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Review article

The Modern Concept of Etiopathogenesis and Diagnosis of Shock

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SUMMARY

Introduction/Aim. Shock is a life-threatening condition that occurs due to a mismatch in the supply and consumption of oxygen, which leads to cell and tissue hypoxia, resulting in cell death and dysfunction of vital organs. The effects of shock are reversible in the early stages, but delay in diagnosis and initiation of treatment can lead to irreversible changes. There are four main categories of shock: hypovolemic, distributive, cardiogenic, and obstructive. The aim of the paper is to present a new perception of viewing the etiopathogenesis and effectively establish the diagnosis of shock.
Etiology. Hypovolemic shock can occur due to hemorrhagic and non-hemorrhagic causes. Distributive shock is divided into septic, systemic inflammatory response syndrome (SIRS), anaphylactic, neurogenic, and endocrine. Cardiogenic shock occurs due to intracardiac causes, while obstructive shock occurs due to extracardiac causes.
Pathogenesis. The pathogenesis of each type of shock is different depending on the etiology. Generally speaking, shock has three phases: compensated, cellular distress phase, and decompensated. When the shock progresses into an irreversible phase, it usually ends with multiorgan failure (MODS) and death.
Clinical presentation. Symptoms may vary depending on the type and stage of shock. The most important changes during this syndrome are at the level of hemodynamics, so the most common clinical signs are hypotension, tachycardia, tachypnea, disturbed mental status, cold extremities, and oliguria.
Diagnosis. The diagnosis of shock is based on history, clinical presentation, physical examination, vital parameters and biochemical analyses, SOFA criteria (sequential organ failure assessment score), acid-base status, diuresis measurement, etc.
Conclusion. Understanding the etiopathogenesis of shock and recognizing its early signs are vital for timely interventions that lead to improved patient outcomes.

Keywords: **shock, hemodynamic disorder, sepsis, etiopathogenesis**

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INTRODUCTION

Shock is a condition manifested as circulatory failure that can lead to life-threatening outcomes (1). Shock is a state of hypoxia in cells, which can cause improper functioning of tissue and organs. Shock can lead to multiorgan failure (MODS) and death, but if timely diagnosis is made, it can be treated with positive outcome (2).

In the beginning, the emphasis was mainly on traumatic hemorrhagic shock, however, shock is now considered a life-threatening condition that occurs due to a mismatch in supply and consumption of oxygen, given that this is a main characteristic of all types of shock. Later, it was determined that various etiopathogenetic factors can progress to this condition. These factors, as well as different therapeutic measures, have led to a new classification of shock that contains four main categories:

- hypovolemic,
- distributive,
- cardiogenic,
- obstructive (3).

EPIDEMIOLOGY OF SHOCK

The three most common types of shock are, in order of frequency, distributive, hypovolemic, and cardiogenic shock. The fourth, the obstructive type, is somewhat rare. Septic shock, a type of distributive shock, is the most common type of all shocks, and carries a mortality rate between 40% and 50% (4). This is shown in Table 1.

Table 1. *Relative frequency of different types of shock*

Shock type	Relative incidence
Hypovolemic	16%
Distributive/Septic	64%/55%
Cardiogenic	15%
Obstructive	2%

ETIOLOGY OF SHOCK

Shock represents one of the most difficult clinical syndromes with a complex list of causes and is potentially fatal without adequate diagnosis and treatment. Numerous etiological factors can contribute to each of the four categories of shock.

Hypovolemic shock is divided in two broad subtypes: hemorrhagic and non-hemorrhagic.

Causes of hemorrhagic shock include:

- traumas and polytraumas (external and internal bleeding due to tissue, organ or blood vessel injuries);
- gastrointestinal bleeding (variceal bleeding, portal hypertensive bleeding, peptic ulcer, diverticulosis and many others);
- vascular etiology (aortoenteric fistula, abdominal aortic aneurysm rupture, tumor eroding into the main blood vessel, etc.);
- bleeding due to inadequate use of drugs (anticoagulants).

Causes of non-hemorrhagic shock include:

- gastrointestinal—vomiting, diarrhea;
- loss of the third space—pancreatitis, cirrhosis, intestinal obstruction;
- renal—endocrine disorders (hypoaldosteronism, diabetes), drug-induced diuresis;
- skin—Stevens-Johnson syndrome, burns, heat stroke, pyrexia (1, 5).

Distributive shock is divided into: septic, systemic inflammatory response syndrome (SIRS), anaphylactic, neurogenic, and endocrine (3).

The 2009 European Prevalence of Infections in Intensive Care Study (EPIC II study) found that Gram-negative bacterial infections (62%), followed by Gram-positive infections (47%), far exceeded other pathogens as the primary cause of sepsis syndrome. The increased prevalence of Gram-positive infections can be attributed to the increased frequency of invasive procedures and hospital-acquired infections. Among the most frequent pathogens are *E. coli, K. pneumoniae, Haemophillus spp, S. pneumoniae, S. pyogenes, S. aureus, N. meningitidis, Pseudomonas spp*, anaerobes (6).

Risk factors for developing sepsis are: malignancy, prolonged hospitalization, liver cirrhosis, immunosuppressive conditions, diabetes, major operations, burns, use of corticosteroids, trauma, the presence of permanent catheters, age, and hemodialysis.

SIRS is a condition of an excessive inflammatory reaction that can be triggered by bacteria, fungi, viruses, parasites, burns, pancreatitis, fat or air embolism, etc (7).

Anaphylactic shock is a condition characterized by a hypersensitivity response which is interfered by immunoglobulin E (IgE). Bronchospasm and cardiovascular collapse are the most severe con-

sequences. Allergens that can cause this are food, drugs (e.g., antibiotics, and NSAIDs), insect stings, etc. (8).

Neurogenic shock occurs in case of trauma to the spinal cord or brain. It includes damage to the autonomic nervous system and vagal tone (9).

Endocrine shock can be present in adrenal insufficiency and myxedema.

Cardiogenic shock occurs due to inadequate functioning of the heart. It leads to reduced cardiac output and hypoperfusion. Various causes contribute to this shock such as:

- cardiomyopathies—fulminant dilated cardiomyopathy, acute myocardial infarction, cardiac arrest, myocarditis;
- mechanical—mitral insufficiency, aortic insufficiency, rupture of papillary muscles, chordae tendineae or aneurysm of the ventricle;
- arrhythmias—tachy- and brady-arrhythmias (10, 11).

Obstructive shock mainly occurs due to extracardiac causes that leads to inadequate minute volume:

- pulmonary vascular—pulmonary hypertension, pulmonary embolism,
- mechanical—pericardial tamponade, tension pneumothorax, restrictive cardiomyopathy (12, 13).

PATHOGENESIS OF SHOCK

The main reason for the occurrence of this condition is hypoxia in the cells, which switches from aerobic to anaerobic metabolism, creating an increase in lactate in the blood. This leads to increased acidosis that reduces organ perfusion, which further leads to tissue hypoxia causing cell death and MODS (14).

Hypovolemic shock is a state where the loss of intravascular lumen causes inadequate organ perfusion. Cardiogenic shock is characterized by a reduction in the pumping capacity of the heart leading to reduced ejection or aggravated filling of the ventricles with blood (15). Obstructive shock appears when there is an obstruction of large blood vessels or the heart itself, which results in an increase in right ventricular afterload and a decrease in left ventricular preload. In these three types of shock, there is a decrease in cardiac output that prevents adequate oxygen transport. In distributive shock, there is decreased peripheral vascular resistance due to immune response and bacterial toxins, which leads to inadequate oxygen extraction.

Generally speaking, shock has the following three phases:

- pre-shock or compensated shock is reflected in the response to hypoxia causing peripheral vasoconstriction, tachycardia and changes in systemic blood pressure;
- shock-the most typical symptoms of shock are caused by early organ dysfunction, which is the result of the progression of the previous phase, as compensation mechanisms prove to be insufficient;
- irreversible organ dysfunction—the final phase that leads to multiorgan failure and death (16).

Compensated phase. Initially, when oxygen supply and arterial pressure are reduced, an adrenergic response is triggered by sympathetic vasoconstriction of most blood vessels, but primarily of the precapillary sphincter, excluding a large part of tissue from the supply. At first, blood flow is being diverted to the heart and brain and perfusion of less important organs is reduced. Beta-adrenergic amines increase cardiac contractility and initiate the release of corticosteroids, renin, and glucose. Increased glucose due to lack of oxygen in the cells causes further production of lactate, while renin-angiotensin-aldosterone system and antidiuretic hormone are the cause of fluid conservation (17).

Cellular distress phase. It is characterized by the formation of lactate in the cells due to the lack of oxygen, which results in the loss of ATP and countering the effects of catecholamines by creating vasodilation. Constriction of the postcapillary sphincter and inclusion of AV shunts occurs. A decrease in cardiac output causes compensatory tachycardia, however, when energy reserves are used up, heart failure occurs with an additional decrease in cardiac output and stroke volume (18).

Decompensation phase. At the level of microcirculation, leukocytes and endothelium interact and destroy proteoglycans and glycosaminoglycans attached to the endothelial membrane, causing microvascular dysfunction with capillary leakage syndrome and vasodilatation of the precapillary sphincter (19). At the cellular level, mitochondrial damage occurs with consequences on blood vessels (20). Neurohumoral mediators are consumed, and hypoxic tissues hardly create new mediators, while adrenergic receptors become insensitive due to down-regulation (21).

A combination of all the listed factors can lead to progressive dysfunction of two or more organs or life-threatening damage called multiorgan dysfunction syndrome (MODS). MODS is characteristic of every shock in later stages, but it is most likely in septic shock (22).

During septic shock, inflammatory and coagulation cascades are activated in areas of hypoperfusion. These areas activate the immune system and release harmful substances (reactive oxygen, proteolytic enzymes), as inflammatory mediators (cytokines, leukotrienes, tumor necrosis factor). All this triggers a cascade reaction that results in the production of a strong vasodilatator agent (nitric oxide) (23).

In septic shock, vasodilation of blood vessels leads to hypotension. Despite normal blood pressure and cardiac function, localized vasodilation can cause focal cellular hypoxia. In addition, excess nitric oxide is converted to free radicals, such as peroxynitrite. These free radicals can damage mitochondria and reduce ATP (adenosine triphosphate) production. All this can significantly increase microvascular permeability, allowing fluid and sometimes plasma proteins to end up in the interstitial space. In the gastrointestinal tract, this can translocate enteric bacteria, which can lead to metastatic infections (23).

The main process is endothelial dysfunction that can cause vasodilation and disturbance in the macro- and microcirculation, leading to an increase in vascular permeability (24).

Bacterial toxins lead to hemolysis of erythrocytes and accumulation of hemoglobin, which, along with damaged tubular epithelium, leads to acute renal failure. Alveocapillary membrane damage and non-cardiogenic pulmonary edema also occur. All the mentioned mechanisms lead to MODS.

Lungs are mostly highly affected, where elevated membrane permeability causes alveolar infiltration and inflammation. Due to the progression of hypoxia, acute lung injury (ALI) can occur, and if the progression continues, it can lead to acute respiratory distress syndrome (ARDS). Renal hypoperfusion may lead to acute tubular necrosis. Typical signs of renal failure are oliguria, anuria, and an increase in nitrogenous products (23).

Coronary hypoperfusion together with mediators (tumor necrosis factor and interleukin-1) can decrease contractility, which reduces cardiac output, further worsening of myocardial perfusion and arrhythmias can occur, causing a vicious cycle that often culminates in death (15). Due to hypoperfusion in the gastrointestinal tract, ileus and bleeding may be manifested. Hepatocellular necrosis can be developed along with elevation of transaminases and decreased production of coagulation factors (25). All this can lead to disseminated intravascular coagulopathy (DIC) (26).

CLINICAL PRESENTATION OF SHOCK

Clinical presentation may be various depending on the severity of the disease. Changes that occur during this syndrome are at the level of hemodynamics, so the most common clinical signs indicating shock are hypotension, tachycardia, tachypnea, disturbed mental status, cold extremities, changes in the skin color, anuria, acidosis, and elevated lactate level (27, 28). Table 2 shows hemodynamic changes in different types of shock.

Table 2. *Hemodynamic changes in different types of shocks*

Type of shock	Hemodynamic changes
Hypovolemic	↓ preload ↑ SVR ↓ CO
Distributive	↓ preload ↓ SVR ↓ / ↑ CO
Cardiogenic	↑ preload ↑ SVR ↓ CO
Obstructive	↓ preload ↑ SVR ↓ CO

*SVR- systemic vascular resistance; CO- cardiac output

All the abovementioned may be present in hypovolemic shock, as well as orthostatic hypotension, pallor, flattened jugular veins, and bleeding (29).

The septic shock is usually associated with a various clinical picture, but the initial sings are:

- fever;
- temperature > 38 °C or < 6 °C;
- tachycardia with a heart rate > 90 beats per minute in adult patients or less than two standard deviations for age in pediatric patients;

• tachypnea with respiratory rate > 20 breaths per minute in adult patients or more than two standard deviations for age in pediatric patients (30).

Severe sepsis involves multiple organ dysfunction and includes the following signs and symptoms:

• cardiovascular system—hypotension, cyanosis, chest pain, suffocation, Beck's triad (pericarditis with tamponade), petechiae;

• respiratory system—cough, dyspnea, tachypnea, chest pain;

• gastrointestinal system—vomiting, diarrhea, blood in the stool, purulent stool, abdominal pain, ileus, stress ulcers;

• urinary system—oliguria, anuria, hematuria, pyuria;

• nervous system—disorders of consciousness, meningeal signs, headache, photophobia, stiff neck (28, 31).

Septic shock is diagnosed as a clinical condition associated with infection and vasopressor requirement to maintain a mean arterial pressure of 65 mmHg or greater and a serum lactate level greater than 2 mmol/L (> 18 mg/dl) in the absence of hypovolemia (32).

It is characterized by two phases:

• compensated hyperdynamic (warm) phase, and,

• decompensated hypodynamic (cold) phase.

In the compensated phase of shock, blood pressure is maintained due to peripheral vasodilation and cardiac output is preserved with tachycardia, but other signs may be present (rapid capillary refill, warm extremities, strong pulse). If fluid resuscitation and vasoactive support are properly managed, healing can occur (31).

As shock goes into the decompensated phase, hypotension and a drop in cardiac output occur, so patients have cold extremities, delayed capillary refill (longer than three seconds). Following the worsening of condition, shock can become irreversible, which can result in multiorgan dysfunction syndrome and death (30). Table 3 shows the diagnostic criteria for SIRS.

Table 3. *Systemic inflammatory response syndrome (SIRS)*

Temperature > 38 °C or < 36 °C	1
Heart rate > 90/min	1
Respiratory rate > 20/min or $PaCO_2$ < 32 mm Hg	1
Leukocyte number > 12 000/mm^3 or < 4000/mm^3 or > 10% immature forms	1

The clinical picture of anaphylactic shock varies significantly from patient to patient depending on the dose, the site of antigen entry and the degree of sensitization of the individual. Hypotension, flushing, urticaria, tachypnea, hoarseness, macroglossia, edema of the face and oral mucosa, inspiratory stridor may develop in patients with a positive history of exposure to a known allergen (medications, food, stings). The fatal outcome occurs most often due to thromboembolic complications, ventricular dysfunction or heart rhythm disorders (33).

Cardiogenic shock should be suspected if the patient has chest pain, convergent arterial blood pressure, late inspiratory cracks or the presence of arrhythmias with the presence of cold extremities, agitation, disturbances of consciousness and oliguria (15).

Clinical picture of obstructive shock is nonspecific (tachycardia, tachypnea, oliguria and disturbances of the state of consciousness). Patients with subcutaneous emphysema, auscultatory silent breathing, deviation of the trachea to the healthy side on X-ray, enlarged jugular veins, as well as information about trauma, mechanical ventilation or cystic lung disease may have tension pneumothorax. Aortic dissection is characterized by chest or abdominal pain, while pericardial effusion includes dyspnea, Beck's triad, and pulse paradox (12).

SHOCK DIAGNOSIS

The diagnosis of shock is based on history, physical examination, clinical presentation, vital parameters, biochemical analyses, SOFA criteria (sequential organ failure assessment score), SIRS criteria, acid-base status, blood count, hemodynamic monitoring, diuresis measurement, chest X-ray, blood culture, and other samples depending on the need (urine, bronchoalveolar lavage, stool, manure, etc.) (34). Diagnostic criteria are complex and consist of several general clinical and laboratory parameters. Table 4 provides a more detailed explanation of MODS diagnostic criteria and Table 5 shows the criteria for quick evaluation of the SOFA score.

All patients should continuously have cardiopulmonary monitoring in intensive care units. It is

necessary to evaluate the function of all organs, through various tests and examinations. These include the mental status assessment with Glasgow Coma Scale (GCS), lung function via arterial blood gasses (ABG), heart function with invasive hemodynamic monitoring, renal function via diuresis measurement and metabolism with lactate measurement. In addition, a complete blood count with leukocyte formula analysis is needed, as well as complete biochemical tests of liver, kidney, cardiac biomarkers, a panel for disseminated intravascular coagulopathy (coagulation screening and d-dimer) and acid-base status (30).

Determining the level of CD14 or presepsin can help in the diagnosis of sepsis, considering that it is significantly related to the severity of the clinical presentation and the prognosis of the disease. Presepsin is generated as the body's response to bacterial infection, although it is not entirely clear how presepsin is produced in the body. Research shows that presepsin could be a diagnostic biomarker for sepsis with high sensitivity and specificity. Interleukin-6 (IL-6) is a molecule that helps cells communicate during the body's response to infection. It has been suggested that measurement of IL-6 levels during sepsis can be helpful in identification of patients with sepsis and initiation of adequate treatment (35).

Determination of C-reactive protein and procalcitonin can help in differentiating between viral and bacterial sepsis, as bacterial sepsis shows a higher trend of these proteins. Compared to CRP, procalcitonin has a higher diagnostic value. Namely, the levels of procalcitonin correlate well with the severity of the clinical picture in sepsis, while on the other hand, the decrease in the level of this biomarker indicates an effective therapeutic course and adequately implemented antibiotic therapy.

Table 4. *Multiorgan dysfunction syndrome (MODS) diagnostic criteria*

General indicators: • Temperature (> 38.3 °C); • Hypothermia (basal temperature < 36 °C); • Heart rate > 90/min; • Tachypnea; • Altered mental state; • Significant edema or positive fluid balance (> 20 ml/kg during 24 h); • Hyperglycemia (plasma glucose> 140 mg/dl or 7.7 mmol/l) in the absence of diabetes.
Inflammatory indicators: • Leukocytosis (leukocyte number > 12,000 μl^{-1}); • Leukopenia (leukocyte number < 4000 μl^{-1}); • Normal number of leukocytes with more than 10% immature forms; • C-reactive plasma protein with more than two standard deviations (SD) above the normal value; • Plasma procalcitonin with more than two SD above normal value:
Hemodynamic variables • Arterial hypotension (systolic pressure < 90 mmHg, mean arterial pressure < 70 mmHg, or reduction in systolic pressure > 40 mmHg in adults).
Indicators of organ failure: • Arterial hypoxemia (PaO2/FiO2 < 300); • Acute oliguria (urine output < 0.5 ml/kg/h for at least 2 hours despite adequate fluid replacement); • Creatinine increase > 0.5 mg/dl or 44.2 μmol/l; • Coagulation disorders (INR > 1.5 or aPTT > 60 s); • Ileus (absent sounds of peristalsis); • Thrombocytopenia (platelet count < 100,000 μl); • Hyperbilirubinemia (total bilirubin in plasma > 4 mg/dl or 70 μmol/l).
Indicators of tissue perfusion: • Hyperlactatemia (> 2 mmol/l), • Reduced capillary refill.

Table 5. SOFA *score of organ failure assessment*

Respiratory rate ≥ 22/min	1
Change in mental status	1
Systolic blood pressure ≤ 100 mmHg	1

Disruption of antithrombin III can indicate a septic condition in the body up to 72 hours before the clinical manifestation of the disease, while a decrease in fibronectin indicates the existence of sepsis and is a bad prognostic sign (26, 35).

An X-ray of the chest can be used to reveal pneumonia, acute respiratory distress syndrome (ARDS), or tension pneumothorax. Cardiac ultrasound can be useful in the differential diagnosis of shock and resuscitation of hypotensive patients (36). MSCT of the pulmonary arteries is the gold standard for the detection of thromboembolic events (37). CT scanning can be used to detect abdominal abscess, intestinal perforation, ischemia, or aortic dissection.

CONCLUSION

In summary, a comprehensive understanding of the etiopathogenesis of shock is crucial for effective diagnosis and management. Accurate diagnosis hinges on a thorough clinical assessment, integration of patient history, and the utilization of advanced diagnostic tools. Recognizing the early signs and symptoms of shock allows for timely interventions that can significantly improve patient outcomes. As medical science continues to evolve, ongoing research into the pathophysiological processes of shock at the molecular level will enhance our diagnostic capabilities and treatment strategies, ultimately leading to improved recovery rates.

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Savremeni koncept etiopatogeneze i dijagnostike šoka

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SAŽETAK

Uvod. Šok predstavlja stanje koje ugrožava život, a nastaje usled neusklađenosti ponude i potrošnje kiseonika; to dovodi do hipoksije ćelija i tkiva, koja uzrokuje smrt ćelija i disfunkciju vitalnih organa. Premda su efekti šoka u ranim fazama reverzibilni, odlaganje dijagnoze i započinjanja lečenja može dovesti do nepovratnih promena, uključujući multiorgansku insuficijenciju (MODS) i smrt. Postoje četiri glavne kategorije šoka: hipovolemijski, distributivni, kardiogeni i opstruktivni. Cilj ovog rada bio je da predstavi novu percepciju sagledavanja etiopatogeneze i efikasno postavljanje dijagnoze šoka.

Etiologija. Uzroci nastanka hipovolemijskog šoka mogu biti hemoragijski i nehemoragijski. Distributivni šok se deli na septički šok, sindrom sistemskog inflamatornog odgovora (SIRS), anafilaktički, neurogeni i endokrini šok usled razlika u njihovoj etiopatogenezi. Do kardiogenog šoka dovode intrakardijalni uzroci, dok se opstruktivni šok javlja usled ekstrakardijalnih faktora.

Patogeneza. Patogeneza svakog podtipa šoka je različita i zavisi od načina njegovog nastanka. Uopšteno govoreći, šok ima tri faze: kompenzovanu fazu, fazu celularnog distresa i dekompenzovanu fazu. Kada šok pređe u ireverzibilnu fazu, dolazi do multiorganskog oštećenja i smrti.

Klinička slika. Simptomi mogu varirati u zavisnosti od vrste i stadijuma šoka. Najvažnije promene koje se dešavaju u ovom sindromu tiču se hemodinamike. Naime, najčešći klinički znaci koji upućuju na šok jesu: hipotenzija, tahikardija, tahipneja, poremećen mentalni status, hladni ekstremiteti, modra koža i oligurija.

Dijagnoza. Dijagnoza šoka se zasniva na anamnezi, kliničkoj slici, fizikalnom pregledu, vitalnim parametrima i biohemijskim analizama. Važnu ulogu u postavljanju dijagnoze imaju i skor procene sekvencijalnog otkazivanja organa (engl. *sequential organ failure assessment score* – SOFA *score*), acido-bazni status, krvna slika, hemodinamski monitoring, merenje diureze, hemokultura i dr.

Zaključak. Razumevanje etiopatogeneze šoka i njegovo rano prepoznavanje omogućavaju pravovremenu terapiju i poboljšavaju ishod bolesti.

Ključne reči: **šok, hemodinamski poremećaji, sepsa, etiopatogeneza**

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Review article

Treatment of Burn Injuries in Children

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SUMMARY

Introduction. Burns represent one of the leading causes of morbidity and mortality in children.
Aim. Aim of this review is to gain better understand of the pathophysiological changes and assessment of the severity of burn injuries in different ages of pediatric patients, which may help in early implementation of appropriate therapeutic procedures and improvement of the outcome of these patients.
Literature review. Children are more likely to develop wider and deeper burns, greater fluid and heat loss in comparison to adults. Therefore, the initial assessment of the TBSA and the depth of the burns in children are crucial for their further treatment. The most important approach in the treatment of children with burn injuries includes the management of airways, effective fluid resuscitation, pain control, and prevention of infection.
Conclusion. In the current review we sought to provide recommendations that might help improve the assessment of the severity of burns in children, which may be important for improving their recovery and reducing mortality rate.

Keywords: **burn injury, children, treatment**

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INTRODUCTION

Burn injury in children is the leading cause of mortality, especially in low- and middle-income countries. According to the World Health Organization, burn injuries are the cause of death in 180,000 cases annually, and the fifth most common cause of non-fatal injuries in childhood (1). With advancement in the treatment of these patients, the mortality has decreased over time by 48.1%, and now amounts to 3.8% (2, 3). Pediatric population is an especially vulnerable group for the development of burn injury due to their natural curiosity, limited understanding of risk, and physical environment. This is why burns at home caused by hot liquids and flame are the most common on the upper limbs (4). Initial accurate assessment of the TBSA in burn injuries of children may be crucial for further decision making about their hospitalization and treatment (5). About 2.5% of children with more than 20% of TBSA burns and second-degree burns required admission to hospital (2, 6). Despite significant progress in the treatment of children with burn injuries, children of younger age, with burns like scalds and inhalation injuries, with over 41% of TBSA, and who are hospitalized in lower volume centers, are still associated with high morbidity and mortality, (1, 3, 6-10). The reason for the high mortality in children lies in the untimely recognition of the severity of burn injury and inadequate treatment of these patients. That can be explained with their greater sensitivity to fluid and heat loss and development of wider and deeper burns even in short-term skin exposure compared to adults (2). Also, the implementation of primary prevention programs would be helpful in reducing the incidence of burn injuries in very young children and teenagers (11, 12).

The focus of this review is on better understanding the pathophysiological processes and accurate assessment of the severity of burn injuries, which can provide the timely implementation of appropriate therapeutic procedures in children. That would improve the initial resuscitation, perioperative care, procedural sedation and pain relief as well as reduce the morbidity and mortality of pediatric patients.

PATHOPHYSIOLOGICAL CHANGES IN CHILDREN WITH BURN INJURIES

Better understanding of pathophysiology of burn injury is very important for effective management of pediatric patients. Burn injuries result in local and systemic responses. Local response involves the zone of coagulation, stasis, and hyperemia. In the zone of coagulation, the strongest damage effect occurs with irreversible tissue loss. The zone of stasis is characterized by vasoconstriction and a decrease of tissue perfusion. The perfusion of this zone may significantly improve with initiation of resuscitation, which would prevent the additional deepening and expansion of the burn wound. In the zone of hyperemia, the tissue perfusion is increased and the tissue's ability to recover is great unless there is severe sepsis or prolonged hypoperfusion of tissue (13). Systemic effects of burn injury are expected in children with TBSA over 30%. Cardiovascular changes involve increased capillary permeability with loss of fluids into the interstitial compartment, peripheral and splanchnic vasoconstriction, and decreased myocardial contractility. These changes lead to systemic hypotension and hypoperfusion of organ systems. Respiratory changes in the form of bronchoconstriction are caused by inflammatory mediators, which may lead to respiratory distress syndrome. In addition, the basal metabolic rate in children with burns may multiple by three. In the setting of splanchnic hypoperfusion, early enteral nutrition may have limited effects on the prevention of catabolism and maintain gut integrity (13).

Immunological changes occur in damaged cells with the release of inflammatory mediators (histamine, bradykinin, vasoactive amines, prostaglandins, leukotrienes, etc.). Thus, histamine released from mast cells increases membrane and vascular permeability, while serotonin and thromboxane A2 increase pulmonary vascular resistance and mesenteric vasoconstriction (14). Due to vasodilatation and increased permeability of blood vessels, there is a significant loss of intravascular fluid, protein into the interstitial space and the development of hyperemia and edema. Thus, tissues that are in the immediate vicinity of the necrosis zone can

be additionally damaged in conditions of hypotension, hypoxia and infection, which may additionally increase the necrosis zone after burn injury (13). In addition to inflammatory mediators, hormones such as catecholamines, cortisol and glucagon are also released. These hormones lead to proteolysis, lipolysis, gluconeogenesis, glycogenolysis, and loss of lean body mass and body fat. Catecholamines increase gluconeogenesis, glycogenolysis, lipolysis, production of acute phase reactants, thermogenesis, and cardiac output. Released cortisol leads to hyperglycemia in the first 24 hours of burns as well as proteolysis with simultaneous reduction of protein synthesis (15). All this leads to a negative nitrogen balance, loss of muscle mass up to 50% and slow rehabilitation of children with burns (16). Because of the profound immunological and metabolic changes in severely burned children, complications such as infection, growth arrest, and loss of lean body mass may develop (17).

Released inflammatory mediators, as well as physiological characteristics of children, make these patients more sensitive to fluid and heat loss compared to adults. This is due to an almost 3-fold higher TBSA to body mass ratio, as well as thinner layers of skin and insulating subcutaneous tissue in younger children compared to older children and adults. Because of that, younger children are at greater risk of developing a wider and deeper burn even with short-term skin exposure to heat compared to older children and adults (2). On the other side, temperature regulation in very young children is based in part on non-shivering thermogenesis, which further increases their metabolic rate, oxygen consumption, and lactate production. Therefore, these patients require more intensive fluid replacement and maintenance of normothermia than adult patients. When replacing fluids, it should be taken into account that children younger than one year are more prone to developing hyponatremia due to their larger blood volume, greater loss of sodium through urine, and the inability to concentrate urine (18). These patients are also prone to airway obstruction due to their anatomical features such as smaller relative diameter, shorter mandible and trachea, larger tongue and adenoid, and anteriorly displaced pharynx. Due to anatomical features of the airway and released inflammatory mediators, pediatric patients with inhalation burns may be at greater risk for bronchospasm (19-21).

FIRST AID IN CHILDREN WITH BURN INJURIES

Providing first aid in the form of cooling may be useful in children with TBSA of burns less than 10% and in the absence of shock (22). This type of first aid was implemented in 86.1% of patients, while in others it was not implemented or information about it was missing. Short-term cooling with water was done in 80.2% of patients, while recommended cooling longer than 20 minutes was applied only in 12% of patients (23). This long-term cooling of the burned surface was associated with a reduction in the depth of the burn, as well as in the time required for re-epithelialization of those burned surfaces (24, 25). However, the final outcome of these patients did not depend on the length of cooling of the burned surface, but on the surface and depth of the burns, as well as the mechanism of their injury (26). In chemical burns with corrosive agents, it is necessary to remove contaminated clothing from the surface of the skin and dilute the chemical agent by irrigating it with water (27).

ASSESSMENT OF BURN INJURIES IN CHILDREN

Initial assessment of burn injury in children is crucial for further treatment of these patients. Thus, in the case of minor burns, after dressing the burn wound with dry sterile gauze with the goal of reducing the risk of hypothermia and infection, the patient can be referred for home treatment. However, 2.5% of pediatric patients with burn injury require admission to specialized centers which are intended for the hospital treatment of burns (6). The indication for direct admission to the hospital is based on the following criteria: burns with partial involvement of the skin thickness and TBSA greater than 10%, with complete involvement of the skin thickness greater than 2% of TBSA, burns of the face, hands, genitals, perineum or major joints, circumferential burns of the extremities, all electrical burns caused by high voltage of electric current or low voltage in selected cases, chemical and inhalation burns, burns in patients with already existing comorbidities (diabetes, immunosuppression), which can complicate treatment and increase mortality, suspected abused children, as well as children whose parents are unable to take care of them (22, 28).

During the initial examination, detailed anamnestic data on the mechanism of injury and assessment of the depth and TBSA of the burn should be obtained. The measurement of the TBSA of burns is crucial for initial management, particularly for fluid resuscitation in the first hours after injury. There are several methods of measuring the TBSA of burns in use. The most accurate method for the assessment of TBSA of burns in children is achieved using the Lund and Browder table, which takes into account the age of children (29, 30). The Wallace rule of nines is used for the rapid assessment of TBSA of burns in adult patients. However, this method is not suitable for children, due to significant overestimates of TBSA burns (31). An alternative rule of thumb for rapid assessment of the burned area is the palm of the patient, which represents 1% of the body surface (13). Despite various methods of assessing TBSA, the problem of estimating burn size in children still persists. Overestimating the burn size is present in 70% of cases, underestimating in 15%, while a correct estimation is present only in 15% of children (29). Overestimating may lead to inappropriate resuscitation and administration of fluids over what's required (30). Also, it should be kept in mind that a definitive assessment of the burn depth may only be obtained after 48 to 72 hours from the occurrence of burn injury to children, due to the dynamic nature of the damage and the possibility of the burn deepening during this period. Thus, the assessment of burn depth in children appears to require continued training and education of initial burn providers (29, 32).

TREATMENT OF BURN INJURIES IN CHILDREN

The initial approach in the treatment of children with burn injury involves the management, of airways, fluid resuscitation, and pain control (5). Assessment of airways and possible intubation is especially important in children with burns of the face or neck, symptoms of airway obstruction as well as in children with greater TBSA burn. The intubation process should be considered in patients with deeper and circular burn on the neck, signs of inhalation injury to the airways (stridor, hoarseness, black sputum, respiratory distress, damage to the hairs in the nose, swelling of the face) and oropharyngeal region (soot in the mouth, intraoral edema, and erythema), and burns over 40% of TBSA (22, 33), due to their tendency to develop airway obstruction. The reason for that are anatomical and physiological characteristics of airways and rapid developing of secondary edema after inhalation injury or after intensive fluid resuscitation in the first 48 hours after burn injury in children. The size of the endotracheal tube in children can be determined using the formula, tube size = 4 + (age in years/4) or based on the diameter of the patient's little finger. In case of difficult intubation, it is necessary to provide a video laryngoscope and equipment for emergency tracheotomy (12, 20, 22). Children with smoke injuries, besides airway obstruction may also develop pulmonary edema, decreased pulmonary compliance, ventilation-perfusion mismatch as well as carbon monoxide or cyanide intoxication. The diagnosis of smoke injury may be confirmed through anamnestic data, physical exam, and bronchoscopy. Therefore, these patients often require supportive measures, treatment of pulmonary infection and ventilatory support (20, 33). In case of children with carbon monoxide and cyanide intoxication, the administration of high-flow oxygen and hydroxocobalamin are recommended (22).

Loss of circulating volume is proportional to the severity of the burn. Thus, in case of children with minor burns, oral hydration may be sufficient. However, in children with TBSA of burns greater than 10%, initial fluid resuscitation with 20 mL/kg of intravenous crystalloid solution is needed (5, 22). In early hypovolemic phase, within the first hour of burn injury occurrence, balanced solution such as Ringer's lactate is used for all age groups of children. Its overall amount is calculated based on the Parkland formula (volume mL = 4 × body weight (kg) × % TBSA of burns) (34) and the Galveston formula (volume mL = 5000 mL/m^2 TBSA burns + 2000 mL/m^2 TBSA of Ringer-lactate + Albumin and 5% dextrose). Modified Parkland formula is used in children with a body mass below 20 kg, according to which, in addition to the calculated amount of fluid in keeping with the percentage of burned surface, a certain amount of maintenance fluid should be added. Maintenance of fluid involves the administration of Ringer's lactate solution and 5% dextrose in a volume of 4 mL/kg/h for patients up to 10 kg body weight, plus 2 mL/kg/h for children with body weight between 10 and 20 kg, plus 1 mL/kg/h for every kg above 20 kg of body weight. The reason for that are reduced glycogen storages in younger children and their tendency towards hypoglycemia.

Also, glucose solutions can compensate for the initial hypermetabolic response to burn, characterized by increased energy expenditure, stroke volume, cardiac output, and hyperthermia (14). Of the total amount of calculated fluids, half should be given in the first eight hours after the burn injury, and the remaining half in the next 16 hours. This concept shows its ineffectiveness in patients with greater fluid loss than the ones that are calculated for a given time period, thus emphasizing the importance of hourly titration of fluids to provide satisfactory amount of urine output in the burn patient (22). Therefore, reassessment of fluid status, every 1-2 hours, within the first 24 hours after burn injury is needed.

Administration of colloids can significantly reduce crystalloid overuse, tissue edema, and length of hospitalization in patients with severe burns (35, 36). Therefore, human albumins are recommended in patients with TBSA burns of 15-45% by Legard et al. (22) or over 30% by Dittrich et al. (35) after the first six hours of fluid resuscitation. Other solutions reducing fluid volume have been largely abandoned, such as dextran due to coagulopathy, fresh frozen plasma because of transmissible diseases, and hypertonic saline because of increased mortality and renal failure (22, 37). Until now, different formulas have been used to replace hypertonic solutions or colloids, with different results in the treatment of these patients. This indicates that it is difficult to develop a standardized method of fluid replacement in children with burns because of its dynamic nature and the need to titrate fluids based on evidence of end-organ perfusion.

The effectiveness of fluid resuscitation in children with burns is evaluated based on the achievement of satisfactory end-organ perfusion. Resuscitation fluids should be titrated to provide a urine output of 0.5–1 mL/kg/h, which is in contrast to the previous practice of 1–2 mL/kg/h (15, 36, 38). Depending on the urine output, the amount of fluids should be increased by 10-20% in case of lower target value, or decreased by 10-20% in case of higher target value. Any delay in fluid replacement may increase the risk of acute renal failure, multiorgan dysfunction, prolonged hospitalization, and increase mortality of these patients (10). Pediatric burn patients are also prone to developing acute kidney injury (AKI) not only in the setting of insufficient fluid replacement but also in the setting of rhabdomyolysis or drug-induced kidney injury. AKI occurs in 30 to 50% of burn patients. There are identified risk factors for AKI development such as age, wider and full-thickness of TBSA of burns, flame burn, inhalation injury, burn severity index on admission, organ failure assessment score on admission, baseline level of urea and creatinine, multiple surgeries, sepsis, compartment syndrome, and prolonged PICU hospitalization (39-41). Moreover, the occurrence of AKI in patients with burns can increase mortality by six times compared to patients who did not develop this injury (42-44). Also, a burn in the first 24 hours after the injury can cause a hypermetabolic inflammatory response, the release of catecholamines and other stress hormones and the consequent occurrence of refractory tachycardia, increased cardiac output and increased oxygen consumption. When these patients are anesthetized, catecholamine depletion and cardiovascular collapse may occur. In these clinical settings, the selection of the induction agent for anesthesia should be considered carefully, and inotropic and vasoactive drugs should be administered additionally. Children with burns greater than 10% of TBSA are more prone to greater heat loss due to a higher ratio of TBSA to body mass as well as thinner skin and insulating subcutaneous tissue, compared to adults. Therefore, constant measurement of body temperature, maintaining of environmental temperature at 30-32 ºC, administration of warm intravenous fluids and the use of heating mattresses for parts of the body that are not covered with gauze and bandages is needed to avoid hypothermia (45). In children with TBSA burn over 25% TBSA, systemic edema may be expected within the first 4 to 36 hours of injury, leading to circulatory shock, reduced cardiac output, and organ hypoperfusion. Thus, hemodynamic monitoring such as pulse oximetry, ECG, non-invasive blood pressure measurement can be necessary and at the same time difficult, especially in children with greater burns. In these cases, monitoring of invasive blood pressure and cardiac output via esophageal Doppler is recommended. The placement of a urinary catheter is mandatory in children with burns greater than 15% TBSA to assess hourly diuresis. In addition, continuous monitoring of laboratory parameters such as blood counts, electrolytes, and lactate may be useful in the assessment of burn severity and determination of resuscitation endpoints during the treatment of burn shock (46).

Treatment of burns in children requires adequate pain management. Inadequate treatment of

pain can increase stress, anxiety and fear in patients, which will further intensify the pain. Acute pain can interfere not only with a child's physical activity, but also with their recovery process. Chronic pain can lead to long-term physical dysfunction and impairment of children's quality of life, as well as psychological consequences after the end of burn treatment. This leads to constant emotional stress, disturbed mood, sleep and appetite, school failure and fear of further medical services. In the essence of inadequate treatment of pain lies a poor understanding of pain pathophysiology and its complex nature. Therefore, the treatment of pain remains one of the most challenging medical issues (17, 47). It is believed that multimodal pain management can be effective in eliminating pain in children with burn injury. In addition, analgesic medications have to be titrated on the basis of validated comfort and analgesia assessment scales (22). Opioids represent a key analgetic for pain control in children with burns. However, individual use of opioids can cause some side effects such as nausea, constipation, drowsiness, pharmacological tolerance, addiction during short-term or long-term use. Therefore, combining opioids with non-steroidal anti-inflammatory drugs (NSAIDs) can reduce the need for opioids. The most often used combination is paracetamol and morphine, which can be substituted for oxycodone in case of tolerance (48, 49). Although the use of NSAIDs can reduce the need for opioids, they should be used with caution in severe burns because of their side effects (renal toxicity, gastric ulceration, and antiplatelet effects). Recent data indicates that the use of ketamine in combination with other analgesics may be more effective in pain treatment than a single administration of opioids (22). In addition, ketamine-dexmedetomidine, ketamine-propofol, propofol-remifentanil, propofol-fentanyl, and ketamine-midazolam may be useful in reducing procedural pain and anxiety during wound care procedures in children (50). Other drugs that may be useful in relieving pain are gabapentin and alpha 2 agonists (clonidine and dexmedetomidine). However, despite this, a high intensity of pain (7 out of 10) was recorded during the treatment of patients with burns (51). Thus, combining non-pharmacological techniques such as hypnosis, distraction techniques, relaxation and distraction with analgesics reduced the intensity of pain significantly in stabile burn patients who require frequent wound care procedures (52-56).

Infection is the leading cause of morbidity and mortality in children with severe burns. With the progression of the wound infection to sepsis, death occurs in about 55% of patients (57). The reason for that is an impaired protective function of the skin as the first line of defense for pediatric patients against infection. Risk factors that increase the susceptibility of these patients for infections are: burns with greater TBSA and depth, as well as inhalation injuries (58, 59). Simultaneously, these patients have an altered thermoregulation and metabolic homeostasis, which, along with local capillary damage, local and systemic vasodilatation, makes them more sensitive to fluid loss, tissue edema, invasion of infectious microbes and infection (57, 60, 61). Dysregulation of the immune response in these patients makes them susceptible also to urinary tract infections, pneumonia, and central venous catheter infections (59). If sepsis occurs in the early phase of treatment, gram-positive bacteria should be considered as a cause of infection. Late sepsis is predominantly caused by gram-negative drug-resistant bacteria such as multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. With the identification of the source of infection, treatment should be started as soon as possible with a de-escalation antibiotic in order to reduce the degree of antibiotic resistance (61, 62). In addition to antibiotics in the treatment of burn children with sepsis, vasopressors can also be used. This is due to pediatric patients normally having a higher cardiac output compared to adults, as well as a limited ability to modulate contractility, therefore an increase in stroke volume is often impossible when needed, especially in sepsis. The vasopressor epinephrine proved to be more useful in the treatment of septic shock in children due to improving cardiac output and tissue oxygen delivery, compared to norepinephrine (63). In addition, thromboprophylaxis should be routinely prescribed in the initial phase of severe burn patients and in the patients with placed central venous catheter (22).

During the surgical treatment of children with burns, significant blood loss is possible. Therefore, these patients often require more intensive restitution of blood and blood products, which may amount to more than one estimated circulating blood volume. Sometimes, blood loss can be difficult to assess, especially in children with a large TBSA burn (64). The formula that can be used to calculate the percentage of blood loss during surgical intervention is as follows: 3 x body weight (kg) x % TBSA

in burns. In case of patients with anemia, it is recommended to correct the hemoglobin level below 7 mg/dL. With the use of restrictive strategy, blood replacement was reduced by 50% in the operating room and by 25% outside the operating room or by one third in another study (65, 66). With the application of this strategy, the duration of mechanical ventilation and blood utilization of these patients was significantly reduced, but without affecting mortality, organ dysfunction, wound healing or the occurrence of infectious complications (66).

Other types of treatment include nutritional support, which should be started 12 hours after burn injury occurrence. In addition, oral and enteral nutrition have advantages over parenteral nutrition. With high carbohydrate, low fat enteral nutrition together with pharmacological agents such as growth factors, insulin, propranolol and oxandrolone, and increased nutrient intake is ensured, catabolism and malnutrition are prevented, reparative processes are stimulated, which overall shortens hospitalization, ensures successful recovery and rehabilitation of these patients (67, 68).

CONCLUSION

Burn injury in children is associated with high mortality. With better understanding of pathophysiology and assessment of the severity of burns, especially at younger age, effective management and treatment of pediatric patients may be achieved. Therefore, adequate management of airways, fluid replacement, multidisciplinary approach in pain relief, and prevention of infection may provide the best possible chance for quick and successful recovery of pediatric patients as well as an optimal quality of life afterwards.

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Human and animal rights and informed consent

This paper is a review article, so it was not research involving human participants and/or animals nor there was a need of an informed consent.

Conflict of interest

The authors declare no competing interests.

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Lečenje opekotina kod dece

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SAŽETAK

Uvod. Opekotine predstavljaju jedan od vodećih uzroka morbiditeta i mortaliteta kod dece. Cilj. Cilj ovog preglednog rada bio je da omogući bolje razumevanje patofizioloških promena i procene težinu opekotina kod dece različitog uzrasta, što može pomoći u ranoj primeni odgovarajućih terapijskih procedura i poboljšanju ishoda lečenja ovih pacijenata.
Pregled literature. Verovatnoća da će doći do razvoja širih i dubljih opekotina, većeg gubitka tečnosti i toplote veća je kod dece nego kod odraslih. Stoga, početna procena površine i dubine opekotina kod dece ključna je za njihovo dalje lečenje. Najvažniji pristup u lečenju dece sa opekotinama obuhvata upravljanje disajnim putevima, efikasnu reanimaciju tečnostima, kontrolu bola i prevenciju infekcije.
Zaključak. U ovom preglednom radu nastojali smo dati preporuke koje bi mogle pomoći u poboljšanju procene težine opekotina kod dece, budući da bi to moglo biti važno za poboljšanje procesa njihovog oporavka i smanjenje stope mortaliteta.

Ključne reči: **opekotine, deca, lečenje**

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Review article

Retinitis Pigmentosa Genes Implicated in the Population of America: A Systematic Review

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SUMMARY

Introduction/Aim. Retinitis pigmentosa (RP) is a diverse group of inherited retinal diseases characterized by the gradual degeneration of rod and cone photoreceptors in the retina. RP is primarily inherited, with numerous genetic mutations implicated in its pathogenesis. The aim of this study was to summarize the findings of studies related to genes implicated in retinitis pigmentosa, in autosomal dominant (adRP), autosomal recessive (arRP), and X-linked RP (xlRP) patients in America.
Material and Methods. In this comprehensive search of literature via the Medline/PubMed database, SciELO, Redalyc, ScienceDirect, and Google Scholar (English/Spanish), 75 articles between 2010-2020 were reviewed; the final analysis was based on 21 articles.
Results. The main gene mutations found in America for adRP were RHO (rhodopsin) and PRPF31 (pre-MRNA processing factor 31); for arRP, USH2A (usherin 2A) and EYS (eyes shut homolog); and for xlRP, RPGR (retinitis pigmentosa GTPase regulator) and RP2 (retinitis pigmentosa 2).
Conclusion. Most of the genes currently found worldwide to cause RP were present in America, with similarities and differences with other populations in Asia and Europe.

Keywords: **autosomal dominant, autosomal recessive, inheritance pattern, retinitis pigmentosa, X-linked**

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INTRODUCTION

Retinitis pigmentosa (RP) is a diverse group of inherited retinal diseases (IRDs) characterized by the gradual degeneration of rod and cone photoreceptors in the retina. RP is one of the leading causes of visual impairment, affecting approximately 1 in 3,000–7,000 individuals globally, though prevalence varies by region. Orphanet (1) reports a global prevalence of 1-5 per 10,000 people. The primary symptoms include night blindness and narrowing of the visual field, often progressing to a concentric pattern known as tunnel vision. In the early stages, rod photoreceptors are primarily affected; as the disease advances, cone involvement leads to deficits in visual acuity, color perception, and spatial detail recognition. Typical fundus findings include bone spicule pigmentation, thinning of retinal vessels, and a waxy appearance of the optic disc. Retinal changes are also evidenced by abnormal electroretinogram results and structural alterations detectable via optical coherence tomography. RP can present in two forms: syndromic, where it is part of a broader systemic condition, and non-syndromic RP, where it affects only the eyes. Clinical manifestations vary depending on the specific genetic mutations involved (2-4).

RP is primarily inherited, with numerous genetic mutations implicated in its pathogenesis. Over 3,000–4,000 mutations across more than 80 genes are associated with non-syndromic RP (2-4), while mutations in 31 genes are linked to the syndromic form (5). Usher syndrome and Bardet-Biedl syndrome are the most frequently associated syndromic conditions. The majority of RP cases are caused by monogenic mutations (2), typically affecting photoreceptors or the retinal pigment epithelium (6).

Based on the mode of inheritance, RP is classified as autosomal dominant (adRP), autosomal recessive (arRP), X-linked (xlRP), mitochondrial, or of unknown inheritance pattern (2, 5). Certain genetic mutations associated with RP are also linked to other IRDs. Globally, the most frequently reported genes associated with RP include USH2A (usherin), RHO (rhodopsin), and RPGR (retinitis pigmentosa GTPase regulator) (7), particularly adRP. The primary genes implicated in adRP are RHO, RPRF (rapidly progressive renal failure), PRPH2 (peripherin 2), RP1 (retinitis pigmentosa 1), IMPDH1 (Inosine-5'-monophosphate dehydrogenase 1), and PRPF8 (pre-mRNA processing factor 8). For arRP, the most commonly involved genes include USH2A, ABCA4 (ATP-binding cassette subfamily A member 4), PDE6A (phosphodiesterase 6A), PDE6B (phosphordiesterase 6B), and RPE65 (retinoid isomerhydrolase). In xlRP, RP1 and RPGR are the principal genes (2).

Although the genetic characteristics and mutations in RP have been extensively documented worldwide, there is limited and outdated information available for the Americas, particularly Latin America, with some studies being over a decade old. Currently, there is no approved treatment for RP, which highlights the importance of identifying the specific causative gene in affected individuals. This is especially relevant as pharmaceutical companies and research institutions are increasingly focusing on clinical trials aimed at developing therapies to treat or slow the progression of the disease. This review aims to summarize the genes associated with adRP, arRP, and xlRP in the Americas.

MATERIAL

Search

This systematic review was carried out between October and December 2020. Studies published from 2012 to 2020 that reported genetic mutations associated with RP in North American and Latin American populations were considered eligible for inclusion. The review outlines the current state of research on the genetic characterization of RP, drawing from academic databases including MEDLINE, PubMed, SciELO, Redalyc, ScienceDirect, and Google Scholar. Articles published in English and Spanish were included. Based on the selected studies, several tables were created to organize genetic information and list the authors of relevant publications.

The search strategy was structured around the topics of interest, methodologies used, and target populations. The following search terms and descriptors (MeSH, DeCS) were used: “retinitis pigmentosa,” OR “retinosis pigmentaria,” OR “inherited retinal dystrophies,” OR ("retinitis pigmentosa/epidemiology"[Mesh], OR "retinitis pigmentosa/etiology"[Mesh], OR "retinitis pigmentosa/genetics"[Mesh], OR "retinitis pigmentosa/statistics and numerical data"[Mesh]), AND “retinitis pigmentosa/genetics,” OR “retinitis pigmentaria/ genética,” AND “retinitis pigmentaria/autosomal dominant,”

AND "retinitis pigmentaria/autosomal recessive," AND "retinitis pigmentaria/X-linked," AND "retinitis pigmentaria/non-syndromic," "retinitis pigmentaria/America," OR "retinosispigmentaria/America". These terms were applied to full-text searches or topic-based queries, depending on the options available in each database.

Ethics

The study protocol was approved by the Ethics Research Committee of Ciprés Grupo Médico (2020-09-02), Toluca, Mexico. Informed consent was not required, as the data were derived from previously published studies.

Eligibility criteria

This review included studies that identified genes associated with syndromic or non-syndromic RP in patients from the American continent. Studies were required to have clearly defined clinical diagnostic criteria for RP (3) and to use reliable and conclusive genetic testing methods. Additional relevant articles cited within selected studies were also retrieved. Reference lists were reviewed to identify any additional publications not captured by the initial search.

Studies were excluded if they focused on mitochondrial or unknown inheritance patterns of RP or reported findings related to other IRDs. Articles that did not meet the inclusion criteria based on their titles and abstracts were also excluded.

RESULTS

General findings

Out of 75 studies initially identified, 21 articles were included in the review, while 54 were excluded after evaluating their abstracts and full texts. Of the selected articles, 57% originated from North America, 14% from Colombia, 10% each from Brazil and Cuba, and 5% from Mexico and Venezuela, respectively. The specific disorders covered are detailed below.

Autosomal dominant RP

Eighteen genes were identified in association with the adRP inheritance pattern. The RHO (rhodopsin) gene was reported in four countries: Brazil, Colombia, Mexico, and North America. The SNRP200 gene was found in Brazil and North America. BBS1, PRPF31, and PRPF8 were reported in both Brazil and Mexico, while TOPORS was identified in Mexico and North America. In Brazil and North America, the highest number of adRP-related genes (61% and 50%, respectively) was reported, followed by Mexico (33%) and Colombia (17%). Among the most frequently reported genes were RHO, PRPF31, and SNRP200. In syndromic forms, the BBS1gene was identified in 7.23% of cases (Table 1). A detailed list of syndromic and non-syndromic RP-related genes identified in the American population is provided in Table 2.

Table 1. *Autosomal dominant retinitis pigmentosa mutations in America*

Gene	Probands (n)/Country	Percentage
CRX (Cone rod homeobox).	1 (Brazil) (8)	1.20
PDE6B (Phosphodiesterase 6 B)	5 (Brazil) (8)	6.02
PROM1 (Prominin 1)	2 (Brazil) (8)	2.41
RIMS1 (Regulating synaptic membrane exocytosis 1)	1 (Brazil) (8)	1.20
ROM1 (Retinal outer segment membrane protein 1)	1 (Brazil) (8)	1.20
BBS1 (Bardet-Biedl syndrome 1)	6 (Brazil) (8)	7.23
RP1 (Retinitis pigmentosa 1)	1 (Mexico) (9)	1.20
IMPG1interphotoreceptormatrixproteoglycan 1	1 (North America) (10)	1.20
IMPDH1 (Inosine monophosphate dehydrogenase 1)	1 (Canada) (11)	1.20
	1 (North America) (10)	-
EFTUD2 elongation factor Tu GTP binding domain containing 2	(North America) (12)	0.00
RHO (Rhodopsin)	6 (Brazil) (8)	24.10
	11 (Canada) (11)	
	N/R (Colombia) (13)	
	3 (Mexico) (14)	

SNRP200 (Putative U5 small nuclear ribonucleoprotein 200 k Da helicase)	4 (Canada) (11)	9.64
	4 (Brazil) (8)	
PRPH2 (Peripherin 2)	3 (Canada) (11)	7.23
	N/R (Colombia) (13)	
	3 (Brazil) (14)	
PRPF31 (Pre-mRNA processing factor 31)	2 (Canada) (11)	22.89
	6 (Brazil) (9)	
	3 (Mexico) (9)	
	5 (North America) (10)	
	3 (North America) (15)	
NR2E3 (nuclear receptor subfamily 2 group E member 3)	2 (Brazil) (8)	4.82
	2 (Mexico) (9)	
NRL (Neural retina leucine zipper)	1 (Mexico) (9)	1.20
	N/R Colombia (13)	
PRPF8 (Pre-mRNA processing factor 8)	3 (Brazil) (8)	4.82
	1 (Mexico) (9)	
TOPORS (TOP1 binding arginine/serine-rich protein)	1 (Mexico) (9)	2.41
	3 (Canada) (11)	
	83	100.00

N/R: not reported

Table 2. *Autosomal dominant retinitis pigmentosa. Syndromic and no syndromic genes identified in the American population*

Gene	Nucleotide variant	Protein variant-exon	# Families	# patients/n	Country
CRX (Cone rod homeobox).	Not reported	Not reported	1,159	1,246 (121 with RP) n =1	Brazil (8)
	122 G → A 436_447del	Arg41Gln Leu146_Pro149del	200	n=2	North America (8)
RDS			200	n=18	North America (8)
RP1			200	n=7	North America (8)
PDE6B (Phosphodiesterase 6 B)	Not reported	Not reported	1,159	1,246 (121 with RP) n= 5	Brazil (8)
PROM1 (Prominin 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=2	Brazil (8)
RIMS1 (Regulating synaptic membrane exocytosis 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (9)
ROM1 (Retinal outer segment membrane protein 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (10)
BBS1 (Bardet-Biedl syndrome 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=6	Brazil (11)
RP1 (Retinitis pigmentosa 1)	c.2029C>T	p. Arg677Ter	Not reported	143, n=1	Mexico (10)
IMPG1interphotoreceptor matrix proteoglycan 1	c.T1823C	p. L608P	Not reported	35, n=1	North America, Hispanic probands (12)
IMPDH1 (Inosine monophosphate dehydrogenase 1)	c.954G>C	p.Q318H, exon 9	60	60, n =1	Canada (French Canadian Population) (8)
	c.612_614delTTC	p. K206del	Not reported	35, n=1	North America, Hispanic probands (11)
	676 G → A	Asp226Asn	200	n=5	North America (13)

EFTUD2 elongation factor Tu GTP binding domain containing 2	Not reported	p. Arg220Cys p. Ile80Leu p. Thr272Ala	Not reported	Not reported	North America (14)
RHO (Rhodopsin)	Not reported	Not reported	1,159	1,246 (121 with RP) n=6	Brazil (11)
	c.403C>G c.809G>T c.1031A>C c.151G>C c.541G>A c.553T>G	p.R135G, Exon 2 p.S270I, Exon $ p.Q344P, Exon 5 p.G51R, Exon 1 p.E181K, exon 3 p.C185R, exon 3	60	60, n=11	Canada (French Canadian Population) (8)
	genomic Localization: 3q21-q24	Rhodopsin	Not reported	Not reported	Colombia (11)
	c.491C>A c.557C>G	p. Ala164Glu p. Ser186Trp	Not reported	143, n=3	Mexico (13)
			200	n=53	North America (14)
SNRP200 (Putative U5 small nuclear ribonucleoprotein 200 k Da helicase)	c.2122G>A c.2041G>T c.3260C>T	p.V708I, exon 16 p.R681C, exon 16 p.S1087L, exon 25	60	60, n=4	Canada (French Canadian Population) (11)
	Not reported	Not reported	1,159	1,246 (121 with RP), n=4	Brazil (8)
PRPH2 (Peripherin 2)	c.554T>C	p.L185P, exon 1	60	60, n=3	Canada (French Canadian Population) (9)
	6p21.2-cen	Peripherin	Not reported	Not reported	Colombia (10)
PRPF31 (Pre-mRNA processing factor 31)	54618847del C c.862C>T	54618847del C p.R288W, exon 9	60	60, n=2	Canada (French Canadian Population) (15)
	Not reported	Not reported	1,159	1,246 (121 with RP), n =6	Brazil (8)
	c.682G>C c.866_879 del GGAAAGCGGC CCGG	p. Ala228Pro p. Arg289ProfsTer30	Not reported	143, n=3	Mexico (9)
	c.A172T c.866_879 del GGAAAGCGGC CCGG c.322+ 4_322+ 7delAGTG chr19:54632400, C> A	p.K58 p. R289Pfs*30) 5´terminal of intron 5	Not reported	35, n=5	North America (9)
			200	n=11	North America (13)
NR2E3 (Nuclear receptor subfamily 2 group E member 3)	Not reported	Not reported	1,159	1,246 (121 with RP), n=2	Brazil (8)
	c.166G>A	p. Gly56Arg	Not reported	143, n= 2	Mexico (9)
NRL (Neural retina leucine zipper)	c.148 T>C	p. Ser50Pro	Not reported	143, n=1	Mexico (9)
	14q11.2	NRL	Not reported	Not reported	Colombia (11)
PRPF8 (Pre-mRNA processing factor 8)	Not reported	Not reported	1,159	1,246 (121 with RP), n=3	Brazil (8)
	c.6928 A>G	p. Arg2310Gly	Not reported	143, n=1	Mexico (8)
			200	n=6	North America (8)
TOPORS (TOP1 binding arginine/serine-rich protein)	c.2554_2557delGA GA	p. Glu852GlnfsTer13	Not reported	143, n=1	Mexico (8)
	c.2666A>C c.2474_2475insA	P. H889R p. Y825X	60	60, n= 3	Canada (French Canadian Population) (8)

Autosomal recessive RP

In cases of arRP, 45 genes were identified. The USH2A gene was reported in four countries: Brazil, Colombia, Cuba and Mexico. Additionally, the genes CDHR1, CERKL, CRB1, MERTK, PDE6A, and RDH12 were detected in both Brazil and Mexico. The WDR19 gene was identified in Brazil and North America, while ABCA4 was reported in Mexico and North America. Brazil accounted for the highest number of arRP-related genes (53.4%), followed by Mexico (41.8%), North America (21%), Cuba (11.6%), and Colombia (4.6%). In Venezuela (2.33%), the MYO7A gene was associated with the Usher syndrome. The most frequently reported gene mutations in arRP were USH2A, EYS, and MYOTA (Table 3). A summary of the syndromic and non-syndromic genes identified in the American population is presented in Table 4.

Table 3. *Autosomal recessive retinitis pigmentosa mutations in America*

Gene	Probands (n)/Country	Percentage
BBS1 (Bardet-Biedl syndrome 1)	1 (Brazil) (8)	0.53
BBS2 (Bardet-Biedl syndrome 2)	1 (Brazil) (8)	0.53
CDHR1 (cadherin related family member 1)	1 (Brazil) (8)	0.53
CNGA1 (cyclic nucleotide-gated channel subunit alpha 1)	1 (Brazil) (8)	0.53
CNGB1 (cyclic nucleotide-gated channel subunit beta 1)	1 (Brazil) (8)	0.53
EYS (Eyes shut homolog)	1 (Brazil) (8)	8.42
	15 (North America)(16)	
GPR98†	1 (Brazil) (8)	0.53
HGSNAT (heparan-alpha-glucosaminide N-acetyltransferase)	2 (Brazil) (8)	1.05
RP1 (retinitis pigmentosa 1)	7 (Brazil) (8)	3.68
DHDDS dehydrodolichyldiphosphate synthase subunit	3 (North America) (17)	1.58
MKS1 (transition zone complex subunit 1)	1 (Brazil) (8)	0.53
PRPH2 (Peripherin 2)	1 (Brazil) (8)	0.53
ABHD12 (Abhydrolase domain containing 12, lysophospholipase)	1 (Brazil) (8)	0.53
MYO7A (Myosin7A)	8 (Brazil) (8)	5.79
	3 (Venezuela) (18)	
TULP1 (TUB like protein 1)	1 (Brazil) (8)	0.53
SAG S-antigen visual arrestin	N/R (North America) (19)	0.00
WDR19 (WD repeat domain 19)	1 (Brazil) (8)	0.53
	1 (North America) (10)	0.53
CDH23 (Cadherin -related 23)	N/R (Cuba) (20)	0.00
ADGRV1 (adhesion G protein-coupled receptor V1)	N/R (Cuba) (20)	0.00
PCDH15 (protocadherin related 15)	4 (Cuba) (20)	2.11
RP1 retinitis pigmentosa 1	1 (North America) (10)	0.53
ABCA4 (ATP binding cassette subfamily A member 4)	N/R (Mexico) (9)	1.05
	1 (North America) (10)	
	1 (North America) (21)	
ARL6 (ADP ribosylation factor like GTPase 6)	N/R (Mexico) (9)	0.00
PCARE (photoreceptor cilium actin regulator)	N/R (Mexico) (9)	0.00
CLN3 (CLN3 lysosomal/endosomaltransmembrane protein, battenin)	1 (Mexico) (9)	0.00
RDH5 (retinol dehydrogenase5)	1 (Mexico) (9)	0.53
RP2 (retinitis pigmentosa 2)	2 (Mexico) (9)	0.00

RPE65 (retinoidisomerohydrolase RPE65)	1 (Mexico) (9)	0.53
SPATA7 (spermatogenesis associated 7)	1 (Mexico) (9)	1.05
BBS10 (Bardet-Biedlsyndrome 10)	1 (Colombia) (22)	0.53
GNAT1 (G protein subunit alpha transducin 1)	1 (Mexico) (9)	0.53
IFT140 (intraflagellar transport 140)	1 (Mexico) (9)	0.53
IMPG2 (interphotoreceptor matrix proteoglycan 2)	1 (Mexico) (9)	0.53
FAM161A (FAM161 centrosomal protein A)	4 (North America)(17)	0.53
USH2A (Usherina 2A)	21 (Brazil) (8)	42.63
	7 (Colombia) (23)	
	N/R (North America) (24)	
	26 (Colombia) (23)	
	11 (Cuba) (20)	
	11 (Cuba) (25)	
	4 (Mexico) (10)	
	1 (North America) (9)	
CLRN1 (Clarin 1)	2 (Cuba) (20)	1.58
	1 (Brazil) (8)	
CDHR1 (cadherin related family member 1)	1 (Brazil) (8)	1.05
	1 (Mexico) (9)	
CERKL (Ceramide Kinase Like)	7 (Brazil) (8)	4.21
	1 (Mexico) (9)	
CRB1 (Crums cell polarity complex component 1)	5 (Brazil) (8)	3.68
	2 (Mexico) (9)	
MERTK (MER proto-oncogene, tyrosine kinase)	6 (Brazil) (8)	3.68
	1 (Mexico) (9)	
PDE6A (Phosphodiesterase 6 A)	2 (Brazil) (8)	2.11
	2 (Mexico) (9)	
PDE6B (Phosphodiesterase 6 B)	1 (North America) (10)	0,53
RDH12 (Retinol dehydrogenase 12)	3 (Mexico) (9)	2.63
	1 (Brazil) (8)	
	1 (North America) (10)	
	190	100

N/R: Not reported

Table 4. *Autosomal recessive retinitis pigmentosa. Syndromic and no syndromic genes identified in the American population*

Gene	Nucleotide variant	Protein variant-exon	# Families	# patients/n	Country
BBS1 (Bardet-Biedl syndrome 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (8)
BBS2 (Bardet-Biedl syndrome 2)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (8)
CDHR1 (cadherin related family member 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (8)
CNGA1 (cyclic nucleotide-gated channel subunit alpha 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (8)
CNGB1 (cyclic nucleotide-gated channel subunit beta 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=3	Brazil (8)
EYS (Eyes shut homolog)	Not reported	Not reported	1,159	1,246 (121 with RP) n=16	Brazil (8)
GPR98†	Not reported	Not reported	1,159	1,246 (121 with RP)	Brazil (16)

				n=1	
HGSNAT (heparan-alpha-glucosaminide N-acetyltransferase)	Not reported	Not reported	1,159	1,246 (121 with RP) n=2	Brazil (8)
RP1 (retinitis pigmentosa 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=7	Brazil (8)
DHDDS (dehydrodolichyldiphosphate synthase subunit)	Not reported	p.Lys42Glu		275, n=3	North America, Jewish ancestry (8)
MKS1 (transition zone complex subunit 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (17)
PRPH2 (Peripherin 2)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (8)
ABHD12 (Abhydrolase domain containing 12, lysophospholipase)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (8)
MYO7A (Myosin7A)	Not reported	Not reported	1,159	1,246 (121 with RP) n=8	Brazil (8)
	c.6079_6081del	p.H2027del	1	12, n=3	Venezuela (8)
TULP1 (TUB like protein 1)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (8)
SAG S-antigen visual arrestin	c.440G>T	p.Cys147Phe	300	12 families	North America (8)
WDR19 (WD repeat domain 19)	Not reported	Not reported	1,159	1,246 (121 with RP) n=1	Brazil (19)
	c.G3533A c.A2561C	p.R1178Q p.K854T	Not reported	35, n=1	North America (8)
CDH23(Cadherin – related 23)	c.7730_7734delTCAGT c.1624G>T	p. Phe2577Serfs∗28 p. Glu542∗	11	11	Cuba (10)
ADGRV1 (adhesion G protein-coupled receptor V1)	c.15448_15449delCT c.15448_15449delCT	p. Leu5150Hisfs∗6 p. Leu5150Hisfs∗6	11	11	Cuba (20)
PCDH15 (protocadherin related 15)	c.3661C>T	p. Gln1221∗	11	11, n= 4	Cuba (20)
RP1 retinitis pigmentosa 1	c.C1625G c.C4105T	p.S542* p.Q1369*	Not reported	35, n=1	North America, Hispanic probands (20)
ABCA4(ATP binding cassette subfamily A member 4)	c.4919G>A	p. Arg1640Gln	Not reported	143	Mexico (10)
	c.T6179G c.G6089A ()	p.L2060R p.R2030Q)	Not reported	35, n=1	North America, Hispanic probands (9)
ARL6 (ADP ribosylation factor like GTPase 6)	c.373dupA	p. Ile125AsnfsTer7	Not reported	143	Mexico (10)
PCARE (photoreceptor cilium actin regulator)	c.947delA	p. Asn316MetfsTer7	Not reported	143	Mexico (21)
CLN3 (CLN3 lysosomal/endosomaltransmembrane protein, battenin)	c.266G>A	p. Arg89Gln	Not reported	143, n=1	Mexico (9)
RDH5(Retinol dehydrogenase5)	c.839G>A	p. Arg280His	Not reported	143, n=1	Mexico (9)
RP2 (retinitis pigmentosa 2)	NC_000023.10 (NM_006915.2): c. (?_−1) _ (768+1_769−1) del (exon 1–2 deletion) c.969+2T>G		Not reported	143, n=2	Mexico (9)
RPE65 (retinoidisomerohydrolase RPE65)	c.405T>A	p. Asn135Lys	Not reported	143, n=1	Mexico (9)
SPATA7 (spermatogenesis	c.322C>T	p. Arg108Ter	Not	143, n=1	Mexico (9)

associated 7)			reported		
BBS10 (Bardet-Biedlsyndrome 10)	c.39_46delGGCGTTGC	p. Ala14GlyfsTer79 (A14Gfs*79)	1	1 n=1	Colombia (9)
GNAT1(G protein subunit alpha transducin 1)	c.282delT	p. Ala95HisfsTer9	Not reported	143, n= 1	Mexico (9)
IFT140(intraflagellar transport 140)	c.1451C>T and c.2786delC	p. Thr484Met and p. Thr929SerfsTer21	Not reported	143, n= 1	Mexico (22)
IMPG2(interphotoreceptor matrix proteoglycan 2)	c.3093_3097dupTGGAG and c.2038delG	p. glu1033ValfsTer13 and p. Glu680SerfsTer21	Not reported	143, n=1	Mexico (9)
FAM161A **FAM161 centrosomal protein A**	c.1113 C. G/+ c.1133 T. G/+ c.1153 C. G/+ c.1391 A. G/+ c.1355_6delCA/c.1355_6delCA	p. Asp371Glu/+ p. Leu378Arg/+ p. Gln385Glu/+ p. His464Arg/+ p. Thr452SerfsX3/p. Thr452SerfsX3	Not reported	273, n=4	North America (9)
USH2A (Usherina 2 A)	Not reported	Not reported	1,159	1,246 (121 with RP), n= 21	Brazil (9)
	c.2229delG	Exon 13	Not reported	37, n=7	Colombia (17)
	c.545_56del AA c.775_76delAG C.847_48el GA c.921_22dupGCCA c.1012_16delCTCT c.1256G>A (TGT>TAT) c.1679delC c.1876C>T (CGA>TGA) c.2075C>A(TGC>TGA) c.2100delG c.2276G>T(TGC>TTC) c.2299delG c.2761delC c.2898delG c.3149_50delCA c.4223C>T(CAA>TAA) c.4338_39delCT c.4510_11insA	K182fs S259fs E284fs H308fs L342fs C419F P560fs R626X C691X G700FS C759F E767fs L921fs L967fs Q1063fs Q1408X L1447fs R1504fs	Not reported	275, n=275	North America (8)
	g.129G>T c.1000CT; p.R334W c.2299delG; pE767fs polymorphisms c.504A>G; p.168T c.931A>T; p.D644V c.4252-24_13delCTTT c.4457G>A; p. R1486K		Not reported	26, n=26	Colombia (23)
	c.2299delG c.1841-2A>G	p. Glu767Serfs∗21 p. Gly614Aspfs∗6	11	11	Cuba (24)
	c.2299delG	Not reported	Not reported	40, n= 11	Cuba (23)
	c.11387C>T c.907C>A and c.5218delA c.2332G>T and c.5836 C>T	p. Pro3796Leu p. Arg303Ser and p. Ile1740PhefsTer10 p. Asp778Tyr and p. Arg1946Ter	Not reported	143, n= 4	Mexico (20)

	c.11156 G>A and c.13348 C>T	p. Arg3719His and p. Pro4450Ser			
	c.G12575A c.C13664T	p.R4192H p.P4555L	Not reported	35, n=1	North America, Hispanic probands (25)
CLRN1(Clarin 1)	c.619C>T c.619C>T	p. Arg207∗	11	11, n= 2	Cuba (10)
	Not reported	Not reported	1,159	1,246 (121 with RP), n=1	Brazil (9)
CDHR1 (cadherin related family member 1)	Not reported	Not reported	1,159	1,246 (121 with RP), n=1	Brazil (20)
	c.963G>C and c.2041−2A>C	p. Gln321His	Not reported	143, n=1	Mexico (8)
CERKL (Ceramide Kinase Like)	Not reported	Not reported	1,159	1,246 (121 with RP), n=7	Brazil (8)
	c.1633_1636dupATCA c.847C>T c.424_427delAATT and c.1032_1039dupTGGGTTCT	p. Ser546AsnfsTer21 p. Arg283Ter c.424_427delAATT p. Asn142Ter and p. Ser347LeufsTer77	Not reported	143, n=1	Mexico (9)
CRB1 (Crums cell polarity complex component 1)	Not reported	Not reported	1,159	1,246 (121 with RP), n= 5	Brazil (8)
	c.2290C>T c.1125C>G	p. Arg764Cys c.1125C>G p. Tyr375Ter	Not reported	143, n=2	Mexico (9)
MERTK (MER proto-oncogene, tyrosine kinase)	Not reported	Not reported	1,159	1,246 (121 with RP), n =6	Brazil (8)
	c.2531G>A	p. Arg844His	Not reported	143, n=1	Mexico (9)
PDE6A (Phosphodiesterase 6 A)	Not reported	Not reported	1,159	1,246 (121 with RP), n=2	Brazil (8)
	c.2302G>T c. 1705 C>A c.1684 C>T	p. Glu768Ter p. Gln569Lys p. Arg562Trp	Not reported	143, n=2	Mexico (8)
PDE6B(Phosphodiesterase 6 B)	c.703delC	p.L235Wfs*33	Not reported	35, n=1	North America, Hispanic probands (9)
RDH12(retinol dehydrogenase 12)	c.446T>C c.295C>A c.446T>C	p. Leu149Pro p. Leu99Ile p. Leu149Pro	Not reported	143, n=3	Mexico (8)
	Not reported	Not reported	1,159	1,246 (121 with RP), n=1	Brazil (9)
	c.C146A c.C295A	p.T49K p.L99I	Not reported	35, n=1	North America, Hispanic probands (10)

X-linked RP

In this review, four genes associated with xlRP were identified. RP2 and RPGR were reported in Brazil and North America, while RP3 and CHM were found only in North America. RPGR had the highest frequency of occurrence, followed by RP2 (Table 5). The syndromic and non-syndromic genes identified in the American population are listed in Table 6.

Table 5. *X-linked retinitis pigmentosa mutations in America*

Gene	Probands (n)/Country	Percentage
RPGR (retinitis pigmentosa GTPase regulator)	19 (Brazil) (8)	86.36
	N/R (North America) (26)	
	1 (North America) (10)	
	N/R (North America) (27)	
RP2 (Retinitis pigmentosa 2)	2 (Brazil) (8)	9.09
	N/R (North America) (26)	
	N/R (North America) (27)	
CHM (Rab escort protein)	1 (North America) (10)	4.55
OFD1 (RP23)	N/R (North America) (28)	0.00
	22	100

N/R: not reported

Table 6. *X-linked retinitis pigmentosa. Syndromic and no syndromic genes identified in the American population*

Gene	Nucleotide variant	Protein variant-exon	# families	# patients/n	Country
RP2 (retinitis pigmentosa 2)	Not reported	Not reported	1,159	1,246 (121 with RP), n=2	Brazil (8)
	409-411del 650-351del 515insG 670insC IVS1+3A→G 82C→G 200G→A 353G→A 565T→C	Ile37del, exon2 Phe117fsTer 155 exon 2 Ser172fsTer173, exon 2 Arg225fsTer234, exon 2 - - - Tyr27Ter, exon 1 Cys67Tyr, exon 2 ARG118His, exon 2 Leu188pro, exon 2	234	n=171	North America (26)
	c.688_692del del EX04-flanking	p. Lys230Glnfs*3 del EX04-flanking	56	n=19 Families	North America (10)
RPGR (retinitis pigmentosa GTPase regulator)	Not reported	Not reported	1,159	1,246 (121 with RP), n=19	Brazil (8)
	8C→A IVS1-15A→G 212C→T 1146T→A 1223G→T IVS10+16A→G 1333G→A 1350A→G 1354A→G 1426A→G IVS12-101t→A IVS12-100 1-bpins IVS12-97T→C	Promoter Intron 1 Exon 3 Exon 10 Exon 10 Intron 10 Exon 11 Exon 11 Exon 11 Exon 11 Intron 12 Intron 12 Intron 12 Intron 12	234	n=185	North America (26)

	IVS12-93 2-bpins IVS12+11A→G 1635-1637del 1657C→T 1746G→A IVS16-137T→A IVS17+46C→T IVS18-11T→C	Intron 13 Exon 14 Exon 14 Exon 14 Intron 16 Intron 17 Intron18			
	c.2333delA	p.E778Pfs*83	Not reported	35, n=1	North America, Hispanic probands (27)
	c.194G > A c.297_306del c.865A > G c.934þ1G > T c.1377_1378del c.1573-8A > G c.1636G > T c.2188G > T c.2212G > T c.2218G > T c.2340del c.2405_2406del c.2442_2445del c.2517_2518del c.2625dupA c.2763_2764del c.3106del	p. Gly65Asp p. Leu100Glnfs*30 p. Ile289Val Splicing p. Leu460Ilefs*2 Splicing p. Glu546X p. Gly730X p. Gly738X p. Glu740X p. Ala781Argfs*34 p. Glu802Glyfs*32 p. Gly817Lysfs*2 p. Glu841Glyfs*237 p. Gly876Argfs*203 p.Glu922Glyfs*156 p.Glu1036Lysfs*53	56	n=19 Families	North America (10)
CHM Rab escort protein	c.116+1G>A	(p.?)	Not reported	35, n=1	North America, Hispanic probands (28)

Simple forms of RP

A total of 21 studies conducted in Mexico and North America reported 14 gene mutations linked to sporadic cases of RP. In cases with undetermined inheritance patterns, USH2A and RPE65 were the most frequently identified genes. ABCA4, RP1, RDH12, RPE65, and USH2A were associated with arRP, SNRP200 with adRP, and RPGR with xlRP in the Mexican population (Table 7). A complete list of syndromic and non-syndromic genes identified in the American population is provided in Table 8.

Table 7. *Mutations for sporadic cases in America*

Gene	Probands (n)/Country	Percentage
USH2A (Usherin 2 A)	1 (Mexico) (9)	4.76
	3 (North America) (10)	14.29
RPE 65 (retinoidisomerohydrolase RPE65)	3 (Mexico) (9)	14.29
ABCA4 (ATP binding cassette subfamily A member 4)	1 (Mexico) (9)	4.76
CFAP410 (cilia and flagella associated protein 410)	1 (North America) (10)	4.76
CERKL (Ceramide Kinase Like)	1 (Mexico) (9)	4.76
IDH3B (isocitrate dehydrogenase (NAD (+)) 3 non-catalytic subunit betas)	1 (Mexico) (9)	4.76
IFT140 (intraflagellar transport 140)	1 (Mexico) (9)	4.76
RDH12 (Retinoldehydrogenase 12)	2 (Mexico) (9)	9.52
RHO (Rhodopsin)	1 (North America) (10)	4.76
RP1 (Retinitis pigmentosa1)	1 (Mexico) (9)	4.76
RPGR (retinitispigmentosa GTPase regulator)	1 (Mexico) (9)	4.76
	1 (North America) (10)	4.76
PRPF8 (pre-mRNA processing factor 8)	1 (North America) (10)	4.76
PDE6B (Phosphodiesterase 6 B)	1 (North America) (10)	4.76
SNRNP200 (Putative U5 small nuclear ribonucleoprotein 200 kDa helicase)	1 (Mexico) (9)	4.76
	21	100.00

N/R: not reported

Table 8. *Simple retinitis pigmentosa. Syndromic and no syndromic genes identified in the American population*

Gene	Nucleotide variant	Protein variant-exon	# families	# patients/n	Country
ABCA4 (ATP binding cassette subfamily A member 4)	c.1417_1420dupATTA and c.5196+1G>A	p. Thr474AsnfsTer4	Not reported	143, n=1	Mexico (9)
CFAP410(cilia and flagella associated protein 410)	c.G218C c.G364C	p.R73P p.D122H	Not reported	35, n=1	North America, Hispanic probands (10)
CERKL (Ceramide Kinase Like)	c.847C>T	p. Arg283Ter	Not reported	143, n=1	Mexico (9)
IDH3B (isocitrate dehydrogenase (NAD (+)) 3 non-catalytic subunit betas)	c.857G>A	p. Gly286Glu	Not reported	143, n=1	Mexico (9)
IFT140 (intraflagellar transport 140)	c.386T>G c.1377G>A	p. Leu129Trp p. Trp459Ter	Not reported	143, n=1	Mexico (10)
RDH12 (retinol dehydrogenase 12)	c.295C>A c.295C>A and c.697G>C	p. Leu99Ile p. Leu99Ile and p. Val233Leu	Not reported	143, n=2	Mexico (9)
RHO (Rodopsin)	c.C408A	p.Y136*	Not reported	35, n=1	North America, Hispanic probands (9)

RP1 (retinitis pigmentosa1)	c.3150delA	p. Lys1050AsnfsTer7	Not reported	143, n=1	Mexico (9)
RPE 65 (retinoid isomerohydrolase RPE65)	c.131G>A and c.61delG c.386 C>T and c.1067dupA c.95−2A>T	p. Arg44Gln and p. Glu21AsnfsTer10 p. Thr129Ile and p. Asn356LysfsTer9 Not reported	Not reported	143, n=3	Mexico (9)
RPGR (retinitis pigmentosa GTPase regulator)	c.1859_1860delAG	p. Lys620ArgfsTer9	Not reported	143, n=1	Mexico (10)
	c.G494A	p. G165D	Not reported	35, n=1	North America, Hispanic probands (9)
PRPF8 (pre-mRNA processing factor 8)	c.C5041T	p. R1681W	Not reported	35, n=1	North America, Hispanic probands (9)
PDE6B (Phosphodiesterase6 B)	c.G704C ()	p. R235P	Not reported	35, n=1	North America, Hispanic probands (10)
USH2A (Usherin 2 A)	c.12575G>A and c.3629T>C	p. Arg4192His and p. Leu1210Pro	Not reported	143, n=1	Mexico (10)
	c.G12575A c.T9799C c.1841-2A>G c.G8254A c.T12443C	p.R4192H p.C3267R (p.?) p.G2752R p. L4148P	Not reported	35, n=3	North America, Hispanic probands (10)
SNRNP200 (Putative U5 small nuclear ribonucleoprotein 200 kDa helicase)	c.3260C>T	p. Ser1087Leu	Not reported	143, n=1	Mexico (9)

DISCUSSION

RP is the most prevalent form of retinal dystrophy (3, 29, 30) and is characterized by significant genetic heterogeneity. Mutations in the same gene can lead to varying clinical presentations across different individuals (31). Additionally, the frequency of specific RP-related mutations varies between populations (32).

This review of RP genetic characterization in American populations identified Brazil as the country with the highest number of reported genes, while most of the included publications originated from North America. The arRP had the largest number of associated genes reported, followed by adRP, and then sporadic (simple) RP. In contrast, only three genes were identified in association with xlRP.

Among RP subtypes, adRP generally has a more favorable prognosis compared to others (32). In this review, the RHO and PRPF31 genes were found in four and three American countries, respectively. Mutations in PRPF31 were also reported as the third most common cause of adRP in several countries, including China, France, India, Japan, and the USA. Similarly, mutations in RHO, PRPF31, and RP1 were documented in both Indian and Belgian populations (33-35).

Although new mutations and genes associated with RP subtypes continue to be discovered each year through ongoing research (3), RHO mutations remain significant—accounting for approximately 30% of cases in Americans of European descent and 10% in Chinese patients (32). Additionally, RHO mutations have been reported in individuals from Israel, Palestine (36), Spain (37), Korea (38), Sweden, and Iran. A study on adRP in Italian families, consistent with this review's findings, identified RHO as the most frequently involved gene, with mutations found in 16% of the families. The second most commonly implicated gene in Italy was RP1, whereas PRPF31 mutations were not reported in that cohort (39). Similarly, a study from France found RHO and PRPH2 to be the most frequent genes involved in

adRP, while PRPF31 and RP1 were less commonly reported (40).

There are an estimated 55 genes believed to contribute to arRP, accounting for about 2–5% of all RP cases (40, 41). USH2A mutations are a major cause of non-syndromic arRP globally. This review identified five countries with the highest occurrence of USH2A, which is also recognized as the leading genetic cause of the Usher syndrome—a condition that affects both vision and the auditory–vestibular system (3, 42). In Brazil, USH2A and MYO7A were the most commonly identified genes among RP patients (8). Another relevant gene is ABCA4, reported in both Brazil and North America. While primarily associated with inherited retinal dystrophies distinct from RP, ABCA4 mutations are commonly found in patients with Stargardt disease and, to a lesser extent, in cone-rod dystrophy and RP cases (43).

A study conducted in the Jerusalem region, known for its high consanguinity rate, found that 63% of cases had an arRP inheritance pattern, particularly in the Arab Muslim cohort. The most frequently identified genes were DHDDS, FAM161A, and EYS (44). These results differ from those of this review, where USH2A is the primary cause of RP in arRP cases.

In a study of a four-generation British family with adRP, mutations in the RDH12 gene were detected. In the USA, this gene was associated with arRP and sporadic RP cases (45). In a Japanese cohort, EYS was identified as the most common gene, affecting 44.5% of diagnosed patients, which contrasts with the findings in this review (46).

A longitudinal study in France, spanning 21 years, examined 21 families (33.3%) and found mutations in both alleles for MERTK, RDH12, RP1, RPE65, ABCA4, PDE6A, and CNGB1, while nine families had a single heterozygous mutation in USH2A, BBS1, LRAT, and RPE65 (47). These results differ from the most common genes identified in this review.

A study on IRDs in France, where RP was the most common, found mutations for xlRP in RP2 (7.7%), RPGR (76.9%), and unidentified mutations (15.4%) (47). Six loci were implicated in xlRP, the most severe form of RP. As observed in this review, RPGR and RP2 are the main genes associated with xlRP (found in 70–90% and 6–20% of cases, respectively). These mutations also cause xlRP in the Japanese population, with significant visual impairment (48). RPGR mutations are present in 30% of Americans of European descent and 10% of Chinese patients (5, 32). Additionally, in 30–60% of xlRP cases, 17 mutations in the ORF15 region of RPGR were implicated (41).

Table 9. *Functions of genes associated with adRP, arRP, xlRP, and simplex RP*

Inheritance pattern	Gene	Function
Autosomal dominant	*RHO*	Light absorption (50)
	PRPF31	RNA splicing (51)
Autosomal recessive	*USH2A*	Development and maintenance of cells of the inner ear and retina (52)
	EYS	Maintenance of the integrity and protein trafficking of photoreceptor cells (52)
	MYO7A	Renewal of the outer photoreceptor disc, distribution, and migration of Retinal pigment epithelial melanosomes and phagosomes, regulation of opsin transport (51)
X-linked	*RPGR*	Exact function unknown (53)
	RP2	Maintenance of Golgi cohesion and targeting of proteins to the plasma membrane (54)
Simplex	*USH2A*	Development and maintenance of cells of the inner ear and retina (52)
	RPE65	Regeneration of the visual pigment necessary for both rod and cone-mediated vision (55)
	RDH12	Part of the visual cycle, reduction of all-trans and all-cisretinoids (56)

adRP: Autosomal dominant retinitis pigmentosa, arRP: Autosomal recessive retinitis pigmentosa, xlRP: X-linked retinitis pigmentosa, and simple retinitis pigmentosa

Simplex or isolated cases refer to patients who do not have affected first-degree relatives and no reports of more distant family members being affected. For these cases, USH2A and RPE65 were identified as the most common causative genes for RP. This aligns with a study on Spanish families, where USH2A was found to be more prevalent; however, no mention of RPE65 mutations was made (49). Table 9 summarizes the main physiological functions that may be affected by gene mutations and lists the most frequently implicated causative genes associated with the different inheritance patterns of RP.

Finally, several patient organizations exist in the region, with some of the most well-known being Alianza de Retinosis Pigmentaria Argentina (ARPA), Fundación Argentina de Retinosis Pigmentaria (FARPA) (https://retinosis.org/fundacion-argentina-de-retinosis-pigmentaria), Asociación Colombiana de RP (ACORP), Asociación Retina Brasil, Asociación Retina Sao Paulo (http://retinaiberoamerica.org/retina-sao-paulo), undación Lucha contra la Retinosis Pigmentaria in Chile (FUNDALURP) (http://fundalurp.cl), Fundación Retina República Dominicana, Fundación de Retinitis Pigmentosa in Puerto Rico (https://retinitispigmentosapr.com), and Asociación Nacional de Retinosis pigmentaria in Mexico (https://www.facebook.com/retinosispigmentariamexico). These organizations are part of Retina Iberoamerica and Retina Internacional. They work to improve patient access to research, facilitate participation in controlled clinical trials worldwide, and provide emotional support and basic information about the disease (56).

The main limitation of this review was the lack of available literature from all American countries, particularly from Latin America. Additionally, some studies reported the presence of gene mutations but did not specify the number of affected probands.

CONCLUSION

Most of the genes currently identified as causing RP worldwide are also present in America. In the American population, the most common genes associated with adRP are RHO, PRPF31, and SNRPN200. The most frequent genes linked to arRP are USH2A, EYS, and MYO7A; for xlRP, RPGR and RP2 are implicated, while in simplex forms, USH2A, RPE65, and RDH12 are the primary genes involved.

There are similarities in the genetic characterization of RP between America and European countries, particularly for adRP and xlRP, although there are notable differences when compared to Asian populations, which show a greater variety in the presentation of gene mutations.

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Competing interests

Authors declare no conflicts of interest.

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Geni povezani sa retinitisom pigmentozom u populaciji Amerike: sistematski pregled

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SAŽETAK

Uvod/Cilj. Retinitis pigmentoza (RP) predstavlja heterogenu grupu naslednih bolesti mrežnjače, koje karakteriše postepena degeneracija štapičastih i kupastih foto-receptora. Bolest se primarno nasleđuje, a u njenoj patogenezi učestvuje velik broj različitih genskih mutacija. Cilj ovog istraživanja bio je da sumira rezultate studija koje su ispitivale gene povezane sa retinitisom pigmentozom kod bolesnika sa autozomno dominantnim (adRP), autozomno recesivnim (arRP) i X-vezanim (xlRP) obrascem nasleđivanja na području Amerike.

Materijal i metode. Sprovedena je sveobuhvatna pretraga literature u bazama podataka *Medline*/*PubMed, SciELO, Redalyc, ScienceDirect* i *Google Scholar* (na engleskom i španskom jeziku). U pregledu je analizirano sedamdeset pet radova objavljenih između 2010. i 2020. godine, a u finalnu analizu uključena je dvadeset jedna studija.

Rezultati. Najčešće mutacije gena identifikovane kod bolesnika sa adRP-om u Americi bile su RHO (rodopsin) i PRPF31 (faktor prerade pre-mRNK 31), a kod bolesnika sa arRP-om USH2A (ušerin 2A) i EYS (homolog proteina „zatvorenih očiju"). Mutacije gena RPGR (regulator GTP-aze za retinitis pigmentozu) i RP2 (retinitis pigmentoza 2) bile su dominantne kod bolesnika koji su imali xlRP.

Zaključak. Većina gena koji su širom sveta identifikovani kao uzročnici RP-a pronađena je i kod bolesnika u Americi, s tim što su uočene određene sličnosti i razlike u poređenju sa populacijom u Aziji i Evropi.

Ključne reči: **autozomno dominantno, autozomno recesivno, obrazac nasleđivanja, retinitis pigmentoza, X-vezano nasleđivanje**

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Original article

Vascularization, Proliferative Activity and the p53 Status in Glioblastomas

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SUMMARY

Introduction/Aim. Glioblastomas (GBMs) are among the most vascularized human tumors and the presence of microvascular proliferation is one of the diagnostic hallmarks of these malignancies. The aim of the present study was to investigate the extent of vascularization and its relation to proliferative activity and the p53 status in GBMs.
Methods. Tissue samples from 100 selected primary GBMs were analyzed by immunohistochemistry for the expression of CD34 in vascular endothelial cells and Ki-67 antigen (using the MIB-1 antibody) and p53 in tumor cells. The microvessel density (MVD), a measure of the extent of tumor vascularization, was evaluated in CD34-immunostained sections in three hot spots and presented as the mean for each tumor specimen.
Results. We found that the high MVD was more frequent in tumors showing the high MIB-1-labeling index (MIB-1 LI) as compared to those with the low MIB-1 LI, but the difference was not statistically significant. Also, the extent of vascularization did not differ significantly between p53-negative and p53-positive tumors. Both the level of MVD and the proportion of GBMs with low versus high MVD did not differ significantly in relation to the expression levels of p53 (low vs. high or overexpression). No association was found between MVD and tumor cell MIB-1 LI and the p53 status in primary GBMs.
Conclusion. These data suggest that the effect of p53 on primary GBM vascularization failed to detect possibly due to the influence of certain factors, including the presence of other or additional molecular alterations in the tumor cells and the hypoxic microenvironment of tumors. They also support the hypothesis that the effect of p53 on angiogenesis may be tumor-type specific.

Keywords: **glioblastoma, microvessel density, angiogenesis, immunohistochemistry, MIB-1 proliferation index, p53**

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INTRODUCTION

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults and has a poor prognosis (1, 2). Despite advances in therapy, most patients with GBM die within two years (2, 3), and the overall five-year survival rate is only 5.5% (1). Glioblastomas (GBMs) are highly invasive, hypoxic and hypervascular in nature, and are among the most vascularized human tumors (4). The presence of microvascular proliferation (MVP) is one of the diagnostic hallmarks of GBM (5). Tumors can use several mechanisms to acquire blood supply, including co-option of the pre-existing vasculature by tumor cells, angiogenesis, vasculogenesis, intussusception and vasculogenic mimicry (6). The formation of new blood vessels via the process of angiogenesis (the sprouting of vessels from the pre-existing ones) in GBMs is thought to be crucial for their growth (7). In addition, neovascularization can be supported by other mechanisms such as vasculogenesis (the recruitment of bone marrow-derived circulating endothelial progenitor cells, which differentiate and incorporate into the tumor vessels) (8, 9), and vasculogenic mimicry, in which GBM stem cells transdifferentiate into vascular endothelial cells (10, 11) and vascular mural cells (12).

Neovascularization of brain tumors (particularly in GBMs) is thought to be driven mainly by vascular endothelial growth factor (VEGF) signaling via its vascular endothelial growth factor receptor 2 (VEGFR2) (13). VEGF, a major pro-angiogenic factor, is expressed at high levels in these tumors (4). In GBMs, both tumor hypoxia and genetic alterations (such as EGFR amplification and inactivation of p53, PTEN) together induce the expression of VEGF and other pro-angiogenic factors via hypoxia-inducible factor-1 (HIF-1), resulting in the angiogenic response (7). The HIF-1-independent mechanisms are also implicated in the tumor vessel formation (4, 7). However, the newly-formed vessels are structurally and functionally abnormal (6, 13). Consequently, tumors develop multiple regions of hypoxia and the ensuing foci of palisading necrosis that are linked with adjacent florid MVP (14).

The p53 tumor suppressor protein plays the crucial role in protecting against neoplastic transformation. The p53 protein functions, at least in part, as a transcription factor regulating the expression of target genes that have an important role in mediating cell-cycle arrest, DNA repair, apoptosis, and senescence (15). In addition, the p53 protein has also been attributed to an angiogenesis-regulating function via interference with HIF-1α (the subunit of the heterodimer HIF-1 and a central responder to hypoxia), downregulation of pro-angiogenic factors, including VEGF and basic fibroblast growth factor (bFGF), and upregulation of angiogenesis inhibitors, including thrombospondin-1 (TSP-1), brain-specific angiogenesis inhibitor-1 (BAI-1) and collagen propyl-4-hydroxylase α2 (P4HA2) (16).

Ohgaki et al. reported that mutations of the TP53 gene occurred in two-thirds of precursor low-grade diffuse astrocytomas, having a crucial role in the development of secondary GBMs derived thereof, whereas in primary (de novo) GBMs, TP53 mutations are less frequent (< 30% of cases) (17). The incidence of p53 protein accumulation (nuclear immunoreactivity for p53) is also lower in primary than in secondary GBMs (18).

The aim of the present study was to investigate the extent of vascularization and its relation to proliferative activity and the p53 status in glioblastomas.

MATERIAL

Patients and tissue samples

A series of one hundred selected adult patients with primary GBM (WHO grade IV) which had been diagnosed at the Center for Pathology, Niš, Serbia, between 2004 and 2011, was included in this retrospective study. Patient selection was based on the availability of paraffin-embedded tissue and corresponding clinicopathologic data. None of the GBM patients had undergone radiotherapy or chemotherapy before surgical intervention. All tumor tissue samples were obtained by resection. Tumor specimens from each case were reviewed to confirm the diagnosis of GBM, before inclusion in the study, according to the WHO criteria (5).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections were stained with standard hematoxylin and eosin (H&E) for morphologic analysis (the presence of tumor tissue and histopathological criteria of malignancy, including MVP). Representative pa-

raffin blocks for immunohistochemistry (IHC) were selected based on H&E-stained sections. IHC was done on 5-μm-thick deparaffinized sections using the following primary monoclonal antibodies: anti-CD34 (clone QBEnd 10, 1:50, Dako), anti-Ki-67 (clone MIB-1, 1:50, Dako), and anti-p53 (clone DO-7, 1:60, Dako). After the microwave pretreatment, the sections were incubated overnight at 4 °C with the diluted primary antibodies. Detection of immunestaining was performed using standard labeled streptavidin-biotin peroxidase technique (LSAB2 Kit/HRP, Dako) according to the manufacturer's instructions, and diaminobenzidine was used as chromogen. The sections were then counterstained with hematoxylin and mounted.

Evaluation of staining results

The staining results of IHC were evaluated by two investigators (I.D. and D.T.) independently. When the evaluation was different, the final decision was made by consensus. The number of immune-positive endothelial and tumor cells was counted using light microscope (Leica, Germany). Cytoplasmic staining for CD34 and nuclear staining for Ki-67 and p53 were interpreted as being immune-positive.

The MIB-1 labeling index (MIB-1 LI) was obtained by manually counting the positively stained tumor cell nuclei in the areas of their highest density (immunoreactivity for Ki-67 antigen). A total of 1000 tumor cell nuclei were evaluated in each specimen and the percentage of labeled nuclei relative to the total number of tumor cell nuclei was calculated. The mean MIB-1 LI for all GBMs investigated served as the cut-off value.

The p53-positive tumor cell nuclei were also determined by counting 1000 tumor cells in the most stained areas for each specimen. GBMs with more than 10% of p53 stained tumor cell nuclei were estimated as p53-positive, otherwise as negative (19). Immunostaining for p53 in more than 50% of tumor cells (19, 20) was considered as high expression (or overexpression) of the p53 protein, otherwise as low.

Measuring microvessel density

The extent of GBM vascularity was determined quantitatively by measuring microvessel density (MVD). The intratumoral MVD was measured in the CD34 immunostained GBM sections, as described elsewhere (21). The areas of highest vascularization ("hot spots") were selected by scanning the tumor sections at low magnification (x 40 and x 100). MVD was than determined by counting manually all immunolabeled vessels on a x 200 magnification field. In addition to recognizable microvessels, any brown staining of endothelial cell or endothelial cell cluster clearly separated from adjacent microvessels, tumor cells or other connective tissue elements was regarded as a single countable microvessel. Due to the uneven distribution of vascular structures in GBM tissues, the microvessels were counted in three selected fields (hot spots) in each specimen. MVD was expressed as the mean of three hot spots (the mean number of microvessels per x 200 field for each case). The mean MVD for all tumor specimens served as the cut-off: the level of MVD was classified as low if less than the mean, otherwise as high.

Statistical analysis

The statistical analysis was performed using SPSS 16.0 software. All data except for p53 are presented as mean ± standard deviation. The association between MVD and MIB-1 LI and the p53 protein status was determined using the Chi-squared test or the Student's t-test. P values of < 0.05 were considered to be statistically significant.

RESULTS

A series of 100 selected adult patients with primary GBM who underwent tumor resection, without prior adjuvant therapy, were included in the present study. There were 64 males and 36 females. The age of patients ranged from 33 to 78 years with a mean age of 61 years. All tumors analyzed showed microvascular proliferation (endothelial cell proliferation of the various degree was detected on H&E stained sections and by immunostaining of serial sections for Ki-67 antigen and CD34 antigen) (Figure 1). MVP with the formation of glomeruloid vascular structures was observed in most GBM cases (approximately 70%), which were unevenly distributed in tumor tissues.

Microvessel density was a preferred parameter to quantify the extent of vascularization in tumors. In the present study, MVD was evaluated in the CD34-immunostained tumor sections. CD34-positive staining was observed in vascular endo-

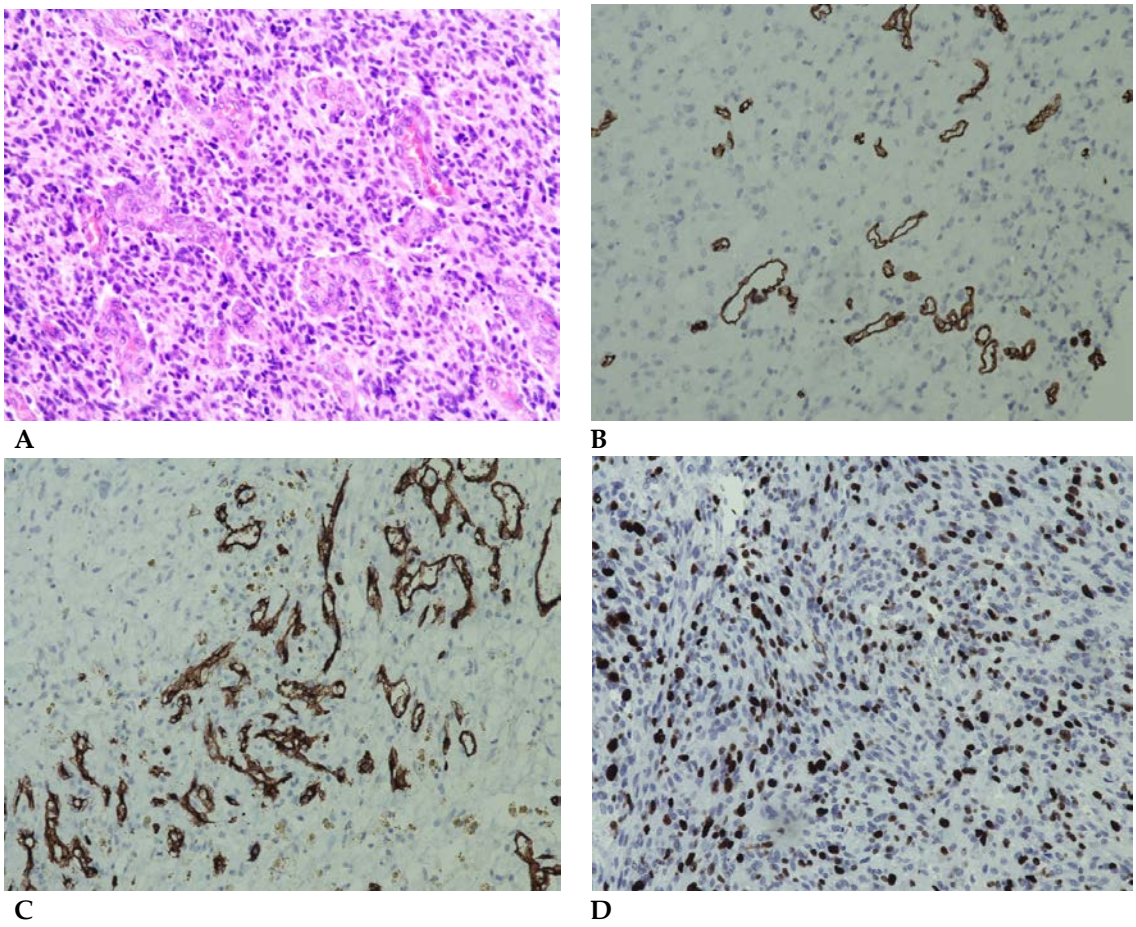


Figure 1. *The presence of microvascular proliferation illustrated by H&E (A) and CD34 immunostained sections (B) of primary glioblastomas. Different microvascular formations illustrated by immunostaining for CD34 (C). Primary glioblastoma with a high MIB-1 labeling index (D). Original magnification x 200 (A-D).*

Table 1. *Association of MVD with MIB-1 labeling index and the p53 status in primary glioblastomas (n = 100)*

Variables	Low MVD		High MVD		P value*
	< 101 (n = 52)		≥ 101 (n = 48)		
MIB-1 LI					
< 27	30	57.7	22	42.3	0.324
≥ 27	22	45.8	26	54.2	
p53 protein status					
p53- negative	22	44.9	27	55.1	0.233
p53- positive	30	58.8	21	41.2	

MVD: microvessel density
Data presented as n (%)
*Chi-squared test

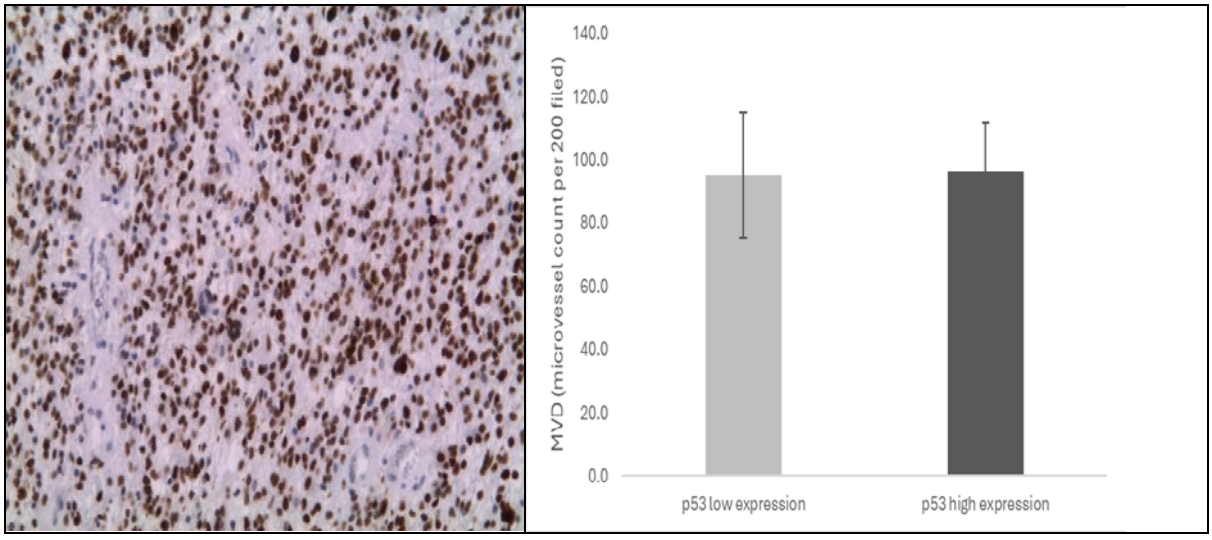
thelial cells, including microvessels, single endothelial cells and endothelial cell clusters (Figure 1B and C). The mean MVD for all primary GBMs was 101.55 ± 23.32 per x 200 microscopic field.

MVD and MIB-1 labeling index and p53 status

The proliferating fraction of tumor cells was evaluated using the MIB-1 antibody for Ki-67 antigen that is expressed in all phases of the cell cycle except GO. The MIB-1 labeling index was expressed as a percentage of positively labeled tumor cell nuclei per total nuclei counted. The mean MIB-1 LI was 27.15 ± 9.41 %. Using the cut-off value of MIB-1 LI, 52% of GBMs having a MIB-1 LI lower than 27% (Table 1). In this group, 30 tumors had the low level of MVD, while 22 had the high level of MVD. Additionally, in the group with the high value of MIB-1 LI (≥ 27%), 22 tumors showed the low level of MVD, while 26 had the high level of MVD (Figure 1, Table 1, Figure 1D). Therefore, the high MVD was more frequent in GBMs with the high MIB-1 LI, whereas the low MVD was more frequent in GBMs with the low MIB-1 LI. However, the difference was statistically not significant (p = 0.324). Consistently, intratumoral microvessel density was not associated with tumor cell MIB-1 labeling index.

Based on the findings that various human cancers carrying TP53 mutations or p53 protein accumulation were more vascularized than those with wild-type p53, and the data that p53 inhbits angiogenesis (16), we investigated the expression of p53 in primary GBMs to evaluate its effect on the extent of vascularization in these tumors. The monoclonal antibody for p53 used in the present study detects both wild- type and mutant p53 proteins. Of initially selected GBM samples with more than 10% of p53 immunostained tumor cell nuclei, 51% of tumors were identified as p53-positive. We found that the proportion of GBMs with a high MVD was larger in the p53-negative group as compared to those in the p53-positive group, but this difference was not statistically significant (p = 0.233) (Table 1).

To further evaluate the effect of the p53 protein status on the GBM vascularization, we excluded p53-negative tumors since they showed the EGFR overexpression (our unpublished data), as known that EGFR amplification and protein overexpression to promote tumor angiogenesis (GBM vascularization). Additionally, we selected tumors with more than 50% of immunostained tumor cell nuclei and this was considered as high p53 expression (or overexpression) (Figure 2A), otherwise as low. The high p53 expression was found in 22 % of GBMs investigated (Table 2). The comparison of MVD (< 101 vs. ≥ 101) with the expression levels of p53 (low vs. high) showed no significant difference



A B

Figure 2. *Primary glioblastoma with a high p53 expression (> 50% tumor cells exhibit the nuclear p53 accumulation). Original magnification x200 (A). MVD (microvessel density) values in tumors with low p53 expression and those with high p53 expression (B). Data are presented as mean ± standard deviation*

Table 2. *Association between MVD and the p53 expression in primary glioblastomas*

Variable	Low MVD		High MVD		P value*
	< 101 (n = 30)		≥ 101 (n = 21)		
p53 expression					
p53 - low	17	58.6	12	41.4	1.000
p53 - high	13	59.1	9	40.9	

MVD: microvessel density
The expression of p53 was divided into two groups: low and high expression (> 50% of immunostained tumor cell nuclei)
Data presented as n (%)
*Chi-squared test

(p = 1.000). In addition, results from the comparative microvessel counts in a low p53-expressing and a high p53-expressing primary GBM specimens failed to show a significant difference (p = 0.856, Figure 2B). Thus, no association was found between intratumoral MVD and the p53 status in primary GBMs.

DISCUSSION

GBMs are highly invasive, aggressive and vascularized malignancies. Despite advances in neurosurgery, radiotherapy and chemotherapy, the prognosis for patients with GBM remains poor (2). The temozolomide (TMZ) chemotherapy and the presence of O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation in GBMs confer with better tumor response to treatment and the survival advantage (22). Both the tumor suppressor protein p53 and MGMT are involved in DNA repair after the chemotherapy or radiotherapy that may contribute to drug resistance (23).

Microvascular proliferation is the defining histopathological phenotype of both primary and secondary GBMs (5, 24). In a series of primary GBMs investigated, the mean intratumoral MVD was found to be high, which reflects the extent of tumor vascularization. The results of our study are in agreement with previously reported data (25-27). Kiesel et al. found that the mean MVD was high in GBM samples with strong fluorescence induced by 5-ALA (5-aminolevulinic acid), which corresponded to compact tumors (27).

Several studies have investigated the proliferative activity in GBMs, especially MIB-1 (Ki-67) labeling index, but mean values of MIB-1 LI varied broadly (28-31). In the present study, the mean MIB-1 LI for primary GBM cases was among the highest as reported in the literature (20, 27-29). In addition, the cut-off value of MIB-1 LI in our study is close to the value reported by others (29). We also analyzed the relation of MVD to MIB-1 LI. The proportion of GBMs with the high MVD was not significantly different between the group with the high MIB-1 LI and the group of tumors with the low MIB-1 LI. There was also no significant difference between the low MVD and MIB-1 LI values (low vs. high). These data demonstrate that there is no association between MVD and tumor cell MIB-1 labeling index in a series of primary GBMs investigated. This finding is in agreement with data in previous studies (20, 25, 32). Since the tumor angiogenesis is a highly complex process that has both a genetic and hypoxic regulation (involving both the tumor cells and the associated microenvironment), it is possible that the extent of tumor vascularization does not correlate with the tumor cell proliferation as noted in the present study and studies mentioned above.

In addition to the crucial tumor suppressive function, the p53 protein has been attributed a role in the regulation of tumor angiogenesis (16). Mutation of the TP53 gene is common in GBMs, seen in 60-70% of secondary GBMs and 25-30% of primary GBMs (33). These TP53 mutations are associated with a poor prognosis for overall survival in GBM patients (23). Most TP53 mutations in GBMs are missense mutations (34), which lead to stabilization and nuclear accumulation of mutant p53 proteins, but the incidence of p53 protein accumulation is more frequent than TP53 mutations are (18). As previously reported (23), this discrepancy may be explained by the complex formation of p53 protein with other proteins (oncoproteins) that stabilize or

modify it. Therefore, the cut-off value (> 50%) for p53 was used in the present study, considering that the tumors with possible TP53 mutations show a high positive ratio (> 50%) for p53 (35). TP53 mutations are most common in the DNA-binding domain, and generally result in loss-of-function, gain-off-function and dominant -negative (mutational) effect for p53 (33). Several studies have demonstrated that mutant p53 possesses gained (oncogenic) functions, contributing to tumor growth and progression (23, 36). Also, TP53 mutation may decrease the chemosensitivity of GBM to TMZ by increasing MGMT expression (23).

To evaluate the effect of the p53 status on vascularity in GBMs, we analyzed the relation between MVD and p53 expression (p53-negative vs. p53-positive tumors, and ones with low vs. high p53 expression). The proportion of GBMs with a high MVD was larger in the p53-negative group as compared to the p53-positive, but this difference was not statistically significant. We found that the level of MVD in tumors with a high p53 expression (> 50%), which was suggestive for the presence of TP53 mutations, was not significantly higher than that in tumors with a low p53 expression. Additionally, the proportion of GBMs with a high MVD was not statistically different between low p53- and high p53-expressing tumors.

The present study revealed that the extent of vascularization in primary GBMs was not associated with the p53 protein status. These data are in line with previous findings from other studies (20, 37). Berger et al. investigated the value of the TP53 mutational status on the extent of vascularization in primary GBMs, and were found that neither total area nor total number of vascular structures differed significantly between p53 wild-type and p53 mutant tumors (37). Additionally, among the investigated angiogenesis-related target genes (VEGF, bFGF,TSP-1, BAI1, P4HA2), only P4HA2 mRNA was found to be upregulated by wild-type p53 overexpressed in LN-308 GBM cells but without increasing in protein levels, indicating that the p53/P4HA2-mediated anti-angiogenic pathway is defective in GBM cells, which is opposite to H1299 cancer cells (37). These data suggest that the effect of p53 on tumor angiogenesis may be cell or tumor-type specific and challenge the view of p53 as an angiogenesis-regulating factor in GBM (37).

Notably, in a study of diffuse low-grade astrocytomas, it was observed that MVD and absolute vessel number were increased in TP53 mutated tumors in comparison to their TP53 wild-type counterparts, indicating that p53 exerts an angiogenesis-inhibiting function in diffuse low-grade astrocytomas (38). Given that the TP53 mutations stand for an early event in the progression of astrocytomas (17, 18) and the formation of new blood vessels (neovascularization) characterizes the phenotype of GBMs (5), it seems that the influence of p53 on tumor angiogenesis may decrease during malignant progression with accumulated of additional molecular alterations in tumor cells and tumor hypoxia.

GBMs are known to be highly vascularized tumors with potent angiogenic activity (4,7). One common feature in the transition from low-grade or anaplastic astrocytomas to secondary GBM is a dramatic increase in MVP (24). An equivalently robust MVP is observed in primary GBM (24). Accordingly, several studies have demonstrated that MVD increased with an increase in the atrocytoma grade, being the highest in grade IV tumors (21, 25, 32, 39). The MVD was also observed to increase with an increase in pathologic grade of gliomas (predominantly astrocytomas) investigated (40). However, studies concerning the prognostic value of MVD in malignant astrocytomas (mainly GBMs) disclosed controversial results. Some studies revealed that intratumoral MVD was an independent prognostic factor (21, 39). In contrary, other studies revealed no prognostic value of MVD (41-43).

Besides the high MVD, GBMs usually present with a regionally heterogeneous vascularization. Therefore, in the present study, the MVD was evaluated in three hot spots and presented as the mean for each tumor specimen. The vascular patterns have been recognized as prognostic factor for GBMs (20). A large study of primary GBMs revealed a significant correlation of vascular patterns with patient outcome (20). It was observed that prominent classic (predominantly capillary-like) vascular pattern and low content of bizarre vascular pattern (predominance of glomeruli/garland/vascular clusters) was an independent factor for longer survival (20). It was also observed that in tumors with prominent classic vascular pattern, MVD was significantly higher though the MIB-1 LI did not differ significantly. In a systematic study of two large GBM series, both vascular parameters (MVD and vascular patterns) were evaluated retrospectively by multiple observers (42). MVD and vascular patterns were not

correlated with patient outcome. The authors also concluded that poor observer agreement limits the clinical utility of MVD and vascular patterns as prognostic factors and recommended that these vascular parameters need to validated (42).

The data concerning p53 suggest that it is not only involved in regulating tumor angiogenesis (16) but it can also been potentially implicated in tumor response to anti-angiogenic therapy (AAT) (44). Experimental studies in mice bearing tumors derived from TP53-/- colorectal cancer cells were showed to be less responsive to AAT than mice bearing isogenic TP53+/+ tumors (44). Moreover, the study concerning p53 showed that p53-positive microvascular proliferation cells exhibit TP53 mutations (identical to those in tumor cells) and tend to be clustered histopathologically in GBM tissues (35). These findings are in keeping with previous studies showing that GBM stem cells are capable of transdifferentiation into endothelial cells (10, 11) and mural cells (12) to promote vasculogenic mimicry (VM). It has been shown that the endothelial-like cells carry the same genetic alterations as tumor cells within GBM (such as TP53 mutation or EGFR amplification) (10, 11) and that anti-angiogenic treatments such as VEGF blockade can only partially inhibit this vasculogenic mimicry (11). Interestingly, one study showed that in high-grade astrocytomas, the level of MVD was lower in VM-positive tumors than those in VM-negative tumors (43). Findings from previous studies suggest that VM as an alternative mechanism in GBM vascularization may reduce responsiveness to AAT. Anti-angiogenic therapies were used (as monotherapy or combined with chemoradiotherapy) mainly against VEGF and its receptors to normalize the tumor vasculature in patients with GBM, however, with no significant survival benefit to patients (13, 45).

CONCLUSION

In the present study of 100 selected primary GBMs, the mean intratumoral MVD evaluated in three hot spots was found to be high, which reflects the extent of vascularization in these malignancies. No association was found between MVD and tumor cell MIB-1 labeling index and the p53 status. The extent of vascularization did not differ significantly between p53-negative and p53-positive tumors or between ones expressing low and high levels of p53. These findings suggest that the effect of p53 on primary GBM vascularization failed to detect possibly because of the influence of certain factors, including the presence of other or additional molecular alterations in the tumor cells and the hypoxic microenvironment of tumors. However, our study has some limitations since it was retrospective and obtained data concerning the p53 status based on immunohistochemistry. In this context, further studies will be required.

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Vaskularizacija, proliferativna aktivnost i p53 status u glioblastomima

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SAŽETAK

Uvod/Cilj. Glioblastomi (GBM) spadaju u najviše vaskularizovane tumore kod ljudi, a prisustvo mikrovaskularne proliferacije predstavlja jedno od dijagnostičkih obeležja ovih maligniteta. Cilj ove studije bio je da ispita stepen vaskularizacije u odnosu na proliferativnu aktivnost i p53 status GBM-a.
Metode. Imunohistohemijski je u uzorcima tkiva 100 odabranih primarnih GBM-a analizirana ekspresija CD34 u vaskularnim endotelnim ćelijama, Ki-67 antigena (primenom MIB-1 antitela) i p53 u tumorskim ćelijama. Mikrovaskularna gustina (MVG), mera stepena vaskularizacije tumora, određivana je na CD34-imunobojenim presecima u trima „vrućim tačkama" i prikazana kao prosečna vrednost za svaki uzorak tumora.
Rezultati. Visok MVG je bio češći nalaz u tumorima sa visokim MIB-1 indeksom nego u onima koji su imali nizak MIB-1 indeks, ali razlika nije bila statistički značajna. Ni razlika u stepenu vaskularizacije između p53-negativnih i p53-pozitivnih tumora nije bila značajna. Nivo MVG-a i proporcija GBM-a sa niskim odnosno visokim MVG nisu se značajno razlikovali u odnosu na nivo ekspresije p53 (nizak odnosno visok). Nije zapažena povezanost MVG sa MIB-1 indeksom tumorskih ćelija i p53 statusom u primarnim GBM-ima.
Zaključak. Dobijeni podaci ukazuju na to da efekat p53 na vaskularizaciju primarnih GBM-a nije bio detektovan; možda su na to uticali određeni faktori, uključujući prisustvo drugih ili dodatnih molekularnih alteracija u tumorskim ćelijama i hipoksične mikrosredine tumora. Takođe, ovi rezultati podržavaju hipotezu o tome da pomenuti efekat p53 može biti specifičan za tip tumora.

Ključne reči: **glioblastom, mikrovaskularna gustina, angiogeneza, imunohistohemija, MIB-1 proliferativni indeks, p53**

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Original article

Hepatorenal Toxicity of Different Doses of Ketorolac Administration in Adult Male Rats: A Preclinical Study

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SUMMARY

Background/Aim. Ketorolac is a potent non-steroidal anti-inflammatory drug (NSAID) that can inhibit cyclooxygenase activity and prostaglandin synthesis, thereby reducing pain and inflammation. The aim of this study was to investigate the hepatorenal toxicity of ketorolac administration in adult male rats.
Methods. Twenty-four adult male Wistar rats were randomly assigned to three groups (n = 8 per group): a control group receiving normal saline (1 mL/kg), a low-dose ketorolac group (10 mg/kg), and a high-dose ketorolac group (20 mg/kg). The animals were maintained under standard housing conditions for three weeks after the last treatment. Blood samples were collected under anesthesia, and serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (Cr) were measured using commercial kits and a BT 1000 Biotectica analyzer.
Results. Compared to the control group, the low-dose ketorolac group did not show a significant increase in ALT and AST levels, but the high-dose ketorolac group exhibited a significant elevation in these hepatic enzymes ($P < 0.05$). Both the low-dose and high-dose ketorolac groups demonstrated a significant increase in BUN and Cr levels compared to the control group, with the high-dose group showing a more pronounced elevation in these renal parameters ($p < 0.05$).
Conclusion. The findings of this study suggest that high-dose ketorolac administration can induce hepatotoxic and nephrotoxic effects, as evidenced by the increased levels of liver and kidney function markers in adult male rats. These results highlight the importance of careful monitoring and dose optimization when using ketorolac in clinical settings.

Keywords: **ketorolac, hepatorenal toxicity, kidney, liver, rat**

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INTRODUCTION

Ketorolac is a powerful non-steroidal anti-inflammatory medication that is extensively utilized for the treatment of moderate to severe pain (1, 2). As an inhibitor of cyclooxygenase (COX) enzymes, ketorolac blocks the synthesis of pro-inflammatory prostaglandins, thereby reducing pain and inflammation (3-4). While the analgesic and anti-inflammatory properties of ketorolac make it a valuable therapeutic option, non-steroidal anti-inflammatory drugs (NSAIDs) in general are known to carry risks of adverse effects, particularly involving the gastrointestinal, cardiovascular, and renal systems (5-7). The hepatotoxic and nephrotoxic potential of ketorolac has been a topic of concern in clinical practice. Several case reports and observational studies have documented instances of ketorolac-induced liver injury and acute kidney injury, often in the setting of prolonged administration or pre-existing comorbidities (8-11). However, the precise dose-dependent nature of ketorolac's hepatorenal toxicity has not been extensively characterized in controlled preclinical investigations.

Animal models, such as the adult male rat, provide a valuable platform to systematically evaluate the toxicological profile of pharmaceutical agents under standardized experimental conditions (12, 13). By employing this approach, researchers can elucidate the threshold doses at which ketorolac may exert detrimental effects on the liver and kidneys, thereby informing safer clinical use of this non-steroidal anti-inflammatory drug (NSAID). The present study aimed to investigate the dose-dependent hepatorenal toxicity of ketorolac administration in an adult male rat model.

METHODS

Study design and animal grouping

This was a controlled preclinical study that examined the dose-dependent hepatic and renal toxicity of ketorolac in an adult male rat model. The study included 24 adult male Wistar rats weighing 200-250 grams. The animals were randomly assigned to three groups (n = 8 per group), using a computer-generated randomization scheme. The control group (C) received 1 mL/kg of normal saline; the low-dose ketorolac group (LDK) received 10 mg/kg of ketorolac, whereas high-dose ketorolac group (HDK) received 20 mg/kg of ketorolac.

Animal housing and care

The experimental animals were housed in a regulated environment within the animal research facility, which was maintained at a temperature of 24 ± 2 degrees Celsius and operated on a 12-hour light/dark cycle. The rats had ad libitum access to typical rodent diet and drinking water for the full length of the study.

Drug administration and sample collection

The assigned treatments were administered daily via oral gavage for three weeks. One week after the last dose, the animals were anesthetized, and blood samples were collected via cardiac puncture using sterile syringes. The blood samples were permitted to coagulate, after which they were centrifuged at 1500 revolutions per minute for 10 minutes to separate the serum component. The serum samples were then stored at a temperature of -20 degrees Celsius until subsequent analysis could be performed.

Biochemical analyses

Serum levels of the following parameters were measured using commercial kits (Pars Azmoun, Iran) and a BT 1000 Biotectica auto-analyzer: AST, ALT, BUN, Cr.

Ethical considerations

The study was approved by the Ethics Committee of the university (IR.SHMU.REC.1397.113). The guidelines for the Care and Use of Laboratory Animals were followed. Only male animals were used in the study to eliminate potential confounding factors related to pregnancy.

Statistical analysis

Normality of the continuous variables was assessed using the Shapiro-Wilk test. One-way ANOVA compared mean liver and kidney function parameters across the three groups, with post-hoc LSD analysis identifying significant differences. P-

values less than 0.05 were considered statistically significant.

RESULTS

The mean weight of rats in the C group was 212.37 ± 11.01 g, in the LDK group it was 212.12 ± 3.79 g, and in the HDK group it was 214.5 ± 3.96 g, with no statistically significant difference among the three groups (p = 0.63).

Liver function markers

The AST and ALT levels are presented in Figure 1. One-way ANOVA revealed a statistically significant difference in mean AST (F (2.21) = 15.72, p < 0.001) and ALT (F (2.21) = 18.84, p < 0.001) levels across the three groups. Post-hoc analysis using the LSD test showed that the mean ALT level in the C group was 24.12 U/L, 39.16 U/L in the LDK group, and 78.25 U/L in the HDK group. Compared to the C group, the ALT level in the HDK group was significantly higher (p < 0.001). Similarly, the mean AST level in the C group was 28.32 U/L, 44.23 U/L in the LDK group, and 93.55 U/L in the HDK group. Compared to C group, the AST level was significantly elevated in the HDK group (p < 0.001). There were no statistically significant differences in AST or ALT levels between the LDK and C groups.

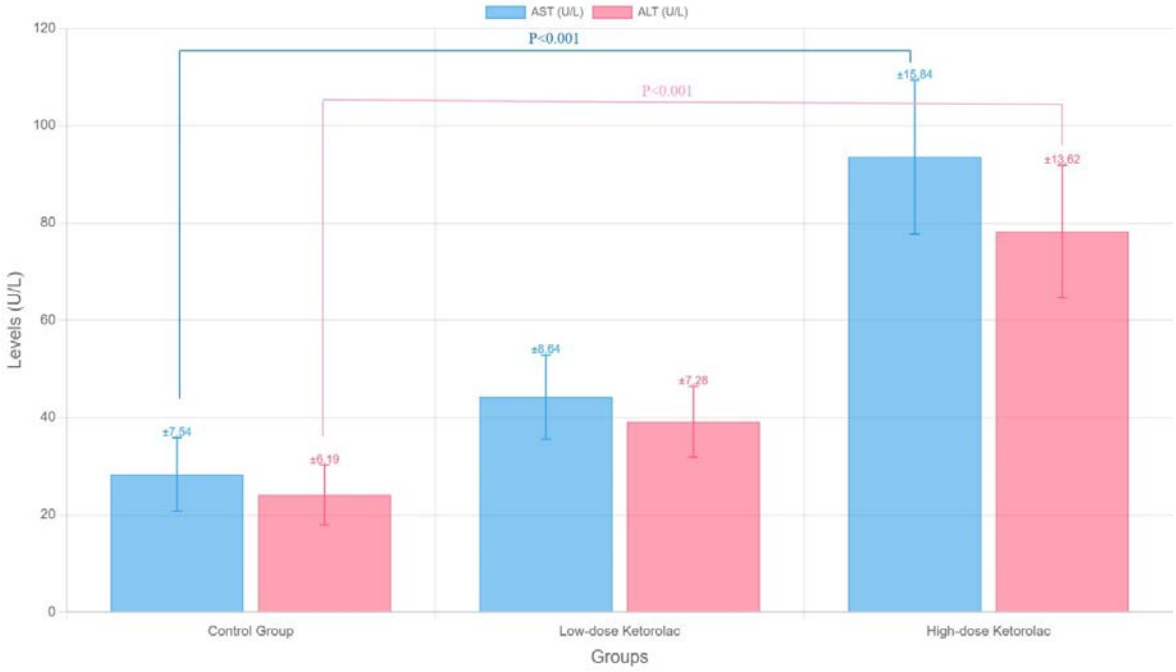

Figure 1. *Serum AST and ALT levels in the three groups*

Kidney function markers

The serum levels of BUN and Cr are shown in Figure 2. One-way ANOVA demonstrated significant differences in mean BUN (F (2.21) = 22.41, p < 0.001) and Cr (F (2.21) = 19.76, p < 0.001) across the three groups. Post-hoc analysis using the LSD test revealed that the mean BUN level in the C group was 16.14 mg/dL, 41.15 mg/dL in the LDK group, and 79.36 mg/dL in the HDK group. Both the LDK group and the HDK group had significantly higher BUN levels compared to the C group (p < 0.001). Additionally, the BUN levels were observed to be significantly elevated in the HDK treatment group in comparison to the LDK group (p = 0.002). The mean Cr level in the C group was 0.21 mg/dL, 0.42 mg/dL in the LDK group, and 0.96 mg/dL in the HDK group. Both the LDK group and the HDK group had significantly higher Cr levels compared to the C group (p < 0.001). Additionally, the HDK group exhibited significantly higher Cr levels compared to the LDK group (p = 0.005).

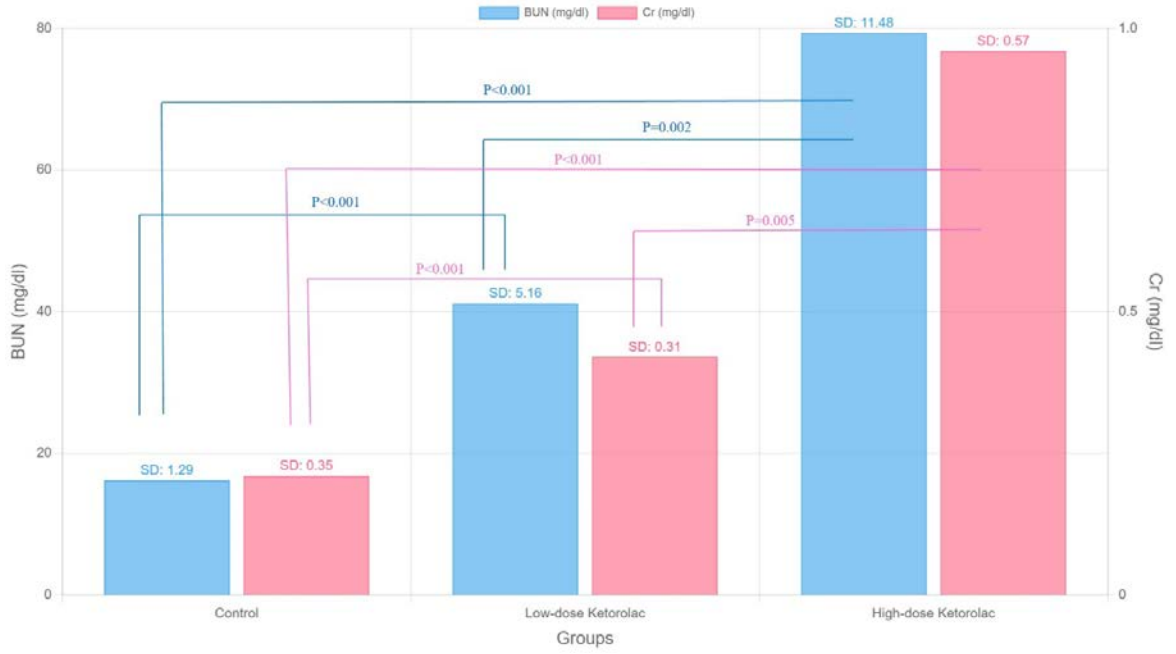

Figure 2. *Serum BUN and creatinine levels in the three groups*

DISCUSSION

The present study provides important insights into the hepatorenal toxicity of ketorolac, a commonly prescribed NSAID, in an adult male rat model. The results demonstrate that HDK administration significantly impairs both liver and kidney function, while LDK primarily affects renal parameters. A significant increase in serum AST and ALT levels observed in the HDK group compared to the C group is a clear indicator of hepatocellular injury. Liver enzymes such as AST and ALT are released into the bloodstream upon damage or dysfunction of hepatocytes, the primary functional cells of the liver (14-16). The observed elevation in these biomarkers suggests that HDK exposure can lead to disruption of the structural and functional integrity of hepatic tissue, potentially through mechanisms involving oxidative stress, mitochondrial dysfunction, and inflammatory responses (17-20). The lack of significant changes in liver enzymes between the control group and the LDK group implies that the lower dose of the drug may not be sufficient to induce overt hepatic toxicity in this animal model. This finding is consistent with the known dose-dependent nature of NSAID-induced liver injury, where higher doses are more likely to trigger hepatocellular damage (21-28). The observed differential effects on liver function between the low and high-dose groups highlight the importance of considering the appropriate dosage when evaluating the safety and tolerability of ketorolac in both preclinical and clinical settings.

Regarding the assessment of renal function, the present study demonstrated that both LDK and HDK groups exhibited significantly elevated levels of BUN and creatinine, two well-established biomarkers of kidney injury and dysfunction. Moreover, the HDK group exhibited even greater increases in BUN and creatinine compared to the LDK group, indicating a dose-dependent nephrotoxic effect of the drug. The observed elevations in BUN and creatinine levels are consistent with the known nephrotoxic potential of NSAIDs, including ketorolac (29-36). The underlying mechanisms by which ketorolac can impair renal function are multifaceted and involve disruptions in the homeostasis of renal blood flow, glomerular filtration, and prostaglandin synthesis (37-39). Specifically, ketorolac's inhibition of cyclooxygenase (COX) enzymes, which play a critical role in the regulation of renal hemodynamics and electrolyte balance, can lead to vasoconstriction, reduced glomerular filtration rate, and impaired urine output, ultimately resulting in the accumulation of waste products such as BUN and creatinine (40-42).

The dose-dependent nature of the renal effects observed in this study suggests that the degree of

ketorolac-induced nephrotoxicity is closely related to the administered dose. This finding is particularly important, as it highlights the need for careful dosage selection and monitoring when prescribing ketorolac, especially in patients with pre-existing renal impairment or those at higher risk of developing NSAID-associated kidney injury. The underlying mechanisms responsible for the hepatorenal toxicity of ketorolac are not entirely clear, and several potential biological pathways have been hypothesized to explain this observed phenomenon. One key mechanism involves the induction of oxidative stress and inflammatory responses by ketorolac, which can lead to cellular damage and dysfunction in both the liver and kidneys (42, 43). Ketorolac's inhibition of prostaglandin synthesis may also play a role, as prostaglandins are crucial mediators of renal blood flow, glomerular filtration, and tubular function (44). Additionally, ketorolac and its metabolites may directly interact with cellular components, such as mitochondria, and disrupt essential metabolic pathways, contributing to the observed hepatic and renal toxicity (43). The interplay between these various mechanisms, including oxidative stress, inflammation, and metabolic disturbances, likely contributes to the overall hepatorenal toxicity profile of ketorolac.

This study has several limitations that should be acknowledged. First, the investigation was limited to two specific doses of ketorolac (10 mg/kg and 20 mg/kg), which may not fully capture the complete dose-response relationship. Including additional intermediate and lower doses could provide a more comprehensive understanding of the threshold for hepatorenal toxicity. Second, while biochemical markers of liver and kidney function were assessed, no histopathological analyses were conducted to confirm cellular-level changes or identify specific patterns of tissue injury. Such data could provide deeper mechanistic insights into the toxic effects of ketorolac. Finally, this preclinical study was conducted in a controlled laboratory environment, which may not fully replicate the complex clinical scenarios encountered in human patients, particularly those with comorbidities or concurrent medication use.

CONCLUSION

In conclusion, the present study provides compelling evidence that HDK administration can induce significant hepatotoxicity, as evidenced by the elevated serum levels of liver enzymes AST and ALT. Furthermore, both LDK and HDK groups exhibited dose-dependent renal toxicity, as indicated by the increased BUN and creatinine levels. These findings underscore the importance of carefully considering the potential hepatorenal side effects associated with ketorolac therapy and the need for appropriate dosage adjustments and close monitoring in clinical settings, especially in patients with pre-existing liver or kidney disorders. Future studies exploring the underlying molecular mechanisms and exploring potential protective strategies may help elucidate the complex pathways involved in ketorolac-induced hepatorenal toxicity.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Toksičnost različitih doza ketorolaka u jetri i bubrezima odraslih pacova muškog pola: pre-klinička studija

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SAŽETAK

Uvod/Cilj. Ketorolak je potentni nesteroidni antiinflamatorni lek (engl. *nonsteroidal anti-inflammatory drug* – NSAID) koji može inhibirati aktivnost ciklooksigenaze i sintezu prostaglandina, čime smanjuje bol i upalu. Cilj ove studije bio je da istraži hepatorenalnu toksičnost primene ketorolaka kod odraslih pacova muškog pola.

Metode. Dvadeset i četiri odrasla pacova muškog pola soja Wistar nasumično su podeljena u tri grupe (n = 8 po grupi): kontrolnu grupu koja je primala fiziološki rastvor (1 mL/kg), grupu koja je dobijala nisku dozu ketorolaka (10 mg/kg) i grupu koja je dobijala visoku dozu ketorolaka (20 mg/kg). Životinje su čuvane pod standardnim uslovima tri nedelje nakon poslednjeg tretmana. Uzorci krvi su uzeti dok su pacovi bili pod anestezijom, a nivoi alanin aminotransferaze (ALT), aspartat aminotransferaze (AST), uree u krvi (engl. *blood urea nitrogen* – BUN) i kreatinina (Cr) mereni su komercijalnim kompletima i analizatorom BT 1000 *Biotectica*.

Rezultati. U poređenju sa kontrolnom grupom, grupa koja je primala nisku dozu ketorolaka nije pokazala značajan porast nivoa ALT-a i AST-a, dok je grupa koja je primala visoku dozu ketorolaka pokazala značajan porast ovih hepatičnih enzima ($p < 0,05$). I u grupi sa niskom dozom i u grupi sa visokom dozom ketorolaka zabeležen je značajan porast nivoa BUN-a i Cr-a u poređenju sa kontrolnom grupom. U grupi koja je dobijala visoku dozu ketorolaka porast ovih renalnih parametara bio je izraženiji ($p < 0,05$).

Zaključak. Rezultati ove studije ukazali su na to da primena visoke doze ketorolaka može imati hepatotoksične i nefrotoksične efekte; to je potvrdilo zabeleženo povećanje nivoa markera funkcije jetre i bubrega kod odraslih pacova muškog pola. Ovakvi rezultati ističu važnost pažljivog praćenja i optimizacije doza prilikom primene ketorolaka u kliničkim uslovima.

Ključne reči: **ketorolak, hepatorenalna toksičnost, bubrezi, jetra, pacov**

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Original article

N-Terminal Pro-Brain Natriuretic Peptide Superiority for Prognosis of Major Adverse Cardiovascular Events in Patients with Acute Myocardial Infarction without Heart Failure

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SUMMARY

Introduction/Aim. Many markers are used to evaluate the prognosis in patients with acute myocardial infarction (AMI). Researches are focused on available markers with high sensitivity and specificity. The aim of our study was to evaluate the prognostic value of N-terminal pro brain natriuretic peptide (NT-proBNP) and its superiority compared with other prognostic markers in patients with AMI.

Patients and methods. Sixty-six patients with the diagnosis of AMI were enrolled in the study. The evaluated variables were: symptoms, cardiovascular risk factors, laboratory analyses (including NT-proBNP), GRACE risk score, electrocardiography, left ventricular ejection fraction (LVEF) and coronary angiography. One- and six-month major adverse cardiovascular events (MACE) included: reAMI, heart rhythm disorders, acute heart failure, stroke, fatal event.

Results. Patients with one-month and six-month MACE were older, had anterior AMI, higher levels of NT-proBNP, urea, creatinine, lower LVEF, creatinine clearance (CCr) and hemoglobin level. NT-proBNP is an independent predictor of short-term (p = 0.002) and long-term (p = 0.000) prognosis. Its cut point of 1,467 pg/ml is a significant independent predictor of one-month MACE and cut point of 996 pg/ml is a significant independent predictor of six-month MACE.

Conclusion. NT-proBNP is a strong short-term and long-term predictive marker in AMI patients without heart failure.

Keywords: **prognosis, acute myocardial infarction, NT–proBNP**

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INTRODUCTION

Acute myocardial infarction (AMI) is an important cause of heart failure with preserved and reduced left ventricular ejection fraction despite improved treatment options (1). This makes the evaluation of prognostic markers in patients with AMI still relevant research. Galectin-3 (Gal-3) is a significant prognostic marker (2), however, its determination is not available in many centers. Researches are focused on available, cheap variables with high sensitivity and specificity. Biochemical analyzes continue to attract the most attention because of its availability and simple determination. N-terminal pro brain natriuretic peptide (NT-proBNP) is now used as a standard biochemical analysis in many coronary units. Our research is focused on the evaluation of prognostic significance and superiority of NT-proBNP compared with other available variables in patients with AMI without acute heart failure regarding one- and six-month major adverse cardiovascular events (MACE).

AIM

The aim of our study was to evaluate the prognostic value of NT-proBNP and its superiority compared with other prognostic markers in patients with AMI.

PATIENTS AND METHODS

Patients and study design: We enrolled 66 patients with AMI, hospitalized between January and July 2009 in the Coronary Unit, Clinical Center Kragujevac, Serbia. The local Ethics Committee approved the study and all patients signed written informed consent. We did not include the patients with end-stage chronic renal disease, acute heart failure, chronic obstructive pulmonary disease, patients under 18 years of age and patients who did not want to participate in the study. Criteria for AMI were set according to the European Society of Cardiology (ESC) guidelines (3). The evaluated variables were as follows:

1) Symptoms: chest pain, exhaustion;

2) Cardiovascular (CV) risk factors: age, gender, tabacco, dyslipidemia (HLP), diabetes mellitus (DM), arterial hypertension (HTA), obesity, known coronary artery disease (CAD), stroke, family history of CAD, emotional stress;

3) Electrocardiography on admission for AMI localization (anterior-inferior);

4) Biochemical analysis on admission: hemoglobin, glucosae, C-reactive protein (CRP), urea, creatinine, creatinine clearance (CCr), body mass index (BMI), cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, troponin, NT-proBNP (usung ELFA technique);

5) Echocardiography on admission and determination of LVEF using the Simpsons method;

6) Coronary angiography performed during hospitalization, including the identification of culprit artery and the number of diseased coronary vessels;

7) MACE after one and six months included: reAMI, heart rhythm disorders, heart failure, stroke, fatal event. Data about MACE were recorded using telephone, standard visits and medical database in case of no response to previously mentioned visists.

Statistical analysis

The prognostic values of the examined variables were evaluated using the X^2 test and Mann-Whitney U test. Plasma concentrations of continuous variables were described as the median and interquartile range (25^{th} to 75^{th} percentile). Binary logistic regression (univariate and multivariate) and

receiver-operating characteristic (ROC) curves were used to evaluate the prognostic NT-proBNP superiority compared to other markers. A p value (two-tailed) < 0.05 was considered statistically significant.

RESULTS

The study population consisted of 49 males and 17 females. Median age of patients was 64.45 ± 10.361 years, 64 ± 10.17 years for males and 65.76 ± 10.81 years for females. ECG: 33 patients had ST-elevation myocardial infarction (STEMI), and 33 patients had non-ST elevation myocardial infarction (NSTEMI); 32 (48.48%) patients had inferior AMI, 33 (50%) patients had anterior AMI and 1 (1.52%) patient did not have ECG abnormalities. Coronary angiography was performed in 61 patients: left coronary artery (LCA) was the culprit artery in 38 (57.57%) patients and right coronary artery (RCA) in 23 (34.85%) patients; 26 (39.39%) patients had one-vessel and 35 (53.03%) patients had multi-vessel disease.

One-month MACE: We verified one-month MACE in 22 (33.33%) patients: 18 males and 4 females. Patients with one-month MACE were older, had anterior AMI, higher levels of NT-proBNP, urea, creatinine, lower LVEF, CCr, and hemoglobin level (Table 1 and 2).

Table 1. *Baseline characteristics of the study's patients stratified by the presence of one-month major adverse cardiovascular events (MACE) including death*

Variable	On admission (N)	One-month MACE (N)	χ^2 (p)
Gender (male)	49	18	0.86
Exhaustion	56	17	0.25
Chest pain	63	21	1.00
Emotional stress	62	21	1.00
Family history	39	15	0.48
Arterial hypertension	44	16	0.73
Diabetes mellitus	21	10	0.18
Dyslipidemia	7	2	1.00
Previous coronary artery disease	15	8	0.13
Stroke	4	1	0.25
Smoking	44	14	0.82
Ventricular arrhythmia	39	14	0.62
STEMI/NSTEMI	33/33	12	0,79
Localization: anterior/inferior	32/33	14/8	**0.046**
Culprit artery: LCA/RCA	38/23	15/4	0.091
Multi-vessel/one vessel disease	26/35	12/8	1.00

STEMI—ST elevation myocardial infarction, NSTEMI—non-ST elevation myocardial infarction, LAD—left anterior descendent artery, RCA—right coronary artery

Table 2. *Patient characteristics according to the presence of one-month major adverse cardiovascular events (MACE) including death*

Variable	Without MACE	MACE	t* or Z** (p)
	mean ± SD	mean ± SD	
Age (years)	62.39 ± 10.429	68.59 ± 9.096	**0.021***
LDL (mmol/L)	3,681 ±1.177	3.366 ± 0.778	0.267*
Weight (kg)	81.47 ± 12.665	83.71 ± 11.529	0.495*
BMI (kg/m²)	27.53 ± 3.189	26.28 ± 3.086	0.141*
Creatine clearance	104.62 ± 36.40	72.63 ± 30.55	**0.001***
NT-proBNP (pg/ml)	658.00 (315.00-1107.00)	2424.00 (1808.00- 2557.00)	**0.000****
Hemoglobin (g/L)	141.00 (130.0-149.00)	132.00 (119.00-140.00)	**0.038****
Urea (mmol/L)	5,60 (4,10-7,00)	8.00 (6.90-10.00)	**0.000****
Creatinine (mmol/L)	78.00 (65.00-86.00)	100.00 (85.0-140.00)	**0.000****
LVEF (%)	50.00 (45.00-55.00)	45.00 (40.00-45.00)	**0.004****
Glucose (mmol/L)			0.896**
CRP (mmol/L)			0.071**
Troponin (pg/ml)			0.547**
Cholesterol (mmol/L)			0.608**
HDL cholesterol (mmol/L)			0.137**
CKMB (mmol/L)			0.427**
Heart rate (beats/min)			0.499**

Values are presented as mean ± SD or median with interquartile range (IQR). Two-tailed unpaired t-test (normalized distribution; t(p)) or Man-Whitney (non-normalized distribution; Z (p)), and χ^2 (p). BMI—body mass index, NT-proBNP—NT-pro brain natriuretic peptide, LVEF—left ventricular ejection fraction, CRP—C reactive protein, CKMB—creatine kinase isoenzime MB

Univariate binary logistic regression of MACE occurrence revealed potential predictors (Table 3). In a multivariate model, NT-proBNP was an independent predictor for one-month MACE in patients with AMI without heart failure (Table 3).

Using the ROC curve analysis (Figure 1), we found that NT-proBNP and creatinine levels had good discriminatory ability (AUC > 0.7) in selecting patients with one-month MACE occurrence; area = 0.868, p < 0.0005 and area = 0.775, p < 0.0005, respectively (Graph 1A and 1B). NT-proBNP is a better marker for one-month MACE (Z = 10.10, p < 0.0005) (Graph 1C). The optimal cut-off of NT-proBNP for predicting one-month MACE is 1,467 pg/ml with sensitivity 81.8%, specificity 86.4%, PPV 75.0%, and NPV 90.5%.

Six-month MACE: The number of patients with MACE after six months was significantly increased up to 31 patients (46.9%) (McNemar test, p = 0,0005): 22 males and 9 females. Patients with six-month MACE were older, had anterior AMI, culprit LCA, higher levels of NT-proBNP, urea, creatinine, lower LVEF, CCr, and hemoglobin level (Table 4 and 5).

Univariate binary logistic regression of MACE occurrence revealed potential predictors (Table 6). In a multivariate model, NT-proBNP was an independent predictor of six-month MACE in patients with AMI without heart failure (Table 6).

Using the ROC curve analysis (Figure 2), we found that NT-proBNP and creatinine concentrations had good discriminatory ability (AUC > 0.7) in selecting patients with six-month MACE occurrence; AUC = 0.892, p < 0.0005 and area = 0.714, p < 0.003, respectively (Figure 2A and 2B). NT-proBNP is a better marker for one-month MACE (Z = 18.69, p < 0.0005) (Figure 2C). The optimal cut-off of NT-proBNP for predicting the six-month MACE is 996 pg/ml, with sensitivity 80.6%, specificity 80.0%, PPV 78.1%, and NPV 82.3%.

Table 3. *Univariate and multivariate logistic regression for all significant univariate variables predicting major adverse cardiovascular events (MACEs) or death at one month follow-up*

Variable	Univariate		Multivariate	
	P	OR (95% CI)	p	OR (95% CI)
Age	**0.025**	1.065 (1.008–1.124)		
NT pro BNP	**0.000**	1.002 (1.001–1.003)	**0.002**	1.002 (1.001–1.003)
Hemoglobin	0.054	0.966 (0.932–1.001)		
Urea	0.665	1.000 (1.000–1.000)		
Creatinine	**0.001**	1.049 (1.020–1.079)	0.06	1.036 (0.998–1.075)
LVEF	**0.012**	0.919 (0.861–0.982)		
Triglicerides	0.065	0.557 (0.330–1.036)		
Creatinine clearance	**0.002**	0.966 (0.954–0.988)		

NT-proBNP –NT—pro brain natriuretic peptide, LVEF—left ventricular ejection fraction

ROC curve—receiver operating characteristic curve

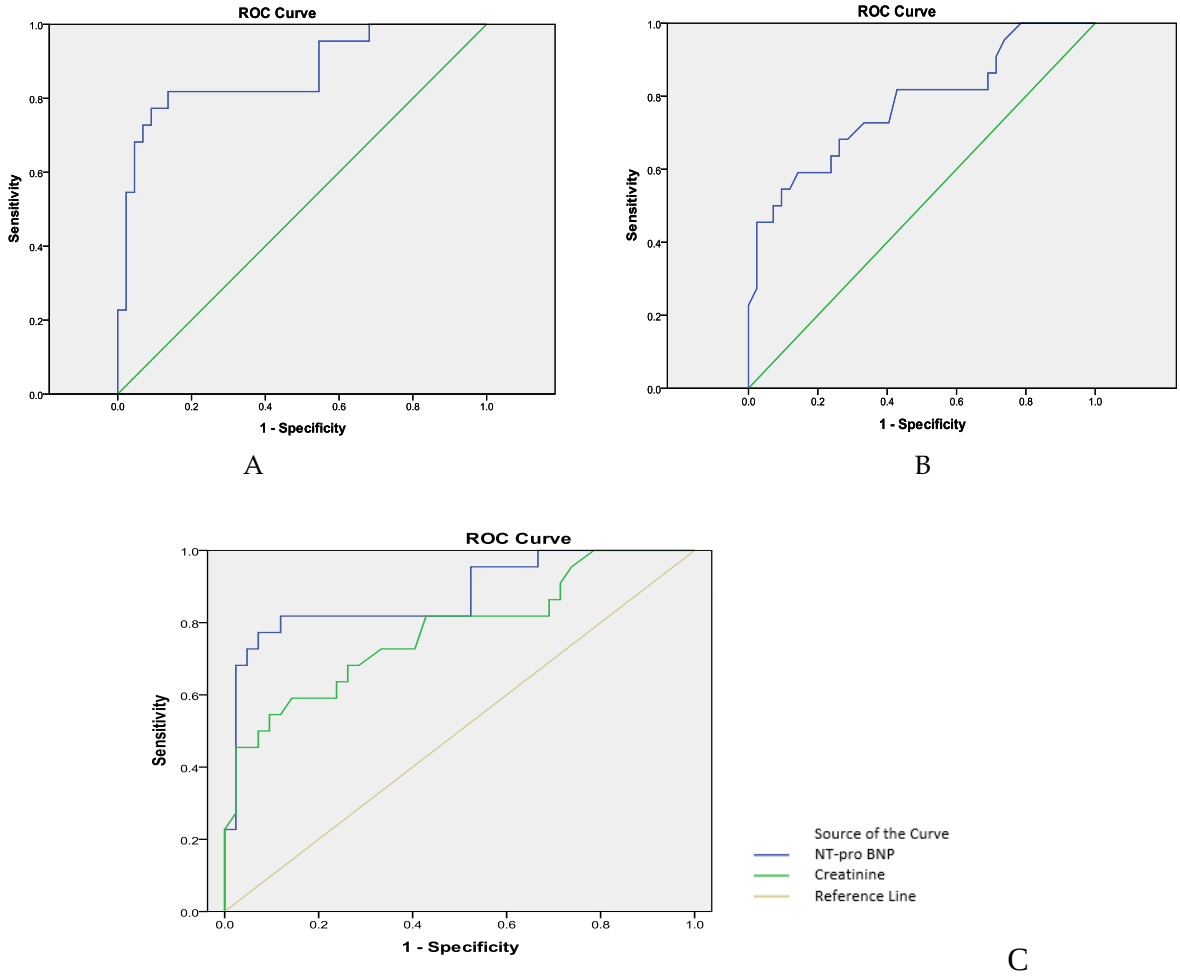


Figure 1. *The ROC curve analysis of NT-proBNP (A) and creatinine (B) in the identification of AMI patients with likelihood of one-month occurrence of MACE or death, and comparison of these two markers (C)*

Table 4. *Baseline characteristics of the study's patients stratified by the presence of six-month major*

adverse cardiovascular events (MACE) including death

Variable	On admission (N)	Six-month MACE (N)	χ^2 (p)
Gender (male)	49	22	0.77
Exhaustion	56	26	0.73
Chest pain	63	29	0.22
Emotional stress	62	30	1.00
Family history	39	20	0.68
Arterial hypertension	44	24	0.18
Diabetes mellitus	21	10	0.06
Dyslipidemia	7	4	0.70
Previous coronary artery disease	15	10	0.16
Stroke	4	2	0.44
Smoking	44	21	1.00
Ventricular arrhythmia	39	16	0.71
STEMI/NSTEMI	33/33	18	0,32
Localization: anterior/inferior/lateral	32/33	19/12	**0.012**
Culprit artery: LCA/RCA	38/23	22/5	**0.013**
Multi-vessel/one vessel disease	26/35	17/10	0.059

STEMI—ST elevation myocardial infarction, NSTEMI—non-ST elevation myocardial infarction, LAD—left anterior descendent artery, RCA—right coronary artery

Table 5. *Patient characteristics according to six-month MACE occurrence*

Variable	Without MACE	MACE	t* or Z** (p)
	mean ± SD	mean ± SD	
Age (years)	61.1±10.461	67.55±9.295	**0.021***
LDL (mmol/L)	3.624±1.2265	3.513±0.8758	0.683*
Hight (cm)	172.38±8.521	175.70±8.514	0.125*
Weight (kg)	80.88±13.126	83.70±11.225	0.363*
BMI (kg/m²)	27.14±3.248	27.093±3.170	0.94*
Creatinine clearance	107.11±39.29	78.54±39.02	**0.002***
NT-proBNP (pg/ml)	541.00 (273.00-927.00)	2034.00 (1495.00- 2503.00)	**0.000****
Hemoglobin (g/L)	141.00 (133.0-150.00)	132.00 (119.00-140.00)	**0.002****
Urea (mmol/L)	5.50 (4.10-7.00)	7.60 (6.30-9.40)	**0.003****
Creatinine (mmol/L)	75.00 (65.00-86.00)	90.00 (81.00-121.00)	**0.003****
LVEF (%)	50.00 (45.00-55.00)	45.00 (40.00-50.00)	**0.024****
Glucose (mmol/L)			0.602**
CRP(mmol/L)			0.153**
Troponin (pg/ml)			0.842**
Cholesterol (mmol/L)			0.679**
HDL cholesterol (mmol/L)			0.213**
CKMB (mmol/L)			0.451**
Hart rate (beats/min)			0.325**

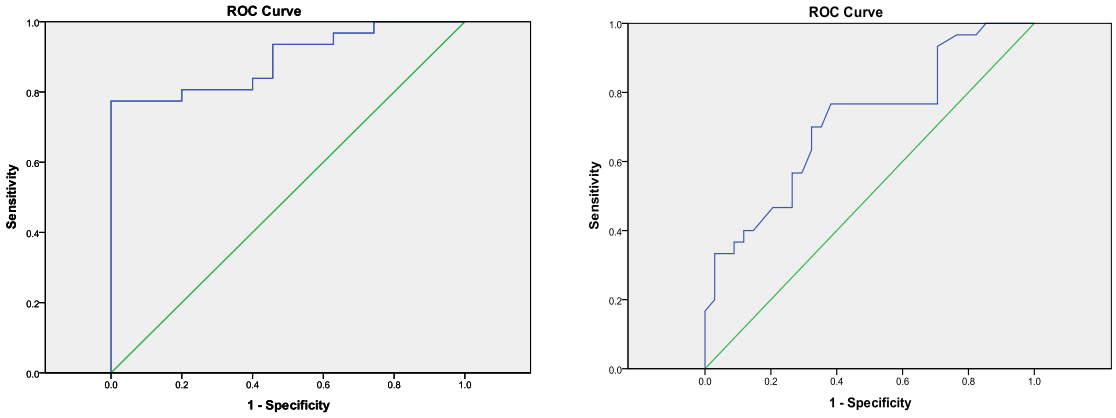
Values are presented as mean ± SD or median with interquartile range (IQR. Two-tailed unpaired t-test (normalized distribution; t(p)) or Man-Whitney (non-normalized distribution; Z (p)).
BMI—body mass index, NT-proBN—NT-pro brain natriuretic peptide, LVEF—left ventricular ejection fraction, CRP—C reactive protein, CKMB—creatine kinase isoenzime M

Table 6. *Univariate and multivariate logistic regression for all significant univariate variables predicting major adverse cardiovascular events (MACEs) or death at six months follow-up*

Variable	Univariate		Multivariate	
	p	OR (95% CI)	p	OR (95% CI)
Age	**0.025**	1.060 (1.007 – 1.115)		
NT-proBNP	**0.000**	1.002 (1.001 – 1.004)	**0.00**	1,003 (1,001 - 1,004)
Hemoglobin	0.007	0.948 (0.912 – 0.985)		
Urea	0.544	1.000 (1.000 – 1.000)		
Creatinine	**0.004**	1.020 (0.997 – 1.043)		
LVEF (%)	**0.034**	0.934 (0.877 – 0.995)		
Triglicerides	0.761	0.939 (0.624-1.411)		
Creatine clearance	**0.004**	0.973 (0.955-0.991)		
Distal LAD	0.085	1.020 (0.997-1.043)		
Culprit artery	**0.037**	0.413 (0.180/0.949)		

NT-proBNP—NT-pro brain natriuretic peptide, LVEF—left ventricular ejection fraction, LAD— left anterior descendent artery

ROC curve—receiver operating characteristic curve



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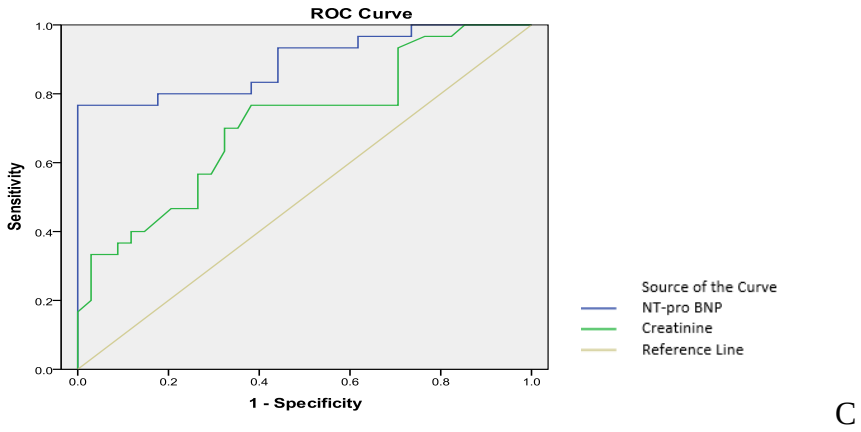

Figure 2. *The ROC curve analysis of NT-proBNP (A) and creatinine (B) in the identification of AMI patients with likelihood of six-month occurrence of MACE or death, and comparison of these two markers (C)*

DISCUSSION

NT-proBNP is the most usable marker for acute heart failure. Due to the presence of the heart failure in a wide range of diseases, the evaluation of this marker, available in almost every coronary unit, draws the attention of scientists. Our results showed higher NT-proBNP concentrations in the first hour of AMI symptoms and its level was related to infarct size (4). Worse short-term prognosis of AMI patients was detected in those with higher NT-proBNP concentrations measured in the first sample and without later rapid decline, and in those with higher NT-proBNP level three days after AMI (5) and persistently higher NT-proBNP levels in multiple measurements after AMI (6).

Our study measured the NT-proBNP level up to 24 hours of the onset of symptoms, and only patients with AMI without acute heart failure were evaluated. Patients with one-month and six-month MACE were older, with anterior AMI, culprit LCA, higher levels of NT-proBNP, urea, creatinine, lower levels of LVEF, CCr, and hemoglobin. Our results showed a connection between NT-proBNP levels and risk of re-AMI, heart failure, heart rhythm disorders, stroke and death, one and six months after AMI. A predictive value of NT-proBNP for short-term and long-term MACE in AMI patients was independent of clinical characteristics, biochemical analyses, LVEF, left ventricular wall localization or culprit artery.

The reported one-month and six-month MACE rates of 33.33% and 46.9%, respectively, in our study, were similar to the results of other reported studies. Variations in MACE rates can be explained by different length of follow-up, adverse events included in MACE, and a type of included patients (7-10).

Previously reported studies showed an impact of NT-proBNP on prognosis in patients with AMI. Wang J and colleagues made biomarker-based risk model using the baseline NT-proBNP and other biomarkers for one-year MACE. In this study, the patients with MACE were older, had higher prevalence of arterial hypertension, diabetes mellitus, congestive heart failure, history of AMI, and stroke. The best prognostic marker was NT-proBNP, the values of which were similar to our results (11). One study in Korea has developed machine learning-based model for the prediction of outcomes in AMI patients. The primary outcome was one-year all-cause death; secondary outcomes included CV deaths, one-year and three-year MACE. This study singled out the best predictors for the primary outcome: peak troponin I ($p = 0.048$), cholesterol level (0.047) and NT-proBNP level (0.039) (12). Another study with 1,105 AMI patients treated with PCI evaluated the association between NT-proBNP levels and three-year MACE. They found that patients with adverse events—all-cause death, AMI recurrence, and re-hospitalization due to heart failure had the highest concentration of NT-proBNP (13). The study of Platelet Inhibition and Patients Outcomes (PLATO

trial) showed an independent association between NT-proBNP and adverse outcomes (14). Prospective ARNI vs ACE Inhibitor Trial to Determine Superiority in Reducing Heart Failure Events After Myocardial Infarction (PARADISE-MI) found a connection of NT-proBNP with adverse events in AMI patients (15). A meta-analysis of 19 studies performed up to June 2021 confirmed a connection between higher concentration of NT-proBNP and adverse events (all cause death and MACE) (16). Compared to our study, all of them measured NT-proBNP concentration in AMI patients independently of the presence of acute heart failure which can be a contributing factor.

The evaluation of prognostic significance of NT-proBNP was performed among patients with other forms of ischemic heart disease. One study measured NT-proBNP in patients with MI with non-obstructive coronary arteries (MINOCA) and without heart failure. They showed a connection between higher NT-proBNP concentrations and risk of re-hospitalizations (17). Evaluations in patients with unstable angina and NSTEMI were evaluated in: The Fast Assessment in Thoracic Pain (FAST) study, The Global Utilization of Strategies To open Occluded arteries-IV (GUSTO IV) and Fast Revascularization during Instability in Coronary artery disease (FRISC II) trial. The FAST (755 patients; follow up 40 months) study found a connection between higher NT-proBNP concentrations and mortality (18). In the GUSTO-IV trial (7,800 patients with acute coronary syndromes without ST-segment elevations (ACS-NSTE); follow up—one year) NT-proBNP was the strongest independent predictor of mortality (19). The FRISC II trial (2,019 patients, follow up—two years) compared an early invasive to non-invasive strategy in patients with unstable angina. NT-proBNP was independently associated with mortality (20).

All of the above-mentioned studies corroborate our findings; however, the majority of these measured NT-proBNP plasma concentration in patients with ischemic heart disease independent of the presence of acute heart failure, followed up on patients over both short-term and long-term periods, and included varied adverse outcomes. The exclusion of patients with acute heart failure made our study different, being thus one step ahead of others. Our study shows the significance of NT-proBNP measurements in AMI patients, early after admission and identifies patients with high risk for adverse events. As we previously reported, Gal-3 plasma concentration in patients with AMI has a high long-term prognostic value (2). However, the availability of Gal-3 is not comparative to NT-proBNP in clinical practice. This available marker in every coronary unit can be used as a standard marker for one- and six-month prognosis for patients with AMI.

Identification of high-risk patients for MACE through simple NT-proBNP determination early after admission could be a step forward in the prognosis of AMI patients and prevention in the post-AMI period. The main limitation of our study is a small number of patients. Increasing the number of patients with different forms of ischemic heart disease and using national registries for ACS will be helpful for further research. Determination of NT-proBNP levels in different time of ACS onset and presence/absence of acute failure can determine the best time of sampling for short- and long-term prognosis.

CONCLUSION

The NT-proBNP plasma concentration measured in first 24 hours of the onset of symptoms is a strong independent short-term and long-term predictive marker in AMI patients without heart failure.

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Superiornost N-terminalnog promoždanog natriuretičkog peptida u prognozi velikih neželjenih kardiovaskularnih događaja kod bolesnika koji su doživeli akutni infarkt miokarda bez srčane dekompenzacije

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SAŽETAK

Uvod/Cilj. Kod bolesnika sa akutnim infarktom miokarda (AIM) ispituje se prognostički značaj brojnih markera. Istraživači se fokusiraju na one koji su široko dostupni i imaju visoku senzitivnost i specifičnost. Cilj ove studije bio je da utvrdi prognostički značaj N-terminalnog promoždanog peptida (NT-proBNP) i njegovu superiornost nad ostalim markerima koji se koriste kod bolesnika sa AIM-om.

Pacijenti i metode. Studija je obuhvatila 66 bolesnika sa AIM-om. Pri evaluaciji u obzir su bile uzete sledeće varijable: simptomi, kardiovaskularni faktori rizika, laboratorijske analize (uključujući NT-proBNP), elektrokardiografija, ejekciona frakcija leve komore (LVEF) i koronarna angiografija. Jednomesečni i šestomesečni veliki neželjeni kardiovaskularni događaji (engl. *major adverse cardiovascular events* – MACE) uključivali su: ponovljeni AIM, poremećaje srčanog ritma, akutnu srčanu insuficijenciju, moždani udar, smrtni ishod.

Rezultati. Bolesnici sa jednomesečnim i šestomesečnim MACE-om bili su stariji, imali su infarkte prednjeg zida, povišene nivoe NT-proBNP, uree, kreatinina, niži LVEF, CCr (engl. *creatinine clearance* – CCr) i hemoglobin. NT-proBNP je bio nezavisan prediktor kratkoročnog (p = 0,002) i dugoročnog (p = 0,000) MACE-a. Vrednost NT-proBNP koja je na prijemu iznosila 1467 pg/ml pokazala se kao nezavisan prediktor jednomesečnog MACE-a, a vrednost od 996 pg/ml kao nezavisan prediktor šestomesečnog MACE-a.

Zaključak. NT-proBNP je kod bolesnika koji su doživeli AIM bez srčane dekompenzacije snažan prediktor MACE-a, i kratkoročno i dugoročno.

Ključne reči: **prognoza, akutni infarkt miokarda, NT-proBNP**

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Original article

Acute Skin Toxicity in Breast Cancer Patients Following Different Fractionation Regimens of Postoperative Radiotherapy

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SUMMARY

Introduction/Aim. Breast cancer (BC) represents a globally significant health issue, with incidence rates varying worldwide. Radiotherapy is crucial in treating BC, however, it can cause adverse effects, including skin reactions. The aim of this research was to evaluate the impact of two different radiotherapy fractionation regimens on the occurrence and severity of acute skin toxicity in BC patients.
Methods. The prospective study involved 44 patients who underwent postoperative radiotherapy. The patients were randomly divided into two groups: one group received hypofractionated regimen (40.05 Gy in 15 fractions over three weeks), while the other group received the standard fractionated regimen (50 Gy in 25 fractions over five weeks). The patients in this study were monitored weekly for acute skin toxicity throughout the duration of radiotherapy and following the completion of treatment.
Results. The patients receiving the standard fractionated regimen experienced a higher frequency and intensity of acute skin reactions, including erythema, dry desquamation, and moist desquamation. Skin reactions of grade I and II were particularly prominent in the patients receiving 50 Gy. Although the patients receiving hypofractionated radiotherapy had less severe skin reactions, mild skin changes did occur, although they were generally less prominent.
Conclusion. The study points to the need for a careful selection of fractionation regimens in postoperative breast radiotherapy. Additionally, this study contributes to the understanding of the relationship between different radiotherapy modalities and the occurrence of acute skin toxicity, providing guidelines for optimizing treatment in BC patients.

Keywords: **breast cancer, breast-conserving surgery, radiotherapy, radiodermatitis**

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INTRODUCTION

Malignant breast tumors (BC) represent a global health problem today since they are the most frequently diagnosed malignant disease in women worldwide (1). According to the World Health Organization, it is estimated that there were over 2.3 million new cases of BC in 2020, accounting for 11.7% of all new cases, with significant impacts on the healthcare system and individuals (2). The incidence varies from country to country, with higher prevalence in developed countries compared to developing ones, which is attributed to differences in lifestyle, hygienic-dietary habits, access to preventive measures, and genetic predisposition (3). The high morbidity and mortality rates (3, 4) associated with BC were the stepping stones to efforts to enhance therapeutic approaches with a view to reducing incidence and improving treatment outcomes.

A multidisciplinary therapeutic approach involves the application of surgery, chemotherapy, hormone therapy, targeted therapy, and radiotherapy to achieve the best possible outcome for patients. Radiotherapy is an essential component in the modern therapeutic approach, aimed at controlling the disease from its earliest stage to metastatic phases (5, 6). Despite its justified and almost constant use, it can be accompanied by a range of acute adverse effects including fatigue, skin reactions, pain, stress, and reduced quality of life.

During radiotherapy, healthy tissue, including skin, is inevitably exposed to radiation doses that can lead to varying degrees of damage (7). Acute skin toxicity encompasses a broad spectrum of manifestations, from mild erythema and skin dryness to more severe forms such as moist desquamation, ulceration, and necrosis (1, 8). These skin reactions can significantly affect the quality of life of patients, causing pain, discomfort, and even social isolation due to aesthetic consequences. Moreover, severe skin reactions may require treatment interruption or delay, which can compromise treatment efficacy.

The fractionation regimen of radiotherapy is one of the most important factors influencing the occurrence and severity of acute skin toxicity (9, 10). Standard regimens involve the application of lower radiation doses over a longer period of time, such as the widely used regimen of 25 fractions over five weeks, with a daily dose of 2Gy, which may result in fewer skin reaction (11). In contrast, hypofractionated regimens, which involve higher doses of radiation over a shorter period, offer certain advantages such as shorter treatment duration and greater convenience for patients but may lead to more prominent skin reactions during treatment (12). Research on different fractionation regimens and their association with acute skin toxicity is crucial for treatment optimization, with studies giving contradictory conclusions, making it difficult to establish general recommendations.

AIMS

This study aims to assess the impact of two commonly recommended fractionation regimens of postoperative radiotherapy on the occurrence and degree of acute skin toxicity in BC patients, comparing conventional fractionation (CF) with hypofractionated radiotherapy (HF).

PATIENTS AND METHODS

Patient selection

Patients involved in this prospective study underwent their active treatment by means of postoperative radiotherapy between October 2023 and March 2024, at the Radiotherapy Daily Hospital of the Oncology Clinic at the University Clinical Center Niš. They were informed about the study during their initial consultation. Detailed explanations regarding the study's purpose and methods were provided, and written consent was obtained from all participants. This study received an approval from the Ethics Committee of the University Clinical Center Niš, under the number 30414/5. Written informed consent was obtained from all patients included in the study.

The selection included women over 40 years of age with pathologically confirmed ductal carcinoma *in situ* (DCIS) or invasive breast cancer, stage Tis-T2, N0-N1a, M0. General data about patients, tumor characteristics, and previous treatments (tumor type, grade, resection margin status, hormonal status, previous neoadjuvant or adjuvant chemotherapy, concomitant use of targeted or hormonal therapy) were obtained from medical records. Patients were matched by gender, age, and disease stage. All patients had previously undergone breast-conserving surgery. The surgical procedure included palpation-guided lumpectomy for palpable tumors, while for non-palpable tumors wire-guided or ultra-

sound-guided lumpectomy was used, with negative resection margins on the definitive histopathological specimen. Axillary region surgery included sentinel lymph node biopsy (SN) or axillary lymph node dissection (ALND). Adjuvant chemotherapy, targeted, or hormonal therapy was administered according to the histological characteristics of each patient's tumor and in accordance with national guidelines (13).

The radiotherapy treatment plan included whole breast irradiation (WBI) without adding additional fields to cover regional lymph nodes and tumor bed, so as to make the study groups homogeneous.

Exclusion criteria included the presence of synchronous malignancies, a history of previous breast cancer treated with radiotherapy, bilateral breast cancer, previous radiotherapy in the current radiation area, use of statins in personal therapy, radically operated breast tumors, axillary node status greater than N1a, male gender and unwillingness of the patient to undergo regular follow-ups and check-ups at the radiotherapy ambulance.

Patients were randomly selected to receive either hypofractionated whole breast irradiation (HF-WBI) with a therapeutic dose of 40.05 Gy in 15 fractions over three weeks or standard fractionated whole breast irradiation (CF-WBI) with a therapeutic dose of 50 Gy in 25 fractions over five weeks.

Preparation and method of radiotherapy administration

Preparation for radiation treatment included simulation using a General Electric CT scanner, with patients positioned in a supine position with their arms raised above their head, utilizing appropriate immobilization equipment, specifically an extended wing board in this case. The clinical target volume (CTV) of the breast to be treated was defined using radiopaque markers, as a precondition for easier delineation of target volumes. Delineation of target volumes and organs at risk (OAR) was performed according to national and international guidelines (13-15). The target volume of interest referred to the remaining post-surgery breast tissue, with delineation of the contours of OAR in close anatomical proximity. A protocol margin of 0.7 mm was added to cover inter/intra-fractional errors.

Patients were scheduled to undergo three-dimensional conformal radiotherapy (3DCRT) using megavoltage tangential fields, with beforehand directed planning technique, with the potential addition of segmental fields in order to enhance dose homogeneity. The dose ranged from 95% to 105% of the prescribed dose within the clinical target volume, and the dose to structures outside the treated breast was limited according to the care standard. After the planning phase, therapeutic plans were reviewed, which included the evaluation of dose distribution, color wash dose representation, and dose-volume histograms in order to ensure the appropriateness of the radiotherapy plan. Radiotherapy treatment commenced between 21 and 63 days after the last surgical intervention or the final cycle of adjuvant chemotherapy.

Assessment of skin toxicity

The patients in this study were monitored weekly for acute skin toxicity throughout the duration of radiotherapy. Following the completion of treatment, they were monitored weekly for up to eight weeks since the beginning of the treatment. The next check-up was scheduled three months after the treatment. Additional toxicity assessments were conducted at the discretion of the physician and/or based on the patient's needs. Acute skin toxicity is defined as skin reaction in the irradiated area of the breast occurring during the radiotherapy course or within two months after its completion. The data were noted down as part of personal medical history, and the assessment of skin damage and classification of these acute skin reactions as a result of radiotherapy administration were conducted using the Radiation Therapy Oncology Group (RTOG) scale (16), which includes criteria for the assessment of morbidity caused by radiation. In order to classify the effects of radiotherapy, these criteria categorize the severity of skin reactions into five grades: grade 0 (no reaction), grade 1 (mild erythema, dry desquamation, epilation, or reduced sweating), grade 2 (moderate, rapid erythema, exudative dermatitis in the form of plaques, and moderate edema), grade 3 (exudative dermatitis with involvement of skin folds, and intense edema), and grade 4 (ulceration, bleeding, or necrosis).

Statistical analysis

Data are presented as frequency distributions expressed as percentages or mean values with stan-

dard deviations. Normality distribution was confirmed by Kolmogorov–Smirnov test. The comparison of mean values was done using the Student's t-test, while in case of categorical variables, the Chi-squared or Fischer's exact test was used. Values of $p < 0.05$ were considered to be statistically significant. The statistical package SPSS (version 21.0, IBM Corp, 2012; NY, USA) was used for data processing.

RESULTS

The research involved monitoring and assessing acute skin toxicity in 44 patients with BC. These patients were divided into two groups of 22 patients each: the Gy40 group, which received 40.05 Gy dose in 15 fractions over three weeks, and the Gy50 group, which received 50 Gy dose in 25 fractions over five weeks. The research results, including the socio-demographic characteristics of the patients with BC who participated, are given in Table 1.

Table 1. *Sociodemographic characteristics of the breast carcinoma*

	Group I – Gy40 (n=22)	Group I – Gy50 (n=22)	p value
Age (mean ± SD)	61.9 ± 8.5	50.2 ± 10.4	> 0.05
Type of carcinoma			
Ductal	17	16	> 0.05
Lobular	1	5	
Ductal-lobular	4	1	
Disease stage			
T1 N0	11	16	> 0.05
T2 N0	11	6	
Type of chemotherapy			
Neoadjuvant	4	2	> 0.05
Adjunctive	7	3	> 0.05
Target	4	0	*0.036*
Hormonal	20	21	> 0.05

The average age of patients in the Gy40 group was 61.9 years, while in the Gy50 group it was 50.2 years. Statistical analysis did not reveal a significant difference in age between the groups ($p > 0.05$).

Regarding the histopathological confirmation of BC, 17 patients in the Gy40 group and 16 patients in the Gy50 group had patohistologically verified ductal BC. Lobular BC was detected in one patient in the Gy40 group and in five patients in the Gy50 group. Ductal-lobular carcinoma was diagnosed in four patients in the Gy40 group and in one patient in the Gy50 group. Analyzing the histopathological types of carcinoma in the studied groups, no statistically significant difference was found (Table 1). In addition, there was no statistically significant difference in disease stage ($p > 0.05$), with both groups including patients with early-stage BC (Table 1).

The research results obtained by the analysis of the applied systemic treatments, including chemotherapy, targeted therapy, and hormonal therapy in neoadjuvant and adjuvant approaches, did not show a statistically significant difference between the groups, except in the subgroup receiving targeted therapy ($p = 0.036$). Targeted therapy in the neoadjuvant approach, and subsequently adjuvant up to one year according to care standard, was administered to four patients in the Gy40 group, whereas there were no patients in the Gy50 group who underwent this therapeutic approach.

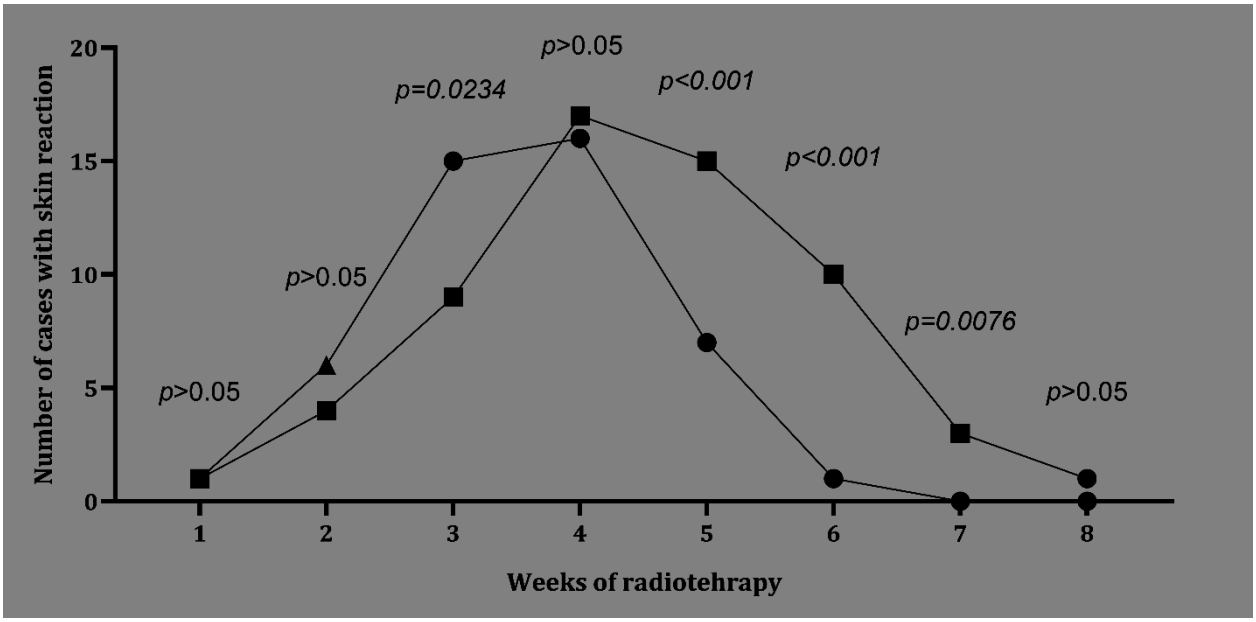

Figure 1. *Frequency of cases with skin reaction to different radiotherapeutic modalities during an eight-week period – Gy40 (square) and Gy50 (circle)*

The frequency of skin reactions over the 8-week period is shown in Figure 1. Throughout this period, patients were monitored for dose differences between modalities, with a slightly higher frequency observed in the group receiving the standard dose regimen of 50 Gy in 25 fractions compared to the hypofractionated regimen of 40.05 Gy in 15 fractions. Statistically significant increases in skin lesions were noted in the 3rd, 5th, 6th and 7th week in the patients receiving the standard dose regimen of TD 50 Gy.

In addition to assessing the incidence of acute skin reactions, the research also evaluated the severity of superficial skin damage, in particular the occurrence of radiodermatitis of grade I (erythema), grade II (dry desquamation), and grade III (moist desquamation) among patients receiving different radiotherapy modalities in the Gy40 and Gy50 groups (Table 2).

Type I was the most common and was observed more frequently in the patients treated with 50 Gy compared to those treated with the hypofractionated regimen of 40.05 Gy (Figure 2). This frequency was statistically significantly higher in weeks 5 and 6 of therapy.

Table 2. *Statistical comparison between the total skin reaction occurrence in patients treated with different radiotherapeutic modalities*

	Group I – Gy40	Group I – Gy50	p value, Chi-square
Type I	46	60	0.043, 6.292
Type II	2	13	
Type III	0	2	

Type II was significantly more prevalent in the Gy50 group compared to the Gy40 group, with significant differences in the frequency of occurrence of these reactions in weeks 5 and 6.

Type III was recorded only in two patients who received 50 Gy. However, a small sample size limits the possibility to achieve statistical significance compared to the Gy40 group, where no patients exhibited this type of reaction (Figure 2).

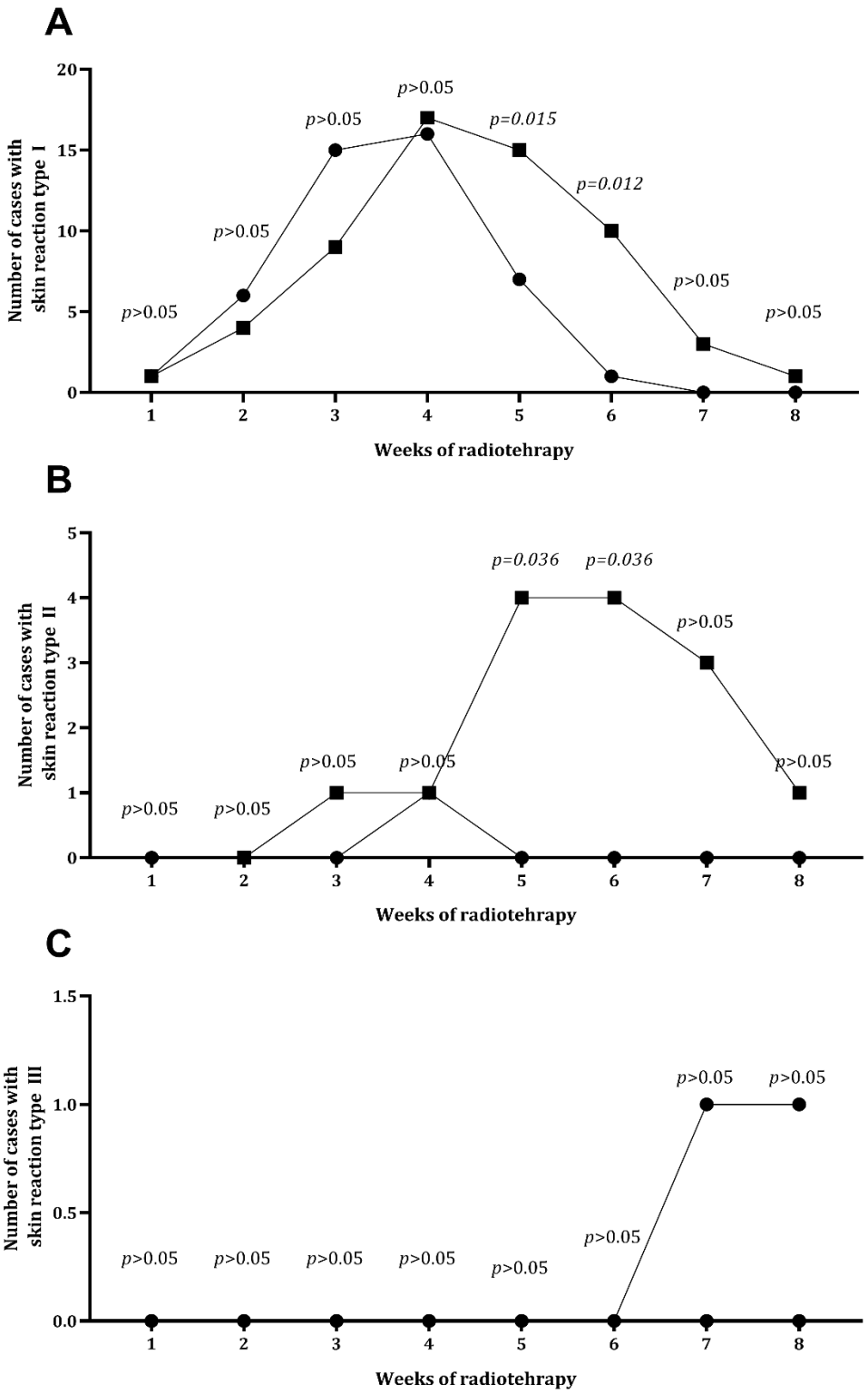

Figure 2. *Frequency of cases with different degree (I – A; II – B; III – C) of skin reaction in relation to different radiotherapeutic modalities during an eight-week period – Gy40 (circle) and Gy50 (square)*

DISCUSSION

Breast-conserving surgery (BCS) combined with postoperative radiotherapy has become the standard approach for treating early-stage BC. However, in order to be applied, it must be medically justified based on the disease stage, histopathological characteristics of the malignant cells, and patient preferences (17, 18). The rationale behind this treatment stems from numerous studies showing improved outcomes in clinical practice in terms of local control, distant disease relapse control, and overall survival (19-21). Without compromising oncological outcomes, this mode of treatment results in better cosmetic outcomes, which is crucial for the patient's quality of life (22-24). The introduction of ionizing photon radiation into clinical practice, generated in radiotherapeutic devices like linear electron accelerators, has enabled the broad application of radiotherapy following lumpectomy. This has enabled the elimination of residual cancer cells in the breast, significantly reducing the risk of locoregional recurrences and contributing to the extension of survival for breast cancer patients (25, 26). This treatment is particularly important for patients with invasive BC, when lymph nodes are also affected or following mastectomy, especially for those with high risk of disease recurrence (27, 28).

The results of this study indicate that the patients were approximately of the same age, with the exception of those in the Gy50 group, who were slightly younger on average, which is in accordance with the care standard in our country. It is also important to note that individual patient factors such as skin type, age, comorbidities, and genetics can significantly impact the occurrence of acute skin toxicity. Some patients may be naturally more resistant to radiation, while others may be more prone to toxicity regardless of the fractionation regimen. It has been highlighted in multiple studies that radiation chemically damages the skin, and the use of statins has been identified as a potential initiator of radiodermatitis (29). The patients involved in this study did not receive this therapy in order to make a better assessment.

No statistically significant differences in cancer type or disease stage between the groups were found, suggesting that patients in both groups had similar basic tumor characteristics. This is important for the study's homogeneity as it allows valid comparisons of the effects of different radiotherapy doses. On the other hand, it is important to emphasize that a significant difference in the use of targeted therapy between the groups was noted. This finding may impact study outcomes, as targeted therapy might have a synergistic effect with radiotherapy (30), which could be considered a study limitation.

The frequency of skin reactions was higher in the Gy50 group, which is expected given the higher radiation dose. However, the observed difference was not drastic, which may indicate good tolerance of higher doses of radiotherapy or the effectiveness of protective measures applied during the treatment, such as the use of moisturizers and skin care products. A study by Lee et al. reported a similar incidence, with 97.3% of cases of radiodermatitis among 111 women with BC in South Korea, although the radiotherapy technique used was not specified (31). Having taken this into consideration, it should be noted that in order to make the group homogenous, all patients in this study underwent three-dimensional conformal radiotherapy (3DCRT) on the treated breast volume, using the opposing tangential fields and additional segmental fields if necessary, for better dose distribution. Patients whose treatment plans required additional fields for the treatment of lymphatics and boost doses on the tumor bed were excluded from the study.

Initial research conducted after the introduction of hypofractionated postoperative breast radiotherapy brought about some uncertainty regarding this protocol's benefits (32), as it could be associated with significant acute and late toxic effects, such as soft tissue necrosis and fibrosis. These concerns and conclusions were likely due to limited knowledge in the field of radiobiology, resulting in the application of lower doses per fraction. Consequently, there was apprehension regarding acute complications with new dose regimens including the application of higher daily doses than the standard ones, as ionizing radiation inevitably affects the skin even when targeted at the tumor-affected tissue. On the other hand, more significant skin toxicity was observed in a study conducted in Brazil, where 100% of 86 women developed skin reactions after radiotherapy with a linear accelerator, receiving a total dose of 50.4 Gy (daily dose of 180 cGy) (33), which to some extent aligns with the results of this study.

Analyzing clinical data, Qi XS et al. obtained encouraging results indicating that the α/β ratio in BC cells is low, providing a basis for introducing

hypofractionated regimens into clinical practice (34). Such an approach proved to be useful during crisis situations like the COVID-19 pandemic, given the increasing number of patients, limited medical personnel, and restricted technical capabilities of the health centers. Studies like the Standardization of Breast Radiotherapy (START-B) and the Ontario Cooperative Oncology Group (OCOG) also highlighted equivalent tumor control with better cosmetic outcomes and late toxicity when using hypofractionated regimens of 40 to 42.5 Gy with daily doses greater than 2 Gy compared to traditional regimens (26, 35). Consequently, hypofractionated radiotherapy for BC has become part of the care standard in our institution, alongside standard protocols involving the application of a therapeutic dose of 50 Gy over five weeks.

This research also assessed the severity of damage during routine check-ups conducted from the first to the eighth week, employing the RTOG scale for skin toxicity. The results show a statistically significant difference in the occurrence of grade I radiodermatitis, specifically the occurrence of erythema in the group treated with standard fractionation of 50 Gy. This is a familiar outcome, as erythema is one of the most common acute skin manifestations of the applied treatment (36). Given that the tissue has a high proliferative index, which makes it highly radiosensitive, this outcome is justifiably one of the most frequent complications of radiotherapy (37). Encouraging results have been obtained by means of comparative analysis which indicates that patients in the hypofractionated group treated with 40 Gy experienced fewer acute adverse effects, manifested as skin reactions, which is in accord with recent clinical research (38). A large multicenter study found that hypofractionation regimen improved patient comfort and reduced dermatitis incidence in patients undergoing postoperative radiotherapy for BC. Consistent with these studies, the obtained results also suggest that the hypofractionated regimen could lower the risk of radiation dermatitis compared to the standard fractionation applied until now (39).

The absence of significant differences between HF and CF regimens suggests that the choice of fractionation regimen should be based on other factors, such as patient comfort, treatment duration, logistical requirements, and patient preferences. Hypofractionated regimens, due to their shorter treatment duration, may be preferable for patients who favor a shorter treatment period or face logistical challenges with longer treatment. Considering these research results along with reduced treatment costs and increased patient convenience (40-42), hypofractionation is justifiably part of the leading radiotherapy guidelines and protocols as a superior and cost-effective treatment option.

CONCLUSION

Despite significant advancements in medicine and technology, the number of breast cancer patients continues to rise, necessitating further research and development of effective strategies for prevention, early detection, and treatment, as well as improving the quality of life for patients after treatment. The current challenge is to minimize morbidity caused by radiotherapy while preserving efficacy at the same time. This study contributes to a better understanding of the incidence of acute adverse effects of postoperative radiotherapy for breast cancer and aids in shaping clinical practice recommendations with a focus on reducing acute toxicity and improving tolerance of hypofractionated radiotherapy treatments. The results suggest that, despite differences in radiotherapy dose, there are no significant differences in the basic characteristics of patients, and that higher doses of radiotherapy are associated with slightly higher incidence of skin reactions. It is important to emphasize that a careful selection of daily dose and total dose of radiotherapy can significantly impact the balance between treatment efficacy and minimizing side effects. Thorough consideration of this balance will aid in better understanding regarding treatment planning for individual patients.

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Akutna kožna toksičnost kod pacijentkinja sa karcinomom dojke nakon različitih režima frakcionisanja postoperativne radioterapije

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SAŽETAK

Uvod/Cilj. Karcinom dojke predstavlja značajan zdravstveni problem na globalnom nivou, sa incidencijom koja varira širom sveta. Savremeni terapijski protokoli uključuju multidisciplinarni pristup koji kombinuje hirurgiju, hemoterapiju, hormonoterapiju, ciljanu terapiju i radioterapiju. Radioterapija je ključna u tretmanu karcinoma dojke, ali može izazvati neželjene efekte, među kojima su i reakcije na koži. Cilj ovog istraživanja bio je da se proceni uticaj dvaju različitih režima frakcionisanja radioterapije na pojavu i težinu akutne kožne toksičnosti kod pacijentkinja sa karcinomom dojke.

Pacijenti i metode. Prospektivna studija je obuhvatila 44 pacijentkinje koje su podvrgnute postoperativnoj radioterapiji. Pacijentkinje su bile nasumično raspoređene u dve grupe: jedna grupa je tretirana hipofrakcionisanim režimom (40,05 Gy u 15 frakcija tokom tri nedelje), dok je druga grupa primala standardni frakcionisani režim (50 Gy u 25 frakcija tokom pet nedelja). Kod pacijentkinja je u toku tretmana na nedeljnom nivou vršena procena akutne kožne toksičnosti.

Rezultati. Rezultati istraživanja su pokazali da su kod pacijentkinja koje su primale standardni frakcionisani režim učestalost i intenzitet akutnih kožnih reakcija, uključujući eritem, suvu deskvamaciju i vlažnu deskvamaciju, bile veće. Reakcije kože gradusa I i II bile su naročito izražene kod pacijentkinja iz grupe koja je primala 50 Gy. Mada su pacijentkinje koje su primale hipofrakcionisanu radioterapiju imale manje ozbiljne kožne reakcije, pojavile su se blage promene na koži, koje su generalno bile slabije izražene.

Zaključak. Ova studija je ukazala na to da je potrebno pažljivo odabrati režim frakcionisanja u postoperativnoj radioterapiji dojke. Takođe, doprinela je razumevanju odnosa između različitih radioterapijskih modaliteta i pojave akutne kožne toksičnosti, pružajući pritom smernice za optimizaciju tretmana kod pacijentkinja sa karcinomom dojke.

Ključne reči: **rak dojke, poštedna operacija dojke, radioterapija, radiodermatitis**

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Original article

The Association between 4-Hydroxyglutamate and Pre-Eclampsia

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SUMMARY

Introduction/Aim. Pre-eclampsia is a major cause of perinatal mortality. Glutamate plays a critical function in the promotion of a healthy pregnancy. Therefore, the aim of this study was to determine the extent of the correlation between pre-eclampsia and 4-hydroxyglutamate acid.
Methods. This is a case-control study that was conducted on a sample of 100 pregnant women in the third trimester. The study group consisted of 26 cases of mild pre-eclampsia and 24 cases of severe pre-eclampsia, while the control group consisted of 50 normotensive pregnant women. In addition to human 4-hydroxyglutamate, which was measured by ELISA, liver function test, renal function test, uric acid, serum lactate dehydrogenase (LDH), complete blood picture, and urine albumin were performed for all patients.
Results. The mean level of 4-hydroxyglutamate was significantly higher in the case group (mean ± SD = 243 ± 59 pg/ml) than in the control group (mean ± SD = 90 ± 45 pg/ml), with a range of 29–351 pg/ml vs 4–185 pg/ml and a p-value of < 0.0001/ml. Severe pre-eclampsia patients had higher mean 4-hydroxyglutamate levels (257.88 ± 43.436 pg/ml) than mild cases (229.77 ± 68.789 pg/ml), although the difference was non-significant. A 4-hydroxyglutamate level of ≥ 142.5 was shown to be a highly sensitive (92%) and specific (85.2%) indicator of pre-eclampsia using the receiver operator characteristics curve. A 4-hydroxyglutamate level of ≥ 142.5 led to a 5.75-fold higher risk of pre-eclampsia.
Conclusion. The level of 4-hydroxy glutamate was increased significantly in pregnant women with pre-eclampsia compared to healthy women and could be used as a predicting marker with high sensitivity.

Keywords: **pre-eclampsia, glutamate, laboratory markers**

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INTRODUCTION

Glutamate and glutamine are amino acids that are substantially consumed and highly prevalent in the neonate during the final phases of gestation. Glutamine is involved in the metabolism of carbon and nitrogen in fetuses and has a significantly higher concentration ratio in the plasma of fetuses than in their mothers, surpassing other amino acids in humans and mammals (1).

Pre-eclampsia (PE) is often regarded as a placental disorder since it manifests in individuals with hydatidiform moles and the only effective treatment is the delivery of the placenta. Effective implantation of the embryo and the subsequent development of the placenta requires proper spiral arteries remodeling via trophoblastic invasion. Emerging evidence indicates that an insufficient energy supply may result in abnormal implantation and superficial spiral artery remodeling, which disrupts the metabolome of the placenta and fetus. This disruption can lead to placental ischemia-induced pre-eclampsia, which is followed by pervasive alterations in the metabolome. Therefore, PE is linked to many metabolic problems such as dyslipidemia, hyperuricemia, hyperglycemia, and insulin resistance (2–4). It is widely accepted that the regulation of glutamate and glutamine transfer in the placenta is essential for the success of a healthy pregnancy (5).

The function of glutamate in the PE has not been thoroughly examined. Sovio et al. (5) found that when measured in the first trimester, it could serve as a reliable predictor of the occurrence of pre-eclampsia. In this study, we aimed to determine the association between 4-hydroxyglutamate acid and pre-eclampsia in the third trimester.

PATIENTS AND METHODS

This research is a case-control study carried out at the Department of Obstetrics and Gynecology of Baghdad Teaching Hospital during the period between February and October 2022. Administrative approvals were granted from the Council of Iraqi Board of Medical Specialization: registration number (EAC–1248) on January 5, 2022. Also, the approval of the Department of Obstetrics and Gynecology at Baghdad Teaching Hospital was obtained: registration number (8557) on December 21, 2021.

The study included a sample of 100 pregnant women in the 3rd trimester ≥ 28 weeks of gestation. The women were divided into two groups. Group 1: The case group consisted of 50 pregnant women with pre-eclampsia, with 26 cases being mild and 24 cases being severe. Group 2: The control group consisted of 50 normotensive individuals.

The inclusion criteria for study groups were singleton pregnancy in the third trimester (28 to 39 weeks) with pre-eclampsia in mild or severe form with a viable fetus. Women with gestational age less than 28 weeks, and those with chronic diseases (chronic hypertension, diabetes, thyroid disease, renal disease, liver disease, autoimmune disease, hematological diseases, cardiovascular diseases), multiple pregnancies, smoker, chronic drug users, eclampsia at presentation, intrauterine fetal death were excluded from the study. All patients were informed about the nature of the study and verbal consent was taken from them.

Patients in both groups underwent general examination, vital signs (systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate, respiratory rate, body temperature) were checked as well as abdominal and obstetric examination. A mercurial sphygmomanometer was employed to measure the blood pressure (BP) after the patient had been seated for a period of time. In the event that the measured BP was 140/90 or higher but less than 160/110, the BP would be reassessed after approximately four hours. A urine sample would be collected to test for albumin if the reading remained the same or increased. The patient would be diagnosed with pre-eclampsia and enrolled in the study if the result was positive. For those with BP of ≥ 160/110, a urine sample for albumin would be requested without delay and when positive, the patient would be confirmed with pre-eclampsia. Subsequently, a blood sample would be collected. The level of S. 4-hydroxy-L-glutamic acid was performed for all patients in addition to other laboratory investigations such as proteinuria, protein/creatinine ratio, liver function test (AST, ALT, S. albumin), complete blood count (CBC), uric acid, LDH, renal function test (BUN, S. creatinine).

Demographic and clinical data were collected through the distribution of a well-designed questionnaire including maternal age, educational level, occupation, residency, parity, and gestational age, which was confirmed by early pregnancy ultrasound as well as abdominal and obstetrical ultrasound (Philips HD 11 ultrasound machine) performed by a radiologist at the time of admission.

Blood sample collection: Five milliliters of venous blood were obtained from the participants prior to the administration of any medication. The entire blood sample was collected with anticoagulant containing tubes, such as citrate or EDTA. The tubes were centrifuged at a speed of 2,000 to 3,000 rpm for 20 minutes after being maintained at ambient temperature for a period of 10 to 20 minutes. Retrieved plasma samples were frozen at -4 °C and thawed prior to measurement.

Human 4-hydroxyglutamate (4OHGlu) was measured using a designated ELISA Kit Catalogue Number: SL3706Hu) and loaded into the analyzer. The assay range provided by the manufacturer is 4 pg/ml-200 pg/ml

Statistical analysis: All statistical analyses were conducted using IBM-SPSS (USA Chicago) and data were presented in the form of counts, percentages, mean, standard deviation (SD), minimum (Min), and maximum (Max).

The association between categorical variables was assessed by Chi-square or Fisher exact test, while the Student's t-test or Mann-Whitney u test was used to compare continuous variables, as suitable. The receiver operating characteristic (ROC) curve was utilized to determine the optimal cut-off points, following the Yoden J index test, for estimating the area under the curve (AUC), sensitivity (SN), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), test accuracy (Acc), and relative risk of each variable.

RESULTS

The demographic data of study groups are illustrated in Table 1. There was no significant difference between the study and control groups.

The case group had significantly more irregular antenatal care visits, although there was no statistically significant difference between educational level and residency, as shown in Table 2.

As depicted in Table 3, both baseline systolic and diastolic blood pressures were significantly higher in the case group compared to the control group. Further, investigations showed that the values of liver function test were significantly higher in the study group as compared to the control.

Table 1. *Demographic data*

Variables	Case		Control		P Value
	Mean	SD	Mean	SD	
Age	29	9	28	8	0.508
Gravidity	4	3	5	3	0.268
Parity	2	2	3	2	0.233
Miscarriage	1	1	1	1	0.789
GA	33	7	34	7	0.409

* GA: gestational age

Table 2. *Antenatal care, education distribution*

Variable		Case	Control	Total	P value
		No. (%)	No. (%)	No. (%)	
ANC (antenatal care)	**Irregular**	47 (94)	33 (66)	80 (80)	**< 0.0001**
	Regular	3 (6)	17 (34)	20 (20)	
Educational level	**Illiterate**	10 (20)	14 (28)	24 (24)	0.106
	Primary	19 (38)	24 (48)	43 (43)	
	Secondary	14 (28)	11 (22)	25 (25)	
	University	7 (14)	1 (2)	8 (8)	

*ANC: antenatal care; No.: number

Table 3. *Distribution of vital signs and relation between different parameters in studied groups*

Variables	Case		Control		P value
	Mean	SD	Mean	SD	
Systolic BP	158	22	126	12	**< 0.0001**
Diastolic BP	97	21	68	15	**< 0.0001**
PR	94	25	92	24	0.689
Variables	Control		Mild	Severe	P value
	Mean ± SD		**Mean ± SD**	**Mean ± SD**	
AST (aspartate transaminase)	38.04 ±9.52		76.15 ±24.51	86.88 ±22.43	**< 0.0001**
ALT (alanine transaminase)	37.92 ±9.91		78.58 ±21.08	92.25 ±21.07	**< 0.0001**
BUN (blood urea nitrogen)	21.78 ±5.38		27.58 ±3.74	28.83 ±4.33	**< 0.0001**
Creatinine	1.25 ±0.34		1.85 ±0.19	1.89 ±0.22	**< 0.0001**
Uric acid	5.82 ±0.93		6.94 ±0.82	6.95 ±0.89	**< 0.0001**
S. albumin	3.85 ±0.47		3.14 ±0.41	3.28 ±0.49	**< 0.0001**
LDH	178.74 ±34.65		260.58 ±46.17	284.38 ±47.58	**< 0.0001**
Platelet	258.26 ±66.79		93.46 ±17.84	89.46 ±16.8	**< 0.0001**
Protein/Creatinine ratio	21.84 ±4.4		35.95 ±4.69	34.87 ±4.88	**< 0.0001**

*BP: blood pressure; PR: pulse rate; *n= number, *SD = standard deviation, *P = probability, *AST = Aspartate transaminase, *ALT = Alanine transaminase, *BUN = Blood urea nitrogen, *S = serum, *LDH = Lactate dehydrogenase, *Cr = Creatinine

Table 4. *Distribution of 4-hydroxy glutamate*

	Mean	Standard deviation	P. value
Case	**243**	**59**	**< 0.0001**
Control	**90**	**45**	**< 0.0001**

Table 5. *Distribution of 4-hydroxyglutamate according to pre-eclampsia severity*

Preeclampsia	4- hydroxyglutamate			
	No.	Mean	SD	P value
Severe	24	257.88	43.436	0.089
Mild	26	229.77	68.789	

Similarly, LDH, BUN, S. creatinine uric acid, and protein/creatinine ratio were all significantly higher in the pre-eclampsia group, whereas platelet and albumin were lower in pre-eclampsia cases compared to control.

The mean level of 4-hydroxyglutamate was significantly higher in the case group (mean ± SD = 243 ± 59 pg/ml) when compared with the control (mean ± SD = 90 ± 45 pg/ml), while the range was (29–351 pg/ml vs 4–185 pg/ml), with a p-value of < 0.0001, as shown in Table 4.

Regarding severity of preeclampsia there was no difference in level of 4 hydroxy glutamate between mild and severe preeclampsia as shown in Table 5.

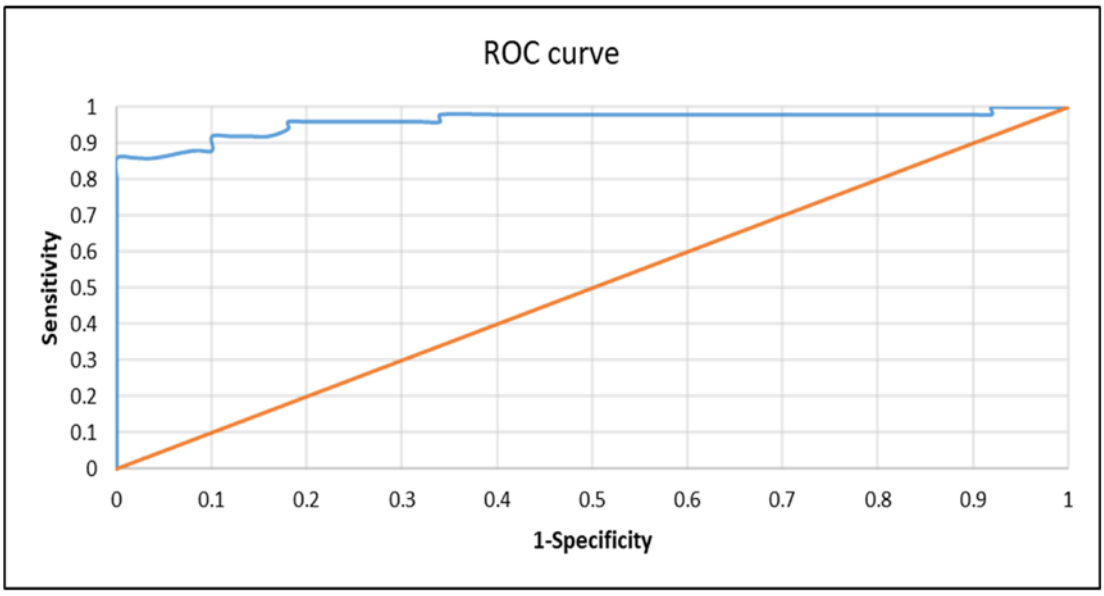

Figure 1. *ROC curve analysis*

Table 6. *Predictive ability of 4-hydroxyglutamate*

Parameter	Level
AUC	0.963
95% CI	0.922-1.000
Cutoff point (pg/ml)	≥ 142.5
Sensitivity	92%
Specificity	84%
PPV	85.2%
NPV	91.3%
Accuracy	88%
Odd ratio	5.75
95% CI	3.031-10.908

The receiver operator characteristics curve revealed that a 4-hydroxyglutamate level of ≥ 142.5 was a highly sensitive (92%) and specific (85.2%) marker of pre-eclampsia. Patients with a 4- hydroxyglutamate level of ≥ 142.5 had a 5.75-fold increased the risk of pre-eclampsia, as shown in Figure 1 and Table 6.

DISCUSSION

Treatment of hypertensive diseases during pregnancy is a distinctive challenge due to the fact that therapeutic interventions affect both the mother and the embryo simultaneously, occasionally resulting in a conflict between their well-being. Pre-eclampsia has the potential to rapidly progress to severe complications, which may lead to the death of both the mother and the infant (6).

Metabolomics is an essential element of system biology, with a primary emphasis on the analysis of fluids, including blood, urine, and excrement. It examines the small molecule metabolites present in various metabolic pathway matrices and their resulting products. In contrast to other "omics" techniques, metabolomics is a potent methodology that enables the direct evaluation of cellular/tissue biochemical activity and state by measuring metabolites and their concentrations, hence providing a pre-

cise representation of the molecular phenotype (7). Lipidomics, a specific branch of metabolic profiling (8) that has been associated with the onset and progression of a variety of diseases, including cardiovascular metabolic syndrome (9), is intricately linked to the dysregulation of lipid metabolism. Metabolomics and lipidomics have the potential to provide new and valuable information for a more comprehensive understanding of pre-eclampsia. Additionally, they may disclose the complex molecular processes that contribute to the development of pre-eclampsia.

The association of pre-eclampsia with maternal age, first pregnancy, and low socioeconomic class were reported previously (10–14), however, this was not evident in the current study probably due to the small sample size. Similarly, we did not observe an association between pre-eclampsia and previous miscarriage, whereas a meta-analysis of 68,185 patients concluded that previous miscarriage may lead to placental dysfunctional disorder causing pre-eclampsia (15). Regular antenatal care may provide a primary preventive approach for high-risk patients such as lifestyle modification and low doses of aspirin (16). This may explain the significant association between pre-eclampsia and irregular antenatal care visits in the current study which is in keeping with what has been reported by Briceño-Pérez et al. (16).

The most interesting observation reported about 4-hydroxyglutamate is that, in contrast to other protein biomarkers, like PAPP-A and PIGF, the maternal blood level of 4-hydroxyglutamate during the first trimester is significantly associated with the risk of developing pre-eclampsia, and this association is independent of maternal characteristics (17).

Collagen, whether ingested or synthesized in the body, is the primary source of 4-hydroxyproline, which is converted into 4-hydroxyglutamate in the mitochondria. Increased collagen turnover and 4-hydroxyproline release are the causes of the higher 4-hydroxyglutamate levels. Maintaining a healthy pregnancy also depends on the placenta's ability to regulate glutamate and glutamine exchange (1).

The mean level of 4-hydroxyglutamate was significantly higher in the case group than the control in the third trimester. In Sovio et al.'s study (5) which included 519 participants, they compared the level of 4-hydroxyglutamate in 194 women with pre-eclampsia with 325 normotensive women at 12, 20, 28, and 36 weeks of gestation and found that 4-hydroxyglutamate was universally elevated (from week 12 to 36) in cases of pre-eclampsia, and it was considered as a predictor for the development of pre-eclampsia when measured as early as the 12th week.

In the current study, it was determined that 4-hydroxyglutamate is a sensitive and specific marker of pre-eclampsia. AUC values of 0.963 were associated with a 5.75-fold increased risk of pre-eclampsia at the level of 142.5 pg/ml. At the 12th week of gestation, Sovio et al. determined that the AUC was 0.673 (5). However, the 4-hydroxyglutamate exhibitted no significant predictive ability for severity prediction in the current study. In their comparison of 104 cases of pre-eclampsia with 100 normotensive women, Zhao et al. (14) (n = 204) found that the sensitivity for the prediction of pre-eclampsia was 84.2%, with a specificity of 68% for mild pre-eclampsia and 75% and 86% for severe pre-eclampsia.

The elevated level of 4-hydroxyglutamate may be attributed to the effect of pre-eclampsia by the activation of gluconeogenesis with the formation of glutamine products, especially 4-hydroxyglutamate, that are normally transported across the placenta (18). The presence of inadequate oxygen supply in cells and placenta, known as a hypoxic condition, leads to a state similar to placental oxidative stress in pre-eclampsia. This is supported by the observed increase in markers of hypoxia (oxoguanine DNA glycosylase 1, hypoxia-inducible factor 1 alpha subunit) and pre-eclampsia (soluble fms-like tyrosine kinase 1) both in laboratory experiments and in living organisms (19).

In cases of pre-eclampsia, renal function tests (BUN and serum creatinine) were both elevated. This indicates that high blood pressure has a detrimental impact on the kidneys, further compounded by proteinuria, which in turn affects filtration gradients. Charles et al. (14) (n = 144) observed a comparable outcome when they compared 72 cases of pre-eclampsia and 72 normotensive women in terms of renal function and electrolytes. They discovered that deteriorating renal function was associated with an increase in the severity of pre-eclampsia. As suggested by Bellos et al. in their meta-analysis, uric acid levels are significantly elevated in pre-eclampsia cases (20). This elevation is a result of vascular injury that is induced by pre-eclampsia, which in turn activates oxidative stress and inflammatory cascades. The meta-analysis included 196 studies and constituted 39,540 women.

They discovered that serum uric was further elevated in cases of severe pre-eclampsia, eclampsia, and HELLP syndrome. The serum albumin was significantly lower in pre-eclampsia cases due to two factors: first, the urinary loss of albumin as a result of pre-eclampsia, and second, the body is under stress in the pre-eclampsia condition, which causes a decrease in albumin, a negative acute phase reactant, as a result of increased consumption, decreased production, and increased loss to the interstitial space, resulting in edema. Shi et al. (n = 618) discovered a comparable outcome when they compared 309 women with pre-eclampsia and 309 normotensive women (21). They discovered that an excessive decrease in serum albumin (from the baseline at the early third trimester) toward the end of pregnancy was associated with an increased risk of pre-eclampsia complications. In cases of pre-eclampsia, serum lactate dehydrogenase is substantially elevated. This may be attributed to a variety of factors, including tissue ischemia (placental ischemia), increased glycolysis (as a response to stress), and endothelial injury. Saleem et al. (n = 60) also discovered a comparable outcome when they compared the serum LDH levels of 30 women with pre-eclampsia and 30 normotensive women. They determined that LDH is a robust marker and predictor of pre-eclampsia (22).

CONCLUSION

The level of 4-hydroxyglutamate was increased significantly in pregnant women with pre-eclampsia in comparison to healthy women and could be used as a predicting marker with high sensitivity.

DECLARATIONS

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

Dr. Noor A. Fawzi: concepts, design, literature search, clinical studies, data analysis, statistical analysis, manuscript preparation and manuscript review.

Prof. Wasan Wajdy: concepts, design, data analysis, manuscript preparation and manuscript review.

Ethics approval and consent to participate

Prior to data collection, a statement of patient by written informed consent to participate in the study as specified in the Declaration of Helsinki was sought from each patient and the information gathered was kept anonymous. Personal names were substituted with identifying numbers. The information was securely stored on a laptop protected by a password, and the data was solely used for research objectives while maintaining confidentiality.

Administrative approvals were granted from Council of Iraqi Board of Medical Specialization: registration number (EAC- 1248) on January 5, 2022. Also, the Approval of the Department of Obstetrics and Gynecology at Baghdad Teaching Hospital: registration number (8557) was obtained on December 21, 2021.

Patient consent for publication

A statement of consent for publication was obtained from the patient according to the principles of the Declaration of Helsinki.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools (to be included only when AI tools are used): none.

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Povezanost 4-hidroksiglutamata sa preeklampsijom

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SAŽETAK

Uvod/Cilj. Preeklampsija je jedan od glavnih uzroka perinatalnog mortaliteta. Glutamat ima ključnu ulogu u održavanju zdrave trudnoće. Stoga, cilj ove studije bio je da se utvrdi stepen povezanosti između preeklampsije i 4-hidroksiglutamatne kiseline.

Metode. Ovo je studija tipa slučaj-kontrola, sprovedena na uzorku od 100 trudnica u trećem trimestru trudnoće. Ispitivana grupa sastojala se od 26 trudnica sa blagom preeklampsijom i 24 trudnice sa teškom preeklampsijom. Kontrolnu grupu činilo je 50 normotenzivnih trudnica. Svim pacijentkinjama je određen nivo 4-hidroksiglutamata (metodom ELISA). Takođe, urađeni su testovi funkcije jetre i bubrega i analiza kompletne krvne slike i određeni nivoi mokraćne kiseline, laktat dehidrogenaze (LDH) u serumu i albumina u urinu.

Rezultati. Prosečan nivo 4-hidroksiglutamata bio je značajno viši u grupi ispitanica (srednja vrednost ± SD = 243 ± 59 pg/ml) nego u kontrolnoj grupi (srednja vrednost ± SD = 90 ± 45 pg/ml), sa opsegom 29–351 pg/ml u poređenju sa 4–185 pg/ml, i vrednošću p < 0,0001/ml. Pacijentkinje sa teškom preeklampsijom imale su više prosečne vrednosti 4-hidroksiglutamata (257,88 ± 43,436 pg/ml) nego one sa blagim oblikom preeklampsije (229,77 ± 68,789 pg/ml), premda ta razlika nije bila statistički značajna. Nivo 4-hidroksiglutamata ≥ 142,5 pokazao se kao visoko osetljiv (92%) i specifičan (85,2%) pokazatelj preeklampsije prilikom korišćenja ROC (engl. *receiver operator characteristics*) krive. Nivo 4-hidroksiglutamata ≥ 142,5 bio je povezan sa 5,75 puta većim rizikom od razvoja preeklampsije.

Zaključak. S obzirom na to da je nivo 4-hidroksiglutamata bio značajno viši kod trudnica sa preeklampsijom nego kod zdravih trudnica, može se koristiti kao prediktivni marker sa visokom osetljivosti.

Ključne reči: **preeklampsija, glutamat, laboratorijski markeri**

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Original article

The Application of Artificial Intelligence in the Healthcare System Management in the Republic of Serbia: Enhancing Efficiency, Predictive Capacity, and Decision-Making

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SUMMARY

Introduction/Aim. Artificial intelligence (AI) offers transformative potential in healthcare management by enhancing predictive analytics, optimizing resource allocation, and supporting clinical decision-making. This study aims to examine the applications of AI in Serbian healthcare institutions, focusing on improving operational efficiency and patient outcomes.
Methods. The research employed a cross-sectional survey conducted among 450 healthcare professionals from various levels of healthcare in Serbia (primary, secondary, and tertiary). Data were collected via an online survey during October and November 2024. Statistical analysis included methods such as ANOVA and regression analysis to evaluate the impact of AI on diagnostic accuracy, resource optimization, and patient satisfaction.
Results. The study found that AI implementation positively impacts diagnostic accuracy (88% of respondents), resource optimization (82%), and patient satisfaction (79%). Differences were observed between urban and rural areas, as well as between public and private healthcare institutions. Major challenges identified include the lack of training (75%), data privacy concerns (68%), and limited infrastructure (70%).
Conclusion. The study confirms that AI holds significant potential to improve healthcare in Serbia, particularly in urban and private institutions with better infrastructure. However, addressing challenges related to training, data privacy, and infrastructure is crucial, especially in rural areas. A phased approach to AI implementation is recommended, focusing initially on diagnostics and resource management to maximize healthcare performance.

Keywords: **artificial intelligence, healthcare management, predictive analytics, decision support systems, resource optimization, Serbia**

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INTRODUCTION

Artificial intelligence (AI) is increasingly recognized as a critical tool for transforming healthcare. By enabling data-driven insights, streamlining clinical processes, and enhancing patient outcomes, AI has shown significant promise in improving healthcare delivery (1, 2). As healthcare systems face growing pressures due to aging populations, rising healthcare costs, and increased chronic disease prevalence, AI provides a solution for meeting these demands without overextending healthcare resources (3). The COVID-19 pandemic has further underscored the need for adaptive, resilient healthcare systems, where AI applications have facilitated rapid data analysis, vaccine distribution, and patient monitoring (4, 5).

Countries with well-established digital infrastructure, such as the United States, Germany, and Japan, have successfully integrated AI in various healthcare applications, including radiology, genomics, and patient management (6). However, for countries like Serbia, where resources are limited, implementing AI presents unique challenges and opportunities. The Serbian healthcare system comprises a mix of public and private institutions with significant disparities between urban and rural healthcare access. These inequalities are particularly evident in rural areas, where healthcare facilities often lack specialized medical personnel and advanced equipment (7). Addressing these disparities with AI could bridge the gap, offering equal healthcare quality across different regions (8).

Despite these potential benefits, AI integration in healthcare is complex and requires considerable investments in infrastructure, workforce training, and regulatory adaptation (9). Serbia faces specific obstacles, including limited technological infrastructure, budgetary constraints, and a need for skilled personnel who can operate and manage AI systems. Additionally, the ethical implications of AI, such as data privacy, transparency, and algorithmic fairness, are critical considerations in healthcare contexts, where patient trust and safety are paramount (10). This study explores the potential impact of AI on the Serbian healthcare system, focusing on key areas such as predictive analytics, diagnostics, and resource management. The findings aim to support policymakers in understanding AI's benefits and challenges and inform strategies for its effective implementation.

The aim of the study was to provide foundational insights for policymakers on the integration of AI within Serbia's healthcare system. It focuses on detailing both the potential enhancements AI can bring in terms of diagnostic accuracy, resource optimization, and patient outcomes, as well as the challenges faced, including infrastructure, ethical considerations, and the need for skilled personnel. The study aims to guide strategic decisions for effective adoption and implementation of AI technologies to enhance the healthcare system's overall performance in Serbia.

LITERATURE REVIEW

The theoretical framework for AI in healthcare is based on computational intelligence, data science, and machine learning (ML) theories, which enable AI systems to analyze data, make predictions, and support clinical decision-making processes (11, 12). ML models are pivotal in AI's healthcare applications, as they allow systems to recognize patterns within data, adapt to new information, and improve predictive accuracy over time.

Predictive analytics and patient flow management

Table 1 (13-16) shows predictive analytics in healthcare and utilizes historical and real-time data to forecast patient inflows, aiding administrators in resource allocation and reducing bottlenecks (17). This capability is essential for Serbian hospitals, especially in urban centers, where high patient volumes often strain resources. AI-based predictive tools can inform hospital administrators of expected patient surges, enabling them to optimize staffing, bed assignments, and other critical resources to minimize patient wait times and improve care quality (18, 19).

Table 1. *Key predictive analytics applications in healthcare*

Application	AI Capability	Benefits	Reference
Patient admission prediction	Predicting daily admissions	Improves resource allocation	(13)
Readmission risk estimation	Identifying high-risk patients	Reduces readmissions	(14)
Length of stay forecasting	Estimating patient length of stay	Optimizes bed management	(15)
Emergency demand prediction	Forecasting emergency cases	Enhances emergency preparedness	(16)

Table 2. *AI-driven resource optimization applications in healthcare*

Resource optimization area	AI approach	Outcome	Reference
Staff scheduling	Reinforcement learning	Reduced wait times and burnout	(24)
Equipment utilization	Predictive analytics	Maximized use of medical equipment	(25)
Inventory management	Demand forecasting	Optimized supply chain processes	(26)
Facility maintenance	Predictive maintenance models	Reduced downtime for critical assets	(27)

Diagnostic precision and clinical support

AI enhances diagnostic accuracy across multiple medical fields, including radiology, pathology, and oncology. Deep learning models, such as convolutional neural networks (CNNs), are particularly effective in image-based diagnostics, helping to identify abnormalities such as tumors, fractures, and cardiovascular issues with precision comparable to human specialists (20, 21). Research has demonstrated that AI algorithms can detect early-stage cancer in medical imaging, contributing to earlier intervention and potentially better patient outcomes (22). In Serbia, these tools are invaluable for rural clinics, allowing healthcare providers in underserved areas to access diagnostic support remotely, thereby mitigating the disparity between urban and rural healthcare quality (23).

Resource optimization

Table 2 (24-27) shows that efficient resource utilization is essential for healthcare systems that face financial and logistical constraints. AI in resource optimization leverages machine learning algorithms to manage hospital resources such as staff, equipment, and supplies, ensuring that each is allocated effectively based on patient needs and institutional capacities (28). In Serbian hospitals, resource optimization is particularly critical given the frequent budget constraints and the high demand for services. By automating routine tasks, AI can help healthcare administrators reduce waste, lower operational costs, and improve service delivery (29).

Ethical considerations and data privacy

AI's reliance on extensive data raises ethical and privacy concerns in healthcare. Protecting patient privacy is a top priority, as sensitive data is integral to AI's operation. Healthcare institutions must implement stringent data governance frameworks to secure data and prevent breaches (30). Additionally, the interpretability of AI decisions, particularly with complex algorithms, is crucial to maintain transparency and accountability in medical settings (31). AI governance in healthcare must ensure that decisions are justifiable and free from biases, which can impact patient care and ethical standards (32).

Research underscores the wide-ranging applications of AI in healthcare, highlighting its role in enhancing diagnostic precision, improving patient outcomes, and optimizing operational efficiency. Studies show that AI tools can reduce diagnostic errors, streamline workflows, and support clinical decision-making, but also emphasize the challenges in infrastructure, data security, and ethical governance (33, 34). AI's role in supporting telemedicine is also critical, particularly in geographically isolated or underserved areas, as it provides remote diagnostic capabilities and bridges gaps in healthcare access (35). The literature emphasizes the need for tailored approaches in resource-constrained countries like Serbia, where AI applications must be adapted to the local context and healthcare priorities (36). Table 3 gives a summary of findings from AI healthcare literature.

METHODS

The study appears to utilize a cross-sectional survey design. This type of study design involves collecting data at a single point in time, or over a short period, to analyze current attitudes, beliefs, or practices within a specific population—in this case, healthcare professionals across various institutions in Serbia. The survey assesses perceptions and barriers related to the adoption of AI in healthcare settings, providing a snapshot of current opinions and issues at the time the data was collected. This method is effective for gaining an overview of the current state of AI integration and identifying the key factors influencing its implementation in healthcare.

Sampling and data collection

The study involved an online anonymous survey of 450 healthcare professional in primary, secondary and tertiary level of healthcare from diverse

Table 3. *Some findings from AI healthcare literature*

Country/Region	AI implementation focus	Outcome	Reference
India	Telemedicine	Enhanced access in rural areas	(37)
Brazil	Resource management	Decreased operational costs	(38)
China	Predictive analytics	Improved patient flow management	(39)
UK	Diagnostic support	Increased diagnostic accuracy	(40)

Table 4. *Demographics of survey respondents by institution type and role*

Institution type	Physicians (%)	Nurses (%)	Admin staff (%)	Tech/IT specialists (%)
Urban	45	30	15	10
Rural	35	40	20	5
Public	42	38	15	5
Private	48	28	12	12

Serbian healthcare institutions in the period from October to November 2024, covering urban and rural regions, public and private sectors, which is shown in Table 4. The survey collected both quantitative and qualitative data on AI's perceived impact and adoption barriers. Additionally, in-depth interviews were conducted with key stakeholders to gain insights into specific infrastructural and operational challenges associated with AI implementation.

Qualitative characteristics of the study

1. In-depth research approach: The paper conducts a thorough investigation into how AI can transform healthcare system management in Serbia, utilizing statistical methods such as ANOVA and regression for data analysis.

2. Multidisciplinary focus: The study incorporates theories from computational intelligence, data science, and machine learning, providing a comprehensive overview of how AI can improve diagnostics, resource management, and clinical decision-making.

3. Emphasis on ethical and infrastructure issues: The research highlights the importance of ethical considerations and infrastructural challenges in the integration of AI into healthcare, which are crucial for successful implementation.

4. Practical implications and policy recommendations: Beyond theoretical analysis, the paper offers concrete recommendations for policymakers, including the need for infrastructure development, workforce training, and data management.

5. Primary data utilization: The study uses survey data collected from 450 healthcare professsionals across various institutions in Serbia, providing firsthand relevant data that supports the findings.

Quantitative characteristics of the study

1. Statistical analysis: The research employs advanced statistical techniques, including analysis of variance (ANOVA) and regression analysis, to quantify the impacts of AI adoption on healthcare metrics such as diagnostic accuracy, patient satisfaction, and operational efficiency.

2. Empirical data collection: The study features a structured survey targeting a large sample of healthcare professionals, which gathers both quantitative and qualitative data on the perception and barriers to AI adoption.

3. Data-driven insights: The analysis of survey results offers numeric values that demonstrate the perceived benefits and challenges of AI in healthcare, allowing for a quantitative assessment of AI's impact across different types of healthcare institutions.

4. Extensive referencing: The study includes a robust reference list that quantifies the depth of literature review and research grounding, reflecting a rigorous academic approach.

5. Predictive analytics applications: Specific tables within the study detail the various applications of predictive analytics in healthcare, providing quantitative data on the capabilities and benefits of AI technologies in managing patient admission, risk estimation, and resource allocation.

Based on the qualitative and quantitative characteristics outlined in the study, the survey questions incorporated into the research likely encompassed various aspects of AI adoption in healthcare, aiming to gather both detailed opinions and measurable data from healthcare professionals.

Qualitative questions

1.Experiences and perceptions:

- How do you perceive the impact of AI on the clinical decision-making process in your institution?

- Can you describe any specific instances where AI has significantly influenced patient outcomes at your facility?

2. Barriers to AI integration:

- What are the main challenges you face in integrating AI technologies into your daily practices?

- Can you discuss any ethical concerns you have regarding AI use in healthcare?

3. Future expectations:

- What are your expectations regarding the future role of AI in healthcare management?

- How do you envision the overcoming current obstacles to AI adoption?

4. Training and education needs:

- What kind of training or educational programs do you believe are necessary to enhance AI adoption among healthcare professionals?

Quantitative questions

1. Perception of benefits:

- On a scale of 1 to 10, how would you rate the impact of AI on improving diagnostic accuracy in your institution?

- To what extent do you agree that AI has optimized resource allocation at your healthcare facility? (1 = strongly disagree, 5 = strongly agree)

2.Adoption levels:

- How extensively has AI been adopted in your institution's operational processes? (Multiple choice: not at all, partially, extensively)

- What percentage of your clinical procedures incorporate some form of AI?

3. Infrastructure and support:

- Rate the adequacy of the current infrastructure to support AI technologies in your institution. (1 = very inadequate, 5 = very adequate)

- How sufficient is the IT support for troubleshooting AI applications in your healthcare setting? (1 = not sufficient, 5 = very sufficient)

4. Training and preparedness:

- Have you received any formal training related to AI? (yes/no)

- If yes, rate the effectiveness of this training in preparing you to work with AI technologies. (1 = not effective, 5 = very effective)

These qualitative and quantitative questions would help gather a comprehensive understanding of the perceptions, experiences, and actual measurable impact of AI within healthcare settings, facilitating a deeper analysis of both the subjective and objective aspects of AI integration in healthcare.

Statistical analysis

The study employed ANOVA to analyze differences in AI impact perceptions across institution types as it is shown in Table 5, while multiple regression analysis quantified the relationship between AI adoption and performance metrics such as diagnostic accuracy, patient satisfaction, and operational efficiency as it is shown in Table 6. Correlation analysis provided further insight into associations between AI utilization levels and institutional outcomes (41).

Table 5. *AI Impact scores by institution type (urban vs. rural)*

Institution type	Diagnostic accuracy score	Resource efficiency score	Patient satisfaction score
Urban	4.8	4.5	4.6
Rural	4.2	4.0	4.1

Table 6. *Perceived impact of AI on diagnostic accuracy by professional role*

Professional role	High impact (%)	Moderate impact (%)	Low impact (%)
Physicians	70	20	10
Nurses	60	30	10
Administrative staff	40	45	15
Technicians & IT	75	20	5

RESULTS

Descriptive statistics and initial observations

Survey data revealed that 88% of respondents perceive AI as beneficial for diagnostic accuracy, with urban institutions showing a higher degree of confidence due to enhanced infrastructure.

ANOVA analysis

ANOVA analysis highlighted significant variations in AI perception based on institution type, with urban and private institutions reporting higher scores in AI's impact on diagnostic accuracy and operational efficiency than rural and public institutions.

Table 7 presents the analysis of variance (ANOVA) results that explore the differences in perceptions of AI's impact across different types of institutions (e.g., urban vs. rural). The table highlights significant statistical differences in AI's perceived effects on variables such as diagnostic accuracy and resource efficiency. Key metrics reported include the mean scores, F-values, and p-values for each category:

- Diagnostic accuracy: This row shows the mean scores for diagnostic accuracy as perceived by respondents from different institution types. Higher scores indicate a more positive perception of AI's impact on diagnostic accuracy. The F-value and p-value test the statistical significance of the difference between groups, indicating whether these differences are likely due to chance.

- Resource efficiency: Similarly, this section displays the mean scores for how AI is perceived to impact resource efficiency. The statistical measures again help to establish whether the observed differences across institution types are statistically significant.

Table 8 further examines the differences in AI perception scores, breaking them down into both institution type (e.g., urban vs. rural) and the specific aspect of healthcare they impact, such as diagnostic accuracy, resource optimization, and data security. The table provides a detailed look at how these perceptions vary, with mean scores listed for each subgroup alongside their corresponding F-values and p-values, which assess the statistical significance of the results:

- Diagnostic accuracy: Compares the mean scores of diagnostic accuracy perceptions between urban and rural healthcare institutions.

- Resource optimization: Analyzes differences in how urban and rural institutions perceive AI's role in optimizing healthcare resources.

- Data security: Examines the variance in scores related to AI's impact on data security between different regions.

Both tables are crucial for understanding how the integration of AI is perceived differently across various healthcare settings. They provide empirical evidence that can guide targeted interventions and policies to address the specific needs and concerns of different healthcare providers.

Table 7. *ANOVA results by the key AI impact variables*

Variable	Institution type	Mean score	F-value	p-value
Diagnostic accuracy	Urban	4.8	8.21	0.0005
	Rural	4.2		
Resource efficiency	Public	3.8	6.75	0.0008
	Private	4.5		

Table 8. *ANOVA results for AI perception scores by institution type and region*

Factor	Mean score (Urban)	Mean score (rural)	F-value	p-value
Diagnostic accuracy	4.8	4.2	9.17	0.0007
Resource optimization	4.5	4.1	7.32	0.0009
Data security	3.9	3.4	6.12	0.0032

Table 9. *Regression analysis for key performance indicators*

Outcome variable	Predictor variable	Beta coefficient	p-value	R^2
Diagnostic accuracy	AI training level	0.72	0.0002	0.64
Resource utilization	Institution type (Private)	0.58	0.0008	0.57
Patient satisfaction	Degree of AI integration	0.56	1	0.52

Table 10. *Regression analysis for predictors of AI adoption willingness*

Predictor variable	Beta coefficient	p-value	R^2
Perceived benefit to efficiency	0.68	0.0001	0.61
Training accessibility	0.45	2	0.45
Data privacy confidence	0.52	0.0005	0.53

Regression analysis

Regression analysis showed a strong positive relationship between AI adoption and diagnostic accuracy, patient satisfaction, and operational efficiency.

Table 9 presents the results of a regression analysis that quantifies the relationship between AI adoption and key performance metrics in healthcare settings, such as diagnostic accuracy, resource utilization, and patient satisfaction. This table uses predictor variables (such as the level of AI training and the degree of AI integration) to determine how they influence various outcome variables:

- Diagnostic accuracy: This section of the table shows the beta coefficient, p-value, and R^2 value for the predictor variable "AI training level." A positive beta indicates a direct relationship where higher levels of AI training correlate with improvements in diagnostic accuracy. The R^2 value explains the proportion of variance in diagnostic accuracy that is predictable from the AI training level.

- Resource utilization: Similar analysis is done for "Institution type" as a predictor of how effectively resources are utilized in healthcare facilities, showing the impact of private versus public institution types on resource utilization.

- Patient satisfaction: This part assesses the influence of the "Degree of AI integration" on patient satisfaction levels, indicating how deeper integration of AI into healthcare processes might enhance patient experiences.

Table 10 focuses on the willingness of healthcare institutions to adopt AI technology. It explores various predictors that could influence this willingness, such as perceived benefits to efficiency, accessibility of training, and confidence in data privacy:

- Perceived benefit to efficiency: This row displays how the belief that AI can enhance operational

efficiency impacts the willingness to adopt AI technologies. The beta coefficient shows the strength and direction of this relationship.

- Training accessibility: Analyzes how the availability of AI-related training influences adoption willingness, suggesting that better access to training could positively affect attitudes towards AI adoption.

- Data privacy confidence: Examines whether confidence in the ability to protect patient data influences the willingness to adopt AI, with the beta coefficient indicating the impact of data privacy concerns on decision-making about AI adoption.

Both tables use statistical methods to provide insights into the factors that drive the adoption of AI in healthcare settings, revealing the direct and measurable impacts of training, institutional type, and perceptions on AI's integration and the overall effectiveness of healthcare services. These analyses are crucial for understanding barriers to AI adoption and for developing strategies to encourage its broader acceptance and use in the healthcare industry.

Summary of survey findings

Survey responses indicated that while AI is generally perceived positively, there are concerns related to infrastructure limitations, training inadequacies, and data privacy, especially in rural areas.

Figure 1 provides a summary of survey findings regarding the perceived benefits and challenges associated with the implementation of artificial intelligence (AI) in healthcare.

On the left side of the figure, the percentages of respondents who identified key benefits of AI are shown, while the right side highlights the main challenges reported by the participants.

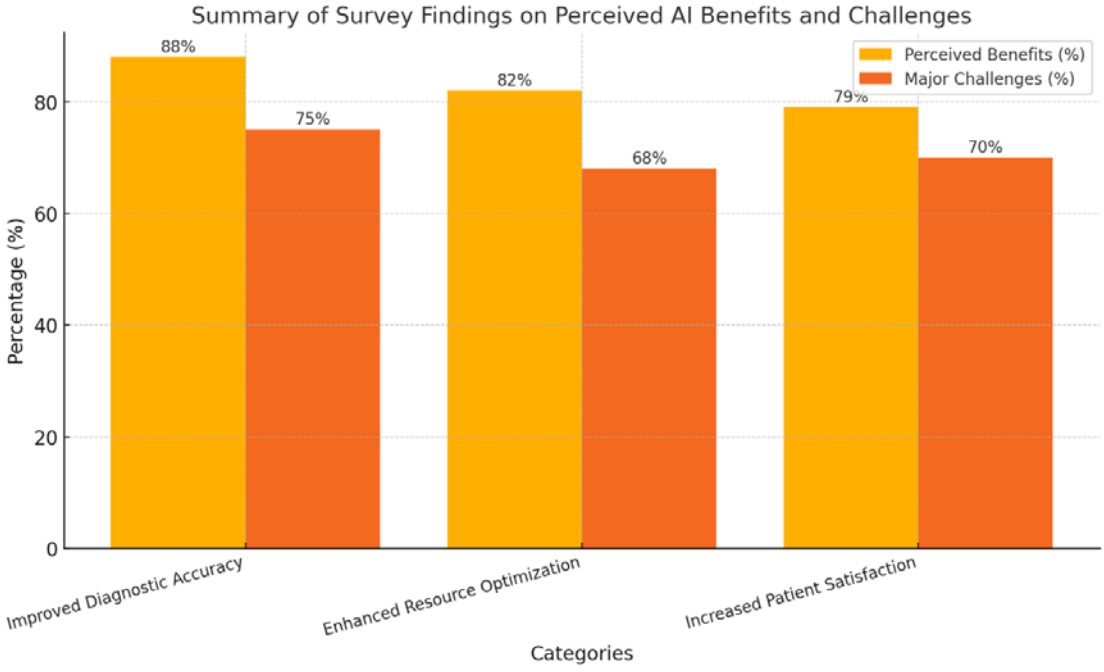

Figure 1. *Summary of survey findings on perceived AI benefits and challenges*

Perceived benefits

The majority of respondents (88%) emphasized an improved diagnostic accuracy as the most significant benefit of AI, showcasing a strong trust in its application for improving the diagnosis precision. Similarly, enhanced resource optimization was recognized as a benefit by 82% of respondents, reflecting the ability of AI to streamline healthcare processes. Lastly, 79% of participants noted that AI contributes to increased patient satisfaction, demonstrating its potential to improve the overall patient experience.

Major challenges

Among the challenges, the lack of training programs was the most prominent, reported by 75% of respondents. This indicates a gap in the availability of educational resources and skills needed for effective AI adoption. Additionally, 70% of participants pointed to insufficient infrastructure as a significant barrier, which highlights the need for technological upgrades in healthcare facilities. Data privacy concerns were also a key issue, with 68% of respondents expressing apprehension about how AI handles sensitive patient information.

This figure underscores both the transformative potential of AI in healthcare and the critical obstacles that need to be addressed to fully realize its benefits. It serves as a foundation for developing strategies that balance these opportunities and challenges, ensuring the successful integration of AI technologies into healthcare systems.

Table 11 presents a summary of the survey findings regarding the perceived benefits and major challenges associated with AI adoption in healthcare. The table lists the benefits perceived by healthcare professionals, such as improved diagnostic accuracy, enhanced resource optimization, and increased patient satisfaction. Alongside each benefit, a percentage indicates how many respondents recognize that particular advantage. The table also identifies the major challenges—like lack of training programs, data privacy concerns, and insufficient infrastructure—highlighting the percentage of respondents who view these as significant barriers to AI adoption. This format provides a clear, quantitative measure of the general consensus on AI's impact and the obstacles to its broader integration.

Table 12 explores the relationship between the benefits of AI and their correlation with patient satisfaction. This table presents various AI benefits, such as improved diagnostic accuracy and reduced wait times, and shows their correlation coefficients with patient satisfaction metrics. The significance levels (p-values) are also provided to assess the statistical significance of these correlations. A positive correlation coefficient indicates that as the benefit increases (e.g., better diagnostic accuracy or reduced wait times), there is a corresponding increase in patient satisfaction. This table is crucial for understanding how specific improvements attributed to AI can directly affect patient experiences and satisfaction in healthcare settings.

Together, these tables provide a comprehendsive statistical overview that helps to quantify and analyze the perceptions of AI's benefits and challenges, as well as the tangible impacts on patient satisfaction within healthcare systems.

Table 11. *Summary of key challenges in AI adoption by institution type*

Challenge	Public sector (%)	Private sector (%)	Total (%)
Insufficient training programs	76	54	65
Data privacy concerns	72	68	70
Infrastructure limitations	85	47	66

Table 12. *Correlation analysis of AI benefits and perceived patient satisfaction*

AI benefit variable	Patient satisfaction correlation (r)	Significance (p-value)
Diagnostic accuracy improvement	0.72	0.0004
Reduced wait times	0.65	1
Enhanced resource utilization	0.63	0.0012

DISCUSSION

The findings suggest that AI has significant potential to enhance healthcare in Serbia, particularly in urban and private institutions with better infrastructure. However, the disparities between urban and rural institutions in terms of AI impact perception point to the need for targeted investment in rural healthcare infrastructure. The observed positive relationship between AI integration and performance metrics—such as diagnostic accuracy and patient satisfaction—reinforces the value of AI in improving healthcare outcomes and operational efficiency. Yet, barriers like limited training programs, privacy concerns, and infrastructure constraints must be addressed for Serbia to fully leverage AI's potential in healthcare (42).

Practical implications

Table 13 provides a concise summary of the frequency of key barriers to AI adoption as identified by survey respondents in the healthcare sector. This table lists specific barriers such as lack of training, data privacy concerns, and insufficient infrastructure. For each barrier, a frequency percentage is shown, indicating how often each issue was mentioned by respondents across all surveyed institutions.

- Lack of training: This barrier refers to the perceived deficiency in AI-related training and education among healthcare professionals. The percentage shown represents how many respondents feel that inadequate training is a significant obstacle to effective AI integration.

- Data privacy concerns: This entry highlights concerns related to the handling and protection of patient data when using AI systems. The percentage indicates the proportion of healthcare professionals who see data privacy as a critical issue that needs addressing before AI can be fully embraced.

- Insufficient infrastructure: This barrier points to the lack of necessary technological and physical infrastructure to support AI technologies effectively. The percentage reflects the view among respondents that their current facilities are not adequately equipped to handle the integration and operation of advanced AI systems.

This table is crucial for understanding the prevalence of each identified barrier, providing insights into what factors are perceived as the most significant impediments to the adoption and effective use of AI within healthcare environments. It guides stakeholders on where to focus their efforts to improve the readiness and acceptance of AI technologies in healthcare settings.

Future research directions

Future studies could examine the long-term impact of AI integration on Serbian healthcare outcomes. Additionally, research on the ethical implications of AI, particularly regarding transparency and bias, would be valuable to guide responsible AI adoption in healthcare.

CONCLUSION

This study confirms that AI holds transformative potential for healthcare in Serbia by improving diagnostic accuracy, operational efficiency, and patient satisfaction. However, effective AI implementation requires strategic investments in infrastructure, comprehensive training programs, and strong ethical frameworks to ensure data privacy and fairness. Policymakers in Serbia are encouraged to adopt a phased approach to AI integration, beginning with high-impact areas such as diagnostics and resource management, while gradually expanding AI's scope as infrastructure and expertise develop.

The proposed changes in policy and infrastructure are critical for overcoming the existing barriers and maximizing the potential of AI in healthcare.

1. Increase funding for AI in rural areas: This recommendation emphasizes the need to reduce disparities in healthcare quality between urban and rural areas. Investing in AI in rural areas can enable better diagnostics and resource management, leading to more uniform healthcare quality across the country.

2. Implement AI-specific training programs: The introduction and enhancement of educational programs that provide healthcare professionals with the necessary knowledge and skills to effectively use AI tools are proposed. This includes everything from basic computer literacy to advanced courses on machine learning and data analytics.

3. Establish data governance protocols: Protecting patient privacy and data integrity is essential in the context of AI in healthcare. Therefore, it is crucial to establish strict protocols that regulate the col-

lection, processing, and storage of health data, ensuring that AI-based decisions are transparent and fair.

These changes are designed to address both the technical and ethical aspects of implementing AI in the healthcare system, with the goal of creating a reliable, efficient, and equitable environment that benefits both healthcare workers and patients.

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Primena veštačke inteligencije u upravljanju zdravstvenim sistemom u Republici Srbiji: povećanje efikasnosti, prediktivnog kapaciteta i donošenja odluka

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SAŽETAK

Uvod/Cilj. Veštačka inteligencija (engl. *artificial intelligence* – AI) nudi transformativni potencijal u upravljanju zdravstvenom zaštitom, budući da poboljšava prediktivnu analitiku, optimizuje alokaciju resursa i podržava kliničko donošenje odluka. Cilj ove studije bio je da ispita primenu AI-ja u zdravstvenim ustanovama u Srbiji, fokusirajući se pritom na poboljšanje operativne efikasnosti i ishoda lečenja bolesnika.
Metode. Istraživanje je sprovedeno kao studija preseka koja je podrazumevala anketu sprovedenu među 450 zaposlenih u različitim nivoima zdravstvene zaštite u Srbiji (u primarnoj, sekundarnoj i tercijarnoj). Podaci su prikupljeni sprovođenjem onlajn ankete u oktobru i novembru 2024. godine. Da bi se procenio uticaj primene AI-ja na dijagnostičku preciznost, optimizaciju resursa i zadovoljstvo pacijenata, urađena je statistička analiza, za koju se se koristile metode kao što su ANOVA i regresiona analiza.
Rezultati. Rezultati istraživanja su ukazali na pozitivan uticaj primene AI-ja na preciznost dijagnostike (88% ispitanika), optimizaciju resursa (82%) i zadovoljstvo pacijenata (79%). Uočene su razlike između urbanih i ruralnih sredina, kao i između javnih i privatnih zdravstvenih ustanova. Najveći izazovi identifikovani u istraživanju odnose se na nedostatak obuke (75%), zabrinutost za privatnost podataka (68%) i ograničenu infrastrukturu (70%).
Zaključak. Ova studija je potvrdila da AI ima značajan potencijal kada je reč o unapređenju zdravstvene zaštite u Srbiji, posebno u urbanim i privatnim ustanovama sa razvijenijom infrastrukturom. Međutim, neophodno je prevazići izazove u vezi sa obukom, privatnošću podataka i infrastrukturom, naročito u ruralnim sredinama. Preporučuje se da se implementaciji AI-ja pristupi u fazama, i to tako što će se fokus najpre usmeriti na dijagnostiku i menadžment resursa kako bi se ostvarila najveća moguća efikasnost zdravstvenog sistema.

Ključne reči: **veštačka inteligencija, menadžment u zdravstvu, prediktivna analitika, sistemi za podršku odlučivanju, optimizacija resursa, Srbija**

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Original article

Rhabdomyosarcoma in Children in the Five-Year Period in Oncology Unit in a Pediatric Teaching Hospital

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SUMMARY

Introduction/Aim. Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in pediatric population and adolescents. Limited data is available on the characteristics of RMS in Iraqi pediatric patients. The aim of the study was to examine the clinical and histological aspects of RMS in Iraqi children, with a focus on their response to treatment, prognosis, and survival.

Methods. A retrospective cohort study was conducted at the Oncology Unit of Children's Welfare Teaching Hospital, Medical City, Baghdad, Iraq and included patients who were newly diagnosed with RMS and received treatment during the period between January 1, 2015, and December 31, 2019. The patients were followed up from the time of diagnosis until October 1, 2020.

Results. A total of 59 patients were included with a median age of 3.5 years ranging between 1–12 years, with a male-to-female ratio of 3.2:1. The most frequent clinical presentation was urine retention in 15 patients (25.4%), followed by proptosis in 14 (23.7%) patients.

However, the main sites of involvement were the head and neck in 23 (39%) patients, followed by the trunk and the genitourinary tract observed in 17 (28.8%) and 15 (25.4%) patients, respectively. The alveolar type, found in 11 (18.7%) patients, was the most prevalent histological variety, followed by the embryonal type reported in 42 (71.3%) patients, whereas six patients (9.7%) had other varieties. Of the 49 patients included in the prognosis assessment, 18 patients (36.7%) had a complete response, 20 patients (40.8%) died, and 11 patients abandoned treatment. The average duration of disease recurrence was 21.3 months, with a 7% recurrence rate. The overall survival rate was 36.7%, with a mean survival duration of 14.1 months.

Conclusions. Pediatric RMS in Iraq is primarily prevalent in males. There is a significant delay in disease diagnosis from the onset of the symptoms. A high rate of advanced-stage disease may relate to patients reluctant to discontinue treatment. The low disease-free survival rate is due to impediments that hindered the effectiveness of therapy.

Keywords: **rhabdomyosarcoma, follow up, pediatrics, Iraq**

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INTRODUCTION

Rhabdomyosarcoma (RMS) is mesenchymal tumor that can arise in nearly any part of the body; however, the trunk, extremities, and the head/neck regions are the most frequently affected. RMS is the most prevalent soft tissue sarcoma in pediatric and adolescent populations (1), with an estimated 350 new cases annually. The maximum incidence is observed in the 0–14 age group, where it accounts for 5.8% of all malignant solid tumors in this age group (2, 3). A diminished occurrence peak is observed during the early to mid-adolescent stage, with approximately 66% of cases being detected in individuals under the age of six. A marginal preference for males has been discerned, with a male-to-female ratio that is between 1.3 and 1.5. Male predication has been associated with the patient's race. It has been shown that RMS prevalence in African-American girls is approximately half that of Caucasian girls, while the prevalence in males is comparable between the two racial groups (4). In contrast to predominantly Caucasian population, the incidence of a specific phenomenon appears to be lower among individuals of Asian descent (4).

Although RMS can be observed in various regions of the body, there are identifiable patterns that link the primary site, histology, and age at which the diagnosis is made. Younger infants have a higher incidence of head and neck RMS. The embryonal subtype is the predominant subtype in cases where these malignancies manifest in the orbit. A unique variant of embryonal RMS predominantly observed in neonates is that it originates within the bladder or vagina wall. However, in older adolescents, it may have a manifestation in the nasopharynx. The alveolar histological subtype is frequently associated with tumors of the extremities, which are more prevalent among adolescents (5).

We have limited data on the characteristics of RMS in Iraqi pediatric patients. Without providing information about the particular incidence based on age, the Iraqi Cancer Registry listed RMS as the fourth most prevalent type of cancer affecting the urinary bladder of Iraqi males (6). The aim of this study was to examine the clinical and histological aspects of RMS in children from Iraq, with a focus on their response to treatment, prognosis, and survival.

METHODS

A retrospective cohort study was conducted at the Oncology Unit of Children's Welfare Teaching Hospital, Medical City, Baghdad, Iraq and included patients who were newly diagnosed with RMS tumor and received treatment during the period between January 1, 2015, and December 31, 2019. The study was approved by the Ethical Committee of Children's Welfare Teaching Hospital. Patients without histopathology reports or incomplete clinical or follow up information were excluded from the study.

A thorough examination of hospital and outpatient medical records, notes, and laboratory data was conducted to determine demographic information, including age, gender, place of residence, duration of symptom onset, clinical manifestation, date of diagnosis, and the results of diagnostic investigations. The patients underwent a variety of clinical investigations, such as complete blood count (CBC), renal function test (RFT), liver function test (LFT), chest x-ray (CXR), abdominal ultrasound, computed tomography (CT) of the thorax, CT of the primary site lesion, magnetic resonance imaging (MRI) of the primary site lesion, and (when feasible) bone marrow aspiration and biopsy. All patients were staged in accordance with the TNM staging system (RMS staging based on TNM classification) (7, 8) and were treated according to the standard United Kingdom Children's Cancer Study Group (UKCCSG) rhabdomyosarcoma protocol.

The patients were followed up from the time of diagnosis until October 1, 2020. Event-free survival (EFS) was defined as the duration from the start of treatment to the onset of disease progression/relapse, loss to follow-up, or mortality from any cause. The time from the commencement of treatment to mortality from any cause was referred to as the overall survival (OS).

Statistical analyses

An analysis of the data was conducted using Statistical Package for the Social Sciences (SPSS), version 13. Quantitative data was presented as mean and median, while qualitative data was represented in terms of frequency and percentage. The Chi-square test was employed to evaluate the relation-

ships between event-free survival and independent variables under investigation. The Kaplan-Meier method was employed to illustrate the survival curves. P-values that were equal to or less than 0.05 were considered significant.

RESULTS

The study comprised a total of 59 patients who met the inclusion criteria. The median age was 3.5 years, with a range of 1-12 years. The male population was predominant, with a male-to-female ratio of 3.2:1. The median duration from the onset of symptoms to diagnosis was three months, with a range of 0.5-24 months and a mean of 3.8 months (Table 1).

Table 1. *Demographic and clinical characteristics of rhabdomyosarcoma patients (n = 59)*

Characteristics	No.	%
Age (years)		
< = 1	3	**5.1**
1-5	42	**71.2**
> 5	14	**23.7**
Sex		
Males	45	**76.3**
Females	14	**23.7**
Duration from onset to diagnosis		
< 1 month	4	**6.8**
1-4 months	39	**66.1**
> 4 months	**16**	**27.1**

The largest number of patients were recorded in Baghdad (42.4%), followed by Karbala (11.9%), and Anbar (6.8%), as illustrated in Table 2.

The most frequent clinical presentation was urine retention, which was observed in 15 (25.4%) cases, followed by proptosis in 14 (23.7%) cases, as illustrated in Table 3.

However, the primary site of involvement was the head and neck in 23 patients (39%), followed by the trunk and the genitourinary tract in 17 (28.8%) and 15 (25.4%) cases, respectively, while extremities were affected in 4 (6.8%) cases, as indicated in Table 4.

The mean haemoglobin level was 10.2 g/dl, with a range of 4-14 g/dl. In 47 patients (79.7%), a chest x-ray of 10 patients (16.9%) revealed thoracic involvement. Only one patient underwent a bone marrow aspiration examination, which revealed bone marrow involvement, as shown in Table 5.

Table 2. *The residence of rhabdomyosarcoma patients (n = 59)*

Governorate	No.	Percent
Baghdad	25	**42.4**
Karbala	7	**11.9**
Anbar	4	**6.8**
Dyala	4	**6.8**
Dywania	3	**5.1**
Kurkok	3	**5.1**
Wasit	3	**5.1**
Babelyon	2	**3.4**
Basrah	2	**3.4**
Nasria	2	**3.4**
Salah Aldin	2	**3.4**
Samawa	1	**1.7**
Najaf	1	**1.7**
Total	**59**	**100.0**

* Mousal, Misan, Kurdistan—no referred cases

Table 3. *Clinical presentation of patients with rhabdomyosarcoma (n = 59)*

Main clinical presentation	No.	%
Urine retention or overflow incontinence	15	**25.4**
Proptosis	14	**23.7**
Local mass and pallor fever	11	**18.6**
Lymphadenopathy	9	**15.3**
Upper way obstruction	4	**6.8**
Abdominal mass	4	**6.8**
Scrotal swelling	1	**1.7**
Mass and ear discharge	**1**	**1.7**

Table 4. *The main sites involved by rhabdomyosarcoma (n = 59)*

Site	No.	%
Head and neck	23	**39**
Trunk	17	**28.8**
Genitourinary tract	15	**25.4**
Extremities	**4**	**6.8**

The diagnosis was established for all patients through histological examination of the mass. A histological examination by immunohistochemical study was conducted on 14 patients at the Department of Pathology at Rome University. Twelve patients had the same diagnosis as the histopathological study in Iraq. The diagnosis was modified in two of the fourteen patients. Embryonal type in 42 patients comprised the main histological variety, accounting for 71.3 percent. The alveolar type was found in 11 patients (18.7%), whereas other varieties were reported in and 6 (9.7%) patients. Four patients had soft tissue sarcoma, one patient had botryoid RMS, and one patient had undifferentiated RMS, as shown in Table 6.

We found that the largest number of patients were in stage III disease (23, 39%), followed by stage I (18, 30.5%), II (11, 18.6%) and IV (7, 7.9%), respectively, as shown in Table 7.

Of 59 patients, 10 were excluded from the treatment analysis. These patients had a histological type other than alveolar or embryonal, had been treated at other centers, refused treatment, or passed away before commencing treatment.

The UKCCSG protocol was implemented for all 49 patients. The mass was surgically removed from three patients, while only two patients underwent radiotherapy with chemotherapy.

The patient was followed from the time of diagnosis until October 1, 2020, with an average follow-up period of 16 months. The prognosis of 49 patients after treatment is depicted in Table 8. Of these, 18 patients (36.7%) had a complete response, 20 patients (40.8%) died, and 11 patients abandoned treatment (the majority of whom were lost to follow-up).

Sepsis was the primary cause of mortality in 10 patients, accounting for 50% of the cases, as illustrated in Table 9.

Table 5. *Initial laboratory results of rhabdomyosarcoma patients (n = 59)*

Item	No.	Percentage (valid %)
HB		
< 10 g/dl	23	**39 (39.7)**
> 10 g/dl	35	**59.3 (60.3)**
Not Recorded	1	**1.7**
CXR		
Normal	47	**79.7 (82.5)**
Metastasis	10	**16.9 (17.5)**
Not recorded	2	**3.4**
Bone marrow examination		
Normal	57	**96.6 (98.3)**
Involved	1	**1.7 (1.7)**
Not recorded	1	
		1.7

Table 6. *Histopathology types (n = 59)*

TYPE	No.	%
Embryonal	42	71.3
Alveolar	11	18.7
Others	6	9.7

Table 7. *Tumor staging of rhabdomyosarcoma patients (n = 59)*

STAGE	No.	%
I	18	30.5
II	11	18.6
III	23	39
IV	7	11.9

Table 8. *Outcome in rhabdomyosarcoma patients (n = 49)*

Outcome	No. of patient	%
Death	20	40.8
Alive	18	36.7
Lost	11	22.4
Total	49	100.0

Table 9. *Causes of death in rhabdomyosarcoma patients (n = 20)*

Cause of death	No.	%
Sepsis	10	50
Progressive disease—died at home	4	20
Fits and apnea	2	10
renal failure (obstructive uropathy)	2	10
Bleeding	1	5
Upper air way obstruction	1	5
Total	20	100.0

The average duration of disease recurrence was 21.3 months, with a 7% recurrence rate. The overall survival rate was 36.7%. Furthermore, the event-free survival was determined to be 16 months, as illustrated in Figure 1. The Kaplan-Meier study of the overall survival according to the clinical stage of the disease is depicted in Figure 2. The p-value is 0.23, indicating that there is no significant difference between clinical staging and survival time (not significant).

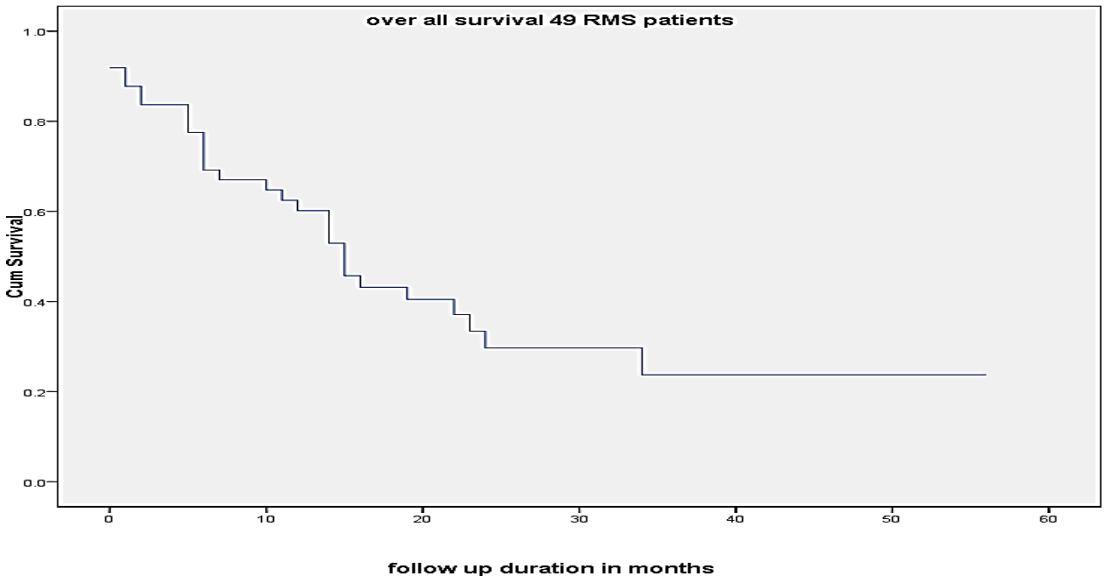

Figure 1. *Event-free rhabdomyosarcoma patients (n = 49)*

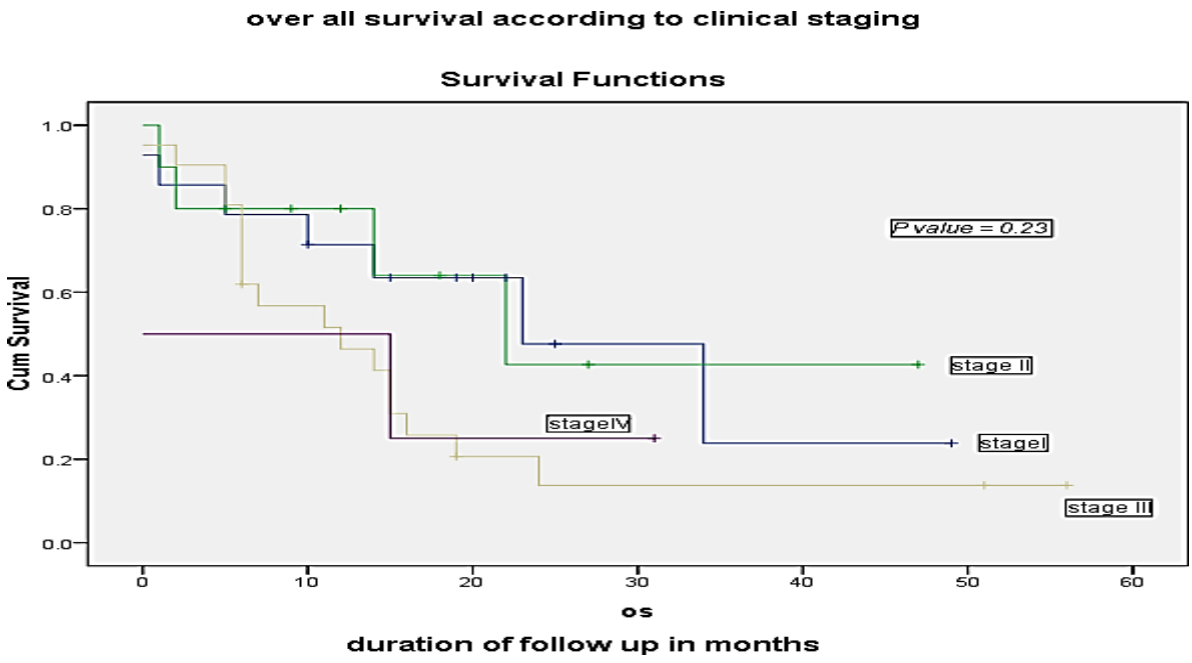

Figure 2. *Overall survival according to clinical staging of RMS patients*

DISCUSSION

Although RMS is the most frequent soft tissue sarcoma encountered in children, its overall incidence is low, accounting for 4.71 per million children and adolescents under 20 years of age in the United States (9). Racial differences in the clinicodemographic characteristics of this tumor have an area of debate (10, 11). This study evaluated the clinicodemographic features of pediatric RMS in Iraqi patients, providing information about patients' outcomes.

We observed an evident male predominance with a ratio of 3.21:1, which is higher than the figure reported previously in Iraq—2:1 (12) and the neighboring country Turkey—1.8:1 (13). It is also higher than what was reported in other countries—e.g., United States—1.5:1 (14) and Guatemala—1.3:1 (15). However, an Egyptian study reported higher male predominance with a ratio of 6.5:1 (16). Gender disparity has been previously reported in several studies. Research demonstrated that the prevalence of RMS in African-American girls is approximately half that of Caucasian girls, while the prevalence in males is comparable between the two racial groups (4).

The median age in the current study was 3.5 years, with a range of 1 to 12 years. This is comparable to the median age of 3.4 years in the Kadhem study from Iraq (12), but it is lower than the median age of 6 years in the Anitelon study from Guatemala (15) and the median age of 4.5 years in the Karkas study from Turkey (13). It is also higher than the median age of 3 years in the Al Sherbiny study from Egypt. (16). This may be contingent upon the age limit of each center and the quantity of the sample that was collected.

The average duration from the onset of symptoms to the diagnosis of RMS was 3.8 months, with a range of two weeks to two years. This duration exceeds the average duration of one month reported in the study conducted by other researchers (13). These findings suggest a delay in the diagnosis and referral of RMS cases. The most frequent clinical presentation in the current investigation was urine retention, which accounted for 25.4% of cases. Eye proptosis was present in 23.7% of cases, while Kadhim's study from Iraq demonstrated that ocular proptosis was the most common clinical manifestation (12). Consistent with the literature where the head and neck were reported as the most frequent site accounting for 40% of pediatric RMS (14), 39% of patients in our study had tumors in the head and neck. Similar findings were documented in other Iraqi studies (12, 17), while the Turkish study reported only 31.4%, and another wester study reported this involvement in 20% of cases (13, 18).

The dominant histological type of RMS in the current study was embryonal accounting for 71.3% of all cases. This is similar to a previous local study which reported 79% of embryonal histological type (17), which is higher than in other studies in which embryonal histological type accounted for 46% (15, 19) only. By contrast, a Turkish study reported a higher rate of embryonal RMS up to 80% (13).

We found that 23 (39%) patients were in stage III of the disease. In the literature, the rate of stage III disease ranged between 17–76% (12, 13, 16, 18). Delays in diagnosis and referral for reasons related to doctors and patients may explain this relatively high rate of advanced-stage disease. Additionally, lung metastasis seems to be underestimated in the current study (16.9%), which is likely to be due to a shortage of imaging facilities.

The UKCCSG treatment protocol was implemented in all 49 patients in the current study. Eighteen (36.7%) patients achieved complete remission, which is lower than the rate reported by other studies (45%) (15), (58.8%) (13). The mortality rate in the current study was 40.8%, which is comparable to that reported by other studies (41.2%) (13). The recurrence rate in our study was 14.2%, with a mean time of 21.3 months. Other studies reported a higher rate of recurrence reaching up to 52, which was related to patients' reluctance to complete treatment.

CONCLUSION

The pediatric RMS in Iraq is primarily prevalent in males. There is a significant delay in disease diagnosis from the onset of symptoms. A high rate of advanced-stage disease may relate to patients reluctant to discontinue treatment. A low disease-free survival rate is due to impediments that hinder the effectiveness of therapy, which is similar to reports of developing nations.

Conflict of interest

No conflict of interest.

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Rabdomiosarkom kod dece praćen u petogodišnjem periodu u Onkološkoj jedinici Pedijatrijske nastavne bolnice

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SAŽETAK

Uvod/Cilj. Rabdomiosarkom (engl. *rhabdomyosarcoma* – RMS) predstavlja najćešći sarkom mekih tkiva u pedijatrijskoj populaciji i kod adolescenata. Dostupni su ograničeni podaci o karakteristikama RMS-a u pedijatrijskoj populaciji Iraka. Cilj ove studije bio je da se ispitaju klinički i histološki aspekti RMS-a kod dece u Iraku, sa posebnim osvrtom na odgovor na terapiju, prognozu i stopu preživljavanja.
Metode. Sprovedena je retrospektivna kohortna studija u Onkološkoj jedinici Pedijatrijske nastavne bolnice pri Medicinskom centru u Bagdadu u Iraku. U studiju su uključeni bolesnici sa novodijagnostikovanim RMS-om koji su primali terapiju u periodu od 1. januara 2015. do 31. decembra 2019. godine. Bolesnici su praćeni od trenutka uspostavljanja dijagnoze do 1. oktobra 2020. godine.
Rezultati. U studiju je uključeno ukupno 59 bolesnika prosečne starosti 3,5 godina (raspon od jedna godine do 12 godina); odnos muškog i ženskog pola bio je 3,2 : 1. Najćešća klinička manifestacija RMS-a bila je urinarna retencija, koja se javila kod 15 (25,4%) bolesnika, a potom proptoza koja se javila kod 14 (23,7%) bolesnika. Najčešće zahvaćene regije bile su regije glave i vrata, kod 23 (39%) bolesnika, potom regija trupa utvrđena kod 17 (28,8%) bolesnika, dok je zahvaćenost urogenitalnog trakta bila utvrđena kod 15 (25,4%) bolesnika. Alveolarni tip RMS-a dijagnostikovan je kod 11 (18,7%) bolesnika, dok je embrionalni tip bio prisutan kod 42 (71,3%) bolesnika. Ostale histološke varijante zabeležene su kod 6 (9,7%) bolesnika. Od 49 bolesnika uključenih u procenu prognoze, kod 18 (36,7%) bolesnika zabeležen je kompletan odgovor na terapiju, dok je 20 (40,8%) preminulo, a 11 bolesnika prekinulo terapiju. Prosečno vreme do recidiva bolesti iznosilo je 21,3 meseca uz stopu recidiva od 7%. Ukupna stopa preživljavanja iznosila je 36,7%, sa prosečnim preživljenjem od 14,1 meseca.
Zaključak. Rabdomiosarkom kod dece u Iraku prvenstveno se javlja kod muške dece. Uočeno je značajno kašnjenje u dijagnostici bolesti od trenutka pojave simptoma. Visoka stopa uznapredovalih stadijuma bolesti može se dovesti u vezu sa odustajanjem bolesnika od terapije. Niska stopa preživljavanja osoba bez simptoma bolesti rezultat je faktora koji su ometali efikasnost terapije.

Ključne reči: **rabdomiosarkom, praćenje, pedijatrija, Irak**

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Original article

Physicians' Personality Traits as Predictors of Empathy in the Health Context

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SUMMARY

Introduction/Aim. Empathy is pivotal in healthcare, enhancing patient-provider relationships and healthcare outcomes. This study explores how empathy relates to the Big Five personality traits among Serbian physicians.

Methods. A cross-sectional survey of 304 Serbian physicians (37.5% male, 62.5% female; mean age 37.38 years) was conducted using online questionnaires from May to August 2023. The Jefferson Empathy Scale (JSE) measured empathy levels, and the Big Five Inventory (BFI) assessed personality traits (Openness to Experience, Conscientiousness, Extraversion, Agreeableness, Neuroticism). Statistical analyses, including descriptive statistics and regression using SPSS, examined associations between personality traits and empathy scores.

Results. Significant correlations between empathy and specific personality traits were found, suggesting a relationship between personality traits and empathetic behavior among physicians. Agreeableness (β = 0.298) and Openness to Experience (β = 0.133) emerged as significant positive predictors of empathy.

Conclusion. Understanding how personality traits are related to empathy is crucial for enhancing patient care and professional development in healthcare. This study underscores the need to integrate empathy-promoting strategies into medical training to cultivate compassionate healthcare providers.

Keywords: **empathy, Big Five personality traits, healthcare providers, medical education**

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INTRODUCTION

Empathy is a fundamental aspect of emotional intelligence, which involves the capacity to recognize, understand and appreciate someone else's emotions, experiences, and perspectives. Therefore, empathy is a foundational aspect of healthy and positive social interactions. It is crucial in social relations for numerous reasons, such as building trust and rapport, strengthening relationships, effective communication, conflict resolution, emotional support, strengthening community and social bonds, enhancing leadership skills, promoting prosocial behavior, etc. Overall, it enables individuals to see beyond their own perspectives, appreciate the experiences of others, and create a more compassionate and understanding world (1, 2).

In a health context, empathy is a cornerstone of patient-centered care, where healthcare providers prioritize understanding the emotions and concerns of their patients. Dealing with health issues can be emotionally taxing. Empathetic healthcare providers can help alleviate anxiety and stress by providing emotional support and understanding. It helps create a more trusting and supportive environment for patients, leading to better healthcare outcomes (3).

Differing levels of empathy can be attributed to several factors, including individual personality (4). Personality encompasses the distinct combination of qualities, behaviors, and thought patterns that make an individual unique. The Big Five model is widely used in personality psychology and has practical applications in personality assessment. This model includes five broad dimensions of personality traits: openness to experience, conscientiousness, extraversion, agreeableness, and neuroticism (5). Openness to experience reflects a person's openness to new experiences, ideas, and intellectual curiosity. Individuals high in openness tend to be imaginative, creative, and open-minded, while those low in openness are more conventional and prefer routine and familiarity. Conscientiousness relates to a person's level of self-discipline, organization, and responsebility. Highly conscientious individuals are dependable, punctual, and goal-oriented, while those low in conscientiousness may be more spontaneous and less focused on long-term planning. Extraversion refers to a person's level of sociability, assertiveness, and preference for social interaction. Extroverts are outgoing, energetic, and enjoy being around others, whereas introverts are more reserved, introspective, and often prefer solitary activities. Agreeableness reflects a person's tendency to be compassionate, cooperative, and considerate of others' feelings. People high in agreeableness are empathetic and nurturing, while those low in this trait may be more competitive and assertive. Neuroticism, also called emotional stability, measures the extent to which a person experiences negative emotions such as anxiety, stress, and mood swings. Individuals high in neuroticism tend to be more emotionally sensitive and prone to experiencing distress, while those low in neuroticism are more emotionally resilient and stable (6, 7).

A multi-center four-country study revealed substantial evidence, linking specific personality traits patterns to empathy. Specifically, agreeableness and conscientiousness emerged as the most significant predictors of affective and cognitive empathy (8). According to a study conducted with university students, it was found that agreeableness, openness, conscientiousness, and extraversion could serve as potential indicators of empathy (9). In another study involving medical students, researchers identified a significant correlation between empathic concern and agreeableness (10). Despite the Big Five model of personality being a useful framework for explaining individual variations in human experience and behavior, its association with measures of empathy remains not entirely understood (5).

On the other hand, the recent study from Krasnov et al. (11) found that empathy did not integrate with emotional intelligence traits in the personality profiles of medical doctors during the COVID-19 pandemic. It suggests that distancing from personal experiences in interpersonal relationships may be necessary for doctors to regulate their professional responsibilities during such emotionally charged times.

The aim of the study was to investigate the associations between empathy and the Big Five personality traits among Serbian physicians. Specifically, our aim was to investigate if we can predict the level of empathy in physicians based on personality traits.

MATERIAL AND METHODS

This cross-sectional study was conducted via an online questionnaire between May and August 2023. The sample consists of 304 Serbian physicians.

Sample characteristics

The study included a total of 304 participants, with a gender distribution of 114 males (37.5%) and 190 females (62.5%). Age ranged from 25 to 66 years old, with a mean of 37.38 years.

Instruments

Jefferson Empathy Scale (JSE)

The JSE was employed as a validated instrument to assess empathy levels among study participants. It is a self-report questionnaire developed to assess an individual's empathic tendencies. Scoring of the JSE involves calculating a total empathy score based on the participant's responses to the individual items. The responses are scored on a scale from 1 to 7. The total score is used to categorize participants into different levels of empathy.

Big Five Inventory (BFI)

The BFI is a widely adopted psychometric tool used to measure an individual's personality traits across five broad dimensions: Openness to Experience, Conscientiousness, Extraversion, Agreeableness, and Neuroticism. It consists of a series of items, each designed to assess a specific aspect of an individual's personality. Respondents are asked to rate their level of agreement or disagreement with each statement on the Likert scale. On a Likert scale, the first point typically represents the lowest level of agreement or intensity, such as "strongly disagree" or "never," while the last point represents the highest level, like "strongly agree" or "always." The total score is used to categorize participants into different types of personality.

Statistical analysis

Descriptive statistics were computed to summarize participant characteristics such as marital status, number of children, workplace sectors, and medical specializations. Independent samples t-tests were conducted to examine potential differences in empathy scores across gender, prior communication skills training, recent psychological support seeking, and types of medical specialization. Additionally, regression analysis was performed to investigate the predictive relationship between personality traits (extraversion, agreeableness, conscientiousness, neuroticism, openness to experience) and empathy scores. SPSS (Statistical Package for the Social Sciences) was utilized for all statistical analyses.

Ethical considerations

The study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Novi Sad, Serbia. Informed consent was obtained from all participants.

RESULTS

The study included a total of 304 participants, with a gender distribution of 114 males (37.5%) and 190 females (62.5%). Age ranged from 25 to 66 years old, with a mean of 37.38 years. Further sociodemographic details are presented in Table 1.

Based on the sociodemographic data presented in Table 1, the majority of participants are either married or in a committed relationship, have no children, and are employed within tertiary healthcare institutions. Additionally, their employment is primarily within the public sector of the Republic of Serbia. Notably, a significant portion of the sample is either currently enrolled in a specialization program or has already completed one. The distribution of participants across these specializations is further detailed in Table 2.

Among the total participants, only 86 (28.3%) reported receiving any form of communication skills training during their education. When asked about perceived adequacy of time for reliable patient diagnosis and/or treatment, 134 participants (44.1%) responded affirmatively. Furthermore, 47 participants (15.5%) out of the total sample sought psychotherapy or some form of psychological support within the past six months (Table 1).

Table 1. *Sociodemographic data*

		N	%
Marital status	Divorced	15	4.9 %
	Single	53	17.4 %
	Married	149	49.0 %
	In a relationship	86	28.3 %
	Widowed	1	0.3 %
Number of children	Three or more children	7	2.3 %
	Two children	67	22.0 %
	One child	59	19.4 %
	No children	171	56.3 %
Workplace	Primary healthcare institution	70	23.0 %
	Secondary healthcare institution	60	19.7 %
	Tertiary healthcare institution	174	57.2 %
Employment sector	Public sector	252	82.9 %
	Private sector	26	8.6 %
	Both public and private sectors	26	8.6 %
Work location	Republic of Serbia	289	95.1 %
	Bosnia and Herzegovina	8	2.6 %
	Montenegro	2	0.7 %
	Croatia	1	0.3 %
	Germany	4	1.3 %
Specialization	Unspecialized (general practitioner)	42	13.8 %
	Currently undergoing specialization	149	49.0 %
	Completed specialization	113	37.2 %
Communication skills training	Yes	86	28.3 %
	No	218	71.7 %
Sufficient time for patients?	Yes	134	44.1 %
	No	170	55.9 %
Personal psychotherapy (past 6 months)	Yes	47	15.5 %
	No	257	84.5 %

Prior to conducting the main analyses, descriptive statistics for the study variables were assessed, as presented in Table 3. Based on the skewness and kurtosis values, the normality of the data distributions for these variables can be inferred. This fulfills the prerequisite for conducting further analyses. Cronbach's alpha is reported for each dimension, ranging from 0.647 (Neuroticism) to 0.806 (Extraversion), indicating acceptable to good internal consistency for the scales.

To assess potential differences in empathy scores between genders, participants with prior communication skills training and those without, individuals who sought psychological help in the last six months and those who did not, and participants in surgical and non-surgical specialties, independent samples t-tests were conducted for each variable (Table 4). An independent samples t-test revealed a significant difference in empathy scores between males (M = 5.11, SD = .79) and females (M = 5.33, SD = .69); t (302) = -2.457, p = .015. Females reported significantly higher empathy scores than males. An independent samples t-test did not reveal a significant difference in empathy scores based on the personal psychotherapy, communication skills and type of specialization (Table 4).

To investigate how personality traits contribute to explaining empathy, a linear regression

Table 2. *Specializations*

	N	%
Surgical specializations	**45**	**17.3**
Anesthesiology, Reanimatology and Intensive Care Medicine	26	10.0
Anesthesiology, Reanimatology and Intensive Care Medicine, Clinical Pharmacology	1	0.4
Pediatric Surgery	4	1.5
Neurosurgery	1	0.4
General Surgery	1	0.4
General Surgery, Abdominal Surgery	1	0.4
Orthopedic Surgery and Traumatology	4	1.5
Orthopedic Surgery and Traumatology, Pediatric Surgery	1	0.4
Plastic, Reconstructive and Aesthetic Surgery	3	1.2
Urology	3	1.2
Vascular Surgery	1	0.4
Non- surgical specializations	**215**	**82.7**
Dermatology and Venereology	4	1.5
Child and Adolescent Psychiatry	1	0.4
Child Neurology	1	0.4
Epidemiology	5	1.9
Physical Medicine and Rehabilitation	16	6.2
Gynecology and Obstetrics	5	1.9
Hygiene	4	1.5
Infectious Disease Medicine	2	0.8
Internal Medicine	58	22.3
Medical Oncology	1	0.4
Public Health	1	0.4
Clinical Biochemistry	5	1.9
Clinical Pharmacology	2	0.8
Medical Microbiology	1	0.4
Neurology	5	1.9
Ophthalmology	11	4.2
General Medicine	7	2.7
Otolaryngology (Ear, Nose & Throat)	5	1.9
Pathology	1	0.4
Pediatrics	31	11.9
Pediatrics, Child Neurology	1	0.4
Psychiatry	28	10.8
Radiation Oncology	7	2.7
Radiology	6	2.3
Social Medicine	2	0.8
Sports Medicine	1	0.4
Emergency Medicine	3	1.2

Table 3. *Descriptive statistics for study variables*

	Min	Max	M	SD	Skewness	Kurtosis	α
Empathy	1.60	6.60	5.25	.73	-.611	.943	.775
Extraversion	1.13	5.00	3.60	.69	-.326	.089	.806
Agreeableness	1.00	5.00	4.04	.60	-.706	1.364	.788
Conscientiousness	1.00	5.00	3.90	.57	-.802	2.445	.790
Neuroticism	1.50	4.50	2.89	.56	-.050	-.076	.647
Openness to Experience	1.40	4.60	3.58	.51	-.373	.531	.665

Table 4. *Independent samples t-tests for empathy scores*

		$\bar{x}$	SD	t	p
Gender	Male	5.11	.79	-2.457	.015
	Female	5.33	.69		
Personal Psychotherapy (Past 6 Months)	Yes	5.39	.70	-1.459	.146
	No	5.22	.74		
Communication skills training	Yes	5.28	.72	-.441	.660
	No	5.24	.74		
Type of specialization	Surgical	5.23	0.706	.177	.860
	Non-surgical	5.25	0.726		

Table 5. *Regression coefficients for predicting empathy*

	B	**β**	**p**
Extraversion	.068	.064	.304
Agreeableness	.364	.298	.000
Conscientiousness	.041	.032	.625
Neuroticism	-.037	-.028	.621
Openness to Experience	.190	.133	.047

analysis was conducted. Personality traits were entered as predictors, and empathy served as the criterion variable. The results of the analysis were statistically significant ($R = .451$; $F(5,298) = 15.18$; $p < .001$). The model explained 20.3% of the variance in empathy scores. Individual contributions of the predictor variables to the criterion are presented in Table 5. Agreeableness ($\beta = 0.298$) and Openness to Experience ($\beta = 0.133$) emerged as significant positive predictors of empathy.

DISCUSSION

Medical treatment is not only about medical knowledge and technical skills but also about recognizing and addressing the emotional and psychological aspects of a patient's experience, leading to more compassionate and effective healthcare delivery. The foundation for establishing a therapeutic physician-patient relationship hinges on the physician's capacity to empathize with the patient (4).

The study included a diverse sample of healthcare professionals, predominantly from tertiary healthcare institutions within the public sector in the Republic of Serbia. This distribution reflects the broader healthcare landscape in the region, where a majority of practitioners are engaged in specialized fields within public institutions.

The findings revealed a notable gender difference in empathy scores, with females reporting

significantly higher levels of empathy compared to males. This result aligns with the existing literature suggesting that women tend to exhibit greater empathy in interpersonal interactions. Women generally exhibit greater empathy in interpersonal interactions due to a combination of biological, social, and cultural factors, including differences in brain structure, hormonal influences, and gender-specific socialization processes. This difference may have implications for patient care and provider-patient relationships within healthcare settings, highlighting potential areas for targeted training or intervention to enhance empathetic communication across genders (12).

Contrary to expectations, participation in communication skills training did not significantly affect empathy scores among healthcare professionals in our study. This finding contrasts with previous research indicating that communication training can improve empathy and patient outcomes. The discrepancy could be attributed to variations in training content, duration, or the individualized nature of empathy development (13, 14).

Similarly, the analysis did not find a significant difference in empathy scores between healthcare professionals who had sought personal psychotherapy or psychological support within the past six months and those who had not. This suggests that personal psychological well-being, at least within the timeframe examined, may not directly translate into heightened empathetic abilities in professional contexts among healthcare providers (15, 16).

Our study explored the relationship between empathy and personality traits, revealing that agreeableness and openness to experience were significant predictors of higher empathy scores. These traits emphasize characteristics such as altruism, cooperativeness, and receptiveness to new ideas and experiences. Traits like altruism, cooperativeness, and openness to new experiences foster empathy by promoting prosocial behavior, enabling a better understanding of others' feelings and needs, and facilitating emotional connections through active listening and compassion. The findings support previous research suggesting that personality plays a crucial role in shaping empathetic behaviors (17, 18).

The study's findings are subject to several limitations, including potential sample bias, reliance on self-reported data, and the constraints of a cross-sectional design that limits causal inferences.

The results of this study carry several practical implications for healthcare training and practice. Firstly, the gender difference in empathy underscores the importance of gender-sensitive training approaches that cater to the unique communication styles and empathetic tendencies of both male and female healthcare providers. Secondly, the non-significant effect of communication skills training on empathy suggests a need for reassessment and potential enhancement of existing training programs. Incorporating more experiential and context-specific training modules could better equip healthcare professionals with the skills necessary to navigate complex patient interactions empathetically. Lastly, future research could delve deeper into longitudinal studies to explore how changes in personal and professional circumstances affect empathy over time among healthcare professionals. Additionally, investigating the role of organizational culture and workplace environment in fostering empathetic behaviors could provide valuable insights into enhancing patient-centered care delivery.

CONCLUSION

The study identified significant associations between empathy levels and specific personality traits among Serbian physicians. These insights underscore the importance of integrating personality-aware interventions in medical education to foster empathy among healthcare providers. Understanding these dynamics can lead to enhanced patient-centered care and better healthcare outcomes overall. Further research could explore additional factors affecting empathy in healthcare settings, contributing to more effective training programs and supportive environments for both providers and patients.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Osobine ličnosti lekara kao prediktori empatije u zdravstvu

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SAŽETAK

Uvod/Cilj. Empatija u zdravstvu veoma je važna za poboljšavanje odnosa između bolesnika i pružalaca zdravstvenih usluga, kao i za krajnje rezultate zdravstvene zaštite. U ovoj studiji ispitivalo se kako je kod srpskih lekara empatija povezana sa velikih pet faktora ličnosti.
Metode. Sprovedeno je transverzalno istraživanje koje je obuhvatilo 304 srpskih lekara (37,5% muškaraca, 62,5% žena; prosečna starost 37,38 godina); oni su popunjavali onlajn upitnike od maja do avgusta 2023. godine. Jefferson skala empatije (engl. *Jefferson Scale of Empathy* – JSE) merila je nivo empatije, dok je Skala velikih pet faktora ličnosti (engl. *Big Five Inventory* – BFI) služila za ocenjivanje osobina ličnosti (otvorenost ka iskustvu, savesnost, ekstraverzija, saradljivost, neuroticizam). Statističke analize, uključujući deskriptivnu statistiku i regresionu analizu pomoću *SPSS* softvera, istražile su veze između osobina ličnosti i empatije.
Rezultati. Utvrđene su značajne korelacije između empatije i specifičnih osobina ličnosti, što ukazuje na povezanost između osobina ličnosti i empatičnog ponašanja lekara. Saradljivost (β = 0,298) i otvorenost ka iskustvu (β = 0,133) pokazale su se kao značajni pozitivni prediktori empatije.
Zaključak. Razumevanje povezanosti osobina ličnosti sa empatijom ključno je za unapređenje nege bolesnika i profesionalnog razvoja u zdravstvu. U ovoj studiji istaknuta je potreba za integracijom strategija koje podstiču empatiju u medicinsko obrazovanje kako bi zdravstveni radnici stekli više znanja o empatičnim procesima.

Ključne reči: **empatija, velikih pet faktora ličnosti, pružaoci zdravstvenih usluga, medicinsko obrazovanje**

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Case report

Doxycycline Hyclate-Induced Esophageal Injury Associated with Inappropriate Drug Use

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SUMMARY

Introduction. Drug-induced esophageal injuries are rare in clinical practice. Doxycycline is the most common antibiotic that can damage the esophagus.
Case report. We present a 26-year-old woman who visited a gastroenterologist because of difficulty swallowing and pain during swallowing. After laparoscopic treatment of endometriosis, the gynecologist prescribed her doxycycline capsules of 100 mg daily, in duration of five days, in order to prevent infection. The patient took a doxycycline hyclate capsules with a small amount of water, at night, before going to bed. After the third day of therapy, the patient developed difficulty swallowing, which progressed to odynophagia until the end of therapy. A performed endoscopy showed ulceration involving almost the entire circumference of the esophageal lumen in the middle part of the esophagus, which was suspected malignant neoplasm. Virological analyzes and tumor markers were within normal limits. Pathohistological examination of ulceration biopsy was without any signs of malignancy. After a month of proton pump inhibitors (PPIs) therapy, the patient was symptom free at the control examination, and the endoscopic finding was normal.
Conclusion. It is difficult to distinguish endoscopically extensive doxycycline-induced esophageal ulcerations caused by esophageal cancer. In addition, the pathological finding is not specific. The anamnesis of inadequate use of the drug is important and all patients taking doxycycline must be given detailed instructions about the appropriate administration methods.

Keywords: **doxycycline, esophagus, ulcer, endoscopy**

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INTRODUCTION

Drug-iduced esophageal injuries are rare in clinical practice. However, about 100 types of medications that can cause esophageal injuries have been described in the refrence literature (1). Doxycycline is the most common antibiotic that can damage the esophagus (2), especially in the mid-esophageal segment (3). Esophageal lesions caused by doxycycline mostly appear as mild esophagitis and sometimes as ulceration with a generally benign course. However, extensive ulcerations after doxycyline intake have been reported so far (4). It is difficult endoscopically to distinguish extensive doxycycline-induced esophageal ulcerations from esophageal cancer. A few cases of doxycycline-induced ulceration mimicking esophageal cancer have been described in the literature (2, 4).

Older people are more prone to drug-induced esophageal injuries as a result of decreased esophageal motility and saliva production, cardiac enlargement, and multiple drugs intake (1, 2). However, doxycyline-related esophageal injury frequently occurs in young women with no history of esophageal dysfunction. The reason is an incorrect intake of doxycycline with a small amount of water or just immediately before going to sleep (5, 6).

CASE REPORT

We present a case of a recently treated 26-year-old woman who visited a gastroenterologist due to difficult and painfull swallowing. Two weeks before, the patient underwent laparoscopic treatment for endometriosis due to infertility. The gynecologist prescribed her doxycycline hyclate capsules of 100 mg daily, in duration of five days, to prevent infection. The patient took a doxycycline capsules with a small amount of water, at night before going to bed. After the third day of therapy the patient developed difficulty swallowing, which progressed to odynophagia until the end of therapy. Physical examination, blood count and laboratory analysis were within normal limits. Proximal endoscopy showed ulceration involving almost the entire circumference of the esophageal lumen (Figure 1) in the middle part of the esophagus, suspected of malignant neoplasm. Cold and liquid diet and proton pump inhibitors (PPIs) two times a day were prescribed. Virological analyzes were performed since viral esophagitis gives a similar clinical and endoscopic picture. Anti-herpes simplex virus antibodies, anti-varicella-zoster virus antibody and anti-cytomegalo virus antibody were negative. Due to an endoscopic finding similar to esophageal cancer, tumour markers were checked. The levels of the carcinoembryonic antigen (CEA), squamous cell cancer antigen (SCC-Ag), CA 19-9, CA 125 were within normal limits. Pathohistological examination of ulceration biopsy specimens stained with standard haematoxylin-eosin (HE) indicated squamous epithelium with mixed inflammatory infiltrate, granulation tissue with numerous neutrophils and eosino-

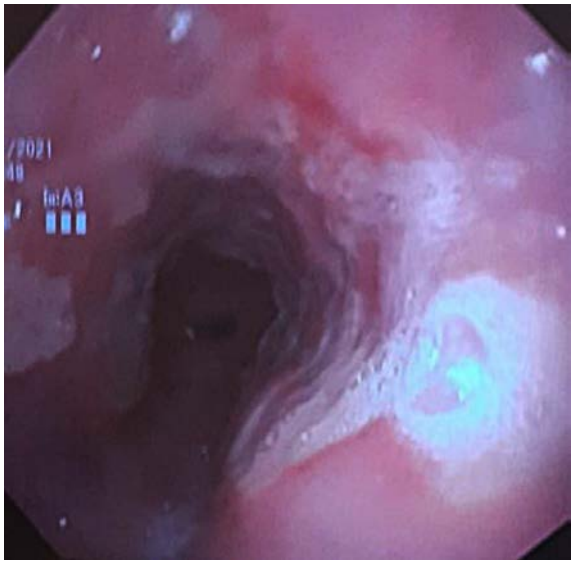

Figure 1. *Endoscopic appearance of the ulcer at the level of the mid-esophagus. An upper endoscopic examination revealed deep ulceration at the level of the mid-esophagus*

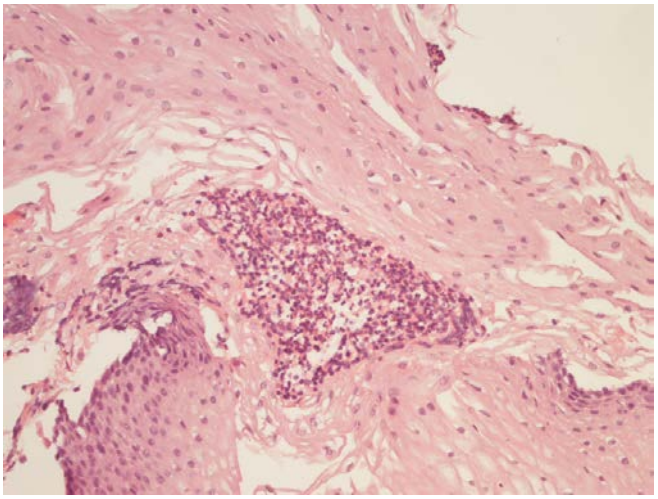

Figure 2. *Squamous epithelium with inflammatory infiltrate (HE x 100)*

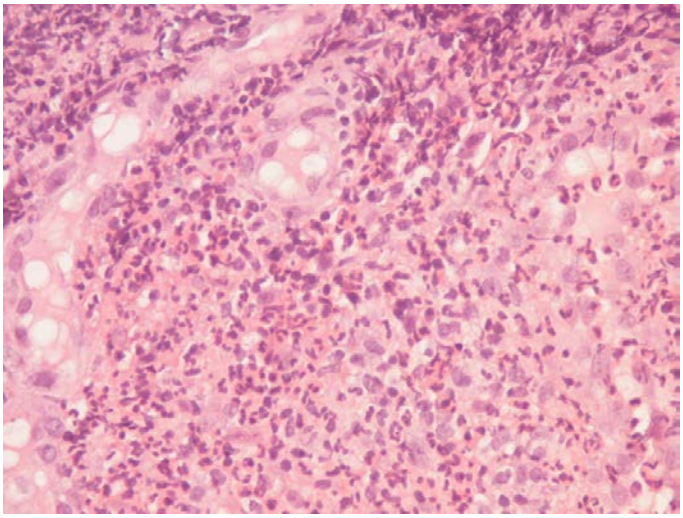

Figure 3. *Granulation tissue with numerous neutrophils and eosinophilic leukocytes (HE x 200)*

philic leukocytes, without any signs of malignancy (Figure 2 and 3).

After a month of PPIs therapy at the control examination, the patient was symptom free, and the endoscopic finding was within normal limits.

DISCUSSION

Approximately 100 types of medications are reported to cause esophageal injuries (2). Drug-induced esophageal injuries are classified by their etiology into four categories: (I)—injury resulting from acidic antibiotics; (II)—chemical esophagitis caused by bisphosphonates such as alendronate; (III)—hyperosmotic injury caused by potassium chloride or quinidine, and (IV)—distal esophagitis associated with non-steroidal anti-inflammatory drugs in patients with gastroesophageal reflux disease (5). Tetracyclines are a group of bacteriostatic antibiotics that provide a broad-spectrum of activity against both Gram-positive and Gram-negative infections (7). Doxycycline hyclate is the most common antibiotic that can damage the esophagus—it contributed to 27% of all cases reported in the world literature (6). A frequent use of doxycycline hyclate capsulses cause more damage to esophageal epithelium than the same drug taken in tablets. This is also more common with the use of doxycycline hyclate compared to doxycycline monohydrate (8).

The mechanism of esophageal mucosal injury may be explained by doxycycline acidic efect, gelatinous sticky capsules, increased mucosal concen-

trations and intracellular toxicity (1, 9, 10). Also, dissolved doxycycline molecules can cause an inhibition of protein synthesis, which further leads to esophageal epithelial injuries, such as ulcers (11, 12). Endoscopy is considered as a method of choice for detecting drug induced esophageal injuries. Doxycycline-induced esophageal ulceration varied in size, depth, and number. Those are usually discrete, confluent, linearbroad band-formed and butterfly-shaped ulcers partially covered with pseudomembranes (1). Doxycyline hyclate tends to cause the damage in mid-esophageal segment in 66% of cases (3, 6). There has been reported one case of doxycycline-induced esophageal ulcers and tissue fragility mimicking esophageal cancer simultaneously in the lower and mid-esophageal segment (4). Extensive ulcerations may cause endoscopists to suspect esophageal cancer and may be the reason for many additional analyses. In such cases, it is necessary to exclude esophageal carcinoma by histology. Pathological findings on endoscopic biopsies specimens are not specific.

We used the Naranjo probability scale, which can indicate a probable adverse drug reaction (13). The score was 8, which is indicative of probable adverse drug reaction. The adverse effect associated with doxycycline treatment may contribute to poor infection treatment outcomes and development of bacterial resistance.

All patients taking doxycycline must be given detailed instructions about the adequate medication administration to prevent esophageal injury. It is recommended to swallow doxycycline capsule whole, with at least 100 ml of water, while sitting or standing (upright for at least 90 seconds after swallowing). Doxycycline is best taken in the morning, if possible, or at least one hour before bedtime. It is important that the patient remain in the upright position for at least 30 minutes after taking doxycycline to prevent the irritation of the throat and esophagus. Recommendation is to substitute doxycycline monohydrate for doxycycline hyclate to prevent irritation of esophagus and start taking a PPI, which can be helpful in blocking the acid that can cause further irritation and prevent healing.

CONCLUSION

We presented a case of an uncommon doxycycline hyclate-induced extensive esophageal ulceration mimicking esophageal cancer. Esophageal injury was serious and adverse effect was caused by inappropriate use of doxycycline. Therefore, it is important that all patients taking doxycycline must be given detailed instructions about the appropriate administration methods and their adherence to prescribed regimens. Doxycycline monohydrate has an advantage over doxycycline hyclate in the prevention of irritation of the esophagus.

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Conflict of interes

The authors declare that they did not have any competing interest.

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Oštećenje jednjaka izazvano neadekvatnom upotrebom doksiciklin-hiklata

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SAŽETAK

Uvod. Oštećenja jednjaka izazvana lekovima retka su u kliničkoj praksi. Antibiotik koji najčešće može dovesti do oštećenja jednjaka jeste doksiciklin.
Prikaz slučaja. U ovom radu je predstavljen slučaj dvadesetšestogodišnje žene koja se javila gastroenterologu zbog otežanog gutanja i bolova pri gutanju. Nakon laparoskopskog lečenja endometrioze ginekolog joj je propisao terapiju kapsulama doksiciklina od 100 mg dnevno u trajanju od pet dana, kako bi se sprečila pojava infekcije. Bolesnica je uzimala kapsule doksiciklin-hiklata sa malom količinom vode uveče pre spavanja. Posle trećeg dana terapije došlo je do pojave otežanog gutanja, koje je do kraja terapije prešlo u odinofagiju. Prilikom endoskopije uočena je ulceracija koja je zahvatala skoro čitav lumen srednjeg dela jednjaka; sumnjalo se da je reč o malignoj neoplazmi. Virusološke analize i tumor markeri bili su u granicama normale. Patohistološki pregled biopsije ulceracije nije pokazao znakove maligniteta. Nakon mesec dana terapije inhibitorima protonske pumpe (IPP), bolesnica na kontrolnom pregledu nije pokazivala nikakve simptome, a endoskopski nalaz je bio normalan.
Zaključak. Endoskopski je teško razlikovati ekstenzivne ulceracije jednjaka izazvane doksiciklinom od maligne neoplazme jednjaka. Takođe, patološki nalaz nije specifičan. Veoma je važna anamneza o neadekvatnoj upotrebi leka. Svim bolesnicima koji uzimaju doksiciklin treba dati detaljna uputstva o odgovarajućem načinu primene leka.

Ključne reči: **doksiciklin, jednjak, ulceracija, endoskopija**

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Case report

Giant Epithelial Cysts of the Spleen in Children: Report of Two Cases and Mini Literature Review

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SUMMARY

Introduction. Cystic changes of the spleen are one of the rare clinical entities in the pediatric population. The paper presents a partial splenectomy in children performed by open approach as a safe surgical treatment of giant epithelial splenic cysts.
Case report. Two cases of giant epithelial cysts of the spleen in children are presented. Medical documents and data referring to age, clinical features, findings on ultrasonography and magnetic resonance imaging (MRI), performed surgery, and follow-ups were collected and analyzed retrospectively. Both of the patients complained of abdominal pain; the diagnosis was made on ultrasound and confirmed by MRI. Open cystectomy with partial resection of the spleen was performed in both cases. There were no complications and no recurrence during the follow-up period. The literature review revealed that up to date only seven authors have published papers on this topic, and different treatment methods and results have been reported.
Conclusion. Although very rare, splenic cysts should always be considered in the differential diagnosis of non-specific pain or cystic formations in the upper left quadrant of the abdomen. Taking into account the immunological role of the spleen, tissue preservation surgery should be the main goal. Open partial splenectomy which was performed in both patients was without intra- or postoperative complications and uneventful follow-up, and could be one of the safe management options.

Keywords: **epithelial cysts, spleen, children**

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INTRODUCTION

Cystic changes of the spleen are one of the rare clinical entities in the pediatric population. Literature data shows an incidence of 0.5–2%, where half of the total number occurs in the age under 15 years (1). Based on the presence of an epithelial lining, all cystic changes of the spleen are divided into true or primary cysts that are characterized by the presence of an epithelial lining and pseudocysts or secondary cysts that do not have an epithelial lining. By further division, primary cysts can be divided into non-parasitic or congenital and parasitic (2). The most common cause of primary cysts of the spleen is human hydatid disease or echinococcosis, commonly seen in Africa and Central America. However, non-parasitic cysts are much less common and usually occur in Europe and North America (3). The exact pathophysiological mechanism of the occurrence of these cysts has not yet been clearly defined and there are several different theories such as mesothelial invagination theory, lymph space theory, and endodermal inclusion theory (4-6). Although diagnosing splenic cysts are not such a challenge, the management of these cysts could be challenging. Considering the lack of an adequate amount of data, there is still no clearly defined standard for the treatment of these changes in the pediatric population. This study aims to present a partial splenectomy in children performed by open approach as a safe surgical treatment of giant epithelial splenic cysts.

CASE ONE

Medical documents and data referring to age, clinical features, findings on ultrasonography and MRI, performed surgery, and follow-ups were collected and analysed retrospectively.

The first patient was a twelve-year-old girl who presented to the pediatric surgeon due to the existence of a painless lump in the left hypochondriac region. An ultrasound examination was performed and a cystic lesion in the spleen was revealed. A magnetic resonance imaging of the abdomen findings confirmed a large splenic cyst measuring 105 x 76 x 85 mm (Figure 1). Tumor

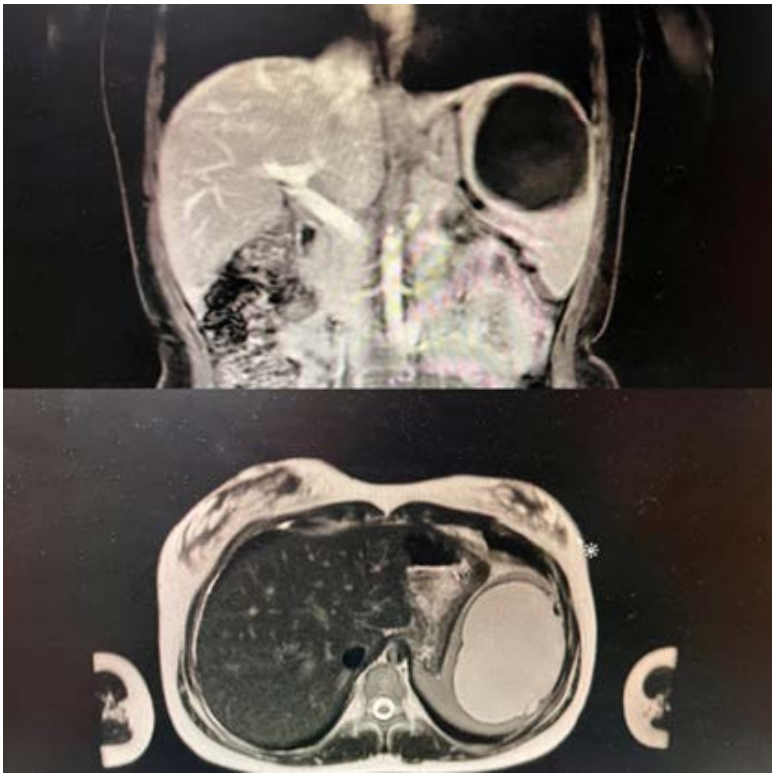

Figure 1. *Magnetic resonance imaging – the first patient*

markers were increased: CEA (carcinoembryonic antigen) was 8.4, and Ca 19-9 was 152.3. Other laboratory findings were within normal values. Cystic echinococcosis was excluded using a serology ELISA test (enzyme-linked immunosorbent assay). Preoperatively, the patient was given a pneumococcal vaccine in case that splenectomy was needed.

Open cystectomy with partial resection of the spleen was performed. Initially, 450 ml of fluid was evacuated from the cyst. The Ulttacision Harmonic Scalpel (Ethicon) was used for spleen resection. Haemostasis was controlled with SURGICEL ™Absorbable Hemostat (Ethicon) and interrupted sutures with Vycril 3/0. No transfusion was required. There were no postoperative complications, evidence of bleeding, or related readmissions. No long-term antibiotics were given after discharge from hospital. The final diagnosis was based on the pathological study of operative specimens.

A unilocular, emptied cystic structure was submitted for pathohistological diagnosis. Micromorphological analysis of the sampled tissue on histological sections revealed a cyst with relatively thick walls. The inner part of the cyst was lined with squamous epithelium. Cellular atypia and pathological mitoses were not observed. The expression of CEA and CA 19-9 was observed by immunohistochemical analysis (Figure 2 and 3).

Regular follow-ups, firstly after a month and afterward every three months for physical and ultrasound examination, and blood cell count in the outpatient department were performed. No cyst recurrence was observed.

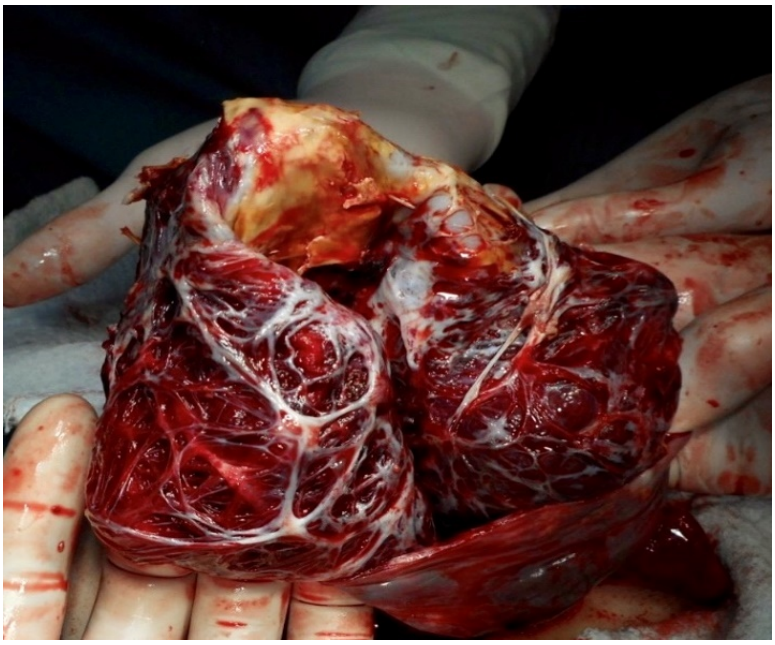

Figure 2. *The inner side of the splenic cyst*

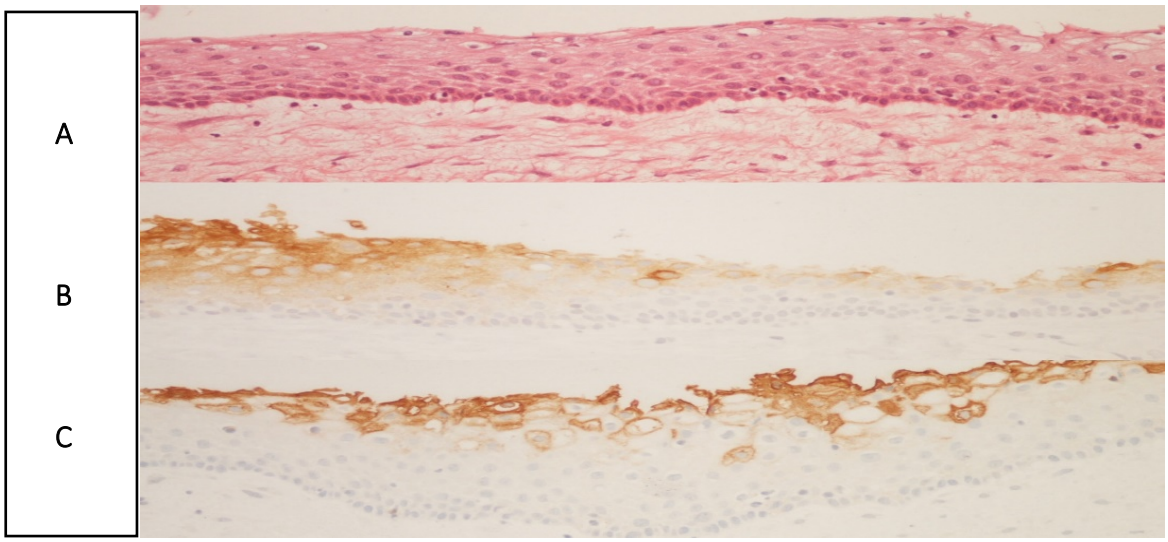

Figure 3. *Histopathological and immunohistochemical analysis. A - H&E staining (magnification x 40); B - CEA expression (magnification x 40); C - Ca 19-9 expression (magnification x 40)*

CASE TWO

The second patient was a ten-year-old girl who had experienced episodes of abdominal pain a few months before the physical examination. The pain was present periodically, especially when she was physically active. A huge cyst in the spleen was first detected by ultrasound, and later, the patient underwent an abdominal MRI examination when a gigantic splenic cyst was described with dimensions 143 x 114 x 135 mm (Figure 4.) All laboratory findings and tumor markers were in the normal range except Ca 19-9 which was 47.3. Echinococcosis was excluded preoperatively, and immunization against the bacteria *Streptococcus pneumonia* was conducted.

Although the cystic lesion was gigantic, and 850 ml of fluid was evacuated from the cyst at the beginning of the surgical procedure, open cystectomy with partial splenectomy was performed and the lower pole of the spleen was preserved (Figure 5). Resection and haemostasis were done in the same manner as previously described in the first case. There were no complications during the surgery or postoperatively, and there was no need for transfusion also. The diagnosis of the epithelial splenic cyst was confirmed on histopathological examination. There was no evidence of cystic rests or recurrence during the follow-up period.

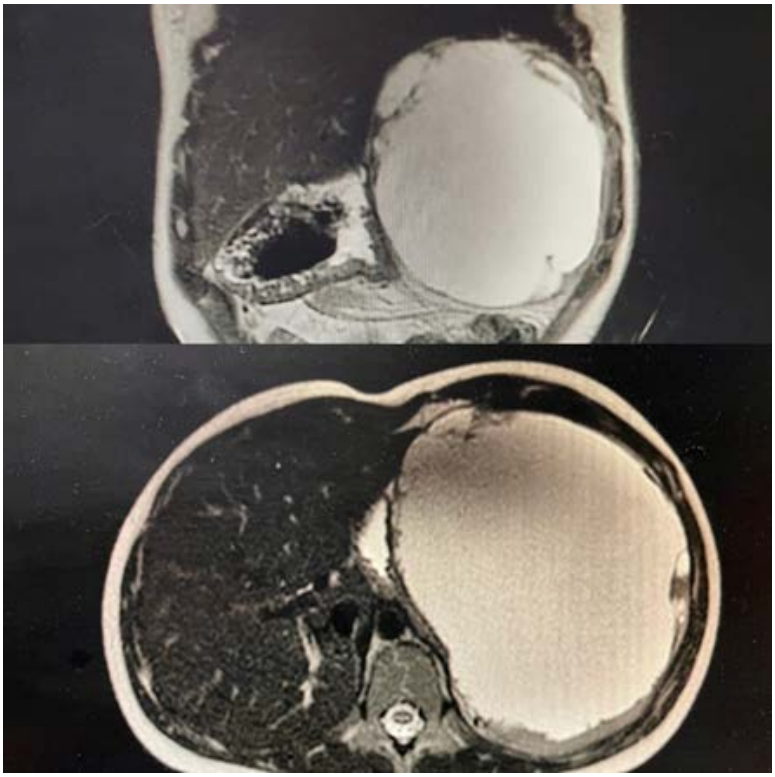

Figure 4. *Magnetic resonance imaging – the second patient*

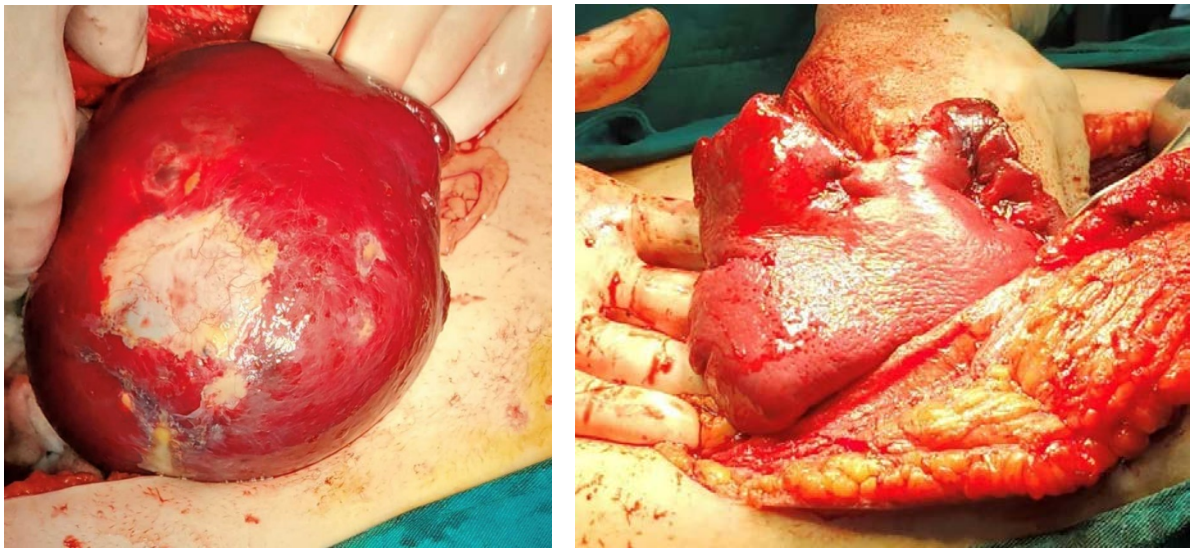

Figure 5. *A - Macroscopic appearance of the cyst; B - Resected spleen after cystectomy and partial splenectomy*

MINI LITERATURE REVIEW

A mini literature review was conducted on this topic. English and Serbian literature was searched on PubMed, Medline, and Kobson databases for terms: cysts, spleen, children, and surgery. Data were entered in a Microsoft Excel (Microsoft Corporation) spreadsheet containing the author's name, year of publication, number of patients, (age), types of performed surgery, complications, and if there were rests of the cysts. All papers that matched our inclusion criteria, containing data on children who were treated due to epithelial splenic cysts were included. Patients with parasitic cysts and literature reviews were exculded . Results are given in Table 1.

Table 1. *Authors, year of publication, patients, and type of surgery are represented in the table*

Author	Year of publication	Number of patients	Gender (male and female)	Age (years)	Open/Laparo-scopic surgery	Type of surgery	Other	Complications	Remnant/ Recurrence
Tsakayannis De	1995	19	M10/F9	0-17	14 open surgeries	Splenectomy 9 Partial splenectomy 2 Cystectomy 3	5 cysts were found on autopsy	/	/
Gezer HO	2015	22	M13/F9	0-14	9 open, 1 laparoscopic surgeries	Partial splenectomy 2 Cystectomy 5	12 ultrasound Follow-up	/	/
Zvizdic Z	2012	1	M0/F 1	10	1 open surgeries	Cystectomy 1	/	/	/
Hassoun J	2007	42	M19/F23	0-18	2 open, 6 laparoscopic surgeries	Splenectomy 3 Partial splenectomy 2 Cystectomy 3	32 ultrasound follow up (in 4 patients aspiration was perforemd)	/	/
Pang W	2009	10	M8/F2	5-15	5 open surgeries	Splenectomy 4 Partial splenectomy 1	Other 5 patients were in angioma group	/	/
Czauderna P	2006	50	M26/F 24	1-17	26 open, 24 laparoscopic surgeries	Partial splenectomy 26 Cystectomy 9 Deroofing 9	/	2 massive bleeding	Eight remnants of the cyst after laparoscopy
Kimber C	1998	6	M2/F4	8-16	6 open surgeries	Splenectomy 1 Partial splenectomy 5	/	/	/
Marjanovic Z	2008	5	M2/F3	13-24	5 open surgeries	Splenectomy 1 Partial splenectomy 4	/	/	/.. 0

DISCUSSION

Splenic cysts in children are not common and clinical presentation and symptomatology are mostly non-specific and depend on the size of the cyst. Smaller cysts are usually asymptomatic, while larger cysts can be presented by a palpable abdominal mass, abdominal pain, vomiting, coughing, or some complications in the form of infection, rupture, and subsequent hemorrhage. Continuous or inconsistent abdominal pain, or radiating left shoulder pain is a consequence of capsule distension or the consequence of dislocation of other abdominal organs (7–9). The increase in the volume of the cyst may be due to the proliferation of the epithelial lining and its secretion or bleeding (10), or it may be due to a difference between the osmotic pressure of the surrounding tissue and the inside of the cyst (11). A palpable mass was present in the first and periodical abdominal pain in the second patient.

The vast majority of splenic cysts are detected accidentally by different imaging methods, most often after abdominal trauma. Diagnosis is commonly made by ultrasound examination. Multislice spiral computed tomography (MSCT) and MRI can give concrete data about the morphology of the cyst, whether it is uni- or multilocular, information about the relationship with the surrounding structures, the content inside the cyst, the existence of pathological communication, the localization of the spleen, so the surgeon can preoperatively make the optimal operative approach (12). Both of our patients underwent an ultrasound examination first. The diagnosis of splenic cysts was confirmed later on MRI. Regarding biomarkers, serum carbohydrate antigen (CA 19-9) can be elevated, and it should be measured preoperatively and postoperatively to exclude recurrence. Also, carcinoembryonic antigen (CEA) with CA 19-9 should be used for differentiating benign cystic lessions from mucinous cystic neoplasm of the pancreas (13). These markers were elevated in both of our patients.

The management of splenic cysts in children includes several options such as observation, aspiration with or without sclerotherapy, deroofing, cystectomy, partial or total splenectomy, and open or laparoscopic approach. Some authors recommend regular follow-up with control ultrasound examinations of cysts smaller than 5 cm as spontaneous regression is described, however, cysts larger than 5 cm should be treated surgically (14, 15). Another type of non-surgical invasive treatment is percutaneous aspiration with or without scleratherapy. The main problem of this type of treatment is recurrence (16, 17). The literature is scarce about the type of treatment in the pediatric population, as it is presented in Table 1. Up today, only 145 pediatric splenic cyst cases have been published. Nowadays, taking into account the immune role of the spleen, tissue preservation surgery is the main goal. Total splenectomy is performed if splenic cysts are located in the hilum of the spleen, in the presence of very large or multiple cysts, and in case of excessive intraoperative bleeding (18). The minimal percentage of tissue required for the normal immunological function of the spleen is 25% (19), therefore, recommended surgical options include partial splenectomy, total cystectomy, deroofing, or marsupialization of cysts. Open partial splenectomy is a relatively safe procedure, without recurrence after this type of surgical treatment (20, 21). Tsakayannis et al. published that all of their patients treated before 1971 underwent splenectomy, and patients treated afterward had hemisplenectomy or cystectomy (22). Also, Pang et al. reported that the majority of their patients had total splenectomy, and one had partial splenectomy (23). Calisti et al. reported five children treated with open total cystectomy, which means peeling the cystic wall off the splenic tissue. After a follow-up period of 6 months to 10 years, they did not show recurrence and had no major complications except in one case (24). A case similar to our second patient in age, gender, cyst dimensions, and treatment was reported by Zvizdic and Karavdic (25). In the last decades, laparoscopy has become the method of choice for many surgeons. The main advantages of laparoscopy include shorter hospital stays, faster recovery, less postoperative pain, and better cosmetic outcomes, but there are also longer operative times increasing operative costs, and high recurrence rates as disadvantages of this method. Hassoun et al. reported recurrence after laparoscopic cystectomy in one patient (26); also, Sinha et al. reported 50% of recurrence after cystectomy. They believed that the high rate of recurrence is connected with the laparoscopic approach (27). Nowak et al. described laparascopic technique for decapsulation, which includes suctioning out the cyst, opening and excising the cystic wall with LigaSure or Harmonic Scalpel device which is more safe as there is less blood loss compared to excising of spleen parenchyma. One should bare in mind that

any remaining fragment of cyst wall could leed to reccurance of the cyst (28). Laparoscopic fenestration is also a safe method for the treatment of superficially located cysts. This method involves the resection of the cyst wall and the creation of permanent communication between the cystic cavity and the peritoneum, however, there is a high rate of recurrence after this technique. Czauderna et al. reported recurrence in 7 of 9 patients treated with laparoscopic fenestration or deroofing (29).

CONCLUSION

Although very rare, splenic cysts should always be considered in the differential diagnosis of non-specific pain or cystic formations in the upper left quadrant of the abdomen. After the diagnosis, further therapeutic treatment depends on the size of the cyst, and therefore, cysts of smaller dimensions (< 5 cm) should be monitored with regular ultrasound examinations, while for larger cysts there are several different therapeutic modalities. Taking into account the immunological role of the spleen, tissue preservation surgery should be the main goal. Open partial splenectomy which was performed in both patients without intra or postoperative complications and uneventful follow-up could be one of the safe management options.

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Gigantske epitelne ciste slezine kod dece: prikaz dvaju slučajeva i mini-pregled literature

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SAŽETAK

Uvod. Cistične promene slezine predstavljaju retku kliničku pojavu u pedijatrijskoj populaciji. Cilj ovog rada bio je da predstavi otvorenu parcijalnu splenektomiju kod dece kao bezbedno hirurško lečenje gigantskih epitelnih cista slezine.
Prikaz slučaja. Prikazana su dva slučaja džinovskih epitelnih cista slezine kod dece. Retrospektivno su prikupljeni i analizirani medicinski dokumenti i podaci koji se odnose na godine starosti, kliničke karakteristike, nalaze sa pregleda ultrazvukom i magnetnom rezonancom, urađene operacije i praćenja. Oba bolesnika su se žalila na bolove u trbuhu. Dijagnoza je postavljena ultrazvučnim pregledom i potvrđena snimanjem magnetnom rezonancom. I u jednom i u drugom slučaju urađena je otvorena cistektomija sa delimičnom resekcijom slezine. Nije bilo komplikacija i recidiva za vreme praćenja. Pregled literature ukazao je na to da je samo sedam autora objavilo radove o ovoj temi, kao i da su prikazane različite metode lečenja i rezultati.
Zaključak. Mada su veoma retke, ciste slezine uvek treba uzeti u obzir prilikom diferencijalne dijagnoze nespecifičnog bola ili cističnih formacija u gornjem levom kvadrantu trbuha. S obzirom na imunološku ulogu slezine, operacija usmerena na očuvanje tkiva treba da bude glavni cilj. Otvorena parcijalna splenektomija kojoj su bili podvrgnuti i jedan i drugi bolesnik bila je bez intraoperativnih ili postoperativnih komplikacija i neometanog praćenja, te bi mogla biti jedna od sigurnih opcija lečenja.

Ključne reči: **epitelne ciste, slezina, deca**

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Case report

Splenic Abscess due to *Salmonella Enteritidis* after Abdominal Trauma Resolved by Interventional Radiological Methods: A Case Report

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SUMMARY

Introduction. Splenic abscess is a very rare extra-intestinal complication of Salmonella infection in the post-antibiotic era with the incidence between 0.14% and 2%. It usually follows bacteremia due to varied etiologies such as trauma, infective endocarditis, intravenous drug abuse, immunodeficiency status (AIDS, diabetes mellitus).
Case report. An 82-year-old woman presented with complaints of upper abdominal pain, fever and nausea for two weeks following abdominal trauma. Computed tomography scan of the abdomen showed hypodense lesion measuring 110 × 80mm (CCxLL), with minimal peripheral contrast enhancement, diagnosed as a splenic abscess.
The patient underwent an ultrasound and X-ray guided percutaneous needle aspiration to collect a sample of pus for microbiological analyses, and in the next step, percutaneous drainage was performed.
***Salmonella enteritidis* was isolated from the culture; the isolate was sensitive to ampicillin, ciprofloxacin, and third-generation cephalosporins. The initially started empiric therapy with amikacin was replaced by cefriaxone. After one month, the patient was discharged for home treatment. During the six-month follow-up, there were no recurrent symptoms and a follow-up CT scan showed a normal-sized spleen with thin, low-density zones under the capsule-sequels of inflammation.**
Conclusion. Only a few cases of splenic abscess caused by *Salmonella enteritidis* have been described in the literature and they were mostly treated with splenectomy. This case of a rare splenic abscess due to *Salmonella enteritidis* was treated successfully with a combination of percutaneous drainage, prolonged antibiotic therapy, and intensive care.

Keywords: ***Salmonella enteritidis*, splenic abscess, percutaneous drainage**

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INTRODUCTION

Splenic abscess is a very rare extra-intestinal complication of Salmonella infection in the post-antibiotic era with the incidence ranging between 0.14% and 2% (1). It usually follows bacteremia due to varied etiologies such as trauma, infective endocarditis, intravenous drug abuse, immunodeficiency status (AIDS, diabetes mellitus) (2-5). Splenic abscesses usually present as unilocular and solitary lesions raging in size from 1 to 18 cm. Numerous microorganisms may be found in splenic abscesses such as staphylococci, streptococci, anaerobic organisms, *Mycobacterium tuberculosis* and fungi, while cultures remain sterile in 11% of cases. Salmonella is responsible for 15% of splenic abscesses (2, 4). Currently, splenectomy is the gold standard in the treatment, while interventional radiological methods can be considered as an alternative therapy, especially in the case of a solitary abscess. We report a case of splenic abscess due to *Salmonella enteritidis* after abdominal trauma successfully treated with percutaneous drainage and antibiotic therapy.

CASE REPORT

An 82-year-old woman presented with complaints of upper abdominal pain, fever and nausea for two weeks following abdominal trauma. Laboratory investigations revealed a raised total leukocyte count, anemia, and elevated C-reactive protein (111 mg/dl). Computed tomography scan of the abdomen showed hypodense lesion measuring 110 x 80mm (CCxLL) with minimal peripheral contrast enhancement, diagnosed as a splenic abscess (Figure 1). The patient underwent an ultrasound and X-ray guided percutaneous needle aspiration with free-hand technique using an 18 Gauge needle to collect a sample of pus for microbiological analyses. In the next step, an 8.5F multisidehole pigtail catheter was placed in collection by performing the Seldinger technique (Figure 2 and 3). The patient was in pronation under analgosedation and with local anesthesia on the site of punction. In next 24 hours, 300 ml of purulent fluid was drained and about 100 ml each day in the next 3-4 days, and then the catheter was extracted. *Salmonella enteritidis* was isolated

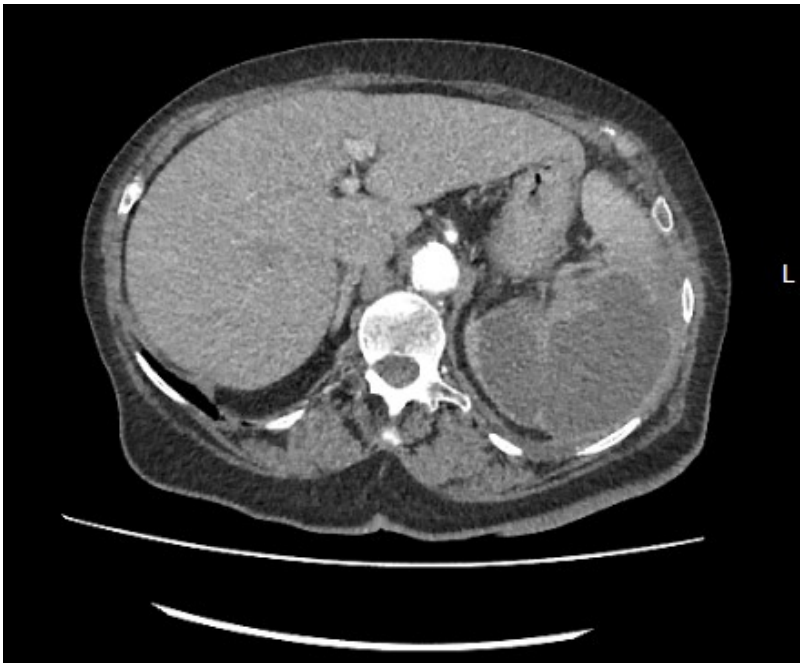

Figure 1. *Multidetector computed tomography, an axial image—splenic abscess*

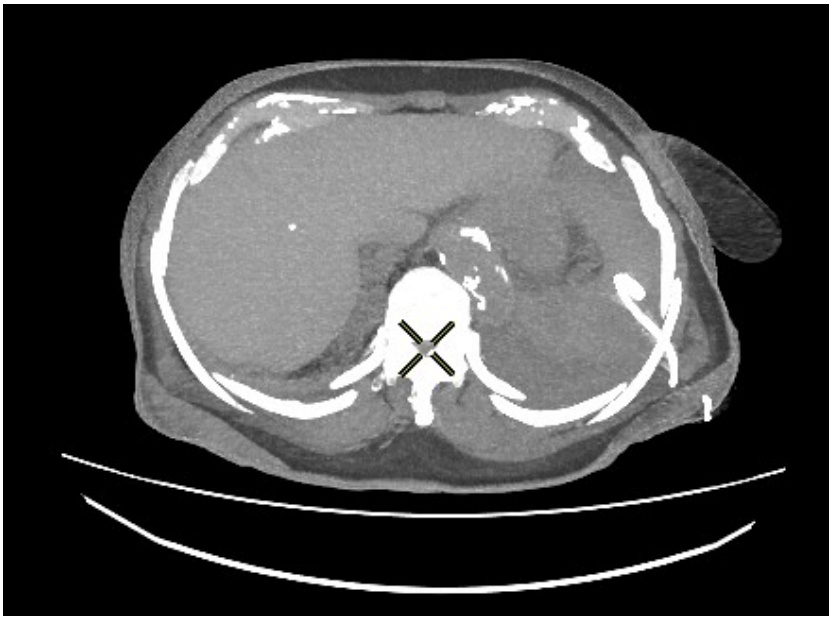

Figure 2. *Multidetector computed tomography—an axial image after placing a drainage catheter*

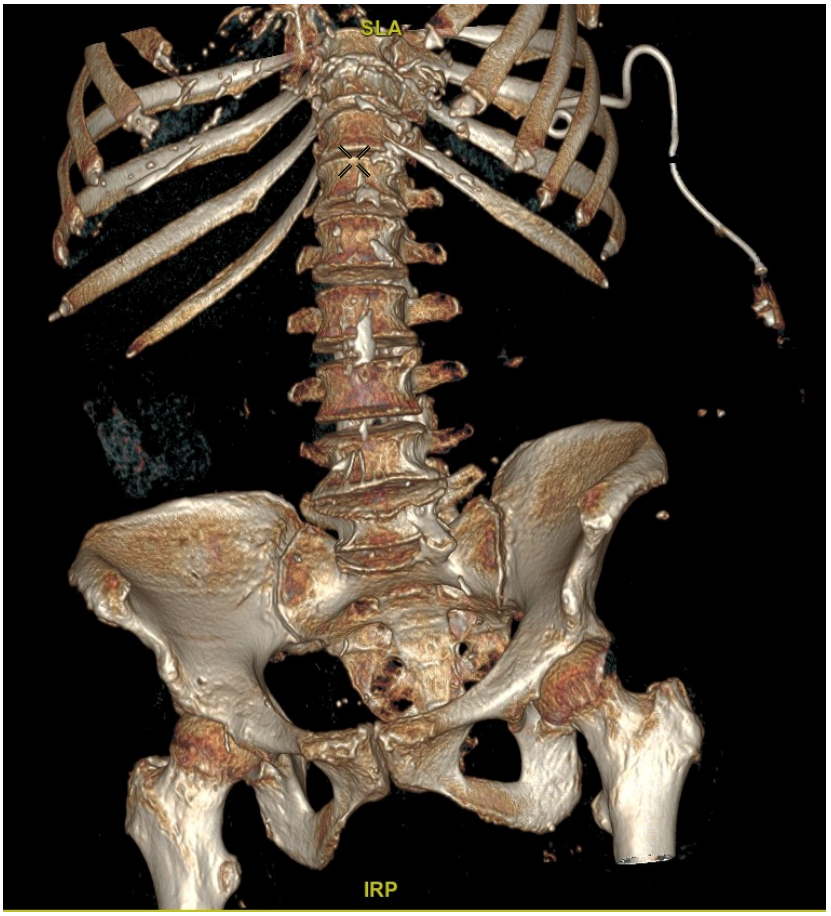

Figure 3. *Multidetector computed tomography—a virtual reality image after placing the drainage catheter*

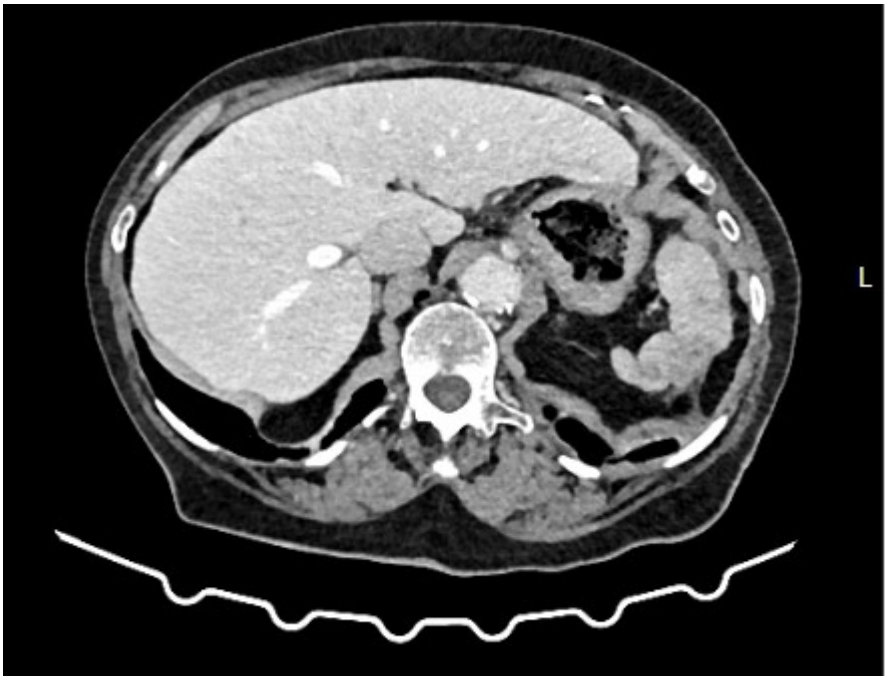

Figure 4. *Multidetector computed tomography—an axial image after drainage the splenic abscess*

from the culture, and the isolate was sensitive to ampicillin, ciprofloxacin and third- generation cephalosporins. The initially started empiric therapy with amikacin was replaced by cefriaxone. After one month, the patient was discharged for home treatment. During the six-month follow-up, there were no recurrent symptoms and a follow-up CT scan showed a normal-sized spleen with thin, low-density zones under the capsule-sequels of inflammation (Figure 4).

DISCUSSION

Salmonella spp. is a bacterial genus that belongs to the Enterobacteriaceae family, present in the intestinal tract of healthy people and animals, but can generate abdominal symptoms after consumption of contaminated food (such as contaminated eggs or meat) (4). These symptoms include diarrhea, nausea, vomiting, abdominal pain and/or fever. Complications may occur in up to 7% of cases and extraintestinal infections are observed in up to 4% of the patients (6). Some serotypes such as *Salmonella typhimurium* and *Salmonella enteritidis* show a greater tendency to cause bacteraemia (2). Splenic abscesses caused by Salmonella spp are uncommon with the incidence between 0.14% and 2% (3). Splenic abscess is associated with high morbidity and mortality if not diagnosed and treated in time. Usually, they stand for the complication of bacteremia (in 49% cases) in the context of trauma, immunosuppression (HIV, diabetes, sepsis, etc.), infectious endocarditis, embolization, use of intravenous drugs or haemoglobinopathies (sickle cell anaemia) (2). In our case, the splenic localization of the abscess may be the consequence of bacteremia following abdominal trauma after falling from ladder. Ultrasonography and CT scan are the gold standard for early diagnosis (3-5), while splenectomy is the gold standard for treatment; ultrasound or CT assisted-percuatneus drainage and antimicrobial therapy can be considered as therapeutic alternatives (1, 3, 4). Although splenectomy is the gold standard, in our case, we opted for a minimally invasive radiological method of treatment due to accompanying comorbidities (atrial fibrillation, arterial hypertension and hypothyroidism) along with the impaired general condition of the patient. Performing a surgical intervention under general anesthesia in this case carries a high risk of complications with longer postoperative recovery.

CONCLUSION

Only a few cases of splenic abscess caused by *Salmonella enteritidis* have been described in the literature and they were mostly treated with splenectomy (2, 4, 7) and only in one case with antibiotic treatment alone (6). This case of rare splenic abscess due to *Salmonella enteritidis* following trauma was treated successfully with a combination of percutaneous drainage, prolonged antibiotic therapy, and intensive care (Figure 5).

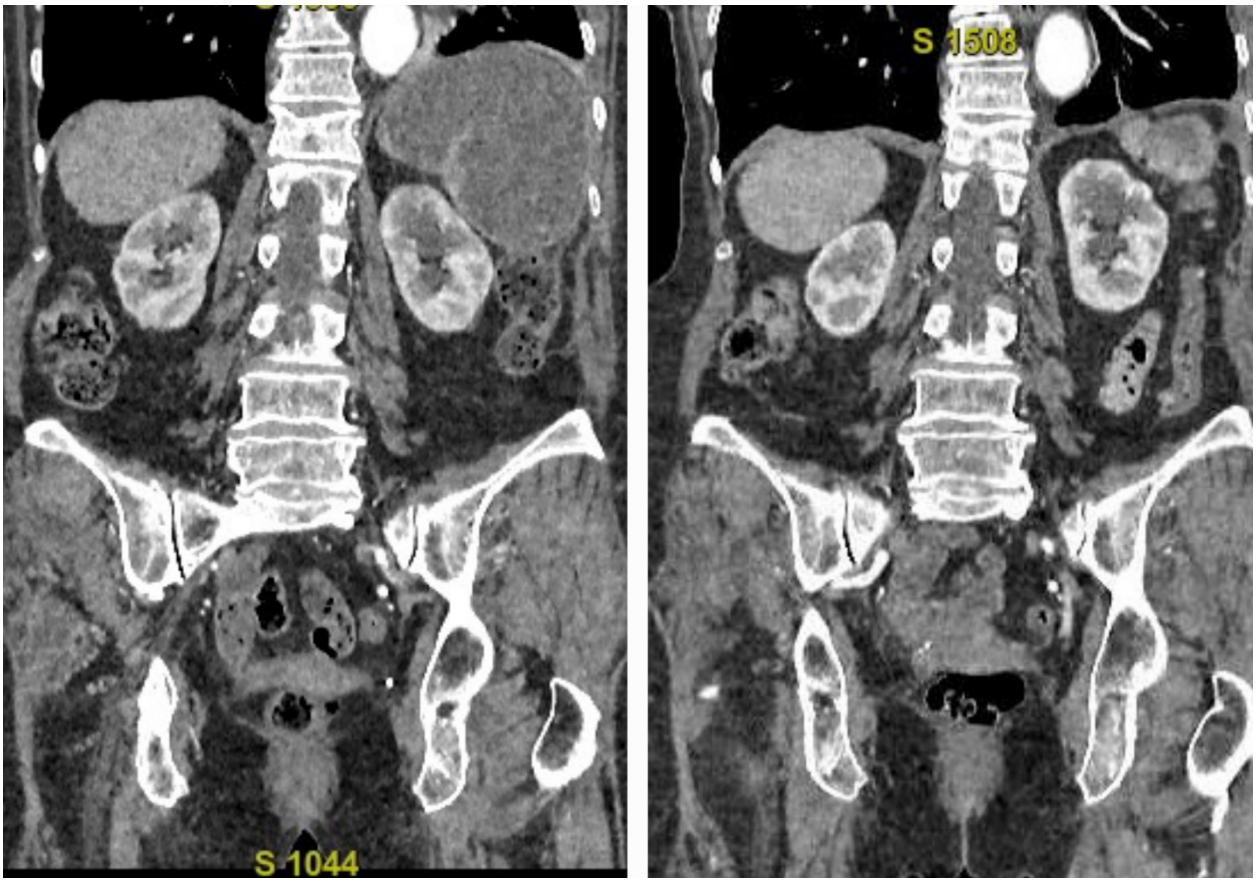

Figure 5. *Multidetector computed tomography—coronal images compared before and after percutaneous drainage of the splenic abscess*

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Apsces slezine izazvan salmonelom enteritidis nakon abdominalne traume izlečen interventnim radiološkim metodama: prikaz slučaja

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SAŽETAK

Uvod. Apsces slezine je veoma retka ekstraintestinalna komplikacija infekcije bakterijom *Salmonella enteritidis* u postantibiotskoj eri, sa incidencijom između 0,14% i 2%. Uglavnom prati bakteriemiju nakon traume, infektivnog endokarditisa, intravenske primene narkotika i imunodeficijencije (AIDS, dijabetes melitus).

Prikaz slučaja. Opisan je slučaj osamdesetdvogodišnje bolesnice sa tegobama u vidu bolova u gornjim delovima abdomena, mučninom i groznicom koji su trajali dve nedelje nakon traume abdomena. Prilikom CT pregleda uočena je hipodenzna lezija dimenzija 110 mm x 80 mm (CCxLL) sa minimalnim perifernim kontrastom, koja je dijagnostikovana kao apsces slezine. Urađena je perkutana aspiracija, koja je bila ehosonografski vođena i pod kontrolom radioskopije, kako bi se uzorkovao sadržaj kolekcije za mikrobiološku analizu, a zatim je obavljena perkutana drenaža. Iz kulture je izolovana *Salmonella enteritidis*, senzitivna na ampicilin, ciprofloksacin i cefalosporine treće generacije. Inicijalno empirijski započeta terapija amikacinom zamenjena je terapijom ceftriaksonom. Bolesnica je posle mesec dana puštena na kućno lečenje. Prilikom praćenja, koje je trajalo šest meseci, nisu zabeleženi recidivni simptomi. Kontrolni CT pregled je pokazao da je slezina normalne veličine, sa tankim zonama niske gustine ispod kapsule koje su bile posledice upale.

Zaključak. U literaturi je opisano samo nekoliko slučajeva apscesa slezine izazvanih salmonelom enteritidis, koji su uglavnom lečeni splenektomijom. U ovom radu je predstavljen slučaj retkog apscesa slezine izazvanog salmonelom enteritidis koji je uspešno izlečen kombinacijom perkutane drenaže, produžene terapije antibioticima i intenzivne nege.

Ključne reči: ***Salmonella enteritidis*, apsces slezine, perkutana drenaža**

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