE IN SPORT

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IZDRŽLJIVOST U SPORTU

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ABSTRACT

Cardiorespiratory endurance, which is also known as aerobic endurance, is the ability of the whole body to sustain prolonged exercise involving relatively large muscle groups. Muscle endurance is defined as the ability of a muscle group to execute repeated contractions over a period of time that is sufficient to cause muscular fatigue or as the ability to maintain a specific percentage of the maximum voluntary contraction for a prolonged period of time. Each version of muscle endurance plays a unique role in sport activities, and each has a special importance to various athletes. Cardiorespiratory endurance is thought to be the most important component of physical fitness. Low endurance capacity leads to exhaustion, even in sports and activities characterised by low dynamics. The combination of spiroergometric testing (with appropriate testing protocols) and the measurements of lactate thresholds is believed to be the gold standard in the assessment of cardiorespiratory endurance. The generated parameters are then used to define the training objectives, to preparefor a precise training plan and program, and to evaluate the effects of the subsequent training effect evaluation. Muscular endurance is specific for each group of muscle, type , and velocity of contraction. Due to the specificity of muscular endurance, a universal assessment of the muscular endurance of the whole body has not yet been developed. Isokinetic and isoinertial dynamometry and numerous field tests are used to assess muscular endurance. By understanding and monitoring endurance in athletes, the training workload during the preparation period and the the pre-competition and competition seasons can be implemented and corrected. The achievement and maintenance of optimal fitness should be regarded as a dynamic concept that requires continual monitoring that is aided by the modern methods of functional diagnoses.

Keywords: *endurance, cardiorespiratory, muscle, athletes, training.*

SAŽETAK

Kardiorespiratorna izdržljivost ili aerobna izdržljivost je sposobnost čitavog tela da održava dugotrajnu fizičku aktivnost i uključuje relativno velike mišićne grupe. Mišićna izdržljivost predstavlja sposobnost mišića odnosno mišićne grupe da izvrši ponavljanje kontrakcije kroz period vremena dovoljan da izazove mišićni zamor ili sposobnost da održi specifični procenat maksimalne voljne kontrakcije u toku dužeg vremenskog perioda. Svaka ima jedinstven udeo u sportskim aktivnostima i svaka se razlikuje po svojoj važnosti, kod različitih sportista. Kardiorespiratorna izdržljivost se smatra najvažnijom komponentom fizičke pripremljenosti. Nizak kapacitet izdržljivosti vodi ka zamoru, čak i u sportovima i aktivnostima niže dinamike. Kao zlatni standard za ispitivanje kardiorespiratorne izdržljivosti smatra se kombinacija spiroergometrijskog testiranja (uz primenu adekvatnog protokola testiranja) i određivanja nivoa laktatnih pragova. Dobijeni parametri se zatim koriste za definisanje ciljeva treninga i izradu preciznog plana i programa treninga, kao i za naknadnu evaluaciju efekata treninga Mišićna izdržljivost je specifična za svaku mišićnu grupu, tip i brzinu kontrakcije. Zbog toga ne postoji univerzalna procena mišićne izdržljivosti celog tela. Za procenu mišićne izdržljivosti koriste se metode izokinetičke i izoinercijalne dinamometrije, kao i brojni terenski testovi. Razumevanje i praćenje izdržljivosti kod sportista omogućava izradu i korekciju individualnih zona trenažnog opterećenja u odgovarajućim ciklusima pripremnog perioda tokom predtakmičarske i takmičarske sezone. Postizanje i održavanje optimalnog stanja treniranosti treba shvatiti kao dinamički koncept koji zahteva stalno praćenje, primenom savremenih metoda funkcionalne dijagnostike sportista.

Ključne reči: *izdržljivost, kardiorespiratorna, mišići, sportisti, trening.*

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BACKGROUND

Endurance is a term in exercise physiology that describes two separate but closely related phenomena: cardiorespiratory endurance and muscular endurance. Each type of endurance plays a unique role in sport activities, and each has a special importance to different athletes. Endurance is a basic necessity for competition in certain sports (e.g., distance running, cycling, endurance swimming, cross-country skiing, etc.), but it is also an essential component of a large number of otherteam sports (e.g., football, rowing, tennis, basketball, etc.).

Cardiorespiratory endurance, or aerobic endurance, is the ability of the whole body to sustain prolonged exercise involving relatively large muscle groups (1). Cardiorespiratory endurance is related to the development of the ability of the cardiovascular and respiratory systems to maintain an oxygen supply to the muscles engaged in prolonged physical activity as well as to the muscle's ability to obtain the necessary energy by way of aerobic processes. This is the reason why the terms cardiorespiratory and aerobic endurance are commonly used as synonyms. With endurance training, more oxygen per unit of time can be supplied and used in activated muscles. These training improvements allow an athlete to be engaged with a greater intensity in a physical activity requiring endurance, without a deterioration in the quality of performance.

Muscle endurance is defined by the American College of Sports Medicine (ACSM) as the ability of a muscle group to execute repeated contractions over a period of time that is sufficient to cause muscular fatigue or the ability to maintain a specific percentage of the maximum voluntary contraction for a prolonged period of time (2). Therefore, muscle endurance is specific to certain muscles or muscle groups. In the routine practice of sport training, the most common criterion for muscle endurance is the number of resistance exercise repetitions that are performed until the moment of muscle failure.

A tThe individual endurance of an athlete is the baseline for the improvement of his/her ability, and improvements can result through training, including the maintenance of competitive abilities by competing in competitions (3), the avoidance of detraining due to a low training workload, and the avoidance of an overtraining syndrome and/or possible injuries due to a high of a training workload (4).

ADAPTATIONS TO ENDURANCE TRAINING

Endurance improvement through regular aerobic training is the result of numerous adaptations to a training stimulus. Some adaptive processes occur within the muscles themselves, providing a more efficient transport and use of oxygen and energetic substrates. Other important changes occur in the cardiovascular system, improving the blood supply to and within muscles. Adaptive changes in a body during endurance training, including increased left ventricle dimensions, reduced peripheral blood vessel resistance, and increased blood volume, enable increased values of stroke volume during submaximal and maximal physical activity and at rest. Muscle blood flow is increased with endurance training due to an enlargement of the capillary network (by the formation of new and opening of the existing capillaries), a more efficient blood flow distribution, and an increase in blood volume. Increased blood volume is caused by an increase in blood plasma volume, which leads to a reduction in blood viscosity, facilitating circulation and oxygen supply (5). Endurance training results in lower blood pressure values during submaximal physical exercise. During maximal intensity physical exercise, the ,systolic pressure is elevated and the diastolic pressure is reducedas compared to the pre-training values (6).

Endurance training induces an increase in the number and size of mitochondria in the muscle fibres. The activity of many oxidative enzymes is increased as a consequence of adaptation to training. These muscular changes, combined the oxygen transport system adaptation, increases the capacity of oxidative metabolism and cardiorespiratory endurance (7). An adapted skeletal muscle contains more glycogenas compared to an unadapted muscle. Moreover, adapted muscles contain more fat in the form of triglycerides. The activity of many enzymes involved in the beta (β) oxidation of fats increases with training, resulting in increased levels of free fatty acids (8). These changes result in the increased use of fats as an energy source, the saving of glycogen, and, thus, the createdevelopment of favourable conditions favorable for prolonged physical activity without exhaustion.

CARDIORESPIRATORY ENDURANCE

According to many published papers and textbooks on the science of sport, cardiorespiratory endurance is thought to be the most important component of physical fitness. Endurance is the athletes' best defence against exhaustion. Low endurance capacity leads to exhaustion, even in sports and activities characterised by low dynamics. For each athlete, regardless of their discipline or activity, fatigue is the main obstacle to optimal performance. Even slight fatigue may disturb the ability of an athlete to perform because of reduced muscle strength, prolonged reaction and movement times, reduced agility and neuromuscular coordination, reduced velocity of the entire body, and reduced concentration and nimbleness. The cardiorespiratory endurance of an athlete is determined by a large number of factors (9, 10), with the most important being age, gender, body mass, genotype, physical activity (degree of fitness), and acute and past diseases, among others.

Regardless of the degree of preparedness of a cardiovascular system to supply the working muscles with an adequate amount of blood, endurance is hampered if the

respiratory system is unable to transport enough oxygen to satisfy the oxygen demand. The function of the respiratory system usually does not limit the ability to perform physical activities because ventilation can be increased to a higher degree than can cardiovascular function (11). The respiratory system undergoes specific adaptations during endurance training to enhance its efficiency. Pulmonary ventilation is usually not considered to be a limiting factor when performing physical, endurance exercises (12). However, some evidence suggests that in a highly trained athlete (13), at a certain point, the capacity of the pulmonary system for oxygen transport may be insufficient to satisfy the oxygen demand and insufficient to keep pace with the capacity of an adapted cardiovascular system.

The generally accepted parameters to assess cardiovascular endurance are the maximal oxygen uptake (VO_{2max}), lactate (aerobic) threshold, maximal lactate steady state (anaerobic threshold), and exercise economy.

Maximal oxygen uptake (VO_{2max}) is the maximum capacity of an individual's body to transport and use oxygen during exercise or physical activity. VO_{2max} is widely accepted as the single best measure of cardiovascular endurance.

The lactate (aerobic) threshold is defined as the increase of physical exercise at which point anaerobic glycolysis is markedly activated in the engaged muscles, and the lactic acid concentration is elevated as compared compared to the resting values (14). This threshold most commonly occurs at an intensity of 40 - 60% VO_{2max} and when thea lactic acid blood concentration of approximately 1,5 -2 mmol/l.

The maximal lactate steady state (MLSS) or anaerobic threshold is defined as the intensity of physical exercise at which stable states of VO_2 and lactic acid in the blood can still be achieved and reflects a balance between the accumulation and degradation of lactic acid (15). MLSS is most commonly reached at an intensity of approximately 80-90% VO_{2max} (in untrained healthy subjects; 65 - 70% VO_{2max} is reached in elite athletes up to 95% VO_{2max}) and when the lactate blood concentration is approximately 3-5 mmol/L.

Exercise economy is used to express the oxygen consumption required to perform a given exercise workload, whether the exercise is running, cycling, or any other endurance activity (16). Differences in the VO_2 between individuals at similar exercise workloads illustrate the individual variation found in exercise economy.

With the above parameters, the intensity at the lactate threshold and MLSS can be expressed in terms of running speed (on a treadmill in km/h) or power (on a bicycle ergometer in watts, kpm/min or km/h; in watts on sport-specific ergometers, such as swimming, rowing, kayak and other similar activities). In practice, the generated results are expressed as the percentages of the achieved maximal oxygen uptake (%VO_{2max}), of the maximal speed achieved in the test (%Vmax), of the maximal achieved intensity in the test (%Pmax), and so on.

The tests used to measure cardiorespiratory endurance are classified in different ways related to: the test character (specific and non-specific), the performance mode (continued and discontinued), the type of workload (fixed and progressive workload), and the performance place (laboratory and field tests). The question of the appropriate test choice has been addressed in numerous studies and is especially important in light of the advances in technology and the increased availability of particular field methods (heart rate monitors, GPS devices, calculators of energy expenditure, etc.). Traditionally, dosed test workload ergometers (arm crank or cycle ergometers) and treadmills have been most commonly used in laboratories. More specific ergometers for certain sports (swimming, rowing, kayaking, ski-running, skating, etc.) have been increasingly used as well.

The use of arm cranks or cycle ergometers in laboratory testing with precise workload dosing allows for the use of additional invasive and non-invasive diagnostic methods (17). Due to the sedentary position of the exerciser and the defined cyclic repetition of pedalingpedalling, the risk of injury is low during the testing exercises. The shortcoming of this test type is its lack of the sufficient involvement of large muscle groups such that local muscle endurance and, not general cardiorespiratory endurance, commonly limits the maximal achievable test results (18).

As a treadmill enables natural movements (walking or running) with the engagement of large muscle groups and without the requirement of specific skills of the examinee, it can be used for different examinee profiles. Comparative studies have shown that the measured values of VO_{2max} during treadmill testing were 5-15% higher as compared compared to cycle ergometer values (19). However, the differences in measured VO_{2max} in laboratory treadmill tests may be caused by the test protocols, limiting inter-laboratory comparisons. Test protocols most commonly differ in the duration of particular workload stages, dynamics of workload increases during the tests, changes in treadmill inclination during the test, and so on. Continued progressive exercise tests are more commonly used and are performed until the maximum exhaustion of an athlete is reached by increasing the workload through increasing the treadmill speed, inclination, or a combination of the two. Test protocols with markedly rapid increases in workload and shorter total durations commonly do not register maximal VO_2 values due to muscle failure. On the other hand, long test protocols may also result in slightly lower registered VO_{2max} values due to increased body temperature, significant dehydration, muscle pain, and loss of motivation of the examinee (20, 21). In certain cases, it is necessary to choose a specific test protocol that is most appropriate to the characteristics of the sport in question (3). The use of sport-specific ergometers in laboratory testing enables more precise endurance measurements due to a more true to life reproduction of a dynamic stereotype that is characteristic of the sport and enables better mechanical efficacy (with lower energy consumption) during the test execution.

The above has led to national Olympic committees appointing only one laboratory to perform a functional diag-

nosis and to serve as a national reference institution to test all athletes in the preparation for the Summer or Winter Olympic Games. However, unresolved issues in laboratory testing and the measurement of VO_{2max} for sport competition purposes remain, including increased air resistance in open sport arenas and increased energy consumption with increasing treadmill inclination and changes in biomechanical parameters during the execution of a particular test protocol (the length and frequency of steps, the angular velocity and amplitude in major joints, the activation of particular muscle groups, etc.) (22, 23). An optimal laboratory test should provide most of the parameters required for a proper assessment of the level of physical fitness, definition of individual training areas, and control of the training workload. The combination of spiroergometric testing (with appropriate testing protocols) and the measurements of the lactate thresholds is believed to be the gold standard in the assessment of cardiorespiratory endurance. The generated parameters are then used to define the training objectives, to prepare a precise training plan and program, and to evaluate the effects of the subsequent training effect evaluation for (3, 24). The most obvious shortcoming of such an approach is its high economic price, limiting its use to only the top athletes and preventing its use by young talented athletes for whom training program corrections would represent the method to achieve top results.

MUSCULAR ENDURANCE

The ability of a muscle group to execute repeated contractions or to maintain a specific percentage of its maximum voluntary contraction depends on a number of factors: the contractile and metabolic characteristics of the muscle fibres, the position of the fibres during action, the activation of motor units, present fitness, etc. (25). Neuromuscular adaptation during sport training enables greater endurance to be demonstrated (26) and involves neurologic adaptation (more efficient motor unit activation), an enlargement of the transverse section of muscles, and changes in cellular metabolites. The magnitude of these changes depends on the initial status, type of muscular contraction, intensity and extent of training workload, number of series, selection and sequence of exercises, duration of rest, frequency of training, and movement velocity, among others. (27).

Muscular endurance is specific for each group of muscles, type , and velocity of contraction. As a result, there is no universal assessment for the muscular endurance of the whole body. The isokinetic and isoinertial dynamometry methods and numerous field tests are used to assess muscular endurance. During laboratory isokinetic tests, the angular velocity of a joint is controlled and kept constant; however the linear velocity of the active muscle group is not kept constant . Angular velocity increases at the beginning of each movement until the target velocity is reached. Further acceleration is not possible, and the movement is maintained at a constant velocity until the end of the planned movement. The advantages of isokinetic dynamometry in the assessment of muscular endurance include an optimal workload, an excellent confidence test, and a computer-aided assessment of muscular function. In addition, more advanced isokinetic systems have accessories to allow for the testing of different muscle groups, which is not possible in the associated joints. Except for the isolated movement tests, various manual activities (e.g., lifting and handling of various sport equipment) can be simulated on adapted isokinetic dynamometers with special accessories. The use of standardised protocols for isokinetic dynamometry is essential because of the related methodological issues, such as the positioning and motivation of the examinees during the test. Factors influenced by the positioning of the examinee and segment-joint stabilisation during the test include the muscle group length, contribution of elastic components, effective torque, development of angular velocity, and inhibitory effects of antagonistic muscle groups (28, 29). Therefore, it is essential that these factors are standardised to assure the validity of inter-test comparisons. The major shortcomings of isokinetic dynamometry are a limited movement velocity, the absence of natural movements, and the high equipment costs. Isokinetic testing of an isolated joint does not require natural movements; therefore, and as such,the examinees have to be precisely instructed and examiners have to be well acquainted with the testing system and test requirements. An additional shortcoming of isokinetic dynamometers is their inability to achieve movement velocities specific for a particular sport activity.

In practice, the combination of isoinertial dynamometry and field tests is most commonly employed. Isoinertial dynamometry is a method that enables the simultaneous and precise measurement of a large number of parameters and is aided by various kinematic and ballistic measurement systems. The use of adequate equipment and standardised testing protocols enables the precise measurement of the effective force, distance covered, or movement duration. Depending on the device, a single or several parameters can be measured, and it is possible to calculate the data necessary for high quality diagnosis of muscular endurance using a computer (30). Isoinertial field tests are tests in which a constant external load is resisted during the entire exercise, such as free-weight exercises. In practical work, the term isoinertial reflects a test that determines the ability of an examinee to resist a maximal external load (31). A test with a single maximal repetition (*1-repetition maximum–1RM*) represents the maximum load resisted throughout the range of motion in a controlled way and with good posture (32). The biggest shortcoming of these field tests is the lack of appropriate validity and objectivity and the fact that such movements (e.g., bench press or squats, movements during push–ups, sit–ups, pull–ups, etc.) have little resemblance to most of the movements in sport activities.

CONCLUDING REMARKS

By understanding and monitoring endurance in athletes, the training workload during the preparation period and the pre-competition and competition seasons can be implemented and corrected. The ua the the The achievement and maintenance of optimal fitness should be regarded as a dynamic concept that requires continual monitoring that is aided by the modern methods of functional diagnoses. The specific individual diagnosis of the endurance of an athlete is the basis for the preparation of a precise training plan and program and for the subsequent evaluation, correction, and improvement of the applied means and training methods. Using the generated parameters, training should be planned to result in better muscular endurance via specific neuromuscular adaptations. Moreover, properly guided endurance training should raise the lactate threshold and the level of maximal lactate balance via an increased oxygen supply to the active muscles and a a reduced lactate production in the active muscles, resulting in delayed fatigue in the athlete.

REFERENCES

1. Wilmore JH, Costill DL, Kenney LW. Physiology of sport and exercise. 4th ed. Champaign, IL: Human Kinetics, 2008.
2. Humphries RB, Dugan E, Doyle T. Muscular Fitness. In American College of Sports Medicine, eds. ACSM's Resource Manual for Guidelines for Exercise Testing and Prescription. 5th ed. Philadelphia: Lippincott William & Wilkins, 2006: 206-24.
3. Radovanovic D, Ponorac N, Ignjatovic A, Stojiljkovic N, Popovic T, Rakovic A. Specific alterations of physiological parameters in competitive race walkers. Acta Physiol Hung 2011; 98(4): 448–54.
4. Radovanovic D, Bratic M, Nurkic M, Cvetkovic T, Ignjatovic A, Aleksandrovic M. Oxidative stress biomarker response to concurrent strength and endurance training. Gen Physiol Biophys 2009; S28: 205–11.
5. McGuire DK, Levine BD, Williamson JW, et al. A 30-year follow-up of the Dallas Bed Rest and Training study II. Effect of age on cardiovascular adaptation to exercise training. Circulation 2001; 104(12): 1358–66.
6. Levy WC, Cerqueira MD, Abrass IB, Schwartz RS, Stratton JR. Endurance exercise training augments diastolic filling at rest and during exercise in healthy young and older men. Circulation 1993; 88(1): 116–26.
7. Timmons JA. Variability in training-induced skeletal muscle adaptation. J Appl Physiol 2011; 110: 846–53.
8. Yeo WK, Carey AL, Burke L, Spriet LL, Hawley JA. Fat adaptation in well-trained athletes: effects on cell metabolism. Appl Physiol Nutr Metab 2011; 36(1): 12–22.
9. Bassett DR, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med Sci Sports Exerc 2000; 32: 70–84.
10. Coyle EF. Integration of the physiological factors determining endurance performance ability. Exerc Sport Sci Rev 1995; 23: 25–63.
11. Radovanovic D, Bratic M, Nurkic M, Stankovic N. Recovery of dynamic lung function in elite judoists after short-term high intensity exercise. Arch Budo 2011; 7(1): 21–26.
12. di Prampero PE (2003). Factors limiting maximal performance in humans. Eur J Appl Physiol 2003; 90: 420–9.
13. Wagner PD. A theoretical analysis of factors determining $\dot{V}O_{2max}$ at sea level and altitude. Respir Physiol 1996; 106: 329–43.
14. Barstow TJ, Casaburi R, Wasserman K. O_2 uptake kinetics and the O_2 deficit as related to exercise intensity and blood lactate. J Appl Physiol 1993; 75(2): 755–62.
15. Faude O, Kindermann T, Meye T. Lactate threshold concepts: how valid are they? Sport Med 2009; 39(6): 469–90.
16. Daniels JT. A physiologist's view of running economy. Med Sci Sports Exerc 1985; 17(3): 332–8.
17. Djordjevic D, Cubrilo D, Puzovic V, et al. (2012). Changes in athlete's redox state induced by habitual and unaccustomed exercise. Oxid Med Cell Longev 2012; doi:10.1155/2012/805850
18. Radovanovic D, Aleksandrovic M, Stojiljkovic N, Ignjatovic A, Popovic T, Marinkovic M. Influence of physical training on cardiorespiratory endurance in preadolescent age. Acta Med Mediane 2009; 48(1): 37–40.
19. Verstappen FT, Huppertz RM, Snoeckx LH (1982). Effect of training specificity on maximal treadmill and bicycle ergometer exercise. Int J Sports Med 3(1): 43–6.
20. Pollock ML, Bohannon RL, Cooper KH, et al. Comparative analysis of four protocols for maximal treadmill stress testing. Am Heart J 1976; 92(1): 39–46.
21. Kang J, Chaloupka EC, Mastrangelo MA, Biren GB, Robertson RJ. Physiological comparisons among three maximal treadmill exercise protocols in trained and untrained individuals. Eur J Appl Physiol 2001; 84(4): 291–5.
22. Pereira MA, Freedson PS. Intraindividual variation of running economy in highly trained and moderately trained males. Int J Sports Med 1997; 18(2): 118–24.
23. Riley PO, Dicharry J, Franz J, et al. A kinematics and kinetic comparison of overground and treadmill running. Med Sci Sports Exerc 2008; 40(6): 1093–110.
24. Radovanovic D, Okicic T, Ignjatovic A. Physiological profile of elite women water polo players. Acta Med Medianae 2007; 46(4): 48–51.
25. Komi PV. Strength and power in sport. 2nd ed. London: Blackwell Science, 2003.
26. Ignjatovic A, Radovanovic D, Stankovic R, Markovic Z, Kocic J. Influence of resistance training on cardiorespiratory endurance and muscle power and strength in young athletes. Acta Physiol Hung 2011; 98(3): 305–12.

27. Radovanovic D, Ignjatović A. Physiological basis of force and strength training (In Serbian). Nis: Faculty of Sport and Physical Education University of Nis, 2009.
28. Osternig LR. Isokinetic dynamometry: implications for muscle testing and rehabilitation. Exerc Sport Sci Rev 1986; 14: 45–80.
29. Baltzopoulos V, Brodie DA. Isokinetic dynamometry. Applications and limitations. Sports Med 1989; 8(2):101–16.
30. Ignjatovic A, Markovic Z, Radovanovic D. Effects of 12-week medicine ball training on muscle strength and power in young female handball players. J Strength Cond Res 2012; 26(8): 2166–73.
31. Desqorces FD, Berthelot G, Dietrich G, Testa MS. Local muscular endurance and prediction of 1 repetition maximum for bench in 4 athletic populations. J Strength Cond Res 2010; 24(2): 394–400.
32. Shimano T, Kraemer WJ, Spiering BA, et al. Relationship between the number of repetitions and selected percentages of one repetition maximum in free weight exercises in trained and untrained men. J Strength Cond Res 2006; 20(4): 819–23.

COMPARATIVE ANALYSIS OF THE CHEMICAL COMPOSITION OF HELIANTUS TUBEROSUS L. GROWING IN SERBIA AND ROMANIA

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KOMPARATIVNA ANALIZA HEMIJSKOG SASTAVA HELIANTUS TUBEROSUS L. SA PODRUČJA SRBIJE I RUMUNIJE

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ABSTRACT

Helianthus tuberosus L. (Jerusalem artichoke) *belongs to the Asteraceae family, genus Heliathus, and is a native species of Serbia and Romania. The most valuable constituent is inulin, which together with other constituents forms the unique content of its plant material. Inulin has the effect of reducing the risk of cardiovascular diseases; it also has beneficial effects on different gastrointestinal conditions by serving as a prebiotic and has been shown to be important in the prevention and/or the alleviation of the progression of osteoporosis. The aim of this study was the to compare the chemical compositions of* ***H. tuberosus*** *L. tubers growing in two different geographic areas on the Balkan peninsula, namely in Serbia and Romania. We have determined the content of the eight main components in both herbal samples: total proteins, flavonoids, polyphenolic carboxylic acids, reducing sugars, total carbohydrates, antioxidants, inulin and ascorbic acid,. Furthermore, we calculated a caloric value offor each of the herbal samples. The levels of all the investigated compounds, with the exception of the total carbohydrates and antioxidants, were slightly higher in the plants growing in Romania than in the plants growing in Serbia. Because the differences in the contents of both plant groups are very small, we propose that both materials could be considered as a suitable raw plant material for further processing. The caloric value of the plants growing in Serbia was found to be slightly higher than the caloric value of the ones growing in Romania. Both herbal samples are good sources of inulin and represent valuable raw plant material for further processing.*

Keywords: *topinambur, chemical composition, inulin, Jerusalem artichoke*

SAŽETAK

Helianthus tuberosus L. (Jerusalimska artičoka) *pripada rodu Heliathus iz familije* ***Asteraceae****. Raste kao autohtona vrsta na teritorijama Srbije i Rumunije. Ovu biljnu vrstu karakteriše jedinstven hemijski sastav, a kao najdragoceniji konstituens izdvaja se inulin. Povoljan uticaj inulina na zdravlje ljudi uključuje smanjen rizik za kardiovaskularne bolesti, prebiotski efekat, prevenciju i/ili ublažavanje osteoporoze. Cilj studije je bio poređenje hemijskog sastava krtola* ***H. tuberosus*** *L. sa dva različita staništa na Balkanskom poluostrvu, Srbije i Rumunije. Određen je sadržaj osam glavnih komponenti biljnih sirovina sa oba geografska područja: ukupni proteini, flavonoidi, polifenolne karboksilne kiseline, redukujući šećeri, ukupni ugljeni hidrati, antioksidanti, inulin i askorbinska kiselina i izračunata kalorijska vrednost osušenog biljnog materijala. Sadržaj svih ispitivanih jedinjenja osim ukupnih ugljenih hidrata i antioksidanata je bio nešto viši u biljnoj sirovini poreklom iz Rumunije. Postoje jako male razlike u sastavu biljnih sirovina sa pomenutih staništa, pa se može smatrati da su oba biljna materijala pogodna za dalju obradu. Kalorijska vrednost čičoke koja raste u Srbiji je nešto viša od biljne vrste koja raste u Rumuniji. Obe biljne sirovine su dobri izvori inulina i predstavljaju dragoceni biljni materijal za dalju obradu.*

Ključne reči: *čičoka, hemijski sastav, inulin*

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INTRODUCTION

*Helianthus tuberosus L. (*Jerusalem artichoke*)* belongs to the *Asteraceae* family, genus Heliathus. Its origin was located in northern and central parts of the USA, but it was transferred to Europe in the seventeenth century. J. artichoke grows as a native species in Serbia and Romaniaand is and is known by the traditional names cicoka and topinambur. The differences in the characteristics of the wild and the hybrid J. artichoke species have been investigated at the Institute of Field and Vegetable Crops in Novi Sad, Serbia and at the Research Institute for Cereals and Technical plants in Calarasi, Romania (1).

The tubers of this plant have been used in the human diet. The J. artichoke tuber is composed of up to 80% water, 15% carbohydrate, and approximately 8% protein. It has almost no starch, whereas mono-and polyunsaturated fatty acids are found in trace amounts (1). The tubers are a good source of vitamins (2), minerals (3) and dietary fibre. The most important carbohydrate is inulin, which may be present in 8-21% of the plant's fresh weight (2, 4). Inulin is the most valuable constituent, which together with other constituents forms the unique content of this plant material. Inulin is a carbohydrate formed from fructose units. Fructose chains are linear and linked by a β (1-2) – linkage, and one terminal glucose unit is linked by an α (1-2) - linkage. It has been shown that inulin has numerous beneficial effects on human health. Inulin reduces the risk of cardiovascular disease, which is achieved by lowering the levels of triglycerides (5, 6). It acts beneficially in different gastrointestinal conditions by possessing a prebiotic role (7), and inulin has been shown to be important in the prevention and/or alleviation of osteoporosis progression (8).

The quality of the plant material depends on the geographic area and its climate characteristics as well as the soil type. J. artichoke grows better on relatively infertile land than most crops (1), but fertile soil is required for obtaining high yields and large tuber sizes (9).

The aim of this study was the to compare the chemical composition of *H. tuberosus* L. tubers grown at two different geographic areas on the Balkan peninsula, namely in Serbia and Romania.

MATERIAL AND METHODS

Plant material

J. artichoke tubers were collected when ripe from the Sumadija region of Serbia in the period between October - December 2010. The plant material of Romanian origin was collected on Hofigal's lowland fields in 2011. Preparation of all plant material included washing, peeling, cutting into slices and drying in a mechanical drier at a controlled temperature (40°C). The last step was grinding the dried tubers into a fine powder.

Analyses of plant chemical composition

We have determined and compared the content of the following constituents: total proteins, flavonoids, polyphenolic carboxylic acids, reducing sugars, total carbohydrates, antioxidants, inulin and ascorbic acid. Total proteins in the plant material were determined using the standard Kjeldahl method, which determinates the nitrogen content in the sample (10). Flavonoids were expressed as % of rutin. The content of flavonoids was determined the use ofusing a UV / VIS spectrophotometer; employing the standard curve method, and the absorbances of the herbal extract dilutions were measured at 430 nm (11). The polyphenol carboxylic acid content was expressed as the per cent of caffeic acid. The UV/VIS spectrophotometric method was used, and the absorbances of the ethanolic extract were measured at 660 nm (11).

The content of reducing sugars was determined using the Fehling method of the reduction of copper (II) ions from an alkaline solution of $CuSO_4$ complex to copper (I) ions, which forms a brick red copper (I) oxide precipitate (12). Total carbohydrate content was expressed as a per cent of glucose. The total carbohydrates in J. artichoke were determined using the standard Anthrone method, which is based on the hydrolysis of carbohydrates into simple sugars by dilute hydrochloric acid. The dehydration of glucose produces hydroxymethyl furfural, which after reacting with the Antron reagent, could be measured at 630 nm (13). Determination of the antioxidant content was expressed as g of Trolox/g. Antioxidant activity was determined using the spectrophotometric CUPRAC method. This method measures the absorbance of Cu (I) - neocuproine (2,9-dimethyl-1,10 - phenanthroline) at 450 nm, obtained by the reduction of antioxidant compounds by the CUPRAC reagent (Cu (II) neocuproine) (11).

For the determination of the inulin content, we used the spectrophotometric method. Hydroxymethyl furfural, formed by the hydrolysis of inulin in an acidic medium, reacts with resorcinol and produces a red coloured product. Absorbance was measured at 520 nm. A standard inulin solution was used for the preparation of the calibration curve (14). The ascorbic acid content (‰) was determined after titration with Tillman's reagent. The method is based on the oxidation of ascorbic acid to dehydroascorbic acid, while the Tillman's reagent is reduced to its leuko-base (15).

The caloric value of the J. artichoke was calculated using the following formula:

Kcal = 4 x (% proteins + % total carbohydrates) + 9 x (% lipids) (16).

RESULTS

We have determined the contents of the eight main components in both herbal samples. The analysis of the plant material from Serbia showed the following results: 19,70 % of total proteins, 0,31 % of flavonoids, 0,21 % of polyphenol carboxylic acids, 9,85 % of reducing sugars, 34,83 % of total carbohydrates, 1,37 g Trolox/g of antioxidant, 24,7 % of inulin and 74,3 ‰ of ascorbic acid. We have obtained slightly different results from Romania's plant material. The Romanian J. artichoke showed the follow-

ing results: 21,10 % of total proteins, 0,37 % of flavonoids, 0,22 % of polyphenolic carboxylic acids, 12,79 % of reducing sugars, 28,79 % of total carbohydrates, 1,33 g Trolox/g antioxidant, 25,40 % of inulin and 81,15 ‰ of ascorbic acid. The comparative analysis of the J. artichoke chemical compositions from Serbia and Romania is shown in Table 1.

No	Analysis	*H. tuberosus* Serbia	*H. tuberosus* Romania
1	Total proteins %	19,17	21,10
2	Flavonoids %	0,31	0,37
3	Polyphenol carboxylic acids %	0,21	0,22
4	Reducing sugars %	9,85	12,79
5	Total carbohydrates %	34,83	28,79
6	Antioxidant g Trolox/g	1,37	1,33
7	Inulin %	24,70	25,40
8	Ascorbic acid ‰	74,30	81,15

Table 1. The comparative results of the J. artichoke chemical compositions from Serbia and Romania

The caloric value of J. artichoke growing in Serbia was 218,70 Kcal, and that of J. artichoke growing in Romania was 204,10 Kcal. Hence, the caloric value of J. artichoke growing in Serbia is greater than that of the one from Romania.

DISCUSSION

Our results indicate that the levels of all investigated compounds, with the exception of the total carbohydrates and antioxidants, were slightly higher in the plants growing in Romania than in the plants growing in Serbia. Because the differences in the contents of both samples are very small, we propose that they both could be considered as suitable raw plant material for further processing. J. artichoke has been extensively used as a source of carbohydrates, especially inulin. Therefore, this study complements current research data on J. artichoke growing in the Balkans, and it may substantially contribute to the production of different J. artichoke extracts because selecting a raw plant material for production depends on the chemical content of the plant material and the desired content of the final product.

Plants rich in inulin have recently been thoroughly investigated recently due to the prebiotic effect of inulin (17, 18). This study confirms that J. artichoke is a significant source of inulin, thatwhich could be found in plants growing in countries on the Balkan peninsula – Serbia and Romania. Our results showing that both samples contained up to 25% of inulin confirmed that both plant materials could be used as valuable sources of inulin because the obtained values for inulin are slightly higher than the literature data, which ranges up to 21% (1). The caloric value of the plants growing in Serbia was found to be slightly higher than the caloric value of those plants growing in Romania, and this difference could be associated with the higher content of total carbohydrates present in the Serbian plants. The slightly higher levels of antioxidants in the plants growing in Serbia could not be associated with its content of flavonoids, polyphenol carboxylic acids or ascorbic acid as the main contributors to the antioxidant activity because the levels of these compounds were slightly lower in the plants growing in Serbia than in Romania. Therefore, the authors suggest further investigation of the antioxidant potential of the J. artichoke growing on the Balkan peninsula, and an improvement in sampling by increasing the number of different locations sampled in both countries.

J. artichoke growing on the Balkan peninsula in both Serbia and Romania is a good source of inulin and represents a valuable raw plant material for further processing, although the plants growing in Romania are slightly more suitable due to their chemical composition.

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REFERENCES:

1. Kays SJ, Nottingham SF. Biology and Chemistry of Jerusalem Artichoke *Helianthus tuberosus* L. New York: Taylor & Francis Group, 2007, 53 - 7.
2. Van Loo J, Coussement P, De Leenheer L, et al. On the presence of inulin and oligofructose as natural ingredients in the Western diet. Crit Rev Food Sci Nutr 1995; 35: 525-52.
3. Cieslik E. Mineral content of Jerusalem artichoke new tubers. Zesk Nauk AR Krak 1998; 342: 23 – 30.
4. Roberfroid MB. Dietary fibre, inulin and oligofructose. A review comparing their physiological effects. Crit Rev Food Sci Nutr 1993; 33: 103-48.
5. Tungland BC. Fructooligosaccharides and other fructans: structures and occurrence, production, regulatory aspects, food applications and nutritional health significance. ACS Symp Ser 2003; 849: 135-52.
6. Kaur N, Gupta AK. Applications of inulin and oligofructose in health and nutrition. J Biosci 2002; 27(7): 703-14.
7. Watzl B, Girrbach S, Roller M. Inulin, oligofructose and immunomodulation. Br J Nutr 2005; 93: 49-55.
8. Coudray C, Bellanger J, Castglia-Delavaued C, et al. Effects of soluble or partly soluble dietary fibers supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. Eur J Clin Nutr 1997; 513: 75-380.
9. Kosari N, Cosentino GP, Wieczorek A, et al. The Jerusalem artichoke as an agricultural crop. Biomass 1984; 5: 1-36.

10. Hedge J.E and Hofreiter B.T. Carbohydrate Chemistry, 17th edition. New York: Academic Press, 1962.
11. Council of Europe (COE) - European Directorate for the Quality of Medicines (EDQM). European Pharmacopoeia 6th Edition. 2007.
12. Trajkovic J, Miric M, Baras J, et al. Analiza zivotnih namirnica. Beograd: Tehnolosko-Metalurski fakultet, 1983.
13. Stanimirovic S. Bromatologija. Beograd: Farmaceutski fakultet Univerziteta u Beogradu, zavod za bromatologiju, 1988.
14. Sadasivam S, Manickam A. Biochemical Methods, 3rd edition. New Age International, 2007; 13-14.
15. Rosner L and Bellows J. Ascorbic Acid Oxidase in Determining Vitamin C in Lens and Aqueous Humor. Proc Soc Exp Biol Med 1936; 34: 493-4.
16. Niketic – Aleksic G. Tehnologija voca i povrca. Beograd: Poljoprivredni fakultet, 1982, 25-7.
17. Gibson GR, Probert HM, Loo JV, et al. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 2004; 17(2): 259-75.
18. Dewulf EM, Cani PD, Claus SP, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. Gut 2012. *In press*.

TREATMENT WITH AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA PATIENTS – A 10-YEAR SINGLE CENTRE EXPERIENCE

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TERAPIJA BOLESNIKA SA MULTIPLIM MIJELOMOM AUTOLOGOM TRANSPLANTIRACIJOM MATIČNIH ĆELIJA-DESETOGODIŠNJE ISKUSTVO JEDNOG MEDICINSKOG CENTRA

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ABSTRACT:

Background - *Multiple myeloma is a malignant neoplasm of plasma cells. Autologous stem cell transplantation (ASCT) has become the first line of therapy because of the low transplant-related mortality and its ability to prolong event-free survival, which results in improved quality of life. High–dose therapy (HDT) with ASCT should be part of the primary treatment in newly diagnosed patients under the age of 65 with adequate performance status and organ function.*

Aim: *The aim of this study is to present our ten-year experience in treating multiple myeloma patients with ASCT.*

Materials and methods: *Over a 10 -year period, we have performed 35 courses of HDT and consecutive ASCT in 31 patients with multiple myeloma (4 tandem transplantations). In this study, we retrospectively analysed the epidemiological characteristics of this group of patients.*

Results: *There were 14 female and 17 male patients. The median patient age was 52 years (range 43-64). The conditioning regimen used was high-dose Melphalan in doses of 200 mg/m^2, and the dose used in the second (tandem) transplantation was 140 mg/m^2. The median count of infused CD34+ cells was 3.65x10^6/kg. As a source of added stem cells, we used phlebotomy in 3 patients. The median period from diagnosis to transplantations was 10 months. Of 31 patients, 21 (67%) are currently alive and 10 (33%) have died (3 renal failure, 3 multi-organ failure, 2 infections, and 2 fatal cerebral bleeding). The disease-free survival was 24 months.*

Conclusions: *ASCT offers better survival and quality of life compared to patients treated only with standard chemotherapy.*

Keywords: *multiple myeloma, stem cell transplantation*

SAŽETAK

Multipli mijelom je maligna bolest plazma ćelija. Autologna transplantacija matičnih ćelija (ASCT) postala je prva linija terapije, uglavnom zbog niske smrtnosti i produženog preživljavanja , tako da dovodi do poboljšanja kvaliteta života. Terapija visokim dozama citostatika (HDT) sa ASCT treba da budu deo primarnog lečenja kod novootkrivenih pacijenata do 65 godina starosti uz adekvatan opšti klinički status.

Cilj: *Cilj ovog rada je da predstavi deset godina našeg iskustva u lečenju obolelih od multiplog mijeloma putem ASCT.*

Materijal i metode: *Tokom desetogodišnjeg perioda, obavili smo 35 tretmana HDT i konsekutivnog ASCT kod 31 bolesnika sa multiplim mijelomom (4 Tandem transplantacije). U ovoj studiji smo analizirali retrospektivno epidemiološke karakteristike ove grupe pacijenata.*

Rezultati: *Odnos ženske i muške populacije iznosi 14:17, prosečne starosti: 52 godine (od 43-64). Visoke doze Melphalana u dozama 200mg/m2 korišćene su kao kondicioni režim, u drugoj (tandem) transplantaciji 140mg/m2. Srednji broj unetih CD34 + ćelija bio je 3,65 k106/kg. Kao izvor dodatih matičnih ćelija, koristili smo flebotomiju kod 3 bolesnika. Srednji period od dijagnoze do transplantacija bio je 10 meseci. Od 31 bolesnika, 21 (67%) su živi, 10 (33%) je umrlo (3 od bubrežne insuficijencije, 3 od multiorganske disfunkcije, 2 od infekcije, 2 od fatalnog cerebralnog krvavljenja. Preživljavanje bez ponovnih simptoa bolesti je 24 meseca.*

Zaključci: *ASCT obezbeđuje bolje rezultate za opstanak i kvalitet života u poređenju sa pacijentima lečenim standardnom hemoterapijom.*

Ključne reči: *multipli mijelom, transplantacija matičnih ćelija*

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INTRODUCTION

Multiple myeloma is a malignant haematological neoplasm of plasma cells that accumulate in bone marrow, leading to bone destruction and marrow failure (1). The disease develops in 1–4 per 100,000 people per year. The disease is more common in men and is twice as common in blacks as it is in whites (2). With conventional treatment (MP regimen), the prognosis is 3–4 years, and it may be extended to 5–7 years or longer with advanced treatments. Multiple myeloma is the second most common haematological malignancy (13%) and constitutes 1% of all cancers. It was estimated that multiple myeloma would account for 19,920 new cancer cases in the United States in 2008. This figure includes 11,190 cases in men, 8,730 cases in women, and 10,690 deaths. The median age of myeloma patients at diagnosis is 69 years for men and 72 years for women (3). High-dose chemotherapy with autologous stem cells transplantation should be part of the primary treatment in newly diagnosed patients up to the age of 65 years with adequate performance status and organ function (Grade A; level Ib) according to the British Council of Haematology and UK Myeloma. The diagnostic criteria according to Durie and Salmon are used to confirm diagnosis (4). A bad prognosis is associated with partial or complete deletion of chromosome 13. Melphalan and prednisone (MP) have remained the standard therapy for decades, and the median survival with this therapy was approximately 3 years. High-dose therapy (HDT) and Autologous Stem Cell Transplantation (ASCT) havse been used in the management of myeloma since the efficacy of high dose melphalan in the treatment of high-risk myeloma and plasma cell leukaemia was first reported more than 20 years ago (5). ASCT has become the first line standard of care in patients deemed suitable for transplant. ASCT is the standard of care because of the low transplant-related mortality and the prolonged event-free survival (EFS), which results in improved quality of life (6). Treatment with high-dose therapy and single autologous stem cell transplantation is a category I recommendation of the National Comprehensive Cancer Network. In young patients, the impact of dose intensity has been demonstrated, and single HDT supported with ASCT using a conditioning regimen with Melphalan alone should be considered as a standard of care (7). Double transplantation can be proposed to patients failing to achieve a very good partial response after the first stem cell transplantation (BCSH and UKMF Guidelines on the Management and Diagnosis of Multiple Myeloma Sept 2010). Stem cells are now almost exclusively derived from peripheral blood following stimulation with growth factors with or without chemotherapy. The optimal regimen for mobilising peripheral blood stem cells (PBSC) is unclear, but cyclophosphamide (1.5 to 4 g/m^2) with G-CSF is widely used. Purging harvested stem cells with monoclonal antibodies and/or CD34+ stem cell selection does reduce contamination with tumour cells but does not influence the relapse risk (8). High-dose melphalan (200 mg/m^2) remains the standard conditioning prior to ASCT in first transplants, while a reduced dose of 140 mg/m^2 is used in second transplantation. Recent studies have shown that the dose of melphalan can be increased to 220 mg/m^2 (9), with improved PFS compared with historical controls, or to 240–300 mg/m^2 in combination with amifostine. However, this approach is associated with increased toxicity (10). The addition of total body irradiation (TBI) results in increased toxicity with no improvement in response rate or PFS, whereas combination chemotherapy increases the toxicity (11,12,13). Bortezomib has shown synergistic effects with melphalan without prolonged hematologic toxicity. The recently reported IFM phase 2 study enrolled 54 untreated patients to receive bortezomib (1 mg/m2x 4) and melphalan (200 mg/m^2) as a conditioning regimen. They reported a response ≥VGPR in 70% of patients and a 32% CR. There were no toxic deaths observed, and there was minimal peripheral neuropathy. Different studies have reported that the combination of chemotherapy plus new drugs can induce 70-90% partial response and 30-40% complete response (14,15,16). The introduction of lenalidomide (analogue of thalidomide), bortezomib, and other novel agents have been extensively investigated to improve the duration of response (17,18,19). According to guidelines from the British Haematology Council and International Myeloma Forum published in September 2010, the following recommendations are supported: HDT with ASCT should be part of the primary treatment in newly diagnosed patients up to the age of 65 years with adequate performance status and organ function (Grade A; level Ib); HDT with ASCT should be considered in patients aged >65 years with good performance status (Grade B; level IIa); and conditioning with melphalan alone, without TBI, is recommended (Grade B; level IIa). The usual dose is 200 mg/m^2, but the dose should be reduced in older patients (over 65-70 years) and those with renal failure.

MATERIALS AND METHODS

Over the 10-year period from September 2000 to September 2010haematology, 195 stem cell transplantations were performed at our institution (University Clinic of Haematology, Medical Faculty, Skopje) on various haematological malignant and non-malignant diseases (AML: 109, ALL: 7, CML: 9, CLL: 1, NHL: 15, HD: 15, Myelofibrosis: 1, Ewing sarcoma: 1, Aplastic anaemia: 2, Multiple myeloma: 35). Allogeneic transplantation from HLA identical siblings were used to treat 56 patients, and autologous transplantation was used to treat 139 patients. Peripheral blood stem cells were used in 165 transplantations, and bone marrow was used in 30 transplantations. In 31 patients with multiple myeloma, we performed 35 high-dose chemotherapy and autologous stem cells transplantations (4 tandem transplantations) from peripheral blood stem cells. The high-dose conditioning regimen consisted of 200 mg/m^2 Melphalan in first transplantation, while the second (tandem) transplantation used a dose

Patient	Age	Gender	Ig	Bence-Jones	Renal failure	Fracture	Ro.Th	Months Dg./Tx	Months A/D
V.A	64	M	IgG	Negative	No	Th12/L1	Yes	6	A=92
T.S.	45	F	IgG	Negative	No	No	No	4	A=96
N.V.1	43	M	IgG	Negative	No	Th12/L1	Yes	5	A=61
V.Z.1	53	F	IgG	Negative	No	Hip	Yes	11	A=58
V.Z.2	53	F	IgG	Negative	No	No	Yes	17	A=64
G.Z.1	51	F	IgG	Negative	No	No	No	7	A=63
G.Z.2	51	F	IgG	Negative	No	No	No	10	A=66
S.J.	46	F	IgG	Negative	No	No	No	5	A=102
A.Dz.	50	M	IgG	Negative	No	L2	No	22	A=75
N.V.2	43	M	IgG	Negative	No	Th12/L1	Yes	8	A=69
S.S.	43	F	IgA	Positive	Yes	Negative	No	12	A=6
Z.G.	50	M	IgA	Positive	Yes	L2L3	Yes	5	A=6
A.L.	43	M	IgG	Positive	Yes	No	No	10	A=8
M.T.	50	M	IgG	Negative	No	Yes	Yes	6	A=16
M.Sh.	52	M	IgG	Negative	No	Yes	Yes	8	A=12
G.D.	48	F	IgG	Negative	No	No	No	5	A=15
Kr.Kos.	57	M	IgG	Positive	No	No	No	9	A=25
Lj.M.	56	M	IgG	Positive	Yes	No	No	6	A=24
M.D.	48	M	IgG	Negative	No	No	No	12	A=24
Ka.K.	52	F	IgG	Positive	Yes	No	No	6	A=27
S.B.	48	F	IgG	Positive	Yes	Th9	Yes	20	A=32
E.L.	60	M	IgG	Negative	No	No	Yes	11	A=34
A.T.	60	F	IgG	Negative	No	No	No	6	A=6
N.O.1	50	M	IgG	Negative	No	No	No	4	A=36
N.O.2	50	M	IgG	Negative	No	No	No	4	A=40
M.M.	47	M	Kappa	Positive	Yes	No	No	4	D=50
P.N.	60	F	IgA	Negative	No	No	No	12	D=62
A.C.	57	F	IgG	Negative	No	No	No	44	D=127
B.J.	60	M	IgA	Negative	No	L1	Yes	6	D=66
Z.S.	59	M	IgG	Negative	No	No	No	12	D=42
Da.K	63	M	IgG	Negative	No	No	No	20	D=45
Di.Ko	46	M	IgG	Negative	No	No	No	8	D=36
T.K.	42	F	IgG	Negative	No	Th12	Yes	15	D=44
D.L.	52	F	IgG	Positive	Yes	L2	Yes	22	D=36
A.L.	56	F	IgG	Positive	Yes	Yes	No	8	D=54

Table 1. Patient epidemiological and clinical data

of 140 mg/m^2 Melphalan. All patients were treated in a sterile room conditioned with HEPA filtration. In our study, we analysed 31 patients with multiple myeloma diagnosed according to Salmon and Durie diagnostic criteria. All patients were diagnosed and treated with induction therapy to reach remission at our hospital using various chemotherapeutic regimens. The standard chemotherapy regimen in our hospital is the VAD protocol in patients eligible for autologous transplantation. VAD (Vincristine, Adriamycin, Dexamethasone) is administered in 4 cycles every 28 days. Patients who achieved complete remission received priming therapy with Endoxan 2.0-4.0 g/m^2 plus G-CSF for peripheral stem cell mobilisation and harvesting. Non-responders received Thalidomide+Dexamethasone as a second line therapy for a 5-month period. As a third-line therapy, we used Bortezomib (Velcade). The median period from diagnosis to transplan-

tation in our group of patients was 10 months (from 4-44 months). Usually, we performed 2 apheresis procedures (1-3) to harvest adequate numbers of CD 34+ cells. A Baxter CS 3000 cell separator was used in 40 procedures, and a Cobe Spectra was used in 35 procedures. DMSO, Earhle's medium, and autologous plasma were used as a cryoprotectant. The cryopreservation was performed using a controlled Air Space Freezing system. The cryopreserved stem cells were stored at -172°C. The high-dose Melphalan was administered on day -2 and day -1 in 30-minute infusions through the central venous line. Approximately 24 hours after chemotherapy was completed, the autologous stem cells were thawed in a sterile bath and re-infused into the patient. As an anti-infective prophylaxis, every patient received Ciprofloxacin 1,0 gr/day, Difluconazol, or Itraconazole 200 mg/day, i.v. Acyclovir 1500 mg/day., and i.v. immunoglobulins 0,1/kg once a week. G-CSF was introduced from day +1 until neutrophil recovery. This research study was performed in accordance with the Declaration of Helsinki and was approved by the Local Ethics Committee. All patients signed a consent form.

RESULTS

Over a 10-year period at our hospital, we treated 31 patients with multiple myeloma. In 4 patients, we performed tandem transplantation (at a period from 4-6 months after the first transplantation). The dose of Melphalan was 200 mg/m^2 in the first and 140 mg/m^2 in the second transplantation. The median count of re-infused CD34+ cells was 3,65 x 10^6/kg b.w. (from 2,0 – 12,5). Engraftment was established on day +11 (from day +7 to day +16). The median number of days for G-CSF use was 10 days (from 7-14 days). During the first 30 days after transplantation, which is the period of aplasia, there were only a few infective complications in our group of patients. The early transplant related mortality (until day +100) was 0. The most common complication after transplantation was mucositis. There was Grade IV mucositis present in 8 patients. There were central venous catheter infections with coagulase negative staphylococcus in 5 patients. The fatal outcomes were due to renal failure in 3 patients (50, 62, and 127 months after transplantation), multi-organ failure in 3 patients (66, 42, and 45 months after transplantation), infections in 2 patients (one with aggressive hepatitis B virus infection 36 months after transplantation, and one with pneumonia 44 months after transplantation), and 2 fatal cerebral bleeding (36, 54 months after transplantation). All of the patients died with active relapse of myeloma disease.

DISCUSSION

Multiple myeloma was the most frequent indication for which autologous stem cell transplantation is performed (20). However, autologous stem cell transplantation is not curative, and most patients relapse within a median of 3 years. The median survival after transplantation in our

Number of patients	31
Number of transplantations	35 (4 tandem transplantations)
Age	52 years (43-64)
Gender	Male: 17 (55%) Female: 14 (45%)
Myeloma types	IgG =30(85%); IgA=4(11%) Light chain=1(4%)
Bence-Jones proteinuria	10 (28%)
Lytic bone lesion with fracture	13 (37%)
Previous radiotherapy	13 (37%)
Period from Diagnosis to transplantation	10 (4-44 months)
Living 21 patients (67%)	6-102 months after transplantation
Deceased 10 patients (33%)	36-127 months after transplantation

Table 2. Summary of patient characteristics treated with autologous transplantation

	Median	Range
number of apheresis	2	1 - 3
counts of infused CD34+ cells	3,65	2,0 – 12,5
days of G-CSF	10	7 – 14
day of engraftment	+11	+7 - +16
blood transfusions	2	0 – 6
platelet transfusions	6	0 – 18

Table 3. Graft characteristics

Febrile episodes	10
Central venous catheter infections	5
Mucositis	8
Pneumonia	2
Neutropenic enterocolitis	2

Table 4. Early post-transplant complications

group was 38 months (from 36 months to 127 months after transplantation). The superiority of autologous stem cell transplantation over conventional chemotherapy was first demonstrated by The Intergroupe Francophone du Myelome (IFM) (21). In multiple myeloma, the standard high-dose therapy is single agent Melphalan at a dosage of 200 mg/m^2. Attempts to improve this regimen with conventional drugs or total body irradiation have failed to improve the response rate but have increased both haematologic and non-haematologic toxicities. A synergistic effect between bortezomib and melphalan has been demonstrated. Allogeneic stem cell transplantation was introduced in the treatment of multiple myeloma 25 years ago, but the toxicity was very high, with a transplant related mortality in excess of 50% in studies including heavily pre-treated patients (22,23,24,25). Allogeneic SCT using conventional conditioning and HLA-matched sibling donors can result in long-term survival and may have a role in younger patients, but it is an option for only a very few selected patients. One of the main problems with allografting using conventional conditioning was the high transplant-related mortality (TRM). However, there is now evidence from both the EBMT and individual centre studies that this has improved in the last 10 years. The 2-year TRM has fallen from 46% before 1994 to 30% since 2000 (Russell et al, 2000; Gahrton et al, 2001). This may reflect transplantation earlier in the course of the disease, improved supportive care and/or careful patient selection. Several well-designed, non-randomised studies show little benefit from allografts in the progressive disease/relapse situation (Einsele et al, 2003; Kröger et al, 2004). Patients transplanted in first remission have a 60% chance of entering CR, and one-third of these patients are in a persistent molecular remission with a very low risk of relapse (Corradini et al, 2003). Therefore, allografts should be performed in first chemo-sensitive phase. The potential benefit of this outcome may justify the risks of allogeneic SCT in patients up to 50 years of age, particularly for patients early in their disease. Therefore, allogeneic stem cell transplantation should not be proposed to patients older than 50 years of age. The combination of high-dose chemotherapy and autologous stem cell transplantation is an effective strategy to treat multiple myeloma patients and appears superior to standard chemotherapy. Novel agents, such as lenalidomide, bortezomib, and other treatments, have improved the survival of these patients. Introducing these agents earlier in the course of the disease together with autologous stem cell transplantation will improve the duration of remission.

REFERENCES

1. Attal M, Harousseau JL, Stoppa AM, et al. A prospective randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. N Engl J Med. 1996;335:1844–1845.
2. Child JA, Morgan GJ, Davies FC, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med. 2003;348:1875–1883.
3. Palumbo A, Bringhen S, Petrucci MT, et al. Intermediate-dose melphalan improves survival of myeloma patients aged 50 to 70: results of a randomized controlled trial. Blood. 2004;104:3052–3057.
4. Fermand JP, Katsahian S, Divine M, et al. High-dose therapy and autologous blood stem-cell transplantation compared with conventional treatment in myeloma patients aged 55 to 65 years: long-term results of a randomized control trial from the Group Myelome-Autogreffe. J Clin Oncol. 2005;23:9227–9233.
5. Blade J, Rosinol L, Sureda A, et al. High-dose therapy intensification compared with continued standard chemotherapy in multiple myeloma patients responding to the initial chemotherapy: long-term results from a prospective randomized trial from the Spanish cooperative group PETHEMA. Blood. 2005;106:3755–3759.
6. Barlogie B, Kyle RA, Anderson KC, et al. Standard chemotherapy compared with high-dose chemoradiotherapy for multiple myeloma: final results of the phase III US Intergroup trial S4321. J Clin Oncol. 2006;24:929–936.
7. Moreau P, Facon T, Attal M, et al. Comparison of 200 mg/m^2 melphalan and 8Gy total body irradiation plus 140 mg/m^2 melphalan as conditioning regimens for peripheral blood stem cell transplantation in patients with newly diagnosed multiple myeloma. Final analysis of the IFM95-02 randomized trial. Blood 2002;99:731–735
8. Barlogie B, Jagannath S, Desikan KR, et al. Total therapy with tandem transplant for newly diagnosed multiple myeloma. Blood. 1999;93:55–65.
9. Attal M, Harousseau JL, Facon T, et al. Single versus double autologous stem cell transplantation for multiple myeloma. N Engl J Med. 2003;349:2495–2502.
10. Cavo M, Zamagni E, Cellini C, et al. Single versus tandem autologous transplants in multiple myeloma: Italian experience. J Clin Oncol 2007;25:2434–2441.
11. Fermand JP. Single versus double high-dose therapy supported with autologous blood stem cell transplantation using unselected or CD34 enriched ABSC: results of a two by two designed randomized trial in 230 young patients with multiple myeloma [abstract]. Hematol J. 2005;90:40.
12. Sonneveld P, Van Der Holt B, Sergeren CM, et al. Intensive versus double intensive therapy in untreated multiple myeloma. Updated analysis of the prospective phase III study Hovon 24-MM [abstract]. Hematol J. 2005;90:37–38.
13. Goldschmidt H. Single versus double high-dose therapy in multiple myeloma: second analysis of the trial GMMG-HD2 [abstract]. Hematol J. 2005;90:38.
14. Greipp PR, San Miguel JS, Durie BG, et al. International staging system for multiple myeloma. J Clin Oncol. 2005;23:3412–3420

15. Avet-Loiseau H, Attal M, Moreau P, et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myélome. Blood. 2007;109:3489–3495.
16. Shaughnessy JD, Zhan F, Burrington BE, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood. 2007;109:2276–2284.
17. Cavo M, Zamagni E, Tosi P, et al. Superiority of thalidomide and dexamethasone over vincristine-doxorubicin-dexamethasone as primary therapy in preparation for autologous transplantation for myeloma. Blood. 2005;106:35–39.
18. Macro M, Divine M, Uzunhan Y, et al. Dexamethasone plus Thalidomide compared to VAD as a pre-transplant treatment in newly diagnosed multiple myeloma [abstract]. Blood. 2006;108:Abstract no. 57.
19. Rajkumar SV, Blood E, Vesole D, et al. Phase III trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma. J Clin Oncol. 2006;24:431–436.
20. Goldschmidt H, Sonneveld P, Breitkreuz I, et al. Phase III study on the effect of thalidomide combined with high-dose melphalan in myeloma patients up to the age of 65 years [abstract]. Blood. 2005;106:Abstract no. 424.s
21. Harousseau JL, Attal M, Leleu X, et al. Bortezomib plus dexamethasone as induction treatment prior to autologous stem cell transplantation in patients with newly diagnosed multiple myeloma. Haematologica. 2006;91:1498–1505.
22. Crawley C, Iacobelli S, Björkstrand B, et al. Reduced-intensity conditioning for myeloma: lower nonrelapse mortality but higher relapse rates compared with myeloablative conditioning. Blood. 2007;109:3588–3594.
23. Badros A, Barlogie B, Siegel E, et al. Improved outcome of allogeneic transplantation in high-risk multiple myeloma patients after non-myeloablative conditioning. J Clin Oncol. 2002;20:1295–1303.
24. Kroger N, Schwerdtfeger R, Kiehl M, et al. Autologous stem cell transplantation followed by a dose-reduced allograft induces high complete remission rate in multiple myeloma. Blood. 2002;100:755–760.
25. Maloney D, Molina A, Sahebi F, et al. Allografting with nonmyeloablative conditioning following cytoreductive autografts for the treatment of patients with multiple myeloma. Blood. 2003;101:3447–3454.
26. Garban F, Attal M, Michallet M, et al. Prospective comparison of autologous stem cell transplantation followed by dose-reduced allograft (IFM 99-03 trial) with tandem autologous stem cell transplantation (IFM 99-04 trial) in high-risk de novo multiple myeloma. Blood. 2006;107:3474–3480.
27. Bruno B, Rotta M, Patriarca F, et al. A comparison of allografting with autografting for newly diagnosed myeloma. N Engl J Med. 2007;356:1110–1120.
28. 4. B G M Durie, J-L Harousseau, J S Miguel, at all. International uniform response criteria for multiple myeloma *Leukemia* (2006) 20, 1467–1473. doi: 10.1038/sj.leu.2404284

DEPRESSIVE SYMPTOMS IN MEDICAL STUDENTS

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SIMPTOMI DEPRESIJE KOD STUDENATA MEDICINE

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ABSTRACT

Mental disorders represent a growing problem in the student population. There has been a recent increase in the prevalence of depressive symptoms among medical students. The objective of this study was to determine the frequency of depressive symptoms in medical students to provide insight into this problem in our country . In total, 131 fourth-year medical students were included in the study. To determine rates of depressive symptoms in the student population, we used the BDI IA. Symptoms of depression were found in 26.7% of students. The most common levels of depression were mild and moderate. The mean value of the BDI scores was 7.51±7.62. The high level of depressive symptoms found in medical students highlights the need for more comprehensive insight and follow up of this problem in the student population.

Key words: *medical students, depressive symptoms, mental health*

SAŽETAK

Mentalne bolesti i poremećaji predstavljaju sve veći zdravstveni problem u studentskoj populaciji. Cilj ove studije je bio da utvrdimo učestalost depresivnosti kod studenata medicine u našoj zemlji i time damo bolji uvid u ovu problematiku kod nas. U studiji je učestvovao 131 student četvrte godine medicine. Za utvrđivanje depresivne simptomatologije kod studenata korišćena je Bekova skala za samoprocenu depresivnosti. Simptomi depresivnosti nađeni su kod 26.7% studenata. Najčešće se radilo o depresivnosti blagog i umerenog intenziteta. Srednja vrednost na BDI skali bila je 7.51±7.62. Visok nivo depresivnosti nađen kod studenata medicine upućuje na potrebu za sveobuhvatnijim sagledavanjem i praćenjem ove problematike u studentskoj populaciji.

Ključne reči: *studenti medicine, depresija, mentalno zdravlje*

INTRODUCTION

Previous studies have shown that mental disorders represent a growing problem in the student population (1-3). Medical education is often considered to be very stressful due to its duration and the nature of the topic studied (4). The frequency of depression in medical students is greater compared to that in the general population and varies from 10-25% according to various studies (5-10).

The epresence of depression in medical students is associated with a greater risk of suicide (11,12), increased use of benzodiazepines (13) and a poorer quality of life (14). The incidence Depression among students is accompanied by a decrease in academic performance, i.e., a lower average grades (15). Depressed medical students, especially those with a higher depression score, find that their opinion is less respected than the opinions of others and that they are viewed as less capable when compared to other students (12). Unrecognised and untreated depression during medical studies seems to have subsequent consequences, as indicated by a higher rate of depression and suicide among doctors compared to other professionals (16).

Despite the increased frequency and obvious consequences of various psychological problems, medical students are generally unwilling to seek adequate professional help. They would much rather seek self-treatment or ask for help from family members and friends (17,18). The reason for this behavior is that mental illness is regarded as a form of weakness and is associated with a potential negative influence on future career development (19). As a consequence, often neither mental healthcare providers nor members of the public have proper insight into the fre-

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quency of mental problems in students. In this study, we aimed to establish the frequency of depression in medical students in our country .

PATIENTS AND METHODS

Patients

This research was conducted as an observational cross-sectional study from May to June 2010. A total of 131 fourth-year students, representing more than half of all students (response rate = 54%) at the Medical Faculty in Kragujevac participated in the study. Students participated in the research voluntarily and anonymously.

Methods

To measure depression, we used Beck's Depression Inventory (BDI), a 21-item self-report questionnaire. The BDI is a well-known scale used for the self-assessment of depression in clinical and non-clinical populations (20). Currently, the BDI-II is most widely employed, but we used the BDI-IA. We did thisused this version because the results presented in this paper are a part of larger study that, began at a time during whichwhen the BDI-II version was not widely used and because this version is free. The BDI-II version was validated in 2011 on a Serbian student sample. A study showed that the psychometric characteristics of this version were in agreement with the literature (internal consistency was 0.87) (21). The cut-off points for the BDI were as follows: 0-9, no depression; 10-15, mild depression; 16-19, mild to moderate depression; 20-29, moderate to severe depression; and 30-63, severe depression (22). The various symptoms of depression can be grouped into a limited number of clusters. The structure of BDI consists of several factors (23,24,25). Some of these factors were established in the BDI-I . The first factor, the affective cluster (the sum of scores on items 1, 4, 10, 11, and 12 from the BDI), represents the core elements of depression and are represented by the following symptoms: 1, sadness; 4, dissatisfaction; 10, crying episodes; 11, irritability; and 12, social withdrawal. The second factor, the cognitive cluster (items 2, 3, 5, 6, 7, 8, 9, 13, 14, and 20), addresses the following cognitive symptoms: 2, pessimism; 3, sense of failure; 5, guilt; 6, expectation of punishment; 7, self-dislike; 8, self-accusation; 9, suicidal ideation; 13, indecisiveness; 14, change in body image; and 20, somatic preoccupation. The third factor, the somatic cluster (items 15, 16, 17, 18, 19, and 21), assesses the presence of the following symptoms: 15, slowness ; 16, insomnia; 17, fatigue; 18, change in appetite; 19, loss of weight; and 21, loss of sexual interest (25).

Statistical methods

The data are expressed as the mean±standard deviation and percentage (%). To establish the difference in the frequency of depression with respect to gender, the *Chi-squared* test was used. The *Mann-Whitney* test was used to determine differences in levels of depression between male and female students (the scores for the various items are not normally distributed in these groups). Analyses were performed with the *Statistical Package for the Social Sciences* (SPSS), version 13.0. P-values ≤ 0.05 were considered statistically significant.

RESULTS

In the examined student sample, 47.3% (62) of participants were male.

Symptoms of some type of depression were found in 26.7% (35) of the students (Table 1). Of the males, 25.8% (16) were depressed, and of the females, 27.5% (19) were depressed. The mean BDI score was 7.51±7.62 (Table 2). There was no statistically significant difference in the frequency (p=0.823) and level (p=0.921) of depression among male and female students.

Depression level (BDI)	% (N*)
No depression (0-9)	73.3% (96)
Mild depression (10-15)	14.5% (19)
Mild to moderate depression (16-19)	3% (4)
Moderate to severe depression (20-29)	7.6% (10)
Severe depression (30-63)	1.5% (2)

Table 1: Number of examinees by category on the BDI scale (*number of examinees)

	BDI
Men	8.16±9.17
Women	6.93±5.90
Total	7.51±7.62

Table 2: Mean scores on the BDI scale

Cluster	Score
Affective	2.56±2.67
Cognitive	2.66±3.46
Somatic	2.28±2.86
BDI total	7.51±7.62

Table 3: Cluster analysis of depressive symptoms

DISCUSSION

In our sample, the depression was identified in 26.7% of participating studentswho participated in the sample. This frequency is 2-3 times greater than that notedreported for in the general population (26). Studies in which the BDI was used to reportdetermine the prevalence of depression have reported a wide range of results (Table 3). In addition-Additionally, our findings waswere similar to the results of many studies in which the BDI or similar diagnostic in-

struments were used (5, 9, 10, 13, 27-31), including studies that have been carried out in the same region. A study of medical students in the Republic of Macedonia indicated that 10.4% of students had a BDI score greater than 17 (13). Twelve per cent of students in the present study had a BDI score greater than 16. In a study conducted in adolescents in Croatia, 9.7% of adolescents fulfilled the criteria for a moderate or severe depressive episode (32). In our sample, the prevalence was 9.1% of students.

Study	Cut-off	Frequency
Zoccolillo et al. (5)	BDI>9	22%
Mancevska et al. (13)	BDI≥17	10.4%
Tija et al. (9)	BDI≥8	15.2%
Clark et al. (10)	BDI>14	25%
Givens et al. (33)	BDI >8	24%
Hendryx et al (34)	BDI>9	19%
Leão PB (35)	BDI≥11	20%

Table 4: Findings of several studies examining depression in medical students using the 21-item BDI

The mean BDI score in this sample of medical students was similar to that noted in studies ofon medical students in Macedonia (8.3±7.4) (13) and heterogeneous student groups from Novi Sad, Serbia (32).

Regarding factor analysis of the scale, the scoring structure is variable among both different samples and similar samples . The affective, cognitive and somatic components are evident in various combinations in most studies (36). The cluster analysis in our study showed that every factor equallycontributed equally to the BDI score. A study that used the same cluster model in a population of medical student in Sao Paolo showed similar results. The authors collected BDI scores during the students' basic, intermediate and internship periods and found that the total BDI scores were highest during the internship period (11.7) and lowest during the intermediate period (7.0). The principal cluster responsible for the BDI score was the affective cluster (25). We analysed students in their fourth year, a year that represents the intermediate period of medical education. Analysing the characteristics of depressive symptoms among medical students is particularly relevant forto assisting medical professionals in addressing the different patterns of depression noted in this population and developing specific coping strategies.

No difference in the frequency or intensity of depression with regard to gender was established in this study sample. This finding is surprising in light of the fact that the frequency of depression in the general population is greater in women (37) and that similar results have beenthis gender difference has been reported in other studies involving medical students (3, 12, 38). However, gender differences appear during adolescence (39), and at that time, the differences are not yetoccursfixed, as they are later in life (40). Thus, we could also interpret our findings in that manner. Another implication of our finding is that the causes of depression in medical students are of such a nature that both men and women are equally sensitive to them. ThishasSimilar results have already been reported for medical students in other studies (9).

The limitations of our study includeing the following: self-assessment scales were used for establishing the frequency of depression and suicidal risk, the investigated sample was relatively small, and the number of measured variables was also small. Future research in a similar population that includes a larger number of variables should be conducted to replicate our results, especiallyparticularly the absence of a significant difference in scores between genders.

CONCLUSION

With a 26.7% prevalence of depression, which is more than twice as high as that in the general population, medical student represent a population that is vulnerable to developing depressive mood disorders. The results of our study emphasise the need to develop programs to support and assist students in their health environment, with the objective identifying depression early to prevent future consequences.

LITERATURE

1. Mowbray CT, Megivern D, Mandiberg JM et al. Campus mental health services: Recommendations for change. Am J Orthopsychiat 2006; 76: 226-37.
2. Anonimno. American College Health Association National College Health Assessment Spring 2006 Reference Group data report (abridged). J Am Coll Health. 2007; 55: 195-206.
3. Mackenzie S, Wiegel JR, Mundt M, Brown D, Saewyc E. Depression and suicide ideation among students accessing campus health care. Am J Orthopsychiatry 2011; 81: 101-7.
4. Firth-Cozens J. Medical student stress. Med Educ 2001; 35: 6-7.
5. Zoccolillo M, Murphy GE, Wetzel RD. Depression among medical students. J Affect Disord 1986; 11: 91-6.
6. Assadi SM, Nakhaei MR, Najafi F, Fazel S. Mental health in three generations of Iranian medical students and doctors A cross-sectional study Soc Psychiatry Psychiatr Epidemiol 2007; 42: 57-60.
7. Seliger K, Brähler E. Mental health of students of medicine-an empirical study. Psychotherapeut 2007; 52: 280–86.
8. Dahlin M, Joneborg N, Runeson B. Stress and depression among medical students: a cross-sectional study. Med Educ 2005; 39: 594-604.
9. Tjia J, Givens JL, Shea JA. Factors associated with undertreatment of medical student depression. J Am Coll Health 2005; 53: 219-24.

10. Clark DC, Zeldow PB. Vicissitudes of depressed mood during four years of medical school. JAMA 1988; 260: 2521-8.
11. Tyssen R, Vaglum P, Grønvold NT, Ekeberg O. Suicidal ideation among medical students and young physicians: a nationwide and prospective study of prevalence and predictors. J Affect Disord 2001; 64: 69-79.
12. Schwenk TL, Davis L, Wimsatt LA. Depression, stigma, and suicidal ideation in medical students. JAMA 2010; 304: 1181-90.
13. Mancevska S, Bozinovska L, Tecce J, Pluncevik-Gligoroska J, Sivevska-Smilevska E. Depression, anxiety and substance use in medical students in the Republic of Macedonia. Bratisl Lek Listy 2008; 109: 568-726.
14. Klemenc-Ketis Z, Kersnik J, Eder K, Colaric D. Factors associated with health-related quality of life among university students. Srp Arh Celok Lek 2011; 139: 197-202.
15. Hysenbegasi A, Hass SL, Rowland CR. The impact of depression on the academic productivity of university students. J Ment Health Policy Eco 2005; 8: 145-51
16. Hem E, Haldorsen T, Aasland OG, Tyssen R, Vaglum P, Ekeberg O. Suicide rates according to education with a particular focus on physicians in Norway 1960-2000. Psychol Med 2005; 35: 873-80.
17. Eisenberg D, Golberstein E, Gollust SE. Help-seeking and access to mental health care in a university student population. Med Care 2007; 45: 594-601.
18. Hooper C, Meakin R, Jones M. Where students go when they are ill: how medical students access health care. Med Educ 2005; 39: 588-93.
19. Chew-Graham CA, Rogers A, Yassin N. 'I wouldn't want it on my CV or their records': medical students' experiences of help-seeking for mental health problems. Med Educ 2003; 37: 873-80.
20. Beck AT, Ward C, Mendelson M. Beck Depression Inventory (BDI). Arch Gen Psychiatry 1961; 4: 561–71.
21. Novović Z, Mihić L, Tovilović S, Jovanović V, Biro M. Psychometric characteristics of the Beck depression inventory on a Serbian student sample. Psihologija. 2011; 44(3):225-243.
22. Timotijević I, Paunović VR. Instrumenti kliničke procene u psihijatriji. Beograd, Institut za mentalno zdravlje; 2003.
23. Beck AT, Steer RA, Brown GK. Beck Depreession Inventory. 2nd editon manual. San Antonio: The Psychological Corporation; 1996.
24. Ward LC. Comparison of factor structure models for the Beck Depression Inventory-II. Psychol Assess 2006; 18: 81-8.
25. Baldassin S, Alves TC, de Andrade AG, Nogueira Martins LA. The characteristics of depressive symptoms in medical students during medical education and training: a cross-sectional study. BMC Med Educ. 2008 Dec 11;8:60.
26. Copeland JR, Beekman AT, Dewey ME, et al. Depression in Europe. Geographical distribution among older people. *Br J Psychiatry* 1999; 174: 312-21.
27. Kongsomboon K. Psychological problems and overweight in medical students compared to students from Faculty of Humanities, Srinakharinwirot University, Thailand. J Med Assoc Thai 2010; 93: S106-13.
28. Ball S, Bax A. Self-care in medical education: effectiveness of health-habits interventions for first-year medical students. Acad Med 2002; 77: 911-17.
29. Ahmadi J, Kamel M, Ahmed MG, Bayoumi FA, Moneenum AA. Dubai Medical Collegestudents' scores on the Beck Depression Inventory. IRCMJ 2008; 10: 169-72.
30. Honney K, Buszewicz M, Coppola W, Griffin M. Comparison of levels of depression in medical and non-medical students. Clin Teach 2010; 7: 180-4.
31. Lewinsohn PM, Hops H, Roberts RE, Seeley JR, Andrews JA. Adolescent psychopathology: I. Prevalence and incidence of depression and other DSM-III-R disorders in high school students. J Abnorm Psychol 1993; 102: 133-44.
32. Degmecić D, Filaković P. Depression and suicidality in the adolescents in Osijek, Croatia. Coll Antropol. 2008; 32: 143-5.
33. Givens JL, Tjia J. Depressed medical students' use of mental health services and barriers to use. Acad Med. 2002; 77: 918-21.
34. Hendryx MS, Haviland MG, Shaw DG. Dimensions of alexithymia and their relationships to anxiety and depression. J Pers Assess. 1991 Apr;56(2):227-37.
35. Leão PB, Martins LA, Menezes PR, Bellodi PL. Well-being and help-seeking: an exploratory study among final-year medical students. Rev Assoc Med Bras 2011; 57: 379-86.
36. Vanheule S, Desmet M, Groenvynck H, Rosseel Y, Fontaine J. The factor structure of the Beck Depression Inventory-II: an evaluation. Assessment. 2008;15:177-87.
37. Blazer DG, Kessler RC, McGonagle KA, Swartz MS. The prevalence and distribution of major depression in a national community sample: the National Comorbidity Survey. Am J Psychiatry 1994; 151: 979-86.
38. Roh MS, Jeon HJ, Kim H, Han SK, Hahm BJ. The prevalence and impact of depression among medical students: a nationwide cross-sectional study in South Korea. Acad Med 2010; 85: 1384-90.
39. Ignjatović-Ristić D, Lazić LJ, Petrović D. Depressive disorder/symptoms in adolescence. World Journal of Biological Psychiatry 2001; 2 (suppl 1)
40. Hankin BL, Abramson LY, Moffitt TE, Silva PA, McGee R, Angell KE. Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study. J Abnorm Psychol 1998; 107: 128-40.

SERUM DEPRIVATION INDUCES APOPTOTIC CELL DEATH IN THE THESC CELL LINE

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SERUMSKA DEPRIVACIJA INDUKUJE ĆELIJSKU SMRT APOPTOZOM U THESC ĆELIJSKOJ LINIJI

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ABSTRACT

Uterine myomas are comprised of smooth muscle cells from blood vessel walls of the uterus and fibroblasts, and constitute the basic components of fibroids. The aim of our study was to evaluate the cytotoxic and apoptotic effects of serum deprivation on fibroblasts originating from the ThESC myoma cell line. Cell viability, morphological changes and the percentage of apoptotic cells were determined in the presence and absence of serum. The experimental group was cultured in medium lacking serum for 24- and 48-hour periods, and the control group was cultured in complete medium. Cell viability was evaluated using the MTT assay. Changes in cell morphology were investigated using native microscopy. The percentage of apoptotic cells was determined using ethidium bromide/acridine orange staining. There was a time-dependent and statistically significant decrease in cell viability in the experimental group when compared to the control group. Cells in the experimental group displayed morphological changes that are characteristic of apoptosis. These changes were not detected in the control group. In the experimental group, there was a statistically significant increase in the percentage of apoptotic cells, while this percentage was not statistically significant in the control group. The obtained results suggest that the time-duration of serum deprivation directly correlates to the induction of apoptosis in the ThESC cell line.

Key words: *fibroma, apoptosis, serum deprivation, viability*

SAŽETAK

Miomi uterusa izgrađeni su od glatkih mišićnih ćelija zidova krvnih sudova uterusa i fibroblasta koji sačinjavaju osnovnu komponentu fibroida. Cilj našeg istraživanja bilo je ispitivanje citotoksičnog i apototičnog efekta serumske deprivacije na fibroblaste koji vode poreklo iz mioma, ThESC ćelijska linija. Vijabilnost ćelija, morfološke promene kao i procenat apoptotičnih ćelija određivani su u prisustvu i odsustvu seruma. Eksperimentalna grupa ćelija bila je uzgajana u medijumu koji nije sadržao serum u toku 24 i 48 časova, dok je kontrolna grupa ćelija uzgajana u kompletnom medijumu. Vijabilnost ćelija bila je određivana pomoću MTT testa; morfološke promene detektovane su pomoću nativne mikroskopije dok je procenat apoptotičnih ćelija bio određen bojenjem pomoću etidijum bromid/akril oranžom. Primećeno je statistički značajano smanjenje vijabilnosit ćelija eksperimentalne grupe u poređenju sa kontronom grupom ćelija. Ćelije eksperimentalne grupe pokazivale su morfološke promene karakteristične za apotozu. Ove promene nisu detektovane u kontrolnoj grupi ćelija. Statistički značajno povećanje u procentu apoptotičnih ćelija primećeno je u eksperimentalnoj grupi, dok u kontrolnoj grupi procenat apoptotičnih ćelija nije ispoljavao statističku značajnost. Dobijeni rezultati ukazuju da je dužina serumske deprivacije u direktnoj korelaciji sa indukcijom apoptotične smrti u ThESC ćelijskoj liniji.

Ključne reči: *fibroidi, apoptoza, serumska deprivacija, vijabilnost*

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INTRODUCTION

Uterine fibroids (myomas) are the most common pelvic tumours in women and the most common indication for hysterectomy. They occur primarily during the reproductive years (1). Fibroids are benign tumours that develop within the walls of uterine blood vessels and consist of smooth muscle cells and fibrous connective tissue (2). Their size varies from tiny nodules of approximately 10 mm to large tumours of greater than 20 cm. Fibroids can be found protruding into the uterine cavity (submucosal), within the uterine wall (intramural), beneath the uterine serosa (subserosal), a nd, in rare instances, attached to abdominopelvic structures (parasitic) (3). Symptoms caused by fibroids include prolonged or heavy menstrual bleeding, pelvic pressure or pain, and, in rare cases, reproductive dysfunction (4). This is the most common gynaecological problem experienced by women, with clinical significance in 20–40% of women of childbearing age (2). Fibroid growth primarily depends on the hormone state of the organism. The ovarian steroid hormones oestrogen and progesterone stimulate fibroid growth (5). Other studies pinpoint diet, stress and ecological factors as significant in the aetiology of these tumours (6, 7). Fibroids are composed of smooth muscle cells and fibroblasts, which, according to some authors, originate from myometrial cells undergoing mitotic changes. As a result of these changes and myofibroblast formation, both oestrogen and progesterone have proliferation effects on these cells after binding to their receptors. The ThESC cell line is a human endometrium fibroblast–like cell (HESC) line obtained from an endometrial myoma that was immortalised with human telomerase reverse transcriptase (hTERT). Telomerase is an enzyme shown to confer unlimited replicative capacity to normal cells without causing the deregulation of normal growth control. The immortalised HESC line (ThESC) is karyotypically, morphologically, and phenotypically similar to the primary parent cells, and it is a powerful and consistent resource for in vitro research (8). The ThESC cell line is cultivated in basal growth medium supplemented with foetal bovine serum (FBS), which is also referred to as foetal calf serum (FCS). Serum provides a broad spectrum of macromolecules necessary for in vitro cell growth, such as glucose, albumin and other energy molecules. FBS is a very complex mixture of a large number of constituents, such as growth factors, hormones, lipoid substances, carrier proteins, and attachment and spreading factors (9). Compared to plasma, FBS shows a better mytogenic effect (stimulation of proliferation) because it contains growth factors referred to as insulin-like growth factor I (IGF-I) and insulin-like growth factor II (IGF-II) (10). Lipoids are essential for cell growth. Also present in FBS are cholesterol, fatty acids, phospholipids and triacylglycerols. The maintenance of normal processes involved in cell proliferation, maturation and differentiation requires the presence of hormones found in FBS, such as insulin, cortisone, triiodthyronine, thyroxine and PTH (9). Proteins found in serum, including vitronectin and fibronectin-like molecules, have important roles in cell attachment and cell adhesion (11). Iα-I is a serum protein that stabilises the cumulus extracellular matrix (12). Serum also provides beneficial factors to the culture environment, including energy substrates, vitamins, amino acids, and binding and transport proteins (13, 14). In addition to the above-mentioned functions, serum is important for the early expression of some genes encoding regulatory proteins, and serum components are known to activate protein kinase C (PKC) (15, 16). Serum also enables the maintenance of optimal pH and the elimination of the toxic products from the medium (9). Serum deprivation is one of many methods used to induce programmed cell death, or apoptosis (17, 18, 19). Previous research has demonstrated that cells in serum-deprived media undergo numerous changes that lead to apoptosis. After 2 hours of serum deprivation, some cultures show a retraction of the cytoskeleton, the rounding of cells, and the compaction of nuclear chromatin along the nuclear periphery (20). All of these morphological changes can be observed in cells in the very early stages of apoptosis. Serum depletion has a time-dependent effect on the number of apoptotic cells (20, 21). Morphological changes occur in the entire cell, and the dynamics of both protein synthesis and degradation change (22). As the protein dynamics change, so do the dynamics of DNA synthesis and degradation. Further serum deprivation results both in DNA fragmentation and slower DNA synthesis. Changes in the genetic material cause a decrease in the mitotic potential of serum-deprived cells. Short periods of 24 hours of serum deprivation significantly decrease the proportion of cells in mitosis, and after 48 h of serum deprivation, only 2% of cells are able to undergo mitosis (23). Prolonged treatment with serum-deprived medium induces massive DNA fragmentation in cells, which results in decreased viability in culture (24, 25, 26). All of the described changes that occur in the absence of serum are hallmarks of apoptosis. Prolonged serum deprivation finally results in programmed cell death. Apoptosis in treated samples is significantly higher when compared to control cells cultivated in serum-supplemented medium. The percentage of apoptotic cells depends on the cell culture, type of serum, and volume of serum in the medium. However, most studies indicate that apoptosis is enhanced 2 to 8 times in serum-deprived cells. After 24 h of treatment, the percentage of apoptosis is 5 times higher when compared to the number of spontaneous apoptotic cells in the control group, while the ratio in the cells treated for 48 hours is 8 times higher when compared to control cells. The data demonstrate that the percentage of apoptosis induced by serum deprivation is time-dependent. To date, studies have shown that serum deprivation induces apoptosis in a caspase-dependent manner (17, 18, 21, 26, 27).

MATERIALS AND METHODS

In our experiments, we used the ThESC cell line (ATCC®: CRL-4003tm) of human fibroblasts derived from a uterine myoma that have been immortalised with human telomerase reverse transcriptase (hTERT). The cells were cultured and maintained in DMEM complete growing medium containing 4.5 g/L of glucose, 2% L-glutamine (2 mM), 1% penicillin/streptomycin, 1% non-essential amino acids, 1% insulin transferrin supplement and 10% FBS in a controlled environment at 37°C and 5% CO_2. Cells were washed three times in 1xPBS before treatment. The cells were divided into two groups: the experimental group (FBS-deprived DMEM medium) and the control group (complete DMEM medium). The experimental group was treated with serum-deprived medium for 24 and 48 h and was then assayed using the MTT viability test and acridine orange/ethidium bromide staining. The MTT assay was used to determine the effect of serum starvation on the viability of experimental cells in comparison to the control cells. The percentage of viable cells in the experimental group was calculated in comparison to the viable cells in control group. To evaluate changes in morphology, cells were examined using phase-contrast microscopy following serum starvation for 24 and 48 h. To visualise early and late apoptotic changes, the staining procedure for both control and treated cells was performed using both 0.01 % acridine orange and ethidium bromide. The aim of our study was to investigate the effect of serum starvation on the viability of the human uterine myoma cells *in vitro* and its potential role in apoptosis.

RESULTS

We used the MTT assay to evaluate the effect of serum deprivation on the viability of ThESC cells. After treatment, the percentage of viable cells in the treatment group was calculated in comparison to the control group. Our results show that the serum-starved cells experienced a time-dependent and statistically significant decrease in viability when compared to the untreated cells. Furthermore, our results show a 9 times greater percentage of viable cells in the group of cells that were serum-starved for 24 h compared to the group that was serum-starved for 48 h (Figure 1.). This result correlates to the literature findings in which serum provides a broad spectrum of molecules necessary for the *in vitro* growth of cells (9).

1.

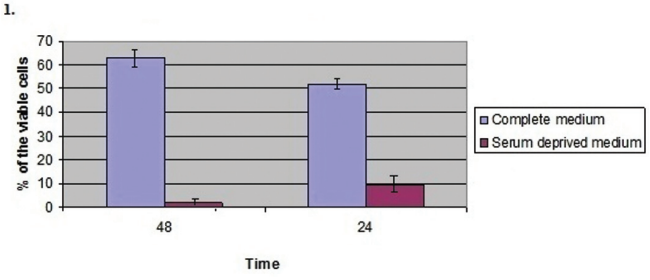

Figure 1. The percentage of viable ThESC cells after 24- and 48-h periods of serum starvation compared to the control cells.

Following the MTT assay, phase-contrast microscopy was performed to determine the morphological changes that occurred in serum-starved cells. Data presented in the literature demonstrates that serum is crucial for cell attachment and cell adhesion (11) and that the Iα-I protein present in serum stabilises the cumulus extracellular matrix (12). These data suggest that serum provides molecules that are essential for maintaining normal cell morphology. To compare our findings to the available literature data, the morphological changes of ThESC cells were evaluated after serum deprivation (Figure 2.). Our results show that the morphological changes after serum-deprivation corresponded to the literature results and our MTT assay results. Control cells maintained normal morphology, including a normal nuclear shape and the appearance of the cytoplasmic skeleton. Control cells exhibited normal and undisrupted continuity of all features, including the integrity of the cell membrane, actin filaments, and the shape of the nuclear membrane,. Following 24 h of serum deprivation, cell morphology was slightly altered when compared to the control cells. Both the shape of the nucleus and the continuity of the cytoskeleton were different in the absence of FBS in the growth medium (Figure 2.). Chromatin condensation and disruption in normal cell morphology were obvious. After 48 h of serum starvation, the cells exhibited distinct morphological changes that clearly affected the nucleus, including both chromosomal defragmentation and nuclear shrinkage. The changes in cell morphology corresponded to the time of serum deprivation. The described changes are clear indicators of on-going apoptotic changes in the serum-deprived cells (Figure 2.).

2.

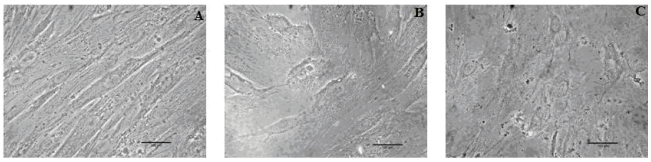

Figure 2. Morphological changes of ThESC cells following both 24- and 48-h periods of serum starvation. A. Normal morphology of control cells grown in complete medium. B. Partially disrupted morphology of cells grown in serum-deprived conditions after 24 hours. C. Completely disrupted cell morphology of the experimental group after 48 hours of serum deprivation.

We wanted to further analyse if the results obtained using the MTT assay and the observed morphological changes corresponded to the apoptotic changes as determined using ethidium bromide-acridine orange staining (Figure 3.). Ethidium bromide is used to identify cells that are in the final stages of apoptosis. To determine which of the treated cells exhibited early or late apoptotic changes, ethidium bromide-acridine orange staining was performed. The EB/AO combined stain causes live cells to fluoresce green, while apoptotic cells display distinctive red-orange fluorescence. The control cells (24 and 48 h) displayed clear green fluorescence, which confirmed their viability. However, treated cells displayed fluorescence that ranged from light orange (24 h) to intense red (48 h). These results correspond to the previously described early and late apoptotic changes (Figure 3.).

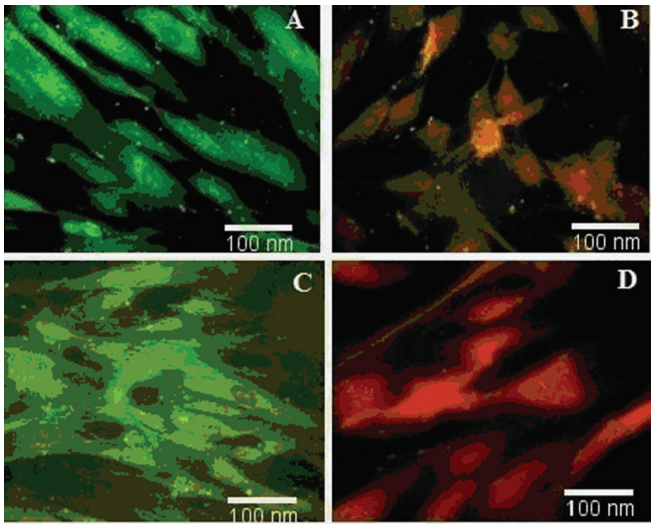

Figure 3. Morphological changes in ThESC cells were visualised using 0.01% acridine orange and ethidium bromide staining. A. Control cells grown in complete medium for 24 h. B. Morphological changes of cells after 24 h of serum starvation. The arrows indicate defragmented DNA and a complete loss of normal morphology. C. Control cells grown in complete medium for 48 h. D. Morphological changes of cells after 48 h of serum starvation, showing complete degradation of the nucleus. The observed morphological changes correspond to the typical changes for cells undergoing the late stages of apoptosis.

DISCUSSION

In the present study, we evaluated the effect of serum deprivation in a cultured human endometrium fibroblast-like cell line that was obtained from an endometrial myoma and immortalised with human telomerase reverse transcriptase. Serum provides viability for cells in culture due to the presence of tropic factors. In accordance with previous studies (12, 13, 20, 21, 23), our results showed a large increase in the number of apoptotic cells after serum deprivation for both 24 and 48 hours when compared to untreated cells that were grown in medium supplemented with FBS. First, we investigated the influence of serum starvation on cultured ThESC cells using the MTT assay. Our results clearly suggest that serum-starved cells displayed a decrease in viability compared to untreated cells. The decrease in viability of serum-starved cells occurred in a time-dependent manner. These findings correlate to those obtained in other studies (21, 28) and confirm our hypothesis that serum provides a broad spectrum of molecules necessary for the *in vitro* growth of cells. The decrease in the number of viable cells was much larger after 48 h. The number of the viable cells was reduced by five times after the first 24 hours and by nine times after the next 24 hours of treatment. This result suggests that cells undergo apoptosis more frequently after serum starvation for longer than 24 hours than when they are serum-starved for less than 24 hours. These results also indicate that approximately 10% of cells can overcome serum withdrawal for 24 hours but that a much smaller number of cells can overcome prolonged starvation. As trophic factor deficiency (29, 30, 31) is known to induce apoptosis, we analysed cell morphology using phase-contrast microscopy. The phase-contrast microscopy results correlated with literature data (12, 13, 20, 21, 23) and the results we obtained using the MTT assay. Images taken after 24 hours of serum starvation showed a significant number of deformed cells with membrane shrinkage and differences in chromatin condensation . The loss in the normal shape of the cells indicated that the cytoskeleton was also interrupted and that actin filaments had lost their structure and function. Some cells had almost normal shapes, which indicated that they still had not undergone apoptosis. Their nuclei were condensed, but mostly without shrinkage of the nuclear membrane. After 48 h of treatment, cells displayed a total loss of structure and membrane continuity. The chromatin was more condensed, and products of defragmentation were observed. Additionally, nuclei were smaller after 48 hr of treatment when compared tothan after 24 h of treatment. Cells did not have distinct shapes, which indicated a total loss of cytoskeletal structures. We concluded that after 24 h of serum starvation, cells lose their membrane integrity but mostly keep their nuclear shape, while after 48 hours of starvation, most cells lose both membrane and nuclear integrity. Our results correlate with the results of previous studies (20, 32). The MTT assay and phase-contrast microscopy demonstrated that ThESC cells undergo apoptosis after serum starvation. Our next step was to confirm the viability of these cells by using staining to differentiate viable cells from apoptotic cells. To confirm our hypothesis and visualise the early and late apoptotic changes, we used an acridine orange/ethidium bromide staining procedure. We used ethidium bromide/acridine orange viable staining, which causes live cells to fluoresce green and dead cells to fluoresce orange to red. The shade of red fluorescence depends on the apoptotic phase of the cell. Green fluorescence of control cells indicates that cells in the untreated control group are viable. Cells that had been treated for 24 h fluoresced light red, which indicated that these cells were in an early phase of apoptosis. Dark red fluorescence in cells that had been treated for 48 h indicated that these cells were in a late phase of apoptosis. The light green colour of control cells after 48 h can be explained by the fact that cells had already used most of the components of the medium and, as a result, their viability was decreased. This correlates to the intensity of fluorescence in apoptotic cells. This staining confirmed our conclusion that serum starvation induces apoptosis in ThESC cells. These results correlate to the data obtained using the MTT assay and phase-contrast microscopy. The serum deprivation of ThESC cells for various durations resulted in characteristics typical of apoptosis, such as chromatin condensation, chromosomal defragmentation, and nuclear shrinkage. Although DNA fragmentation has been considered a hallmark of apoptosis, there is a consensus on the dispensability of this characteristic in certain cell types under defined conditions (33, 34). Conceivably, the regulation and kinetics of DNA degradation in the fibroblast-like cell line may be different from that of thymocyte (35, 36) or lymphocyte (20, 37) models.

The serum-deprived cells also demonstrated several other characteristic morphological features of apoptotic cell death (20), including rounding, loss of cell-to-cell contact, cellular condensation, and the preservation of membrane integrity and organelle structures. Our findings are consistent with studies using other cell lines (20, 21, 28). However, unlike the study of G. V. Kulkarni and C. A. G. McCulloch, we detected DNA fragmentation. The previously described apoptotic changes correlate with the intensity of red-orange fluorescence observed by EB/AO staining. Our results agree with previous studies that suggest that serum starvation causes the release of cytochrome c from the mitochondria with a loss of mitochondrial membrane potential (38). Thus, our findings suggest a hypothesis by which serum deprivation results in the apoptotic death of ThESC cells through mitochondrial pathways. If our assumption is correct, the results would be consistent with previous studies demonstrating that the release of cytochrome c activates caspase-9, which then activates caspase-3 and leads to DNA fragmentation (39, 40, 41). This pathway represents the classic mitochondrial or intrinsic pathway to apoptosis. Similar results have been obtained in other studies (42, 43). Serum deprivation may also be associated with the activation of caspase-8, which represents a key player in the extrinsic or death receptor-mediated pathway of apoptosis (44). Although this and other similar factors have not yet been investigated in ThESC cells, the serum deprivation of ThESC cells could also result in the activation of the extrinsic pathway of apoptosis. Further studies are needed to delineate the exact mechanism and pathway of apoptosis in serum-starved ThESC cells.

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REFERENCES

1. Nowak RA. Novel therapeutic strategies for leiomyomas: targeting growth factors and their receptors. Environmental Health Perspectives. 2000; Volume 108, Supplement 5, pages 849-853
2. Strinic Tomislav et al. Uterine Artery Embolisation as Nonsurgical Treatment of Uterine Myomas. Obstet Gynecol. 2011; Article ID 489281, 4 pages doi:10.5402/2011/489281
3. Victor Gomel MD and Andrew I. Brill MD Reconstructive and Reproductive Surgery in Gynecology, First Edition. 2010; Elizabeth L. Taylor, Elizabeth A. Pritts, William H. Parker, and David L. Olive.; chapter 19, page 326 (doi: 10.3109/9781841847573)
4. Stewart EA. Uterine fibroids. Lancet. 2001; 357(9252):293-8.
5. Flake GP, Andersen J, Dixon D. Etiology and pathogenesis of uterine leiomyomas: a review. Environmental Health Perspectives. 2003; 111:1037-1054. http://dx.doi.org/10.1289/ehp.5787
6. Nowak RA Identification of new therapies for leiomyomas: what in vitro studies can tell us. Clin Obstet Gynecol. 2001; 44(2):327-34.
7. Shannon K. Laughlin, Jane C. Schroeder, Donna Day Baird. New Directions in the Epidemiology of Uterine Fibroids. Semin Reprod Med. 2010; 28(3):204-17
8. Krikun G. et al. A novel immortalized human endometrial stromal cell line with normal progestational response. Endocrinology, 2004; vol. 145 no. 5 2291-2296
9. Gerhard Gstraunthaler. Alternatives to the Use of Fetal Bovine Serum: Serum-free Cell Culture. Altex, 2003; volume 20, issue 4, pages 275-281
10. Annemarie Honegger and Rene E. Humbel. Insulin-like Growth Factors I and I1 in Fetal and Adult Bovine Serum. The Journal of Biological Chemistry, 1986; Vol. 261, No 2, pp. 569-575
11. Edward G. Hayman, Michael D. Pierschbacher, Shintaro Suzuki, Erkki Ruoslahti. Vitronectin—A major cell attachment-promoting protein in fetal bovine serum. Cell biology, 1983; Vol. 80, pp. 4003-4007
12. Lin Chen, Simon J. T. Mao, and William J. Larsen. Identification of a Factor in Fetal Bovine Serum That Stabilizes the Cumulus Extracellular Matrix. The Journal of Biological Chemistry, 1992; Vol. 267, No. 17, Issue of June 15, pp. 12380-12386
13. J.R. Dobrinsky, L.A. Johnson and D. Rath. Development of a Culture Medium (BECM-3) for Porcine Embryos: Effects of Bovine Serum Albumin and Fetal Bovine Serum on Embryo Development. *Biology of Reproduction.* 1996; **vol. 55** no. 5 1069-1074
14. Stephan J. Reshkin. et al. Phosphoinositide 3-Kinase Is Involved in the Tumor-specific Activation of Human Breast Cancer Cell Na/H Exchange, Motility, and Invasion Induced by Serum Deprivation. The Journal of Biological Chemistry. 2000; 25;275(8):5361-9.
15. Mara Fiorani, Orazio Cantoni, Andrea Tasinato, Daniel Boscoboinik, Angelo Azzi. Hydrogen peroxide-and fetal bovine serum-induced DNA synthesis in vascular smooth muscle cells: positive and negative regulation by protein kinase C isoforms. Biochim Biophys Acta. 1995; 19;1269(1):98-104.
16. Barbara A. Christy, Lester F. Lau, and Daniel Nathans. A gene activated in mouse 3T3 cells by serum growth factors encodes a protein with "zinc finger" sequences (transcription factors/serum response element). *PNAS.* 1988; **vol. 85** no. 21 7857-7861
17. Chang-Qing Zhao, Da Liu, Hai Li, Lei-Sheng Jiang and Li-Yang Dai. Interleukin-1β enhances the effect of serum deprivation on rat annular cell apoptosis. Apoptosis. 2007;12(12):2155-61.

18. Esther Potier, Elisabeth Ferreira, Alain Meunier, Laurent Sedel, Delphine Logeart-Avramoglou, and Hervé Petite. Prolonged Hypoxia Concomitant with Serum Deprivation Induces Massive Human Mesenchymal Stem Cell Death. Tissue Eng. 2007;13(6):1325-31.
19. Barbara Ahlemeyer, Anja Möwes, Josef Krieglstein. Inhibition of serum deprivation- and staurosporine-induced neuronal apoptosis by Ginkgo biloba extract and some of its constituents. Eur J Pharmacol. 1999; 19;367(2-3):423-30.
20. G. V. Kulkarni and C. A. G. McCulloch. Serum deprivation induces apoptotic cell death in a subset of Balb/c 3T3 fibroblasts. J Cell Sci. 1994;107(Pt 5):1169-79.
21. Weiquan Zhu, Jinghai Chen, Xiangfeng Cong, Shengshou Hu, Xi Chen. Hypoxia and Serum Deprivation-Induced Apoptosis in Mesenchymal Stem Cells. Stem Cells. 2006; 24(2):416-25.
22. Graciela Fuertes, Jos'e Javier Mart´in De Llano, Adoraci´on Villarroya, A. Jennifer Rivett and Erwin Knecht. Changes in the proteolytic activities of proteasomes and lysosomes in human fibroblasts produced by serum withdrawal, amino-acid deprivation and confluent conditions. Biochem J. 2003; 375(Pt 1): 75–86.
23. W.A. Kues, M. Anger, J.W. Carnwath, D.Paul, J.Motlik, and H. Niemann. Cell Cycle Synchronization of Porcine Fetal Fibroblasts: Effects of Serum Deprivation and Reversible Cell Cycle Inhibitors. Biology of Reproduction. 2000; vol. 62 no. 2 412-419
24. Rosario Maroto and J. Regino Perez-Polo. BCL-2-Related Protein Expression in Apoptosis: OxidativeStress Versus Serum Deprivation in PC12 Cells. J Neurochem. 1997; 69(2):514-23.
25. Hee-Yong Kim, Mohammed Akbar, Audrey Lau, and Lisa Edsall. Inhibition of Neuronal Apoptosis by Docosahexaenoic Acid (22:6n-3) Role Of Phosphatidylserine In Antiapoptotic Effect. J Biol Chem. 2000; 275(45):35215-23.
26. Hans-Peter Gerber. et al. Vascular Endothelial Growth Factor Regulates Endothelial Cell Survival through the Phosphatidylinositol 3-Kinase/Akt Signal Transduction Pathway. J Biol Chem. 1998; 273(46):30336-43.
27. Supriya Jayadev et al. Role For Ceramide In Cell Cycle Arrest. The Journal of Biological Chemistry. 1995; 270, 2047-2052.
28. Alicia A Goyeneche, Jacquelyn M Harmon and Carlos M Telleria. Cell death induced by serum deprivation in luteal cells involves the intrinsic pathway of apoptosis. Reproduction. 2006; 131(1):103-11.
29. Takamatsu Manabu, Fujita Tsunenori, and Hotta Hak. Suppression Of Serum Starvation-Induced Apoptosis By Hepatitis C Virus Core Protein. Kobe J. Med.Sci. 2001; 47,97/112
30. Kummer J. L., Rao P. K., and Heidenreich K. A. Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. Journal of Biological Chemistry. 1997; 272, 20490-20494.
31. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science. 1995; Vol. 270 no. 5240 pp. 1326-1331
32. Chantal J. Schamberger, Christopher Gerner, Christa Cerni. Caspase-9 plays a marginal role in serum starvation-induced apoptosis. Exp Cell Res. 2005; 302(1):115-28.
33. Cohen, G. M., Sun, X.-M., Snowden, R. T., Dinsdale, D. and Skilleter, D. N. Key morphological features of apoptosis may occur in the absence of internucleosmal DNA fragmentation. Biochem J. 1992; 286(Pt 2): 331–334.
34. Collins, R. J., Harmon, B. V., Gobe, G. C. and Kerr, J. F. Internucleosomal DNA cleavage should not be the sole criterion for identifying apoptosis. Int. J. Rad. Biol. 1992; Vol. 61, No. 4 , Pages 451-453
35. Cohen, J. J. and Duke, R. C. Glucocorticoid activation of calcium dependent endonuclease in thymocyte nuclei leads to cell death. J Immunol. 1984; 132(1):38-42.
36. Yonish-Rouach, E., Resnitzky, D., Lotem, J., Sachs, L., Kimchi, A. and Oren, M. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature. 1991; 352(6333):345-7.
37. Colotta, F., Polentarutti, N., Sironi, M. and Mantovani, A. Expression and involvement of c-fos and c-jun protooncogenes in programmed cell death induced by growth factor deprivation in lymphoid cell lines. J Biol Chem. 1992; 267(26):18278-83.
38. Irma Charles. et al. Serum Deprivation Induces Apoptotic Cell Death of Transformed Rat Retinal Ganglion Cells via Mitochondrial Signaling Pathways. Invest Ophthalmol Vis Sci. 2005; 46(4):1330-8.
39. Li Y, Schlamp CL, Nickells RW. Experimental induction of retinal ganglion cell death in adult mice. Invest Ophthalmol Vis Sci. 1999; 40(5):1004-8.
40. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell. 1996; 86(1):147-57.
41. Liu X, Zou H, Slaughter C, Wang X. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. Cell. 1997; 89(2):175-84.
42. Xinjianp Peng, Takeshmi Aruo, Hiroyam Atsuo, Shigekti Akekida, and Jun Deguchi. Serum Deprivation-Induced Apoptosis in Cultured Porcine Granulosa Cells Is Characterized by Increased Expression of p53 Protein, Fas Antigen and Fas Ligand and by Decreased Expression of PCNA. Endocr J. 1998; 45(2):247-53.
43. Gustincich S. and Schneider C. Serum deprivation response gene is induced by serum starvation but not by contact inhibition. Cell Growth Differ. 1993; 4(9):753-60.
44. Zhang B, Hirahashi J, Cullere X, Mayadas TN. Elucidation of molecular events leading to neutrophil apoptosis following phagocytosis: cross talk between caspase 8, reactive oxygen spe cies, and MAPK/ERK activation. J Biol Chem. 2003; 278(31):28443-54.

SUDDEN CARDIAC DEATH IN HAEMODIALYSIS PATIENTS - ASSESSMENT OF RISK FACTORS AND PREVENTION

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IZNENADNA SRČANA SMRT BOLESNIKA NA HEMODIJALIZI: PROCENA RIZIKA I PREVENCIJA

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ABSTRACT

Haemodialysis patients are at high risk for sudden cardiac death (SCD), with the annual mortality rate from SCD ranging from 5-7%. The risk factors for SCD on haemodialysis include left ventricular remodelling, coronary disease, heart failure, rapid electrolyte and fluid shifts during dialysis sessions and secondary hyperparathyroidism. Echocardiography and standard electrocardiography can help identify patients at risk for SCD. The strategy for preventing SCD in haemodialysis patients includes primary (medicamentous treatment and adjusting dialysis parameters) and secondary (coronary revascularisation and placement of cardioverter defibrillator) treatment planning.

Key words: *sudden cardiac death, haemodialysis, treatment plan*

SAŽETAK

Bolesnici koji se leče ponavljanim hemodijalizama imaju visok rizik od iznenadne srčane smrti. Jednogodišnja stopa iznenadne srčane smrti kod ovih bolesnika iznosi 5-7%. U faktore rizika za nastanak iznenadne srčane smrti spadaju: preoblikovanje leve komore srca, koronarna arterijska bolest, srčana slabost, brza izmena elektrolita i tečnosti u toku seanse hemodijalize, i sekundarni hiperparatireoidizam. Ehokardiografija i standardna elektrokardiografija omogućavaju izdvajanje bolesnika koji imaju povećan rizik od iznenadne srčane smrti. Strategija za sprečavanje nastanka iznenadne srčane smrti kod bolesnika koji se leče ponavljanim hemodijalizama sastoji se u primeni primarnog i sekundarnog plana lečenja. Primarni plan lečenja uključuje medikamente i prilagođavanje uslova hemodijalize bolesniku, dok sekundarni plan lečenja uključuje revaskularizaciju koronarnih arterija i ugradnju implantabilnog kardioverter defibrilatora.

Ključne reči: *iznenadna srčana smrt, faktori rizika, hemodijaliza, plan lečenja*

INTRODUCTION

Sudden cardiac death (SCD) is unexpected death due to cardiac causes occurring within one hour of symptom onset (1). Although the most frequent cause of SCD is coronary artery disease, other contributing factors in maintenance haemodialysis (HD) patients include left ventricular remodelling, volume overload, disturbed electrolyte balance, rapid electrolyte and fluid shifts and secondary hyperparathyroidism (1, 2). The annual mortality rate from SCD in HD patients is 5-7% (2).

Aetiopathogenesis of sudden cardiac death

Risk factors and cardiovascular diseases

Uraemia in general and HD in particular carry numerous risk factors for the development of cardiovascular disease. These include high arterial blood pressure, multiple lipid abnormalities, oxidative stress, microinflammation, hyperhomocysteinaemia, anaemia, high shunt blood flow and secondary hyperparathyroidism (3-5). All these factors are responsible for the development of atherosclerosis, arteriosclerosis and cardiomyopathy (5).

Atherosclerosis occurs when fatty plaques build up in the intimal layer of arterial walls, narrowing the lumen of the vessel. Atherosclerosis of the coronary arteries causes ischaemic heart disease (6). Arteriosclerosis is a condition in which the artery wall thickens due to media thickening, calcification and remodelling of the smooth muscle cells of the tunica media into osteoblasts-like cells. Hardening of the arterial walls in arteriosclerosis decreases their elasticity and causes pressure overload to the left ventricle (3-7). Cardiomyopathy occurs due to left ventricular pressure and/or volume overload, which causes left ventricular wall thickening, decreased myocardial vascular-

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sation, myocardial scarring and alterations of the small blood vessels in the myocardial interstitium. Clinical manifestations of cardiomyopathy include heart failure, myocardial hypersensitivity to ischaemia, prolonged ventricular repolarisation (prolonged QT interval), increased risk for ventricular arrhythmias and SCD (5, 8, 9). Left ventricular hypertrophy, coronary artery disease and disturbed systolic function of the left ventricle (left ventricular ejection fraction < 35%) represent major risk factors for SCD in maintenance HD patients (5, 8, 9).

Risk factors for sudden cardiac death related to haemodialysis

Rapid electrolyte shift

Haemodialysis *per se* is a risk factor for arrhythmias due to rapid the electrolyte shifts and cardiovascular instability (intradialytic hypotension) that can occur with this treatment (10-13). Rapid potassium and calcium shifts during HD sessions and low levels of electrolytes in the dialysate increase the risk for cardiac arrhythmia (10-13). The use of potassium-free or low-potassium dialysates ($[K^+]d$ = 1.0 mmol/L), particularly at the beginning of a dialysis session, removes potassium quickly, causing a rapid decline in the patient's serum potassium concentration, which may lead to a disturbance of ventricular myocardial repolarisation, an increase in the corrected QTc interval (QTd) dispersion and increased risk for ventricular arrhythmias (10-13).

Calcium ions, however, play an important role in myocardial and arterial contractility. The use of low-calcium ($[Ca^{2+}]$ = 1.25 mmol/L) dialysates decreases ionised serum calcium and prolongs and increases the dispersion of the QTc interval, thus contributing to ventricular arrhythmias and SCD (10-13).

Educating patients to maintain an optimum predialysis potassium level of 4.6-5.3 mmol/L and individual adjustment of dialysis parameters decreases the risk of SCD death in HD patients (13).

Rapid fluid shifts (haemodynamic instability)

Dialysis-induced hypotension is represented by a sudden drop of systolic blood pressure below 90 mmHg or of at least 30 mmHg during HD (13, 14). Acute intradialytic hypotension reduces coronary blood flow and may lead to myocardial ischaemia, disturbances of regional myocardial motility and ventricular arrhythmia even in the absence of coronary heart disease (15). Haemodynamic instability during HD sessions can be prevented by the early identification of patients at higher risk for hypotension and by the individual adjustment of dialysis parameters. Patients at risk are those with malnutrition and hypoalbuminaemia, left ventricular hypertrophy, diabetes and diseases of the autonomous nervous system. Individualised dialysis strategies to obtain haemodynamic stability include the adequate choice of dialysers, adjusting the dialysate temperature, the use of sodium-modelling profiles, the careful adjustment of ultrafiltration and sequential dialysis (13-15).

Interdialytic restriction of fluid and dietary sodium intake (recommended maximum intake 2.0 g/24 h) and an interdialytic weight gain of less than 4% of the dry body weight decrease left ventricular overload, haemodynamic stress and the risk for ventricular arrhythmias (16).

Identifying patients at risk for sudden cardiac death

All HD patients have increased risk for SCD (17-19). The plan for the early detection of patients at risk for SCD should include the following: clinical assessment of the patient (anamnesis and physical examination), echocardiographic examination (measuring QT/QTc interval, measurement of QTc interval dispersion, variation of T wave amplitude and duration), measuring heart rate variability (HRV) and determining serum troponin T and troponin I concentrations (20-24).

The QT interval is easily measured and enables the identification of patients at higher risk for SCD. The QT interval is a measure of the time between the start of the Q wave and the end of the T wave on an electrocardiogram (ECG). It represents the time required for the completion of both ventricular depolarisation and repolarisation. The standard clinical correction is to use Bazett's formula to calculate the heart rate-corrected QT interval. Bazett's formula is $QTc=QT/\sqrt{RR}$, where RR is the interval from the onset of one QRS complex to the onset of the next QRS complex, measured in milliseconds. In HD patients, a QTc interval longer than 500 ms suggests an increased risk for ventricular dysrhythmia (24, 25). Furthermore, increased QTc dispersion, as measured on standard ECG, is a useful and reliable predictor of susceptibility to life-threatening ventricular arrhythmias in HD patients. QTc interval variability is the difference between the longest and the shortest QTc interval registered on a standard ECG (25, 26); normal QTc variability is 40 - 50 ms. QTc variability over 50 ms in HD patients suggests higher risk for ventricular arrhythmias and SCD (26).

Heart rate variability is an electrocardiographic parameter of sympathetic and parasympathetic control of the heart rate. A decrease or loss of HRV is associated with increased risk for ventricular arrhythmias and SCD (27, 28). Drugs that induce parasympathetic activity also decrease the rate of SCD in HD patients. ACE inhibitors and beta-blockers enhance the parasympathetic influence on the heart, increase HRV and decrease the risk for SCD in HD patients (27, 28). Nocturnal HD, performed for 8 to 10 hours, 6 times/week, decreases sympathetic activity and increases HRV and the partial pressure of arterial blood overnight compared to the standard HD scheme of 3 times for 4 hours each week (20).

Treatment plan

The plan to reduce the risk for SCD in maintenance HD patients includes primary and secondary measures (29-32). The primary plan requires medicamentous therapy and

individual adjustment of HD parameters, while secondary intervention includes coronary revascularisation and the placement of an implantable cardioverter defibrillator (ICD) (29-32).

Primary treatment plan

Medicamentous therapy

Medicamentous therapy to control risk factors for SCD in maintenance HD patients includes beta-blockers, angiotensin convertase inhibitors, angiotensin receptor blockers, vitamin D active metabolites, calcimimetics and erythropoietin. Beta-blockers decrease the risk for SCD in HD patients by decreasing myocardial sympathetic activity, lowering the rate of ventricular arrhythmias and increasing HRV and baroreceptor sensitivity (28-33). Carvedilol improves left ventricular ejection fraction and survival in heart failure patients with NYHA II and NYHA III on maintenance HD (34, 35). Other treatments are focused on increasing HRV (beta-blockers, angiotensin convertase inhibitors), stabilising arteriosclerotic plaques (anti-aggregation agents and statins) and controlling left ventricular hypertrophy (beta-blockers, angiotensin convertase blockers and erythropoietin) (28-33).

Haemodialysis treatment

A biocompatible high-flux membrane, ultrapure dialysate (bacteria < 0.1 **CFU**/mL and endotoxin < 0.03 **EU**/mL) and an optimal dialysis dose (Kt/V ≥ 1.4) significantly contribute to lowering the mortality rate in HD patients (34). Haemodiafiltration (HDF) achieves optimal transfer for both small (< 5 kDa) and mid-size (5-50 kDa) molecules, including mediators of inflammation, complement factor D and other molecules involved in the development of heart disease (36). The optimal treatment with standard HD or HDF (≥ 3 times per week for ≥ 4 h), with blood flow ≥ 300 mL/min, adequate dietary intake, interdialysis weight gain < 4% and adequate medicamentous treatment (phosphate binders, vitamin D active metabolites, calcimimetics, iron supplements and erythropoietin), should provide a Kt/V ≥ 1.4, mean arterial pressure ≤ 105 mmHg, predialysis serum potassium concentration of 4.6-5.3 mmol/L, serum phosphate concentration of 0.8-1.6 mmol/L, serum calcium concentration of 2.2-2.5 mmol/L, solubility product ≤ 4.4 $mmol^2/L^2$, parathormone levels of 150-300 pg/mL, bicarbonate level ≥ 20 mmol/L, transferrin saturation of 30-50%, serum ferritin concentration of 200-500 ng/mL, haemoglobin concentration of 100-120 g/L and serum albumin concentration ≥ 35 g/L (37).

In patients with disturbed left ventricular systolic function, hypotension and haemodynamic instability limit the achievement of optimal ultrafiltration during an HD session. These patients are candidates for peritoneal dialysis because it provides better haemodynamic stability (38). The treatment plan for haemodynamically unstable patients should also include some of the new HD modalities, such as short daily haemodialysis (SDHD) or nocturnal home haemodialysis (NHD). SDHD is performed 5 to 6 days per week for 1.5-2 hours, whereas NHD is performed while sleeping, usually for 6-8 hours, 4-6 nights per week (38).

Secondary treatment plan

Coronary revascularisation

According to coronarographic findings, HD patients are at either high risk (coronary disease of the main left ventricular artery; three-vessel coronary disease, mild symptoms and decreased left ventricular function; two-vessel disease involving the proximal left coronary artery) or low risk for acute coronary syndrome (single-vessel coronary disease - only one vessel involved and preserved myocardial function) (6, 39, 40). Coronary artery bypass grafting (CABG) decreases the risk of acute coronary syndrome in the high-risk group, whereas patients at low risk are usually treated with drugs, percutaneous transluminal angioplasty (PTCA) or coronary artery stenting (CAS) (6, 39, 40). HD patients have a significantly higher in-hospital mortality rate in the first 30 days following CABG and are at significantly higher risk for postoperative complications (sepsis, respiratory insufficiency and gastrointestinal complications) compared to HD patients after CAS and non-renal patients (41, 42). More studies are needed to determine the criteria for selecting HD patients and adequate coronary revascularisation procedures (41, 42).

Implantable cardioverter defibrillator

ICDs decrease mortality risk by 42% in HD patients surviving cardiac arrest (19, 23, 43). However, only 8% of maintenance HD patients who survive cardiac arrest actually have preventively placed ICDs (19, 23, 42). ICD implantation is associated with a high risk of bleeding, infection and venous subocclusion and occlusion. Therefore, it is typically placed on the side opposite to the vascular access for HD (43). ICDs are placed in HD patients with a left ventricular ejection fraction < 35% following cardiac arrest caused by ventricular dysrhythmias (ventricular tachycardia or fibrillation). More clinical studies are needed to define the criteria for choosing patients for this procedure and to assess its validity, cost-effectiveness and budget impact in a manner similar to other health technologies (43-47).

CASE REPORT

Patient CR, born in 1955, started regular haemodialysis therapy in 1994. (*Insuffitientia renalis chronica terminalis, Status post-nephrectomiam lat.dex. pp calculosis, Haemodialysis chronica regularis*). As a part of uncontrolled secondary hyperparathyroidism (*Secondary hyperparathyroidism, Osteodystrophia chronica renalis, iPTH = 1500 pg/ml),* the patient developed significant vascular (coronary artery) and

valvular calcification, which called for triple aorto-coronary *by-pass* and implantation of a synthetic mitral valve (*Status post-IM inferolateralis aa III, Status post-by-pass AC triplex et implantationem valvulae mitralis aa III*). The patient received regular haemodialysis therapy, erythropoietin, statins and anti-aggregation and anticoagulant therapy with adequate laboratory monitoring. Sudden and rapid deterioration of chronic heart insufficiency (*Myocardiopathia chronica ischaemica decompensata*) and the development of cardiogenic shock (*Shock cardiogenes*) resulted in the patient's death (*Exitus letalis*).

CONCLUSION

Early identification of maintenance HD patients at risk of ventricular dysrhythmias and SCD enables timely and adequate treatment to decrease the risk for SCD mortality and improve survival.

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REFERENCES

1. Green D, Roberts PR. Ventricular arrhythmias and sudden death in patients with chronic kidney disease. J Ren Care 2010; 36(Suppl 1): 54-60.
2. Herzog CA, Mangrum M, Passman R. Sudden Cardiac Death and Dialysis Patients. Semin Dial 2008; 21(4): 300-7.
3. Petrović D, Jagić N, Miloradović V, Stojimirović B. Non-tradicional risk factors for development of cardiovascular complications in haemodialysis patients. Ser J Exp Clin Res 2009; 10(3): 95-102. (in Serbian)
4. Chue CD, Townend JN, Steeds RP, Ferro CJ. Arterial stiffness in chronic kidney disease: causes and consequences. Heart 2010; 96(11): 817-23.
5. Shamseddin MK, Parfrey PS. Sudden cardiac death in chronic kidney disease: epidemiology and prevention. Nat Rev Nephrol 2011; 7(3): 145-54.
6. Petrović D, Miloradović V, Poskurica M, Stojimirović B. Diagnostics and treatment of ishemic heart disease in hemodialysis patients. Vojnosanit Pregl 2009; 66(11): 897-903. (in Serbian)
7. Petrović D, Stojimirović B. Secondary hyperparathyroidism - risk factor for development of cardiovascular complications in patients on hemodialysis. Med Pregl 2010; LXIII(9-10): 674-80. (in Serbian)
8. Petrović D, Stojimirović B. Left ventricular hypertrophy in hemodialysis patients. Med Pregl 2008; LXI(7-8): 369-74. (in Serbian)
9. Petrović D, Miloradović V, Poskurica M, Stojimirović B. Heart failure in haemodialysis patients: evaluation and treatment. Srp Arh Celok Lek 2011; 139(3-4): 248-55. (in Serbian)
10. Beumi M, Coppolino G, Bolignano D, Sturiale A, Campo S, Buemi A, et al. Arrhythmias and Hemodialysis: Role of Potassium and New Diagnostic Tools. Ren Fail 2009; 31(1): 75-80.
11. Severi S, Grandi E, Pes C, Badiali F, Grandi F, Santoro A. Calcium and potassium changes during haemodialysis alter ventricular repolarization: in vivo and in silico analysis. Nephrol Dial Transplant 2008; 23(4): 1378-86.
12. Genovesi S, Dossi C, ViganoMR, Galbiati E, Prolo F, Stella A, et al. Electrolyte concentration during haemodialysis and QT interval prolongation in uraemic patients. Europace 2008; 10(6): 771-7.
13. Pun PH, Lehrich RW, Honecycutt EF, Herzog CA, Middleton JP. Modifiable risk factors associated with sudden cardiac arrest within hemodialysis clinics. Kidney Int 2011; 79(2): 218-27.
14. Santoro A. Cardiovascular dialysis instability and convective therapies. Hemodialysis Int 2006; 10(1): 51-5.
15. Burton JO, Korsheed S, Grundy BJ, McIntyre CW. Hemodialysis-Induced Left Ventricular Dysfunction Is Associated with an Increase in Ventricular Arrhythmias. Ren Fail 2008; 30(7): 701-9.
16. Lopez-Gomez JM, Villaverde M, Jofre R, et al. Interdialytic weight gain as a marker of blood pressure, nutrition, and survival in hemodialysis patients. Kidney Int 2005; 67(Suppl 93): 63-8.
17. Petrović D, Stojimirović B. Cardiovascular morbidity and mortality in hemodialysis patients - epidemiological analysis. Vojnosanit Pregl 2008; 65(12): 893-900. (in Serbian)
18. Petrović D, Obrenović R, Trbojević-Stanković J, Majkić-Singh N, Stojimirović B. Cardiovascular mortality in hemodialysis patients: clinical and epidemiological analysis. J Med Biochem 2011; 30(4): 302-8. (in Serbian)
19. Petrović D, Obrenović R, Trbojević-Stanković J, Majkić-Singh N, Stojimirović B. Hyperphosphatemia - the risk factor dor adverse outcome in maintenance hemodialysis patients. J Med Biochem 2012; 31(3): 239-45. (in Serbian)
20. Kanabay M, Afsar B, Goldsmith D, Covic A. Sudden Death in Hemodialysis: An Update. Blood Purif 2010; 30(2): 135-45.
21. Saravanan P, Davidson NC. Risk Assessment for Sudden Cardiac Death in Dialysis Patients. Circ Arrhythm Electrophysiol 2010; 3(5): 553-9.
22. Petrović D, Jagić N, Miloradović V, Stojimirović B. Clinical importance of biochemical markers of cardiac damage in hemodialysis patients. Ser J Exp Clin Res 2008; 9(1): 5-8. (in Serbian)
23. Petrović D, Stojimirović B. Cardiac troponins: outcome predictors in hemodialysis patients. J Artif Organs 2009; 12(4): 258-63.
24. Green D, Roberts PR, New DI, Kalra PA. Sudden Cardiac Death in Hemodialysis Patients: An In-Depth Review. Am J Kidney Dis 2011; 57(6): 921-9.

25. Gussak I, Gussak HM. Sudden cardiac death in nephrology: focus on acquired long QT syndrome. Nephrol Dial Transplant 2007; 22(1): 12-4.
26. Wu V-C, Lin L-Y, Wu K-D. QT interval dispersion in dialysis patients. Nephrology 2005; 10(2): 109-12.
27. Ranpuria R, Hall M, Chan CT, Unruh M. Heart rate variability (HRV) in kidney failure: measurement and consequence of reduced HRV. Nephrol Dial Transplant 2008; 23(2): 444-9.
28. Chan CT, Levin NW, Chertow GM, Larive B, Schulman G, Kotanko P, and the Frequent Hemodialysis Network Daily Trial Group. Determinants of Cardiac Autonomic Dysfunction in ESRD. Clin J Am Soc Nephrol 2010; 5(10): 1821-7.
29. Furgeson SB, Chonchol M. β-Blockade in Chronic Dialysis Patients. Semin Dial 2008; 21(1): 43-8.
30. De Bie MK, van Dam B, Gaasbeek A, van Buren M, van Erven L, Bax JJ, et al. The current status of interventions aiming at reducing sudden cardiac death in dialysis patients. Eur Heart J 2009; 30(13): 1559-64.
31. Petrović D, Tirmenštajn-Janković B, Živanović M, Nikolić A, Poskurica M, Stojimirović B. Sudden cardiac death in patients on regular hemodialysis. Timoč Med Glas 2010; 35(1-2): 19-26. (in Serbian)
32. McCullough PA, Sandberg KR. Chronic Kidney Disease and Sudden Death: Strategies for Prevention. Blood Purif 2004; 22(1): 136-42.
33. Massy ZA, Kasiske BL. Prevention of cardiovascular complications in chronic renal disease. In: Cardiovascular Disease in End-stage Renal Failure. Loscalzo J, London GM, (eds). New York: The Oxford University Press; 2000: 463-81.
34. Cice G, Ferrara L, Benedetto AD, Russo PE, Marinelli G, Pavese F, et al. Dilated Cardiomyopathy in Dialysis-Benefical Effects of Carvedilol: A Double-Blind, Placebo-Controlled Trial. J Am Coll Cardiol 2001; 37(2): 407-11.
35. Cice G, Ferrara L, D'Andrea A, D'Isa S, Benedetto AD, Cittadini A, Russo PE, et al. Carvedilol Increases Two-Year Survival in Dialysis Patients With Dilated Cardiomyopathy. J Am Coll Cardiol 2003; 41(9): 1438-44.
36. Herzog CA. Can We Prevent Sudden cardiac Death in Dialysis Patients? Clin J Am Soc Nephrol 2007; 2(3): 410-2.
37. Karkar A. Caring for Patients with CRF: Rewards and Benefits. Int J Nephrol 2011: ID 639840.
38. Sood MM, Pauly RP, Rigatto C, Komenda P. Left ventricular dysfunction in the haemodialysis population. NDT Plus 2008; 1(4): 199-205.
39. Williams ME. Coronary Revascularisation in Diabetic Chronic Kidney Disease/End-Stage Renal Disease: A Nephrologist's perspective. Clin J Am Soc Nephrol 2006; 1(2): 209-20.
40. Johnston N, Dargie H, Jardine A. Diagnosis and treatment of coronary artery disease in patients with chronic kidney disease. Heart 2008; 94(8): 1080-8.
41. Rahmanian PB, Adams DH, Castillo JG, Vassalotti J, Filsoufi F. Early and late outcome of cardiac surgery in dialysis-dependent patients: Single-center experience with 245 consecutive patients. J Thorac Cardiovasc Surg 2008; 135(4): 915-22.
42. Nevis IF, Mathew A, Novick RJ, Parikh CR, Devereaux PJ, Natarajan MK, Iansavichus AV, et al. Optimal Method of Coronary Revascularisation in Patients Receiving Dialysis: Systematic Review. Clin J Am Soc Nephrol 2009; 4(2): 369-78.
43. Ito I, Kono K, Shinbo G, Tadokoro K, Abe C, Takemura N, et al. Implantable cardioverter defibrillator in maintenance hemodialysis patients with ventricular tachyarrhythmias: A single-center experience. Hemodialysis Int 2009; 13(1): 48-54.
44. Korantzopoulos P, Liu T, Li L, Goudevenos JA, Li G. Implantable cardioverter defibrillator therapy in chronic kidney disease: a meta-analysis. Europace 2009; 11(11): 1469-75.
45. Roberts PR, Green D. Arrhythmias in chronic kidney disease. Heart 2011; 97(9): 766-73.
46. Kanbay M, Solak Y, Covic A, Goldsmith D. Sudden Cardiac Death in Patients with Chronic Kidney Disease: Prevention Is the sine qua non. Kidney Blood Press Res 2011; 34(4): 269-76.
47. Janković SM. Rituximab for the treatment of rheumatiod arthritis patients with failure to the first biologic drug: Impact on Republic fund for health insurance in Serbia. Racionalna terapija 2013; 5(1):1-7.

INSTRUCTION TO AUTHORS FOR MANUSCRIPT PREPARATION

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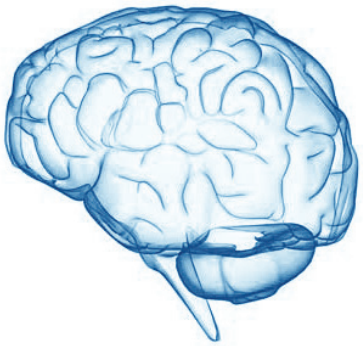

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