ISSN 2671-3497 (Online)

INTERNATIONAL JOURNAL

Institute of Knowledge Management

M E D I S

Vol. 2. 1.

MEDICAL SCIENCES & RESEARCH

ISSN 2671-3497 (Online)

MEDIS – International Journal of Medical Sciences and Research

Volume 2, Issue 1, March 2023.

https://medisij.com

IMPRESSUM

International Journal of Medical Sciences and Research
(MEDIS IJMSR)
(Volume 2, Issue 1 March 2023.)

Editor in chief:
Prof. d-r Robert Dimitrovski

Executive editor:
Prof. dr. Lazar Stošić

Publisher:
Institute of Management and Knowledge
Address: Gjuro Gjonovikj, 11/4 , Skopje 1000, Macedonia
Phone: +389 70 207 370, + 381 63 700 4281

http://medisij.com
E-mail: editor@medisij.com

For publisher:
Prof. d-r Robert Dimitrovski

Print:
GRAFOPROM Bitola

Circulation:
50 copies

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Slobodan, G., Momčilo, C., & Miodrag, Š. (2023). Microbiological activity of silver nanoparticles stabilized with dextran derivatives, 2(1), 1-12. doi: 10.35120/medisij020101g
UDK: 579.67:[546.57:620.3

MICROBIOLOGICAL ACTIVITY OF SILVER NANOPARTICLES STABILIZED WITH DEXTRAN DERIVATIVES

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Abstract: The paper shows the microbiological activity of silver nanoparticles (AgNPs) stabilized with dextran derivatives with carbox-ymethyl dextran (CMD) and dextran sulfate (DS). Non-toxic, green procedures for the synthesis of AgNPs with CMD, DS were developed. The increasing application of silver nanoparticles in many areas, especially favors the so-called non-toxic, i.e. "green" synthesis procedures. Unlike physical and chemical synthetic methods, green synthesis has a number of advantages: lower energy consumption, simple execution, costs are reduced, use of non-toxic chemicals as reducing and stabilizing agents. Nanoparticles AgNPs-DS and AgNPs-CMD showed microbiological activity against the analyzed test microorgan-isms. Silver nanoparticles obtained in this way, due to their stability and preserved antimicrobial activity, can be widely used in various branches of industry. The results of testing the antimicrobial activity of synthesized nanoparticles from the technologi-cal aspects are important; these compounds have potential application in the biomedical field, and simple procedures have many advantages, such as low costs, compatibility for medical and pharmaceutical applications, as well as the production of other commercial products in the food industry.

Keywords: *microbiological activity, silver nanoparticles, green synthesis, food industry.*

Introduction

Nanotechnology is a relatively young, multidisciplinary field in which the knowledge of natural sciences and engineering principles are applied with the aim of obtaining, characterizing and applying nanometer-sized materials (Ramsten, 2014). Materials of nano dimensions have specific characteris-tics. When the number of atoms that make up a material is significantly reduced, the atoms occupy a different arrangement and the distance between the surface atoms changes, which leads to a change in physical and chemical characteristics in relation to materials of the same composition, but with mac-roscopic dimensions (Trajković and Marković, 2010). In the last few decades, metal nanoparticles (gold, silver, platinum, palladium and many metal oxides, and composite materials based on them) have been the focus of many research groups (Krstić, 2013, Nadagouda and Varma, 2007, Liu et al. 2009, Shevchenko et al. 1985, Tolmachev et al.1985).

The size of metal nanoparticles ranges from 1 to 100 nm. Nanoparticles, thanks to their large specific surface area in relation to their volume, as well as their high surface energy, have unique catalytic, electrical, magnetic, optical and mechanical properties. (Horikoshi and Sorpone 2013). Silver nanoparticles are due to their catalytic properties (Jiang et al., 2005) and optical (McFarland and Duy-ne, 2003) properties, as well as antimicrobial activities (Bhattacharya and Mukherjee 2008), attracted the attention of many research groups. They can be synthesized by different procedures - methods: chemical reduction of silver ions (in the presence of a reducing and stabilizing agent) in aqueous solutions (Leopold and Lendl, 2003), Caswell et al., 2003, Chaki et al., 2004) as in non-aqueous solu-tions (Chen and Huang 2002, Wang et al., 2003, Faure et al., 2003), electro chemical method (Johans et al., 2004), reduction of silver ions using ultrasound (Zhang et al. 2004), by photo-induced or cata-lyzed reduction (Shchukin et al., 2003, Zhang et al., 2003, Cozzoli et al., 2004), by microwave-induced synthesis (Komarneni et al., 2002, Yamamoto et al., 2004), by radiation-chemical reduction (Hornebecq et al., 2003, Lihui et al., 2004), micro emulsion method (Zheng et al., 2003, Zheng et al., 2004, Maillard et al., 2002, Maillard et al., 2003) and biochemical reduction of silver ions (Naik et al., 2002, Bhainsa and D'Souza 2006). In all AgNPs synthesis procedures, the basic reaction is the reduc-tion of silver ions to elemental silver. In doing so, one should bear in mind the great tendency towards agglomeration, which reduces the ratio of surface area to volume, and thus changes the properties of nanoparticles. Therefore, when choosing a reducing agent, one must take into account its properties as a protective agent that prevents agglomeration. For this

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purpose, various substances can be used, most often polymers of synthetic or natural origin, especially biopolymers of the polysaccharide type and their derivatives (Krstić, 2013, Nadagouda and Varma, 2007, Liu et al. 2009, Shevchenko et al. 1985, Tolmachev et al.1985, Quelemes et al., 2013,Hamedi et al., 2012, Chudobova et al., 2013b, Garza-Navarro et al., 2013, Prinz et al.,2010). The increasing application of silver nanoparticles in many areas, especially favors the so-called non-toxic, relatively "green" synthesis procedures. In con-trast to physical and chemical synthetic methods, green synthesis has a number of advantages: lower energy consumption, simple execution, costs are reduced, use of non-toxic chemicals as reducing and stabilizing agents, which enables biocompatible and in vivo application of the formed particles (Amooaghaie et al., 2015, Sathishkumar et al., 2009, Bar et al., 2009, MubarakAli et al., 2011). There is an ever-greater interest in green synthetic procedures where plant or other natural products are used as reducing and protective agents. One of the ways to switch to non-toxic syntheses is to use natural products, i.e. water extracts of biomass, as well as polymers of natural or synthetic origin, produced by various microorganisms, as a reducing and stabilizing agent. For biomedical applications, honey has proven to be an attractive green agent, which simultaneously reduces silver and stabilizes silver nanoparticles. Honey has been used for medicinal purposes since ancient times due to its composition and specific characteristics. Honey consists of various carbohydrates (>80%), with fructose and glu-cose being the most abundant, accounting for about 70%, while sucrose (table sugar) represents ~5%. The rest is water, about 20%, while proteins are represented in a small percentage, about 0.3%. During the synthesis of silver nanoparticles in honey, glucose serves to reduce silver, and honey proteins to stabilize nanoparticles. In order for silver reduction to begin, the presence of sodium hydroxide is nec-essary, which facilitates the opening of the glucose ring, which allows the silver ions to oxidize glucose to gluconic acid. During this chemical reaction, silver nanoparticles and gluconic acid are formed, and all other components of honey remain unchanged. The most commonly used biopolymers are poly-saccharides and their derivatives: cellulose and carboxymethyl cellulose, dextran (Berry et al., 2003), CMD and DS, pullulan, alginate, agar, starch (Chairam and Somsook 2008, Chairam et al., 2009), chi-tosan (Park et al., 2005) and glycogen. From synthetic polymers, due to biocompatibility, PVP, PVA, poly(2-hydroxyethyl methacrylate) (PHEMA) and poly(methyl methacrylate) (PMMA) are used. (Stasica et al., 2000, Darwis et al.,2002). The mechanism of reduction of silver ions and stabilization of formed AgNPs by polysaccharides is well explained in the paper (Bankura et al., 2012) on the example of the synthesis of silver nanoparticles in water, using dextran as a reducing and protective agent. Silver nanoparticles cannot be synthesized using a solution of dextran in purified water, because purified water has a weak acidity (pH = 6) that can prevent the reduction of Ag+ to Ag0 in the nanometer range by forming R-O+H2. The presence of a very low concentration of NaOH can liberate the hydroxyl groups of dextran by removing protons and help the formation of silver nanoparticle which is observed by the color change of the solution (Bankura et al., 2012).

Microbiological properties of silver nanoparticles

The microbiological properties of silver have been known for centuries. Thus silverware was used to store water, while compounds containing silver were used in traditional medicine (Armentano et al., 2010, Slawson et al.,1992). In the last few decades, there has been a significant increase in mi-croorganisms that are resistant to existing antibiotics, which has brought silver back into medical use. (Monteiro et al., 2009). Nanoparticles have been shown to have greater microbiological activity than silver ions (Lok et al., 2006). Nanoparticles act at the level of the cell (cytoplasmic) membrane by in-teracting with structural proteins, changing the permeability of the membrane, and then with membrane enzymes, inhibiting their activity. Nanoparticles also penetrate the cell by interacting with DNA, which affects the cell's ability to replicate. In doing so, the nanoparticles also dissolve, releasing silver ions that also have an anti-microbial effect (Morones et al., 2005). Silver nanoparticles have been shown to act on various viruses (Xiang et al., 2011), bacteria (Guzman et al., 2012, Radzig et al., 2013) and fun-gi (Panáček et al., 2009). Chemically synthesized silver nanoparticles in solutions of different saccha-rides showed antibacterial activity against 10 different types of bacteria, whereby the smallest nanopar-ticles in this study with a diameter of 25 nm were the most effective, while the largest nanoparticles with a diameter of 50 nm had the weakest microbiological activity (Panáček et al.,2006). It has also been shown that the shape of nanoparticles significantly affects microbiological activity (Pal et al.,2007). When examining the microbiological activity of silver nanoparticles of different shapes (rod-shaped, spherical and triangular nanoparticles) against E. coli ATCC 10536, it was shown that the most effective nanoparticles were triangular-shaped, while the rod-shaped nanoparticles had the weakest microbio-logical efficiency. Silver nanoparticles are already commercially used for wound dressings (Acticoat, Smith&Nephew, USA), but have potential applications for antimicrobial creams and gels (Jain et al., 2009), as well as for coverings for catheters, drains and

implants (Knetsch and Koole, 2011). Silver nanoparticles are also, due to their strong antimicrobial activity, very attractive components for improv-ing the functionality of wastewater treatment membranes and water filters (Jain and Pradeep 2005). However, the main problem with the application of silver nanoparticles is their rapid release from the surface of the polymer from which the membranes are made (Tseng et al.,2006), therefore, the con-centration of silver in the filtered water is above the permitted concentration of 0.1 mg/l according to the EPA standard (Environmental Protection Agency-EPA). Therefore, the functionality of the membranes is improved by immobilizing the silver nanoparticle inside the polymer fibers from which the membrane is made. In this way, a small percentage of nanoparticles is released from the polymer into the filtered water, and the antimicrobial activity remains unchanged.

Carbohydrates as ligands

Carbohydrates represent a very widespread group of natural products that are synthesized by liv-ing organisms and that occupy an important place in life cycles. They make up about 80% of the mass of the dry substance of plants and about 2% in animals, as energy reserves (eg as starch) or as building materials (cellulose). Polysaccharides have different and, in many cases, complex chemical structures, numerous physiological functions and a wide range of potential applications. The basis of the carbohy-drate structure of polysaccharides is represented by monosaccharide units that can be connected to each other through different positions and orientations, which makes the structural chemistry of poly-saccharides very complex. They can be covalently bound to proteins or lipids and in the form of complex glycoconjugates they form biologically important compounds that have different functions. According to the sources from which they are obtained, they are divided into plant, animal and microorganism poly-saccharides. Polysaccharides are used in industry as thickeners, stabilizers and gelling agents. Also, they are used as agents for removing pollutants from the environment, and due to their biological effects, such as antioxidant, probiotic or antitumor, the interest in polysaccharide chemistry is growing every day (Đaković,1985, Liu et al., 2010a). They can be isolated from different sources (bacteria, fungi, algae and plants), although polysaccharides from algae and higher plants are dominant on the world market. These biopolymers are isolated by direct extraction from biomass and can be used in their native form, while in chemical form they are used in various fields. Thus, for example, dextran, which is a polysac-charide obtained by microbiological synthesis, is a molecular chain of anhydro-D-glucopyranose units connected by α-1,6 glycosidic bonds (in addition to these α-1,6 there may be others, α-1,2, α-1 ,3 and α-1,4). It is used as the most effective substitute for blood plasma, it is used in postoperative therapy to prevent venous thrombosis, as well as for the production of granulated gels in gel chromatography (Nikolić, 2001).

Exopolysaccharide dextran

Dextran is a branched exopolysaccharide consisting of D-glucopyranose residues interconnected by α-1,6 glycosidic bonds in the main sequence, with different content of α-1,2), α-1,3 and α-1,4 branches, and different conformation of the glucopyranose unit (Sarwat et al., 2008) (see Fig. 1). Dextran is a natural product, but it can be synthesized from sucrose by means of certain lactic acid bacteria, the most famous of which are Leuconostoc mesenteroides and Streptococcus mutans. It is used medically as an antithrombotic, to reduce blood viscosity, and as a means of increasing volume in anemia. It is used as a substitute for blood plasma in the treatment of shock caused by loss of body fluids (Rehm, 2010). Polysaccharide dextran has an extraordinary ability to form water-soluble complexes with various biometals (Fe, Cu, Co, Zn, Ca, Mg), as well as other metals (Tb, Al, Cd, Pb, Ni, Mn). (Cakić et al.,2008, Nikolić et al.,2009, Mitić et al., 2009, Nikolić et al., 2008, Mitić et al.,2007, Mitić et al., 2008, Vasko et al., 1971, Barker et al.,1953). Raw dextran cannot be used for clinical purposes, because it has toxic and antigenic properties. For the above reasons, raw dextran is depolymerized and repeatedly fractionated with hydrophilic solvents in order to obtain fractions with the narrowest distribution of molar masses. Due to their toxicity, dextran molecules with a high molar mass cause side reactions in the body, while molecules with a low molar mass are quickly excreted from the body. For clinical purposes, only dextrans with a low degree of branching are used, i.e. those with a content of α-1,6 bonds over 90%. Partially hydrolyzed dextran dissolves in water, formamide, dimethylformamide, dimethylsulfoxide, ethylene glycol, glycerol and in alkali solution. Various alcohols and acetones do not dissolve dextran, but rather precipitate it out of solution.

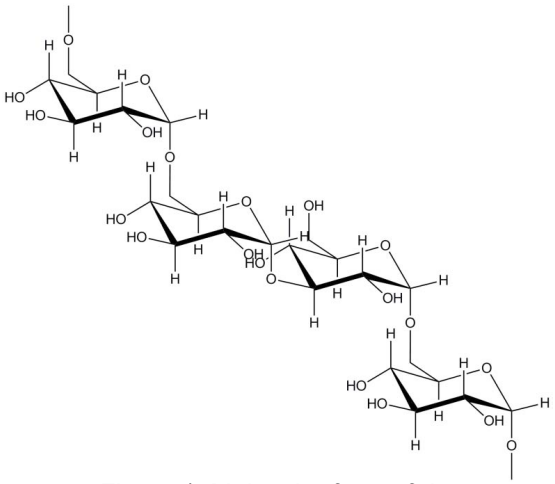

Figure 1. Molecular form of dextran

The reactivity of dextran (but also of other polysaccharides, for example pullulan, inulin, starch, cellulose, agar, agaroid, chondroitin sulfate, etc.) primarily depends on the reactivity of the secondary, equatorially oriented hydroxyl groups of the glucopyranose unit OH at C-2, C-3 and C-4 . Therefore, dextran can build various compounds, among which, due to its biological function and pharmacological activity, esters with inorganic or organic acids, but also complex compounds with biometals are important (Ricketts,1952b, Kentaro and Kotaro 1974). From the group of esters and ethers, the most famous are dextran and cellulose, i.e. and carboxymethyl dextran and carboxymethyl cellulose, as well as dextran sulfate. These compounds are used in pharmacy and cosmetics for the production of many preparations in which they have the function of fillers or, due to their hydrophilicity, for the production of moisturizing creams. Given the presence of carboxyl and sulfo functional groups, they can build complexes with biometals or other derivatives, mostly with Schiff's bases (amino acids), which can be further complexed, say with platinum, and used in cancer therapy, as reducing and stabilizing agents for the synthesis of MNPs or as their carriers (Tao et al., 2011). Certainly the most famous compound of dextran (but also of other polysaccharides, pullulan and inulin) is its polynuclear complex with iron (more correctly with β_2-FeOOH form of iron hydroxide), bearing in mind its wide application in human medicine and veterinary medicine in the treatment of iron deficiency anemia (Cakić et al., 2007).

Dextran derivatives

Carboxymethyl dextran (CMD) is a derivative of dextran, which is obtained by etherification of dextran with halogen acetic acid, (most often chlorine- or bromine-chlorine) in a mixture of alcohol and aqueous sodium hydroxide solution. The synthesis of carboxymethyl dextran is carried out in two stages, the first represents alkalization of dextran, and the second represents carboxymethylation of dextran with chloroacetic acid. CMD dissolves in distilled water and can be precipitated with ethanol.

Dextran sulfate (DS) is an ester of sulfuric acid and dextran. Given that there are three OH groups per glucopyranose unit of dextran, the degree of esterification. In addition to dextran sulfate, inulin sulfates, pullulan sulfates, and others (agar and agaroid, chondroitin sulfate, etc.) are also known (Ricketts 1952a).

Materials and methods

Synthesis of silver nanoparticles (AgNPs) with CMD. In a reactor with a volume of 100 ml, at room temperature, 20 ml of freshly prepared CMD (see Fig. 2) (0.002 M) was first added, and after 10 minutes, with constant stirring (300 rpm), and 10 ml of $AgNO_3$ solution (0.001 M). The suspension was stirred for 2 hours, and then the pH value of the suspension was adjusted to 7 by adding 0.4 ml of NaOH solution (0.001M). The mixture was kept under reflux for 24 hours. The change of white to yellow color of the reaction mixture indicates the formation of AgNPs-CMD nanoparticles. The reaction mixture was then cooled to room temperature, and the AgNPs-CMD nanoparticles were precipitated with 96% ethanol. After standing overnight, the ethanol was decanted, and the precipitate was re-dissolved with redistilled water. Finally, the nanoparticles were precipitated from the solution with 96% ethanol and, after decanting the alcohol, dried for 3 hours at 105°C in a vacuum oven.

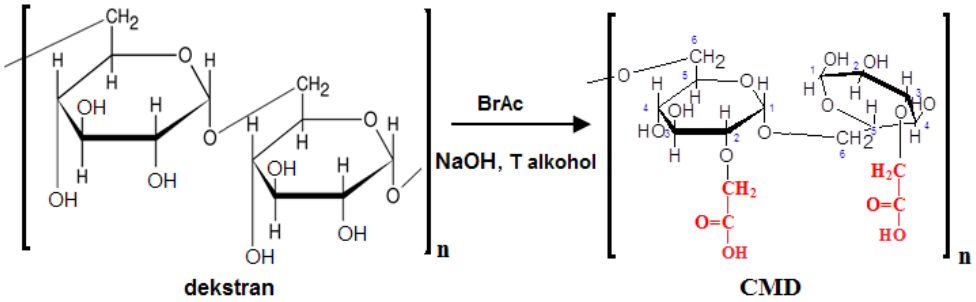

Figure 2. Schematic representation of CMD synthesis

Synthesis of silver nanoparticles (AgNPs) with DS. In a reactor with a volume of 100 ml, at room temperature, 20 ml of freshly prepared DS (see Fig. 3) (0.002 M) was first added, and after 10 minutes, with constant stirring (300 rpm) and 10 ml of AgNO3 solution (0.001 M). The suspension was stirred for 2 hours, and then the pH value of the suspension was adjusted to 7 by adding 0.4 ml of NaOH solution (0.001 M). The mixture was kept under reflux for 24 hours. The change from white to yellow in the reaction mixture indicates the formation of AgNPs-DS nanoparticles shown in Figure 3. The reaction mixture was then cooled to room temperature, and the AgNPs-DS nanoparticles were precipitated with 96% ethanol. After standing overnight, the ethanol was decanted, and the precipitate was re-dissolved with redistilled water. Finally, the nanoparticles were precipitated from the solution with 96% ethanol and, after decanting the alcohol, dried for 3 hours at 105oC in a vacuum oven.

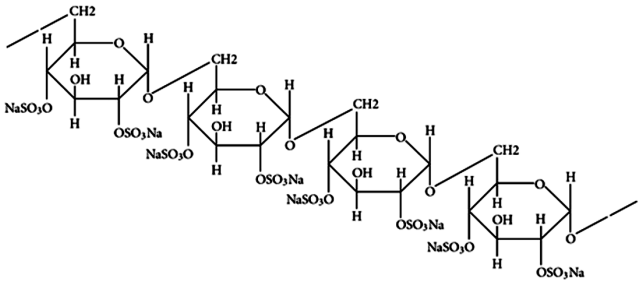

Figure 3. Structural formula of the sodium salt of dextran sulfate DS

Microbiological activity of silver nanoparticles. For testing, standard inoculums of microorganisms were used, which were prepared by sowing the appropriate microorganism culture (100 µl) on a liquid TSB medium (3 ml). After incubation of the inoculum at 37 ºC overnight, the microorganisms were taken from their exponential growth phase (16-18 hours after seeding) and used as such further in the experiment. The method itself is based on adding sterile Erlenmeyer flasks in the sterile atmosphere of the burner in the following order: physiological solution (50 ml), inoculum of the appropriate microorganism (500 µl) and tested silver nanoparticles (1 mg). In order to maintain a sterile environment, the Erlen-meyer flasks were capped and kept at a temperature of 37 ºC in a water bath for 2 h, with agitation. After that, an aliquot of suspension of 1 ml was taken from each Erlenmeyer which was further diluted deci-mally with physiological solution 0.9% NaCl (sterilization for 20 minutes at 121°C). After homogenization of the suspension on a vortex, the density of the suspension was adjusted by comparison with the 0.5 McFarland standard, which corresponds to the number of microorganisms of 1-2x108 CFU/ml (eng. Colony Forming Units) (Andrews, 2005). A 0.1 ml aliquot of the appropriate dilution was transferred to sterile Petri dishes, after which it was covered with melted TSA medium (~20 ml), with mixing. Sterile discs with a diameter of 9 mm were placed on the surface of the seeded substrate and impregnated with 60 µl of AgNPs-CMD, AgNPs-DS nanoparticle solution in concentrations of 0.25 mgcm-3, 0.5 mgcm-3, 1 mgcm-3. Petri dishes were incubated in a thermostat at a temperature of 37 ºC for the next 24 hours (Hamblin and Jori 2011). After incubation, the diameter of the zone of inhibition of the growth of micro-organisms was measured. The following microorganisms were used for the microbiological activity of silver nanoparticles as an indicator of strains: Gram-positive bacteria: Staphylococcus aureus ATCC 25923, Bacillus cereus ATCC 11778, Bacillus luteus in haus strain, Listeria monocytogenes ATCC 15313, Gram-negative bacteria: Escherichia coli ATTC 25922, Pseudomonas aeruginosa ATTC 27853, Klebsiella pneumoniae ATTC

700603, Proteus vulgaris ATTC 8427, and fungus: Candida albicans ATTC 2091.

Results and Discussion

Microbiological activity of AgNPs silver nanoparticles with CMD. A large amount of literature data shows that AgNPs possess significant microbiological activity, which is explained by the accumulation of silver ions from the solution in bacterial membranes, which can lead to cell death (Delić, 2011). Namely, the silver cation can react with thiol groups and proteins in cells, and in addition, it can inactivate en-zymes that are required for normal cellular metabolism (Sondi and Salopek, 2004). The microbiological activity of stabilized AgNPs-CMD was performed using the disk-diffusion method on agar. Clinical iso-lates of bacterial pathogens were used to test the antimicrobial activity of synthesized AgNPs-CMD (B. lutea, B. aureus, B. cereus, E. faecalis, P. aeruginosa, Klebsiella) and fungal strains (C. albicans) as indicators of strains for analysis. The results of testing the antimicrobial activity of the AgNPs-CMD na-noparticles solution are shown in Table 1.

Table 1.
Radial diameter of inhibition zones of tested bacterial and fungal strains

Radial diameter of inhibition (mm)				
AgNPs-CMD				
		G_3 = 0,25 mg/ml	G_4= 0,5 mg/ml	G_5=1 mg/ml
Bacterial strains	*Bacillus lutea*	11	13	20
	Bacillus aureus	12	18	21
	Bacillus cereus	11	12	14
	Enterococcus faecalis	-	-	11
	Pseudomonas aeruginosa	-	-	12
	Klebsiella	13	14	15
Fungal strains	*Candida albicans*	-	-	11

The radial growth of the zone of inhibition increases with increasing concentration of AgNPs-CMD from 0.25 to 1 mg/ml except for strains Enterococcus faecalis in which the zone of inhibition is reached only at a AgNPs-CMD concentration of 1 mg/ml. Fungus Candida albicans is more sensitive to AgNPs-CMD, while an inhibition zone of about 11 mm is achieved at a AgNPs-CMD concentration of 1 mg/ml. AgNPs-CMD nanoparticles showed the highest activity against bacteria B. lutea and B. aureus. The antifungal activity of AgNPs-CMD was analyzed according to the strain C. albicans. Zones of inhibition according to C. albicans are very small. Zone of inhibition B. lutea, B. aureus, B. cereus, E. faecalis, P. aeruginosa and K. pneumoniae were 20, 21, 14, 11, 12 and 15 mm, respectively. AgNPs-CMD at a concentration of 1 mg/ml showed a number of specificities according to their antimicrobial activity (see Fig. 4).

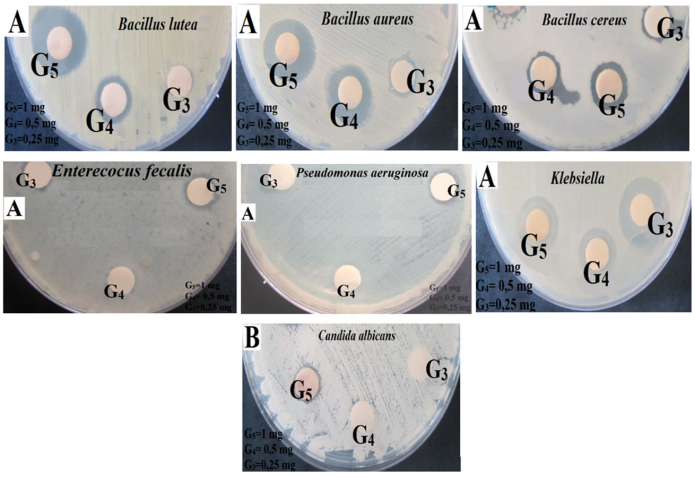

Figure 4. Microbiological activity of AgNPs-CMD nanoparticles of different concentrations against bacterial strains (B. lutea, B. aureus, B. cereus, E. faecalis, P. aeruginosa and Klebsiella) (A) and fungal strain (C. albicans) (B)

Microbiological activity of AgNPs silver nanoparticles with DS. AgNPs-DS solution showed micro-biological activity against bacteria, Staphylococcus aureus, Bacillus cereus, Bacillus luteus in haus strain, Bacillus subtilis, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris which proves the presence of clear zones of bacterial growth inhibition around the disks. The radial diameters of inhibition zones are shown in Table 2.

Table 2.
Radial diameter of inhibition zones for tested bacterial and fungal strains

Radial diameter of inhibition (mm)					
AgNPs-DS					
			G_1 = 0,25mg/ml	**G_2= 0,5mg/ml**	**G_3= 1mg/ml**
Bacterial strains	Gram +	*Staphylococcus aureus*	17	18	19
		Bacillus cereus	16	18	19
		Bacillus luteus in haus strain	20	21	24
		Bacillus subtilis	16	17	19
		Listeria monocytogenes	16	17	18
	Gram -	*Escherichia coli*	17	18	21
		Pseudomonas aeruginosa	23	24	26
		Klebsiella pneumoniae	16	18	19
		Proteus vulgaris	13	14	15
Fungal strain		*Candida albicans*	-	16	-

Inhibition was observed in all analyzed bacterial strains at a concentration of 0.25 mg/ml AgNPs-DS, indicating that the minimum inhibitory concentration for these microorganisms is low. For example, in literature (Dhand et al.,2016) it was stated that the minimum inhibitory concentrations for Escherichia coli and Staphylococcus aureus were about 0.26 mg/l. The highest zones of inhibition were recorded against Pseudomonas aeruginosa and Bacillus luteus in haus strain. The zone of inhibition against these microorganisms for a concentration of 1 mg/ml AgNPs-DS was 26 and 24 mm, respec-tively. Among the bacterial strains, Proteus vulgaris it was the least sensitive to the activity of AgNPs-DS with an inhibition zone of 15 mm, when a concentration of 1 mg/ml AgNPs-DS was used. Testing the activity of different concentrations of AgNPs-DS in relation to all other used bacterial strains showed similar results with inhibition zones in the range of 16-17 mm, 18-19 mm and 18-21 mm for concentra-tions of 0.25, 0.5 and 1 mg/ml AgNPs - DS. Results for Klebsiella pneumoniae, Bacillus luteus in haus strain and Pseudomonas aeruginosa are higher than previously reported for AgNPs-CMD. Antimicrobial activity against Candida albicans was observed only at a concentration of 0.5 mg/ml AgNPs-DS. Low antimicrobial activity of AgNPs against Candida albicans has already been reported for AgNPs stabi-lized by CMD. The mechanism of antimicrobial activity of AgNPs can be explained by the accumulation of silver in bacterial membranes that can lead to cell death. Dissolved Ag cation can react with thiol groups and proteins in cells. Additionally, it can inactivate enzymes that are required for normal cellular metabolism (Dhand et al.,2016). AgNPs-DS at a concentration of 1 mg/ml showed a number of speci-ficities according to antimicrobial activity. But a higher concentration of silver can also be harmful to microbes. Therefore, lower concentrations are much more applicable for this purpose. The lower effec-tive concentrations of AgNPs, which cause an effect on organisms different from the control, range from a few ng/l to tens of mg/l depending on the organism and many other factors. This silver nanoparticle synthesis design has great potential due to its antimicrobial activity.

Conclusions

Synthesized AgNPs-DS and AgNPs-CMD nanoparticles showed microbiological activity against the analyzed test microorganisms. AgNPs-DS and AgNPs-CMD showed no activity on the fungal strain C. albicans, and AgNPs-CMD and on P. aeruginosa in a concentration of 0.5 mg/ml. Mutual comparison of the size of the radial zones of inhibition shows that they are slightly smaller with AgNPs-DS, and the smallest with AgNPs-CMD. Also, the zones of inhibition in all analyzed bacterial strains are larger when

the concentration of AgNPs is 1 mg/ml than for 0.5 mg/ml. The results of testing the antimicrobial activity of the synthesized AgNPs show that they can be used in the biomedical field for the production of var-ious preparations in human medicine, cosmetics, veterinary medicine and the food indus-try.Nanoparticles have been shown to have greater microbiological activity than silver ions. Nanoparti-cles act at the level of the cell (cytoplasmic) membrane by interacting with structural proteins, changing the permeability of the membrane, and then with membrane enzymes, inhibiting their activity. Nanopar-ticles also penetrate the cell by interacting with DNA, which affects the cell's ability to replicate. In doing so, the nanoparticles also dissolve, releasing silver ions that also have an anti-microbial effect. Silver nanoparticles have been shown to act on various viruses, bacteria and fungi. Chemically synthesized silver nanoparticles in solutions of different saccharides showed antibacterial activity against 10 different types of bacteria, whereby the smallest nanoparticles in this study with a diameter of 25 nm were the most effective, while the largest nanoparticles with a diameter of 50 nm had the weakest microbiological activity. It has also been shown that the shape of nanoparticles significantly affects microbiological ac-tivity. When examining the microbiological activity of silver nanoparticles of different shapes (rod-shaped, spherical and triangular nanoparticles) against E. coli ATCC 10536, it was shown that the most effective nanoparticles were triangular-shaped, while the rod-shaped nanoparticles had the weakest microbiological efficiency. Silver nanoparticles are already commercially used for wound dressings (Acticoat, Smith&Nephew, USA), but have potential applications for antimicrobial creams and gels, as well as for coverings for catheters, drains and implants. Silver nanoparticles are also, due to their strong antimicrobial activity, very attractive components for improving the functionality of wastewater treatment membranes and water filters.

Conflict of interests

The authors declare no conflict of interest.

References

Amooaghaie, R., Saeri, M.R., & Azizi, M., (2015), Synthesis, characterization and biocompatibility of silver nanoparticles synthesized from Nigella sativa leaf extract in comparison with chemical silver nanoparticles. Ecotoxicology and environmental safety, 120, 400-408.

Andrews, J.M., (2005), BSAC standardized disc susceptibility testing method. Journal of Antimicrobial Chemotherapy 56 pp. 60.

Armentano, I., Dottori M., Fortunati E., Mattioli S., Kenny J. M., (2010), Biodegradable polymer matrix nanocomposites for tissue engineering: a review. Polymer Degradation and Stability 95: 2126–46.

Bankura, K. P., Maity, D., Mollick, M. M., Mondal, D., Bhowmick, B., Bain, M. K., ... & Chattopadhyay, D., (2012), Synthesis, characterization and antimicrobial activity of dextran stabilized silver nanoparticles in aqueous medium. Carbohydrate polymers, 89(4), 1159-1165.

Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., De, S.P., & Misra, A., (2009), Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids and surfaces A: Physicochemical and engineering aspects, 339(1), 134-139.

Barker, S.A., Bourne, E.J., Stacey, M., & Whiffen, D.H., (1953), Infra-red absorption spectra of dextran and other polyglucosans. Chemistry & Industry, (9), 196-197.

Berry, C.C., Wells, S., Charles, S., Curtis, A.S.G., (2003), Dextran and albumin derivatised iron oxide nanoparticles: influence on fibroblasts in vitro. Biomaterials 24: 4551-7.

Bhainsa, K.C., D'Souza S.F., (2006), Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigates. Colloids and Surface B: Biointerfaces 47: 160-4.

Bhattacharya, R., Mukherjee, P., (2008), Biological properties of "naked" metal nanoparticles. Advanced Drug Delivery Reviews 60: 1289–306.

Cakić, M., Mitić, Ž., Nikolić, G. S., Ilić, L., & Nikolić, G. M., (2008), The investigations of bioactive copper(II) complexes with reduced low-molar dextran. Journal of Spectroscopy, 22(2-3), 177-185.

Cakić, M., Nikolić G., Cvetković D., Ilić Lj., Kompleksi Fe(III) sa oligosaharidima – antianemici. Naučna monografija, (Tehnološki fakultet, Leskovac, 2007).

Caswell, K.K., Bender, C.M., Murphy, C.J., (2003), Seedless, surfactantless wet chemical synthesis of silver nanowires. Nano Letters 3: 667-9.

Chairam, S., Poolperm, C., Somsook E., (2009), Starch vermicelli template assisted synthesis of size/shape-controlled nanoparticles. Carbohydrate Polymers 75: 694–704.

Chairam, S., Somsook, E., (2008), Starch vermicelli template for synthesis of magnetic iron oxide nanoclusters. Journal of Magnetism and Magnetic Materials 320(15): 2039–43.

Chaki, N.K., Sharma J., Mandle A. B.,. Mulla I. S, Pasricha R., Vijayamohanan K., (2004), Size dependent redox behavior of monolayer protected silver nanoparticles (2–7 nm) in aqueous medium. Physical Chemistry Chemical Physics 6: 1304-9.

Chen, D.H., Huang, Y.W., (2002), Spontaneous Formation of Ag Nanoparticles in Dimethylacetamide Solution of Poly(ethylene glycol). Journal of Colloid Interface Science, 255, 299-302.

Chudobova, D., Nejdl, L., Gumulec, J., Krystofova, O., Rodrigo, M. A. M., Kynicky, J., ... & Kizek, R. (2013b). Complexes of silver (I) ions and silver phosphate nanoparticles with hyaluronic acid and/or chitosan as promising antimicrobial agents for vascular grafts. International journal of molecular sciences, 14(7), 13592-13614.

Cozzoli, P.D., Comparelli R., Fanizza E., Curri M.L., Agostiano A., Laub D., (2004), Photocatalytic synthesis of silver nanoparticles stabilized by tio2 nanorods: a semiconductor/metal nanocomposite in homogeneous nonpolar solution. Journal of the American Chemical Society. 126(12):3868-79.
Đaković, Lj., (1985), Koloidna hemija, Tehnološki fakultet, Zavod za izdavanje udžbenika, Novi Sad.
Darwis, D., Stasica P., Razzak M.T., Rosiak J.M., (2002), Characterization of poly(vinyl alcohol) hydrogel for prosthetic intervertebral disc nucleus. Radiation Physics and Chemistry, 63: 539-42.
Delić, D., (2011), Toxicity of silver nanoparticles in duckweed (Lemna minor L.), Sveučilište u Zagrebu, 43.
Dhand, V., Soumya L., Bharadwaj S., Chakra S., Deepika B., Sreedhar B., (2016), Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity." Materials science and engineering. C, 58, 36-43.
Faure, C., Derre A., Neri W., (2003), Spontaneous Formation of Silver Nanoparticles in Multilamellar Vesicles. Journal of Physical Chemistry B, 107: 4738-46.
Garza-Navarro, M.A., Aguirre-Rosales, J.A., Llanas-Vázquez, E.E., Moreno-Cortez, I.E., Torres-Castro, A., & González-González, V., (2013), Totally ecofriendly synthesis of silver nanoparticles from aqueous dissolutions of polysaccharides. International Journal of Polymer Science, 2013.
Guzman, M., Dille J., Godet S., (2012), Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, Biology, and Medicine 8: 37–45.
Hamblin, MR & Jori G., (2011), Photodynamic inactivation of microbial pathogens: Medical and environmental applications, Hamblin MR, Jori G (Eds.), From series: Comprehensive Series in Photochemistry and Photobiology No. 11, The Royal Society of Chemistry, European Society for Photobiology, UK, ISBNs: 978-1-84973-144-7 (Hardback) & 978-1-84973-308-3 (Electronic).
Hamedi, S., Ghaseminezhad, S. M., Shojaosadati, S. A., & Shokrollahzadeh, S., (2012), Comparative study on silver nanoparticles properties produced by green methods, Iranian Journal of Biotechnology, 10(3), 191-197.
Horikoshi, S., Sorpone N., (2013), Microwaves in Nanoparticle Synthesis: Fundamentals and Applications 1. Introduction to Nanoparticles Published Online: 26 APR DOI: 10.1002/9783527648122.ch1
Hornebecq, V., Antonietti, M., Cardinal, T., Treguer-Delapierre, M., (2003), Stable silver nanoparticles immobilized in mesoporous silica. Chemistry of Materials, 15: 1993-99.
Jain, J., Arora, S., Rajwade, J. M., Omray, P., Khandelwal, S., Paknikar, K. M., (2009), Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use. Molecular Pharmacology, 6(5):1388-401
Jain, P., Pradeep T., (2005), Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnology and Bioengineering, 90(1): 59-63.
Jiang, Z.J., Liu C.Y., Sun L.-W., (2005), Catalytic properties of silver nanoparticles supported on silica spheres. Journal of Physical Chemistry B, 109(5), 1730-5.
Johans, C., Clohessy, J., Fantini, S., Kontturi, K., Cunnane, V.J., (2004), Electrosynthesis of polyphenylpyrrole coated silver particles at a liquid– liquid interface. Electrochemistry Communications, 4: 227-30.
Kentaro, M., Kotaro, K., (1974), Flange cover mounting structure; Int C1 Fi6B9/02, Appl. No 287 67; Jap. Pat 120 62A2 (2000) dop. JpP120 626
Knetsch, M.L.W, Koole, L.H., (2011), New strategies in the development of antimicrobial coatings: The example of increasing usage of silver and silver nanoparticles. Polymers 3(1): 340-66.
Komarneni, S., Li, D.S., Newalkar, B., Katsuki, H., Bhalla, A.S., (2002), Microwave-Polyol Process for Pt and Ag Nanoparticles. Langmuir 18: 5959-62.
Krstić, N.S., (2013), Study of the interaction of M(II) biometal ions in model systems with pharmaceuticals and supplements type acids as potential ligands. PhD Thesis. Faculty of Sciences and Mathematics Nish, Serbia.
Leopold, N., Lendl, B., (2003), A new method for fast preparation of highly surface-enhanced raman scattering (SERS) active silver colloids at room temperature by reduction of silver nitrate with hydroxylamine hydrochloride. Journal of Physical Chemistry B., 107: 5723-27.
Lihui, X., Ruimin, Z., Gracien, E.B., Francis, A.O., (2004), Synthesis of silver nano-particles by EB irradiation. Journal of Radiation Research and Radiation Processing 22(2): 69-72.
Liu, C., Lu, J., Lu, L., Liu, Y., Wang, F., Xiao, M., (2010a), Isolation, structural characterization and immunological activity of an exopolysaccharide produced by Bacillus licheniformis 8-37-0-1, Bioresource Technology, 101, 5528–5533.
Liu, M., Yue, X. L., Dai, Z.F., Ma, Y., Xing, L., Zha, Z., Liu, S., Li, Y., (2009), Novel thrombo-resistant coating based on iron-polysaccharide complex multilayers, ACS Applied Materials & Interfaces, 1(1), 113-123.
Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun H., Tam, P.K.H., Chiu, J. F., Che C.M., (2006), Proteomic analysis of the mode of antibacterial action of silver nanoparticles. Journal of Proteome Research 5: 916-24.
Maillard, M., Giorgio, S., Pileni, M.P., (2002), Silver nanodisks. Advanced Materials 14(15): 1084-86.
Maillard, M., Giorgio, S., Pileni, M.P., (2003), Tuning the size of silver nanodisks with similar aspect ratios: Synthesis and optical properties. Journal of Physical Chemistry B, 107(11): 2466-70.
McFarland, A.D., Duyne, R.P.V., (2003), Single silver nanoparticles as real-time optical sensors with zeptomole sensitivity. Nanoletters, 3, 1057- 62.
Mitić, Ž., Nikolić, G. S., Cakić, M., Nikolić, R., & Ilić, L., (2007), Synthesis and spectroscopic characterization of copper (II)-dextran complexes. Russian Journal of Physical Chemistry A, 81(9), 1433-1437.
Mitić, Ž., Nikolić, G. S., Cakić, M., Premović, P., Ilić, Lj., (2009), FTIR spectroscopic characterization of Cu(II) coordination compounds with exopolysaccharide pullulan and its derivatives. Journal of Molecular Structure, 924, 264-273.
Mitić, Ž., Nikolić, G.S., Cakić, M., Development of ATR-FTIR Microspectroscopyc for Analysis of Bioactive Cooper(II)-Polysaccharide Complexes „Shedding Light of Diseanse" Optical Diagnosis for the New Millennium, Conference SPEC 2008, (Sao Jose dos Campos, Sao Paulo, Brazil, 86-88, 2008).
Monteiro, D.R., Gorup, L.F., Takamiya, A.S., Ruvollo-Filho, A.C., Camargo, E.R., Barbos, D.B., (2009), The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. International Journal of Antimicrobial Agents, 34: 103-10.
Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., Yacaman, M.J., (2005), The bactericidal effect of silver nanoparticles. Nanotechnology 16: 2346–53.

MubarakAli, D., Thajuddin, N., Jeganathan, K., & Gunasekaran, M., (2011), Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids and Surfaces B: Biointerfaces, 85(2), 360-365.
Nadagouda, M.N., & Varma, R.S., (2007), Synthesis of thermally stable carboxymethyl cellulose/metal biodegradable nanocomposites for potential biological applications. Biomacromolecules, 8(9), 2762-2767.
Naik, R.R., Stringer, S.J., Agarwal, G., Jones, S.E., Stone, M.O., (2002), Biomimetic synthesis and patterning of silver nanoparticles. Nature materials 1(3): 169-72.
Nikolić, G., (2001), Sinteza, fizičkohemijska svojstva i struktura kompleksa gvožđa (III) sa oligosaharidima, Doktorska disertacija, Tehnološki fakultet, Leskovac.
Nikolić, G.S., Cakić, M., Mitić, Ž., Ilić, B., & Premović, P., (2009), Attenuated total reflectance-fourier transform infrared microspectroscopy of copper (II) complexes with reduced dextran derivatives. Russian Journal of Physical Chemistry A, Focus on Chemistry, 83(9), 1520-1525.
Nikolić,G.S., Cakić, M., Mitić, Ž., & Ilić, Lj., (2008), Deconvoluted Fourier-transform LNT-IR study of coordination copper (II) compounds with dextran derivatives. Russian Journal of Coordination Chemistry, 34(5), 322-328.
Pal, S., Tak K.Y., Song M.J., (2007), Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli. Applied and Environmental Microbiology, 73: 1712–20.
Panáček, A., Kolář, M., Večeřová, R., Prucek, R., Soukupová, J., Kryštof, V., Hamal, P., Zbořil, R., Kvítek, L., (2009), Antifun-gal activity of silver nanoparticles against Candida spp. Biomaterials 30: 6333–40.
Panáček, A., Kvítek, L., Prucek, R., Kolář, M., Večeřová, R., Pizúrová, N., Sharma, V.K., Nevěčná, T., Zbořil R., (2006), Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. Journal of Physical Chemistry B, 110: 16248-53.
Park, J. H., Im, K.H., Lee, S.H., Kim, D.H., Lee, D.Y., Lee, Y.K., Kim, K.M., Kim, K.N., (2005), Preparation and characteriza-tion of magnetic chitosan particles for hyperthermia application. Journal of Magnetism and Magnetic Materials 293: 328-33.
Prinz, E. M., Eggers, R., Lee, H.H., Steinfeld, U., & Hempelmann, R., (2010), Synthesis of drug loaded magnetic nanoparticles and their uptake into immune cells. In Journal of Physics: Conference Series 200(12) p. 122009). IOP Publishing.
Quelemes, P. V., Araruna, F.B., de Faria, B.E., Kuckelhaus, S.A., da Silva, D.A., Mendonça, R.Z., ... & Leite, J.R.S., (2013), Development and antibacterial activity of cashew gum-based silver nanoparticles. International journal of molecular sciences, 14(3), 4969-4981.
Radzig, M.A., Nadtochenko, V.A., Koksharova, O.A., Kiwi, J., Lipasova, V.A., Khmela, I.A., (2013), Antibacterial effects of silver nanoparticles on gram-negative bacteria: Influence on the growth and biofilms formation, mechanisms of action. Colloids and Surfaces B: Biointerfaces 102: 300–6.
Ramsten, J.J., (2014), What is Nanotechnology? Applied Nanotechnology, Second Edition. http://dx.doi.org/10.1016/B978-1-4557-3189-3.00001-4
Rehm, B.H.A., (2010), Bacterial polymers: biosynthesis, modifications and applications, applied and industrial microbiology, Nature Reviews Microbiology, 8, 578– 592.
Ricketts, C.R., (1952a), Interaction of dextran and fibrinogen. Nature, 169(4310), 970-970.
Ricketts, C.R., (1952b), Dextran sulphate-a synthetic analogue of heparin. Biochemical Journal, 51(1), 129-133
Sarwat, F., Qader, S.A.U., Aman, A., Ahmed, N., (2008), Production & characterization of a unique dextran from an indigenous Leuconostoc mesenteroides CMG713, International Journal of Biological Sciences, 4(6), 379–386.
Sathishkumar, M., Sneha, K., Won, S.W., Cho, C.W., Kim, S., & Yun, Y.S. (2009), Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids and Surfaces B: Biointerfaces, 73(2), 332-338.
Shchukin, D.G., Radtchenko, I.L., Sukhorukov, G.B., (2003), Photoinduced reduction of silver inside microscale polyelectrolyte capsules. ChemPhysChem, 4: 1101-3.
Shevchenko, L.I., Lugovaya, Z.A., & Tolmachev, V.N., (1985), Study of the complexing properties of the dextran carboxymethyl ether with transition metal ions in solutions. Polymer Science USSR, 27(9), 2242-2247.
Slawson, R.M.; Van Dyke, M.I.; Lee, H., Trevors J.T., (1992), Germanium and silver resistance, accumulation, and toxicity in microorganisms. Plasmid, 27: 72-9.
Sondi, I., Salopek, B., (2004), Uticaj srebra na E.coli, Journal of Colloid and Interface Science, 27(5), 17-18.
Stasica, P., Rosiak, J.M., Ciach, M., Radek, M., (2000), Approach to construct hydrogel intervertebral discs implants – experimental and numerical investigations. Engineering of Biomaterials 3: 9-14.
Tao, K., Song, S., Ding, J., Dou, H., & Sun, K., (2011), Carbonyl groups anchoring for the water dispersibility of magnetite nanoparticles. Colloid and Polymer Science, 289(4), 361-369.
Tolmachev, V.N., Polovinkina, L.I., & Lugovaya, Z.A., (1985), Study of the acid properties of carboxymethyl dextran. Polymer Science USSR, 27(2), 273-278.
Trajković, V., Marković, Z., (2010), Nanomedicina: Stanje i perspektiva. In book: Biomaterijali. Rakovic D., Uskoković D. (eds), Institut tehničkih nauka Srpske akademije nauke i umetnosti, Beograd, 762-75.
Tseng, C.H., Wang, C.C., Chen, C.Y., (2006), Polypropylene fibers modified by plasma treatment for preparation of Ag nanoparticles. Journal of Physical Chemistry B 110: 4020–9.
Vasko, P.D., Blackwell, J., & Koenig, J.L., (1971), Infrared and raman spectroscopy of carbohydrates: Part I: Identification of O-H and C-H related vibrational modes for D-glucose, maltose, cellobiose, and dextran by deuterium-substitution methods. Carbohydrate Research, 19(3), 297-310.
Wang, X.Q., Itoh, H., Naka, K., Chujo, Y., (2003), Tetrathiafulvaleneassisted formation of silver dendritic nanostructures in acetonitrile. Langmuir, 19: 6242-46.
Xiang, D.X., Chen, Q., Pang L., Zhenga, C.L., (2011), Inhibitory effects of silver nanoparticles on H1N1 influenza A virus in vitro. Journal of Virological Methods, 178, 137–142.
Yamamoto, T., Yin, H.B., Wada, Y., Kitamura, T., Sakata, T., Mori, H., Yanagida S., (2004), Morphology-control in micro-wave-assisted synthesis of silver particles in aqueous solutions. Bulletin of the Chemical Society of Japan 77: 757-61.
Zhang, J., Chen, P., Sun, C., Hu, X., (2004), Sonochemical synthesis of colloidal silver catalysts for reduction of complexing

silver in DTR system. Applied Catalysis A: General 266: 49–54.
Zhang, L.Z,. Yu, J.C, Yip, H.Y., Li, Q., Kwong K.W., Xu A.W., Wong P.K., (2003), Ambient Light Reduction Strategy to Synthesize Silver 316.
Zheng, X.W., Zhu, L.Y., Wang, X.J., Yan, A.H., Xie, Y., (2004), A simple mixed surfactant route for the preparation of noble metal dendrites. Journal of Crystal Growth 260: 255-62.
Zheng, X.W., Zhu, L.Y., Yan, A.H., Wang, X.J., Xie, Y., (2003), Controlling synthesis of silver nanowires and dendrites in mixed surfactant solutions. Journal of Colloid Interface Science 268: 357-61.

Ljupka, A., & Sanja, N. (2023). The power of saliva in diagnostic oral desease, *MEDIS - Medical Science and Research, 2*(1), 13-14. doi: 10.35120/medisij020113l
UDK: 616.316-008.8-074:616.31

THE POWER OF SALIVA IN DIAGNOSTIC ORAL DESEASE

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Abstract: Saliva is an extracellular fluid produced and secreted by the salivary glands in the mouth. In humans, saliva is about 99% water, plus electrolytes, mucus, white blood cells, epithelial cells (from which DNA can be extracted), enzymes (such as lipase and amylase), antimicrobial agents (such as secretory IgA and lysozymes) . Saliva is an important diagnostic method, it helps in determining certain disorders/diseases of the orofacial system. Some bad habits such as smoking increase the secretion of saliva most likely as a result of a defense mechanism of chemical irritation. Of the enzymes, amylase has a role to clean the oral cavity, and also a protective role against caries. Role of saliva in remineralization Remineralization is directly dependent on the presence of ions in saliva. Supersaturation of saliva represents a barrier to demineralization and is a necessary prerequisite for the remineralization process. Also, the degree of saliva secretion depends on the feeling of fear, damage to the salivary glands from radiation to the head and neck, tumor of these glands, thyroid gland deficiency or when using drugs that, as one of the side effects, also have an antisialogogonic effect . Supersaturation of saliva represents a barrier to demineralization and is a necessary prerequisite for the remineralization process. Buffer systems affect the possibility of neutralization of ingested or generated acids. Bicarbonate is the most important buffering system in saliva. Thanks to the physical, chemical and antibacterial properties of saliva. Saliva is an ion reservoir, a buffer and has an antimicrobial effect. It helps in digestion, dissolving food residues, acts as a solvent allowing food to interact with the gustatory papillae, maintains water balance and rinses away food residues. Physical protection - The role of saliva in the physical protection of soft tissues is based primarily on its role of lubrication, soaking the oral mucosa, and at the same time it moistens the food and helps in the formation of the bolus and its swallowing. The clearance of carbohydrates from the mouth takes place in two phases. Fast clearance in the first 6 min, then clearance is slower. The clearance of fluorides is much slower than the clearance of sugars, because fluorides have the ability to bind to hard tissues.

Key words: antimicrobial, demineralization, diagnostic, protection, saliva.
Field: Medical sciences and Health

INTRODUCTION

Saliva is a complex fluid consisting of secretions from the major, major and minor salivary glands. Saliva can be used to diagnose any disease, from the orofacial system, or any bodily disease. In humans, saliva is about 99% water, plus electrolytes, mucus, white blood cells, epithelial cells (from which DNA can be extracted), enzymes (such as lipase and amylase), antimicrobial agents (such as secretory IgA and lysozymes).

MATERIALS AND METHODS

In the past few years, new research has been done proving that salivary biomarkers are an innovation in dentistry to detect certain oral diseases. In addition to biomarkers, it is certainly necessary to apply additional non-invasive diagnostic methods to establish a more accurate diagnosis. Nowadays, liquid biopsy is widely used, which allows to detect even tumor cells and, or fragments of tumor types. With these innovative diagnostic procedures, mutations can be almost perfectly detected in patients already diagnosed with lung cancer.

RESULTS

Saliva is very important in determining oral diseases. The quality of saliva is very important in diagnosis, as well as its quantity. During diagnosis, changes in the secretion of saliva, the formation of bacteria, as well as the analysis of the role of saliva on the occurrence of caries are analyzed. The

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change in saliva secretion can be reversible, but it can also be irreversible, it all depends on numerous pathological factors. In addition, the conclusion is that saliva plays an important role in maintaining the integrity of oral tissues, also saliva has a role in digesting food, as well as in controlling the occurrence of infections, that is, it has a protective role.

DISCUSSIONS

Early detection of the disease is vital for a more successful therapy and a successful outcome of the therapy for a certain oral disease. Of course, early detection has an impact on preventing complications. Saliva is a method for diagnosis and early detection of disease in the orofacial system. Biomarkers, as well as genetic material and proteins, help further in diagnosing diseases. Apart from diagnosing oral diseases, saliva can be used as a diagnostic method for diagnosing other diseases, bodily, autoimmune, for example Sjögren's syndrome, cystic fibrosis, cardiovascular diseases, HIV, diabetes mellitus, caries, periodontopathy and early detection of oral cancer. Today, diagnostic tests are often used in the clinics themselves, and they are very accurate, precise, easy and efficient to use.

CONCLUSIONS

Saliva is a complex fluid consisting of secretions from the major, major and minor salivary glands. Saliva can be used to diagnose any disease, from the orofacial system, or any bodily disease. In humans, saliva is about 99% water, plus electrolytes, mucus, white blood cells, epithelial cells (from which DNA can be extracted), enzymes (such as lipase and amylase), antimicrobial agents (such as secretory IgA and lysozymes).

REFERENCES

Ahmadi Badi S., Bruno S. P., Moshiri A., Tarashi S., Siadat S. D., Masotti A. (2020). Small RNAs in Outer Membrane Vesicles and Their Function in Host-Microbe Interactions. Front. Microbiol. 11:1209. 10.3389/fmicb.2020.01209 - DOI - PMC - PubMed

Akerman L., Casas R., Ludvigsson J., Tavira B., Skoglund C. (2018). Serum miRNA levels are related to glucose homeostasis and islet autoantibodies in children with high risk for type 1 diabetes. PLoS One 13:e0191067. 10.1371/journal.pone.0191067 - DOI - PMC - PubMed

Bhardwaj N., Sena M., Ghaffari G., Ishmael F. (2020). MiR-4668 as a Novel Potential Biomarker for Eosinophilic Esophagitis. Allergy Rhinol. 11:2152656720953378. 10.1177/2152656720953378 - DOI - PMC - PubMed

Hikkaduwa Withanage MH, Gomez Hernandez MP, Starman EE, Davis AB, Zeng E, Lieberman SM. (2021) Dataset – chemokines, cytokines, and biomarkers in the saliva of children with Sjögren's syndrome. Data Brief - PMC - PubMed

Ilea A., Andrei V., Feurdean C.N., Băbțan A.M., Petrescu N.B., Câmpian R.S., et al. (2019) Saliva, a magic biofluid available for multilevel assessment and a mirror of general health-A systematic review. Biosensors. 9(1): 10.3390/bios9010027 - DOI - PMC - PubMed

Mofors J., Arkema E.V., Bjork A. et al. (2019) Infections increase the risk of developing Sjögren's syndrome. J Intern Med. 285:670–80. - PubMed

Sultan A.S., Kong E.F., Jabra-Rizk M.A. (2018) The oral microbiome: A lesson in co-existence. PLoS Pathog. 14(1):e1006719 10.1371/journal.ppat.1006719 - DOI - PMC - PubMed

Sroussi H.Y., Epstein J.B., Bensadoun R.J., Saunders D.P., Lalla R.V., Migliorati C.A., et al. (2017) Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. Cancer Med. 6(12):2918–31. 10.1002/cam4.1221 - DOI - PMC - PubMed

Shah S. (2018) Salivaomics: The current scenario. J Oral Maxillofac Pathol. 22(3):375–81. 10.4103/jomfp.JOMFP_171_18 - DOI - PMC - PubMed

Vivino F., Bunya V.Y., Massaro-Giordano G., Johr C.R., Giattino S.L., Schorpion A., et al. (2019) Sjogren's syndrome: An update on disease pathogenesis, clinical manifestations and treatment. Clin Immunol. 22: 10.1016/j.clim.2019.04.009 - DOI - PubMed

Bogomila, C., Anastas, C,. & Stefan, S. (2023). Abdominal aortic aneurysm rupture – case report, *MEDIS - Medical Science and Research, 2*(1), 15-20. doi: 10.35120/medisij020115c
UDK: 616.136-007.64

ABDOMINAL AORTIC ANEURYSM RUPTURE – CASE REPORT

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Abstract: An Abdominal Aortic Aneurysm (AAA) is a localized dilatation and weakening of the abdominal aorta, as it`s infrarenal part is most commonly affected by the disease. Risk of rupture: Size of the AAA is one of the strongest predictors of rupture, as aortic aneurysms above 5,5cm in diameter have a higher risk. Clinical manifestation: Most of the AAAs have no symptoms and are accidently found. Classis symptoms of ruptured AAA (rAAA) are suddenly occurring severe abdominal and/ or back pain, arterial hypotension and pulsatile abdominal mass. Preoperative management: When rAAA is suspected, the patient should be consulted with a vascular surgeon as soon as possible. Aggressive fluid resuscitation should be avoided. Surgical treatment: Open surgery is usually performed via a transperitoneal approach with a midline laparotomy. Depending on the anatomy of the AAA and iliac arteries involvement an aorto-arotal or aorto-bifemoral bypass is constructed. Complications after repair of rAAA: Local - Lower limb(s) ischemia, Ischemia of the colon; Systemic - Cardiac, Pulmonary, Renal, Liver or Multiorgan failure, with 30-day mortality reaching up to 89%.

CASE REPORT: Male patient, 81 years of age, with multiple concomitant diseases. He was diagnosed with AAA 4 months prior to the rupture. The maximal diameter of the AAA was 15,6cm, iliac arteries were not affected. The patient refused the suggested surgical or endovascular treatments. He presented at ER 4 months later with acute pain in the abdomen and back. Clinical status: severe pain in the abdomen, BP 96/57mmHg, Hgb 102 g/l with HCT -0.331 l/l. On the CT-angiography rupture of AAA was verified with massive retroperitoneal haematoma, occluded right renal artery and aneurysm of the left renal artery. Median laparotomy was conducted under common anaesthesia. Aneurysmal neck was clamped above renal arteries, with clamping time – 30 minutes. After reclamping aorto-bifemoral bypass was constructed. Postoperatively the patient was transferred to intensive care unit (ICU). In the course of ICU treatment, the patient was inadequate and lacked spontaneous diuresis. A temporary catheter for haemodialysis was placed and such was initiated. He was transferred in the Clinic of vascular surgery after stabilizing blood oxygen saturation. The patient was inadequate at the time of transfer, with jaundice present. The patient restored bowel passage, hepatoprotectors were administered and physiotherapy was initiated. On the 20th postoperative day, the patient had a rapid decline in the physical status, demonstrated by hypotonia, bradycardia and regardless of the reanimation, died.

DISCUSSION: Ruptured aneurysm of abdominal aorta has high mortality. Despite the immediate surgical treatment and adequate substitution, the patient had lethal outcome.

CONCLUSION: Ruptured abdominal aortic aneurysm has high 30-day mortality, ranging between 22,9%-65,9%. In cases of acute renal and/or liver failure following the surgical treatment and when haemodialysis is needed, mortality rate can reach up to 89%.

***Keywords**: Ruptured Abdominal Aortic Aneurysm (rAAA), Suprarenal cross-clamping, Postoperative complications of rAAA*

INTRODUCTION

An Abdominal Aortic Aneurysm (AAA) is a localized dilatation and weakening of the abdominal aorta, as it`s infrarenal part is most commonly affected by the disease (Golledge, J. 2006). The most common diameter (evaluated by Ultrasound imaging or Computer-tomography (CT) angiography) of infrarenal aorta, defined as AAA is ≥ 30mm or 1,5 times of normal aortic diameter with involvement of all three anatomic layers of the aortic wall (Lederle, F. A. 1997; Roy R.A. 2022).

The most important risk factors are male sex , smoking and old age, whereas interestingly, diabetes mellitus is a negative risk factor for AAA(Golledge, J. 2006; Lederle, F. A. 1997; Singh, K., 2001; Jamrozik, K., 2000. Cornuz J., 2004). Three phases are distinguished in disease progression: development, growth and rupture. Aneurysmal growth can be associated with upregulated matrix-metalloproteinases in aortic wall and increased plasma protein concentration in both the aneurysmal wall and the intraluminal thrombus (Tamarina, N. , 1997; Maguire, E. M., 2019; Behr Andersen, 2018).

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Risk of rupture

Size of the AAA is one of the strongest predictors of rupture, as aortic aneurysms above 5,5cm in diameter have markedly higher risk of rupture (Aggarwal, S., 2011). Estimated annual risk of rupture increases from 3%-15% for AAA with diameter 5,0cm-5,9cm to 30-50% for AAA bigger than 8,0cm (Brewster DC, 2003).

The expansion rate is another important factor in the risk of rupture (Gadowski GR, 1994; Bengtsson H, 1993). Diameter growth of AAA by 0,5cm for six months is considered in high risk of rupture (Hirsch AT, 2006). Continued smoking, uncontrolled hypertension and increased stress on the aortic wall are other factors, increasing the chance of rupture (Brewster DC, 2003).

Clinical manifestation

Most of the AAAs have no symptoms and are accidently found during ultrasonography, CT of the abdomen or magnetic resonance imaging, performed for different purposes. Symptomatic AAAs are with higher chance of rupture (Aggarwal, S., 2011). Abdominal aortic aneurysm symptoms are nonspecific, diffuse abdominal or lower back pain and pulsatile mass in the abdomen (Roy R.A. 2022). For up to 50% of the patients the rupture is the first clinical presentation of the AAA (Jeanmonod D, 2022).

Classis symptoms of ruptured AAA are suddenly occurred severe abdominal and/or back pain, arterial hypotension and pulsatile abdominal mass (Salata K. 2022).

Diagnostic imaging evaluation

Ultrasound imaging: When used in patients with abdominal and lower back pain or other symptom, consistent with ruptured AAA, ultrasound can identify AAA with high sensitivity and specificity(Knaut AL, 2005; Rubano E, 2013). Major disadvantages of this method are that it is operator dependent, also obesity and overlying bowel gas can hinder proper imaging of the aorta (Scott RA, 1991).

Computed tomography: When evidences of retroperitoneal hematoma and AAA on a CT are present, ruptured AAA is almost certain (Weinbaum FI, 1987). Contrast enhanced CT in those cases help plaining open surgical repair or EVAR (Salata K. 2022).

Preoperative management

When rAAA is suspected, the patient should be consulted with a vascular surgeon as soon as possible. Full blood count, biochemistry and haemostasis should be obtained, while the patient is on active monitoring (heart rate, blood pressure, oxygen saturation). At least 2 large venous lines should be placed, as well as Foley catheter. Aggressive fluid resuscitation should be avoided, because permissive hypotension of rAAA patients improves survival (Spahn DR, 2019). Systolic reassure ranging 70-80mmHg, maintaining consciousness, avoiding ST depression and reducing vital organ ischemia should be targeted (Dick F, 2013).

Surgical treatment

Open surgery is usually performed via a transperitoneal approach with midline laparotomy. Alternatively, a left retroperitoneal approach can be used. Aortic cross clamping should be performed below the renal arteries if possible. If the AAA extends above the renal arteries supraceliac clamping may be preferred, increasing rates of renal dysfunction and risk of multiorgan failure (Bown MJ, 2003). Distal clamping of iliac arteries should be assured before opening the aneurysmal sack to reduce the blood loss. After opening the sack rapid suture ligation of the lumbar arteries and inferior mesenteric artery (IMA) is necessary. Depending of the anatomy of the AAA and iliac arteries involvement tube graft is used to construct aorto-arotal bypass (in case of patient and not aneurysmal iliac arteries); Bifurcated graft for aorto-biliac or bifemoral bypass, in case of not patient, stenotic or aneurysmal iliac arteries.

PRBC and FFP transfusion is usually started after aortic clamping. Before declamping of the anastomoses, a close cooperation with anesthesiologists' team is required to avoid rapid decrease of blood pressure. After hematoma evacuation, a primary abdominal closure is usually used.

Complications after repair of rAAA

Local complications:

• Lower limb(s) ischemia due to graft thrombosis or distal embolization require rapid treatment – thrombectomy of the graft and affected limb(s) arteries;

• Ischemia of the colon – it is caused by combination of factors – ligation of IMA, internal iliac arteries occlusion, perioperative hypotension;

• Abdominal compartment syndrome – caused by large retroperitoneal haematomas and tissue oedema. In those cases, decompression laparotomy is recommended.

• Spinal ischemia – rare complication causing lower extremities paraparesis or paraplegia, due to interruption of pelvic blood supply, prolonged aortic clamping and perioperative hypotension.

Systemic complications:

• Cardiac – arrhythmia, congestive heart failure, myocardial infarction and cardiac arrest increase

mortality of rAAA(Johnston KW. 1994)

• Pulmonary – pulmonary failure, pneumonia and other respiratory complications develop in 36-41% of the cases of rAAA, most commonly resulted by chronic obstructive pulmonary disease (Edwards ST, 2014; Desgranges P, 2015)

• Renal – developing due to hypoperfusion, pre-existing renal disease, contrast media administration, suprarenal clamping, in cases of rAAA it can reach incidence of 26-45% (Edwards ST, 2014). The need of postoperative hemodialysis can reach 11-40%, with mortality rate of those patients varying between 76-89% (Edwards ST, 2014; Johnston KW, 1994; Harris LM, 1991)

• Hepatic – Liver failure in patients with rAAA is caused by hypoxic injury and is usually late complication. Unknown pre-existing liver dysfunction, combined with increased metabolic demands, due to hematoma absorption can lead to renal, cardiac, pulmonary and gastrointestinal disfunctions with a mortality rate of 83% (Hermreck AS, 1997; Maziak DE, 1998; Salata K. 2022).

• Multiorgan failure - Clinical data indicate, that a suprarenal clamping and duration of clamping are associated with higher risk of MOF and death (Mehta M., 2005; El- Sabrout RA, 2001). The incidence of MOF can reach up to 64% in cases of rAAA (Visser JJ, 2009).

Inhospital mortality:

Thirty-day mortality of rAAA is ranging from 22,9%-65,9% (Lieberg J, 2018; Karthikesalingam, A., 2014)

CASE REPORT

Male patient, 81 years of age, with several concomitant disease – congestive heart failure, cerebro-vascular disease. The patient was diagnosed with AAA 4 months prior to the rupture. The maximal diameter of the AAA was 15,6cm with length of 15,5cm, iliac arteries were not affected by the disease. The patient refused suggested surgical or endovascular treatments, discharged on conservative treatment.

The patient presented at ER four months later with acute pain in the abdomen and back, sweating and tingles in the limbs.

Clinical status: severe pain in the abdomen, during the examination, adequate psychological behaviour, blood pressure 96/57, Hgb 102 g/l with Haematocrit -0.331 l/l. Emergent consultation with vascular surgeon was conducted. On the CT-angiography rupture of AAA was verified with massive retroperitoneal haematoma, occluded right renal artery and aneurysm of the left renal artery.

After signing the informed consent, an emergent vascular reconstruction was undertaken.

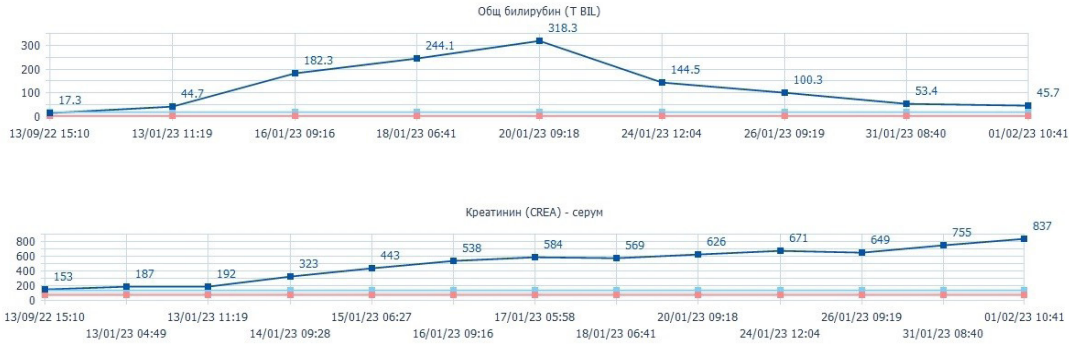
Under common anaesthesia median laparotomy was conducted. Laparotomy revealed huge retroperitoneal hematoma. Aneurysmal neck was clamped above renal arteries, with clamping time – 30 minutes, for constructing the proximal anastomosis. After recalmping an aorto-bifemoral bypass was constructed with silver knitted dacron prosthesis 22/11mm.

Postoperatively the patient was transferred to intensive care unit (ICU) for monitoring with stabile blood pressure and heart rate, varying between RR 110-130/7-80 mmHg, FR 60-75/min. In the course of ICU treatment, the patient was inadequate and lacked spontaneous diuresis. A temporary catheter for haemodialysis was placed for initiating haemodialysis.

He was transferred in clinic of vascular surgery after stabilizing blood oxygen saturation. The patient was inadequate at the time of transfer with jaundice present.

The initiated haemodialysis was continued on regular basis one time/ daily. The patient restored

bowel passage, hepatoprotectors were administered, physiotherapy was initiated.



On the 20th postoperative day, the patient had rapid decline in the physical status, demonstrated by hypotonia, bradycardia and regardless of the reanimation, died.

DISCUSSION

Ruptured aneurysm of abdominal aorta has high mortality (Lieberg J, 2018; Karthikesalingam, A., 2014). Despite the immediate surgical treatment and adequate substitution, the patient had a lethal outcome. Patients with AAA in diameter above 55mm, refusing to undergo conventional or endovascular treatment should be held in supervision as this way local or systemic complications might be avoided.

CONCLUSION

Ruptured abdominal aortic aneurysm has high 30-day mortality, ranging between 22,9%-65,9% (Lieberg J, 2018; Karthikesalingam, A., 2014). In cases of acute renal and/or liver failure following the surgical treatment and when haemodialysis is needed, the mortality rate can reach up to 89% (Edwards ST, 2014; Johnston KW, 1994; Harris LM, 1991, Hermreck AS, 1997; Maziak DE, 1998; Salata K. 2022). Short neck AAA or such involving renal arteries should be intervened after careful planning so the risks of local or systemic complications can be minimized.

REFERENCES

Golledge, J., Muller, J., Daugherty, A. & Norman, P. Abdominal aortic aneurysm: pathogenesis and implications for management. Arterioscler. Thromb. Vasc. Biol. 26, 2605–2613 (2006)

Lederle, F. A. et al. Prevalence and associations of abdominal aortic aneurysm detected through screening. Ann. Intern. Med. 126, 441–449 (1997)

ROY RISHI A., ERIC YATES PRUITT, and GILBERT R. UPCHURCH JR (2022). Aortoiliac Aneurysms: Evaluation, Decision Making, and Medical Management. In P. B. Sidawy AN, Rutherford's Vascular Surgery and Endovascular Therapy. 10th ed. (pp. 914-924). Philadelphia PA: Elsevier.

Singh, K., Bønaa, K. H., Jacobsen, B. K., Bjørk, L. & Solberg, S. Prevalence of and risk factors for abdominal aortic aneurysms in a population- based study: theTromsø study. Am. J. Epidemiol. 154, 236–244 (2001)

Jamrozik, K. et al. Screening for abdominal aortic aneurysm: lessons from a population- based study.Med. J. Aust. 173, 345–350 (2000)

Cornuz J, Pinto CS, Tevaearai H, Egger M. Risk factors for asymptomatic abdominal aortic aneurysmSystematic review and meta- analysis of population- based screening studies. Eur J Public Health. 2004; 14:343–349

Tamarina, N. A., McMillan, W. D., Shively, V. P., & Pearce, W. H. (1997). Expression of matrix metalloproteinases and their inhibitors in aneurysms and normal aorta. Surgery, 122(2), 264–272. https://doi.org/10.1016/s0039-6060(97)90017-9

Maguire, E. M., Pearce, S. W. A., Xiao, R., Oo, A. Y., & Xiao, Q. (2019). Matrix Metalloproteinase in Abdominal Aortic Aneurysm and Aortic Dissection. Pharmaceuticals (Basel, Switzerland), 12(3), 118. https://doi.org/10.3390/ph12030118

Behr Andersen, C., Lindholt, J. S., Urbonavicius, S., Halekoh, U., Jensen, P. S., Stubbe, J., Rasmussen, L. M., & Beck, H. C. (2018). Abdominal Aortic Aneurysms Growth Is Associated With High Concentrations of Plasma Proteins in the Intraluminal Thrombus and Diseased Arterial Tissue. Arteriosclerosis, thrombosis, and vascular biology, 38(9), 2254–2267. https://doi.org/10.1161/ATVBAHA.117.310126

Aggarwal, S., Qamar, A., Sharma, V., & Sharma, A. (2011). Abdominal aortic aneurysm: A comprehensive review. Experimental and clinical cardiology, 16(1), 11–15.

Brewster DC, Cronenwett JL, Hallett JW, Jr, et al. Guidelines for the treatment of abdominal aortic aneurysms. Report of a subcommittee of the Joint Council of the American Association for Vascular Surgery and Society for Vascular Surgery.

J Vasc Surg. 2003;37:1106–17
Gadowski GR, Pilcher DB, Ricci MA. Abdominal aortic aneurysm expansion rate: Effect of size and beta-adrenergic blockade. J Vasc Surg. 1994;19:727–31.
Bengtsson H, Bergqvist D, Ekberg O, Ranstam J. Expansion pattern and risk of rupture of abdominal aortic aneurysms that were not operated on. Eur J Surg. 1993;159:461–7
Hirsch AT, Haskal ZJ, Hertzer NR, et al. ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): A collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): Endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; Trans-Atlantic Inter-Society Consensus; and Vascular Disease Foundation. Circulation. 2006;113:e463–e654
Jeanmonod D, Yelamanchili VS, Jeanmonod R. Abdominal Aortic Aneurysm Rupture. [Updated 2022 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459176/
SALATA K. and LINDSAY T. F. (2022). Ruptured Aortoiliac Aneurysms and their Management. In P. B. Sidawy AN, Rutherford's Vascular Surgery and Endovascular Therapy. 10th ed. (pp. 976-994). Philadelphia PA: Elsevier
Knaut AL, et al. Ultrasonographic measurement of aortic diameter by emergency physicians approximates results obtained by computed tomography. J Emerg Med. 2005;28(2):119–126.
Rubano E, et al. Systematic review: Emergency department bedside ultrasonography for diagnosing suspected abdominal aortic aneurysm. Acad Emerg Med. 2013;20(2):128–138.
Scott RA, Ashton HA, Kay DN. Abdominal aortic aneurysm in 4237 screened patients: Prevalence, development and management over 6 years. Br J Surg. 1991;78:1122–5
Weinbaum FI, et al. The accuracy of computed tomography in the diagnosis of retroperitoneal blood in the presence of abdominal aortic aneurysm. J Vasc Surg. 1987;6(1):11–16.
Spahn DR, et al. The European guideline on management of major bleeding and coagulopathy following trauma: Fifth edition. Crit Care. 2019;23(1):98.
Dick F, et al. Delayed volume resuscitation during initial management of ruptured abdominal aortic aneurysm. J Vasc Surg. 2013;57(4):943–950.
Bown MJ, et al. The systemic inflammatory response syndrome, organ failure, and mortality after abdominal aortic aneurysm repair. J Vasc Surg. 2003;37(3):600–606
Johnston KW. Ruptured abdominal aortic aneurysm: Six- year follow-up results of a multicenter prospective study. Canadian Society for Vascular Surgery Aneurysm Study Group. J Vasc Surg. 1994;19(5):888–900.
Edwards ST, et al. Comparative effectiveness of endovascular versus open repair of ruptured abdominal aortic aneurysm in the medicare population. J Vasc Surg. 2014;59(3):575–582.
Desgranges P, et al. Editor's choice - ECAR (Endovasculaire ou Chirurgie dans les Anevrysmes aorto- iliaques Rompus): A French randomized controlled trial of endovascular versus open surgical repair of ruptured aorto- iliac aneurysms. Eur J Vasc Endovasc Surg. 2015;50(3):303–310
Johnston KW. Ruptured abdominal aortic aneurysm: Six- year follow-up results of a multicenter prospective study. Canadian Society for Vascular Surgery Aneurysm Study Group. J Vasc Surg. 1994;19(5):888–900
Harris LM, et al. Ruptured abdominal aortic aneurysms: Factors affecting mortality rates. J Vasc Surg. 1991;14(6):812–818; discussion 819–820
Hermreck AS, et al. Severe jaundice after rupture of abdominal aortic aneurysm. Am J Surg. 1977;134(6):745–748.
Maziak DE, et al. The impact of multiple organ dysfunction on mortality following ruptured abdominal aortic aneurysm repair. Ann Vasc Surg. 1998;12(2):93–10
Mehta M, et al. Factors associated with abdominal compartment syndrome complicating endovascular repair of ruptured abdominal aortic aneurysms. J Vasc Surg. 2005;42(6):1047–1051
El- Sabrout RA, et al. Suprarenal or supraceliac aortic clamping during repair of infrarenal abdominal aortic aneurysms. Tex Heart Inst J.2001;28(4):254–264
Visser JJ, et al. Prediction of 30- day mortality after endovascular repair or open surgery in patients with ruptured abdominal aortic aneurysms. J Vasc Surg. 2009;49(5):1093–1099
Lieberg J, Pruks L-L, Kals M, Paapstel K, Aavik A, Kals J. Mortality After Elective and Ruptured Abdominal Aortic Aneurysm Surgical Repair: 12-Year Single-Center Experience of Estonia. Scandinavian Journal of Surgery. 2018;107(2):152-157. doi:10.1177/1457496917738923
Karthikesalingam, A., Holt, P. J., Vidal-Diez, A., Ozdemir, B. A., Poloniecki, J. D., Hinchliffe, R. J., & Thompson, M. M. (2014). Mortality from ruptured abdominal aortic aneurysms: clinical lessons from a comparison of outcomes in England and the USA. Lancet (London, England), 383(9921), 963–969. https://doi.org/10.1016/S0140-6736(14)60109-4

Boyko, A., & Velizar, H. (2023). Comparative analysis of conventional and minimally invasive techniques in the treatment of femoral hernias, *MEDIS - Medical Science and Research, 2*(1), 21-24. doi: 10.35120/medisij020121a
UDK: 616.718.4-007.43-089.819

COMPARATIVE ANALYSIS OF CONVENTIONAL AND MINIMALLY INVASIVE TECHNIQUES IN THE TREATMENT OF FEMORAL HERNIAS

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Abstract: Despite the advances in medicine regarding diagnosis and therapeutic options, the treatment of femoral hernias remains a challenge for the surgeon. In the past, this type of hernia was repaired with conventional surgery. Today, mini-invasive techniques are preferred method of choice by many surgeons.

The aim of our study is to compare the results of open and minimally invasive surgery in patients with femoral hernias.

In the present study we include patients with primary unilateral femoral hernias operated in the Surgical Department of EUROHOSPITAL- Plovdiv and the Department of General Surgery of University Hospital "St. George" for the period January 2018 to December 2022. The patients were divided into two groups - conventional and minimally invasive operations. Comparative analysis was performed regarding sex, age, duration of operative intervention, postoperative pain, hospital stay and complications.

33 patients in total were diagnosed and operated for femoral hernias for a period of 5 years. Of these, 16 patients were operated conventionally and 17 underwent laparoscopic surgery. Patients in the minimally-invasive group had a significantly shorter hospital stay (2.1 vs 3.0 days) and less postoperative pain (1.8 vs 3.6 on the 1 to 5 scale). The incidence of the postoperative complications was similar in both groups.

Surgery is the only method of treatment of femoral hernias. Nowadays, minimally invasive techniques became a method of choice compared with conventional surgery. We believe that laparoscopic operations for femoral hernia, performed by experienced surgeon, are effective and safe and could be applied to all type of patients.

Keywords: *Femoral hernia; Laparoscopic surgery; TAPP.*
Field: Medical Sciences and Health

INTRODUCTION

Hernia repair is one of the most frequently performed benign surgical interventions. Annually, over 20 million operations for hernias are performed worldwide. According to NSI data, for 2020 in Bulgaria 12,754 patients were treated electively or emergency with this pathology. Operations for hernias comprise approximately 2.4% of all surgeries for that period of time. (Hammoud M, Gerken J. (2022), NSI (2021)). Femoral hernias, on the other hand, are rare and consist about 3% of all types of hernias. Surgery remains the best method of treatment of these pathology. In the past, herniorrhaphy was considered the gold standard. (Goethals A et al. (2022)) However, nowadays, more preferences are being given to minimally invasive methods. (The HerniaSurge Group. (2018)) Key factors for that are the relatively shorter learning curve and better postoperative results. Preperitoneal or transabdominal approach in the treatment of femoral hernias is still matter of debate among surgeons.

MATERIALS AND METHODS

The aim of our study is to present and analyze our experience in planned and emergency operative treatment of femoral hernias for the period January 2018 to December 2022. Inclusion criteria were patients with primary unilateral femoral hernias and age over 18 years. In all patients, a detailed anamnesis was taken, physical status was examined, standard laboratory tests and ultrasound were performed. According to the urgency of the condition, in some patients an X-ray of the abdomen and a

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CT scan were additionally performed. We recorded the type of primary surgery performed (hernia repair with or without mesh), with or without laparoscopy performed, the length of hospital stay, postoperative pain and complications.

RESULTS

33 patients with femoral hernias were operated in the surgical departments of UMHAT Eurohospital and UMHAT St George for a period of 5 years. The distribution of patients by gender was following: 5 men and 18 women (ratio 1:3,6) . We used a minimally invasive approach in 17 cases (52%). The mean age of the patients in that group was 55.3 years ± 19.4 (range 28-85). For conventionally operated patients it was 47.0 years ± 16.56 (range 32-80) (p = 0.189). Regarding the timing of surgery, 24 operations were elective and 9 were emergency (27%). The average operative time of the miniinvasive surgeries was longer - 59.6 min ± 8.56 (range 45-80). For the open surgeries it was 51 min ± 9.57 (range 30-135) p = 0.683. Regarding postoperative pain (according to the five-point system, where 5 is the strongest pain), lower pain index was reported in the group of laparoscopic operations (1.6 ± 0.62). For the open operations it was 3.2 ± 1.01 (p = 0.013). Comparative analysis that we performed regarding the hospital stay did not show significant differences in both groups (2.1 days ± 0.34 for LS) vs (3 days ± 1.21 p = 0.134 for OP). Regarding the postoperative complications we had 1 wound suppuration and 1 seroma in LS group vs 1 wound suppuration, 1 seroma , 1 hematoma in Open Group (p = 0.217).

We have zero mortality rate for this type of surgical procedures.

Distribution of patients regarding gender and timing of surgery is shown in Fig. 1. Distribution of the patients regarding the type of surgical intervention is shown in Fig. 2.

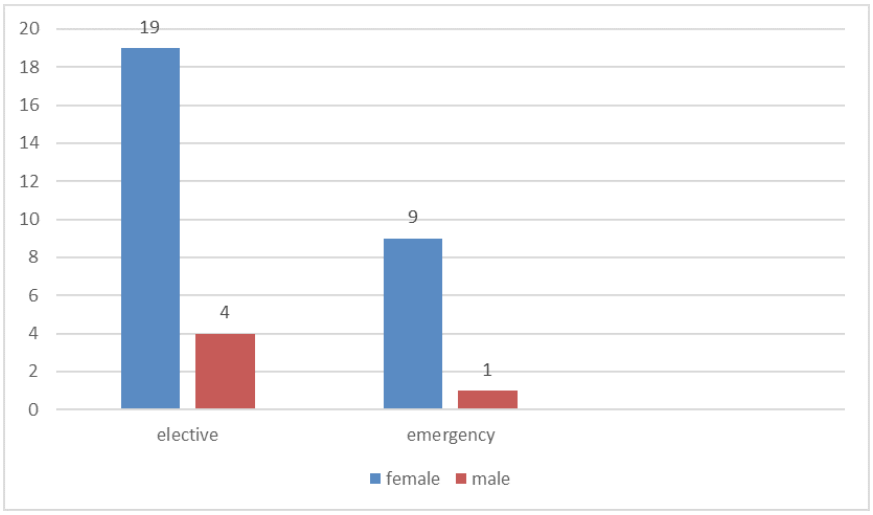

Fig.1 Distribution of patients regarding gender and timing of surgery

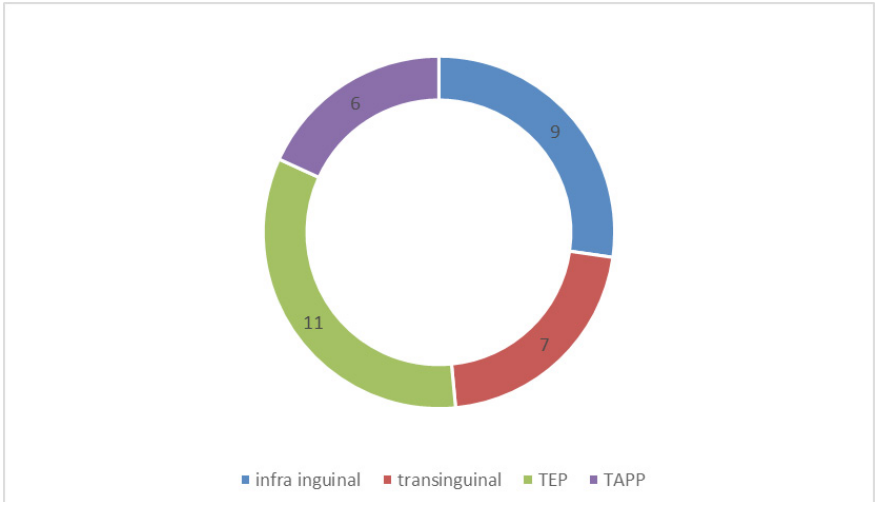

Fig.2 Distribution of the patients regarding the type of surgical intervention

DISCUSSION

The accurate diagnosis and choosing the most appropriate surgical technique remain a challenge for all surgeons. The frequency of femoral hernias reaches 5% of all diagnosed abdominal hernias and takes second place after inguinal hernias. According to the literature, they occur more often in women, which was confirmed in our study as well (n-28; 85 %). Surgery is the only method of definitive treatment for femoral hernias. (van Veenendaal et al.(2020), Coelho et al.(2021), Peitsch WK. (2014)) Three classical approaches are known for this type of pathology: infra-, trans- and suprainguinal. In addition to standard repair with own tissues, there are also tension free techniques with mesh. On the other hand, the diagnostic and therapeutic advantages of laparoscopy make it a surgical method of choice for hernias. (Sorelli P et al..(2009), Białecki J et al.(2021), Lockhart K et al.(2018)) In our series, 52% (n=17) of the patients underwent minimally invasive hernioplasty. A limiting factor in our study was the various teams with their surgical techniques that performed the operations. The most common surgery we performed in our patients was totally extraperitoneal inguinal hernia repair - TEP (33.3%). We did not find any statistically significant differences comparing open and minimally invasive hernioplasty groups regarding gender, age, complications and operative time ($p>0.05$). In our study we found that the laparoscopic procedures were of greater advantage than open surgery in terms of pain intensity and the hospital stay ($p = 0.013$). Due to the incomplete follow-up of our patients operated for femoral hernias an analysis regarding the recurrence rate in both groups was not performed.

CONCLUSIONS

The diagnosis and operative treatment of femoral hernias remain a challenge for all surgeons. Nowadays, minimally invasive techniques became a method of choice compared with conventional surgery. We believe that laparoscopic operations for femoral hernia, performed by experienced surgeon in leading hernia centers, are effective and safe. These types of operations have shorter hospital stay, less pain in the postoperative period, low recurrence rate and could be applied to all type of patients.

REFERENCES

Białecki J, Pyda P, Antkowiak R, Domosławski P. (2021) Unsuspected femoral hernias diagnosed during endoscopic inguinal hernia repair. Adv Clin Exp Med. 30(2):135-138. doi: 10.17219/acem/130357. PMID: 33636062.

Coelho JCU, Hajar FN, Moreira GA, Hosni AVE, Saenger BF, Aguilera YSH, Costa MARD, Claus CMP.(2021) FEMORAL HERNIA: UNCOMMON, BUT ASSOCIATED WITH POTENTIALLY SEVERE COMPLICATIONS. Arq Bras Cir Dig. 15;34(2):e1603. doi: 10.1590/0102-672020210002e1603. PMID: 34669892; PMCID: PMC8521781.

Goethals A, Azmat CE, Adams CT. (2022) Femoral Hernia. Book.StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; https://www.ncbi.nlm.nih.gov/books/NBK535449/

Hammoud M, Gerken J. (2022) Inguinal Hernia. Book. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 30020704.

Lockhart K, Dunn D, Teo S, Ng JY, Dhillon M, Teo E, van Driel ML. (2018) Mesh versus non-mesh for inguinal and femoral hernia repair. Cochrane Database Syst Rev. 13;9(9):CD011517. doi: 10.1002/14651858.CD011517.pub2. PMID: 30209805; PMCID: PMC6513260.

NSI (2021), Zdraveopazvane, p 72. ISSN 1313-1907

Peitsch WK. (2014) A modified laparoscopic hernioplasty (TAPP) is the standard procedure for inguinal and femoral hernias: a retrospective 17-year analysis with 1,123 hernia repairs. Surg Endosc. 28(2):671-82. doi: 10.1007/s00464-013-3208-9. Epub 2013 Sep 17. PMID: 24043647.

Sorelli PG, El-Masry NS, Garrett W V.(2009) Open femoral hernia repair: One skin incision for all. World J Emerg Surg.4(1):3-5.

The HerniaSurge Group. (2018) International guidelines for groin hernia management. Hernia 22, 1–165. https://doi.org/10.1007/s10029-017-1668-x

van Veenendaal, N., Simons, M., Hope, W. et al. (2020). Consensus on international guidelines for management of groin hernias. Surg Endosc 34, 2359–2377 https://doi.org/10.1007/s00464-020-07516-5

Predrad, D. et al. (2023). Treatment of fractures of the distal end of the humerus in children using тhe method of percutaneous osteosynthesis - our findings, *MEDIS - Medical Science and Research, 2*(1), 25-30. doi: 10.35120/medisij020125p
UDK: 616.717.4-001.5-089.84

TREATMENT OF FRACTURES OF THE DISTAL END OF THE HUMERUS IN CHILDREN USING THE METHOD OF PERCUTANEOUS OSTEOSYNTHESIS - OUR FINDINGS

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Abstract: Introduction: Fractures of the distal end (capitulum and trochlea) of the humerus are more common in children than adults. The mechanism of injury demonstrates that the fracture occurs when falling on an outstretched arm, where the protective mechanisms cannot prevent the transfer of energy to the bone, leading to breaking the continuity of the bone, i.e. fracture. Our paper aims to present our experience in percutaneous osteosynthesis of the distal end of the humerus, where we have excellent functional results and 100% healing of the fracture.

Methods: Percutaneous osteosynthesis has been applied to 17 patients. The patients were divided into groups depending on the type of fracture, namely: one group of patients with supracondylar and another group of patients with a condylar fracture of the distal end of the humerus.

Results: It has been proven that percutaneous osteosynthesis of the distal end of the child's humerus led to fracture union in 100% of cases, which is a consequence of the marked stability of the osteosynthetic material. Functional results obtained after 5-6 weeks of rehabilitation are excellent in 99% of cases, except for one patient with a supracondylar fracture where we have a delay of flexion movement by -10 degrees. It should be noted that we had only one complication with a single patient suffering from a dislocation of bone fragments.

Conclusion: If it is possible to perform this method, percutaneous osteosynthesis is the preferred method in treating fractures of the distal end of the humerus among the paediatric population.

Keywords: *fracture, distal humerus, percutaneous osteosynthesis.*
Field: Medical Sciences and Health

Introduction

Fractures of the distal end of the humerus are more common in children than adults. The mechanism of injury demonstrates that the fracture occurs when falling on an outstretched arm, where the protective mechanisms cannot prevent the transfer of energy to the bone, leading to a break in the continuity of the bone, i.e. fracture. Fractures of the distal end of the humerus were divided into supracondylar, condylar, and transcondylar. The supracondylar fracture of the humerus is the most common of all fractures and accounts for 18% of all fractures in paediatrics or 60% of all elbow fractures. The most frequent is the extension type, very often associated with dislocation of the elbow joint(1,2).

Classification of the supracondylar fractures:

Modern literature uses a modified Gartland classification of supracondylar fractures of the distal end of the humerus.

Modified Gartland Classification ofSupracondylar Fractures

Comments

Type I Undisplaced Fat pad present acutely

Type II Hinged posteriorly Anterior humeral line anterior to capitellum

Type III Displaced No meaningful cortical continuity

Type IV Displaces into extension and flexion Usually diagnosed with manipulation under fluoroscopic imaging

Medial comminution Collapse of medial column Loss of Baumann's angle

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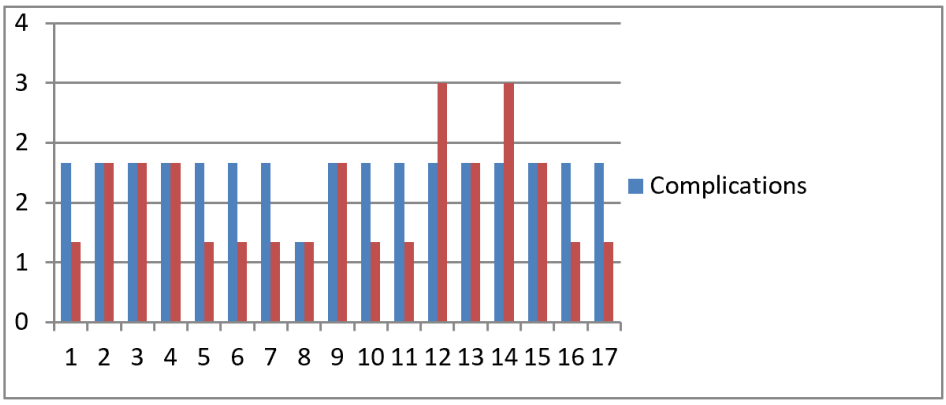
(not truly a separate type)



Picture 1.Classification of the fracture

Transcondylar fractures:

Unlike supracondylar fractures, transcondylar fractures of the distal end of the humerus are less common. The fracture line in this type of fracture passes through both condyles (medial and lateral), with the lower fragment acting as an independent part. Repeatedly this type of fracture is associated with dislocation of the radius and ulna (3,4)

Classification of transcondylar fractures:

Toniolo and Wilkins propose a simple classification of T — transcondylar fractures of the distal end of the humerus based on the degree of dislocation and comminution of the fragments, namely:

· Type I fractures are minimally displaced (Fig. 17-3A, B, C).

· Type II fractures are displaced but do not have comminution of the metaphyseal fragments (Fig. 17-4AB).

· Type III fractures are displaced fractures with comminution of the metaphyseal fragments (Fig. 17-5A-F).

Condylar fractures:

For condylar fractures of the distal end of the humerus, we use the Milch classification based on the presence or absence of a fracture line through (Type 1) or around (Type 2) the ossification center of the capitulum.

THE AIM OF THE PAPER

The paper aims to present our experience in treating fractures of the distal humerus with percutaneous osteosynthesis.

METHODOLOGY

At the Surgery Clinic's Department for Orthopaedic Surgery and Traumatology, Medical Center of KosovskaMitrovica, 27 patients with fractures of the distal end of the humerus were treated. Seven patients were treated non-operatively, and 3 patients were subjected to surgery. In our work, we analyzed 17 patients with a fracture of the distal end of the humerus who were treated with percutaneous osteosynthesis as a definitive treatment method. Indications for percutaneous osteosynthesis are: compromised elbow neurocirculatory report, unsuccessful reduction of Gartland type I fractures, as well as Gartland type II fractures, and unstable and dislocated condylar fractures of the distal end of the humerus. After hospitalization, a clinical and radiological examination was used to assess the stability of the fracture, as well as the need for operative treatment.

Surgical techniques of percutaneous osteosynthesis of the distal humerus

Closed reduction

We start implementing the closed reduction with longitudinal traction and 'milking' of the surrounding soft tissue. The elbow is in slight flexion. This position provides us access to the physiological length of the arm. After that, we approach the correction of varus/valgus angulation and mediolateral translation of the distal fragment of the humerus. If there is also deformation in how the fragments are rotated, we

apply pronation or supination with a varus/valgus component. With pronation, we achieve the correction of internal rotation, while supination helps us correct external rotation. Observing the position of the fragments in the sagittal plane, we apply hyperflexion of the elbow in the extension type of fracture, while in the flexion type, we apply for an extension. We use the "joystick" technique as a reduction option for very unstable fractures (5,6).

Percutaneous osteosynthesis technique

The mobile X-ray machine with an inverted arch is used in percutaneous osteosynthesis of the children's elbow. This technique was first introduced by Charnley in 1954. For osteosynthesis, we use Kirschner needles, which are applied percutaneously, most often from the lateral side, where the medial cortex must also be covered. Using this technique avoids iatrogenic injuries of the ulnar nerve. We apply two or three Kirschner needles, parallel or divergent. With the lateral application of Kirschner needles, the entry point at the level of the capitulum gives us a more stable fixation of the fracture and the stability of the osteosynthetic material compared to the entry point that is directly on the lateral side. (7). If the fracture is very unstable, we can also use the technique of transolecranon application of the needle, in which 4 cortices are included. The Kirschner needles we use for percutaneous osteosynthesis of the distal humerus are 2 mm in diameter. They show much better functional results compared to needles with a diameter of 1mm (8).

Convergent application of needles improves the stability of the fracture and osteosynthetic material but increases the possibility of iatrogenic injuries to the ulnar nerve. It is also possible to use the medial application of needles, but then an incision should first be made on the medial side of the distal humerus in order to protect the ulnar nerve (9,10). Medial application of needles is accompanied by a high risk of nerve injury and requires considerable surgical experience. Therefore, during the application, it is necessary to bend the elbow in the extension position, which moves the nerve to the posterior position.

Open reduction and percutaneous osteosynthesis

Open reduction is applied when the anatomical position of bone fragments cannot be obtained by closed reduction. The soft tissue and vascular structures of the elbow are trapped in the fracture gap between the fragments, making it difficult or impossible to return the fragments to their anatomical position. We also use the open method for all types of open fractures of the distal end of the humerus. Due to the absence of a radial pulse or reduced perfusion of the arm after reduction and fixation, recommendations in literature suggest thatthe dissection of the brachial artery should be performed to detect if there is an interruption of blood flow. However, this method is controversial. For open reduction, we use an anterior approach to the elbow, and as an alternative, a medial, lateral, or posterior approach (11,12).

RESULTS

In our study, there were 9 male patients (53%) and 8female patients (47%). By age structure, 4patients (22%) were under 5years old, 11 patients (64%) were between 5-10 years old, and 3patients (14%) were over 10 years old. Nine patients (53%) had a supracondylar fracture of the distal humerus (2 boys and 7 girls), 6 patients (35%) with a transcondylar fracture (5 boys and 1 girl), and 2patients (12%) with a condylar fracture (2 boys). (Diagram 1)

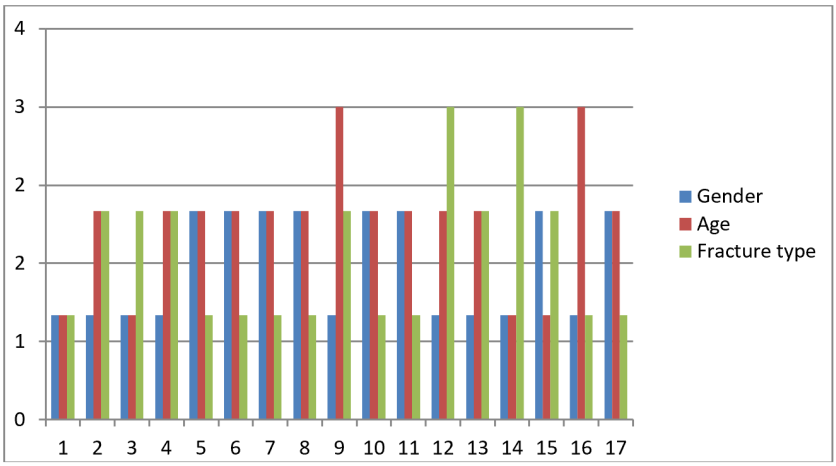


Diagram 1. Gender and age structure of patients and type of fracture.

In diagram 2, we have shown the connection between complications and the type of fracture. Out of 17 patients, we had one complication in a female patient with a supracondylar fracture of the distal end of the humerus. Namely, on the third day after the operation, the osteosynthetic material loosened, after which a revision was performed. (Diagram 2)

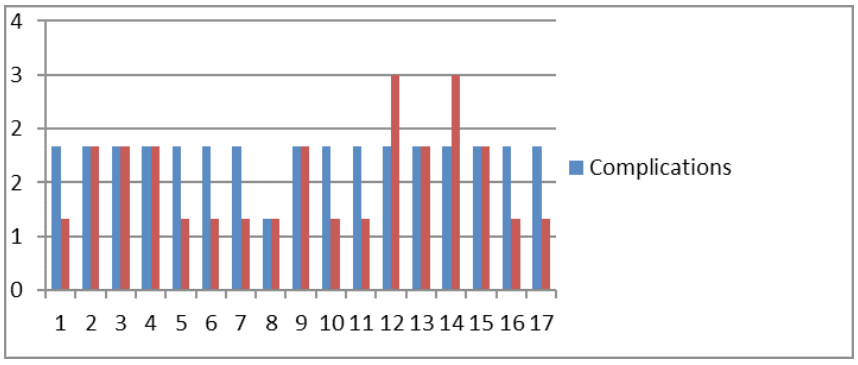


Diagram 2. Fracture type and complications

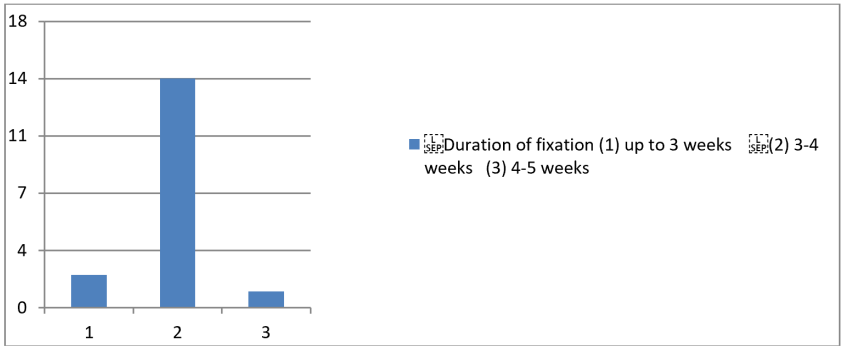


Diagram 3.The duration of the fixation.

In 2 patients (10%), the duration of fixation lasted up to 3 weeks, in 14 patients (85%), the duration lasted between 3 and 4 weeks and in one patient (5%), the duration lasted between 4 and 5 weeks. (Diagram 3)

Movement of the elbow	Supracondylar type of fracture	Transcondylar type of fracture
Extension (N)	Full	Full
Flexion (N)	-10 °	Full
Internal Rotation (pronation)	Full	Full
External Rotation (supination)	Full	Full

Table 1 Movements in the elbow joint after completion of orthopaedic and physical treatment

After completing the operative treatment of the fracture of the distal end of the humerus, excellent functional results have been obtained in 99% of cases. Complete movements of extension, pronation, and supination were present in all patients treated with percutaneous osteosynthesis. In one patient with a supracondylar fracture of the distal end of the humerus, we have a delay of the flexion movement in the elbow joint by -10 degrees. In that same patient, we had a complication earlier in the form of the dislocation of bone fragments. (Table 1)

The average length of rehabilitation for patients with a fracture of the distal humerus is 5-6 weeks.

DISCUSSION

The central outcome of operative treatment of supracondylar and transcondylar fractures is the anatomical reduction and stable fixation without Bowman's angle deformity. It is performed by the method of closed reduction and percutaneous fracture osteosynthesis. Inadequate osteosynthesis and procedural errors are correlated with distal humerus defects. In the literature, several studies are listed, that prove that convergent fixation provides greater stability than divergent fracture fixation, but as a result, has a higher risk of iatrogenic injuries on nervusulnaris. Bloom et al. proved that three divergent Kirschner needles provide the same stability as two convergent ones. Kocher et al. proved that there is no significant difference between the divergent and convergent osteosynthesis techniques in terms of radiographic and clinical outcomes. In a randomized study, Blanco et al. found no significant radiological differences comparing these two surgical techniques (13). In our work, we described the operative treatment of the distal humerus (supracondylar and transcondylar fractures) by the method of percutaneous osteosynthesis, in which we applied the Kirschner needles convergently and covered the second cortex during the process. The clinical results of all 17 patients showed that the osteosynthesis was stable with one complication (fragment dislocation after three days), which is in line with the existing literature. The functional results showed us a 99% success rate of treatment with one contracture in the elbow joint in terms of reducing the degree of flexion.

CONCLUSION

If it is possible to perform this method, percutaneous osteosynthesis is the preferred method in treating fractures of the distal end of the humerus among the paediatric population.

REFERENCES

Ardawatia, G., Waghela, A.B., & Ranade, A.S. (2021). Intraoperative Kirschner Wire Breakage in a Pediatric Supracondylar Humerus Fracture.Cureus.;13(3):e13794. doi: 10.7759/cureus.13794. PMID: 33842167; PMCID: PMC8027960.

Banshelkikar, S., Sheth, B., Banerjee, S., & Maaz, M. (2020), Functional outcome of supracondylar humerus fracture in children with the use of pin configuration as per Bahk classification. J ClinOrthop Trauma;13:78-81. doi: 10.1016/j.jcot.2020.08.012. PMID: 33717880; PMCID: PMC7920002.

Ersan, O., Gonen, E., İlhan, R.D., Boysan, E., & Ates, Y. (2012). Comparison of anterior and lateral approaches in the treatment of extension-type supracondylar humerus fractures in children. J PediatrOrthop B.:121-6. [PubMed]

Gottschalk, H.P,, Sagoo, D., Glaser, D., Doan, J., Edmonds, E.W., & Schlechter, J. (2012). Biomechanical analysis of pin placement for pediatric supracondylar humerus fractures: does starting point, pin size, and number matter? J PediatrOrthop. 32(5):445-51. [PubMed]

Hope, N., & Varacallo, M., (2022) . Supracondylar Humerus Fractures. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; PMID: 32809768.

Iobst, C.A., Bunhor, D., Skaggs, D.L., & Frick, S.L. (2018). Intra-Operative Bone Stability Test. Tech Orthop.;33(4):279-282. doi: 10.1097/BTO.0000000000000278. Epub 2017 Dec 25. PMID: 30542232; PMCID: PMC6250262.

Kasirajan, S., Govindasamy, R., Sathish, B.R.J., & Meleppuram, J.J. (2018). Trans-olecranon fossa four-cortex purchase lateral pinning in displaced supracondylar fracture of the humerus - a prospective analysis in 48 children. Rev Bras Ortop. 53(3):342-349. [PMC free article] [PubMed]

Novais, E.N., Andrade, M.A., & Gomes, D.C. (2013). The use of a joystick technique facilitates closed reduction and percutaneous fixation of multidirectionally unstable supracondylar humeral fractures in children. J PediatrOrthop;33(1):14-9. [PubMed].

Nunes, M.C., Posser, T.D., Israel, C.L., Spinelli, L.F., Calieron, L.G., & Kim, J.H (2019).. Biomechanical Analysis of Two Types of Humerus Supracondylar Fracture Fixation in Anatomical Model. Rev Bras Ortop (Sao Paulo). 261-267. doi: 10.1055/s-0039-1688756. Epub 2019 Jun 27. PMID: 31363279; PMCID: PMC6597430.

Pannu, G.S., Eberson, C.P,, Abzug, J., Horn, B.D., Bae, D.S., & Herman, M. (2016). Common Errors in the Management of Pediatric Supracondylar Humerus Fractures and Lateral Condyle Fractures. Instr Course Lect385-97. [PubMed]

Shah, M., & Agashe, M.V. (2020). Supracondylar Humerus Fractures: Classification Based Treatment Algorithms. Indian J Orthop.;55(1):68-80. doi: 10.1007/s43465-020-00285-2. PMID: 33569100; PMCID: PMC7851217.

Shenoy, P.M,, Islam, A., & Puri, R. (2020). Current Management of Paediatric Supracondylar Fractures of the Humerus. Cureus. ;12(5):e8137. [PMC free article] [PubMed]

Vaquero-Picado, A., González-Morán, G., & Moraleda, L. (2018). Management of supracondylar fractures of the humerus in children. EFORT Open Rev. 3(10):526-540. [PMC free article] [PubMed]

Galina, M. (2023). Evaluation of the therapeutic effects of transcutaneous interferential current therapy and therapeutic exercises on musculoskeletal symptoms in patients with post Covid-19, *MEDIS - Medical Science and Research, 2*(1), 31-38. doi: 10.35120/medisij020131m UDK: 616.71/.74-085.84:[616.98:578.834

EVALUATION OF THE THERAPEUTIC EFFECTS OF TRANSCUTANEOUS INTERFERENTIAL CURRENT THERAPY AND THERAPEUTIC EXERCISES ON MUSCULOSKELETAL SYMPTOMS IN PATIENTS WITH POST COVID-19

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Abstract: The aim of this research was to investigate the therapeutic effectiveness of a multi-component rehabilitation program including: transcutaneous interferential current therapy and therapeutic exercises in patients with musculoskeletal symptoms in post COVID-19 syndrome. Materials and Methods: 62 patients, mean age 55.9(13.99), aged 21 to 79 years, with persistent musculoskeletal symptoms in the recovery phase after experiencing COVID-19 were included in this study. All patients underwent a ten-day treatment course in an outpatient setting, including transcutaneous application of interferential current with four electrodes. The therapeutic parameters were selected with analgesic, trophic and stimulating effects (90-100 Hz, 5min and 0-100 Hz, 15min). Immediately after electrotherapy, kinesitherapy was performed including: breathing exercises, aerobic training, active and resistance exercises for the limbs, postural control exercises and exercises to improve the mobility of the peripheral joints and the axial skeleton. Results were evaluated by: Visual Analogue Scale (VAS) for assessment of back pain, Numerical Rating Scale (NRS) for arthralgia and myalgia assessment, Brief Fatigue Inventory (BFI) – fatigue assessment, functional tests of mobility, Goniometry in the spine, Ott Sign- for measuring the range of motion (ROM) of the thoracic spine, Schober's test to determine a lumbar spine range of motion (flexion), Tom Meyer's test for total hip joint and spine flexion mobility. The results were reported before and after the rehabilitation. The statistical significance level was specified as ($p<0.05$). Results: Musculoskeletal symptoms in the observed patients persisted for an average of 12 weeks, ranging from 7 to 20 weeks, and included: myalgia in 46.8% (29), arthralgia 29.0% (18), pain in various back departments 79.0% (49), fatigue 66.1% (41). Inpatient treatment for acute COVID-19 was performed in 35.5% (22) of patients, and home and outpatient treatment in 64.5% (40). There was a statistically significant reduction (Mean (SD)) after therapy in back pain (VAS) from 4.88(1.5) to 1.93(1.1), myalgia (NRS) from 3.66(1.2) to 1.29(1.1), arthralgia (NRS) from 3.27(1.2) to 0.89 (0.8), and fatigue index (BFI) showed a reduction from 5.73(1.4) to 3.22(1.4). There was an increase in mobility in Ott's symptom from 2.24(1.2) to 2.86(0.9) cm, Schober's test 1.41(1.2) to 2.38(0.9) cm, Tom Meyer's test from - 8.23(6.5) to - 2.46(3.7) cm. Conclusion: In the recovery phase post COVID-19, some of the patients had long-time persistent musculoskeletal symptoms (fatigue, myalgia, arthralgia, back pain). These are a common reason for these patients to be referred for outpatient rehabilitation. In order to design a multi-component rehabilitation programme, it is necessary to take into account individual characteristics and comorbidities. The inclusion of physical modalities such as electrotherapy requires an accurate assessment of the rehabilitation potential and the presence of any counterindications. Therapeutic exercises have been proven to be effective in post COVID-19 patients. The results of this study show that the combination of interferential current therapy and therapeutic exercise can effectively reduce back pain, myalgia, arthralgia, and perception of fatigue. On the other hand, this multicomponent program resulted in improvement in functional tests in the studied patients. Due to the small sample size, it is advisable to continue the study in the future, covering a larger number of patients with long-standing musculoskeletal symptoms as part of post-COVID-19 syndrome, in order to design an effective strategy that can efficiently reduce negative health consequences and improve functional activity in these patients.

Keywords: Post-COVID-19 Rehabilitation, Musculoskeletal symptoms, Transcutaneous electrical nerve stimulation, Interferential current therapy, Therapeutic exercises, Functional activity.

Field: Medical sciences and Health

INTRODUCTION

The emergence of the new corona virus (SARS-CoV-2) in 2019 caused the infection of many people worldwide. (Horton R. (2020)) The potential of the virus to spread rapidly caused the development of a pandemic that required effective acute infection treatment strategy while limiting the size of the

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affected population very quickly. The pandemic has affected and has changed the life style of millions of people around the world (Dos Santos PK. et al. (2022)) At highest risk and potentially most affected were, patients with chronic diseases and the elderly. (Mackenzie & Smith (2020)).

The disease was initially thought to affect primarily the respiratory system, with asymptomatic to mild manifestation. In the severe presentation of SARS-CoV-2 infection, some patients develop severe acute respiratory syndrome in addition to suffering from pneumonia. (Jiang F et al. (2019)) As the pandemic progressed, COVID-19 proved to be a multi-organ and multi-system disease. It progresses with acute, pathological damage to the respiratory system, but also affects the cardiovascular, nervous, kidney, gastrointestinal, and other systems, with musculoskeletal, hepatic, renal, dermatological, psychosocial, and cognitive impairments. (Barker-Davies et al. (2020); Zhang al. (2020)).

These multisystem effects can persist over time and cause the development of prolonged symptoms, characterized as Long COVID-19 lasting from 4 to 12 weeks, or post COVID-19 syndrome, lasting over 12 weeks. Cases have been described in which recovery lasts for more than 6 months. (Azadvari M. et al. (2022); Greenhalgh et al. (2020) Jimeno-Almazán et al. (2021)); Castro JP et al. (2022)) Most common symptoms include: fatigue, post-excersise malaise, breath shortness, headache, neurocognitive complaints such as: brain fog, disorientation, high stress levels, increased anxiety, depression, (Pfefferbaum & North (2020); Fujita et al. (2021); Hou et al. (2020)), difficulty performing activities of daily living, limited work capacity. (Baig (2020)) The musculoskeletal system affection is characterized by symptoms such as fatigue, arthralgia, myalgia, and muscle weakness. Musculoskeletal symptoms are commonly reported in the acute and post-acute phases of COVID-19. (Disser NP. et al. (2020)) These symptoms, may persist for weeks or months after the infection. (Akbarialiabad H. et al. (2021); Dos Santos et al. (2022))

Gradually, the number of patients who have experienced the acute phase of the infection and who have persistent musculoskeletal symptoms is also increasing (Lauwers M. et al. (2022)) These concomitant symptoms, complicate functional recovery and patients often report lower than premorbid functional activity. Physical factors, although considered an adjuvant therapy, would be potentially effective in the recovery period following COVID-19. Conducting rehabilitation, could limit pathological consequences, improve respiratory system functional status (Mollova K. et al. (2022)), reduce chronic pain level, myalgia, and arthralgia, and affect fatigue perception, as well as improve quality of life (Petrova M. et al. (2023))

Rehabilitation programs are most often conducted in outpatient settings (Betschart M. et al. (2021)) after patients have been accurately assessed for rehabilitation potential and comorbidities by a multidisciplinary team of specialists. In case of necessity and concomitant pathology, rehabilitation can also take place in hospital settings (Takeva I. et al. (2021) Negm AM. et al. (2022))

The aim of this research was to investigate the therapeutic effectiveness of a multi-component rehabilitation program including: transcutaneous interferential current therapy and therapeutic exercises in patients with musculoskeletal symptoms in post COVID-19 syndrome.

MATERIALS AND METHODS

62 patients, mean age 55.9(13.99), aged 21 to 79 years, with persistent musculoskeletal symptoms in the recovery phase after experiencing COVID-19 were included in this study. Arthralgia was recorded as pain in any joint for which there was no prior radiologically proven, degenerative, traumatic, or rheumatic disease. Myalgia was recorded as muscle pain that could not be explained by other concomitant disability. Fatigue was recorded according to the patients' subjective description. All patients underwent a ten-day treatment course in an outpatient setting, including transcutaneous application of interferential current with four electrodes. The therapeutic parameters were selected with analgesic, trophic and stimulatory effects (90-100 Hz, 5min and 0-100 Hz, 15min). Immediately after electrotherapy, kinesitherapy was performed including: breathing exercises, aerobic training, active and resistance exercises for the limbs, postural control exercises and exercises to improve the mobility of the peripheral joints and the axial skeleton.

Prior to therapy, patients were informend about the the goals and therapeutic approaches of the rehabilitation. The rehabilitation course was carried out in accordance with the Declaration of Helsinki, after written informed consent and in compliance with current anti-epidemic measures.

Visual analogue scale (VAS) was used to assess the back pain results (0 to 100 mm). Higher score indicates greater pain intensity. Numerical Rating Scale (NRS)) was used to assess arthralgia and myalgia. Pain is measured in whole numbers from 0 "no pain" to 10 "worst possible pain". Brief Fatigue Inventory (BFI) was used to describe fatigue in the past 24 h. The BFI is 9 position scale with 0 to 10 possible answers for each of them. Fatigue scores are as follows: 1-3 (mild), 4-7 (moderate), and 8-10 (severe). Levels of 4 (4.33) or more suggest the need for intervention apart from prevention activities. (Mendoza

TR. et al. (1999)) Ott Sign was used for measuring the range of motion (ROM) of the thoracic spine. From processus spinosis C7, 30 cm along the spine in the caudal direction are measured and marked. The patient then flexes the thoracic compartment and 30 cm are measured again. Normally, the difference between the two measurements is 2 to 4 cm. Schober's test was used to determine a lumbar spine range of motion (flexion). From the patient standing upright, are measured 10 cm from the processus spinosus to L5 cranial and marked. The patient then flexes the spine maximally and the difference between the two measurements is recorded. Tom Meyer's test was used for joint flexion mobility in the spine and hip joints. The patient steps onto a platform and bends over with arms pointing down. The distance from the tip of the third finger to the ground is measured in centimeters. Above the platform the distance is recorded with a '-' sign, below with a '+'. SPSS v. 24.0 is used for statistical processing purposes.

The results were reported before and after the rehabilitation. The statistical significance level was specified as ($p<0.05$).

RESULTS

Musculoskeletal symptoms in the observed patients persisted for an average of 12 weeks (Me(SD)), ranging from 7 to 20 weeks, and included: myalgia in 46.8% (29), arthralgia 29.0% (18), pain in various back departments 79.0% (49), fatigue 66.1% (41). Inpatient treatment of acute COVID-19 was performed in 35.5% (22) of patients, and home and outpatient treatment in 64.5% (40). There was a statistically significant reduction (Mean (SD)) after therapy in back pain (VAS) from 4.88(1.5) to 1.93(1.1) ($p<0.05$), myalgia (NRS) from 3.66(1.2) to 1.29(1.1) ($p<0.05$), arthralgia (NRS) from 3.27(1.2) to 0.89 (0.8) ($p<0.05$), and fatigue index (BFI) showed a reduction from 5.73(1.4) to 3.22(1.4) ($p<0.05$). There was an increase in mobility in Ott's symptom from 2.24(1.2) to 2.86(0.9) cm ($Z=6.174$, $p<0.001$), Schober's test 1.41(1.2) to 2.38(0.9) cm. ($Z=6.515$, $p<0.001$), Tom Meyer's test from - 8.23(6.5) to - 2.46(3.7) cm. ($Z=-6.568$, $p<0.001$). The results are published in Table 1.

Table 1. Dynamics in myalgia, arthralgia, fatigue, and functional tests

Sympoms assessment	Total(n=62) % (n)		Female(n=42) % (n)		Male (n=20) % (n)	
Post COV- 19 period	12 (7-20)		13.5 (7-20)		11.50 (8-17)	
Hospital treatment	35.5 (22)		28.6 (12)		50.0 (10)	
Home treatment	64.5 (40)		71.4 (30)		50.0 (10)	
Sympoms assessment	Before rehab	After rehab	Before rehab	After rehab	Before rehab	After rehab
Back pain	49 (79.0)	49(79.0)	35 (83.3)	35 (83.3)	14 (70.0)	14 (70.0)
VAS (Me±(SD))	4.88±1.5119	1,93±1,19	4.88±1.61	1.914±1.23	4.89±1.28	1.979±1.15
without pain **n (%)**	13 (21.0)		7 (16.7)		6 (30.0)	
Cervical pain 11 (17.7) Cervical and thorax pain 7 (11.3) Lumbar pain 24 (38.7) Back pain 7 (11.3)			Cervical 8 (19.0); Cervical and thorax 6 (14.3); Lumbar 15 (35.7); Back 6 (14.3)		Cervical 3 (15.0); Cervical and thorax 1 (5.0); Lumbar 9 (45.0); Back 1 (5.0)	
Myalgia n (%)	29 (46.8)	28 (45.2)	18 (42.9)	18 (42.9)	11(55.0)	10 (50.0)
NRS (Me±(SD))	3.66 ± 1.21	1.29 ± 1.12	3.44 ± 1.15	1.11 ± 1.08	4.02 ± 1.27	1.60 ± 1.17
without myalgia	33 (53.2)		24 (57.1)		9 (45.0)	
Total 5 (8.1); Cervical 5 (8.1); Cervical and thorax 1 (1.6); Lumbar 9 (14.5); Shoulder and Upper Limb 4 (6.5); Lower limb 5 (8.1)			Total 4 (9.5); Cervical 5 (11.9); Cervical and back 1 (2.4); Lumbar 3 (7.1); Shoulder and Upper limb 1 (2.4) Lower limb 4 (9.5)		Total 1 (5.0) Lumbar 6 (30.0) Shoulder and Upper limb 3 (15.0) Lower limb 1 (5.0)	
Arthralgya	18 (29.0)	18 (29.0)	12 (28.6)	12 (28.6)	7 (35.0)	6 (30.0)
NRS (Me±(SD))	3.27±1.165	0,89 ± 0,76	2.92±1.084	0,67 ± 0,65	3.89±1.107	1,33 ± 0,82
without Arthralgya	44 (67.7)		30 (71.4)		13 (65.0)	
Arthralgya: Shoulder 6 (9.7) Rist/Finger 1 (1.6) Hip 3 (4.8) Knee Joint 6.1 (10)						
Fatigue	41 (66.1)		31 (73.8)		10 (50.0)	
BFI (Fatigue)	5.73 ± 1.43	3.22 ± 1.39	5.76 ± 1.61	3.20 ± 1.52	5.62 ± 0.64	3.26 ± 0.97
without **Fatigue**	21 (33.9)		11 (26.2)		10 (50.0)	

DISCUSSION

As the pandemic of COVID-19 progresses, there are increasing numbers of patients who overcome the acute phase of the disease but continue to have persistent symptoms. These symptoms affect not only the respiratory system but also other organs and systems, including the musculoskeletal system. This calls

for an effective post-covid care strategy (Barker-Davies et al. (2020)) and rehabilitation in order to impove recovery and to reduce the effects of post-covid symptoms. (Betschart M. et al. (2021)) Nowaredays, a number of studies have reported that the main musculoskeletal symptoms include: arthralgia, myalgia, chronic fatigue, pain in various back compartments. (Cipollaro L. et al. (2020); Motaqi M. et al. (2021))

In this study, patients referred for outpatient rehabilitation were found to have more than one musculoskeletal symptom. Almost one third of the patients had arthralgia, about half complained of myalgia, over 60% experienced fatigue, and back pain (summed different areas) occurred in three quarters of the patients. It should be noted that only one third of the patients had a severe acute infection from COVID-19, which was a reason for fospitalisation. Possible reasons for the presence of persistent musculoskeletal symptoms that are still debated are: causes that are a consequence of an excessive immune or inflammatory response (Dos Santos et al. (2022)) (indirect consequences of COVID-19 on skeletal muscle and joints) increased incidence of peripheral thrombosis that disrupts blood flow to the muscles and causes damage to the musculoskeletal system, (Magro C. et al. (2020)) or direct damage from the virus to the endothelium or peripheral nerves. (Cipollaro et al. (Dos Santos et al. (2022)) Furthermore, prolonged immobilization leads to impaired cardiorespiratory function, joint stiffness, contractures, postural instability, etc. Another reason for the presence of muscle weakness could be a consequence of catabolic muscle loss during prolonged immobilization and treatment in the intensive care unit (Hosey & Needham (2020))), or result of the use of steroid drugs or neuromuscular blocking agents (Simpson & Robinson (2020)), or by directly damaging skeletal muscle when SARS-CoV-2 binds to ACE2 receptors and the virus enters the muscle cell. (Dos Santos et al. (2022)) Furthermore, prolonged immobilization leads to impaired cardiorespiratory function, joint stiffness, contractures, postural instability, etc. Acquired muscle weakness and reduction of muscle mass, lead to functional impairment of the musculoskeletal system, delay the recovery process and limit the functional activity of patients. (Simpson & Robinson (2020))

Because of the multi-organ and multi-system impairment caused by COVID-19, rehabilitation of patients with persistent symptoms should address not only respiratory, cardiac or neurological problems, but also the persistent effects on the musculoskeletal system, including: myalgia, peripheral muscle weakness, muscle contractions, arthralgia, and limited mobility in joints, balance and postural disorders, and physical weakness. (Motaqi M. et al. (2021)) The application of a multi-component rehabilitation program is an effective strategy for a range of chronic diseases in elderly patients and those with multiple comorbidities. (Motaqi M. et al. (2021)) However, each patient should receive a program specific to his/her needs, which should be adapted depending on the degree of involvement of the musculoskeletal, cardiopulmonary, etc. systems.

The clinical algorithm includes: a detailed examination of the patient with a precise kinesiological assessment and determination of the rehabilitation potential and the design of a rehabilitation programme including the specific scientifically applied methodologies. When combining them, the aim is to achieve synergism and to avoid antagonism between physical factors. (Koleva I. et al. (2022))

There is much evidence that physical exercise improves respiratory function (Mollova K. et al. (2021); Hockele et al. (2022)), cardiovascular function, reduces mortality, need for hospitalization, and improves patients' functional status. Implementation of exercise and education programs, in patients with cardiopulmonary disease are also effective in improving functional and psychosocial status and improve quality of life. In patients with post COVID-19 syndrome, rehabilitation programs including overall muscle strengthening, (including stationary bicycle training), (Jimeno-Almazán et al. (2021)) occupational therapy sessions that improve daily activity, (Asly & Hazim (2020) as well as strength training (at low loads, low range and repetitions without failure) would also have a positive effect. These improve maximal dynamic strength and skeletal muscle trophicity, and reduce feelings of discomfort, fatigue and stiffness. All these, make the therapeutic exercises, suitable for patients with post-COVID-19 syndrome, who are demotivated to perform physical exercises due to fatigue, deconditioning and low tolerance to exercise and exercise. (Jimeno-Almazán et al. (2021)) On the other hand, the inclusion of therapeutic exercises in the rehabilitation program, leads to a reduction in anxiety and psychoemotional tension. Therapeutic exercises lead to improved quality of life, by increasing functional activity and modulating pain sensation. (Chen et al. (2020))

In this study, in addition to the therapeutic exercise complex in the multi-component rehabilitation program, transcutaneous interferential current stimulation (IFC) was applied to the patients. Interferential current therapy is a non-invasive non-pharmacological treatment used in clinical practice to reduce mainly musculoskeletal pain, edema, and spasticity and to increase muscle strength. (Ozcan J. et al. (2004); Jin H K. et al. (2017) De-La-Cruz-Torres B. et al. (2021); Rampazo ÉP et al. (2022))

IFC, reduces stimulation of cutaneous sensory nerves (Wedenski's theory) below the electrodes and favors effects in deeply located tissues. (Noble GJ. et al. (2000) This effect is probably due to

inhibition of C-nociceptive fibers. Pain reduction, is also mediated by the involvement of a "descending pain suppression mechanism" involving endogenous opioid substances. In terms of influence on muscle, IFC therapy (m. quadriceps femoris), can result in 50% higher activation of the quadriceps compared to that of maximal voluntary contraction (Taradaj J. et al. (2013); Pinfildi CE. et al. (2018)) Youn JI. et al. (2016), found that IFC reduced muscle fatigue and stiffness. Transcutaneously, IFT has been successfully applied to reduce musculoskeletal pain. (Adedoyin et al. (2005)) Musculoskeletal pain, is often associated with fatigue, result of metabolic, energetic and structural changes in the muscles. These changes, are attributed to a lack of oxygen and nutrients supplied by blood vessels, or to altered activity of nerve structures. (Cifrek M. et al, (2009)) Several clinical trials, have shown that IFC is an effective method of pain relief in patients with osteoarthritis for at least 1 week up to 6 months (Itoh K. et al. (2008); Defrin R. et al. (2005); Burch FX. et al. (2008) Adedoyin et al. (2005); Mratskova G. et al. (2021)) In another study, Corrêa J. et al. (2016) recorded a good analgesic effect of IFC in non-specific chronic low back pain (Corrêa J. et al. (2016)). A better analgesic effect of 100Hz frequency in nonspecific low back pain is reported by Almeida N. et al. (2020) and Hussein HM. et al. (2022)) IFC therapy may be effective for reduction of cervical and lumbar spine pain, knee joint osteoarthritis, and postoperative pain. Frequencies in the range of 30-180 Hz are considered to be most effective, with a treatment duration of 20-40 minutes, at a high enough intensity to induce a profound effect in the musculature without causing discomfort (Rampazo & Liebano (2022)).

With regard to the implemented, multi-component rehabilitation program, after completion of the rehabilitation course in the observed group of patients with persistent musculoskeletal symptoms after COVID-19, a fatigue reduction to levels that did not require further intervention beyond prevention activities was found (Mendoza TR. et al. (1999)). There was a reduction in muscle and joint pain levels and improvement in the administered functional tests. On the other hand, it should be mentioned that this study has a number of limitations. The small sample size could have influenced the obtained results. The clinical and functional assessment methods used, also contain an element of subjectivity, which could also affect the results, although these assessment tools are used in routine physiotherapy practice.

CONCLUSION

In the recovery phase post COVID-19, some of the patients had long-time persistent musculoskeletal symptoms (fatigue, myalgia, arthralgia, back pain). These are common reasons for the patients to be referred for outpatient rehabilitation. In order to design a multi-component rehabilitation programme, it is necessary to take into account individual characteristics and comorbidities. The inclusion of physical modalities such as electrotherapy requires accurate assessment of the rehabilitation potential and counterindications. Therapeutic exercises have been proven to be effective in post COVID-19 patients. The results of this study show that the combination of interferential current therapy and therapeutic exercise can effectively reduce back pain, myalgia, arthralgia, and perception of fatigue. On the other hand, this multicomponent program resulted in improvement in functional tests of the studied patients. Due to the small sample size, it is advisable to continue the study in the future, covering a larger number of patients with long-standing musculoskeletal symptoms as part of post-COVID-19 syndrome, in order to design an effective strategy that can efficiently reduce negative health consequences and improve functional activity in these patients.

REFERENCES

Adedoyin, R.A, Olaogun, M.O.B, Oyeyemi, A.L. (2005) Transcutaneous electrical nerve stimulation and interferential current combined with exercise for the treatment of knee osteoarthritis: a randomised controlled trial. Hong Kong Phys J; 23:13-9

Akbarialiabad, H., Taghrir, M. H., Abdollahi, A. et al. (2021). Long COVID, a Comprehensive Systematic Scoping Review. Infection 49, 1163–1186.

Almeida N, Paladini LH, Korelo RIG et al. (2020) Immediate Effects of the Combination of Interferential Therapy Parameters on Chronic Low Back Pain: A Randomized Controlled Trial. Pain Pract. 20: 615–625.

Azadvari, M., Haghparast, A., Nakhostin-Ansari, A. et al. (2022). Musculoskeletal symptoms in patients with long COVID: A cross-sectional study on Iranian patients. Heliyon, 8(8), e10148.

Asly, M., & Hazim, A. (2020). Rehabilitation of post-COVID-19 patients. The Pan African medical journal, 36, 168.

Baig A. M. (2021). Chronic COVID syndrome: Need for an appropriate medical terminology for long-COVID and COVID long-haulers. Journal of medical virology, 93(5), 2555–2556.

Barker-Davies, R. M., O'Sullivan, O., Senaratne, K., et al. (2020). The Stanford Hall consensus statement for post-COVID-19 rehabilitation. British journal of sports medicine, 54(16), 949–959.

Betschart, M., Rezek, S., Unger, I., et al. (2021). Feasibility of an Outpatient Training Program after COVID-19. International journal of environmental research and public health, 18(8), 3978.
Burch F.X, Tarro J.N, Greenberg, J.J, Carroll, W.J. (2008) Evaluating the benefits of patterned stimulation in the treatment of osteoarthritis of the knee: a multi-center, randomized, single-blind, controlled study with an independent masked evaluator. Osteoarthritis Cartilage;16 (8):865-72.
Castro, J. P., Kierkegaard, M., & Zeitelhofer, M. (2022). A Call to Use the Multicomponent Exercise Tai Chi to Improve Recovery From COVID-19 and Long COVID. Frontiers in public health, 10, 827645.
Chen, Z., Lan, W., Yang, G., Li, Y., Ji, X., Chen, L., Zhou, Y., & Li, S. (2020). Exercise Intervention in Treatment of Neuropsychological Diseases: A Review. Frontiers in psychology, 11, 569206.
Cifrek, M., Medved, V., Tonković, S., & Ostojić, S. (2009). Surface EMG based muscle fatigue evaluation in biomechanics. Clinical biomechanics (Bristol, Avon), 24(4), 327–340.
Cipollaro, L., Giordano, L., Padulo, J., Oliva, F., & Maffulli, N. (2020). Musculoskeletal symptoms in SARS-CoV-2 (COVID-19) patients. Journal of orthopaedic surgery and research, 15(1), 178.
Corrêa, J. B., Costa, L. O., de Oliveira, N. T., Sluka, K. A., & Liebano, R. E. (2013). Effects of the carrier frequency of interferential current on pain modulation in patients with chronic nonspecific low back pain: a protocol of a randomised controlled trial. BMC musculoskeletal disorders, 14, 195.
Defrin, R., Ariel, E., & Peretz, C. (2005). Segmental noxious versus innocuous electrical stimulation for chronic pain relief and the effect of fading sensation during treatment. Pain, 115(1-2), 152–160.
De-la-Cruz-Torres, B., Martínez-Jiménez, E., Navarro-Flores, E. et al. (2021). Heart Rate Variability Monitoring during Interferential Current Application in the Lower Back Area: A Cross-Sectional Study. International journal of environmental research and public health, 18(7), 3394.
Disser, N. P., De Micheli, A. J., Schonk, M. M. et al. (2020). Musculoskeletal Consequences of COVID-19. The Journal of bone and joint surgery. American volume, 102(14), 1197–1204.)
Dos Santos et al Dos Santos PK, Sigoli E, Bragança LJG, Cornachione AS. (2022) The Musculoskeletal Involvement After Mild to Moderate COVID-19 Infection. Front Physiol.13:813924.
Fujita K, Inoue A, Kuzuya M, et al. (2021) Mental Health Status of the Older Adults in Japan During the COVID-19 Pandemic. J Am Med Dir Assoc. 22(1):220-221.
Greenhalgh et al. (2020) Greenhalgh T, Knight M, A'Court C, Buxton M, Husain L. Management of post-acute covid-19 in primary care. BMJ. 2020;370:m3026.
Hockele, L. F., Sachet Affonso, J. V., Rossi, D., & Eibel, B. (2022). Pulmonary and Functional Rehabilitation Improves Functional Capacity, Pulmonary Function and Respiratory Muscle Strength in Post COVID-19 Patients: Pilot Clinical Trial. International journal of environmental research and public health, 19(22), 14899.
Hosey MM, Needham DM. (2020). Survivorship after COVID-19 ICU stay. Nature reviews. Disease primers, 6(1), 60.
Hou WK, Lai FT, Ben-Ezra M, Goodwin R. (2020) Regularizing daily routines for mental health during and after the COVID-19 pandemic. J Glob Health.10(2):020315.
Hussein, H. M., Alshammari, R. S., Al-Barak, S. S., (2022). A Systematic Review and Meta-analysis Investigating the Pain-Relieving Effect of Interferential Current on Musculoskeletal Pain. American journal of physical medicine & rehabilitation, 101(7), 624–633.
Itoh, K., Hirota, S., Katsumi, Y., Ochi, H., & Kitakoji, H. (2008). A pilot study on using acupuncture and transcutaneous electrical nerve stimulation (TENS) to treat knee osteoarthritis (OA). Chinese medicine, 3, 2.
Jiang, F., Deng, L., Zhang, L., Cai, Y., Cheung, C. W., & Xia, Z. (2020). Review of the Clinical Characteristics of Coronavirus Disease 2019 (COVID-19). Journal of general internal medicine, 35(5), 1545–1549.
Jimeno-Almazán, A., Pallarés, J.G., Buendía-Romero, Á. et al. (2021). Post-COVID-19 Syndrome and the Potential Benefits of Exercise. Int J Environ Res Public Health. 18(10), 5329.
Jin HK, Hwang TY, Cho SH. (2017) Effect of Electrical Stimulation on Blood Flow Velocity and Vessel Size. Open Med (Wars); 12:5-11.
Koleva I., Yoshinov B., Yoshinov. (2022) Physical medicine and rehabilitation in patients with neurological consequences of COVID-19: Basis principles. Collection of reports. International scientific conference Multidisciplinary Approach to Ensure the Normal Functioning of Health and Public Systems in Pandemics June 22-24, 2022, Stara Zagora
Lauwers M, Au M, Yuan S, Wen C. (2022) COVID-19 in Joint Ageing and Osteoarthritis: Current Status and Perspectives. Int J Mol Sci. 2022;23(2):720.
Mackenzie, J. S., & Smith, D. W. (2020). COVID-19: a novel zoonotic disease caused by a coronavirus from China: what we know and what we don't. Microbiology Australia, MA20013. Advance online publication.
Magro, C., Mulvey, J.J, Berlin, D. et al. (2020) Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res;220: 1–13.
Mendoza, T. R., Wang, X. S., Cleeland, C. S. et al. (1999). The rapid assessment of fatigue severity in cancer patients: use of the Brief Fatigue Inventory. Cancer, 85(5), 1186–1196.
Mollova, K., Valeva, S., Bekir, N., & Uzunova, A. (2021). Pulmonary rehabilitation in post - covid syndrome. Knowledge - International Journal, 49(4), 661–666.
Motaqi M, Hatef B, Askari Ashtiani A, Ghanjal A. (202) 1Musculoskeletal Problems in Patients with COVID-19: A Review Study. Asian J Sports Med;12(3): e111040.
Mratskova G., Dimitrov, N., Goycheva, P., & Petrov, D. (2021). Assesment of the rehabilitation program for patients with knee osteoarthritis and diabetes mellitus type 2. Knowledge - International Journal, 45(4), 853–859.
Negm, A. M., Salopek, A., Zaide, M. et al. (2022). Rehabilitation at the Time of Pandemic: Patient Journey Recommendations. Frontiers in aging neuroscience, 14, 781226.
Noble GJ, Lowe AS, Walsh DM. (2000) Interferential Therapy Review. Part 1. Mechanism of Analgesic Action and Clinical Usage, Physical Therapy Reviews, 5:4, 239-245
Ozcan J, Ward AR, Robertson VJ. A comparison of true and premodulated interferential currents. Arch Phys Med Rehabil. 2004;85(3):409-415.
Petrova M, Takucheva K, Mollova K. (2023) Recovery of post COVID-19 survivors with the methods of sanatorial and SPA

treatmen. Knowledge – International Journal, 55(4), 699-704)
Pfefferbaum, B., & North, C. S. (2020). Mental Health and the Covid-19 Pandemic. The New England journal of medicine, 383(6), 510–512.
Pinfildi, C. E., Andraus, R. A. C., Iida, L. M., & Prado, R. P. (2018). Neuromuscular electrical stimulation of medium and low frequency on the quadriceps femoris. Acta ortopedica brasileira, 26(5), 346–349.
Rampazo, É. P., & Liebano, R. E. (2022). Analgesic Effects of Interferential Current Therapy: A Narrative Review. Medicina (Kaunas, Lithuania), 58(1), 141.
Simpson, R., & Robinson, L. (2020). Rehabilitation After Critical Illness in People With COVID-19 Infection. American journal of physical medicine & rehabilitation, 99(6), 470–474.
Takeva I, Popova I, Ilieva E. (2021) Rehabilitation of patients with COVID-19 and post-COVID symptoms. Physical medicine Rehabilitation Health; 19(3-4):18-26.
Taradaj, J., Halski, T., Kucharzewski, M. et al. (2013). The effect of neuromuscular electrical stimulation on quadriceps strength and knee function in professional soccer players: return to sport after ACL reconstruction. BioMed research international, 2013, 802534.
Youn, J. I., Lee, H. S., & Lee, S. (2016). Determination of effective treatment duration of interferential current therapy using electromyography. Journal of physical therapy science, 28(8), 2400–2403.
Zhang, C., Shi, L., & Wang, F. S. (2020). Liver injury in COVID-19: management and challenges. The lancet. Gastroenterology & hepatology, 5(5), 428–430.

Marija, M., & Bistra, A. (2023). Vitamin D3 oil and water solution, preparation and use in case of deficiency, *MEDIS - Medical Science and Research,* 2(1), 39-42. doi: 10.35120/medisij020139m
UDK: 613.2:577.161.2

VITAMIN D3 OIL AND WATER SOLUTION, PREPARATION AND USE IN CASE OF DEFICIENCY

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Abstract: Vitamins are natural and essential nutrients, which have different biochemical functions and play a major role in growth and development, help in the digestion and utilization of mineral salts and carbohydrates in the body, healing of wounds, stimulate and give strength to the digestive and nervous system, maintenance of healthy bones and tissues, for proper functioning of the immune system and other biological functions. Vitamins generally cannot be synthesized in quantities sufficient to meet the body's needs and therefore must be obtained from the diet or from some synthetic source. Because of this, vitamins are called essential nutrients. Both plants and animals are important natural sources for vitamins. All vitamins can be synthesized or produced commercially and are available for human consumption as pharmaceutical preparations. Vitamins also differ from other biological compound, because relatively small amounts are required to complete their functions. If a vitamin is absent from the diet or is not properly absorbed by the body, it can develop a specific disease due to deficiency.

Keywords: vitamin D3, aqueous solution, oil solution, deficiency.
Field: Pharmaceutical technology

1. INTRODUCTION

Vitamins are regulators of reactions in metabolism, unlike other macronutrients (eg, fats, carbohydrates, and proteins), which are compounds used in reactions regulated by vitamins. Because of this, if a vitamin deficiency occurs, one or more specific metabolic reactions in the cell are blocked and can lead to a disturbance of the metabolic balance in the cell and the whole organism.

Vitamin D is a fat-soluble vitamin that helps the body absorb and retain calcium and phosphorus, which are very important for building bones. Laboratory studies have also shown that vitamin D reduces the growth of cancer cells, can help control infections and reduce inflammation. Many organs and tissues in the body have receptors for vitamin D, suggesting that this vitamin has a large role beyond bone health, while scientists are actively investigating its other functions. Very few foods naturally contain vitamin D, although some are fortified with this vitamin. There are a few that naturally have vitamin D. These are fatty fish such as salmon, tuna and mackerel, beef liver, cheese, mushrooms, egg yolks. Vitamin D can also be obtained from fortified foods. The most commonly added vitamin D is found in milk, breakfast cereals, orange juice, dairy products such as yogurt, and soy beverages.

For most people, the best way to get vitamin D is through a supplement because research shows that you don't get enough from your diet.

1.1 FORMS OF VITAMIN D3

Vitamin D supplements are available in two forms:
• Vitamin D2 (ergocalciferol)
• Vitamin D3 (cholecalciferol).

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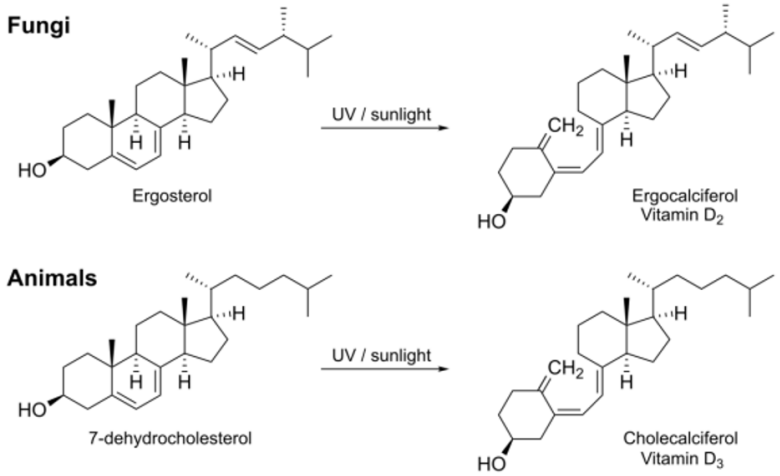

Figure 1. Photochemistry of vitamin D biosynthesis in animals and fungi.

Both are natural forms that are produced in the presence of the sun's ultraviolet-B (UVB) rays, which is why it is also called the "sunshine vitamin", but D2 is produced in plants and fungi, and D3 in animals and humans. The production of vitamin D in the skin is the primary natural source of vitamin D, but many people are deficient because where they live, sunlight is limited in the winter or because they have limited exposure to the sun because they spend most of their time inside. Also, those people who have darker skin tend to have lower levels of vitamin D in their blood because the pigment (melanin) acts as a shadow, reducing vitamin D production.

1.2 RECOMMENDED QUANTITIES

The recommended dietary allowance for vitamin D provides the daily amount needed to maintain healthy bones and normal calcium metabolism in healthy people, assuming minimal sun exposure.

The recommended dietary allowance for adults 19 years of age and older is 600 IU (15 mcg) per day for men and women, and for adults over 70 years of age it is 800 IU (20 mcg) per day. The recommended dietary allowance for adults 19 years of age and older is 600 IU (15 mcg) per day for men and women, and for adults over 70 years of age it is 800 IU (20 mcg) per day.

The term "tolerable upper intake level" is also described in the literature. It is about the maximum daily intake that is unlikely to cause harmful effects on health. The Tolerable Upper Level for vitamin D for adults and children ages 9+ is 4000 IU (100 mcg).

Many people do not meet the minimum daily requirements for vitamin D. The average intake of vitamin D from food and supplements among women aged 51 to 71 years was 308 IU per day, but only 140 IU from food alone (including fortified products). Worldwide, about 1 billion people have inadequate levels of vitamin D in their blood, and deficiencies can be found in all ethnicities and age groups. For example in the United States, about 20% of white adults and 75% of black adults have blood levels below 50 nmol/L. In industrialized countries, doctors are seeing a resurgence of rickets, the bone-weakening disease that was largely eradicated by vitamin D fortification.

1.3 VITAMIN D DEFICIENCY OR VITAMIN D HYPOVITAMINOSIS

It is a disorder caused by a vitamin deficiency that can result either from suboptimal intake of the vitamin or from conditions that prevent the use or absorption of the vitamin in the body.

Vitamin D deficiency can occur for various reasons:

- insufficient intake of vitamin D through diet,
- not enough vitamin D is absorbed from food (malabsorption problem),
- insufficient exposure to sunlight,

• the liver or kidneys do not convert vitamin D into its active form in the body,
when using drugs that reduce the body's ability to convert or absorb vitamin D.

Some people are at greater risk of vitamin D deficiency, for example infants because breast milk is a poor source of vitamin D, then older people because their skin does not produce vitamin D when exposed to the sun as efficiently as younger people, and their kidneys are also less able to convert vitamin D into its active form. Also people with dark skin, which has less ability to produce vitamin D from the sun. Then, people with disorders like Crohn's disease or celiac disease that don't handle fat properly, because vitamin D needs fat to be absorbed. Individuals with increased body weight, because their body fat binds to vitamin D and prevents it from entering the blood, as well as people with osteoporosis, with chronic kidney or liver disease.

Vitamin D deficiency can lead to loss of bone density, leading to osteoporosis and fractures. A severe lack of vitamin D can also lead to other diseases, for example in children it can cause rickets, a disease that causes the bones to become soft and bend.

In adults, severe vitamin D deficiency leads to osteomalacia, which weakens the bones, causing bone pain and muscle weakness. The relationship of vitamin D to several other conditions such as diabetes, high blood pressure, cancer and autoimmune diseases such as multiple sclerosis is also being studied. But experts say more research needs to be done to determine and understand the effect of vitamin D on these conditions.

Due to the great need of vitamin D in infants in the first months of life it is not by chance that for the purpose of this specialist paper we chose the preparation of an aqueous and oily solution of vitamin D3 that is used for prophylactic and therapeutic purposes.

2. MATERIALS AND METHODS

The aqueous solution of vitamin D3was prepared according to the composition given by Vitamins, BASF, Germany. The preparation of the solution itself is performed in the dark because vitamin D is sensitive to light. First, a certain amount of vitamin D3, tocopherol and cremophor RH40 is measured in a tared glass container. They are heated in a water bath at a temperature of 65◦C. In another tared glass container, the amount of distilled water is measured and it is heated in a water bath to 65◦C. After reaching the required temperature, water is added to the vitamin solution and mixed completely. After cooling, the pH of the solution is determined and phosphate buffer is added to pH 7. The amount of the solution is filled in pre-sterilized bottles, the air is replaced with an inert gas, the bottles are closed and packed in the outer paper packaging.

The preparation of the oil solution is in the same way as the water solution, it is carried out in the dark, the prescribed amounts of the given substances are measured in a tared glass container and heated in a water bath at a temperature of 40◦C. After cooling, the solution is added to the indicated amount with neutral olive oil. Filling is done in the same way as the aqueous solution.

The signature of the preparations is carried out with a signature on which is written the name of the medicine, the amount of the active component expressed in I.E./ml, the antioxidant used and the date of manufacture.

During the production of the aqueous and oily solution of vitamin D3in the form of drops for oral use as a galenic preparation, materials and technological procedures are used that ensure their stability.

3. DISCUSSION

The solutions are made according to the general regulations for oral drops, which include certain auxiliary agents such as solubilizer (Cremophor RH40), antioxidant (DL-α-Tocopherol), buffer (phosphate) and are dosed in an inert medium (nitrogen) for greater stability of D3.

For the selection of the formulation in the aqueous solution, we decided on the following composition: Vitamin D3 1,000,000 I.E./g, Cremophor RH40, DL-α-Tochoferol, Phosphate buffer (pH-7), Aqua destilata; while the oil solution has the following composition: Vitamin D31,000,000 I.E./g, DL-α-Tochoferol, Olive oleum neutralisatum.

The aqueous solutions produced are clear, odorless and bitter tasting, and the oil solutions

produced are also clear, yellow oily liquids with a pleasant mild odor and taste.

The stability of the aqueous and oil solutions was determined by monitoring the required parameters and the content of D3, during 3 months kept at two different temperatures: 4◦C and 25◦C.

The obtained results show that during 3 months there are no changes in the clarity and microbiological purity in water and oil solution.

The pH value of the aqueous solution in the solutions kept at two different temperatures is within normal limits because it does not exceed the pH value given in the general regulations for the neutral pH area (6.5-7.5).

The acid number in the oil solutions stored at two different temperatures is also within normal limits because the acid number of Olive oleum is neutral. after Ph.Eur. is max 0.2.

The content of the water and oil solution is within the prescribed permissible limits.

In vivo studies of aqueous and oily solutions of vitamin D3 show satisfactory results. In the group of patients who were prescribed aqueous solutions of vitamin D3for the purpose of protection and treatment from rickets, satisfactory results were obtained and no adverse events were observed.

Likewise, in the group of patients who were prescribed vitamin D3 oil solutions for the purpose of protection and treatment of rickets, satisfactory results were obtained and no adverse events were observed.

4. CONCLUSION

The number of people with vitamin D deficiency is constantly increasing and it is common in all age groups. The importance of this vitamin in overall health and the prevention of chronic diseases is the target of much research. In this paper, two preparations of vitamin D3 4000 I.E./ml were formulated and analyzed as an aqueous and as an oil solution. From the results obtained during the examination, we can conclude that we received two preparations of vitamin D3, professionally prepared, which meet the basic pharmacopoeial requirements and which can be used for the prophylaxis of rickets and for the treatment of rachitic changes.

5. REFERENCES

Bird JK, Murphy RA, Ciappio ED, McBurney MI. Risk of Deficiency in Multiple Concurrent Micronutrients in Children and Adults in the United States. Nutrients. 2017 Jun 24;9(7):655. doi: 10.3390/nu9070655.

Durrant LR, Bucca G, Hesketh A, Möller-Levet C, Tripkovic L, Wu H, Hart KH, Mathers JC, Elliott RM, Lanham-New SA and Smith CP (2022) Vitamins D2 and D3 Have Overlapping But Different Effects on the Human Immune System Revealed Through Analysis of the Blood Transcriptome. Front. Immunol. 13:790444. doi: 10.3389/fimmu.2022.790444

Spiro, A. and Buttriss, J.L. (2014), Vitamin D status and intake in Europe. Nutrition Bulletin, 39: 322-350. https://doi.org/10.1111/nbu.12108

Dominguez LJ, Farruggia M, Veronese N, Barbagallo M. Vitamin D Sources, Metabolism, and Deficiency: Available Compounds and Guidelines for Its Treatment. Metabolites. 2021 Apr 20;11(4):255. doi: 10.3390/metabo11040255.

Mostafa WZ, Hegazy RA. Vitamin D and the skin: Focus on a complex relationship: A review. J Adv Res. 2015 Nov;6(6):793-804. doi: 10.1016/j.jare.2014.01.011. Epub 2014 Feb 8.

Holick MF. Vitamin D deficiency. N Engl J Med. 2007 Jul 19;357(3):266-81. doi: 10.1056/NEJMra070553.

Cashman, K. D. (2022). 100 YEARS OF VITAMIN D: Global differences in vitamin D status and dietary intake: a review of the data, Endocrine Connections, 11(1), e210282. Retrieved Feb 12, 2023

Amrein, K., Scherkl, M., Hoffmann, M. et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr 74, 1498–1513 (2020). doi: 10.1038/s41430-020-0558-y

Zang R, Zhang Y, Zhang H, Zhang X, Lv Y and Li D (2022) Association Between Vitamin D Level and Neonatal Respiratory Distress Syndrome: A Systematic Review and Meta-Analysis. Front. Pediatr. 9:803143. doi: 10.3389/fped.2021.803143

Mosayebi Z, Sagheb S, Mirzendedel M, Movahedian AH. Serum Vitamin D Deficiency in NICU Hospitalized Neonates and Its Association With Neonatal Outcomes. J Family Reprod Health. 2021 Jun;15(2):99-105. doi: 10.18502/jfrh.v15i2.6450.

Neli, G. (2023). Children's rights and access to health care, *MEDIS - Medical Science and Research, 2*(1), 43-48.
doi: 10.35120/medisij020143g UDK: 364.69:342.7-053.2(497.2)"2021/2022"

CHILDREN'S RIGHTS AND ACCESS TO HEALTH CARE

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Abstract: Children have the right to health, to life, to protection and to special care and attention from their parents and from the community in which they live. A number of international acts regulate and regulate these rights, and the national legislation in the country confirms and guarantees them.

A pilot survey on children's and their parents' awareness of children's rights in Bulgaria and their access to health care was conducted among 201 parents, in the period 01.12.2021-01.01.2022. Respondents were asked whether they were aware of children's rights, whether they encountered obstacles to realizing their right to health, as well as whether, in their opinion, children have real access to health care in the country. More than 50% indicate that they are familiar with children's rights, 64.7% answer that children in the country have real access to health care, but 44% share that they encountered certain obstacles when realizing their right to health.

Issues concerning children, their rights and their access to health care affect the interests of individual families, of a given nation, but also of the entire society globally. There are positive trends around the world regarding children's access to health care, but these rights depend not only on the individual and on national legislations, but also on global political decisions and natural disasters that cause refugee flows and put countries in front of new challenges.

Keywords: *children, rights, health care.*
Field: Medical Sciences and Health

1. INTRODUCTION

Children's rights are in fact the human rights of the children themselves but written with the emphasis on the protection and care that children need. All children have the right to live, grow, learn, be heard and to reach their full potential, children have the right to human identity, food, education, health care, judicial protection and contact with their biological parents.

Children's rights have always been included in various declarations, but there has been a need to collect and clarify these rights so that they are more binding. In 1989, the Convention on the Rights of the Child recognized for the first time that children (all persons under the age of 18) are human beings in need of special care and attention.

2. MATERIALS AND METHODS

The aim of this study is to investigate how the rights of children are regulated internationally and nationally and whether their right to health is achievable and applicable in our country.

Documentary, sociological and statistical methods were used. The statistical data package SPSS (Statistical Package for Social Sciences) version 13.0 was used to process the survey data. 201 respondents took part in the survey.

3. RESULTS

International regulation:

According to the UN Convention on the Rights of the Child, he is an individual and the child himself is a subject of human rights. [2] No child is the property of his or her parents, nor of the country in which he or she was born or lives, nor is he or she merely the object of care. In this spirit, Article 5 of the Convention requires parents to provide, in a manner appropriate to the child's development, appropriate guidance and guidance in the process in which he or she exercises his or her Convention rights.

According to Art. 24 of the Convention "States Parties recognize the right of the child to the

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enjoyment of the highest attainable standard of health and of health services for the treatment of diseases and for the recovery of his health". [2] States shall endeavor to prevent any child from being deprived of his or her right of access to health services, to prevent infant and child mortality, to provide all children with the necessary medical care and health care, to combat disease and malnutrition, to provide mothers with appropriate pre- and post-natal care, provide all groups in society, in particular parents and children, with information on the health and nutrition of the child, the benefits of breastfeeding, the hygiene and health of the environment and the prevention of accidents , as well as training and assistance in the use of this information, developing health prevention.

The United Nations Children's Fund (known as UNICEF) was established on December 11, 1946 by the United Nations General Assembly to provide emergency care, food and health care for children affected by World War II, but it continues to a major structure of the United Nations to this day. Its main purpose is to help children in terms of nutrition, protection of their health, as well as in terms of education. The organization operates worldwide.

The European regulation on children's rights and their right of access to health care corresponds to the international one. Article 35 of the Charter of Fundamental Rights of the European Union guarantees individuals the right of access to healthcare. [1] Under EU law, children of migrant citizens who are EU citizens have access to social assistance and health care under the same conditions as local citizens after staying in the host country for three months. Legislation obliges Member States to provide vulnerable migrant children with access to adequate healthcare.

The European Convention on Human Rights does not explicitly guarantee the right to health care or the right to health [4], but the European Court of Human Rights has solid case law in this area. When considering cases in which the lives of children are endangered, it determines the positive obligations of the state to take preventive measures to eliminate life-threatening health risks that are known or should be known to it.

States are obliged to take appropriate measures to set up counseling and education services in order to improve health and develop a sense of individual responsibility for health matters (Article 11 of the European Social Charter).

In 2011, the Committee of Ministers adopted special guidelines for the health of the child. [3] According to Article 19 of the European Convention on the Legal Status of Migrant Workers, migrant workers legally working in another country and their families should have equal access to social assistance and health care.

National regulations:

With the ratification of the Convention on the Rights of the Child in 1991, the Republic of Bulgaria guarantees that it will work to protect the rights of children - to live and grow, to have access to health care, to study, to have their voices heard and to reach full its development potential. The main principles set out in the Convention are the guarantee of the rights of the child, without any discrimination (art. 2), the best interests of the child are paramount (art. 3), the right to life, survival and development. (art. 6) and the child's right to good health and access to health services (art. 24). [2]

The Constitution of the Republic of Bulgaria provides basic rights to its citizens and although they are not explicitly addressed to children, they give rise to legal action against them as well, as the Constitution itself does not allow any restrictions or privileges before the law to its citizens. According to Article 4, para. 2 of the Constitution, the state guarantees the life, dignity and rights of the individual. Citizens have the right to health insurance, guaranteeing them affordable medical care, and to free use of medical care under conditions and by order determined by law (Art. 52, para 1 CRB), as the health insurance of children is covered by the state. According to the Constitution, the state exercises control over all health care institutions, over the provided health services to citizens and children, as well as over the production of medicines, biological products and medical equipment and over their trade (Art. 52, para. 5 CRB). [8] Citizens have the right to a healthy and favorable environment in accordance with the established standards and norms (Article 55 of the CRB).

Questionnaire survey:

A pilot study on the awareness of children and their parents about the rights of children in Bulgaria and their access to health care was conducted among 201 parents in the period 01.12.2021-01.01.2022, and the selection of respondents was random. They were asked whether they were aware of children's rights (Fig. 1) and whether they were aware of the responsibility of parents for non-compliance with the rights of the child (Fig. 2).

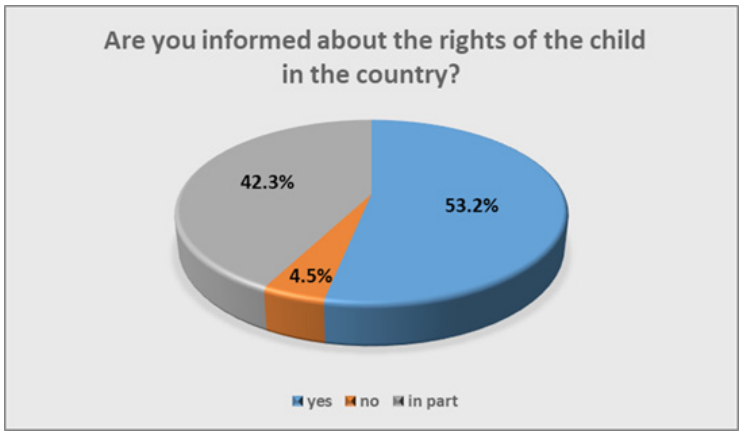

Fig. 1 presents parents' awareness of children's rights in the country.

The results show that more than half answered that they were aware, 42,3% answered that they were partially aware and only 4.5% answered that they were not aware of children's rights.

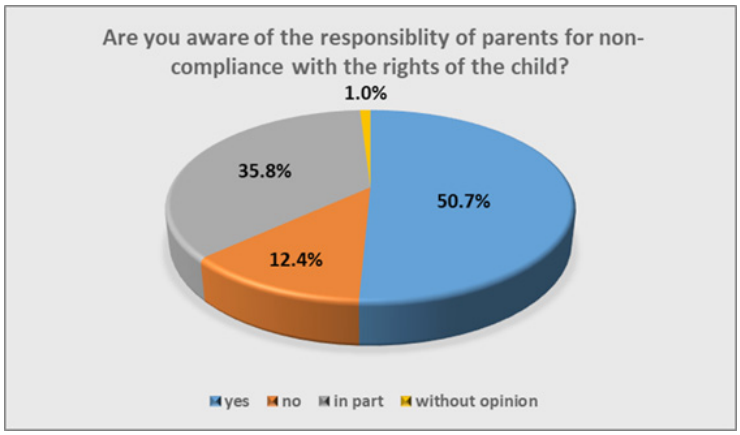

Fig.2 presents parents' awareness of their responsibility for non-compliance with the rights of the child.

50.7% answered that they were aware, 35.8% answered that they were partially aware and only 12.4% answered that they were not aware of their responsibility in case they do not know and do non-compliance with the rights of children.

The respondents were asked whether they had any obstacles to realizing their children's right to health (Fig. 3) and whether according to them children have real access to healthcare in the country (Fig.4)?

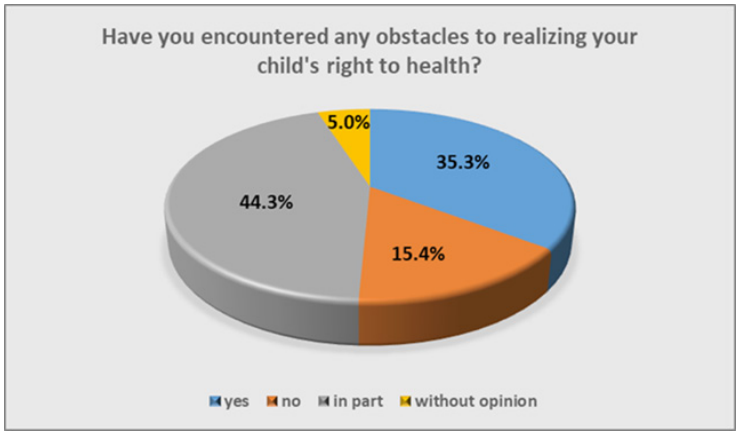

Fig. 3 shows whether parents encountered any obstacles to realize their children's right to health.

44.3% answered that they partly encountered some obstacles to realize their children's right to health, 35.3% answered that they had such obstacles and only 15.4% stated that they did not have any difficulties and obstacles.

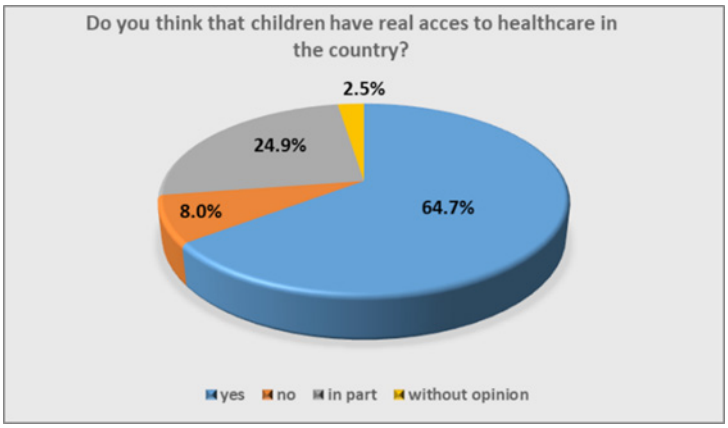

Fig.4 shows the answers of the parents whether, in their opinion, children have real access to health care in the country.

According to 64.7% of the respondents children have real access to health care in the country, only 8% are of the opinion that such access does not exist and 24.9% answered that, according to them, this real access partly exists.

4. DISCUSSIONS

In a national plan, according to the Health Insurance Act, the state has the obligation to insure at its own expense "persons up to the age of 18 and after reaching this age, if they study regularly - until the completion of secondary education, but no later than the age of 22- years old, students included in training through work (dual system of training) for the duration of the training according to the relevant curriculum, as well as students - full-time training in higher schools until reaching the age of 26, and doctoral students in full-time training by state order". [5] According to the same law, health insured persons have the right to receive medical assistance within the scope of the package of health activities, guaranteed by the budget of the National Health Insurance Fund, as well as emergency assistance, wherever they are. These regulations practically guarantee children's access to health care in the country, this access is real, although sometimes not completely satisfactory according to the parents of the children themselves.

Globally, there are also positive trends to improve children's access to health care. In the period 2000-2014, a study was conducted in the USA among 178,038 children aged 0 to 17 years. Trends are examined for health insurance and 5 access indicators: no well-child visit in the year, no doctor's office visit, no dental visit, no usual source of care, and unmet health needs. The survey results show that the proportion of uninsured children decreased from 12.1% in 2000 to 5.3% in 2014, improvement was reported in all 5 indicators of access to health care, and the results show that improvements apply to all children in the country regardless of their ethnicity. [6]

Children and their access to health care are affected not only by time and past government policies, but also by political decisions, wars and refugee crises. Children and adolescents under the age of 18 who are refugees and are not accompanied by their parents represent a special risk group. Additional challenges stem from the specific situation, lack of legal conditions, as well as language and cultural skills on the part of health care providers and unaccompanied refugee minors themselves. [7] Modern times present individual countries with new challenges, but they all have the duty and responsibility to care for all children and ensure their internationally recognized right to health according to the Convention on the rights of the child to the United Nations.

5. CONCLUSIONS

The issue of children's health and their access to health care is extremely relevant even today. Globally, there are still a number of places where healthcare remains a myth not only for parents but also for children. It is necessary to take continuous care by international organizations and individual governments to improve these statistics and to guarantee the rights of children globally, as well as to guarantee their most basic right - the right to life and health.

REFERENCES

Charter of fundamental rights of the European union, (2016/C 202/02)
Convention on the Rights of the Child, Adopted and opened for signature, ratification and accession by General Assembly resolution 44/25 of 20 November 1989, entry into force 2 September 1990, in accordance with article 49
Council of Europe, Committee of Ministers (2011), Guidelines on Child-Based Health Care, 21 September 2011
European Convention on Human Rights, Rome, 4.XI.1950
Health Insurance Act, Pub.SG No. 70 of 19.06.1998
Larson, K., Cull, W.L., Racine, A.D., & Olson, L.M. (2016). Trends in Access to Health Care Services for US Children: 2000-2014. Pediatrics, 138(6), e20162176. https://doi.org/10.1542/peds.2016-2176
Spallek, J., Tempes, J., Ricksgers, H., Marquardt, L., Prüfer-Krämer, L., & Krämer, A. (2016). Gesundheitliche Situation und Versorgung unbegleiteter minderjähriger Flüchtlinge - eine Näherung anhand qualitativer und quantitativer Forschung in der Stadt Bielefeld [The health situation and health care needs of unaccompanied minor refugees - an approximation based on qualitative and quantitative studies from Bielefeld, Germany]. Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz, 59(5), 636–641. https://doi.org/10.1007/s00103-016-2339-2
The Constitution of the Republic of Bulgaria, Pub. SG No. 56 of 13.07.1991 Hayes, J. (2018). The theory and practice of change management. Palgrave.

Nikolay, K. (2023). Vitamin B12 deficiency in continuous metformin treatment, 2(1), 49-52. doi: 10.35120/medisij020149k
UDK: 616.392:577.164.1]:615.252.349.7.065(497.2)"2022"

VITAMIN B12 DEFICIENCY IN CONTINUOUS METFORMIN TREATMENT

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Abstract: As we know type 2 diabetes is a global disease with many complications. The long-term treatment with metformin as a first line of therapy has been proven to cause low levels of cobalamin /vit. B12/. The main purpose of this analysis is to show the frequency of vitamin B12 deficiency among patients treated with biguanides. The RSNC/regional non-profit association/ "Diabetes Care" in Burgas conducted the study. Patients on metformin treatment for 5 to 10 years were included in it. From January to September 2022 was the period when all the data was collected. Information on the duration of metformin treatment was collected through an anamnesis. All patients were tested for vitamin B12 levels and the average value was found to be < 145 pmol/L - according to certified laboratory reference values - from 145 to 569 pmol/l. All participants in this study had laboratory evidence of anemia and were deficient in this vitamin . Vitamin B12 deficiency is preventable and these data show us the need of regular screening in patients on long-term treatment with metformin. This will allow the identification of patients with vitamin deficiency and the addition of treatment with it or/and discontinuation of metformin treatment.

Keywords: *Vitamin B12, deficiency , diabetes mellitus type 2, metformin*
Field: Medical Sciences and Health

INTRODUCTION

Vitamin B 12/cyanocobalamin/is a complex organic molecule consisting of a centrally activated cobalt atom connected by a covalent nitrogen bond to a tetrapyrrole ring and to a cyanide group. Vit. B12 has two active coenzymes: methylcobalamin/with an important role for DNA synthesis/ and 5-deoxy-adenosylcobalamin, responsible for mylinization. Microorganisms/bacteria and fungi/ are the main sources of Vit.B12 in nature. For plants and animals, vitamin B12 is only of exogenous origin. The following products are relatively richer in content: meat, eggs, milk, raw liver, as well as fermenting plant products such as beer, brewer's yeast, pickles. The human body contains only 5 mg. the liver appears to be the largest depot of vitamin B12.(Grudeva P, 2020)

Vitamin B12 ingested with food in the acidic environment of the stomach is released from the proteins and binds to two specific ligands - 1/c intrinsing factor/IF/- glycoprotein produced by the parietal cells of the fundus and 2/c proteins R, called cobaltophilins. which are contained in the gastric juice. The IF-vitamin B12 complex is absorbed in the distal ileum. After passing through the enterocyte, vitamin B12 enters the blood, bound to a protein - transcobalamin, synthesized by the endothelial cells of the vessels. This complex reaches the bone marrow, where it enters the cells by endocytosis. Vit. B12 accumulates as a reserve mainly in the liver/about 2-3 g./- quantities sufficient for 3-4 years. Daily needs are 1-2 mg.(Kerticova S.,2000).

Vitamin B12 is a key micronutrient responsible for DNA methylation and has diverse metabolic roles ranging from lipid metabolism to endothelial dysfunction (McNulty H, Pentieva K, Hoey L, Ward M, 2008). Metformin treatment is the first-line treatment in patients with type 2 diabetes in combination with lifestyle modification according to the current recommendations of the American Diabetes Association (ADA), European Association for the Study of Diabetes (EASD), and the American Association of Clinical Endocrinologists/American College of Endocrinology. These recommendations are based mainly on its glucose-lowering effects, its relatively low cost, and few side effects. Furthermore, unlike other antidiabetic medications, metformin appears to be neutral with respect to body weight. Metformin is a safe treatment for type 2 DM in the over 60 age group(Stanchev P. et all.,2021).

Metformin is suitable as initial therapy in newly diagnosed type 2 diabetes mellitus, in the absence of contraindications.

Positive effects:

-suppresses endogenous hepatic glucose production

-weight neutral with chronic use, with potential for some weight reduction

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-does not increase the risk of hypoglycemia;
-beneficial effects on lipid indicators; -there is evidence of cardiovascular benefits;
- effects regarding protection from carcinogenesis have been established.

Side effects - from the gastrointestinal tract - metallic taste, nausea, vomiting, diarrhea, bloating, flatulence; may lead to low vitamin B12 levels, necessitating periodic monitoring and replacement at low levels, especially in patients with anemia and neuropathy. Contraindications: all conditions associated with severe tissue hypoxia and ischemia - severe heart failure, acute stage of myocardial infarction, liver failure, CKD IV degree (Borisova AM, 2019). It is used as monotherapy or in combination with other drugs (Hirst J.A, Farmer A.J, Ali R, Roberts N.W, Stevens R.J, 2012). However, one of its side effects is a decrease in vitamin B12 levels. There are several pathophysiological theories about how metformin decreases the absorption of vitamin B12. According to one of them, it changes the distribution in the tissues and the metabolism of B12. Also, the liver accumulates B12, as a result of which its circulating levels in the serum decrease(Aroda et all, 2016). Another theory is that metformin slows down the absorption of glucose, which slows down gut motility and allows bacterial overgrowth, which interferes with the complex of intrinsic factors and reduces the absorption of B12 (Beulens et al., 2015). However, the most plausible and generally accepted cause of vitamin B12 deficiency due to metformin-induced malabsorption is interference with calcium-dependent binding of the inducing factor-vitamin B12 complex to the receptor in the terminal ileum (Bauman, W.A., Shaw, S., Jayatilleke, E., Spungen, A.M., Herbert, V., 2000). Another mechanism by which B12 deficiency can be reached is by displacement of calcium from the ileal mucosa by metformin. As a trace element, calcium is required for the B12-intrizinc factor complex.(William, A.and all, 2000). Different cut-off values invariably result in underestimation or overestimation of cases of vitamin B12 deficiency. Vitamin B12 deficiency is defined as serum levels < 148 pmol/L. Levels between 148 and 221 pmol/L are considered borderline and suggest marginal deficiency.(Green R et all, 2017). Metformin reduces circulating B12 levels by about 25% (Beulens JW et all, 2014) Another study in Africa showed that the prevalence of vitamin B12 deficiency, defined by levels <150 pmol/L, in metformin-treated T2DM patients was 28.1%. The authors recommend regular screening for vitamin B12 deficiency in patients on long-term metformin therapy (Ahmed,M.A., Muntingh,G., and Rheeder,P. 2016). In a study conducted in Ghana, the prevalence of vit.B12 deficiency was reported to be 32.1%. The authors reported the negative impact of long-term metformin therapy, its higher daily dose and high body fat content, which increase the possibility of manifestation of cyanocobalamin deficiency. In diabetics taking metformin in a total daily dose of ≥ 1500 mg, for a period of ≥ 6 years, the research team recommends regular and systematic monitoring of vitamin B12 levels(Laing,E.F. et all, 2019). Other authors estimate the deficiency of vitamin B12 at 14.1%. Compared to diabetics without metformin in therapy, the prevalence of its deficiency is significantly higher in the group of patients treated with it. After 4.9 years of metformin use, the authors report, that the chances of developing neuropathy or anemia cannot be predicted(Groot-Kamphuis, D.M. et all, 2013). Among the Bulgarian population of patients with type 2 diabetes mellitus, the status of vitamin B12 was investigated. In the studied population of 308 diabetics, deficiency was found in 34%, low-normal levels in 28%. 37.7% of the group of patients treated with metformin were deficient in vitamin B12. 18% of diabetics on non-metformin therapy were also found to be B12 deficient. The mean level of vit.B12 in the group receiving metformin was 178 pmol/l, and in the group without metformin 235 pmol/l. It turns out that the level of vit. B12 correlates with the average dose of metformin, and not with the duration of therapy. Each increase in the dose of metformin by 250 mg. increases the risk of vitamin B12 deficiency by 15% (Chakarova N. et all.,2019). The influence after a 6-month treatment with metformin on the serum levels of vitamin B12 in type 2 diabetics was also investigated by another Bulgarian group. 18 patients were studied - 9 women and 9 men, with an average age of 47.6 years with an average duration of diabetes of 6.1 years. Plasma vitamin B12 levels were measured at baseline and after 6 months of treatment with an average daily dose of 1500 mg metformin. Regarding vit B12, a certain decrease was registered, which, however, could not reach statistical significance/from 233.3 to 207.4 pmol/l/. Changes in erythrocytes and serum iron were observed.(Boyanov,M., Boneva,Zh.,Tsoncheva,A.,Protich,M., 2003)

Considering the consequences of possible vitamin B12 deficiency, such as megaloblastic anemia and polyneuropathy, screening among patients treated with metformin is appropriate. Follow-up testing is also appropriate. This would identify patients with insufficient serum levels of vitamin B12 and allow for prompt correction of the deficiency. With this in mind, a group of scientists developed a list of criteria that aims to cover the high-risk group of patients treated with metformin - their screening and periodic follow-up. The proposed criteria include:

- guiding clinical signs for vit. B12 deficiency
-patients with type 2 DM with available comorbidities-diabetic peripheral neuropathy, autonomic

neuropathy, or both

- metformin treatment lasting more than 5 years
- the patient over 65 years old
- minimum six months of metformin treatment at a daily dose ≥ 1500 mg and high total cumulative exposure
- antacid medications administered together with metformin for a period of ≥ 12 months
- presence of other diseases and risk factors leading to an increased risk of vitamin B12 deficiency. (Infante,M.,et all. 2021).

MATERIAL AND METHODS

The main purpose of this study was to evaluate the vitamin B12 deficiency and the presence of anemic syndrome in all tested patients. The medical records and laboratory tests were conducted on sixty-one participants who were on treatment with metformin for a period between 5-10 years. All the 61 participants were Caucasian- 41 women and 20 men, respectively.

Regardless of gender, the average age of the participants was 78.73 years/from 48 years. to 93 years/. For women it is 80.26 years/from 48 years to 92 years/, and for men it is 75.6 years/from 50 years to 93 years/.

RESULTS

After processing the data, it was found that the average level of vit.B12 in the studied group of patients was 112.99 pmol/L

DISCUSSION

Vitamin B12 deficiency is less well known and can be a reason for health consequences, such as megaloblastic anemia and peripheral neuropathy. Considering the above data, we are obliged to focus on the necessity of early screening, routine examination, identification and timely correction of the deficiency of this vitamin in diabetics with metformin treatment. Low levels of vitamin B12 is a highly prevalent, yet easily correctable factor that would lead to improved patient care.

CONCLUSION

The present analysis also has some serious limitations, such as the small number of examined patients, the majority of whom fall into the age range over 75 years. Larger studies with more patients are needed to determine whether the deficiency of vit. B12 is a chance finding, whether it is the result of long-term metformin use or it is result of dietary factors.

Acknowledgements

I express my gratitude to the team of RSNC/regional non-profit association/"Diabetes Care" Burgas for the provided data from the screening conducted by them.

REFERENCES

Grudeva-Popova, Zh.(2020) Internal Medicine, p.706-707
Kertikova, S.(2000) Pediatrics- p. 476-477
McNulty, H., Pentieva,K.,Hoey, L.,Ward ,M.(2008) Homocysteine, B-vitamins and CVD. Proc Nutr Soc, 67(2):232–237.
Stanchev, P.,Orbetsova, M.,Kurteva, A.,Becheva, E.(2021) Oral treatment of type 2 diabetes in the elderly MEDINFO 5
Borisova, A.M. (2019) Recommendations for good clinical practice in ZD-BDE, p.39
Hirst, J.A., Farmer, A.J., Ali, R., Roberts, N.W., Stevens, R.J.(2012) Quantifying the Effect of Metformin Treatment and Dose on Glycemic Control. Diabetes Care 35, 446–454. [CrossRef].
Aroda, V., Edelstein, S., Goldberg, R., Knowler, W., Marcovina, S., Orchard,T., Crandall, J. (2016). Long-term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. Journal of Clinical Endocrinology and Metabolism, 101, 1754–1761

Beulens, J., Hart, H., Kuijs, R., Kooijman-Buiting, A. M., & Rutten, G.(2015). Influence of duration and dose of metformin on cobalamin deficiency in type 2 diabetes patients using metformin. Acta Diabetologica, 52, 47–53
Chakarova, N., Dimova, R., Mincheva, R., Grozeva, G., Todorova, A., Serdarova, M., Tankova, C.(2019) Vitamin B12 status in a Bulgarian population of patients with type 2 diabetes 2 Endocrinology 3
Boyanov,M., Boneva,Zh.,Tsoncheva,A.,Protich,M., (2003) Plasma levels of homocysteine, folates and vitamin B12 in patients with type 2 diabetes mellitus treated with metformin ,Endocrinology, issue 4

Bauman ,W.A., Shaw ,S., Jayatilleke, E., Spungen, A.M., Herbert, V.(2000) Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care23(9)1227-31.
William, A.,Shaw, S.,Jayatilleke, E.,Spunger, A.,Herbert, V.(2000) Increased Intake of Calcium Reverses Vitamin B12 Malabsorption Induced by Metformin Diabetes care , Volume 23, Number 9, September
Green, R., Allen, L.H., Bjørke-Monsen, A.L., Brito, A., Guéant, J.L., Miller, J.W., Molloy, A.M., Nexo, E., Stabler, S., Toh, B.H., Ueland, P.M., Yajnik, C.(2017) Vitamin B12 deficiency. Nat Rev Dis Primers; 3: 17040 [PMID: 28660890 DOI: 10.1038/nrdp.2017.40]
Beulens, J.W., Hart,H.E., Kuijs, R., Kooijman-Buiting, A.M., Rutten, G.E.(2014) Influence of duration and dose of metformin on cobalamin deficiency in type 2 diabetes patients using metformin. Acta Diabetol, [Epub ahead of print].
Marwan, A., Ahmed, G. M. and Rheeder,P.(2016) Vitamin B12 deficiency in metformintreated
type-2 diabetes patients, prevalence and association with peripheral neuropathy
Ahmed et all.(2016) BMC Pharmacology and Toxicology 17:44 DOI 10.1186/s40360-016-0088-3
Laing,E.F., Nsiah,P., Anthony,R., Acheampong,E., Asamoah,S.K. , show all (2019) Vitamin B12 deficiency in type 2 diabetic patients on metformin: a cross-sectional study from South-Western part of Ghana Alexandria Journal of Medicine Volume 55, Pages 58-67 | Received 13 May 2019, Accepted 17 Aug 2019, Published online: 12 Sep.
Groot-Kamphuis, D.M.,Dijk, P.R, Groenier, K.H. , Houweling, S.T. , Bilo, H.J.G.,
Kleefstra, N.(2013) Vitamin B12 deficiency and the lack of its consequences in type 2 diabetes patients using metformin The netherlsnds journal of medicine se p t emb e r , v ol . 7 1, n o 7
Infante,M., Leoni,M., Caprio,M.,Fabbri,A.(2021) Long-term metformin therapy and vitamin B12 deficiency: An association to bear in mind World J Diabetes July 15; 12(7): 916-931 DOI: 10.4239/wjd.v12.i7.916 ISSN 1948-9358 (online)

Rumyana, Y. (2023). Unhealthy diet as a behavioral risk factor for socially significant diseases and premature mortality, 2(1), 53-58. doi: 10.35120/medisij020153y
UDK: 613.2.035:616-036.88

UNHEALTHY DIET AS A BEHAVIORAL RISK FACTOR FOR SOCIALLY SIGNIFICANT DISEASES AND PREMATURE MORTALITY

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Abstract: A number of epidemiological studies prove that for the group of chronic socially significant diseases, nutritional factors are more important in cardiovascular diseases, oncological diseases, diabetes and obesity. Quantitative and qualitative nutritional disorders underlie the development of the most important risk factors for disease, disability and premature mortality in modern society. A target task of the programs for a healthy lifestyle is the reduction of habits harmful to health, among which unhealthy eating is included.

The purpose of the present study is to investigate unhealthy eating as a behavioral risk factor related to morbidity and mortality from socially significant diseases in Bulgaria and the other member states of the European Union.

Methodology: The following research methods were used: documentary method - review of: scientific literature; published materials from the Organization for Economic Cooperation and Development on the health profiles of the countries; induction and deduction; comparative analysis; graphical method for visual presentation of the obtained results.

Results and analysis: From the study, it is clear that unhealthy eating as a behavioral risk factor for morbidity and mortality is represented by 17% on average in the European Union. In Bulgaria, 29% of all deaths in 2019, which is the highest share in the EU, are due to irrational and unbalanced nutrition. It is followed by the countries of Central and Eastern Europe. A significant role for this problem is the low intake of fruits and vegetables and the high consumption of sugar and table salt.

Conclusion: The main element of a healthy diet is the intake of fruits and vegetables. Their consumption varies between countries. The most significant benefits of their consumption are due to the reduction of both cardiovascular diseases and the prevention of oncological diseases.

Recommendations: It is necessary to direct the efforts of the society to a correct food policy, a change in the food system and good education of individuals to create their own healthy microenvironment, different from the current toxic and obesogenic environment. Only in this way will the development of the global epidemic of obesity, type 2 diabetes and other socially significant diseases be reduced.

Keywords: *unhealthy diet, behavioral risk factor, socially significant diseases, premature mortality, fruits and vegetables*
Field: 3) Medical sciences and Health

1. INTRODUCTION

A number of epidemiological studies prove that for the group of chronic socially significant diseases, nutritional factors are of great importance in cardiovascular, oncological diseases, diabetes and obesity. Quantitative and qualitative nutritional disorders underlie the development of the most important risk factors for disease, disability and premature mortality in modern society.

The role of dietary factors on elevated blood pressure is well known and proven.

A significant positive effect on its reduction is the reduction of excess weight, reduction of fat intake, increase of fruit and vegetable intake, reduction of salt consumption. According to D. Popova (2015), the way of eating probably exerts long-lasting effects on blood pressure already with the start of improper fetal nutrition, which determines the further trends and magnitude of arterial pressure in children and adults in response to eating habits.

The INTERSALT studies demonstrate an absence of hypertension in some very isolated societies where salt intake is very low and blood pressure does not increase with age. The same studies found a trend toward no weight gain, lower fat intake, and a high intake of fruits and vegetables—three important conditions that help lower blood pressure. [8]

Over the past few decades, the incidence of obesity and diabetes has increased dramatically in all countries of the world. More than one billion people are currently overweight or obese, with more

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obese individuals residing in developing countries than in developed countries. In developed Western countries, recent reductions in the incidence of strokes and coronary heart disease have been achieved despite progressive increases in obesity and diabetes. This fact is explained by the successful reduction of cholesterol levels in these societies in connection with the replacement of dietary saturated and trans-fats with mainly omega-6 polyunsaturated fats. But these fats favor obesity and type 2 diabetes, despite having a beneficial effect on atherosclerotic and thrombotic processes.

For the prevention of cardiovascular and some oncological diseases, the regular intake of fruits and vegetables is recommended as an essential element of a healthy and balanced diet.[6]

Consumption of fruits and vegetables varies widely among countries depending on economic, cultural and agricultural characteristics. Inadequate fruit and vegetable intake is associated with about 14% of gastrointestinal cancer mortality, about 11% of coronary heart disease mortality, and about 9% of stroke mortality worldwide. The most significant benefits of fruit and vegetable consumption are due to a reduction in cardiovascular disease, but fruits and vegetables also prevent cancer. A higher incidence of mortality associated with low fruit and vegetable consumption occurs in middle-developed European countries.[8]

Many countries in the Mediterranean region have different diets, but with some common characteristics. This diet lowers the incidence of coronary heart disease, which is partly due to the increased consumption of monounsaturated fatty acids (mainly from olive oil) and the low consumption of saturated fat. The summarized key components of the Mediterranean diet are as follows:

- abundance of plant foods (fruits, vegetables, potatoes, bread, cereals, legumes, nuts and seeds);
- minimally processed and fresh seasonal foods;
- fresh fruit as a typical dessert;
- olive oil as the main source of fat;
- dairy foods, chicken and fish in low to moderate quantities;
- less than 5 eggs per week;
- red meats rarely and in small quantities;
- wine in low to moderate amounts (1-2 glasses daily for men and 1 glass for women).

The purpose of the present study is to investigate unhealthy eating as a behavioral risk factor related to morbidity and mortality from socially significant diseases in Bulgaria and the other member states of the European Union.

2. MATERIALS AND METHODS

The following research methods were used:

- documentary method - review of: scientific literature; of published materials from the Organization for Economic Co-operation and Development on the health profiles of the countries;
- induction and deduction;
- comparative analysis;
- graphic method for visual representation of the obtained results.

3. RESULTS

From the study, it is clear that unhealthy eating as a behavioral risk factor for morbidity and mortality is represented by 17% on average in the European Union.

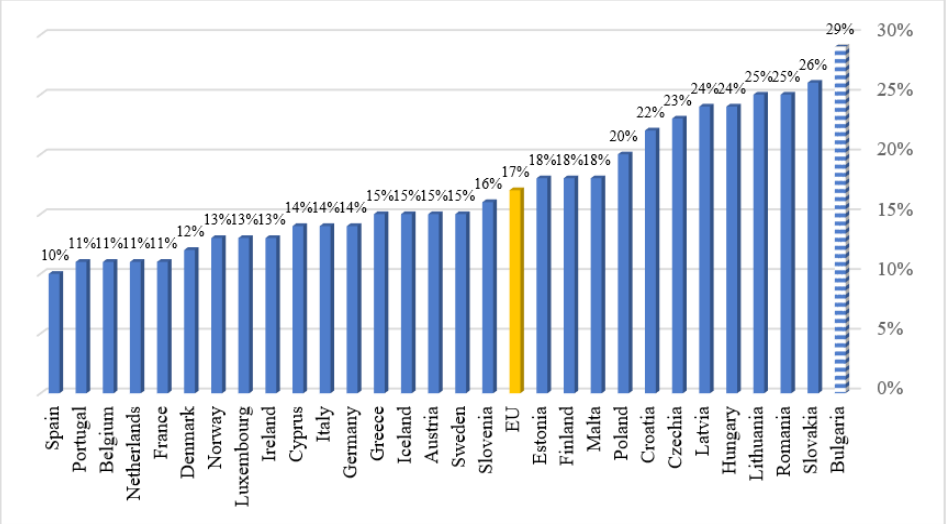

Fig. 1. Relative share of mortality caused by behavioral risks related to unhealthy eating in EU Member States

Sources: OECD, Eurostat, 2021

Notes: 1 The study also includes Norway and Iceland as members of the European Free Trade Association.

2 Nutrition-related risks include 14 components, such as low consumption of fruit and vegetables and high consumption of sugar-sweetened beverages. [7]

Almost half of all deaths in Bulgaria are due to behavioral risk factors, among which unhealthy nutrition occupies a leading place. The problem is particularly significant as it accounted for 29% of all deaths in 2019, the highest proportion among EU countries. A significant role is played by the low intake of fruits and vegetables and the high consumption of sugar and table salt.

As can be seen in figure 1 for the countries of Central and Eastern Europe – Slovakia, Lithuania, Hungary, Latvia, Czech Republic, a quarter of all deaths in 2019 can be attributed to risks related to unhealthy and irrational nutrition. They are followed by Croatia and Poland.

The main risk factor for chronic non-infectious diseases such as diabetes, cardiovascular and some types of oncological diseases is excess weight. A 2022 World Health Organization report notes that obesity levels in Europe are rising due to high consumption of high-calorie foods, trans-fats and saturated fats and increasingly sedentary lifestyles. [9]. And an OECD study, "The Heavy Burden of Obesity", found that obesity could cause more than 320,000 premature deaths a year in EU countries over the next 30 years if no countermeasures are taken (OECD, 2019 [5]).

Adolescent overweight and obesity are a growing public health problem.

According to data from the Organization for Economic Cooperation and Development, the relative share of obesity among adults in Bulgaria in 2019 was 13%, which is the third lowest among EU countries and below the EU average of 16%.

In most EU countries, more than half of adults are overweight or obese. Between 2014 and 2019, obesity rates increased in almost all countries, except France and Luxembourg, where they remained stable. Over this five-year period, the largest increases were observed in Austria, Croatia, Finland, Hungary and Slovakia.

In all EU countries, the proportion of men who are overweight or obese is higher. The gender gap is more significant in Luxembourg and the Czech Republic.

In terms of education, the situation is as follows: people with less education are more likely to be overweight or obese than those with higher education. This difference is particularly pronounced in Portugal and Luxembourg. According to the results of the study, differences in the prevalence of overweight among people with high and low education lead to additional inequality in health and employment. Those suffering from at least one chronic disease associated with being overweight are less likely to be employed; and when they are employed, they are more likely to be absent or less productive than healthy individuals. [5].

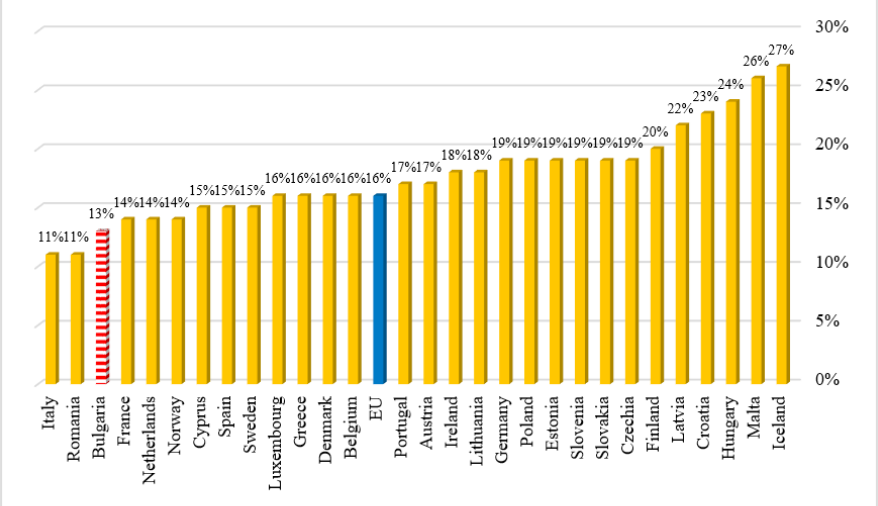

Fig. 2. Relative proportion of adults with obesity

Sources: OECD, Eurostat, 2021

Figure 2 shows the relative share of obese adults in EU countries, including Iceland and Norway - members of the European Free Trade Association. The obesity rate is highest in Iceland, which in 2019 had 27% obesity among the adult population (over 18). Much higher than the EU average (16%) is the adult obesity rate in Malta, Hungary, Croatia, Latvia and Finland. The relative share of obese adults was lowest in Bulgaria (13%), Romania and Italy (11% each).

4. DISCUSSION

During the outbreak of the COVID-19 pandemic, overweight and obesity were particularly at risk for developing more severe symptoms and death. [3,6] The imposition of lockdown and other restrictions on mobility contributed to negative changes in the eating habits of a large part of the population.

To prevent these adverse consequences leading to obesity, countries have begun to pursue policies promoting a healthier lifestyle. [1]. One of these policies is promoting the intake of more fruits and vegetables, which are a basic element of a healthy diet.

The World Health Organization recommends consuming at least 400g (i.e. 5 portions) of fruit and vegetables per day, and in response to this recommendation, the EU launched the “5 a Day” campaign. [6]

In Bulgaria, a small number of adults follow this recommendation. The country has the second lowest fruit intake of all EU countries. According to a survey, three out of five adults say they don’t eat fruit, and 49% don’t eat vegetables every day.

In response to the problems associated with overweight and obesity in adolescents, programs have been introduced to promote healthy eating in school-age children.[7] The situation is similar in Romania, Latvia, Luxembourg and the Czech Republic.

According to Eurostat data from 2019, in EU member states, on average, only 12% of adults report consuming five or more portions of fruit and vegetables per day. Between 1 and 4 portions per day are taken by 55% of the population. Among the countries with such a high intake are Ireland, the Netherlands, Denmark and France. Less than 1 serving per day consumed 33 percent. [6]

Women, as well as people with a higher level of education, eat more fruits and vegetables every day.

Interventions to promote healthy eating include subsidies for fresh fruit and vegetables, regulations to limit foods high in fat, salt and sugar, setting nutrient-based standards in schools and public institutions, reformulating products with high levels of sugar, simple and informative front-of-pack labels and youth nutrition education programs. In this context, the EU Farm to Fork Strategy and the European Cancer Plan have been created, which require a review of EU rules on information provided to consumers. [3]

In 2017, over 950,000 deaths in the EU were linked to an unhealthy diet, and half of people over 18 were overweight. [10]

Policies for prevention and health promotion in Bulgaria have a limited impact. In 2015, the proposal

to introduce a tax on food and drinks with a high content of salt, trans fat, sugar and caffeine did not receive the support of stakeholders and the Council of Ministers. Among the attempts to solve the problem of the growing number of children suffering from overweight or obesity is the project “Healthier children”. [7]

5. CONCLUSION

Irrational and unbalanced nutrition occupies a leading place among behavioral risk factors, causing about half of the deaths in Bulgaria. This mortality can be prevented with good prevention and promotion of a healthy lifestyle, but unfortunately this is not among the priorities of the government policy.

A main element of a healthy diet is the intake of fruits and vegetables. Their consumption varies from country to country. The most significant benefits of their consumption are due to the reduction of both cardiovascular diseases and the prevention of oncological diseases.

6. RECOMMENDATIONS

It is necessary that society’s efforts be directed towards a preventive food policy, a change in the food system and a good education of individuals to create their own healthy microenvironment, different from their current toxic and obesogenic environment. Only in this way will the development of the global epidemic of obesity, type 2 diabetes and other socially significant diseases be reduced.

REFERENSES

European Commission (2020), Farm to Fork Strategy, https://food.ec.europa.eu/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf.

Katz, M. (2021), “Regardless of Age, Obesity and Hypertension Increase Risks With COVID-19”, JAMA Internal Medicine, Vol. 181/3, p. 381, https://doi.org/10.1001/jamainternmed.2020.5415.

Laaninen, T. (2022), Nutrient profiles: A ’farm to fork’ strategy initiative takes shape, European Parliamentary Research Service.

Mantzoros CS, Williams CJ, Manson JE et al. (2006), Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. Am J Clin Nutr 2006; 84:328-335

OECD (2019), The Heavy Burden of Obesity: The Economics of Prevention, OECD Health Policy Studies, OECD Publishing, Paris, https://doi.org/10.1787/67450d67-en.

OECD/European Union (2022), Health at a Glance: Europe 2022: State of Health in the EU Cycle, OECD Publishing, Paris, 118-123, https://doi.org/10.1787/507433b0-en. ISBN 978-92-76-58684-5 (pdf)

OECD/European Observatory on Health Systems and Policies (2021), Bulgaria: Country Health Profile 2021, State of Health in the EU, OECD Publishing, Paris/European Observatory on Health Systems and Policies, Brussels. 7-22, ISBN 9789264361645 (PDF)

Popova, D. (2015), Nutrition and socially significant chronic diseases. Health of the world population, GorexPress, 137-162

WHO Europe (2022), WHO European Regional Obesity Report 2022, World Health Organization Regional Office for Europe, https://apps.who.int/iris/handle/10665/353747.

https://www.europarl.europa.eu/news/bg/headlines/society/20200519STO79425/ot-fermata-do-trapezata-strateghiiata-na-es-za-ustoychivi-khranitelni-verighi