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Normal human microbiota and dysbiosis - implications for health and disease

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Abstract

The normal human microbiota, formerly called the "*microbial flora*," consists of bacteria, fungi, viruses, and parasites that colonise the skin and mucous membranes of the respiratory, gastrointestinal, and genitourinary tracts. The number and diversity of microorganisms varies between different body niches and is greatest in the intestinal tract. The microbiota contributes to the homeostasis of the human organism by preventing colonisation by pathogenic microorganisms, participating in digestive processes and metabolism, and regulating immune functions.

Various environmental and genetic factors can lead to an imbalance in the human microbiota, called *dysbiosis*, which can affect human health. Dysbiosis is usually the result of decreased microbial diversity and a lower number of saprophytic microorganisms, followed by an overgrowth of opportunistic species. The most common diseases directly related to intestinal dysbiosis are antibiotic-associated diarrhoea and pseudomembranous colitis, both of which are associated with the excessive growth of harmful bacteria and *Clostridioides difficile* following broad-spectrum antibiotic therapy.

Dysbiosis is associated with various health conditions or diseases such as acne, psoriasis, eczema, chronic obstructive pulmonary disease, inflammatory bowel disease, obesity, metabolic syndrome, type 2 diabetes, autoimmune diseases and allergies, neurological diseases such as Parkinson's disease, Alzheimer's disease, epilepsy and stroke, depression, anxiety, infertility, preterm birth, and malignancies.

Key words: human microbiota, dysbiosis, commensal bacteria, dysbiosis-associated diseases

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Introduction

The normal human microbiota consists of bacteria, archea, fungi, viruses and parasites that colonise the skin and mucous membranes of humans without causing disease when the host is immunocompetent and in good general health. The human microbiota used to be referred to as the *"physiological microbial flora"*, or "*microflora*", but in the last decade the term "*human microbiota"* has gained acceptance and is widely used amongst healthcare professionals and the scientific community. Bacteria are the most numerous members of the human microbiota. It used to be assumed that their number was ten times higher (1×10^{14}) than that of our own tissue cells (1×10^{13}). According to new findings, however, this ratio is closer to 1:1 (3.8×10^{13} to 3×10^{13}), as red blood cells have been included in the total number of cells in the human body (1). There are an estimated 500–1000 bacterial species that exist in the human body, although the number of subspecies could be orders of magnitude higher (2, 3). Most species of these bacteria colonise the gastrointestinal tract, and nowadays many scientific studies aim to elucidate the role of the gut microbiota in health and disease (4).

The microorganisms that populate the human body interact with it in various ways, and the result of these interactions can be neutral, beneficial, or harmful to the host. Most members of the human microbiota enter into neutral or beneficial interactions with our organism, which can be characterised as saprophytic (neutral), commensal or mutualistic (5, 6, 7). *Saprophytism* (neutralism) is defined as an interaction between microorganisms and the human body in which both organisms coexist without affecting each other. In contrast, *commensalism* is beneficial to the microorganism (referred to as commensal) and neutral to the human organism, and most members of the human microbiota belong to the commensal bacteria (6). An interaction that is beneficial to both the microorganism and the human body is *mutualism*. The bacteria in the microbiota of the gastrointestinal tract were originally commensals, but evolved into mutualists when they began to produce vitamin K, which the human body cannot synthesize on its own (7). A harmful interaction between microorganisms and the human body manifests as *parasitism*, which benefits the microorganism and harms the human. In this type of relationship, the parasite uses the human body to maintain its biological cycle, or it feeds on human cells and thus damages the human organism. This damage is usually gradual, allowing the parasite to coexist in the host's body for a long period of time, which in rare cases can lead to death. Although many species of yeasts, moulds and parasites coexist in the human body, they are not considered strict parasites and thus harmful, as they are part of the normal human microbiota (5).

The microbiota that is always present in the human organism is called the resident microbiota. It consists of microorganisms that have a fixed body niche in one part of the body where they remain indefinitely. The transient microbiota is able to colonise the human organism for a short period of time, as it is quickly suppressed by the resident microbiota or the activity of the immune response. Strictly pathogenic bacteria can also colonise the skin or mucous membranes of the host and form a neutral relationship with it, which is referred to as asymptomatic carriage. The causative agents of pneumonia

and/or meningitis, *Haemophilus influenzae* and *Neisseria meningitidis*, can be isolated from throat swabs in 5% to 40% of healthy people (8, 9).

The colonisation by microorganisms that constitute the human microbiota and the composition of the microbiota depend on numerous exogenous and endogenous factors. It used to be assumed that the uterus is a primarily sterile organ, and that the fetus is sterile until the rupture of the fetal membranes during birth. Recent studies using molecular techniques point to bacterial colonisation of the uterus, placenta, and amniotic fluid that appears to impact fertility and pregnancy (10, 11, 12, 13). Intrauterine colonisation of the fetus by members of the microbiota has also been demonstrated by isolating bacteria from meconium (i.e., the first fetal stool) (10, 13). Mass colonisation of the baby's skin and mucous membranes occurs during birth and continues in the first days after birth, primarily by the mother's microbiota, but also by other microorganisms from the environment. The composition of the microbiota is influenced by the mode of delivery, so that babies born vaginally are dominated by bacteria of the genus *Lactobacillus*, which are found in the microbiota of the maternal vaginal mucosa, while babies born by caesarean section are dominated by bacteria of the genus *Staphylococcus*, which represent the skin microbiota of the mother (14, 15).

In the first days of an infant's life, the composition of the microbiota depends on random exposure to microorganisms that colonise specific sites of the infant's body without competition. Later, the microorganisms that are best adapted to colonise a particular site (called the body niche) prevail and become the dominant species. In the first years of life, the composition of the microbiota is influenced by the type of diet, so that in breastfed infants it is significantly more diverse and dominated by bifidobacteria, while in formula-fed infants it is less diverse, and lactobacilli predominate (15). The composition of the microbiota changes significantly after the transition to solid food, so that around the age of three years a permanent microbiota is formed which is unique to each individual and remains stable in adulthood (16).

Throughout life, human microbiota is influenced by a number of endogenous factors such as gender, age, hormonal status, and general health of the organism, as well as exogenous factors such as personal hygiene (use of soap, deodorant, mouthwash, skin peeling, vaginal rinsing, etc.), diet, intake of certain medications (especially broad-spectrum antibiotics), quality of drinking water, environmental changes, and exposure to toxins or chemical compounds from the environment (2, 17, 18).

Physiological role of microbiota in human organism and dysbiosis

The normal human microbiota has numerous beneficial effects on the human body, although in some cases its presence can be harmful. The positive effects of the microbiota are reflected in the prevention of colonisation by pathogenic microorganisms, participation in digestive processes, and influence on the metabolism and immunity of the host (19).

The prevention of colonisation by pathogenic microorganisms is largely a consequence of the preponderance of members of the human microbiota, resulting in

competition for nutrients and receptors on the surface of epithelial cells to which pathogenic microorganisms would bind. Members of the microbiota also produce small antibacterial molecules called bacteriocins, such as colicin, microcin, nisin, enterocin, lugdunin, lantibiotics and cutimycin, which inhibit growth and colonisation by pathogenic species (20). The skin microbiota produces fatty acids, and the lactobacilli of the vaginal microbiota produce lactic acid, which creates an acidic pH on the mucosa that inhibits the growth of pathogenic microorganisms (21, 22).

In addition to digesting common nutrients such as proteins, lipids and carbohydrates, intestinal bacteria are also involved in the digestion and metabolism of complex carbohydrates that the human body cannot utilise. The gut microbiota has numerous enzymes for the utilisation of dietary fibres such as cellulose, pectin, xylan, lignin, non-starch polysaccharides, starch, and oligosaccharides (fructo-oligosaccharides and galacto-oligosaccharides) that are resistant to host digestive enzymes (23). Fermentation of dietary fibres releases a variety of secondary products, such as gases (hydrogen, carbon dioxide and methane), short-chain fatty acids (acetate, propionate, butyrate, valerate, isovalerate, formate and hexanoate), organic acids (lactate and succinate) and alcohols (ethanol and methanol), which have antibacterial activity or are used by human cells as an energy source (19, 23). Short-chain fatty acids or their deficiency may influence the development of a variety of diseases, from allergies and asthma to cancer, autoimmune diseases, metabolic diseases, glucose homeostasis, including insulin secretion and insulin sensitivity, obesity, and neurological diseases such as multiple sclerosis (24). The intestinal microbiota is also capable of synthesising vitamin K, and B group vitamins thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folates, nicotinic acid, and cobalamin (25).

The human microbiota influences the state of the immune system by constantly providing non-specific antigenic stimuli to immune system cells and stimulating secretory IgA production on mucosal surfaces (4). The importance of this role is reflected in the higher incidence of immunopathological diseases (e.g., asthma) in children exposed to a less diverse microbiota during growth (26).

The negative effects of the human microbiota are reflected in the development of opportunistic infections and the production of carcinogenic and toxic products that can lead to disease, malignancies, and an impaired response to cancer immunotherapy (27). Members of the microbiota may also be involved in mechanisms of antimicrobial resistance, facilitated by horizontal transfer of resistance genes between bacteria or production of enzymes that degrade antibiotics (28).

A balanced microbial ecosystem in the host is called *eubiosis* and ensures normal and healthy functioning of the human body. In contrast to eubiosis, an imbalance in the composition and diversity of the host-associated microbiota, known as *dysbiosis*, is associated with many human diseases. These terms are often used in the context of the intestinal microbiota, as the microorganisms of the gut are the most numerous populations (4). Most health conditions caused by dysbiosis are the result

of the elimination of beneficial microorganisms and/or excessive growth and proliferation of opportunistic pathogens or pathogenic microorganisms that cause a state of acute or chronic inflammation. The use of broad-spectrum antibiotics such as cephalosporins leads to a reduction in the intestinal microbiota and excessive proliferation of the anaerobic bacterium *Clostridium difficile*, which causes a diarrhoea syndrome or pseudomembranous colitis. An imbalance in the oral or vaginal microbiota can also lead to the occurrence of fungal infections caused by *Candida* species (29).

Dysbiosis can be caused by a variety of factors, including poor nutrition, antibiotic use, concomitant infections, stress, and other environmental factors. Restoring a balanced gut microbiota often requires dietary changes, the use of prebiotics and probiotics, and other lifestyle changes aimed at promoting the growth of beneficial bacteria and suppressing harmful ones.

Composition of microbiota in health and dysbiosis-related diseases

With regard to the presence of members of the human microbiota, regions of the human organism are divided into primarily sterile regions, where no microorganisms are present, and colonised regions, which are inhabited by members of the human microbiota. Primary sterile regions include blood, cerebrospinal fluid, pleural, pericardial, and peritoneal fluids, urine from the upper parts of the urinary tract, and all tissues and internal organs. Under normal circumstances, microorganisms are not present in these regions, but they may be present temporarily if the epithelial barrier is breached after trauma or during delivery, and they are removed by the cells of the reticuloendothelial system. Transient bacteraemia lasts for minutes or a few hours and most commonly occurs after manipulation of non-sterile body sites, such as dental procedures, gastrointestinal biopsies, percutaneous catheterization of the vascular system, bladder or common bile duct, and surgical debridement or drainage, i.e., after procedures involving contaminated or colonised skin and/or mucous membranes, and at the onset of acute bacterial infection (30). However, in medical microbiology, transient bacteraemia has no clinical significance as it is usually asymptomatic and cleared by the immune system response and is not an indication for microbiologic diagnosis by means of haemoculture.

The colonised areas of the human organism are the skin and mucous membranes which are in contact with the external environment. The number of microorganisms present in these areas and the diversity of species vary from region to region and depend on the nutritional needs of the microorganisms and the conditions in which their growth is possible. Some microorganisms that are strict aerobes grow on the surface of the skin, whereas facultative and strict anaerobes are most abundant in the gut microbiota. Table I provides an overview of the most common species found in the resident microbiota of colonised parts of the body, and species with pathogenic potential found in carriers (31, 32, 33, 34).

Table I Microbiota of the colonised regions of the human body and potentially pathogenic bacteria

Tabela I Mikrobiota kolonizovanih regija ljudskog organizma i potencijalno patogene bakterije

Colonised part of the human organism	**Resident microbiota (low virulence potential)**	**Major potential pathogens in carriers**
Skin	*Cutibacterium* spp. (formerly *Propionibacterium* spp.*)* *Corynebacterium* spp. Coagulase negative staphylococci	*Staphylococcus aureus*
Oral cavity	*Neisseria* spp. Viridans streptococci *Moraxella* spp. *Peptostreptococcus* spp.	*Streptococcus pyogenes* *Candida albicans*
Nasopharynx	*Neisseria* spp. Viridans streptococci *Moraxella* spp. *Peptostreptococcus* spp.	*Streptococcus pneumoniae* *Neisseria meningitidis* *Haemophilus influenzae* Goup A streptococci *Staphylococcus aureus* (anterior nares)
Stomach and small intestine	*Streptococcus* spp. *Peptostreptococcus* spp. (oral)	/
Colon	*Eubacterium* spp. *Lactobacillus* spp. *Bacteroides* spp. *Fusobacterium* spp. *Enterobacteriaceae* *Enterococcus* spp. *Clostridium* spp.	Enterotoxigenic *Bacteroides fragilis* Enteropathogenic *E. coli* *Pseudomonas* spp. *Candida* spp. *Clostridium* spp. (*C. perfringens, C. difficile*)
Vagina **Prepuberty/menopause**	*Corynebacterium* spp. Staphylococci *Enterobacteriaceae*	*C. albicans*
Reproductive age	*Lactobacillus* spp. Streptococci	Group B streptococci *C. albicans*

Since most colonised regions harbour at least two different bacterial species, normal microbiological findings show a polymicrobial saprophytic microbiota in low numbers, indicating a state of health and no need for antibiotic therapy. However, in the state of dysbiosis, one bacterial species supresses the others and predominates, resulting in the isolation of a monomicrobial pure culture in high numbers, without the presence of a saprophytic microbiota. This microbiological finding indicates a state of infection and should be interpreted carefully along with other clinical signs and symptoms.

The most significant health conditions and diseases related to dysbiosis are presented in Figure 1.

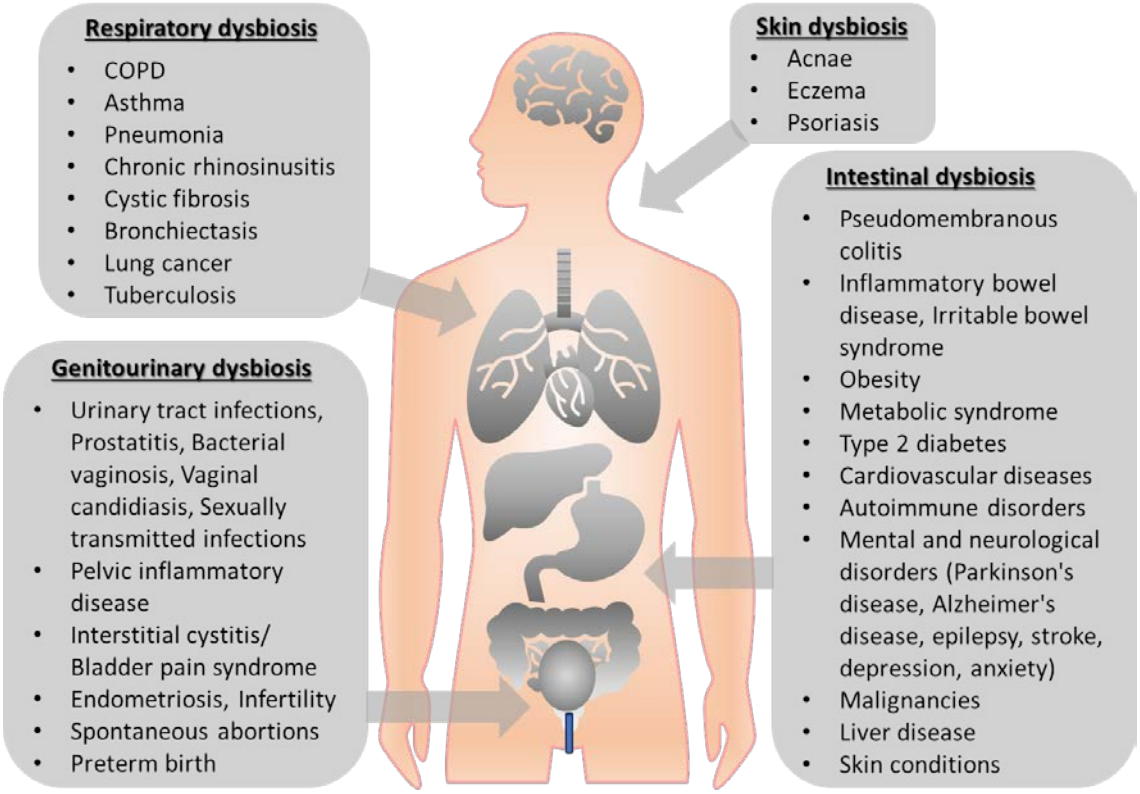

Figure 1. Dysbiosis-related diseases of the skin, respiratory tract, intestinal tract, and genitourinary tract

Slika 1. Bolesti kože, respiratornog, gastrointestinalnog i urogenitalnog trakta koje su povezane sa disbiozom

Gastrointestinal tract

The intestinal microbiota has the greatest diversity and abundance in terms of the number of microorganisms or species in the gastrointestinal tract. The total number of microorganisms per millilitre of intestinal contents increases along the gastrointestinal tract from $10^1 – 10^3$/ml in the oesophagus and stomach, to $10^8 – 10^9$/gramme of contents

in the lower part of the small intestine. The largest number of microorganisms, 10^{12} – 10^{14}/gramme of faeces, is found in the colon and rectum (35). The predominant microbial phyla in the gut are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia, with the most important genera being *Lactobacillus, Bacillus, Clostridium, Enterococcus, Ruminicoccus, Bacteroides, Prevotella.* and *Bifidobacterium* (36).

The oropharynx is colonised by streptococci, diphtheria, oral *Neisseria, Moraxella* spp. and *Actinomyces* spp., and a small number of *Candida* spp. Anaerobes and microaerophilic microorganisms colonise the deeper areas of the gingival crevices and tonsillar crypts. Saliva normally contains a mixed microbiota of about 10^8 microorganisms per millilitre. In the small intestine the resident microbiota is sparse, with the exception of the lower ileum, where a microbiota similar to that of the colon is found. The colon has the most numerous and diverse microbiota in the body. More than 90% of bacteria are anaerobes, mainly members of the genera *Bacteroides, Fusobacterium, Eubacterium,* and *Clostridium,* and the remaining 10% belong to the *Enterobacteriaceae* family (*E. coli, Klebsiella* spp., *Enterobacter* spp.) (19, 35).

The innate and adaptive immune response have the ability to discriminate between commensal and pathogenic bacteria through the activity of pattern-recognition receptors, such as Toll-like receptors, and a fine balance between regulatory T cells and $CD4^+$ effector T cells in the intestinal mucosa. An imbalance in the composition and diversity of the intestinal microbiota due to environmental and genetic factors increases the risk of infection with pathogens and promotes their excessive growth. Overuse and misuse of broad-spectrum antibiotics such as cephalosporins or fluoroquinolones often leads to antibiotic-associated diarrhoea or life-threatening pseudomembranous colitis caused by *C. difficile,* with toxic megacolon, perforation of the colon, and sepsis (37). Microbial dysbiosis can induce chronic inflammation of the intestinal mucosa, mediated by Th_1, Th_2 and Th_{17} cells and cytokines IL-4, IL-5, IFN-γ, IL-13, IL-17, IL-6, IL-8 and IL-22, leading to the development of numerous pathologic conditions of noninfectious aetiology, e.g. gastrointestinal disorders, metabolic disorders (obesity, metabolic syndrome, and type 2 diabetes), immune system disorders, autoimmune diseases and allergies, mental and neurological disorders, skin diseases, and malignancies (18, 19).

Gastrointestinal diseases associated with intestinal dysbiosis include inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). The two most important and common IBDs characterised by chronic inflammation of the gastrointestinal tract, biochemical changes in mucins in the colon, and decreased production of defensins are Crohn's disease and ulcerative colitis. In addition to genetic risk factors for the development of the disease, a reduction in microbial diversity and a decrease in the number of *Faecalibacterium prausnitzii* and *Roseburia hominis* have been demonstrated in patients with Crohn's disease (35). Gut dysbiosis may also contribute to the exacerbation of these diseases (33, 38, 39, 40). A study by Raftery et al. reviewed the association between IBD and chronic obstructive pulmonary disease, and suggested that the gut microbiota may also have an impact on respiratory health (41). However, gut

dysbiosis in COPD has not yet been described, although this would be expected given that environmental and genetic factors leading to microbial dysbiosis and chronic inflammation are present in both the intestinal tract and lungs (41). Irritable bowel syndrome is a functional gastrointestinal disorder characterised by abdominal pain, bloating, and changes in bowel motility. Alterations in the gut microbiota at the level of genera, such as *Coprococcus* spp., *Collinsella* spp., and *Coprobacillus* spp., have been associated with IBS symptoms (42, 43).

Several research papers suggested that altered gut microbiota composition may play a role in obesity by affecting metabolism, inflammation, and dietary energy production (44, 45, 46, 47). A high-fat, high-carbohydrate diet causes *Firmicutes* (*Clostridium* spp.), *Prevotella* spp., and *Methanobrevibacter* spp. to predominate, and beneficial genera and species such as *Bacteroides* spp., *Bifidobacterium* spp., *Lactobacillus* spp., and *Akkermansia* spp. to significantly decrease. Dysbiosis also alters the integrity of the intestinal epithelial barrier, translocation of bacteria and inflammation, expression of starvation hormones, induces dyslipidemia and low-grade chronic inflammation caused by metabolic endotoxemia, leading to obesity and its concomitant diseases (46, 47). Metabolic syndrome includes a number of risk factors such as obesity, hypertension, insulin resistance, and abnormal lipid levels that may eventually lead to the onset of cardiovascular and cerebrovascular diseases and type 2 diabetes (48, 49).

The gut microbiota plays an important role in the gut-brain axis and its bidirectional neurocrine, endocrine, and immune-mediated signalling pathways (50, 51, 52). Gut dysbiosis has been associated with various **neurological diseases** such as Parkinson's disease, Alzheimer's disease, epilepsy, stroke and vascular cognitive impairment, amyotrophic lateral sclerosis (ALS), and even mood disorders such as depression and anxiety (53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68). The composition of the gut microbiota also mediates the anti-seizure effects of the ketogenic diet in patients with severe epilepsy (69).

Among other health conditions, intestinal dysbiosis has been linked to the development of allergies such as asthma and atopic dermatitis, as well as autoimmune diseases such as rheumatoid arthritis, lupus, and multiple sclerosis due to its potential influence on immune responses, inflammation and immune system regulation, increased risk of colorectal cancer due to the production of certain metabolites that trigger genotoxic stress and promote genetic/epigenetic alterations (*Fusobacterium nucleatum* has been associated with cancer, and *Ruminococcus bromii* with a favorable outcome), liver diseases such as nonalcoholic fatty liver disease and alcoholic liver disease, and skin diseases such as acne, eczema, and psoriasis (4, 16, 19, 35).

Skin

As the largest human organ, the skin has multiple functions and serves as the first line of defence against microorganisms and various environmental factors. The skin provides a dry, slightly acidic, and aerobic environment and is colonised by approximately 10^4 to 10^5 microorganisms per cm^2. The composition and number of

microorganisms in the skin's microbiota are determined by the activity of the sebaceous and sweat glands, so moist areas (armpits, perineum, and interdigital spaces) have a richer microbiota than other areas of the skin. The skin microbiota is most numerous in the hair follicles and sebaceous gland excretory ducts, and is easily recovered from these niches after the application of antiseptics or disinfectants. The most common species are staphylococci (*S. aureus* and coagulase-negative staphylococci *S. epidermidis* and *S. warneri*) and *Cutibacterium* spp. (formerly known as *Propionibacterium* spp.), which are scattered over the entire skin surface, whereas diphtheria bacilli of the genus *Corynebacterium* are more common in moist skin folds. *Cutibacterium* spp. grow in sebum and break down skin lipids to fatty acids, which inhibit or kill other bacteria that may colonise the skin. Other microorganisms, such as *Streptococcus* spp. and *Micrococcus* spp., apathogenic bacteria and anaerobes *Clostridium* spp. and *Peptostreptococcus* spp., and fungi *Candida* spp. and *Malassezia* spp., are present to a lesser extent (31, 70).

The conjunctiva of the eye has a strong mechanism against pathogenic bacteria and therefore a very sparse microbiota, mainly derived from the skin. The presence of antimicrobial compounds (lysozyme, defensins, and lactoferrin) and secretory IgA in lacrimal secretions, as well as the action of blinking and rinsing the eye with tears, ensure that the number of bacteria remains low (71).

Various factors can damage the skin barrier and lead to dysbiosis. These factors include varying hygiene practices (excessive use of harsh soaps, antibacterial products, and frequent washing), use of topical or oral antibiotics, certain skin diseases and underlying health conditions, diet and lifestyle, and environmental factors (exposure to pollutants, UV radiation, and other environmental stressors). Dysbiosis of the skin microbiota can manifest itself in various skin conditions such as irritation, itchiness, dryness, redness, inflammation, and susceptibility to infection, and has a strong impact on barrier function and a role in inflammatory skin diseases such as acne, psoriasis, and atopic dermatitis. In psoriasis, *Firmicutes*, and the genera *Corynebacterium* spp., *Cutibacterium* spp., *Staphylococcus* spp., and *Streptococcus* spp., are the most common bacterial strains in the skin microbiota, while *Actinobacteria* are relatively rare. Atopic dermatitis is characterised by a lower diversity of the skin microbiota and a greater number of *Staphylococcus* spp., especially *S. aureus*, and lower numbers of *Cutibacterium* spp., *Corynebacterium* spp., *Streptococcus* spp., *Acinetobacter* spp. and *Prevotella* spp. (72).

Respiratory tract

The upper respiratory tract, the nose and nasopharynx, are colonised with different bacterial species that form the microbiota. The microbiota of the nasopharynx is similar to the microbiota of the skin (*S. epidermidis* and diphtheria bacilli), and 25% to 30% of healthy people carry pathogenic *S. aureus* as a resident or transient microbiota. This microbiota is also similar to the oral microbiota, but potentially pathogenic

microorganisms such as *Haemophilus* spp., *Streptococcus pneumoniae, Neisseria* spp., and *Moraxella* spp. are frequently transmitted here (32).

The lower respiratory tract below the larynx is protected by the action of the ciliary epithelium and the movement of mucus, which expels microorganisms that reach the mucosa of the trachea or large bronchi. The sterile areas of the respiratory tract are the bronchioles and alveoli, which are sterile due to the presence of alveolar macrophages, as well as the paranasal sinuses and middle ear.

Respiratory dysbiosis, bacterial overgrowth, and alterations in the number and function of $CD4^+$ helper T cells, $CD8^+$ cytotoxic T cells, and regulatory T cells populations can promote inflammatory responses in the airways and lungs, leading to acute and chronic respiratory diseases. Bacterial overgrowth of the resident microbiota plays an important role in common lung infections like bronchitis, pneumonia associated with risk factors (community-acquired pneumonia, immunodeficiency-related pneumonia, ventilator-associated pneumonia, SARS-CoV-2-associated pneumonia), acute respiratory distress syndrome, and chronic obstructive pulmonary disease (COPD) (73).

Chronic obstructive pulmonary disease is a group of progressive lung diseases, including emphysema and chronic bronchitis, characterised by breathing difficulty and decreased airflow. Recent studies point to the role of the gut-lung axis in COPD, as dysbiosis of the gut and airway microbiota may contribute to inflammation and exacerbations in COPD patients (74, 75, 76). The most common bacterial isolates from the sputum of COPD patients during exacerbations are phyla *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Fusobacteria*, and the genera *Streptococcus*, which is the most abundant, *Neisseria, Porphyromonas, Haemophilus, Veillonella, Prevotella, Rothia, Pseudomonas, Staphylococcus, Proteus*, and *Moraxella*. Numerous studies indicate that lower microbial diversity is associated with acute COPD exacerbation (76, 77, 78, 79). COPD patients with declining respiratory function have a greater abundance of the *Firmicutes* in the gut microbiota than other patients in whom the phylum *Bacteroidetes* and the genus *Alloprevotella* predominate (75).

Asthma is a chronic inflammatory disease of the respiratory tract that affects 300 million children and adults worldwide. The development of asthma is strongly influenced by environmental and other exogenous factors (allergens, air pollutants), as well as genetic predispositions, which shape the respiratory microbiota, particularly during birth and early childhood (26). Alterations in the respiratory microbiota with an abundance of proteobacteria with genera *Haemophilus* and *Moraxella* in children and adult asthmatics influence airway inflammation and contribute to bronchoconstriction and bronchial hyperreactivity, leading to exacerbation of asthma symptoms (80, 81, 82).

The importance of the respiratory and intestinal microbiota in modulating immune responses also plays a role in other respiratory diseases, including common respiratory infections such as viral infections and pneumonia, chronic rhinosinusitis, cystic fibrosis, bronchiectasis, lung cancer, and tuberculosis (83). An imbalance in the respiratory

microbiota affects susceptibility to viral infections, such as influenza and the common cold, and increases susceptibility to pneumonia-causing pathogens. Chronic rhinosinusitis (CRS) is a persistent inflammation and infection of the nasal passages and sinuses. Dysbiosis in the microbiota of the nose and sinuses is thought to contribute to the development and persistence of CRS (84).

Cystic Fibrosis (CF) is a genetic disorder that results in thick, hyper-viscous mucus production that harbours bacteria and can lead to chronic respiratory infections. The core microbiota of CF patients' lungs shows an overexpression of *Proteobacteria* and *Actinobacteria*, and includes genera *Streptococcus, Prevotella, Rothia, Veillonella, Actinomyces, Fusobacterium, Gemella, Granulicatella, Neisseria, Atopobium*, and *Porphyromonas*. Disorders in the respiratory microbiota may play a role in the progression of respiratory infections in individuals with CF (85).

Among other respiratory diseases, intestinal and respiratory dysbiosis may contribute to recurrent respiratory infections in individuals with bronchiectasis, and the immunomodulatory role of the gastrointestinal microbiota significantly influences the immune response to tuberculosis infection, susceptibility to tuberculosis, and its progression (86, 87). Although the direct relationship with lung carcinogenicity is not fully understood, some studies have investigated the possible role of respiratory dysbiosis in the development and progression of lung cancer (86).

Genitourinary tract

The kidneys, renal pelvis, ureters, and urinary bladder are among the primary sterile regions, while the lower part of the urethra contains 10^3 - 10^4 microorganisms transferred from the perineal skin. The urinary tract microbiome (i.e., urobiome) consists of coagulase-negative staphylococci (*S. epidermidis*), diphtheroids, *Enterococcus* spp, *Streptococcus* spp, and the anaerobes *Bacteroides* spp, *Fusobacterium* spp, and *Peptostreptococcus* spp. (88, 89).

The vaginal microbiota plays an important role in the resistance to colonisation by invading pathogens, which is critical for the prevention of sexually transmitted infections, urinary tract infections, and vulvovaginal candidiasis. The vaginal microbiota varies with age and hormonal status (34, 90). Before puberty and after menopause, it is mixed, nonspecific, and relatively sparse, containing microorganisms belonging to the microbiota of the skin and colon. In the reproductive period, it consists mainly of anaerobic and microaerophilic bacteria. The most abundant bacteria in the vaginal microbiota of reproductive age women are *Lactobacillus* spp. and anaerobic bacteria, including *Gardnerella vaginalis*, *Prevotella* spp., *Mobiluncus* spp., *Ureaplasma urealyticum*, and *Mycoplasma hominis* (91, 92). The vaginal microbiota has been grouped into five types known as community state types I–V, and all five types are dominated by *Lactobacillus* spp. (*L. crispatus, L. gasseri, L. iners, L. jensenii*), polymicrobial microbiota including *Lactobacillus* and bacterial vaginosis-associated bacteria. Types I, III and IV are commonly found in women, while the other two types are rare (93).

The *Lactobacillus* genus bacteria break down glycogen deposited in the epithelial cells of the vagina under the influence of estrogen hormones and metabolize it to lactic acid, which provides the acidic pH of the vaginal secretion (pH 3.5-4.5). This has an inhibitory effect on most microorganisms, with the exception of gram-positive cocci and yeasts, which can survive in an acidic environment (22). *Gardnerella vaginalis, Prevotella* spp. (*Prevotella bivia, Prevotella disiens*), *Porphyromonas* spp., *Peptostreptococcus* spp. and *Mobiluncus* spp. may also be present in small numbers on the vaginal mucosa. These bacteria are the causative agents of bacterial vaginosis, which occurs when the normal vaginal microbiota is disturbed due to a decrease in lactobacilli (94).

Genitourinary microbiota is distinct from other microbial communities in the body and can be influenced by various factors, including sexual activity, hygiene practices, and hormonal changes. An imbalance in the urogenital microbiota contributes to an increased risk of urogenital infections, urinary tract dysfunction, infertility, endometritis, and preterm birth, and may play a role in the development of cervical cancer, among other factors.

Urinary Tract Infections (UTIs) occur when bacteria, often from the gut, enter and multiply in the urinary tract. Changes in the urobiome and vaginal microbiota might impact the susceptibility to UTIs, benign prostatic hyperplasia, urinary incontinence and overactive bladder syndrome, interstitial cystitis/bladder pain syndrome, bladder cancer, and urinary tract infections (88, 89, 95, 96, 97).

Alterations in the urinary and genital microbiota can contribute to the development or exacerbation of prostatitis, as they induce prostate inflammation, which leads to benign prostatic conditions such as prostatitis/chronic pelvic pain syndrome and benign prostatic hyperplasia. The whole human microbiota is capable of influencing systemic hormone levels and may play an important role in the development of prostate cancer that is dually affected by estrogen and androgen levels (98, 99).

Bacterial Vaginosis (BV) is an anaerobic polymicrobial disease characterised by subclinical inflammation of the vaginal mucosa. This can lead to itching, discharge, and an increased risk of other infections. Women with BV have reduced lactobacillus abundance and species diversity, with *L. iners*, *L. rhamnosus*, *L. salivarius*, and *L. reuteri* predominating, and other beneficial species such as *L. crispatus, L. fermentum, L. acidophilu*s, and *L. delbruckii* absent (100, 101).

An imbalance in the vaginal microbiota, especially after broad-spectrum antibiotic therapy, also contributes to vaginal candidiasis caused by *Candida albicans* and other non-albicans species (102). Alterations in the number and distribution of lactobacilli species may also lead to an overgrowth of *Candida* species and cause asymptomatic vulvovaginal candidiasis (101).

The genital microbiota may influence the susceptibility to sexually transmitted infections (STIs) such as chlamydia, gonorrhoea, trichomoniasis, and HPV, and lead to the development of pelvic inflammatory disease (103, 104, 105). Alterations in the

vaginal microbiota and increased incidence of HPV infection have been associated with cervical intraepithelial neoplasia (CIN) and cervical cancer (106, 107). Patients with HPV infection and CIN or cervical cancer were found to be depleted of *Lactobacillus crispatus* and to have an increased diversity of anaerobic microorganisms *Atopobium vaginae, Dialister invisus, Finegoldia magna, Gardnerella vaginalis, Prevotella buccalis,* and *Prevotella timonensis* (108, 109). Studies on HIV virus transmission suggest that lactic acid produced by a *Lactobacillus*-rich microbiota likely inhibits HIV transmission, whereas polymicrobial microbiota (type IV of vaginal secretions) and STIs likely increase HIV transmission (110).

In addition to gynaecological health, the vaginal microbiota has a strong influence on the reproductive health of females (111, 112). The type of predominant species in the vaginal microbiota may have role in endometriosis, fertility and infertility, and outcome of assisted reproduction technologies (113, 114, 115). Altered microbiota could also determine pregnancy outcomes (116, 117). Recurrent spontaneous abortions in the first trimester of pregnancy have been associated with a high prevalence of *Leptotrichia amnionii, Atopobium vaginae* and *Sneathia sanguinegens* (118, 119, 120). Several studies have suggested a possible association between an imbalance in the vaginal microbiota and an increased risk of preterm birth (121, 122, 123). The risk of preterm birth was greater in women with high concentrations of *Atopobium vaginae, Gardnerella vaginalis*, or ureaplasma. An increased risk was associated with the predominance of *Lactobacillus iners, Streptococcus* and *Bifidobacterium* in the vaginal microbiota, compared with the protective effect of *Lactobacillus crispatus* found in women with term births (122, 123).

Conclusion

The human microbiota is an essential and complex ecosystem that profoundly influences various aspects of human health. This complex community of microorganisms which colonise various niches of the human body plays a multifaceted and pivotal role in maintaining health and well-being. Moreover, an imbalanced microbiota is associated with a variety of health conditions and diseases, emphasizing the importance of maintaining and preserving its diversity and balance. Therapeutic approaches to dysbiosis focus on supplementing the normal microbiota with probiotic microorganisms in probiotics, prebiotics, postbiotics, and synbiotics, or on regenerating the intestinal microbiota with fecal transplants. Research in this area continues to advance, promising innovative treatments and interventions that harness the power of the microbiota to improve human health.

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Normalna ljudska mikrobiota i disbioza - implikacije po zdravlje i bolest

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Kratak sadržaj

Normalna ljudska mikrobiota, koja se ranije nazivala „*mikroflora*“, sastoji se od bakterija, gljivica, virusa i parazita koji kolonizuju kožu i sluzokožu respiratornog, gastrointestinalnog i genitourinarnog trakta. Broj i raznovrsnost mikroorganizama variraju između različitih telesnih niša i najveći su u crevnom traktu. Mikrobiota doprinosi homeostazi ljudskog organizma tako što sprečava kolonizaciju patogenim mikroorganizmima, učestvuje u procesima varenja i metabolizma i reguliše imunološke funkcije.

Disbioza je stanje u kome dolazi do neravnoteže sastava mikrobiote usled uticaja različitih egzogenih ili endogenih faktora, što može uticati na ljudsko zdravlje. Ona je najčešće rezultat smanjene raznovrsnosti mikroorganizama i manjeg broja saprofitnih bakterija, što je praćeno prekomernim rastom potencijalno štetnih vrsta. Najčešće bolesti koje su direktno povezane sa crevnom disbiozom su dijareja povezana sa primenom antibiotika i pseudomembranozni kolitis, a obe nastaju kao posledica prekomernog rasta štetnih bakterija i *Clostridioides difficile* nakon terapije antibioticima širokog spektra.

Disbioza je povezana sa različitim zdravstvenim stanjima ili bolestima kao što su akne, psorijaza, ekcem, hronična opstruktivna bolest pluća, inflamatorna bolest creva, gojaznost, metabolički sindrom, dijabetes tipa 2, autoimunske bolesti i alergije, neurološke bolesti kao što su Parkinsonova bolest, Alchajmerova demencija, epilepsija i moždani udar, depresija, anksioznost, neplodnost, prevremeni porođaj i maligni tumori.

Ključne reči: humana mikrobiota, disbioza, komensalne bakterije, bolesti povezane sa disbiozom

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Association of catechol-O-methyltransferase gene polymorphisms with treatment response and levodopa-induced complications in Parkinson's disease: A summary of current knowledge

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Abstract

Catechol-O-methyltransferase (COMT) is one of the cardinal enzymes in the degradation of catecholamines and levodopa. Genetic variants of the *COMT* gene may affect COMT enzyme activity. The most examined *COMT* gene polymorphism is the nonsynonymous single nucleotide polymorphism (SNP) in exon 4 (Val108/158Met; rs4680). This highly functional polymorphism is responsible for fourfold variations in enzyme activity and dopamine catabolism. Recent data suggested that even synonymous SNPs of the *COMT* gene can lead to changes in enzyme activity. Genetically determined COMT activity can affect an individual's response to levodopa therapy and carries the risk of complications from prolonged levodopa use in Parkinson's disease (PD) patients. Identifying at-risk individuals through genetic susceptibility markers could help to prevent the development of levodopa-induced complications in PD.

Key words: Parkinson's disease, levodopa-induced dyskinesia, hallucinations, *COMT* gene, single nucleotide polymorphisms, Val158Met (rs4680)

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Introduction

Parkinson's disease (PD) is a progressive disabling disorder caused by the degeneration of dopaminergic nigrostriatal pathways. It is clinically manifested by the presence of motor (bradykinesia, resting tremor, rigidity, and postural instability) and non-motor symptoms (cognitive decline, behavioral disturbances, autonomic dysfunction, sleep disorders) (1). The dopamine precursor, levodopa, has remained the most effective symptomatic therapy in PD since its introduction (2). Acute side effects of levodopa, such as nausea, vomiting, anorexia, and hypotension, commonly recede after 2 to 3 weeks of drug use (2). However, prolonged treatment with levodopa and disease progression lead to several motor and non-motor complications (2). About 40-50% of PD patients medicated with levodopa develop motor complications, such as motor fluctuations and dyskinesias, 4-6 years following the introduction of levodopa (3). Psychotic symptoms occur in about one-third of PD patients on dopaminergic therapy, usually ten or more years after diagnosis, and significantly impair the quality of life of these patients (4).

Disease progression parameters and clinical variables do not explain inter-individual heterogeneity in response to levodopa, suggesting a complex pathomechanism involving genetic factors. Pharmacogenetics aims to identify genetic factors that could be responsible for variability in treatment response, as well as for the occurrence of complications of chronic dopaminergic therapy. Polymorphisms in various genes implicated in dopamine metabolism and transport have been studied concerning the side effects of prolonged use of levodopa in PD patients, and the *COMT* gene is one of the most examined genes.

Thus, we performed this review to critically discuss current findings and how polymorphisms of the *COMT* gene may affect the treatment response and levodopa-induced complications such as motor fluctuations, dyskinesias, hallucinations, and psychosis in PD patients. An extensive literature search for English-language clinical trials was performed using MEDLINE (through PubMed up to April 2023) and EMBASE databases. The search terms were: "Parkinson's disease," "levodopa-induced dyskinesia," "hallucinations," "catechol-O-methyltransferase" or "*COMT* polymorphism," "single nucleotide polymorphisms," and "Val158Met (rs4680)". We have also searched manually to identify any studies potentially omitted by the database search. We (BR and MS) independently reviewed the titles and abstracts. The inclusion criteria were cross-sectional or longitudinal, the study group were idiopathic PD patients, genetic factors were any SNPs of the *COMT* gene, outcomes were dose-response, motor fluctuation, dyskinesia, hallucinations, and psychosis, and patients were treated with chronic dopaminergic agents (levodopa, dopamine agonists, MAO-B inhibitors and COMT inhibitors). References of reviewed articles and meta-analyses were also checked. The systematic search procedures used are shown in Figure 1.

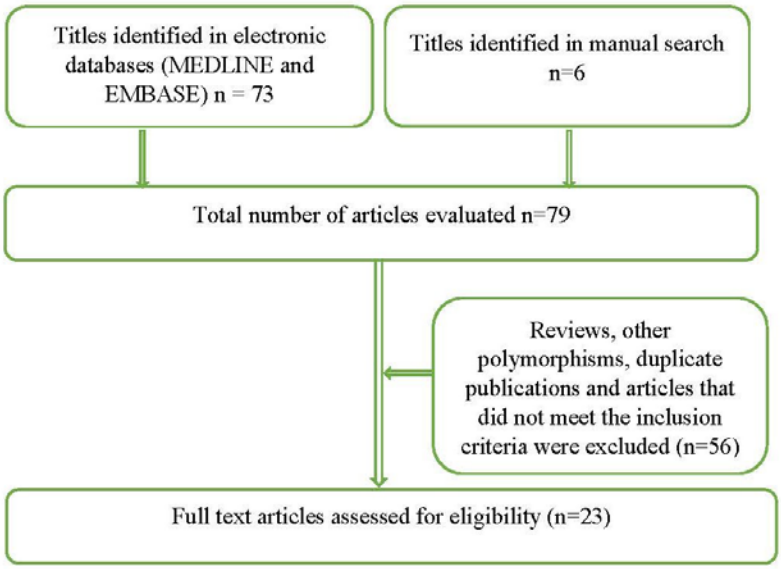

Figure 1. The methodology used
Slika 1. Korišćena metodologija

COMT gene

The human *COMT* gene is localized on the long arm chromosome 22 at the gene map locus of 22q11.2. It consists of six exons (the first two non-coding) and two promoters, P1 and P2, which control expression and are located in exon 3 (5). The *COMT* gene encodes two known isoforms of the COMT enzyme: the shorter, soluble, or cytosolic isoform (S-COMT) and the membrane-associated isoform (MB-COMT) (5, 6). MB-COMT is located in almost all parts of the human central nervous system (CNS). At the same time, S-COMT predominates in most other tissues except the brain (7). COMT enzyme degrades catecholamines, including dopamine and levodopa.

SNP rs4680 polymorphism is the most considered *COMT* gene polymorphism found in the 4th exon of the *COMT* gene. The substitution of valine (Val) to methionine (Met) at position 158, i.e., the replacement of G>A (Val158Met), leads to greater thermolability of the enzyme, resulting in approximately 30% lower enzyme activity. Val with Met can be replaced in the S-COMT but at position 108 (8, 9). COMT genotype distribution varies by ethnic origin. About 25% of Caucasians are homozygous for the low activity variant (Met/Met; AA), 25% are homozygous for the high activity variant (Val/Val; GG), and the remaining ones have the intermediate activity variant (Val/Met; AG) (7). AA genotype COMT is present in only 10% of the Asian population. The SNP rs4680 *COMT* gene has been linked to differences in cognitive abilities (specifically in tasks involving executive functions), mood, pain perception, and response to physical or

emotional stress (8-10). It has been shown that carriers of the GG genotype have an increased risk of developing schizophrenia (6) and a weaker response to olanzapine therapy (11).

However, it has been recognized that the haplotype formed by the four SNPs of the *COMT* gene (rs6269, rs4633, rs4818, and rs4680) has been affected to a greater extent on enzyme activity compared to a single rs4680 polymorphism (12). Therefore, the ACCG, ATCA, and GCGG haplotype carriers have low, medium, and high COMT activity, respectively (12).

Recent data suggested that synonymous SNPs of the *COMT* gene (i.e., rs165728 and rs174699) impact mRNA structure and stability and decrease COMT activity (13).

The association of therapeutic response to levodopa treatment and *COMT* gene SNPs

There are appreciable inter-patient and intra-personal distinctions in the response to levodopa. Genetic variations in the COMT activity contribute to different individual levodopa responses. It could be expected that carriers of the low COMT activity genotype would show a more significant response to levodopa than those with other genotypes. However, studies have shown that different COMT genotypes (14, 15) did not affect the duration or extent of response to a single levodopa dose in PD patients. Bialecka et al. found that daily doses of levodopa were significantly higher for GCGG *COMT* haplotype carriers (rs6269-rs4633-rs4818-rs4680) (16). A recent case-control study investigated the association of eleven SNPs in the *COMT* gene and levodopa response in Chinese PD patients and found that patients with the TT genotype of rs165728 and TT of rs174699 required higher daily levodopa equivalent doses (LEDs) than the patients with CC and CT genotypes (13). Replacement of the T allele with C has been hypothesized to reduce COMT enzymatic activity, decreasing the needed levodopa doses (13). Though rs165728 is located in the 3'-UTR, and rs174699 is located in intron 5, they possibly alter mRNA structure and stability, decreasing COMT activity. In addition, Xiao et al. found that carriers of the T allele of rs4633 and A allele of rs4680 used higher daily LEDs (17).

The association of motor complications of prolonged levodopa treatment and *COMT* gene SNPs

PD patients frequently develop severe and often debilitating motor complications (motor fluctuations and dyskinesia) due to their prolonged therapy. The "wearing off" phenomenon is the predictable recurrence of PD symptoms ahead of the scheduled levodopa dose. The "on-off" phenomenon refers to sudden, sometimes unpredictable changes in PD symptoms, varying between the "on" state (phase of optimal drug action and motor improvement) and the "off" state (phase of returning PD symptoms after the expiration of therapy). Motor fluctuations most likely occur due to pharmacokinetic and pharmacodynamic factors associated with chronic levodopa use in the presence of severe nigrostriatal degeneration (1, 18).

The impact of COMT genotype on the risk of motor fluctuations has been investigated in several studies, with conflicting results (16, 19). A cross-sectional study among Chinese PD patients found that AA homozygotes had a lower risk of developing "wearing-off," suggesting that the rs4680 *COMT* gene might affect susceptibility to "wearing off" (20). However, a meta-analysis showed that allele A of rs4680 increased the risk of "wearing off" (21).

COMT inhibitors are considered the medication of choice for treating motor fluctuation by increasing the "on" state, decreasing the "off" state, and improving motor response (22, 23). Administration of levodopa with a COMT inhibitor maintains a permanent level of levodopa in the plasma and obtains uniform availability of levodopa in the brain. Few trials have examined the potential contribution of the COMT genotype in the clinical response to COMT inhibitors. Corvol et al. have shown that *COMT* polymorphism rules the acute response to COMT inhibitors (24). In this study, the GG rs4680 *COMT* gene genotype was associated with a prolonged entacapone-induced "on" state (24). However, other studies did not show variations in the therapeutic response and toxicity of COMT inhibitors among PD patients with different COMT genotypes. One longitudinal study found no association between rs4680 *COMT* and the duration of "on" and "off" states and disease severity in PD patients during two months of entacapone treatment (25). After entacapone therapy, the mean reduction in daily levodopa dose in each patient was significant in GG and AG carriers, but not in those with the AA genotype of the *COMT* gene (25). Kim et al. also showed that entacapone's efficacy and side effects did not differ among PD patients with different *COMT* genotypes (26).

Levodopa-induced dyskinesia (LID) or involuntary movements are usually clinically manifested as choreiform or dystonic movements and less frequently in ballistic movements and myoclonus. The most common LIDs are "peak dose," which occur when the concentration of levodopa in the blood is at its highest. Dyskinesias present during the entire duration of the levodopa effect are referred to as square wave dyskinesias (1, 18). Biphasic dyskinesias are less frequent and occur when plasma levodopa concentrations fall below or rise above the threshold for therapeutic efficacy. Finally, "off" dyskinesias happen when the concentration of levodopa in plasma is low and manifest through pain and cramps in the legs or foot dystonia. Clinical risk factors for LID include younger age at PD onset, greater severity, longer disease duration, length of levodopa treatment, and higher total levodopa dose (27, 28). However, it is still difficult to explain why dyskinesias do not appear in all patients medicated with high doses of levodopa for an extended period and sometimes occur in those exposed to relatively low levodopa daily doses in a short period (1).

The role of *COMT* gene polymorphisms as a vulnerability factor in LID onset has been explored to a great extent. Previous studies regarding the association of the rs4680 *COMT* gene polymorphism and LID have shown conflicting results. A prospective study conducted in the Netherlands found a twice higher risk among heterozygous PD patients and a 2.81 times higher risk of developing dyskinesia during levodopa therapy among AA carriers of the rs4680 *COMT* gene (19). A possible interpretation of this

result was that a higher incidence of dyskinesia occurred in patients with lower COMT activity due to exposure to higher doses of levodopa. In one study, the AA genotype was more common in patients with motor fluctuations (p = 0.045, OR= 3.82) or dyskinesia (p = 0.030, OR = 4.80) than in controls. Still, this finding was insignificant after Bonferroni's correction (29). Sampaio et al. found that the *COMT* AA genotype (rs4680) was associated with the risk of LID after adjusting for sex and age (OR=5.53; 95%CI 1.5-20.1; p=0.0009) (30). A recent study that included 220 Brazilian PD patients found that AA genotype *COMT* had a 3.8-fold increased risk for LID development after Bonferroni's correction (HR=3.841; 95% CI 1.29–11.37; p=0.012) (31). However, several other clinical studies have not verified the findings that a low-activity allele will have an increased risk of dyskinesia (14, 16, 32-35). The cross-sectional studies with Polish (16) and Italian PD patients (14) found no significant association between *COMT* polymorphism and LID, as well as the extensive survey of 1087 Chinese PD patients (30). The reviewed studies regarding the association between *COMT* polymorphism and LID demonstrated inconsistent results, which may be due to different ethnic groups, methodology used, and outcome definitions. The study with Chinese patients found that the GG genotype was more common in patients with motor fluctuations than in those without them (30). However, a meta-analysis involving 2385 PD patients implied that the AA rs4680 *COMT* may increase the risk of LID in a recessive genetic model for PD patients (36). Pooled ORs and 95% CIs suggested that the AA rs4680 *COMT* was associated with LID (OR=1.39, 95%CI:1.02–1.89, p=0.039) in the recessive model, and this correlation was more evident in Brazilian samples in the analysis stratified by ethnicity. Another recent meta-analysis on ethnicity showed that allele A rs4680 *COMT* is a risk factor for LID development in Asian PD patients. At the same time, using different genetic models, the GG genotype is a risk factor for LID development in non-Asian PD patients (37). Study design and small sample sizes led to the following essential limitations of the reviewed studies. Most of the studies are cross-sectional or retrospective, so they cannot be used to determine causality. Longitudinal studies with large samples would be preferable to provide insight into cause-and-effect relationships.

Ivanova et al. reported an association between four COMT SNPs (rs165774, rs4818, rs4633, and rs4680) and LID in 232 Caucasian PD patients (33). The rare allele of rs165774 was associated with an increased risk of dyskinesia (OR = 1.75; 95%CI 1.14-2.72), while in the case of rs4818, rs4633 and rs4680, the rare allele or homozygote genotype for the rare allele were protective against LID (OR = 0.57; 95%CI 0.34-0.92, OR=0.45; 95%CI 0.23-0.89 and OR=0.46; 95% CI 0.23-0.91, respectively), although the results did not reach statistical significance after adjusting for disease duration (33). No associations were found between combined *BDNF* (Brain-Derived Neurotrophic Factor) Val66Met, *COMT* Val158Met, and T941G *MAO-A* (Monoamine oxidase-A) SNPs and the prevalence or time to onset of LID in PD patients (32). A recent study found no individual associations of *BDNF* Val66Met, *DAT* (Dopamine Transporter) rs397595, and *COMT* rs4680 SNPs, but a potential combined impact of these polymorphisms on the occurrence of levodopa-induced motor complications (38). This

result implicated that a single SNP does not drive the event of motor complications. In addition, the phenotypic expressions of SNPs are probably affected by gene-gene interactions.

The association of non-motor complications of prolonged levodopa treatment and *COMT* gene SNPs

Hallucinations occur in about 8-40% of PD patients on chronic dopaminergic therapy, most often as visual hallucinations with preserved introspection in the initial stages and less frequently as auditory hallucinations (4). Besides hallucinations, the clinical range of psychotic symptoms in PD encompasses minor hallucinations (illusions, presence hallucinations, passage hallucinations) and delusions (4). The pathophysiology of psychotic symptoms in PD is complex and still insufficiently known. Prolonged dopaminergic therapy was long considered to be the primary risk factor for hallucinations in PD. Still, recent studies have shown that antiparkinsonian drugs alone are neither necessary nor sufficient to explain the onset of psychotic symptoms in all patients (39, 40). The appearance of psychotic symptoms in PD was related to older age, disease duration, cognitive disorders, depression, and sleep disorders (4).

Thus far, several attempts have assessed the impact of genetic factors on developing hallucinations in PD. A recent cross-sectional study, which included 234 PD patients, found no association between rs4680 of the *COMT* gene and psychotic symptoms in PD (41). This finding follows a retrospective postmortem study by Camicioli et al. (42) and another study by Creese et al. (43), which included demented PD patients. However, the survey by Radojević et al. showed that, in addition to well-established clinical risk factors for psychosis in PD (dopaminergic drugs, motor status, depression, and anxiety), the GG rs2734849 of the *ANKK1* gene was a potential contributing factor (41). These results suggested that dopamine probably has a limited role in developing psychotic symptoms in PD compared to other neurotransmitters. Besides *COMT*, other genes might increase the risk of treatment complications (43). Moreover, the occurrence of levodopa nonmotor complications could be the result of gene-gene interactions. Namely, haplotypes of *COMT* and *SLC6A3* were associated with the occurrence of visual hallucinations (AT *vs*. GC: OR=0.34; 95%CI=0.16-0.72; p=0.005) (44).

Table I summarizes studies examining the association of *COMT* gene polymorphisms with treatment response and levodopa-induced complications in PD patients.

Table I Studies examining the association of catechol-O-methyltransferase (*COMT*) gene polymorphisms with treatment response and levodopa-induced complications in Parkinson's disease (PD) patients presented in chronological order

Tabela I Studije o udruženosti polimorfizama gena za katehol-O-metiltransferazu *(COMT)* sa terapijskim odgovorom i komplikacijama izazvanim levodopom kod pacijenata sa Parkinsonovom bolesti prikazane hronološki

Study (Year)	Study design/sample	Genes (SNPs)	Outcomes	Main findings
Lee et al. (2001)[15]	Cross-sectional study with 73 Korean PD patients	*COMT* (rs4680)	Motor response after a single levodopa dose challenge test	No association
Lee et al. (2002)[25]	A longitudinal study with 65 Korean PD patients with end-of-dose deterioration	*COMT* (rs4680)	The therapeutic efficacy of entacapone was assessed using the UPDRS score, the daily levodopa dosage, and the patients' diary card.	No association The mean of the percentage reduction of daily levodopa dose for each individual after entacapone treatment was significant in patients with GG and GA rs4680 *COMT*, but not in those with AA genotype.
Watanabe et al. (2003)[29]	Cross-sectional enrolled 121 Japanese PD patients	*COMT* (rs4680)	PD susceptibility Wearing-off Dyskinesia	AA rs4680 *COMT* may be related to an increased risk of wearing-off (p=0.045, OR=3.82) or dyskinesia (p=0.030, OR=4.80) compared with controls, although these differences were not significant after Bonferroni's correction.
Bialecka et al. (2004)[45]	The retrospective study enrolled 95 patients diagnosed with idiopathic PD and treated with levodopa.	*COMT* (rs4680) *MAO-B* (A>G, Intron13)	Levodopa dose	AA rs4680 *COMT* was associated with using doses of levodopa below 500mg during the first five years of treatment.
Contin et al. (2005)[14]	Cross-sectional that included 104 Italian PD patients	*COMT* (rs4680)	Levodopa pharmacokinetic and pharmacodynamic variables and the presence of dyskinesias after standard oral levodopa/benserazide test	No association
Camicioli et al. (2005.)[42]	Retrospective study in 47 autopsy-confirmed cases of PD	COMT (rs4680)	Hallucinations	No association
Bialecka et al. (2008)[16]	Case-control study that included 322 Poland PD patients	*COMT* (rs6269, rs4633, rs4818, rs4680)	Levodopa dose Motor complications	Levodopa doses prescribed for carriers of the high activity haplotype (604.2±261.9mg) were significantly higher than those for noncarriers (512.2±133.5mg, p<0.05).

Corvol et al. (2011)[24]	A randomized cross-over clinical trial with 33 French PD patients	*COMT* (rs4680)	The primary endpoint was the effect of entacapone on the motor response to levodopa. Secondary endpoints were the peak motor response, time to peak, levodopa pharmacokinetics, and COMT activity in red blood cells.	GG rs4680 *COMT* genotype in PD patients enhanced the effect of entacapone on the levodopa pharmacodynamics and pharmacokinetics.
Kim et al. (2011)[26]	Longitudinal study with168 Korean PD patients who had daily "off" duration of ≤2 hours	*COMT* (rs4680)	The efficacy and side effects of entacapone	No association
De Lau et al. (2012)[19]	A longitudinal study among a hospital-based cohort of 219 Dutch PD patients	*COMT* (rs4680)	Dyskinesia	AA rs4680 *COMT* carriers had an increased risk of developing dyskinesias during follow-up in a dose-dependent manner (adjusted hazard ratios for the AG and AA genotypes [compared to GG]: 2.09 [95% confidence interval (CI) 1.07–4.06] and 2.81 [CI, 1.43–5.54], respectively.
Torkaman-Boutorabi et al. (2012)[46]	Cross-sectional study that enrolled 103 Iranian PD patients	*COMT* (rs4680) *MAO-B* (rs1799836)	Levodopa doses	There are no significant differences in genotype distributions when comparing those receiving daily doses of levodopa above 500 mg and below 500 mg in the fifth year of treatment.
Yin et al. (2013)[36]	A case-control study that involved 97 Chinese patients with PD	*COMT* (rs74745580, rs4633, rs6267, rs3838146)	PD susceptibility Severity of disease Levodopa dose Duration of levodopa	The polymorphisms rs4633, rs6267, and rs3838146 were associated with the severity of PD disease, but not with levodopa medication.
Wu et al. (2014)[20]	Cross-sectional study with 259 Chinese PD patients	*COMT* (rs4680)	Wearing off	AA rs4680 *COMT* was related to a decreased risk of wearing off. GG vs. AA rs4680 *COMT* carried a higher risk factor for the wearing-off ($p < 0.001$) [OR=8.84, CI: 4.74-16.39]. GA vs. AA genotype had a higher risk for wearing off (p=0.013) [OR=6.54, CI:1.49-28.57].
Cheshire et al. (2014)[32]	A longitudinal study that involved 285 pathologically confirmed PD cases	*COMT* (rs4680) *MAO-A* (rs6323) *BDNF* (rs6265)	Dyskinesias	No association

Xiao et al. (2017)[17]	A case-control study with 143 outpatient Chinese PD patients	*COMT* (rs4680, rs6269 rs4633, rs4818)	PD severity Levodopa treatment response Wearing-off	TT rs4633, AA rs4680, and the two linked TT/AA rs4633-rs4680 were more frequent in patients with wearing-off, longer disease duration, higher LED, and higher UPDRS scores ($p < 0.05$).
Sampaio et al. (2018)[30]	A retrospective study with 162 PD Brazilian patients on levodopa	*COMT* (rs4680) *MAO-B* (rs1799836)	Therapeutic response to levodopa Dyskinesia	AA rs4680 *COMT* was associated with a risk of dyskinesia after adjusting for sex and age.
Michałowska et al. (2018)[38]	Cross-sectional study with 76 PD patients on chronic levodopa therapy lasting at least three years	*COMT* (rs4680) *DAT* (rs397595) *BDNF* (rs6265)	Motor levodopa-induced complications (on-off and dyskinesias)	There is no association between individual *BDNF*, *DAT*, and *COMT* polymorphisms. The genotype combination of AG *BDNF*, AG *DAT*, and GG *COMT* was correlated with motor complications, and the genotype combination of GG *BDNF*, AA *DAT*, and AA *COMT* showed a lack of motor complications in PD patients.
Kakinuma et al. (2018)[34]	Retrospective study with 110 Asian patients with PD	*COMT* (rs4680) *MAO-B* (rs1799836)	Dyskinesia	No association between COMT and dyskinesia Patients with AG or GG rs1799836 were more likely to have dyskinesia than those with an AA genotype (HR=3.41; 95% CI 1.28–9.10).
Ivanova et al. (2019)[33]	Cross-sectional study with 232 Russian PD patients	*COMT* gene (rs4680, rs6269, rs4633, rs4818, rs769224, rs165774, rs174696)	Dyskinesia	The rare allele of rs165774 was associated with an increased risk of dyskinesia (OR = 1.75 [95% CI 1.14-2.72]). The rare allele or homozygote genotype for the rare allele of rs4818, rs4633, and rs4680 were protective against dyskinesia (OR = 0.57 [0.34-0.92], 0.45 [0.23-0.89] and 0.46 [0.23-0.91], respectively).
Redenšek et al. (2019)[44]	A retrospective cohort study that enrolled 231 PD on levodopa and DAs treatment duration at least three months	*COMT* (rs4680, rs165815) *DRD2* (rs1799732, rs1801028) *DRD3* (rs6280) *SLC22A1* (rs628031) *DDC* (rs921451, rs3837091) *MAOB* (rs1799836), *SLC6A3*	Motor fluctuations Dyskinesia Excessive daytime sleepiness and sleep attacks Visual hallucinations (VHs) Nausea/vomiting Orthostatic hypotension Peripheral edema Impulse control disorders	Carriers of at least one *COMT* rs165815 C allele had lower odds for VHs (OR = 0.34; 95% CI = 0.16–0.72; p = 0.004); Heterozygotes for *SLC22A1* rs628031 and carriers of at least one *SLC22A1* rs628031 A allele had lower odds for dyskinesia (OR = 0.48; 95% CI = 0.24–0.98, p = 0.043 and OR = 0.48;

		(rs393795, rs6347, rs104209) *SLC7A5* (rs1060253, rs1060257) *SLC18A2* (rs14240) SV2C SNP (rs1423099)		95% CI = 0.25–0.92; p = 0.027, respectively) Haplotypes of *COMT* and *SLC6A3* were associated with the occurrence of VHs (AT vs. GC: OR = 0.34; 95% CI = 0.16–0.72; p = 0.005).
Dos Santos et al. (2020)[31]	220 Brazilian PD patients	*COMT* (rs4680) *DRD1* (rs4532), *DRD2* (rs1800497), *DAT1* (rs28363170)	Dyskinesias	AA rs4680 *COMT* had a3.84–fold increased risk for dyskinesia development (HR=3.841; 95% CI 1.29–11.37; p=0.012).
Zhao et al. (2020)[13]	73 Chinese PD patients	*COMT* (rs4680, rs4633, rs769224, rs4646316, rs174699, rs737865, rs4646312, rs933271, rs174675, rs2020917, rs165728	Dyskinesia	TT rs165728 and rs174699 had larger LED than CC and CT genotypes (p=0.01421 for rs165728 and p=0.02302 for rs174699). GG rs4680 had a lower occurrence of dyskinesia than AA and AG (p=0.0196). CC rs4633 had a lower occurrence of dyskinesia than TT and TC (p=0.0429)
Radojević et al. (2021)[41]	A cross-sectional study that included 234 Serbian PD patients on levodopa therapy for at least two years and age at onset > 40 years	*COMT* (rs4680) *DRD2* (rs6277, rs1076560, and rs2283265) *ANKK1* (1800497, rs2734849) *DAT* (VNTR)	Psychosis	TT rs6277 *DRD2* carriers had 2.3 times higher risk (0R=2.302; CI 1.100-4.816, p=0.027) and GG rs2734849 *ANKK1* 2.2 times higher risk (OR=2.203, CI1.073-4.522, p=0.031) for developing psychosis.

PD - Parkinson's disease, COMT – catechol-O-methyltransferase, SNP – single nucleotide polymorphisms, OR -odds ratio, CI- confidence interval, MAO – monoamine-oxidase, DRD2- dopamine receptor 2, BDNF – brain-derived neurotrophic factor, DAT – dopamine transporter, UPDRS – Unified Parkinson's Disease Rating Scale, LED – levodopa equivalent doses, VHs=visual hallucinations

Conclusion

SNP rs4680 of the *COMT* gene has been considered the main reason for individual variation in human COMT activity. However, several studies have failed to demonstrate the relationship between treatment response, long-term levodopa complications, and rs4680 of the *COMT* gene, implying that a single SNP does not drive the event of difficulties. Additionally, interactions between SNPs likely affect mRNA structure and protein translation efficiency, all leading to changes in enzyme activity. Besides *COMT*, other genes affect individual response to dopaminergic drugs and the risk of treatment complications. Increased insight into how genetic variants affect response to levodopa could contribute to personalized antiparkinsonian therapy to maximize symptom control while minimizing severe side effects.

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Udruženost polimorfizama gena za katehol-O-metiltransferazu sa terapijskim odgovorom i komplikacijama izazvanim levodopom kod Parkinsonove bolesti: Rezime sadašnjih saznanja

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Kratak sadržaj

Katehol-O-metiltransferaza (engl. *catechol-O-methyltransferase*, COMT) je jedan od glavnih enzima u razgradnji kateholamina i levodope. Genetske varijante *COMT* gena mogu uticati na aktivnost COMT enzima. Polimorfizam *COMT* gena koji je najviše proučavan je nesinonimni jednonukleotidni polimorfizam (engl. *single nucleotide polymorphism*, SNP) u egzonu 4 (Val108/158Met; rs4680). Ovaj visoko funkcionalni polimorfizam odgovoran je za četvorostruke varijacije u aktivnosti enzima i katabolizmu dopamina. Nedavni podaci sugerišu da čak i sinonimni SNP *COMT* gena mogu da dovedu do promena u aktivnosti enzima. Genetski određene razlike u COMT aktivnosti mogu uticati na odgovor pojedinca na terapiju levodopom i nose rizik od komplikacija dugotrajne primene levodope kod pacijenata sa Parkinsonovom bolešću (PB). Identifikacija osoba u riziku putem markera genetske osetljivosti može pomoći u prevenciji komplikacija izazvanih levodopom kod PB.

Ključne reči: Parkinsonova bolest, levodopa indukovane diskinezije, halucinacije, *COMT* gen, jednonukleotidni polimorfizmi, Val158Met (rs4680)

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Role of lipoprotein lipase variants in metabolic disorders and cardiovascular diseases

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Abstract

Lipoprotein lipase (LPL) is a glycoprotein that is produced and secreted into the interstitial space in various tissues, including the cardiac muscle, adipose tissue, macrophages, and skeletal muscle. LPL activity could be affected by genetic alterations which result in changes in lipid metabolism. This review article only focuses on reporting the recent studies which mainly explain the role of the LPL gene variants in metabolic syndrome and cardiovascular diseases. There are over 100 LPL gene variants, but this review article reported rs1801177, rs118204069, rs118204057, rs118204060, rs118204068, rs268, and rs328 as the most common in metabolic syndrome patients. In cardiovascular diseases, LPL variants rs1801177, rs268 and rs328 were the most prevalent. Therefore, it is suggested that further studies should be conducted to identify the LPL gene variants in other cardiovascular diseases, including cardiac arrhythmia. This review article concludes that LPL deficiency and dysfunction are associated with many diseases, such as obesity, insulin resistance, diabetes, chylomicronemia, atherosclerosis, myocardial infarction, coronary artery disease, and stroke.

Key words: lipoprotein lipase, metabolic syndrome, cardiovascular diseases, pathophysiology, mutation

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Introduction

In 1929, Macheboeuf identified the lipoprotein as a highly lipid-rich formation that is easily soluble in water (1), whereas the human Lipoprotein Lipase (LPL*)* gene was isolated in 1970 (2). LPL, designated with the enzyme commission (EC) number EC 3.1.1.34, is a crucial extracellular enzyme in the metabolism of lipoproteins. It plays a significant role in the maturation of various classes of lipoprotein particles (3). LPL belongs to the mammalian lipase family including hepatic lipase, endothelial lipase, pancreatic lipase, and gastric lipase (4, 5). LPL is secreted from glycoprotein with 55 kDa and synthesized from numerous cell types, including muscle cells, adipocytes, and macrophages (6). The LPL protein plays a crucial role in lipid metabolism as a multifunctional glycoprotein enzyme. Following secretion, LPL attaches to the endothelial surface, facilitating the hydrolysis of triglycerides (TG) in circulating lipoproteins. This process involves the crucial step of eliminating lipoproteins, including those of endogenous origin, such as very-low density lipoproteins (VLDL), and exogenous sources like chylomicrons provided free fatty acids (FFAs) and glycerol for tissue use (7, 8). Another study demonstrated that LPL functions as a cleansing factor by efficiently hydrolyzing TG. LPL affects the serum concentrations of TG and the production of lipoprotein particles, which are processed by hepatic lipase. A recent study has investigated how LPL assists as the ligand for the protein which is associated with the low-density lipoprotein receptor (LDLR) and influences hepatic secretion and VLDL cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) capture (9). Moreover, the retention of VLDL and LDL particles by the subendothelial matrix of the arterial wall is increased by LPL, which promotes the transformation of these lipoproteins into more atherogenic forms (10). LPL activity could be affected by genetic modifications which result in changes in lipid metabolism: for example, an extended half-life of LDL-C, reduced production of high-density lipoprotein (HDL), and reduced hydrolysis of chylomicrons and VLDL-C (11, 12). Moreover, Augustus et al. (13) investigated various physiological roles of LPL using a mouse model. Firstly, they identified cardiac LPL as a crucial modulator of plasma TG levels. Secondly, the decreased uptake of lipoprotein-derived fatty acids led to reduced expression of genes involved in fatty acid oxidation. Thirdly, there was an elevation in cardiac glucose uptake without altering overall glucose homeostasis in the body. Fourthly, remarkably, the insulin-signaling pathway underwent changes, with a reduction in insulin receptor substrate 1 (IRS-1) expression and an increase in insulin receptor substrate 2 (IRS-2) expression. In summary, the authors reported, for the first time, that mice with a tissue-specific deletion of cardiac LPL demonstrated the importance of cardiac LPL in regulating plasma TG levels and clearing postprandial lipoproteins. The products of lipolysis generated by LPL influenced PPAR actions, and LPL activity played a role in metabolic switching between fatty acid uptake and glucose utilization. Moreover, the authors generated mice with acute depletion of LPL in the heart, resulting in similar changes in cardiac gene expression, heart function, and plasma lipids as observed with prenatal loss of

this enzyme in the heart. This confirmed the impact of LPL loss on cardiac function. The study indicated that the loss of this enzyme is not the primary cause of the metabolic and functional alterations seen with chronic LPL loss during development and in prenatal periods. Furthermore, it emphasized the importance of FFA in the adult heart, suggesting that interventions inhibiting the heart's ability to utilize FFA could have adverse effects. Acute loss of LPL could be induced by infection, and previous studies have reported reduced cardiac LPL activity in conditions such as diabetes and starvation. The authors speculated that changes in LPL actions might contribute to acute alterations in cardiac function (14). Atherosclerotic arteries were found to have higher LPL activity compared to normal arteries (15, 16). Considering the genetic, clinical, and biological significance, several investigators have noted the association of the rs328 variant with blood pressure and hypertension (17-19).

The LPL gene is located on the short arm of chromosome 8 and region 21.3 (8p21.3) that comprises 9 introns and 10 exons, encoding a protein consisting of 475 amino acids (20, 21). In 1960, Havel and Gordon reported the first cases of LPL deficiency in idiopathic hyperlipemia patients (22). Gaudet et al. (23) further highlighted that LPL deficiency is a rare inherited disease associated with severe hypertriglyceridemia, chylomicronemia, and the increased risk of recurrent pancreatitis, among other potential complications. Subsequent studies have identified various alteration in the LPL gene that are implicated in diverse metabolic disorders and cardiovascular conditions. Approximately 100 LPL gene variants have been documented, including rs1801177, rs118204069, rs118204057, rs118204060, rs118204068, rs268, and rs328. For instance, rs118204057 involves a G-to-A transition at nucleotide 818 in exon 5 (c.562G>A), resulting in a gly188-to-glu (p.G188E) substitution in the mature protein, and is associated with familial chylomicronemia syndrome characterized by markedly elevated triglyceride levels. Similarly, rs118204060, a C-to-T transition at nucleotide 875 in exon 5 (c.619C>T), leads to an amino acid substitution of leucine for proline-207 (p.P207L), also linked to familial chylomicronemia syndrome. Another variant, rs118204068, involves a G-to-A transition in exon 6 (c.749G>A), causing a substitution of asparagine for aspartic acid at residue 250 (p.D250N), and serves as the basis for familial chylomicronemia and hypertriglyceridemia cases. Additionally, rs268, a nucleotide substitution in exon 6 (c.872A>G), results in an asn291-to-ser substitution (p.N291S) associated with an increased risk of hypertriglyceridemia and cardiovascular diseases (CVDs). The rs118204069 variant, a T-to-C transition in exon 3 (c.257T>C), leads to a trp86-to-arg substitution (p.W86R), contributing to LPL deficiency and familial chylomicronemia syndrome. Finally, rs328 involves a C→G transversion at nucleotide 1595 within exon 9 (c.1339C>T). This alteration transforms the serine 447 codon (TCA) into a premature termination codon (TGA) (p.Ser447X), resulting in the generation of a truncated enzyme lacking the two carboxyl-terminal amino acids (Ser–Gly). The c.1339C>T variation is a common polymorphism with no functional significance, and it is not associated with variations in lipid metabolism risk. Studies

reported these mutations were common LPL mutations in metabolic syndrome (MetS) patients.

Multiple restriction fragment length polymorphisms (RFLPs) have been detected within the LPL gene, including variants associated with *BamHI, PvuII, HindIII, BstNI, BstI, BglII, and XbaI*. Among these, the polymorphisms characterized by the *HindIII* and *PvuII* RFLP sites (located on introns 8 and 6 of the LPL gene, respectively) are the most prevalent and could be linked to significant modifications in plasma lipid levels. The *HindIII* polymorphism results from the occurrence or absence of a T→G transition at position +495 in intron 8 of the LPL gene, and is among the most prevalent polymorphisms. The *PvuII* polymorphism results from a C⇒T transition at the restriction site within intron 6 of the LPL gene, positioned 1.57 kb from the splice acceptor (SA) site. The region encompassing the *PvuII* site shares its homology with the splicing site, resembling the consensus sequence essential for 3′-splicing and lariat structure formation. This suggests that the C497→T (CAG CTG ⇒ TAG CTG) alteration may disrupt the accurate splicing of messenger RNA (mRNA) (24). In addition, these LPL variants, including rs1801177, rs268, rs1801177, and rs328, were also reported in patients affected with cardiovascular disorders. Therefore, this review article focuses exclusively on summarizing recent studies that primarily elucidate the role of these LPL gene mutations in MetS and CVDs, as explained in Figure 1 and Table I.

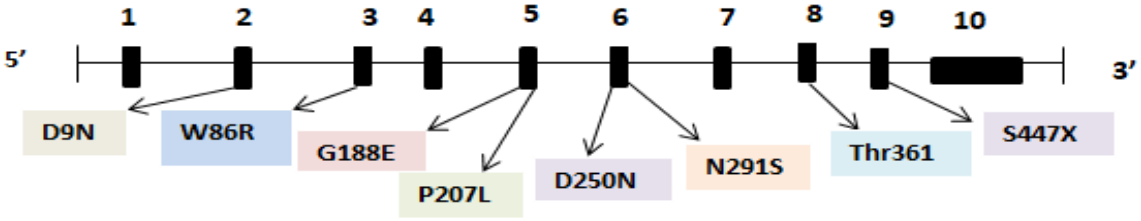

Figure 1. Common variants within the exonic region of the LPL gene resulting in protein substitutions, ultimately contributing to metabolic and cardiovascular disorders

Slika 1. Uobičajene varijante unutar egzonskog regiona LPL gena koje rezultiraju supstitucijama proteina, što na kraju doprinosi metaboličkim i kardiovaskularnim poremećajima

Table I Role of LPL gene variants in metabolic and cardiovascular disorders

Tabela I Uloga varijanti LPL gena u metaboličkim i kardiovaskularnim poremećajima

LPL Variants	Exon/ Intron	Nucleotide Change	Amino Acid Change	Mutation Type	Clinical Significance	Role of LPL variants in disorders
rs1801177	Exon 2	c.106G>A	p.Asp9Asn	Missense	Benign	familial hypertriglyceridemia, familial chylomicronemia, hypertriglyceridemia, atherosclerosis, coronary artery disease,
rs118204069	Exon 3	c.257T>C	p.Trp86Arg	Missense	Pathogenic	hypertriglyceridemia
rs118204057	Exon 5	c.562G>A	p.Gly188Glu	Missense	Pathogenic	familial hypertriglyceridemia, familial chylomicronemia, hypertriglyceridemia
rs118204060	Exon 5	c.619C>T	p.Pro207Leu	Missense	Pathogenic	hypertriglyceridemia
rs118204068	Exon 6	c.749G>A	p.Asp250Asn	Missense	Pathogenic	hypertriglyceridemia
rs268	Exon 6	c.872A>G	p.Asn291Ser	Missense	Benign	familial hypertriglyceridemia, familial chylomicronemia, hypertriglyceridemia, metabolic syndrome, atherosclerosis, coronary artery disease,
rs328	Exon 9	c.1339C>T	p.Ser447X	Nonsense	Benign	dyslipidemia, hypertension, atherosclerosis, obesity, type 2 diabetes, hypertriglyceridemia, coronary artery disease, atherosclerosis
rs316	Exon 8	c.1164C>A	p.Thr361	Silent	Synonymous	atherosclerosis
Pvull	Intron 6	IVS6+1595C>T	No change	NA	NA	myocardial infarction, atherosclerosis
HindIII	Intron 8	IVS6+481T>G	No change	NA	NA	type 2 diabetes, atherosclerosis, myocardial infarction, coronary artery disease, hypertension

NA: Not applicable

Methods

Literature survey and selection criteria

Google Scholar, Science Direct, and PubMed were used to review the literature. Numerous keywords were used for searching the literature, such as lipoprotein lipase, MetS, CVDs, and LPL variants. The language for the review of clinical studies was set to English. This review article only focuses on reporting the recent studies which mainly explained the pathophysiological aspects of the LPL gene variants in MetS and CVDs. The time frame was not limited, even though more recent studies were preferred.

Lipoprotein lipase (LPL) role in metabolic disorders

MetS is a combination of various conditions, such as elevated blood glucose, hypertension, increased serum TG, reduced serum HDL-C, and central obesity. The

presence of MetS is linked to an increased risk of developing type 2 diabetes (T2D) and CVD. MetS has been defined by different organizations, including the International Diabetes Federation, World Health Organization, National Cholesterol Education Programme Adult Treatment Panel III, American Association of Clinical Endocrinologists, and the European Group for the Study of Insulin Resistance (25, 26). LPL is centrally involved in the metabolism of both VLDL and HDL. Various diseases, such as obesity, atherosclerosis, dyslipidemia, insulin resistance (IR), diabetes, chylomicronemia, and Alzheimer's disease, have been associated with LPL dysfunction or deficiency (27). Moreover, the most prevalent variants in the LPL gene include rs118204057, rs1801177, and rs268.

Role of LPL variants in familial chylomicronemia

Familial LPL deficiency is recognized as the most prevalent form of familial chylomicronemia syndrome, formerly referred to as type 1 hyperlipoproteinemia (OMIM# 609708). It follows an autosomal recessive inheritance pattern and is predominantly observed in children, with an approximate prevalence of one in 1,000,000 in the general population of the US (28). Familial LPL deficiency is characterized by severe hypertriglyceridemia, leading to recurrent acute pancreatitis, hepatosplenomegaly, episodes of abdominal pain, and eruptive cutaneous xanthomata. The impaired clearance of chylomicrons from the plasma results in the accumulation of TG in plasma, with concentrations exceeding 111.1 mmol/L in untreated states, giving it a milky appearance. The condition is identified through biallelic pathogenic variants in LPL via molecular genetic testing and is caused by homozygous pathogenic LPL variants (28). Familial chylomicronemia is associated with homozygous mutations, while the heterozygous mutation is significantly prevalent in the general population, ranging from 3-7%. Heterozygous mutations result in up to a 50% reduction in LPL activity, leading to elevated TG levels and reduced HDL-C levels. These lipid profile alterations increase the susceptibility to cardiovascular disease (29). The occurrence of homozygous LPL deficiency is approximately 1 per million individuals, and its primary functions involve the hydrolysis of TG and the peripheral uptake of FFA. Molecular characteristics of this condition encompass significantly reduced or completely absent LPL enzyme activity. The proportion of monogenic variants contributing to this condition is estimated to be 95% (30, 31). Moreover, mutations in the LPL gene result in partial enzyme deficiency, leading to elevated TG levels which form the basis of familial chylomicronemia, characterized by TG levels ranging from 16.7 mmol/L to 44.4 mmol/L, increases in VLDL-C, and decreased levels of LDL-C and HDL-C, manifesting as pure hypertriglyceridemia, with TC levels below 13 mmol/L. Various LPL variants involving amino acid replacements at specific positions of the LPL gene have been identified, such as rs1801177, rs268, and rs118204057 (32). Similarly, Pingitore et al. (33) suggested that two newly identified mutations lead to type 1 hyperlipoproteinemia attributed to LPL gene mutations, resulting in a decrease or absence of LPL secretion, along with a loss of LPL enzymatic activity. Additionally, a mutation in the Apolipoprotein C-II (ApoCII)

gene diminishes the enzymatic activity of LPL, an essential activator of LPL. Interestingly, mutations like R72T in the Apolipoprotein C-II gene result in severe hypertriglyceridemia and recurrent pancreatitis (34).

Role of LPL variants in obesity

Schwartz et al. (35) characterized and defined obesity as an excess of body fat mass. The study by Nuermaimalti et al. (36) illustrated adipogenesis as the process of lipid accumulation and adipocyte differentiation, with the expression of LPL messenger ribonucleic acid (mRNA) often considered an early indicator of adipocyte differentiation. Similarly, Kersten (37) demonstrated that, during adipogenesis, transcription of the LPL gene is stimulated by fatty acids, the adipogenic transcription factor peroxisome proliferator-activated receptor-gamma (PPARγ), and other PPARγ agonists in differentiated adipocytes. Additionally, Wang et al. (38) illustrated that insulin exerts a significant influence on LPL activity in adipose tissues during adipocyte differentiation by enhancing LPL gene transcription. Furthermore, in mature adipocytes, insulin not only elevates the level of LPL mRNA, but also regulates LPL activity through both posttranscriptional and posttranslational mechanisms (38). The LPL variants may influence the concentrations of plasma lipids. In children with uncomplicated obesity, body mass index (BMI) and plasma lipoproteins could potentially impact the distribution of subcutaneous fats (39). Likewise, Huang et al. (40) proposed that central obesity and the levels of serum lipids could be influenced by the LPL gene rs328 variants. This highlights the importance of reducing waist circumference to enhance serum lipids, particularly in individuals with central obesity, especially those with the rs328 genotype. Numerous studies on obesity in both humans and rodents have indicated increased LPL activity in adipose tissue (27). Similarly, obese individuals exhibit higher adipose tissue LPL activity per fat cell compared to lean control subjects (41).

Role of LPL variants in Type 2 Diabetes

LPL activity is frequently diminished in T2D, leading to a reduction in HDL-C levels and an elevation in serum TG levels (42-44). Furthermore, numerous studies have demonstrated the association between genetic variations in LPL and lipid metabolism in individuals with T2D (45-47). Ma et al. (46) documented a correlation between reduced levels of HDL cholesterol and elevated plasma TG levels, along with the presence of the H+ allele (risk allele) of the LPL HindIII polymorphism in Chinese individuals with early-onset T2D. Additional investigations have also indicated a connection between T2D complications and LPL polymorphisms (48-52). Additionally, Ng et al. (51) uncovered an association between rs328 and nephropathy in T2D patients. Moreover, in 2007 Radha et al. (53) demonstrated that polymorphisms in the promoter region, including G53C of the LPL gene, confer protection against T2D. Likewise, Cho et al.'s (54) study concluded that the LPL gene product, which regulates lipid levels in the blood, may be a significant genetic factor influencing the onset of T2D in the Korean population. LPL activity in both the skeletal muscle and adipose tissue is insulin-dependent and varies in diabetes mellitus based on ambient insulin levels and insulin sensitivity (55). Taskinen et al. (55) indicated

that modifications in lipoproteins could influence LPL activity in individuals with diabetes. These modifications encompass low HDL and low LPL activity in conditions of insulin deficiency, high TG and high VLDLs, normal or low VLDLs and increased HDLs in chronically insulin-treated patients with elevated LPL activity, and low HDLs in untreated T2D patients. In white adipose tissue, heightened LPL activity observed in obese and T2D individuals shares a common characteristic – hypertriglyceridemia, which is positively linked to adverse lipid accumulation in tissues (56, 57). Insulin regulates the production and expression of LPL in adipocytes and in the skeletal muscle (58). The levels of pre-heparin LPL decrease in tandem with the worsening of MetS, exhibiting a negative correlation with TG, fasting blood glucose, body weight, and IR, while positively correlating with HDL-C (58). Moreover, individuals with T2D exhibit lower circulating preheparin LPL mass and reduced LPL production compared to their healthy counterparts, along with an inverse correlation between LPL and glycated hemoglobin in T2D (58).

Role of LPL variants in Metabolic Syndrome

Genetic studies have identified numerous variants within the LPL gene, some of which confer protective effects, while others have deleterious consequences. Heparin stimulates the activity of lipoprotein lipase (LPL), leading to increased plasma lipolytic activity and higher levels of free fatty acids in the blood. Assessing post-heparin lipoprotein lipase activities helps identify underlying disorders related to triglyceride and HDL-cholesterol metabolism. In one study, carriers of the rs328 variant exhibit elevated levels of post-heparin LPL activity and increased lipolytic activity. The presence of the rs328 variant is associated with small increases in HDL-C levels, low levels of TG, and a moderate reduction in cardiovascular risk (58). Additionally, carriers of this variant, as reported by Groenemeijer et al. (59), show increased blood glucose and TG levels compared to non-carriers. These findings suggest that the benefits of this mutation may be limited in individuals of normal weight under the assessed conditions (59). Moreover, Daoud et al. (60) determined that distinct genotype frequencies existed between the control and patient groups, although no statistically significant differences were identified between these groups. However, the authors did observe notable variations in plasma levels of TG, LDL-C, TC, and HDL-C in association with the LPL genotype. This observation suggests a correlation between the polymorphisms and lipid profiles in patients with CAD. The interplay of environmental and genetic factors may contribute to the complexity of CAD, potentially influencing the disease onset. Similarly, Goodarzi et al. (61) demonstrated that haplotype structure of the 3' end of the LPL gene was analyzed by genotyping several LPL 3' end single nucleotide polymorphisms (SNPs) in the Mexican American population. Associations between polymorphisms in this region, notably *HindIII*, and surrogate indicators of insulin resistance and atherosclerosis were investigated. *HindIII* variant is associated with dyslipidemia, hypertension, atherosclerosis, and obesity. Additionally, the authors assessed insulin sensitivity in the Mexican American population, finding a direct correlation with variations in the *LPL*

gene through a haplotype-based approach. The authors recommended further investigations in the Mexican American population to delve into the connection between the LPL gene and components of the insulin syndrome. Similarly, Barg (62) elucidated the central role of LPL in the development of MetS and dyslipidemia. The polymorphisms in the LPL gene have been implicated in disturbances of lipid metabolism and the pathogenesis of CAD. Carriers of the X allele of Ser447X polymorphism were associated with a reduced risk of CAD, lower TG levels, and elevated levels of HDL-C. Common LPL mutations such as rs1801177, rs118204069, rs118204057, rs118204060, rs118204068, rs268, and rs328 were identified in patients with hypertriglyceridemia. LPL plays a significant role in various aspects of normal metabolism, including body weight regulation, atherosclerosis, insulin action, and energy balance. Numerous physiological factors and daily conditions, such as fasting and exercise, intricately regulate LPL activity. Moreover, various diseases can impact human metabolism and LPL function. Obesity, osteoporosis, T2D, dyslipidemia, and MetS stand out as prevalent metabolic disorders (63-65). Consequently, MetS is a heterogeneous entity with various synonymous terms, including the Reaven syndrome, plurimetabolic syndrome, atherothrombogenic syndrome, and syndrome X (66-68). Two common LPL gene variants, rs268 and rs328, are associated with MetS due to their impact on low HDL-C and high TG (69). Brunzell et al. (70) demonstrated that individuals with homozygous LPL deficiency, recognized as familial chylomicron syndrome, exhibit severe hypertriglyceridemia, elevated chylomicron levels, and recurrent pancreatitis. However, they did not observe any association with an elevated risk of CAD, as large circulating chylomicrons were incapable of infiltrating the arterial wall (71, 72). Nordestgaard (73) demonstrated that individuals with heterozygous LPL deficiency exhibit impaired lipolysis, resulting in the accumulation of circulating chylomicron remnants and intermediate-density lipoproteins rich in both cholesterol and TG. However, they did not establish a confirmed link to an increased risk of CAD. Conversely, in a cross-sectional study involving CAD case-control studies, Khera et al. (74) reported that gene sequencing identified deleterious alterations in the LPL gene in 188 out of 46,891 individuals (0.4%). These mutations were significantly associated with higher levels of TG and an increased presence of CAD. As per Cagatay et al.'s research (75), the Pvull polymorphism has been associated with reduced levels of HDL-C and elevated TG levels. A meta-analysis has indicated that this polymorphism is correlated with a decreased risk of experiencing a heart attack or myocardial infarction (76). Consequently, it demonstrates a protective effect against cerebrovascular accidents (76).

Role of LPL variants in Atherosclerosis

Increased atherosclerosis and early atherogenic processes are associated with the expression of LPL found on macrophages and other cells within vascular walls. Moreover, overexpression of LPL is correlated with IR and hypertension due to heightened inflammation, vascular remodeling, oxidative stress, sympathetic nervous system activation, vasoconstriction, and sodium retention (77-79). In the Central

European Caucasian population, dyslipidemia in subjects with MetS has indicated that the S1 allele of apolipoprotein C-III (APOC3) SstI polymorphism arising from a substitution of C to G at position 3238 in the 3´ untranslated region of exon 4 in the APOC3 gene, situated on the long arm of chromosome 11, along with the H-allele of LPL HindIII polymorphism, may have a marginal impact on apoB levels (80). The increased risk of premature arteriosclerosis is connected to the accumulation of triglyceride-rich lipoproteins as an independent factor. Among hypertriglyceridemia patients, approximately 20% carry common mutations in the LPL gene, such as rs1801177, rs118204069, rs118204057, rs118204060, rs118204068, rs268, and rs328, which are associated with hypertriglyceridemia. It is advisable to conduct genotyping for these LPL gene mutations, especially in individuals at a high risk of premature arteriosclerosis. Additionally, a significant number of individuals carry silent mutations, including Thr361 (one novel mutation was observed: C1338A in exon 8 of the LPL gene, which is a silent mutation at Thr361), and common mutations, such as rs328, which are associated with less favorable lipid profiles (81).

Role of LPL variants in cardiovascular diseases

Elevated levels of TG are a well-established factor for CVD. LPL plays a crucial role in the hydrolysis of TG, ensuring an adequate supply of fatty acids, primarily to adipose tissue. When there is a deficiency in LPL or an imbalance in tissue-specific LPL activities, this leads to hypertriglyceridemia. Various regulators influence LPL, including angiopoietin-like (ANGPTL) proteins (such as ANGPTL8, ANGPTL4, ANGPTL3) and certain apolipoproteins (including apolipoprotein A5, apolipoprotein C3, and apolipoprotein C2). These regulators collaboratively modulate LPL activity and the utilization of TG (57, 58, 82).

In the Mexican population, Muñoz-Barrios et al. (83) demonstrated an association between the HindIII polymorphism and hypertension. Likewise, Tanguturi et al. (84) reported that individuals with a homozygous genotype for the common allele (H+/H+) of the LPL gene are at an increased risk of experiencing their first myocardial infarction. In contrast, Imeni et al. (85) found no statistically significant association between CAD and the genotypic distribution of the HindIII polymorphism. Additionally, Muñoz-Barrio's study indicated an elevated risk of stroke in individuals with LPL gene variations, particularly in the HindIII polymorphism (83).

Similarly, He et al. (86) explored a reduced risk of stroke in individuals with the HindIII polymorphism carrying the G allele, and this association was observed in both hemorrhagic and ischemic stroke patients. Likewise, in another study (87), an investigation into the association between the distribution of HindIII polymorphism genotypes and the risk of CAD revealed no statistically significant differences between patients with a history of CAD and healthy individuals in Iran.

Likewise, Ma et al. (88) performed a meta-analysis, indicating an increased risk of CAD associated with the LPL rs1801177 polymorphism. However, the LPL HindIII and rs328 polymorphisms demonstrated a protective role against CAD. Additionally, the

authors did not identify any association between the LPL Pvull polymorphism, as well as rs268, and susceptibility to CAD.

Similarly, Xie et al. (89) conducted a meta-analysis on LPL polymorphism and its association with the risk of CAD. The authors concluded that the risk of CAD was associated with the homozygous H+ H+ genotype and H+ allele genotypes of the LPL HindIII polymorphism. Additionally, the risk of CAD was significantly linked to the rs328 XX genotype. However, the risk of CAD was not associated with the Pvull polymorphism. Finally, the authors suggested that the LPL HindIII polymorphism could serve as a potential biomarker for CAD risk.

Similarly, Spence et al. (90) elucidated that the predictor for the baseline carotid plaque area was significantly associated with the LPL rs1801177 genotype, and this association might be influenced by BMI. Furthermore, over a one-year period, plaque progression showed a strong correlation with the rs1801177 genotype. The authors propose that the rs1801177 genotype, as assessed by the progression of carotid plaque area, could be a determinant of atherosclerosis.

Furthermore, Gagné et al. (91) highlighted the connection between genetic variation at the LPL locus and the influence of plasma lipids in modulating the risk of coronary heart disease (CHD). The authors concluded that the rs328 variant could potentially offer significant protection against elevated TG levels, premature CHD, and low HDL-C in the studied subjects. Similarly, Guan et al. (92) examined the association between LPL gene variants rs328, rs1801177, and rs268 polymorphisms and the development of CVDs in children with obesity. In summary, the authors concluded that rs1801177, rs268, and rs328 gene mutations might not be associated with CVD risk in children with obesity.

Conclusion

This review article concludes that LPL deficiency and dysfunction are associated with various disorders, such as obesity, IR, T2D, chylomicronemia, atherosclerosis, myocardial infarction, CAD, and stroke. There are around 100 LPL gene variants, but this review article reported that LPL polymorphisms such as rs1801177, rs118204069, rs118204057, rs118204060, rs118204068, rs268, and rs328 were the common variants present among metabolic syndrome patients. On the other hand, rs1801177, rs268, rs1801177, and rs328 polymorphisms were common in CAD affected patients. Therefore, it is suggested that further studies should be conducted to identify the LPL gene variants in other CVDs, including cardiac arrhythmia.

Conflicts of Interest

The authors declare no conflict of interest.

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Authors' Contributions

All authors contributed to the conception and design of this study. The first draft of the manuscript was written by Sana and Sara Rafaqat. Data collection was performed by Sana and Sara Rafaqat, SS and AK. AK critically revised the manuscript. All authors have read and approved the final version of the manuscript.

Availability of Data

Not applicable.

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Uloga varijanti lipoprotein lipaze u metaboličkim poremećajima i kardiovaskularnim oboljenjima

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Kratak sadržaj

Lipoproteinska lipaza (LPL) je glikoprotein koji se proizvodi i sekretuje u intersticijalni prostor različitih tkiva, uključujući srčani mišić, masno tkivo, makrofage i skeletne mišiće. Aktivnost LPL može biti pod uticajem genetskih modifikacija koje rezultiraju promenama u metabolizmu lipida. Ovaj revijski članak sadrži podatke o nedavnim studijama koje uglavnom objašnjavaju patofiziološke aspekte mutacije gena za LPL u metaboličkom sindromu i kardiovaskularnim bolestima. Zabeleženo je oko 100 mutacija gena za LPL, ali ovaj revijski članak prikazuje rs1801177, rs118204069, rs118204057, rs118204060, rs118204068, rs268 i rs328, koje su najčešći nosioci mutacije gena za LPL kod pacijenata sa metaboličkim sindromom. Kod kardiovaskularnih bolesti, varijante LPL rs1801177, rs268 and rs328 su najučestalije. Potrebne su buduće studije kako bi se ispitale mutacije gena za LPL u drugim kardiovaskularnim bolestima, uključujući srčanu aritmiju. Ovaj revijski članak zaključuje da su deficit i disfunkcija LPL povezane sa bolestima kao što su gojaznost, insulinska rezistencija, dijabetes, hilomikronemija, ateroskleroza, infarkt miokarda, koronarna arterijska bolest i moždani udar.

Ključne reči: lipoproteinska lipaza, metabolički sindrom, kardiovaskularne bolesti, patofiziologija, mutacija

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Human papillomaviruses and cervical cancer from the perspective of the World Health Organisation initiative for cervical cancer elimination

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Abstract

Human papillomaviruses (HPV) are the most common sexually transmitted pathogens worldwide, leading to infections with a wide range of clinical manifestations: from benign conditions to different types of cancer in women and men as well. Cervical cancer is highly correlated with persistent high-risk-HPV (HR-HPV) infection, which is the key factor in emergence of 99.99% of cervical cancer cases. The most effective way to prevent HPV-related cancers is vaccination. There are three available prophylactic HPV vaccines: bivalent, quadrivalent and nonavalent. The nonavalent vaccine is gradually replacing other HPV vaccines in most countries and can be given from year 9, but it is commonly routinely implemented at the

age of 11 to 12. The World Health Organization has recognised cervical cancer as a global threat and has announced the so-called 90-70-90 strategy to reduce and even eliminate cervical cancer. This strategy implies that 90% of girls should be vaccinated by the age of 15, 70% of women should be screened for cervical cancer, and 90% of women diagnosed with cervical disease should receive adequate treatment. Although different treatment options are available: surgery, radiation therapy, chemotherapy, and advanced target therapy using monoclonal antibodies, great efforts are needed to achieve the goals set by the World Health Organization to eliminate cervical cancer.

Key words: high-risk HPV (HR-HPV) infection, cervical cancer, HPV vaccination

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Introduction

Human papillomaviruses (HPV) are small (52-55 nm), icosahedrally symmetric, non-enveloped viruses with circular double strand DNA placed within the capsid made of 72 pentameric capsomers and two structural proteins: L1 (major capsid protein) and L2 (minor capsid protein). HPV are strict human pathogens belonging to the *Papillomaviridae* family, with the affinity to infect cutaneous and mucosal epithelial tissues (1).

More than 200 strains of HPV have been identified, of which approximately 40 types infect the anogenital region (2). Between 5 and 18 of these HPV strains have been classified as high-risk (HR) genotypes. HPV are the most common sexually transmitted pathogens worldwide, with clinical manifestations spanning from benign to malignant processes in women and men. The International Agency for Research on Cancer has recently classified 12 HPV genotypes as carcinogenic, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 (group 1). HPV 68 is also considered probably carcinogenic or group 2a. Additionally, HPV genotypes 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, and 97 have been linked to rare causes of cervical cancer and are classified as possibly carcinogenic (group 2b). Globally, HPV 16 and HPV 18 are responsible for approximately 70% of cervical cancer cases (3).

The virus enters the body via cutaneous or mucosal microlesions and around 85% of the sexually active population (regardless of their sex, gender identity, or sexual orientation) have been infected with HPV during their lifespan. In a great majority of cases, HPV infection is asymptomatic, with 70% of the infected clearing the infection in one year, and 91% clearing the infection in two years (4).

The most common clinical presentations of cutaneous HPV infection are anogenital warts that can appear on the vagina, penis or anus, and are highly associated with low-risk genotypes HPV 6 and HPV 11, which cause 90% of genital warts (5). Genital warts usually cause emotional discomfort for the patient, and the treatment can be long and painful, with frequent recurrent infections.

On the other hand, HPV causes about 5% of cancers worldwide, and it is estimated that 625,600 women and 69,400 men get HPV-related cancer each year (6).

When HPV infection caused by HR oncogenic genotypes is not well controlled by the immune system, it can lead to persistent infection for many years and, if untreated, may cause the development of precancerous and cancerous lesions. HPV infection can cause six types of cancers: it is related to 99.99% cervical cancers, 90% of anal cancers, 50% of penile cancers, 70% of vaginal and vulvar cancers, and 20-60% of oropharyngeal cancers (7, 8). Although early detection of HPV-related cancers can be achieved with appropriate screening methods, the frequency of HPV-related disease is particularly high in developing countries. As the most common HPV-related cancer is cervical cancer, the aim of this paper is to analyse, describe and present the available options for prevention and treatment of this type of cancer. Special attention will be directed towards HPV vaccination as the most effective tool for cervical cancer prevention.

HPV and cervical cancer

Cervical cancer is the most common HPV-related cancer, accounting for 3.1% of all cancers worldwide, and the leading cause of cancer deaths in women in the developing world (with 341,831 deaths annually) (8). It is estimated that the global incidence rate of cervical cancer was 13.3 cases per 100,000 women in 2020, with a mortality rate of 7.2 per 100,000 women (9). The incidence of cervical cancer and mortality rate in 2020 within Europe is presented in Table I (10, 11). Montenegro has the highest incidence and death rate from cervical cancer in the European region, while among the European Union countries the highest incidence and mortality rates have been recorded in Romania, Bulgaria, Estonia, Latvia and Slovakia, respectively (Table I). Serbia is ranked as the fifth country in the European region and the second country in the Balkan region by the incidence and death rate from cervical cancer, according to the available data (Table I). Persistent HPV infection is an essential factor that leads to the development of cervical cancer, which is associated with certain HR types of HPV: genotype HPV 16 is responsible for 55-60% of all cervical carcinomas worldwide, while genotype HPV 18 is recognised to cause around 20% of cervical adenocarcinomas (2, 12). Other oncogenic HPV genotypes which can cause about 25% of cervical carcinomas are 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (13). In Serbia, 12 HR-HPV genotypes have been identified as dominant: 16, 18, 31, 33, 35, 45, 52, 53, 58, 59, 62, 66, with the most frequent genotype being 16, followed by 45, and 31 (14). Persistent infection with oncogenic types is related to the development of premalignant changes or dysplasia of squamous cells in the cervical epithelium (also known as *cervical intraepithelial neoplasia* - CIN). Further on, CIN can slowly progress, forming dysplastic structures that are classified into 3 degrees according to their severity: CIN1 (mild), CIN2 (moderate), CIN3 (severe dysplasia) and, finally, as carcinoma *in situ* and invasive carcinoma. CIN1 denotes mild dysplasia where one-third of the lower epithelium shows dysplasia, CIN2 (or moderate dysplasia) represents a condition where two-thirds of the epithelium is affected, and CIN3 (severe dysplasia) denotes a condition where more than two-thirds of the epithelium is affected (12). The process of HR-HPV carcinogenesis starts with HPV entering microlesions and its integration into the host cell genome. HPV self-replicates, spreads to other epithelial cells, causes irregular growth of infected cells and, subsequently, the virions are released through desquamated cells of the cervical epithelium, enabling further transmission (15). Cervical cancer can be detected by HR-HPV testing, by collecting Pap smears and performing colposcopy (16).

Table I Cervical Cancer incidence and mortality rate across Europe (estimates for 2020) (10, 11)

Tabela I Incidenca karcinoma grlića materice i stopa smrtnosti u zemljama Evrope (procene za 2020. godinu) (10, 11)

Country	Cervical cancer incidence[1]*	Number of new cases in 2020, all ages[2]	Cervical cancer mortality[1]*	Number deaths in 2020, all ages[2]
Montenegro	35.6	113	17	54
Romania	34.2	3 380	18.3	1 805
Bulgaria	28.2	1 009	14.1	503
Estonia	28.1	196	8.88	62
Serbia	27	1 205	14.2	634
Latvia	26.3	267	13.4	136
Slovakia	24.9	698	10.1	284
Hungary	24.7	1 251	9.52	482
Republic of Moldova	22.8	480	11.8	248
Ukraine	20.3	4 756	8.90	2 089
Poland	19.8	3 862	11	2 137
Russian Federation	19.6	15 308	9.64	7 550
Bosnia and Herzegovina	18.6	312	9.14	153
Belarus	16.5	835	7.09	358
Portugal	16.1	865	7.05	379
Croatia	15.8	336	7.06	150
Norway	14.8	397	3.58	96
Czechia	14.1	813	7.32	435
Ireland	13.8	342	4.26	106
Denmark	13.2	384	4.81	140
Greece	13.1	697	5.31	282
Sweden	13	656	3.97	200
Germany	11	4 666	4.90	2 075
North Macedonia	10.9	113	5.95	62
Italy	10.2	3 152	3.26	1 011
France	10	3 379	4.31	1 452

Slovenia	9.96	104	5.17	54
Iceland	9.42	16	2.94	5
Albania	9.41	133	5.24	74
Luxembourg	7.76	24	3.23	10
Austria	8.43	385	3.72	170
Spain	8.23	1 957	3.42	814
Cyprus	7.62	46	5.46	33
Malta	5.91	13	2.27	5

*Per 100.000 women;
[1]Source: https://hpvcentre.net/statistics/
[2]Source: GlobalCan https://gco.iarc.fr

Prophylactic HPV vaccines

The most (cost)-effective way to prevent HPV-related cancers and infections is vaccination.

There are three recombinant HPV prophylactic vaccines available (Table II):

- quadrivalent HPV vaccine Gardasil®4 approved by the *FDA* and *EMA* in 2006;
- bivalent vaccine Cervarix™ approved in 2007 (by the *EMA*) and 2009 (by the *FDA*) and
- nonavalent vaccine Gardasil®9 approved in 2014 (by the *FDA*) and 2015 (by the *EMA*) (8, 17, 18).

Currently available prophylactic HPV vaccines are designed using recombinant DNA technology in different expressions systems - insect cells or *Saccharomyces cerevisiae* – and contain L1 Virus Like Particles (VLPs) of certain HPV genotypes (Table II). These expression systems are used for producing L1 antigens of a specific HPV genotype, as the L1 protein has the ability to spontaneously form the so-called VLP that is highly immunogenic and produces high titers of neutralizing antibodies (8). Five L1 monomers self-assemble to pentamers and form capsomeres, and 72 capsomeres further assemble to VLP (Figure 1, 21). Additionally, VLPs are similar to the original HPV, but do not contain viral DNA nor L2 protein, meaning that VLPs are non-infectious and do not have oncogenic potential. HPV prophylactic vaccines containing L1 VLP of certain HPV genotypes are safe to use and there is no possibility that HPV vaccination may cause HPV infection or malignant transformation of human cells.

All approved HPV vaccines protect against highly oncogenic genotypes HPV 16 and HPV 18, which are the most commonly found ones in cervical cancers (19). A reduction in these genotypes, and also in the incidence of genital warts and precancerous cervical lesions, has been achieved in countries with high vaccination coverage. In addition, these results are also detected in non-vaccinated females and males within

countries with high HPV vaccination rates, indicating that herd immunity can also protect non-vaccinated individuals (22).

Table II Types of prophylactic HPV vaccines and vaccine composition of a 0.5 ml dose of HPV vaccine (adapted from 19, 20)

Tabela II Tipovi profilaktičkih HPV vakcina i sastav HPV vakcine u dozi od 0,5 ml (prilagođeno prema referencama 19, 20)

	Gardasil®4 (Merck & Co, Inc., Rahway, NJ, USA)	**Cervarix™** (GlaxoSmithKline Biologicals)	**Gardasil®9** (Merck & Co, Inc., Rahway, NJ, USA)
Year of FDA approval	2006	2009	2014
Year of EMA approval	2006	2007	2015
Valency	4-Valent	2-Valent	9-Valent
Oncogenic protein subunit component L1 VLP (μg)	HPV 6 (20) HPV 11 (40) HPV 16 (40) HPV 18 (20)	HPV 16 (20) HPV 18 (20)	HPV 6 (30) HPV 11 (40) HPV 16 (60) HPV 18 (40) HPV 31 (20) HPV 33 (20) HPV 45 (20) HPV 52 (20) HPV 58 (20)
Adjuvant	0.225 mg aluminum hydroxyphosphate sulfate	ASO4 (0.5 mg aluminum hydroxide and 50 μg 3-O-desacyl-4″-monophosphoryl lipid A (MPL))	0.5 mg aluminum hydroxyphosphate sulfate
Sodium chloride (mg)	9.56	4.4	9.56
L-Histidine (mg)	0.78	/	0.78
Polisorbate 80 (μg)	50	/	50
Sodium borate (μg)	35	/	35
Sodium dihydrogen phosphate dihydrate (mg)	/	0.624	/
Expression system	*Saccharomyces cerevisiae* (yeast)	Baculovirus-insect cell	*Saccharomyces cerevisiae* (yeast)

Abbreviations: FDA (Food and drug administration); EMA (European Medicines Agency); VLP (Virus Like Particle); AS04 (Adjuvant System 04).

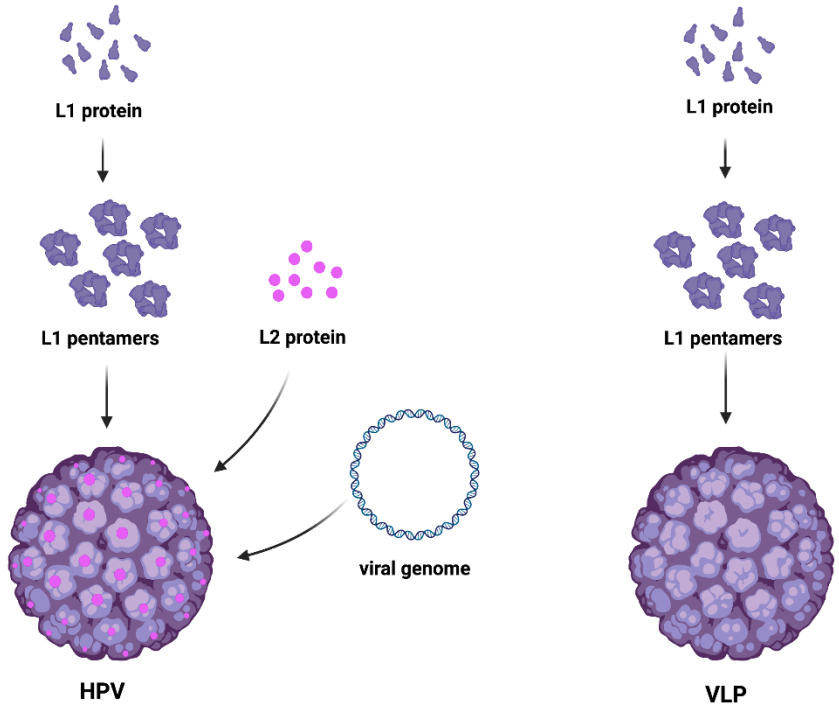

Figure 1. **Comparison of HPV and L1 VLP assembling (created with BioRender.com, adapted from the reference 19)**
Slika 1. **Formiranje HPV i L1 VLP (nacrtano upotrebom BioRender.com programa, prilagođeno iz reference 19)**

Australia was one of the leading countries that were among the first to implement the HPV National Immunisation Programme (NIP) using the quadrivalent HPV vaccine since 2007 for girls, and in 2013 the immunisation programme was extended to boys. This gender-neutral approach is considered vital for disease burden in men, particularly MSM (men who have sex with men) who would not benefit from female-only HPV vaccination. In January 2018, nonavalent HPV vaccine Gardasil®9 replaced the quadrivalent HPV vaccine in Australia (23). The successfully implemented HPV immunisation program in Australia revealed that HPV prevalence in women aged 18-24 declined from 28.7% (before the vaccination programme, from 2005 to 2007) to 2.3% in vaccinated women (from 2010 to 2012) (24). Besides lower HPV prevalence after vaccine introduction, Australia has been one of the leading countries globally in reducing genital warts incidence (25). In addition, a 12 year-long study in Nordic countries (Denmark, Iceland, Norway, and Sweden), where quadrivalent HPV vaccine was introduced, revealed that there were no breakthrough cases of HPV16/18 CIN2 or worse (CIN2+) in 2084 vaccinated women (26).

The nonavalent HPV vaccine is gradually replacing other HPV vaccines in most countries. The highest efficacy of HPV vaccines is among the young population, with the recommendation for vaccination before they become sexually active and before having

contact with HPV. The nonavalent HPV vaccine can be given starting from year 9, but it is best to start with vaccination in children 11-12 years old, and they should get two doses of the HPV vaccine, given 6 to 12 months apart. Children after their 15th birthday need three doses scheduled as 0, 2, and 6 months. The vaccine is indicated according to the EU Summary of product characteristics (SMPC) and local Product Information leaflets for beneficiaries of 9 years of age and above. Routine vaccinations are available according to local jurisdictions through the NIPs or Reimbursement Framework Programs (RFPs). Some countries in the European region, such as Romania and other countries in the EU, provide subsidised programs for adult women from 19 to 45 years of age. In addition, adults between 26 and 45 may consult their physicians regarding the potential benefits of HPV vaccination.

In Serbia, HPV vaccination with nonavalent Gardasil®9 vaccine has been funded by the Republic Fund of Health Insurance for beneficiaries 9-19 years old since June 2022. According to the available data, by the end of 2022, a total of 20,130 doses of Gardasil®9 were administered. The first dose was given to 14,164 people (7,376 to girls and boys 9-14 years old, and 6,788 to people aged 15-19). The second dose was given to 734 people 9-14 years old, while 4,561 second and 618 third doses were given to people aged 15-19 (27).

The vaccine is given as an injection into a muscle, preferably in the shoulder or the thigh, and it is prepared as a suspension for the injection available in vials or prefilled syringes. The side effects are usually mild or moderate and refer to local reactions at the injection site (redness, pain and swelling), and less often fever and headache or fatigue, nausea, muscle pain and syncope (fainting). Although rare, syncope may happen after HPV vaccination, and it is recommended that healthcare professionals administer HPV vaccine while the beneficiary is seated or lying down, and that they wait and observe the beneficiary for 15 minutes to rule out the risk of syncope and possible injury due to a fall. HPV vaccination is contraindicated in pregnant women, people who have had a life-threatening allergic reaction to any component of the HPV vaccine, to a previous dose of the HPV vaccine or yeasts. People with mild infections such as colds may be vaccinated, while vaccination should be postponed in people who are severely ill until they recover (28, 29, 30).

How HPV vaccines work

The immune response induced by natural HPV infection is weak, with very low antibody titres. However, HPV L1 VLPs based prophylactic vaccines have shown great efficacy and effectiveness in clinical trials in countries with high coverage rate. The collected data revealed that inhibitory antibodies (mostly IgG) are the major mediators of vaccine-induced protection. In addition, it is assumed that the specific nature of VLPs is largely attributed to the efficient generation of long-lived antigen-specific antibody-producing cells (31). Several studies have shown that VLPs in HPV vaccine formulations induce effective humoral immune response, and HPV vaccination provides 10- to 100-fold higher antibody titers compared to natural infection (7, 32, 33). Young individuals

aged 9-14 have a better response to HPV vaccination compared to individuals aged 15-24, and antibody titers are stable over time (34). More than 15 years of research has shown that HPV vaccination is safe and effective in reducing HPV-related infections, genital warts and precancerous lesions, and that it provides protection and sustained antibody titers for at least 10 years after vaccination (35).

The exceptional immunogenicity of VLPs based HPV vaccines is largely attributed to the structure of the antigen. VLPs are composed of 360 protein subunits that display a repetitive array of epitopes on their surface and that engage B-cell receptors on naïve B-cells, leading to strong activation of memory B-cells and long-lived plasma cells that produce antibodies for many years (36).

HPV L1 VLPs in vaccines are delivered by intramuscular injection, where dendritic cells of the muscle overtake the antigens and migrate with their cargo to the regional lymph node. This process activates an immune cascade that results in T-cell dependent B cell response and generation of high levels of L1-specific serum neutralizing antibodies and immune memory (37). If natural HPV infection does not involve virus penetration into circulation, the question we may ask is how VLPs based vaccination induces antibodies that reach the HPV infection site, especially women`s genital tract. One possible mechanism is transudation (antibody transport through intact epithelia) of systemic IgG antibodies into the cervicovaginal mucus or direct exudation (passive transfer through damaged epithelia) of systematic antibodies at the site of the infection (38). The very slow progression of natural infection, much slower than for other known viruses, provides an exceptional window for vaccine-induced antibodies to disrupt the process of natural infection (39).

World Health Organization global strategy for the elimination of cervical cancer

In November 2020, the World Health Organization (WHO) announced a global strategy to accelerate the elimination of cervical cancer as a public health problem, a very ambitious global plan whose aim is to reduce the incidence of cervical cancer to less than 4 per 100,000 women worldwide. Why is this global strategy needed? First of all, cervical cancer is a preventable disease, and it can also be adequately treated if detected early. More than 85% of women with cervical cancer are young undereducated women who live in non-developed countries (39). In addition, by the end of 2020, less than 25% of low-income countries and less of 30% lower-middle-income countries had introduced HPV vaccination into their NIP, compared to more than 85% of high-income countries which had implemented NIP in their schedule. To achieve this goal of lowering cervical cancer incidence below 4 per 100,000 women, high coverage of HPV vaccination, screening and treatment of precancerous lesions, and adequate treatment of cervical cancer must be reached by 2030 and maintained at this high level for decades (39).

Therefore, this strategy, known as the Cervical Cancer Elimination Initiative (CCEI) (40), and also as the 90-70-90 strategy, outlines clear targets that must be met by 2030:

- primary prevention – HPV vaccination: 90% of girls will have completed their HPV vaccination course by the age of 15 years;
- secondary prevention - screening: 70% of women will have been screened for cervical cancer using a high-performance test by the age of 35 and again by the age of 45;
- tertiary prevention-treatment: 90% of women diagnosed with cervical disease will have received the treatment (9, 41).

HPV vaccination, as primary prevention, is the most effective long-term intervention for reducing the risk of developing cervical cancer. To achieve 90% coverage of HPV vaccination, different strategic actions are needed: a) securing sufficient and affordable HPV vaccines through appropriate market-shaping interventions; b) increasing the coverage of vaccination (e.g., by implementing school immunization programmes), and additionally, providing monitoring systems or registers in order to track and improve vaccine coverage; c) improving communication and social mobilization efforts, as understanding the social, cultural and other barriers that can affect vaccine acceptance is critical.

The main goal of secondary prevention is to reduce the incidence and mortality rate by identifying and treating women with precancerous lesions. Cytology-based screening has been successfully used with additional diagnostic tests (colposcopy and pathology), but cytology-based programmes have been difficult to implement in low- and middle-income countries. Because of this, initiatives to secure affordable and high-quality diagnostic tests will be prioritized (39).

In addition, comprehensive management of cervical cancer and timely referral of women with suspected or confirmed cervical cancer are crucial for saving lives. Efficient, integrated networks of screening and diagnostic laboratory services are needed, and they will lead to improved access and affordability of screening and treatment, especially in low-income countries (39).

A mathematical model demonstrating the benefits of CCEI implementation by 2030 revealed that the median cervical cancer incidence rate will have fallen by 97% by 2120, preventing more than 74 million new cases of cervical cancer, and 62 million cervical cancer deaths by 2120 (42).

To date, HPV vaccine is part of the NIP for girls in 125 countries and for girls and boys in 47 countries, but this is only about a third of the global population targeted by the 90-70-90 strategy. It is also worrying that global HPV vaccine coverage among girls declined from 20% in 2019 to 15% in 2021, which is far from the goal of the CCEI strategy to vaccinate 90% girls by the age of 15.

In addition, the COVID-19 pandemic disrupted access to preventive strategies, so it remains to be seen whether it is possible to meet the targets of the CCEI or additional efforts will be needed (9).

Treatment of the cervical cancer

One of the goals of the WHO CCEI initiative is to ensure that 90% of women diagnosed with cervical cancer have received appropriate treatment. In 2018, the European Society of Gynecological Oncology (ESGO) jointly published evidence-based guidelines for the management of patients with cervical cancer with the European Society for Radiotherapy and Oncology (ESTRO) and the European Society of Pathology (ESP). In addition, thanks to a large body of new evidence addressing the management of cervical cancer, an update of these evidence-based guidelines was published in 2023 (43).

There are different treatment approaches that depend on the stage of cancer and overall patient health. The selection of treatment plan for cervical cancer nowadays depends on careful preoperative evaluation of pathologic characteristics of the tumour and results of imaging (MRI or expert transvaginal ultrasound). The treatment approach may include one or more different procedures, such as surgery, chemotherapy and/or radiotherapy with external beam radiation therapy and brachytherapy, targeted therapy or immunotherapy (44, 45). Combined therapy – chemoradiotherapy – is the standard of care treatment for locally advanced cervical cancer, and may be effective for many patients; however, the mortality rate is still high (45).

Treatment strategy in early-stage cervical cancer has been modified over the last fifteen years. Radical hysterectomy represents a cornerstone in the treatment of stage IB1 to IIA1. When the surgery is to be performed, careful preoperative evaluation of histopathology, especially the grade and lymphovascular space involvement (LVSI), should be taken into account. Further on, parametrial and/or lymph node involvement diagnosed through imaging methods (MRI or expert transvaginal ultrasound) is another step in planning the treatment protocol. If lymph nodes are radiologically involved, surgery should be abandoned, except for surgical lymph node staging in the paraaortic region. When the sentinel lymph node (SLN) or other lymph nodes are positive on surgical staging, further radical surgery should be abandoned.

After complete pathology of the specimen, the presence of poor prognostic factors such as the size of the tumour, LVSI and depth of stromal invasion (intermediate risk patient) may still demand adjuvant chemoradiotherapy like in the high-risk group of patients (positive lymph nodes, parametria or surgical margins) (45).

Locally advanced cervical cancer should be treated with chemoradiotherapy and brachytherapy. Modern brachytherapy is based on MRI planning that is not available in all countries.

Radiation therapy targets the DNA of cancer cells by high-energy-x rays and it is further divided into external radiation therapy and internal radiotherapy (or brachytherapy) (46).

In those cases where this type of radiotherapy is not developed, the tumour can be pre-treated with neoadjuvant chemotherapy (NACT) for downsizing the tumour. It remains a controversial alternative with no benefits regarding the prognosis (47).

Surgical staging can guide radiation therapy toward the paraaortic region in case of discovery of positive nodes. The debulking can improve sterilisation of the region with radiotherapy. Still, the benefits regarding prognosis after staging and debulking are not completely clarified and demand further randomized studies (43).

Although rare at initial diagnosis, metastatic disease develops in 15-60% of patients with cervical cancer, usually within 2 years of primary treatment. Over the past three decades, the median overall survival (OS) of patients with recurrent and metastatic disease has not improved significantly, despite studies with single-agent or combination chemotherapy. The addition of bevacizumab to chemotherapy (Taxol and Cisplatin) was the only significant advance in the treatment of persistent, recurrent, and metastatic cervical cancer. The results of the GOG 240 study, where the addition of bevacizumab increased median survival by 3.7 months, were the first breakthrough (48).

Chemotherapy is based on drugs used to stop the growth of cancer cells by killing the cells or impacting their ability to divide, and this approach is usually combined with radiotherapy, as mentioned above. Meta analyses from 2017 revealed and confirmed the benefits of concurrent chemoradiotherapy over radiotherapy alone (49).

Thanks to a better understanding of molecular aberrations in cervical cancer, new therapeutic modalities – immunotherapy, including check-point inhibitors, antibody-drug conjugates, and therapeutic vaccines – have appeared in recent years (43).

The addition of pembrolizumab to chemotherapy + bevacizumab in the phase III KEYNOTE-826 study provides a statistically significant improvement in the OS and PFS in persistent, recurrent or metastatic cervical cancer. Pembrolizumab (Keytruda®) is an FDA-approved monoclonal antibody directed against programmed cell death protein 1 (PD-1) which is used for therapy of various cancers, including metastatic or recurrent cervical cancer, following chemotherapy. After a follow-up of 39.1 months, investigators showed that the addition of pembrolizumab to chemotherapy with or without bevacizumab continued to demonstrate clinically a significantly prolonged median OS (26.4 vs 16.8 months; HR: 0.63; P <.0001) and median PFS (10.4 vs 8.2 months; HR: 0.61; P <.0001) in the all-comer population. The median OS was improved in PD-L1 subgroups (PD-L1 CPS ≥1: 28.6 vs 16.5 months, HR: 0.60; PD-L1 CPS ≥10: 29.6 vs 17.4 months, HR: 0.58) (50).

The phase III BEATcc trial of the addition of atezolizumab to the standard of care of bevacizumab and platinum-based chemotherapy as first-line treatment for patients with persistent, recurrent, or metastatic cervical cancer showed that, after a median follow-up of approximately 32 months, the addition of atezolizumab to bevacizumab and chemotherapy improved the median PFS compared with the standard-of-care arm (13.7 vs 10.4 months; HR: 0.62; P = .0001). The PFS benefit in favour of the atezolizumab-containing arm was seen across most subgroups analysed, including age, disease status, chemotherapy backbone, previous chemoradiotherapy, and tumour histology (51).

The antibody-drug conjugate, tisotumab-vedotin, was studied in patients with recurrent and metastatic cervical cancer who had not responded to standard treatment.

Tisotumab vedotin (TivdakTM) is an antibody-drug conjugate (ADC) made of a human monoclonal antibody specific for tissue factor (TF-011) expressed on tumor cells chemically linked to a cancer-killing drug (monomethyl auristatin E; MMAE). In the innovaTV 301 study at the interim primary endpoint of OS, tisotumab vedotin was superior to the investigators' choice of chemotherapy (HR: 0.70; P = .0038). Patients who received tisotumab vedotin experienced a 30% reduction in the risk of death, which is remarkable. At the secondary endpoint PFS was significant (HR: 0.67; P = .0001). The ORR was 17.8% vs 5.2% with tisotumab vedotin vs chemotherapy. Of note, 6 patients (2.4%) in the tisotumab vedotin arm achieved a complete response vs none with chemotherapy (52). Tisotumab vedotin is the first and only ADC recommended for treatment of patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy (53).

One of the latest drugs for the treatment of different solid tumours, including cervical cancer, is Cadonilimab, a PD-1/CTLA-4 bi-specific antibody approved in China in June 2022 for use in patients who progressed on or after platinum-based therapy (54).

Therapeutic cervical cancer vaccines aim to eradicate HPV-infected cells by stimulating cytotoxic T cells against viral/tumour antigens. HPV E6 and E7 oncoproteins are expressed in HPV-related cancers and are ideal targets for a therapeutic vaccine (55).

Many other studies have been established to try and reach a better response rate for these advanced/metastatic and recurrent cervical cancers, such as a phase I NRG GY017 study evaluating 3 total doses of atezolizumab given with chemoradiotherapy (CRT) to patients with high-risk LACC, a randomized phase II trial of pembrolizumab during CRT or after CRT in patients with high-risk LACC, a phase III KEYNOTE A18 trial of pembrolizumab with concurrent chemoradiotherapy (cCRT) vs cCRT alone in patients with newly diagnosed, high-risk, previously untreated LACC, a phase III INTERLACE trial evaluating induction chemotherapy with weekly paclitaxel and carboplatin for 6 weeks followed by chemoradiotherapy (CRT) vs CRT alone in patients with newly diagnosed FIGO 2008 stage IB1N+, IB2, II, IIIB, IVA squamous, adeno, and adenosquamous LACC (56-59).

Although effective treatment options have been established, cervical cancer related mortality remains high, especially in low- and middle-income countries with limited availability of treatment options. Great efforts have to be made to reach the WHO goal of having 90% of women diagnosed with cervical cancer receive appropriate treatment.

However, the main goal is decreasing the incidence of cervical cancer, which would lead to a reduction in the need for treatment in advanced disease stages in the future.

Conclusion

Cervical cancer is a significant part of the global cancer burden in women and the fourth cause of cancer-related deaths in woman. The WHO has recognized the importance of reducing and eliminating cervical cancer and announced a global strategy for cervical cancer elimination (CCEI) in 2020, which includes HPV vaccination, screening, and

appropriate treatment. HPV vaccination is a forefront primary preventive measure, and it has been confirmed that HPV vaccination significantly prevents genital warts and cervical cancer. In order to achieve WHO targets, great efforts are needed in the field of improving HPV vaccination awareness, as well as in promoting the importance of regular screenings in women and the availability of chemotherapy and radiotherapy, particularly in low- and middle-income countries.

Conflict-of-interest disclosure

The authors report no potential conflicts of interest. IRM is an employee of Merck Sharp & Dohme Romania SRL.

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Humani papilomavirusi i karcinom grlića materice iz perspektive inicijative Svetske zdravstvene organizacije za eliminaciju karcinoma grlića materice

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Kratak sadržaj

Humani papilomavirusi (HPV) su među najčešćim uzročnicima seksualno prenosivih patogena i mogu dovesti do različitih kliničkih manifestacija: od benignih stanja do različitih vrsta karcinoma kod žena, ali i muškaraca. Najčešći HPV-posredovan karcinom je karcinom grlića materice koji je u preko 99,99% slučajeva posledica infekcije. Najefikasniji način da se spreči razvoj perzistentne HR-HPV infekcije je vakcinacija. Dostupne su tri profilaktičke vakcine: dvovalentna, kvadrivalentna i devetovalentna. Devetovalentna vakcina pruža najširu zaštitu jer sadrži devet onkogenih HPV genotipova i postepeno zamenjuje ostale vakcine u svim zemljama. Sa vakcinacijom se može krenuti od 9. godine, ali se najčešće rutinski sprovodi kod dečaka i devojčica u uzrastu od 11 do 12 godina. Svetska zdravstvena organizacija je prepoznala karcinom grlića materice kao globalni problem i uvela takozvanu 90-70-90 strategiju u cilju smanjenja stope, pa čak i eliminacije karcinoma grlića materice. Ova strategija podrazumeva da 90% devojčica bude potpuno vakcinisano do 15. godine, 70% žena pristupi redovnom ginekološkom

pregledu do 35. godine i ponovo do 45. godine i 90% žena sa promenama na grliću materice primi adekvatnu terapiju. Iako su dostupne različite terapije poput hirurškog tretmana, radioterapije, hemioterapije i ciljane terapije monoklonskim antitelima, i dalje su potrebni veliki napori da bi se dostigli ciljevi Svetske zdravstvene organizacije.

Ključne reči: HR-HPV infekcija, karcinom grlića materice, HPV vakcinacija

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The role of serum lipid profile in the pathogenesis of arterial hypertension

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Abstract

Hypertension is a key contributor to the high global burden of cardiovascular morbidity and mortality, due to its increasing prevalence worldwide. In clinical practice, dyslipidemia and hypertension often coexist, possibly because they share similar underlying causes, such as endothelial dysfunction and obesity. Consequently, this review article presents the collective findings on the role of lipid profile parameters in arterial hypertension. Individuals with hypertension often have significantly higher mean serum levels of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C), while exhibiting lower mean serum levels of high-density lipoprotein cholesterol (HDL-C) compared to those without hypertension. TC and HDL-C play an important role in the pathogenesis of arterial hypertension. However, there is a lack of studies explaining the link between TG and LDL-C and arterial hypertension. Future studies are necessary to fully elucidate the exact mechanisms by which the mentioned lipid parameters contribute to arterial hypertension.

Key words: arterial hypertension, lipid profile, atherosclerosis

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Introduction

Hypertension is a chronic disorder presented with consistently high blood pressure. It represents an important public health concern and adds to the total burden of cardiovascular (CV) morbidity/mortality worldwide. The world trend of hypertension incidence is rising globally, with an estimated increase to nearly 30% by 2025 (1).

In 2010, nearly 31% of people (i.e. 1.38 billion adults) exhibited hypertension, defined as systolic blood pressure (BP) ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg[2]. Moreover, hypertension prevalence is increasing globally in parallel with unhealthy dietary habits, sedentary lifestyle and obesity (2-6).

Hypertension, as a multifactorial disease, is tightly connected with oxidative stress and inflammation, as well as with endothelial dysfunction, being its major pathophysiological underlying feature (7).

Under physiological condition, antioxidants diminish and/or prevent the harmful effects of the reactive oxygen and nitrogen species (ROS, RNS). Once this antioxidant-pro-oxidant balance becomes exhausted due to overwhelming ROS/RNS accumulation, negative side-effects on target cells occur (7, 8). The activity of endothelial NOS is diminished by ROS/RNS, along with reduced NO synthesis, leading to vasoconstriction (7, 8).

Visceral compartments of the adipose tissue are significant contributors to increased pro-oxidant and pro-inflammatory milieu due to increased secretion of cytokines and adipokines, thus favoring insulin resistant state and atherogenic dyslipidemia, as another underlying feature of hypertension and CV risk (9-11).

Currently, the standard practice to assess the CV risk of an individual involves examining the serum lipid profile, which consists of four key measurements: triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C)[12]. Typically, this test is conducted using a blood sample obtained after fasting (12).

Dyslipidemia refers to an imbalance of lipid biomarkers, including TG, TC, LDL-C, and HDL-C. This condition can lead to serious complications, mostly cardiovascular disease (CVD), which can be influenced by factors like an unhealthy diet, smoking, or genetic predisposition (13). Dyslipidemia and hypertension share the common pathophysiological mechanisms, both of them being major risk factors for CVD, and there is a complex relationship between them (14, 15). These two entities act synergistically concerning the dysfunction of endothelial cells (15).

In hypertension, the dysfunction of endothelial cells forces the onset/progression of the adverse effects of dyslipidemia, and vice versa – the process of hypertension is aggravated due to structural/functional alterations of the vascular wall found in dyslipidemia (15).

Atherosclerosis is a state accompanied by the deposition of fatty plaques in the arteries, leading to modifications in the walls of arteries, i.e. narrowed lumen and stiffened

arteries. Increased LDL-C levels, i.e. LDL oxidation, are the key risk factor for atherosclerosis (15).

Over time, atherosclerosis can reduce the elasticity of the arteries and further increase BP, contributing to the development of hypertension. Atherosclerosis can lead to endothelial dysfunction. This dysfunction can reduce the ability of blood vessels to dilate properly, which can, in turn, raise BP. In clinical practice, it is common for individuals to have both dyslipidemia and hypertension, potentially due to shared underlying causes (16, 17).

Serum lipid and lipoprotein abnormalities are established as an important risk factor for both CVD and essential hypertension. Dyslipidemia is more frequent among individuals with newly diagnosed hypertension (18). Both hypertension and dyslipidemia can cause inflammation within blood vessels, promoting the development and progression of atherosclerosis. One metabolic and inflammatory marker that can predict CV risk is the TG-HDL-C ratio (19-21). According to a previous study, poorly controlled hypertension patients showed a significantly higher TG-HDL-C ratio compared to well-controlled hypertension patients (22). Patients with hypertension who had increased TG levels and LDL-C/HDL-C ratio also faced a higher risk of developing diabetes, with these factors interacting to influence diabetes onset (23).

Patients with hypertension exhibited serum lipid levels that varied depending on age and sex, with non-elderly individuals being more prone to dyslipidemia compared to the elderly. Female patients exhibited higher TG, LDL-C, and TC levels compared to males (24).

Similarly, in a study conducted on an urban population in Bangladesh, the association between serum lipid status and hypertension was investigated (25). The findings indicated that individuals with hypertension had significantly higher levels of TG, LDL-C and TC in comparison with normotensives. Additionally, lower HDL-C was recorded in hypertensive individuals compared to normotensives (25). There were significant associations between TC and systolic BP, as well as TG and diastolic BP. However, systolic and diastolic BP did not show statistically significant associations with the other lipid parameters (26). Nevertheless, it is important to note that there are diverse populations and patterns where dyslipidemia and hypertension may not be consistently linked.

Hence, this review article focuses on the role of lipid profiles in the development of arterial hypertension, an aspect that has not been extensively reported in the literature on hypertension. It specifically summarizes the role of serum levels of TG, LDL-C, HDL-C and TC in the pathogenesis of arterial hypertension.

The role of lipid profile in arterial hypertension

This review article is focused on the influence of serum levels of TC, TG, HDL-C and LDL-C in arterial hypertension. In Figure 1, the lipid profile's contribution to the pathogenesis of arterial hypertension is illustrated. Moreover, a summary of studies which

explained the role of major lipid profile parameters in the development of arterial hypertension is presented in Table I.

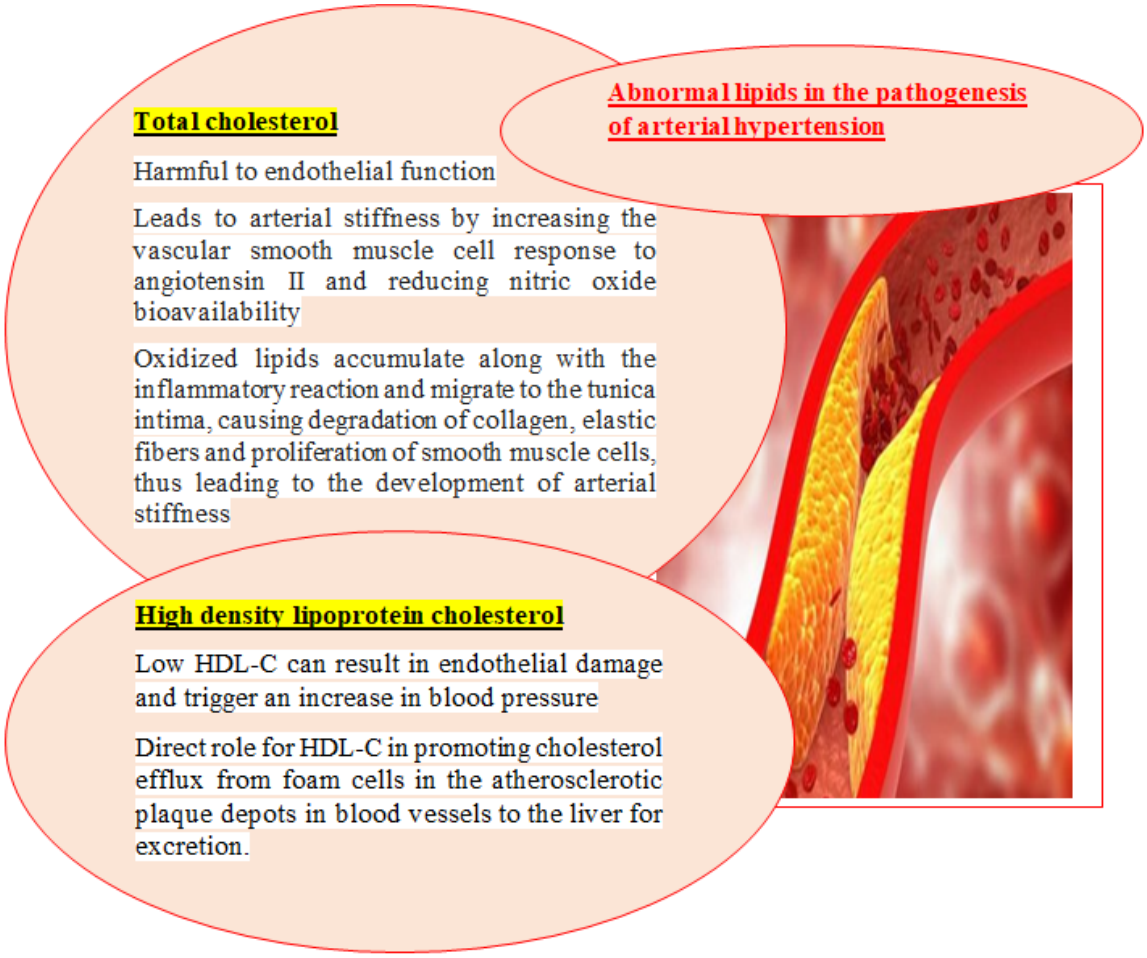

Figure 1. Effects of total cholesterol and high density lipoprotein cholesterol in hypertension development

Slika 1. Uticaj ukupnog holesterola i holesterola iz lipoproteinskih čestica velike gustine na razvoj hipertenzije

Table I Summary of studies which explained the role of major lipid profile parameters in the development of arterial hypertension

Tabela I Sažeti prikaz studija koje objašnjavaju ulogu glavnih parametara lipidnog profila u razvoju arterijske hipertenzije

First author	Lipid profile	The key finding of lipid profile in arterial hypertension
Osuji et al. (18)	TC	Hypertensive patients have been found to have significantly higher serum TC concentrations compared to normotensive individuals.
Anika et al. (26)	TC	A significant association between systolic BP and TC and a significant correlation between TG levels and diastolic BP was found.
Satoh et al. (36)	TC	No significant interactions between blood pressure and TC have been observed for stroke. The joint effect of high BP and high TC levels can enhance the risk of death from coronary heart disease, but not from stroke.
Chen et al. (42)	TC	Arterial stiffness was found to act as a mediator between hyperlipidemia and BP, linking the risk factors of TC and arterial stiffness to high BP.
Tomita et al. (44)	TG	Significant associations were observed between serum TG levels and the onset of hypertension.
Raposeiras-Roubin et al. (45)	TG	Hypertriglyceridemia was related to endothelial inflammation and subclinical atherosclerosis, even in individuals with normal LDL-C levels in subjects with low-moderate CV risk.
Kawamoto et al. (46)	TG	TG levels modulated the correlation between serum uric acid and prehypertension.
Fletcher and Bulpitt (48)	TG, HDL-C	The risk of ischemic heart disease in individuals with low HDL-C and high TG was not directly correlated with systolic or diastolic BP values.
Trimarco et al. (51)	HDL-C	The U-shaped correlation between HDL-C levels and the risk of CV events in hypertensive males were demonstrated.
Chruściel et al. (52)	HDL subfractions	The relationship between total HDL-C and HDL-3 subfraction values and the tendency for hypertension onset in young subjects.
Ivanišević et al. (53)	HDL subfractions	The correlation between hypertension and relative proportion of paraoxonase (PON)-1 on HDL_{3c} subclasses in pregnant women was shown.
Pavithran et al. (54)	HDL-C	Changes in lipid profile, including a lowering of HDL-C, can lead to endothelium damage and increased BP that can contribute to its significant predictive value for coronary heart disease.
Chen et al. (57)	HDL-C	In a large sample-size study that included nearly 63,000 males in China, a positive association between HDL-C and hypertension after adjustment for the BMI was observed.
Al-Jarallah et al. (58)	HDL-C	In rats with normal BP, HDL-C was found to protect against myocardial ischemia/reperfusion damage.
Tsukinoki et al. (60)	LDL-C	The first epidemiological study to investigate the joint impact of LDL-C and BP on different subtypes of CVD in population of Asia.

The role of dyslipidemia in the pathogenesis of hypertension

Changes in lipid metabolism can lead to abnormal concentrations of serum lipids and lipoproteins, which are closely related to hypertension. Dyslipidemia has been shown to have a significant impact on the prognosis of individuals with hypertension (27). Research on the association between hypertension and dyslipidemia, as well as the underlying mechanisms, is growing. Hypertension and dyslipidemia not only share common underlying factors and metabolic abnormalities, but also interact with each other through various mechanisms. Firstly, dyslipidemia contributes to increased BP and vascular endothelial damage. The connection between hypertension and hyperlipidemia is likely mediated by the function of the vascular endothelium. Vascular endothelial activity plays a crucial role in regulating artery contractility. Abnormal lipids can indirectly affect arterial elasticity and lead to hypertension by interfering with the control of vascular endothelial cells (28). Oxidized low-density lipoprotein (ox-LDL) is a crucial component in the impact of hyperlipidemia on vascular endothelial damage. Ox-LDL primarily induces damage and dysfunction in the endothelium, favoring the development of atherosclerosis (24, 28). The process of LDL oxidation is not clearly defined. The scavenger receptors on the macrophages' surface recognize LDL, which further becomes internalized in large amounts and transformed into the foam cells. The expression of adhesion molecules on the surface of cells is induced by ox-LDL. The adhesion of leukocytes and their adherence to the endothelium, as well as their migration into the intima, along with the activation of macrophages to secrete pro-inflammatory mediators and ROS, further aggravate the destabilization of atherosclerotic plaque (29).

The role of total cholesterol (TC) in the pathogenesis of hypertension

Hypertensive patients have been found to have significantly higher serum TC concentrations compared to normotensive individuals. A positive correlation between serum TC and both systolic and diastolic BP was observed in both patients with hypertension and control subjects (18). Similarly, Anika et al. (26) confirmed the association between systolic BP and TC.

Borghi (30, 31) and Pereira (32) presumed that the pathogenic influence of hypercholesterolemia on hypertension may be strongly connected with its impact on peripheral vascular tone and the function of the tissue renin-angiotensin system. Research has also indicated that high TC levels may affect arterial stiffness, suggesting a potential connection between hypercholesterolemia and BP through these factors (32, 33). TC is considered to impair endothelium function, and increased serum TC levels lead to arterial stiffening by enhancing the response of vascular smooth muscle cell to angiotensin II and diminishing the availability of nitric oxide (34, 35).

It has been observed that elevated systolic BP increases the risk of mortality due to intraparenchymal haemorrhage and ischemic stroke, while high TC levels decrease such risk. However, no significant relationship was observed between BP and TC for stroke. In the Asian population, the coexistence of high BP and elevated TC levels can enhance the risk of death from coronary heart disease, which is not the case for stroke (36).

The accumulation of oxidized lipids caused by inflammation and their migration to the tunica intima of blood vessels contribute to the breakdown of collagen, smooth muscle cells proliferation, and the elongation of elastic fibres. These processes collectively contribute to the onset of arterial stiffness (37-39). Moreover, the buildup of lipid plaque in the arteries leads to narrowing, worsening arteriosclerosis, and ultimately resulting in an increase in systolic BP (40-41).

Arterial stiffness was found to act as a mediator between hyperlipidemia and BP, linking the risk factors of TC and arterial stiffness to high BP. To diminish the risk of CVD, clinicians need to pay attention to maintaining optimal blood lipid levels, especially in individuals with hyperlipidemia and hypertension, to delay or alleviate arterial stiffness and the subsequent increase in BP. However, when prescribing statins to individuals with hyperlipidemia, the onset of arterial stiffness and changes in BP need to be monitored. If necessary, strategies for treating or reversing aortic stiffness need to be implemented, as this can enable the prevention of the development of hypertension and manage BP effectively (42).

The role of triglycerides (TG) in the pathogenesis of hypertension

TG, the most common fatty molecules found in organisms, can lead to hypertriglyceridemia when present in high levels in the bloodstream. Hypertriglyceridemia can arise from various pathophysiological conditions and disorders, such as obesity, non-alcoholic fatty liver disease, diabetes mellitus, etc. (43, 44). Even in the absence of high TC or LDL-C levels, elevated TG values are linked with atherosclerosis and increase the CVD risk (5, 21, 45). Hypertriglyceridemia was related to endothelial inflammation and subclinical atherosclerosis, even in individuals with normal LDL-C levels in subjects with low-moderate CV risk (45). In a study conducted on a workplace population in Japan, significant correlations were observed between serum TG levels and the development of hypertension (44). Additionally, Anika et al. (26) found a significant correlation between diastolic BP and TG levels.

The relationship between higher serum uric acid and TG was shown to be an independent indicator of systolic BP, although not of diastolic BP. Elevated serum uric acid levels were correlated with prehypertension in individuals without hypertriglyceridemia, although not in those that exhibited high TG levels. The correlation between serum uric acid and prehypertension was modified by TG levels (46). Other studies have also confirmed the association between serum uric acid levels and higher BP and an independent association between waist-to-hip ratio and systolic BP (47).

In another study involving older white males without pre-existing CVD, a notable finding showed that the risk of ischemic heart disease in individuals with low HDL-C and high TG was not in direct relationship with systolic or diastolic BP levels. Furthermore, the U-shaped correlation between the risk of ischemic heart disease and treated diastolic BP, as previously described, was observed only in men with low HDL-C and high TG (48).

Jeppesen et al. (49) proposed a potential clarification for the paradox in which BP reduction did not lead to the expected decrease in ischemic heart disease risk among hypertensive patients. They suggested that investigators in BP-lowering studies did not account for specific treatment effects in patients with hypertension without and with this particular dyslipidemia (49).

The role of high-density lipoprotein cholesterol (HDL-C) in the pathogenesis of hypertension

HDL is one of the five main categories of lipoproteins, which are complex particles consisting of multiple proteins that transport fat molecules through the body's extracellular fluid. Each HDL particle is composed of an average of 80-100 proteins and is structured by one, two, or three apolipoprotein A proteins. HDL particles grow as they circulate, accumulating fat molecules and carrying hundreds of them per particle (50).

Recent research has shown that high levels of HDL-C in circulation are related to an increased risk of mortality. However, the link between HDL-C and specific CV events has not been studied in hypertensive individuals. Trimarco et al. (51) demonstrated a U-shaped relationship between HDL-C levels and the risk of CV events in hypertensive males.

A low level of HDL-C has long been recognized as a strong predictor of increased CV risk (18). Another study suggested an association between total HDL-C and HDL-3 subfraction levels with the tendency for hypertension development in youngsters (52). Ivanišević et al. (53) showed the correlation between hypertension and relative proportion of paraoxonase (PON)-1 on HDL_{3c} subclasses in pregnant women. Furthermore, Pavithran et al. (54) explained that changes in lipid metabolism, including lower HDL-C, can lead to the damage of endothelium and increased BP, which may contribute to its significant predictive value for coronary heart disease.

In another study, it was suggested that isolated lower HDL-C levels might be a usual lipid abnormality in a specific region of Nigeria, and this condition was exacerbated with the presence of hypertension. HDL-C can assume the role of endothelial damage and BP elevation. Experimental studies indicate that HDL-C takes part in removing cholesterol from atherosclerotic plaque deposits in blood vessels, a process known as reverse cholesterol transport. HDL-C also possesses strong anti-inflammatory and antioxidant properties, which contribute to its protective effects against atherosclerosis (18, 55, 56). Low HDL-C levels have also been associated with the presence of other atherogenic risk factors, according to research (18).

While HDL-C levels were negatively associated with CV events, HDL-C was found to be positively correlated with hypertension, which is recognized as an impairing endothelial function factor. In a large sample-size study that included nearly 63,000 males in China, a positive association between HDL-C and hypertension after adjustment for BMI was observed (57).

Hypertension significantly influences the onset/progression of CVD and can alter the function and composition of HDL-C. However, the precise role of HDL-C in CV problems associated with hypertension has remained unclear. In rats with normal BP, HDL-C was found to protect against myocardial ischemia/reperfusion (I/R) damage. It was uncertain whether enhancing the composition or function of HDL-C in spontaneously hypertensive rats (SHR) would offer protection against myocardial I/R damage. A unique cardioprotective and anti-hypertensive action of HDL-C against myocardial I/R damage in SHR was revealed, and the magnitude of this protection was closely related to the levels of expression of cardiac scavenger receptor class B type-I (SR-BI). Continuous HDL-C therapy preserved the SHR hearts via the process of reduction of inflammation and autophagy (58).

The role of low-density lipoprotein cholesterol (LDL-C) in the pathogenesis of hypertension

LDL-C is a cholesterol which is transported by LDL lipoprotein particles throughout the body in extracellular fluids. It is widely recognized as a primary CVD risk factor and clinical evidence supports the effectiveness of lowering LDL-C levels in reducing atherosclerotic disease events (59). Both LDL-C and BP contribute to the risk of ischemic stroke and coronary artery disease (CAD). Tsukinoki et al. (60) conducted an epidemiological study that was the first to investigate the combined impact of LDL-C and BP on different subtypes of CVD in the population of Asia. Although no significant interaction between LDL-C and BP was found, the data revealed that the CAD risk connected with hypertension or prehypertension was higher in subjects with high LDL-C values compared to those with normal LDL-C values. Hence, it is crucial to control both BP and LDL-C in patients in Japan with hypertension, prehypertension, and high LDL-C values to prevent CAD at an early stage. Additionally, further comprehensive epidemiological research is needed to thoroughly examine the association between LDL-C, BP, and the prevalence of specific CVD subtypes in Asian populations (60). Furthermore, among dyslipidemia outpatients with hypertension in China, low rates of achieving their LDL-C and BP goals were observed, particularly in the departments of endocrinology. Combination therapy was not connected with increased rates of achieving target LDL-C and BP values (61).

It is of utmost importance to note that hypertension is a multifactorial condition influenced by various factors, including genetics, lifestyle, and other comorbid conditions. Dyslipidemia is just one of the many factors that can contribute to the onset or exacerbation of hypertension.

The effect of drugs used for dyslipidemia treatment on blood pressure

Effective management of arterial hypertension often involves addressing multiple risk factors and adopting a universal approach to cardiovascular health (61). Lifestyle changes that include a healthy diet, moderate physical activity, managing stress, and smoking cessation are important strategies for managing both hypertension and

dyslipidemia (62, 63). Additionally, medication may be prescribed when necessary to control lipid levels and BP, reducing the overall risk of CVD.

Statins are crucial medications for the treatment of dyslipidemia, i.e., for lowering LDL-C, due to their efficacy, low cost and safety. The mechanism of action of statins is related to an increase in the expression of LDL receptors at hepatocytes, with consequent LDL-C uptake. The beneficial effects of adding a statin to antihypertensive medications in patients with hypertension has also been confirmed, showing lower BP in these patients (64, 65). Moreover, the favorable properties of statins on BP can be indirectly attributed to their ability to reduce ROS and mediators of inflammation, thus improving endothelium function (65). Statins also downregulate endothelin-1 and angiotensin II-type one receptors and enhance nitric oxide (NO) bioavailability, thus inhibiting the proliferation of vascular smooth muscle cells, improving endothelial-dependent vasodilation and reducing the stiffness of large arteries (65).

Cholesteryl ester transfer protein (CETP) enables the transfer of TG from particles that contain apoB to HDL particles, as well as cholesteryl esters from HDL particles. CETP inhibitors have been shown to have more beneficial properties on LDL-C reduction than on HDL-C increase (66). Namely, the first generation of these drugs (i.e., dalcetrapib, torcetrapib) mostly exhibited off-target effects or increased HDL-C, whereas their second generation (i.e., evacetrapib, anacetrapib) exhibited beneficial properties in LDL-C reduction and were proven to be safe and effective in reducing CV risk. CETP inhibitors have also been shown to improve insulin sensitivity and glucose tolerance, and diminish the risk of new-onset diabetes (66). However, limited unfavorable effects on BP by CETP inhibitors were shown in the first generation of these drugs (67). Anacetrapib led to a mean increase of systolic BP of 0.7 mmHg (68), whereas a mean increase of systolic BP of 0.6 mmHg was shown with dalcetrapib (69). On the other hand, evacetrapib showed no effect on BP (70). Further studies are needed to confirm the properties of CETP inhibitors and clinical trials are currently ongoing (67).

Conclusion

The relationship between hypertension and dyslipidemia is influenced by various factors, including genetics, diet, physical activity, and lifestyle choices. Therefore, the development of these conditions is multifactorial and individual responses can vary. Lipid profile has a significant role in the pathogenesis of hypertension due to its influence on the development of atherosclerosis, which can lead to the narrowing and stiffening of arteries, which in turn can increase BP. This review article emphasized the importance of lipid panels, including TG, LDL-C, HDL-C and TC, in the development of arterial hypertension. Future studies are necessary to elucidate the exact mechanisms by which these lipid profiles contribute to the development of arterial hypertension. It is suggested that managing lipid profiles through lifestyle modifications (e.g., diet and exercise) and medications (when necessary) can help to reduce the risk of developing hypertension and its complications. Monitoring lipid levels is an important aspect of CV assessment and management, as it can provide valuable insights into an individual's overall risk of hypertension and related CVDs.

Conflicts of Interest

The authors declare no conflict of interest.

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Authors' Contributions

All authors contributed to the conception and design of this study. The first draft of the manuscript was written by Saira Rafaqat. Data collection was performed by all three authors. A. Klisić critically revised the manuscript. All authors have read and approved the final version of the manuscript.

Availability of Data

Not applicable.

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Uloga lipida u serumu u patogenezi arterijske hipertenzije

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Kratak sadržaj

Hipertenzija je glavni doprinoseći faktor u pojavi kardiovaskularnog morbiditeta i mortaliteta, zahvaljujući porastu prevalence ovog poremećaja. U kliničkoj praksi, dislipidemija i hipertenzija su često udružena stanja, vjerovatno zahvaljujući zajedničkim patofiziološkim karakteristikama, tj. disfunkciji endotela i gojaznosti. Shodno navedenom, ovaj revijski članak predstavlja zbirni prikaz uloge lipidnog profila u arterijskoj hipertenziji. Osobe sa arterijskom hipertenzijom često imaju više vrednosti ukupnog holesterola i triglicerida, više vrednosti koncentracije holesterola niske gustine, a niže vrednosti koncentracije holesterola velike gustine, u poređenju sa osobama koje nemaju hipertenziju. Ukupni holesterol i koncentracija holesterola niske gustine igraju značajnu ulogu u patogenezi arterijske hipertenzije, ali je nedovoljno studija koje ispituju povezanost triglicerida i koncentracije holesterola velike gustine u patogenezi arterijske hipertenzije. Buduće studije su potrebne kako bi rasvijetlile ulogu dislipidemije u hipertenziji.

Ključne reči: arterijska hipertenzija, lipidni profil, ateroskleroza

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Comparative spectrophotometric determination of 3-hydroxyflavone based on zinc and aluminium complexes and their antioxidative profiles

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Abstract

Flavonoids, as plant-derived compounds, were essential active components in traditional medicine for centuries. Their potential or confirmed effects include antiviral, antimutagenic, anti-inflammatory, antibacterial, vasodilatory, and anticancer properties. The promotion of a plant-based diet, along with the benefits of consuming flavonoids, has recently become increasingly attractive. 3-Hydroxyflavone (3HF) is the structural spine of flavonols, an important subgroup of flavonoids. Although 3HF itself does not exist in plants *per se*, it exerts many of its effects because of its characteristics that allow it to prevent free radical generation. This work is focused on the characterization of 3HF complexes with zinc(II) and aluminium(III) ions (Zn-3HF and Al-3HF, respectively). Besides this, a simple, fast, and low-priced spectrophotometric method for 3HF determination, with very low LOD and LOQ, based on Zn-3HF and Al-3HF formation, was established. A slight advantage is given to the modification with Al^{3+} ion on pH 4.91, due to very low LOD and LOQ values of 1.83×10^{-7} $molL^{-1}$, and 5.50×10^{-7} $molL^{-1}$, respectively, and a high correlation coefficient, R =0.99986. Furthermore, the antioxidant ability of Zn-3HF, Al-3HF, and parent 3HF was examined by the ABTS and DPPH tests. They brought the Zn-3HF complex to the fore as a potential antioxidative agent.

Key words: spectrophotometry, 3-hydroxyflavone, zinc complex, aluminium complex, antioxidative capacity

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Introduction

1. Flavonoids

Herbal drugs are the oldest form of medicines and are used to treat many diseases. This kind of application is possible because of their active ingredients. For some herbal drugs, the chemical nature of the active ingredients is unknown; however, for a large number of compounds isolated from plants, the structure has been defined, and their action has been confirmed. Pharmacologically active ingredients of plants are classified as secondary plant metabolites. One of those ingredients are flavonoids (1).

Flavonoids are extensively spread in plants. So far, more than 8,000 flavonoids have been isolated from plants, and this number is still growing. As the structure of flavonoids became more complex and diverse with the evolution of plants, their role in plants became increasingly important (2). They represent the most important plant pigments; if they are not colored by themselves, they appear as co-pigments. Thus, colorless flavones and flavonols complement the color of anthocyanins. Due to the characteristic absorption spectrum, they help plants attract insects. As integral parts of plant enzyme systems, they are responsible for developing metabolic processes. In addition, they prevent infections of plant tissue by bacteria, yeasts, or viruses (phytoalexin function), and protect plants from excessive UV radiation (1).

Besides great importance for plants, flavonoids are significant for animals and humans. The effects they exhibit are primarily based on their antioxidant action, and it has been unequivocally confirmed that they have antiviral, antimutagenic, anti-inflammatory, antibacterial, vasodilatory, and anticancer effects (3). The pronounced ability of flavonoids to complex with ions of transition metals is one of the mechanisms that allow the accumulation of metals in peripheral tissues, which reduces their harmful effect and enhances the defense mechanism against herbivores and pathogenic organisms. This ability was employed for the quantification of flavonoids in various samples, as well as for the detection of trace metals (4).

2. 3-Hydroxyflavone

3-Hydroxyflavone (3HF) is a chemical compound representing the main structure of flavonols, a widely spread subgroup of flavonoids (Figure 1). However, although this compound itself is not found naturally in plants, 3HF can be practical as a model molecule because of its excited-state intramolecular proton transfer (ESIPT) effect (5), in membrane (6) or intermembrane proteins (7) studies. Generally, 3HF derivates prevent the production of free radicals and can therefore be helpful as antioxidant and protective molecule substances (8).

Therefore, the necessity of syntheses of 3HFs in a shorter time period and as high as possible yields has resulted in many innovative methods. The one proposed by Gonduz et al. (9) considers a simple purification and provides the syntheses of 3HFs with a hydroxyl group on the phenyl ring in just one step, which is a significant improvement compared to the current four steps available in the literature, with longer reaction time and a lower yield.

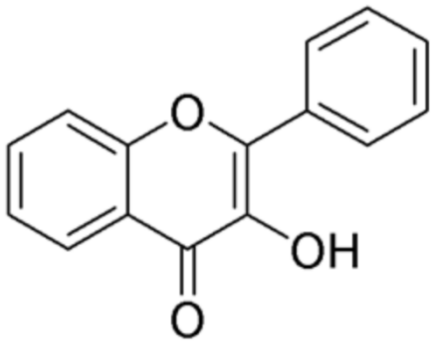

Figure 1. 3-Hydroxyflavone
Slika 1. 3-hidroksiflavon

3-Hydroxyflavone is soluble in methanol and ethanol, while its insolubility in water can be improved by encapsulation in cyclodextrins (4). One of the main characteristics of almost all flavonoids is the formation of complexes with metal ions, due to the existence and appropriate layout of one or more ortho hydroxyl phenolic groups, or phenolic groups with a carbonyl group. Since they possess an α-hydroxycarbonyl group, flavonols exhibit a very high affinity to bind metal ions, especially compared to other flavonoids (Figure 2) (10, 11).

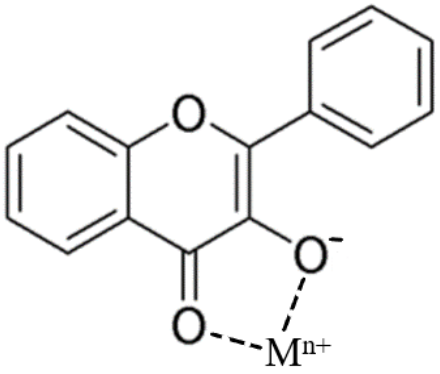

Figure 2. 3-Hydroxyflavone binding site
Slika 2. Mesto vezivanja 3-hidroksiflavona sa metalom M^{n+}

Many studies have examined the effects of 3-hydroxyflavone and its structural analogs. One such study dealt with the activation of the pregnane H receptor (PHR), which belongs to a group of nuclear receptors that regulate the expression of genes for many biological processes. It has been shown that 3HF activates this receptor, which may be helpful in the treatment of diseases that depend on the PHR receptor, such as inflammatory bowel disease (12).

It has also been shown that 3HF inhibits the metastasis of human osteosarcoma cells and reduces tumor growth *in vivo*. This may lead to a clinical trial of osteosarcoma chemotherapy (13).

Like other flavonoids, 3-hydroxyflavone also exhibits antioxidative characteristics. This molecule complexes with metal ions that can induce oxidative stress and is known to suppress the cytotoxicity of lipid peroxides (especially hydroperoxide of linoleic acid) (14).

Adverse effects of 3-hydroxyflavones are manifested when this molecular species is present in increased concentration, which can cause pro-oxidative action, manifested by an increase in the intracellular concentration of free oxygen radicals (ROS) and possible cell damage (15).

Although very similar, the study by Sengupta et al. (16) presented the antioxidant properties of two isomers, 3-hydroxyflavone (3HF)- and 7-hydroxyflavone (7HF), against nicotine-associated oxidative stress and injury in cultured renal proximal tubule cells, and correlated their antioxidant properties with their chemical structure. The data elucidated that although both 3HF and 7HF protect renal cells from NIC-associated cytotoxicity, the mechanism of their action is different (16).

Some very inventive applications of 3HF-based compounds are related to carbon monoxide (CO) as an endogenous signaling molecule that influences various biological processes. The therapeutic potential of CO is hindered by its intrinsic toxicity, and its administration therefore carries a possible risk. Photoactivatable CO-releasing molecules (photoCORMs) are an excellent tool to overcome the side effects of untargeted CO administration and provide precise release control. Thus, the study of Russo et al. (17) reported the CO release mechanism of several flavonol derivatives, previously developed as an efficient photoCORM. The study aimed to examine how to enhance the efficiency of CO photorelease from flavonols, and how to minimize photochemical side-reactions, such as self-photooxygenation. Moreover, the study reported the toxicity of tested flavanols on hepatic HepG2 cells *in vitro* as a significant fact for future possible application.

Intending to find a more comprehensive application of 3HF, researchers have focused on its complexes with metal ions. The syntheses of 3-hydroxyflavone complexes with aluminum and zinc are already reported in the literature (18, 19). In this paper, besides their characterization, the validation of a developed spectrophotometric determination of 3HF based on complexes formed with zinc (Zn-3HF) and aluminum (Al-3HF) is presented. Furthermore, their antioxidant capacity is tested, and more positive issues of 3HF complexes of both tested ions are evaluated.

Experimental

1. Materials and Instruments

3-Hydroxyflavone, KCl, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) trolox, DPPH (2,2-diphenyl-2-picrylhydrazyl), (Sigma-Aldrich), methanol, acetic acid, CH_3COONa, $AlCl_3$, $ZnCl_2$, (Merck), all p.a. purity grade, were used.

The stock solutions of $ZnCl_2$ (1×10^{-3} $molL^{-1}$) and $AlCl_3$ (1×10^{-4} mol L^{-1}) were prepared by dissolving $ZnCl_2$ and $AlCl_3$ in redistilled water. The stock solution (1×10^{-4} mol L^{-1}) of 3HF was prepared by dissolving the appropriate mass in 70% methanol (V/V), and after sonication for 15 minutes, it was stored in a refrigerator.

Acetate buffers (in 70% methanol V/V) with different pH values, previously prepared according to Perrin et al. (20), were used for appropriate spectrophotometric measurements.

Spectrophotometric absorption spectra recording was performed by a *Beckman DU 650 Spectrophotometer* with 1 cm quartz cells. pH-metric measurements were performed by the Thermo Scientific-Orionca with a combined glass electrode (sensitivity ± 0.01 pH).

2. Methods

2.1. Spectrophotometric determination of 3HF

The study of complex formation between 3HF and metal-ions started by testing the dependence of absorbance intensity on pH in acetate buffers (in 70% methanol (V/V)) of different pH values (20). To ensure that quantitative complex formation was achieved, the procedure considered the concentration of metal ions in the mixture was 1 x 10^{-6} mol L^{-1}, while the concentration of 3-hydroxyflavone was twenty times higher, 2 x 10^{-5} mol L^{-1}.

For calculating the stability constant of the complex, β_2, modified Bjerrum's method was used (21).

The stoichiometry of the complexation reaction for both complexes was investigated using the equimolar solution variation method (22). Considering the overall equilibrium of metal ion M^{m+} (Zn^{2+}or Al^{3+}) and *n* ligands (L= 3HF), presented as M^{m+} + nL = $[ML_n]^{m+}$, where *n* can be determined from the plot of the absorbance as a function of the mole fraction, *x*, of the added ligand, in the maximum, *n* is

$$n = \frac{x_{max}}{1-x_{max}}. \quad (1)$$

The calibration curve method was used for spectrophotometric determination of 3HF, requiring prepared solutions containing a constant concentration of $ZnCl_2$ or $AlCl_3$ and different concentrations of 3HF in acetate buffer (in 70% methanol (V/V)) at an appropriate pH (Zn-3HF at 7.99 and Al-3HF at 4.90). The series of seven standard solutions in the range $1 \times 10^{-6} – 2 \times 10^{-5}$ mol L^{-1}, in the presence of the excess of Zn(II) ion or Al(III) ion, 3×10^{-5} mol L^{-1}, were prepared. The blank was acetate buffer in 70% methanol (V/V) 7.99/4.90.

The obtained data were used to calculate analytical validation parameters for spectrophotometric methods for both complexes according to relevant literature (23, 24).

The limit of detection (LOD) was calculated from the equation:

$$\text{LOD} = 3.3\ Sb/a \quad (2)$$

where: *Sb* – standard deviation in intercept; *a* – slope of the calibration line, while the following relation was used to calculate the limit of quantification (LOQ):

$$\text{LOQ} = 10\ Sb/a \quad (3)$$

2.2. Antioxidative tests

Experimental measures and conditions for testing the antioxidative ability of 3HF and corresponding zinc and aluminium complexes were performed according to the procedures for the DPPH tests previously reported for chosen flavonoids (25-27), with the solutions of 3HF at a concentration of 5×10^{-6} mol L^{-1} and concentrations of Zn^{2+} and Al^{3+} - ion c = 2.5×10^{-6} mol L^{-1}.

Furthermore, 3HF and its complexes were tested for scavenging free radicals potential by the stable radical reagent 1,1-diphenyl-2-picrylhydrazyl, DPPH. The hydrogen atom or electron donation abilities of the tested complexes and pure 3HF were estimated based on the bleaching of the methanol solution of DPPH, according to previously reported procedures (25, 26). In brief, 1 mL of the tested samples was added to 4 mL of 0.004 % methanol solution of DPPH. After a 30-minute incubation period at room temperature, protected from light, the absorbance was read against a blank at 517 nm. The inhibition of free radicals by DPPH in percentages (% INH) was calculated from the equation (4):

$$INH = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100\% \quad (4)$$

In the formula (4), $A_{control}$ is the absorbance of the solution containing all reagents except the test compound, while A_{sample} is the absorbance of the prepared tested complex or 3HF.

The samples were also checked for their ability to scavenge ABTS free radicals ($ABTS^{\cdot+}$). The stock solution of $ABTS^{\cdot+}$ was prepared as proposed in the literature (28). In brief, according to Pavun (27), the test sample of 3HF was dissolved in 5 mL of $ABTS^{\cdot+}$ solution, mixed thoroughly, diluted to 10 mL with PBS buffer, and incubated in the dark at room temperature. After 3 minutes, the absorbance was read at 734 nm with a PBS buffer as blank. The results were expressed as % INH (percentage of $ABTS^{\cdot+}$ inhibition) according to the equation:

$$\%\, INH = \frac{[A_{ABTS^{\bullet+}} - A_{sample}]}{A_{ABTS^{\bullet+}}} \times 100 \quad (5)$$

where $A_{ABTS^{\cdot+}}$ is the absorbance of $ABTS^{\cdot+}$ solution + 5 mL of PBS buffer, and A_{sample} is the absorbance of the solution in the presence of the sample after 3 min of reaction.

Results

1. Complex formation between 3HF and metal-ion

The dependence of absorbance of the metal-ions complexes of 3HF intensity on pH was examined. Figure 3 represents the absorbance as a function of the pH solution, where a strong pH dependence can be noticed. The maximum absorbance of the Zn–3HF complex is λmax = 400 nm, at pH 7.85 (Figure 3a). The maximum absorbance of the Al–3HF complex is λmax = 343 nm, at pH 4.91 (Figure 3b). The solutions of $ZnCl_2$ and $AlCl_3$ do not exhibit significant absorbance in the range of 250–500 nm.

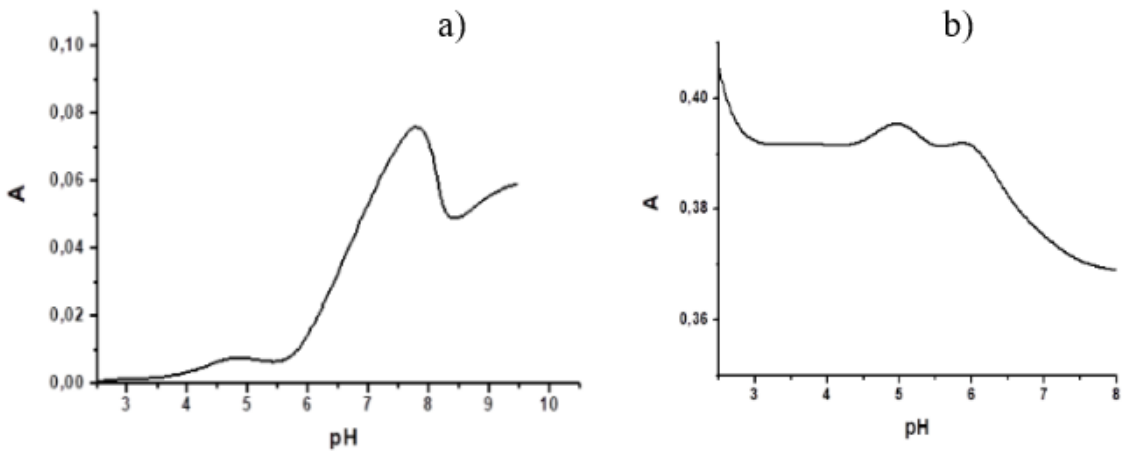

Figure 3. pH dependence of absorbance of Zn-3HF (a), and Al-3HF (b)
Slika 3. pH zavisnost apsorbancije Zn-3HF (a), i Al-3HF (b)

Using an equimolar solution variation, it was determined that 3HF makes a complex with Zn(II) ion at pH 7.98, in the stoichiometric ratio 3HF : Zn^{2+} ion = 2 : 1, with an absorption maximum on λmax = 400 nm (Figure 4a). Al-3HF complex was formed in the stoichiometric relation 3HF : Al^{3+} ion = 2 : 1 as well, but at pH 4.91, with an absorption maximum on λmax = 343 nm (Figure 4b). The compositions of these complexes were also checked by the mole ratio method, confirming the 3HF and M^{m+} (Zn^{2+}or Al^{3+}) ratio is 2 : 1 for both complexes formed at an appropriate pH against 70% methanol (V/V) as a blank.

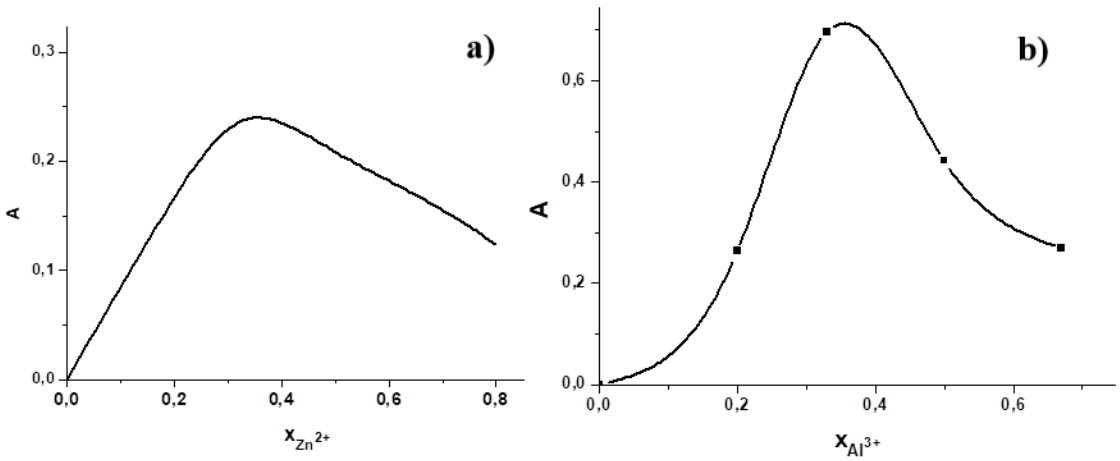

Figure 4. Determination of the 3HF complexes composition by equimolar solutions method: (a) Zn–3HF, and (b) Al–3HF
Slika 4. Određivanje sastava 3HF kompleksa metodom ekvimolarnih odnosa: (a) Zn–3HF, i (b) Al–3HF

2. Spectrophotometric method development

The high values of the stability constants of complexes Zn-3HF ($\log \beta_2 = 14.35$ at pH =7.98) and Al–3HF ($\log \beta_2 = 22.63$ at pH =4.91) allow the quantification of 3HF established on these complexes. The construction of the calibration curve, $A=f(c_{3HF})$ was the same for both metal ions.

For the Zn-3HF complex, the series of solutions were prepared at pH 7.98 in 70% methanol (V/V), and the absorbance was measured at λ= 400 nm.

Good accuracy and reproducibility of the method are reflected in a high correlation coefficient R= 0.9892, with LOD and LOQ calculated as 3.82×10^{-7} mol L^{-1} and 1.15×10^{-6} mol L^{-1}.

In the case of the Al-3HF complex, the solutions were prepared with 70% methanol (V/V), with pH 4.91, while the absorbance was measured at λ= 343 nm. Good linearity of the calibration curve and small scatter of experimental points resulted in a higher correlation coefficient, R =0.99986. The LOD was 1.83×10^{-7} mol L^{-1}, while the LOQ was 5.50×10^{-7} mol L^{-1}.

The accuracy of both developed variations was tested for three different concentrations of 3HF in the range of 2-10 μmol L^{-1}, with five repeated measurements for each. The accuracy and repeatability of the method are reflected in very good recovery and a low coefficient of variation (CV). The obtained analytical parameters are presented in Table I.

Table I Analytical parameters for spectrophotometric determination of 3HF

Tabela I Analitički parametri za spektrofotometrijsko određivanje 3HF

	Method based on Zn-3HF	**Method based on Al-3HF**
pH	7.98	4.91
Wavelength	400 nm	343 nm
Stability	$\log \beta_2 = 14.35$ at pH =7.98	$\log \beta_2 = 22.71$ at pH =4.90
Molar absorption coefficient	3.04×10^4 cm^{-1} mol^{-1} dm^3	3.94×10^5 cm^{-1} mol^{-1} dm^3
Regression equation	$A = (4.32 \pm 0.05) \times10^3 \cdot c - (0.0022 \pm 0.0005)$	$A = (1.82 \pm 0.01) \times10^4 \cdot c + (0.005 \pm 0.001)$
Linearity range	1×10^{-6} mol L^{-1} - 2×10^{-5} mol L^{-1}	1×10^{-6} mol L^{-1} - 2×10^{-5} mol L^{-1}
LOD	3.82×10^{-7} mol L^{-1}	1.83×10^{-7} mol L^{-1}
LOQ	1.15×10^{-6} mol L^{-1}	5.50×10^{-7} mol L^{-1}
Recovery	100.63 %	100.17 %
CV	0.27-0.93 %	0.25-1.00 %

According to the presented data, it can be concluded that a certain advantage can be given to the variation of the spectrophotometric quantification of 3HF developed on its reaction with aluminium. The resulting Al-3HF complex is more stable than Zn-3HF, so the LOD and LOQ values are expected to be lower. In addition, an advantage of this determination is that it is performed in an acidic medium in contrast to a slight base, which is always favored in real samples.

Interestingly, the Zn-3HF complex was previously employed for the spectrofluorimetric determination of zinc traces in tap water, multivitamin tablets/capsules, and hair shampoo, with LOD for Zn^{2+} at 1.5 ppb (29). Herein we used the same complex for 3HF determination, applying simple and low-cost spectrophotometry, and obtained remarkably low values of LOD for 3HF.

As previously shown (25-27), the selectivity of the developed spectrophotometric method concerning the presence of some other flavonoids, including morin, hesperidin, quercetin, or rutin, is ensured by the choice of pH and the wavelength at which the recording is performed.

3. Antioxidative ability

As it is well known, numerous tests can be used to define the antioxidative ability of pure compounds or naturally sourced extracts (30). Which one will be applied depends on many external factors such as pH, solvent, and system characteristics. There is therefore no universal method or universal parameter based on which all compounds could be absolutely compared; it depends on the reaction type being tested and on the mechanism, but this can be overcome by combining several tests.

The spectrophotometric methods used in this study are fast, easy, and widely available, but with somewhat conflicting results due to the influence of some of the factors mentioned above. The results of performed antioxidative tests for 3HF and its complexes are represented in Table II.

Table II Antioxidative activity of 3HF, Zn-3HF and Al-3HF

Tabela II Antioksidativna aktivnost 3HF, Zn-3HF i Al-3HF

Sample	% INH DPPH (30 min)	% INH ABTS (3 min)
3HF	58.9	67.6
Zn-3HF	70.2	77.1
Al-3HF	55.3	59.4

Both applied tests showed that the complexation of 3HF with Zn^{2+} significantly enhances the antioxidant ability. As reported in our previous works, the complexation with Zn^{2+} does not necessarily positively contribute to the flavonoids' antioxidative potential (24-27). ABTS and FRAP tests performed for the complexes of quercetin, morin

and rutin with zinc exhibited about the same or slightly higher antioxidative capacities than pristine flavonoids. In contrast, in the DPPH test there was an increase in activity when zinc was binding with hesperidin and quercetin. None of the three tests recorded the antioxidative activity of the solution of Zn^{2+} itself.

As for the Al-3HF complex, the antioxidant activity (DPPH assay) was slightly lower, but still not significantly different compared to 3HF. The fact that zinc electrode potential is less positive than aluminium might explain such a finding. The results of the ABTS assay showed that the Al-3HF complex is less active than pristine 3HF. This finding agrees with the literature data reported for the complex of Al^{3+} with luteolin (31).

Another direction for future research could be based on the already established effect of 3HF as a safe chelating reagent of cobalt ions, but with low stability of the formed complex (Co-3HF) over time (32). 3HF is not considered a toxic compound (3HF alone caused a significant toxic effect on cells at high concentrations of 1×10^{-3} mol L^{-1}). The stability constants of Zn-3HF, and especially Al-3HF, are considerably higher than those of Co-3HF (32). It can be expected that 3HF can be a candidate for future testing as a chelating reagent for zinc or aluminium, especially having in mind the toxicity of aluminium ions, giving this study additional importance.

Conclusion

Because 3-hydroxyflavone forms stable complexes with Zn^{2+} and Al^{3+} ions, very simple, fast, low-cost spectrophotometric methods for determining 3HF have been developed and validated. The advantage of this eco-friendly method, in addition to its availability, time, and price performance, is reflected in the fact that no toxic solvents are used for the mobile phase, as they often are for HPLC.

A slight advantage could be given to the version with an Al^{3+} ion due to very low LOD and LOQ values and acidic working pH.

At the same time, the significant antioxidant capacity of the Zn^{2+} ion complex of 3HF, confirmed by DPPH and ABTS tests, makes this complex a promising candidate for future investigations in this field. In any case, further experiments for stability and efficiency evaluation of the respective complexes in different environments must not be forgotten.

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Uporedno spektrofotometrijsko određivanje 3-hidroksiflavona bazirano na kompleksima cinka i aluminijuma i njihovi antioksidatni profili

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Kratak sadržaj

Flavonoidi, jedinjenja biljnog porekla, vekovima su bili veoma važne aktivne komponente u tradicionalnoj medicini. Veliki broj njihovih potencijalnih ili već potvrđenih efekata uključuje antivirusna, antimutagena, antiinflamatorna, antibakterijska, vazodilatatorna i antikancerogena svojstva. Promovisanje biljne ishrane, uz isticanje koristi konzumiranja flavonoida, u današnje vreme postalo je sve privlačnije. 3-Hidroksiflavon (3HF) je strukturni stub svih flavonola, važne klase flavonoida. Iako sam 3HF ne postoji u biljkama *per se*, on ispoljava mnoge svoje efekte zahvaljujući osobini da sprečava stvaranja slobodnih radikala. Ovaj rad je fokusiran na karakterizaciju kompleksa 3HF sa jonima cinka(II) i aluminijuma(III) (Zn-3HF i Al-3HF, respektivno). Izvršena je karakterizacija ovih kompleksa i razvijena brza i pristupačna metoda za spektrofotometrijsko određivanje 3HF, na osnovu formiranja kompleksa Zn-3HF i Al-3HF, sa veoma niskim vrednostima LOD i LOQ. Mala prednost je data modifikaciji sa Al^{3+} na pH 4,91 zbog izuzetno niskih vrednosti LOD i LOQ, $1{,}83\times10^{-7}$ mol L^{-1}, odnosno $5{,}50\times10^{-7}$ mol L^{-1}, kao i visokog koeficijenta korelacije, R=0,99986. Pored toga, antioksidativni kapaciteti sintetizovanih kompleksa Zn-3HF i Al-3HF, kao i samog 3HF, ispitani su DPPH i ABTS testovima i doveli su Zn-3HF kompleks u prvi plan za dalja ispitivanja kao potencijalnog antioksidativnog agensa.

Ključne reči: spektrofotometrija, 3-hidroksiflavon, cink kompleks, aluminijum kompleks, antioksidativni kapacitet