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REVIEW ARTICLE

EMERGENCY CONDITIONS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a chronic, neurodegenerative disorder that in certain stages can present a series of acute symptoms and signs, the development of which lasts several hours or days.
Emergencies in PD can be a direct consequence of the pathophysiology of the disease or a secondary consequence of the administration of antiparkinsonian drugs. Urgent conditions in PD can also occur due to falls, infectious diseases, after deep brain stimulation or surgical treatment of other accompanying diseases. This article describes the following emergency conditions: parkinsonism-hyperpyrexia syndrome, dyskinesia-hyperpyrexia syndrome, occurrence of acute psychosis and delirium during PD.
Morbidity and mortality in these disorders are a consequence of the inability to make a timely diagnosis and provide appropriate therapeutic treatment. Timely diagnosis and treatment are very important for reducing the mortality and morbidity rates.

Keywords: Parkinson's disease, emergency conditions, delirium, acute psychosis, parkinsonism-hyperpyrexia syndrome, dyskinesia-hyperpyrexia syndrome

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INTRODUCTION

Movement disorders that require urgent therapeutic treatment are presented with a clinical course that takes several hours or days to develop. Morbidity and mortality in these disorders are a consequence of the impossibility of establishing a timely diagnosis and providing the appropriate therapeutic treatment (1).

Parkinson's disease (PD) is a common neurodegenerative disease characterized by the classic motor features of parkinsonism and progressive loss of dopaminergic neurons in the substantia nigra pars compacta. Clinical challenges are the difficulties to accurately diagnose the symptoms at the earliest stage and manage them at later stages (2).

Although PD is a chronic, neurodegenerative, progressive disorder, the affected individuals may have a series of acute symptoms and signs at certain stages of the clinical picture development (3).

Timely diagnosis and treatment are very important to reduce the mortality and morbidity rate. Emergency conditions in PD can be a direct consequence of the pathophysiology of the disease or a secondary consequence of the use of anti-parkinsonian drugs. Urgent conditions in PD can also occur due to falls caused by postural instability or bronchopneumonia and urinary infections. Emergencies in parkinsonian patients may also occur after deep brain stimulation (DBS) or surgical treatment of diseases not directly related to PD (4).

PARKINSONISM-HYPERPYREXIA SYNDROME

Parkinsonism-hyperpyrexia syndrome (PHS) is a rare complication of PD (5) characterized by hyperthermia, autonomic dysfunction, altered consciousness, severe rigidity, and elevated serum creatine kinase levels. PHS can be caused by infections, a decrease in the dose of a dopaminergic drug, or dehydration.

Potentially life-threatening complications include deep vein thrombosis and pulmonary embolism, aspiration pneumonia, and renal failure (6).

PHS is presented by an acute “akinetic attack”, a severe complication, with an incidence of 0.3% and a mortality of 4% (7). The practice of hospitalizing parkinsonian patients to stop levodopa therapy ("levodopa holidays") was abandoned after reports of severe and potentially fatal PHS mimicking neuroleptic malignant syndrome (8). It may follow a sudden change in dopaminergic medication, although there are various other possible precipitating factors. This syndrome was first observed in patients with advanced PD who underwent “levodopa holidays” to try to limit levodopa-induced motor and neuropsychiatric complications (9). After sudden discontinuation of antiparkinsonian therapy, a syndrome of fever, rigidity, autonomic instability, with elevation of creatine kinase in the serum may occur, which is very similar to neuroleptic malignant syndrome. PHS represents a central hypodopaminergic state (10). This syndrome has been described in PD patients who stopped or reduced antiparkinsonian therapy, as well as in patients treated with DBS where the stimulators were inadvertently turned off (11), and even in patients who did not adequately adjust the doses of antiparkinsonian drugs (12).

PHS usually develops over several days, and can follow the changes in dopaminergic treatment or be caused by trauma, surgery and infections of the lungs, gastrointestinal and urinary tracts, although sometimes there is no obvious cause. In severe cases, patients do not respond to the reintroduction of dopaminergic drugs, parkinsonism rapidly worsens, and they become progressively more immobile and rigid (3).

On examination, patients may present with a delirious state with pronounced global slowness and generalized muscle rigidity. They may develop hyperthermia and elevated serum muscle enzymes, following muscle damage due to severe rigidity. In some patients, dysautonomic features develop, such as tachycardia and unstable blood pressure (13).

The main clinical manifestations are hyperthermia, worsening parkinsonism and elevated creatinine kinase. At least two of the following clinical manifestations are necessary for the diagnosis: altered mental status, autonomic dysfunction, hyperhidrosis, myoclonus, rhabdomyolysis, dystonia, and dehydration. Differential diagnosis should exclude the following conditions: neuroleptic malignant syndrome, serotonin syndrome, dyskinesia-hyperpyrexia syndrome, heatstroke, intracranial infection, autoimmune encephalitis, septic shock, drug intoxication, and thyroid crisis. An alternative syndrome should be considered if an expert physician, based on complete clinical manifestations and ancillary assessments, believes that an alternative condition is more likely than PHS (14).Systemic complications can develop as the akinesia progresses rapidly, including aspiration pneumonia due to

decreased level of consciousness and rigidity and acute renal failure due to rhabdomyolysis and dehydration. Deep vein thrombosis, pulmonary thromboembolism or disseminated intravascular coagulation may also occur (3).

Treatment of PHS depends above all on quick recognition. Early diagnosis is essential. In addition to searching for and correcting the underlying cause, levodopa administration is the basis of treatment (15). Patients must be hospitalized in an intensive care unit for careful monitoring of vital signs, control of metabolic disorders, and supportive measures such as antipyretic therapy, intravenous fluid infusion, electrolyte replacement, and prophylactic anticoagulant administration. The treatment is based on the use of levodopa and dopamine agonists. If antiparkinsonian medications are reduced or discontinued, they should be immediately readministered. The dopaminergic agonists ropinirole or pramipexole can be used. We routinely use levodopa by nasogastric tube administration if necessary (16).

Pulse corticosteroid therapy (1 g daily methyl-prednisolone until symptom improvement) may help, but there is limited evidence of its effectiveness (17).

A double-blind, placebo-controlled study of this disorder suggests that 1g of methylprednisolone per day shortens the time to recovery (18).

If PHS is caused by a reduced dose of dopaminergic drugs, the previous dosing regimen should be immediately reestablished and the oral dose of levodopa gradually increased. In patients with swallowing problems, a nasogastric tube can facilitate the administration of dopaminergic medications. If increasing the levodopa dose fails, apomorphine (intermittent injections or continuous infusion), transdermal rotigotine, or intravenous amantadine sulfate should be tried (13). The most refractory cases may be considered for titrated nasogastric levodopa-carbidopa gel infusion. Other treatments such as oral dantrolene sodium are recommended, but there is no strong evidence of efficacy (9). Patients should have routine checks of serum muscle enzymes, kidney function, and coagulation status. The prognosis can be favourable if adequate treatment is started early, although the majority of patients do not return to their previous functional level (9).

Suggested treatment protocol:

1. Treat the underlying triggers immediately.
2. Provide adequate supportive treatments including vital function support, administration of intravenous fluids, and antipyretic medications.
3. Antibiotic treatment is not necessary, but it should be applied if there is an infectious syndrome.
4. When the diagnosis of PHS is confirmed, dopaminergic drugs should be administered immediately, orally or by nasogastric tube.
5. If a delirious state occurs, it should be treated with intravenous benzodiazepine infusion (taken as needed).
6. If the patient develops multiple organ insufficiency, treatment in the intensive care unit and multidisciplinary treatment should be started immediately (14).

DYSKINESIA-HYPERPYREXIA SYNDROME

Acute hyperpyrexia is a common cause of emergency admission of patients with PD (16). When a patient with PD is referred for examination because of acute hyperpyrexia, an infectious condition is considered first (19, 20).

Dyskinesia-hyperpyrexia syndrome (DHS), an acute complication of PD, was first defined as an emergency in 2010 (21), and is often caused by abuse of antiparkinsonian drugs. In addition, there are a number of other factors that provoke DHS (14), a rare but life-threatening condition with the appearance of severe dyskinesias (dyskinetic status), leading to muscle wasting, rhabdomyolysis, hyperthermia, and confusion (22).

This complication shares some of the clinical characteristics of PHS, but differs in dyskinesias that dominate the clinical picture instead of rigidity. DHS, unlike PHS, should be treated by reducing the dose of dopaminergic drugs, especially dopamine agonists (6). If the correct diagnosis is not established, timely and optimal treatment cannot be given. This can lead to severe consequences for patients with these syndromes (22).

The results of a retrospective literature review revealed a total of 56 PHS and 13 DHS cases, and were more likely to occur in older patients with longer duration of PD. These two syndromes showed a different ratio of women to men, similar mortality and different recovery time. There are significant differences between PHS and DHS, including triggers (abrupt discontinuation of antiparkinsonian drugs vs. antiparkinsonian abuse), symptoms (worsened tremor and rigidity vs. persistent dyskinesia), and treatment (reintroduction vs. drug reduction) (14).

Although hyperthermia in DHS is thought to be the result of massive dyskinetic movements (21), it has also been attributed to dysfunction of central thermoregulation (23).

Many pathological processes in PD can lead to abnormal thermoregulation. Autonomic dysfunction, a common non-motor symptom of PD, can lead to abnormal sweating and cooling of the skin at high temperature (24, 25). The release of dopamine in the hypothalamus, which is impaired in patients with PD, can be increased when the temperature rises (26). Autonomic dysfunction and altered metal status are frequently observed in PHS and DHS patients. However, the pathological mechanisms by which PHS and DHS occur in PD remain unclear.

The two most common provoking factors for DHS are a change or misuse of antiparkinson medication. Excessive dopaminergic stimulation is destructive given that PD patients have a dopamine deficiency in the central nervous system. Other DHS triggers include infection, trauma, and gastroin-testinal disturbance. Clinical manifestations of DHS include hyperthermia, persistent dyskinesia, altered mental status, and, to a lesser extent, autonomic dysfunction, hyperhidrosis, dehydration, and rhabdomyolysis. Treatment of DHS includes supporting vital functions, tapering dopaminergic medications, intravenous fluid infusions, and antipyretics. Thirteen cases of DHS have been described, of which two patients died within days due to pneumonia and renal failure or acute pulmonary edema (27). The remaining eleven patients recovered in 2-10 days (14).

Both PHS and DHS tend to occur in older parkinsonian patients with longer disease duration. DHS mainly occurs in women, PHS is predominantly found in men. The recovery rate for both syndromes is around 80% despite faster recovery in DHS than in PHS patients. Apart from PHS and DHS, an elevated level of creatinine kinase can occur in rhabdomyolysis, myositis, myocardial infarction, muscular dystrophy, etc. The diagnosis of rhabdo-myolysis requires not only a high creatinine kinase level but also elevated myohemoglobin in the blood and urine. Therefore, elevated creatinine kinase alone does not mean rhabdomyolysis, which is present only in a small percentage of PHS and DHS cases. However, a major difference between the two syndromes is that worsening tremors and rigidity predominate in PHS, but persistent dyskinesia is found exclusively in DHS patients. PHS may be caused by abrupt discontinuation of antiparkin-sonian therapy, such as drug withdrawal or loss of DBS stimulator power, whereas DHS is likely caused by the abuse of antiparkinsonian drugs. Accordingly, the primary treatment for PHS is a reintroduction of antiparkinsonian drugs, and for DHS, anti-parkinsonian drug reduction. Other adjunctive treatments are basically similar. Therefore, a careful examination of the drug history and appropriate neurological examinations are essential for the rapid recognition and treatment of the syndrome (14).

Reported case fatality rates are 21.4% for PHS and 15.4% for DHS. Among the 14 cases of deceased patients, there is a total of 12 patients older than 50 years and 12 with more than nine years of PD duration. The causes of death and the number are: hyperthermic coma—three patients, respiratory failure—ten patients, renal failure—seven patients, heart failure—three patients, disseminated intravascular coagulation—two patients and septic shock—one patient. These data suggest that patients of an older age and longer duration of the disease may be more susceptible to the development of multisystem organ failure and a fatal outcome. People with multiple organ failure should start treatment in the intensive care unit and multidisciplinary treatment immediately to reduce the potential mortality (14).

ACUTE PSYCHOSIS IN PARKINSON'S DISEASE

Psychosis is relatively common among patients in the advanced stages of PD and is associated with a certain degree of cognitive dysfunction. Acute onset of psychosis is often provoked by the same agents used to treat motor symptoms of PD (levodopa, dopamine agonists, anticholinergics, amantadine, COMT and MAO-B inhibitors). In addition, other comorbidities, such as respiratory and urinary infections, metabolic or other neurological disorders, can be provocative factors for the development of acute psychosis in PD (28, 29).

Psychotic manifestations usually include visual hallucinations, persecutory delusions, confusion, and psychomotor agitation. Psychosis is perhaps the most common reason for PD patients to be hospitalized. The clinical picture of psychosis begins with visual illusions, misidentifications of real visual timuli. Over time, patients may develop hallucinations that are typically visual, often seeing unfamiliar people or animals. Later on, patients may lose the insight that their hallucinations are not real. Fixed illusions are one of the most severe forms of psychosis. Illusions or visual hallucinations with retained insight are rarely problematic, but progression to loss of insight or fixed delusions can quickly put patients at risk. Patients may react

to their visual hallucinations, feel threatened by them, and react in ways that threaten their own safety or the safety of others, leading to emergency room visits and subsequent hospitalization. It is important to recognize that not all patients follow this stereotypical pattern. Some patients may immediately develop psychosis or delusions without progressing through the other stages (15).

After treatment of potential comorbidities, a gradual withdrawal of potentially related medications should be initiated, starting with anticholinergics, then MAO-B inhibitors, dopamine agonists, amantadine, and COMT inhibitors. It is often necessary to introduce second-generation antipsychotics, such as clozapine or quetiapine. Other options include risperidone or olanzapine, with a significant risk of worsening the motor symptoms (6).

Vaughan and Goldman proposed a useful practical algorithm for treating psychosis in PD (30). Precipitating factors such as systemic disease, metabolic disorders, infection (especially urinary tract infection) or subdural hematoma due to falls should be ruled out first. Anticholinergics, amantadine, monoamine oxidase-B inhibitors, catechol-O-methyl-transferase inhibitors and dopamine agonists should be gradually withdrawn from therapy. Most patients with noninsightful hallucinations cannot tolerate such medications and will do better when treated with levodopa as the sole dopaminergic stimulant. Hallucinations without insight or fixed illusions usually require addition of quetiapine or clozapine. Typical neuroleptics and other atypical neuroleptics (risperidone, aripiprazole, ziprasidone) should be avoided, as these drugs may worsen parkinsonian symptoms (15).

DELIRIUM IN PARKINSON'S DISEASE

Delirium is an acute condition characterized by a fluctuating level of consciousness and orientation. Among hospitalized patients, the prevalence ranges between 10% and 20%. Risk factors for deirium include predisposing factors such as older age, male sex, dementia, visual and hearing impairment, alcoholism, hip fractures and metabolic disorders, while immediate precipitating factors include the use of certain drugs, occult infections, surgery, pain, physical limitations, and admission to intensive care (31). The general treatment of delirium is to identify and address the precipitating factors, while antipsychotics can be used to control the agitated patient (32). Parkinson's disease is an independent risk factor for delirium (33). Treatment of delirium in PD is a unique challenge. Antipsychotics are contraindicated for the treatment of delirium in PD because, in addition to exacerbating parkinsonism, they also present a risk of precipitation of neuroleptic malignant syndrome. Short-acting benzodiazepines may be used if control of an agitated patient is required. Among antipsychotics, quetiapine and clozapine are considered relatively safer for use in PD because of their effects on non-D2 receptors (34, 35).

An accurate and timely diagnosis of delirium is especially crucial because it often stems from a comorbidity that may require specific treatment (36). However, its symptoms (including inattention, disorganized thinking, fluctuating symptoms, visual hallucinations) often overlap with other cognitive disorders associated with PD, which may lead to misdiagnosis (37). The absence of a specific definition of delirium in PD or a reliable complementary test further complicates the establishment of the diagnosis. During the progression of PD, concomitant cognitive impairment becomes increasingly pronounced, which is a risk factor for the development of delirium (38, 39).

Despite several changes in criteria over the past few decades, the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) defines delirium as an acute disturbance of attention, awareness, and cognitive performance with fluctuating features over time (40). Two subtypes of delirium are recognized: hyperactive delirium with agitation represents 25% of cases, while hypoactive delirium with reduced level of consciousness represents 75% of cases (41).

Delirium is conceptualized as a cerebral dysfunction caused by various underlying predisposing factors (aging, cognitive impairment, sensory impairment) and acute triggering factors (drugs, electrolyte and glucose metabolism disorders, uremia, surgery) (42). The presence of multiple predisposing factors reduces the number of triggering factors required to cause delirium. The exact molecular pathways that cause different triggers to converge into a similar syndrome are not yet fully understood. Three main mechanisms are believed to be involved in its pathophysiology: 1. Normal brain functioning relies on significant amounts of energy, and brain metabolic insufficiency—either due to lack of oxygen or glucose—can lead to delirium in various scenarios. 2. Inflammation is a known trigger of delirium and several circulating cytokines

found in patients with delirium, which can have significant effects on the nervous system. 3. Several neurotransmitter deficits have been identified including acetylcholine, dopamine, glutamate, GABA, histamine, and noradrenaline, but none (not even acetylcholine, which is believed to have the strongest implication) has been consistently associated with every case (43). This suggests that a wide range of underlying features and triggers can cause a similar syndrome, although certain mechanisms such as acetylcholine deficiency may be more commonly involved. Ultimately, these mechanisms lead to brain network dysfunction in which several structures are implicated in delirium symptoms, including the hippocampus, thalamus, basal forebrain, and cerebellum as their interconnections (44).

The diagnosis of delirium is clinical, and the Confusion Assessment Method (CAM) is the most widely used and reliable screening tool in clinical practice (45). Once the diagnosis is confirmed, additional diagnostic tests such as laboratory testing, brain imaging, and electroencephalography (EEG) can be helpful in uncovering potential treatable triggers. Traditionally, delirium has been considered a transient condition that resolves once the factors causing it are resolved. However, a growing body of evidence indicates that symptoms can last for months in one-third of patients (46, 47).

PD is associated with an increased risk of developing delirium; however, studies in this area vary considerably in the definition of delirium, in the methods used to diagnose delirium and PD, and in the characteristics of the included patients (48). A recent prospective study, using a standardized diagnostic algorithm based on the DSM-5, estimated the prevalence of delirium of 31% among hospitalized PD patients (49). The susceptibility of parkinsonian patients to develop delirium is thought to stem from the convergence of several overlapping mechanisms between these two conditions: a systemic inflammatory response, neurotransmitter imbalance (including disturbances in the cholinergic system), and alpha-synuclein pathology, which is independently associated with postoperative delirium in cases of gastrectomy (50). Delirium is correlated with cognitive impairment in PD, which increases the likelihood of delirium, emphasizing its importance as a key interacting factor (51).

Clinical features of cognitive impairment in PD and delirium show similarities, including attentional dysfunction, cognitive fluctuations, hallucinations, sleep disturbances, and excessive daytime sleepiness, commonly found in both syndromes (48). Of note, assessment tools to detect delirium, such as the CAM, have not been validated for use in PD, which may lead to misdiagnosis of delirium with long-standing PD symptoms (39).

Delirium is a common and serious complication after DBS, which improves motor complications in patients with advanced PD. Advanced age, cognitive decline, and severity of PD may be risk factors for delirium. The presence of delirium may also affect cognitive function and prognosis. Neurotransmitters such as acetylcholine and dopamine may be involved in the occurrence of delirium. Furthermore, inflammation, effects of microlesion of local nuclei, and brain atrophy may also play a role in the development of delirium after DBS (52).

During the evolution of the clinical picture in PB, the acute appearance of urgent conditions is possible, among which the syndrome of parkinsonism—hyperpyrexia, dyskinesia—hyperpyrexia syndrome, acute psychotic state and delirium have been described. The etiopathogenesis of these acute conditions differs, so the diagnostic and therapeutic approaches are different. Early and adequate recognition of these syndromes is very important for patients' survival and recovery. Lifesustaining measures are mandatory in patients affected by these emergencies and hospitalization in intensive care units is necessary. Survival and recovery of patients depend on appropriate therapeutic procedures that differ in these emergency conditions with the necessary team cooperation of medical professionals.

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Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

REVIEW ARTICLE

BIOLOGICAL PROPERTIES OF BUILDING DENTAL MATERIALS AND CLINICAL CHANGES IN ORAL TISSUES CAUSED BY THEIR APPLICATION: A NARRATIVE REVIEW

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Restoring the morphological and functional integrity of damaged or lost teeth and replacing them with suitable materials remains a significant challenge in modern dentistry. A continuous development of new restorative materials aims to improve mechanical properties, aesthetic outcomes, and longevity of dental restorations, while minimizing adverse biological effects. Biocompatibility represents a fundamental requirement of all dental materials, referring to their ability to perform a specific function in the oral environment without eliciting undesirable local or systemic tissue responses.

The oral mucosa, including the lips, is constantly exposed to numerous physical, chemical, and biological agents that may act as irritants or sensitizers. Given that most dental materials are designed for prolonged intraoral use, their continuous contact with oral tissues can influence mucosal integrity and function. Clinical manifestations of adverse reactions vary in severity and presentation, often depending on the material composition, exposure duration, and individual patient sensitivity.

Local tissue reactions associated with dental materials include conditions such as oral stomatitis, mechanical trauma, thermal and chemical burns, toxic effects, and allergic reactions. Accurate diagnosis and identification of the underlying causative factor are essential for selecting appropriate therapeutic measures and preventing complications. The growing demand for aesthetic and durable restorations underscores the importance of continuous evaluation and improvement of material biocompatibility in contemporary dental practice.

Keywords: building dental materials, biocompatibility, oral changes, treatment

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INTRODUCTION

Restoring the morphological and functional condition of the destroyed and lost teeth and their replacement is a challenge for the application of new, more effective dental materials and the improvement of those already on the market (1, 2). Today, different types of materials are being used in dental practice, which can be roughly divided into two groups: building materials (acrylate polymers, dental composites, ceramics, and metal alloys) and auxiliary materials (plaster, waxes, thermoplastic, impression, and refractory materials) (3–5).

Modern materials in dentistry should strive to ensure optimal functionality with maximum aesthetic effect. In addition to being effective, they clearly need to be safe as well. Biocompatibility testing has become an indispensable factor in the evaluation of every new material, as well as those already in use (6, 7). Any material that performs a specific role in the organism is called a biomaterial and is expected to fulfill the postulate of biocompatibility. Biocompatibility is the ability of a material to perform a specific function in the body after application without causing a response from the host tissue. They are, in fact, expected to be non-toxic, non-allergenic, non-carcinogenic, that is, to be chemically and physically stable (7–10).

The biocompatibility of materials used in dentistry is determined by their chemical composition (release of substances through solubility and corrosion), as well as the roughness of the material's surface. An unwanted reaction of the oral tissue can be a consequence of the toxicity of the applied material, but also the accumulation of infectious material (3, 11, 12). The response of the organism to the material presence is a dynamic process, given that the tissues and the organism change with aging or under the influence of disease. Material in contact with tissue causes a host reaction, but the tissue also causes changes in the material. Their mutual influence is a dynamic process, which changes depending on the environment. Although unwanted tissue reactions to the presence of dental materials are rare, a large number of daily dental treatments increases the possibility of their occurrence (10–13). The task of biocompatibility testing of materials used in dentistry is to remove each of their ingredients that could potentially cause damage to the tissues of the oral cavity or damage to the organism in general (7, 8).

Dental materials have the potential, thanks to their surface structure, to accumulate saliva ingredients, food and drink residues, microorganisms from the oral environment, forming a biofilm that is a collector of infectious content. Parameters that describe the surface of the material, being also important for biological reactions, are roughness, wettability, chemical composition of the material, electric charge, crystal structure, and heterogeneity (14). Building dental materials are found in the oral cavity in an aqueous environment, and depending on their chemical and physical properties, they dissolve slightly. The release of substances from materials, through their solubility or corrosion, also determines their biocompatibility. These potentially toxic substances can damage cells and cause inflammation or allergy, which can be detected by the secretion of proinflammatory cytokines or interglobulin (15).

The health of the oral cavity depends on the integrity of the oral mucosa, because only an intact oral epithelium prevents the penetration of microorganisms and other harmful agents through the mucosa. Good vascularization nourishes the epithelium, so that in case of damage, the morphological integrity of the epithelium is quickly restored. The physical characteristics of the oral epithelium, thanks to the abundance of elastic fibers, tissue hydration, turgor, and density of the oral epithelium, are such that they can withstand stronger pressure and stretching (13, 14). The sensitivity of the oral epithelium plays a very important role in protection, due to the presence of receptors for touch, pain, cold and warm sensations. The attachment epithelium of the gingival sulcus also opposes the penetration of microbes with its structure, the presence of cells–phagocytes, immune reactions, etc. Keratinization enables proper maturation of cells, including proper mitoses and passage of cells through all layers of the epithelium, when desquamation of old cells occurs on the surface of the mucosa (10–14).

The host tissue responds to the presence of dental biomaterials as foreign bodies with a local and systemic reaction and inflammation. The local effect of the material on the host tissue can be the interaction of the material and body fluids, inflammation, toxic action, allergy, infection, changes in wound healing and possible carcinogenesis (11, 12). Effects of tissues on materials are adsorption of proteins on the implant, enzymatic action, degradation of materials, such as physical-mechanical wear, corrosion, and breakage. Systemic effects would be allergic reactions, systemic toxic reactions, and changes in organ systems (14, 15).

In dentistry, local reactions to the presence of biomaterials primarily occur on the pulp, periodontal tissue, and oral

mucosa. They are related both to the release of potentially toxic substances and to the reaction of tissues to the presence and action of microorganisms on the material surface (16).

DENTAL MATERIALS THAT CAN CAUSE CHANGES IN ORAL TISSUES

Dental acrylates

Acrylates are transparent materials that can be easily colored and shaped to accurately replicate lost teeth, gingiva, and skin. Based on their composition, acrylate materials are polyesters of acrylic acid (17). Their primary use is in the fabrication and repair of denture bases, artificial teeth, removable orthodontic appliances, obturator prostheses and epithesis, various splints, as well as temporary crowns and bridges. Additionally, they serve as auxiliary materials for making custom impression trays, bite rims, and bite templates. Acrylates are also components of adhesive cements and modified glass ionomer cements (18, 19).

Technological advancements have enabled the enhancement of acrylate properties to better meet professional demands. Special attention is given to improving the biological characteristics of acrylates due to reported adverse reactions in both patients and dental staff, caused by potentially toxic substances released during preparation and use (19, 20).

The biological activity of acrylates can be considered in two aspects: their interaction with tissues and environmental agents, and the release of potentially toxic substances that can cause local and systemic side effects. The interaction of acrylate materials with the environment is largely determined by their surface design, particularly roughness. Irregular surfaces are prone to the accumulation of plaque, pigments, food and drink residues, and decayed oral tissue (20–23). Improving the surface structure of acrylates can reduce microbial accumulation. One suggested modification is incorporating phosphate or carboxyl groups into the PMMA structure. Such copolymers acquire a negatively charged surface, which attracts positively charged antimicrobial proteins in saliva, preventing Candida species adsorption and growth. However, these modifications may reduce the mechanical strength of the acrylate (24, 25).

The biocompatibility of different materials is assessed by their cytotoxicity (impact on cell viability) and cytostaticity (impact on cell proliferation) in laboratory conditions. After polymerization, acrylates release certain amounts of potentially toxic substances into saliva, where they dissolve and act upon oral tissues. Their toxic effects are mainly due to unpolymerized acrylate components, unreacted system ingredients, and polymerization by-products (26). Although these substances are highly toxic in concentrated form, their amount dissolved in saliva is generally very small and depends on the material's ability to release them. Residual monomers (such as MMA, BuMA, EMA, and UDMA) or crosslinkers (EGDMA, IBMA, etc.) are considered the main contributors to acrylate toxicity and allergenicity due to incomplete polymerization. Yoshii et al. reported that BuMA and EMA polymers exhibit greater cytotoxicity compared to PMMA. To address allergies to conventional acrylates, new hypoallergenic variants have been developed where MMA is replaced with diurethane dimethacrylate, polyurethane, polyethylene terephthalate, or polybutylene terephthal-ate (20–27).

In most cases, adverse effects of acrylate materials are acute, localized, and resolve quickly upon the removal of the causative agent. Clinically, they manifest as cheilitis, stomatitis, burning sensations of varying intensity, and candidiasis. More severe allergic reactions, such as erythema multiforme, have been reported in response to acrylate dentures. Rarely, chronic prosthetic stomatitis in the form of fibrous hyperplasia occurs in elderly patients. Very rarely, chronic irritation caused by acrylates may lead to oral cancer development (27–30).

Dental composites

Dental composite materials are a realistic realization of the aspiration to compensate for lost dental tissue so that the anatomical and morphological restoration of teeth is not only functional but also aesthetically acceptable. Composite materials are expected to chemically and micromechanically bond to hard dental tissues, creating a unique morphological and functional unit with the remaining dental substance (31). Dental composites structurally compensate for defects caused by tooth decay, fracture, or erosion, and are used to produce fillings, inlays, onlays, overlays, and aesthetic veneers. In addition, composites are used in dental prosthetic therapy for the cementation of highly esthetic ceramic restorations, and in orthodontics for fixing brackets of orthodontic appliances (32, 33).

The profession has set high demands on composite materials:

mechanical (the material must be strong and hard enough, durable, and stable), aesthetic (absolute similarity to the dental substance in terms of color, transparency, and texture) and biological (the material must not act irritatingly or toxic on the pulp and other oral tissues) (34-37). The quality of the composite restoration and its longevity depend on the physical and mechanical properties of the material used. Desirable material properties are hardness, strength, elasticity, as well as resistance to bending, tearing, torsion, and wear. Mechanical properties define the ability of the composite not to change its shape and volume under the influence of load forces (38).

The analysis of biological characteristics of composite materials goes in two directions, to evaluate the release of different components from these materials and their local or systemic interaction with different tissues. Composites can have a negative effect on the surrounding tissues in two ways: by elution of bioactive molecules, primarily residual monomer, and by temperature rise during the binding process (38, 39). The residual monomer release can lead to pulp damage, mucosal irritation, contact dermatitis, and allergic reactions. The allergic reaction of the oral tissue is most often of a local nature, although the possibility of systemic damage is not excluded. The local tissue reaction to the released components of the composite is most often manifested as epithelial proliferation, such as lichenoid change (40). More significant are the changes that the unbound composite can cause on the dental pulp, which gradually range from hyperemia, through inflammation, to tissue necrosis. Systemic effects are also possible, and the ways of spreading are through the oral mucosa, diffusion into the pulp through the dental tubules, absorption of volatile ingredients in the lungs and ingestion of components that are released in saliva. Therefore, it is necessary to prevent unwanted reactions of the unpolymerized composite by optimal polymerization under clearly defined conditions (41–43).

Dental ceramics

Dental ceramics are the material of choice for all types of fixed dental restorations, whether bonded to metal alloy surfaces through oxide formation (porcelain-fused-to-metal, PFM) or used independently as all-ceramic restorations (44, 45). Dental ceramics are usually called inorganic structures that contain a compound of oxygen with one or more metallic or semi-metallic elements such as aluminum, calcium, lithium magnesium, phosphorus, potassium, silicon, sodium, zirconium, and titanium (46, 47).

To be considered as an optimal material, dental ceramics should have the following criteria: not to have a harmful effect on tissues and the body, to be electrochemically stable and not to corrode, to replace the missing tissues with color and appearance, to be easily and simply processed and shaped, that they are stable and create a chemical bond with the metal (48, 49). Dental ceramics are used for making inlays, onlays, veneers, artificial crowns, bridge structures, and factory extensions. Zirconia and glass ceramics are used in periodontology and oral implantology (50).

The biocompatibility of ceramic systems in dentistry can be reduced by the fact that food residues and microorganisms can accumulate on their surface, which consecutively leads to potential infection of the oral tissues with which they come into contact (51, 52). For the material to be considered biologically acceptable, it is necessary that it has such a surface design that it reacts as little as possible with tissue and agents from the environment. The uneven ceramic surface is a predilection place for the accumulation of biofilm, so it can be considered a favoring factor in the occurrence of periodontal diseases, caries, and infections of the oral mucous membrane. Different types of ceramics exhibit varying potentials for biofilm accumulation, with zirconia ceramics demonstrating the lowest propensity for such deposition (53–57).

Dental alloys

Metals in dentistry have multiple purposes. In dental practice, combinations of metals with other elements, dental alloys, are used, with the aim of favoring the desired characteristics of the obtained materials and reducing or eliminating unfavorable ones (58–60).

Dental alloys are in constant contact with oral tissues, so their biological properties are extremely important. The biological stability of alloys must be taken conditionally, and it depends on the chemical composition of the alloy, the crystal structure and homogeneity of the solid solution, the compactness of the surface layers, and the integrity of the protective film (61, 62). The dental alloys biocompatibility is determined by their ability to adhere to biofilm and decay products of the oral environment in a highly corrosive environment such as the oral cavity (63).

The biological reaction of the organism to dental materials is mainly based on the interaction of substances released from the material and relevant biological molecules. The most important biological characteristic of dental alloys is corrosion resistance, which implies the behavior of the material in a liquid medium (64–67). Over time, metal corrosion increases the surface roughness, as well as biofilm adherence, potentiates the release of ions and weakens the restoration construction. The origin of dental alloys corrosion is chemical or electrochemical. The tendency towards electrochemical corrosion arises from the material electrical potential. Two various metals or alloys, immersed in an electrolyte, form a galvanic battery (68, 69). The consequences of electrochemical corrosion are various and can be pain or unpleasant sensations due to galvanic current, a metallic taste in the mouth and, very rarely, changes in the shape of restorations. Dissolved metal can be deposited on soft tissue, creating aesthetically unacceptable oral tissue "tattoos", with a potential harmful effect on their structure (70–72).

LOCAL TISSUE REACTION TO THE PRESENCE OF DENTAL MATERIALS

Biocompatibility of dental building materials refers to the ability of the material to fulfill the desired function, without any unwanted local or systemic effects in the recipient or user of the therapy. However, clinical practice shows that certain toxic ingredients can lead to unwanted changes of a local or, much less often, systemic character (73, 74). Depending on the percentage of toxic substances, these substances could leave the dental restorations and diffuse into the saliva, with which they act on the oral mucous membrane (75).

The mucous membrane of the oral cavity, including the lips, is constantly exposed to many potentially irritating and sensitizing substances. Exposure to certain dental materials can cause reactions in the oral cavity, ranging from mild to severe (76). Most dental materials are designed for long-term use; consequently, prolonged exposure may affect the oral mucosa, leading to a variety of clinical changes and symptoms. Changes to the mucous membrane disappear quickly if their cause is removed (77, 78).

After placing a dental prosthesis, a large number of bacteria in the oral cavity increases further. Namely, between the dental prosthesis and the jaw, a small space is created in which there are perfect conditions for the development of bacteria. Deposits on dentures, such as bacterial plaque, fungi, calculus, and food residues, can be responsible for the occurrence of prosthetic stomatitis, angular cheilitis, inflammatory fibrous, halitosis, dental caries in partial denture wearers, mucositis and peri-implantitis in wearers of mobile superstructures on implants (77–80).

Inflammation of the oral mucosa–oral stomatitis

Stomatitis is an inflammation of the mucous membrane of the mouth, including the inner surface of the mouth, cheeks, gingiva, tongue, and throat. Stomatitis can be acute, chronic, moderate, or severe. Inflammation of the oral mucosa is often secondary because it arises from traumatic damage caused by foreign substances (81). All chemical, mechanical, and thermal factors that compromise the integrity of the oral mucosal surface contribute to the development of this condition (82).

Reactions in soft oral tissues can be caused by materials used to restore damaged and lost teeth. For now, it is unknown whether the materials themselves have a toxic effect or whether the products of bacterial plaque that accumulate on teeth and restorations have such an effect (83).

Inflammation presents with hyperemia and edema. The condition involves the entire oral mucosa, including the gingiva. Marked erythema is observed, the mucosa is swollen, the tongue is coated, and the lips are enlarged. The gingiva is inflamed, with interdental papillae appearing enlarged and prone to bleeding even with minimal contact (82–84).Salivary secretion is increased, and halitosis is commonly reported. Patients frequently experience a sensation of oral decay or a bitter taste in the mouth. Regional lymph nodes are enlarged and tender on palpation. Mild fever, reduced appetite, and exacerbation of pain during eating or speaking may also be present. Common symptoms of an infection of the oral mucous membrane are burning and pain, along with a disturbance in the sense of taste, dryness, difficulty in swallowing, etc. These symptoms are not easy to interpret, as they are subject to subjective interpretation by patients (84, 85).

ORAL TISSUE DAMAGES

Mechanical damages

Damage of the oral mucosa can be caused by physical, chemical, or thermal factors. Damage caused by the physical forces is a consequence of the force action on the oral tissues. The forces that act can be small forces of long duration or cumulative forces, such as those that lead to the formation of a traumatic ulcer. A traumatic ulcer (ulcus decubitalis) can be caused by the action of unrepaired teeth, food, self-biting, orthodontic appliances, and bad mobile prostheses. The cause of the ulcer is visible and recognizable, and the diagnosis can also be established by taking anamnesis (86).

Materials employed in the treatment of oral mucosal surfaces are becoming increasingly common in patients wearing removable dental prostheses. After prosthetic therapy and rehabilitation with mobile restorations, because of increased pressure of the prosthesis on the oral mucosa, damage in the form of erosions and ulcerations may occur. These changes can also occur when wearing inadequate crowns or denture hooks (87). The changes are manifested by pain, which makes it difficult for the patient to adapt to prosthetic replacement. Clinically, a solitary ulcer is visible, covered with a whitish-yellowish fibrin deposit. In deeper changes, mucosal necrosis with infiltrated and raised edges has been seen. The pain appears on provocation (88).

Thermal damages

The oral mucosa is sensitive to the effects of low and high temperatures, and damage is more common under the effects of high temperatures. Heat damages are the most often localized on the tongue and the palate mucous membrane. Heat damage is most often accidental–it occurs due to the use of hot food and drinks, while in prosthetics it can occur when tissue is printed with heated thermoplastic materials (89). They can also occur due to overheating during direct modeling of restorations in the patient's mouth or when determining interjaw relationships, or excessive heating of instruments. Thermal damage also occurs when the prosthesis is placed directly in the mouth, i.e., during polymerization, when heat is released (90).

Clinically, the condition manifests as a red or whitish painful enanthema, which can undergo desquamation, leading to the formation of erosion. In larger tissue damage, necrosis may occur, and in smaller wounds, it may regress spontaneously within a week. The changes in the tissues depend on the length of the action and the temperature (91).

Damages caused by thermal factors are in the form of burns, which can be of the first, second and third degree. First-degree burns are painful, mild injuries that manifest as smooth surfaces. Second degree burns are more severe damage. Redness and edema are more pronounced, and vesicles or bullae may also appear. After spraying bullae, painful eroded surfaces remain. Third-degree burns are very severe damage, which also affect the deeper oral soft tissues. Necrosis may also occur in places of damage. Healing is slow, and after healing, scars remain (90–92).

Chemical damages

Chemical damage to the oral mucosa is caused by the local application of caustic agents or by rinsing the mouth with some liquids in a larger quantity or higher concentration, as well as by treatment in dental practice. The most common localization is on the mucous membrane of the cheek and gingiva (93, 94).

Restorative dental materials, antiseptic substances and endodontic substances are the most common causative agents that are used daily by dentists during routine treatment. Among all dental materials, those in liquid state most often cause chemical damage, because they are difficult to manipulate. Some mouthwashes (if they contain alcohol) can damage the mucous membrane (chlorhexidine, hydrogen peroxide, etc.) (95). The drugs (cocaine, amphetamine) can cause oral lesions. Chemicals are a rare cause of damage to the oral mucosa compared to other damages. Acidic and alkaline substances and their salts cause severe damage to the membrane of the oral mucosa, acting through various pathological mechanisms. Acids cause coagulation necrosis, forming a scar that limits penetration into deeper layers (95–97). Alkaline substances cause damage by liquefaction necrosis, which leads to erosion that progressively spreads. A higher concentration of substances and their longer action lead to greater tissue damage (98).

The clinical condition depends on the composition and concentration of the chemical agent. In the oral cavity, chemical substances can cause various erosive lesions, which range from simple desquamation to complete destruction of the oral mucosa with marked spread through

the basal membrane into the submucosa (97, 98). The wounds are usually irregular in shape, whitish in color and covered with pseudomembranes. The lesions are painful. The affected mucous membrane is covered with a whitish membrane which is the result of necrosis. Necrotic epithelium easily peels off, leaving a red surface that bleeds easily. The diagnosis is made based on clinical appearance and history (97–99).

Oral tissue damages by electric power

Different materials in the mouth, under cer-tain conditions, can lead to complications by generating power, which has no physical electrical source but can endanger the oral tissue. This type of power is called galvanic power–battery, and it belongs to damages by electric power, but of a milder intensity, which occurs due to the incompatibility of some metals in the alloy itself. It is created in the mouth during prosthetic treatment of patients. The generation and intensity of the power depends on the potential and distance of the metal, as well as on the electrochemical reaction of the saliva. The production of electricity is higher if the metal surface of the prosthetic restorations is less polished (78, 79).

Galvanism occurs in the oral cavity when the patient has two or more alloys that interfere with each other and lead to the appearance of symptoms of primary or secondary burning mouth syndrome, because the generated power leads to the appearance of sensations in the form of burning, numbness, bitterness, and similar reactions. It is accompanied by a feeling of metallic taste and increased secretion. There are usually no objective changes on the oral mucosa. This can be explained by the fact that the generated power is of very mild intensity, so that it is not able to cause inflammation or lesion of the epithelium. Pigmented spots may appear on the places of prosthetic metal restorations. Long-term irritation of the oral epithelium caused by a galvanic power of lower intensity can cause increased keratinization of the epithelium. The mucous membrane in those places becomes hard, dry and changes color, becoming white. In this way, true leukoplakia can also occur (100).

Oral tissue damages by toxic materials

There are countless substances that have a toxic effect and cause damage to oral tissues. The most common toxic substances that cause oral damage are heavy metals (mercury, lead, thallium) and metalloids (arsenic, antimony, bismuth) (101). Toxic damage to oral tissues can be caused by phosphorus, copper, silver, iodine, and others. Toxic substances enter the oral cavity: directly, via hematogenous route and saliva. Toxic oral damage depends on the type and nature of the toxic substance, but also on a whole range of local and general factors. The occurrence and severity of poisoning is also influenced by the general state of health, age, diet, sanitary culture, etc (102, 103).

Building dental materials are found in the oral cavity in an aqueous environment, and depending on their chemical and physical properties, they dissolve slightly, but still. These potentially toxic substances can damage cells and cause inflammation or allergy, which can be detected by the secretion of proinflammatory cytokines or interglobulin (104). The most used alloy is titanium, followed by gold and chrome-cobalt alloy, as well as ceramic materials. Composites that are in direct contact with fibroblasts can also have a cytotoxic effect. As for acrylates, the potential cytotoxic effect can be explained by direct contact between the material and host cells. In this case, the cellular reaction can not only be reflected by the tissue's cytotoxic response, but its strength also depends on the characteristics of the material's surface. Cold polymerization of acrylic denture bases shows a greater toxic effect on the cellular structure when compared to heat and light polymerization of acrylate denture bases (102, 103). The cytotoxic effect can also occur due to non-polymerized components and released ions from some metals (nickel, palladium) that can act on soft tissue cells (epithelial, cystoblasts, and macrophages).

The surface roughness of the material increases the degree of inflammation. If the chemical or electrochemical corrosion is greater, the symptoms of the disorder may be stronger. The pain may occur, as well as unpleasant sensations of gal-vanic power, a metallic taste in the mouth and, very rarely, changes in the shape of the restoration itself. Bad aesthetic tattoos of oral tissues can sometimes occur, due to the deposition of metals in soft tissues. In most cases, primary chronic inflammation can occur during the toxic effect of the material, and depending on the material, it can be minimal, and in some cases even moderate (101–105).

Allergic reactions in the oral cavity to dental materials

Allergy (allos-different, ergon-reaction) is a reaction between an allergen and an antibody. It is rare but not impossible for an allergic reaction on the oral mucosa to occur because of the presence of materials from which the prosthetic plate is made, the use of other restorative materials, mouthwashes, for maintaining oral and prosthetic hygiene, etc. (106).

There is often an allergic reaction to prosthetic materials such as acrylates (methacrylates). In such cases, an allergic reaction to acrylates occurs due to the residual methyl methacrylate monomer. The patients feel itching, burning, and if the epithelium is damaged, there is also pain, which intensifies when eating and speaking. If the swelling affects the pharynx, especially the larynx, swallowing and breathing problems may occur (106, 107). Patients experience unpleasant breath because oral hygiene is difficult or absent. Allergy to prosthetic materials develops after inserting or fixing dental restorations made of metal, acrylic and other materials. Hypersensitivity to certain prosthetic materials is characterized by the development of a certain type of allergic reaction.

Allergic stomatitis is characterized by polymorphic clinical manifestations, the nature and extent of which are determined by the degree of sensitization. Changes can affect only certain parts of the oral cavity or all its parts. Changes in the oral mucosa are manifested in the form of allergic contact stomatitis, swelling, enanthema, erosions, blisters, or ulcerations. It is often observed in the presence of metal structure of prostheses, cement, application of toothpastes and mouth rinses, paints, and other materials used in dentistry (108). These materials are steel, cobalt, silver, gold, nickel, manganese, chrome, zinc, amalgam, and others. If we talk about products made of precious metals (gold, silver, platinum), they rarely cause an allergic reaction (107).

Allergic cheilitis can occur because of contact allergy with materials used for prosthetic restoration. The mucous membrane can become sensitive, with the appearance of inflammation, most often to dental materials and devices, such as metal and acrylate fillings, crowns, and prostheses. Cheilitis is characterized by enanthema and edema of the lips, more frequently involving the upper lip. The condition typically develops acutely and is associated with prominent subjective symptoms, including a sensation of lip tightness, pruritus, and impaired mobility of the affected region. Vesicles and bullae may appear, the spraying of which creates eroded lesions, often accompanied by severe pain. After such changes, crusts can also form (78, 79, 109).

Oral lichenoid reactions are conditions with a clearly identified etiology. The most common causative factors are hypersensitivity to dental restorative materials, amalgam, composites, dental acrylates, metals, and accumulated dental plaque. They are caused by the appearance of a contact hypersensitivity lesion and can be diagnosed with a patch test, which shows positive results. Clinical manifestations and history are of particular importance for diagnosis. The reaction to dental materials is a type IV delayed hypersensitivity reaction (110). The lichenoid reaction consists of slightly raised white lines that create islands on the oral mucous membrane. Clinically, they appear as white or enanthematous lesions, usually associated with delicate peripheral white striae. Sometimes there are ulcers in their middle. Erosions like lichen planus erosions often occur. The lesions are localized at the point of contact with the materials applied in the oral cavity. It is characteristic that the lesions occur strictly on the cheek mucous membrane, which is in direct contact with the restorative material, while there are none on the opposite side. They appear as unilateral lesions. The diagnosis is usually made only based on clinical examination (79, 111).

THERAPY OF ORAL TISSUE CHANGES CAUSED BY DENTAL MATERIALS

Stomatitis

A well-established diagnosis is a prerequisite for good therapy and the absence of complications. Treatment of stomatitis depends on the cause. The oral mucous membrane inflammation is treated with antibiotics, local antifungals, removal of all local irritations and rehabilitation of the oral cavity, with frequent local application of antiseptic drugs (76).

Mechanical damage

In these cases, with the application of antiseptics, it is crucial to adapt the prosthesis, if possible. Otherwise, it is recommended to create a new one. Treatment can be carried out by removing the causative factors. A decubitus ulcer heals in 7 to 14 days. If the change does not regress

during this period, an ulcer caused by cancer, tuberculosis or syphilis should be ruled out (77, 112).

Thermal damage

For minor damage, no therapy is required, or laser therapy can be used. In severe cases, preventive antibiotics are recommended, as well as local antiseptics (78).

Chemical damage

The best treatment for chemical damage to the oral cavity is prevention. Superficial damage heals very quickly, in a week or two. In the case of larger damage, good results are given by the local application of corticosteroids. Surgical therapeutic procedures and antibiotics are used in rare cases. Inflammation of the mucous membrane is treated with antibiotics, removing all local irritations, and rehabilitating the oral cavity, with frequent local application of antiseptic drugs (97, 98, 113).

Electrical damage

These damages can be avoided if the same material is used during prosthetic treatment, and the generation of galvanic power is excluded. During repeated prosthetic care, in which material of the same potential is used, all changes are completely lost, and the mucous membrane takes on a normal appearance. In this case, it is very important to choose materials for prosthetics, as well as for implantology, including timely removal of defects and replacement of old prostheses (80).

Toxic damage

Inflammation of the oral mucous membrane is treated with antibiotics, removing all local irritations, and rehabilitating the oral cavity. In the case of larger damage, good results are given by the local application of corticosteroids (104, 105).

Allergy therapy

The therapy of allergies manifested on oral tissues is complex and complicated. The basic approach to treatment is exclusion of allergens. Other therapy is symptomatic, such as coating the lips with antihistamines, less often with corticosteroids, with the aim of alleviating the patient's subjective complaints. In severe cases, but rarely, corticosteroids are used parenterally. The solution to metal allergy is the production of metal-free restorations. Metal-free ceramic crowns have a base of zirconium oxide instead of metal. They are characterized by the absence of allergic reactions and exceptional aesthetics (106–109).

Lichenoid reactions

Therapy is based on replacement of restorative material, as well as polishing and smoothing of fixed materials, as well as good oral hygiene. Sometimes the success of the therapy can be achieved with the local application of steroids. Replacing materials that cause a reaction with hypoallergenic materials leads to a state of complete healing and the possibility of further monitoring of clinical symptoms, which can be more easily controlled (110, 111, 114).

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Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

REVIEW ARTICLE

ACUTE EFFECTS OF VARIOUS EXERCISE MODALITIES ON GLYCEMIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES: A SYSTEMATIC REVIEW

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Type 2 diabetes mellitus (T2DM) is characterized by impaired glycemic control, which increases the risk of cardiovascular and metabolic complications. Exercise is a key non-pharmacological intervention known to improve blood glucose regulation, but the acute effects of different exercise modalities on glycemic control in T2DM remain unclear. The aim of this systematic review was to critically analyze and synthesize the existing body of research on the acute effects of various exercise modalities on glycemic control in patients with T2DM.

This systematic review included studies involving adults (≥ 18 years) with T2DM where structured exercise program is the primary or significant intervention, assessing outcomes related to glycemic control (HbA1c, and fasting glucose). A comprehensive search was conducted across two electronic databases (Web of Science and PubMed) using structured search terms like "acute", "exercise", "type 2 diabetes", and "glycemic control". Study selection involved two independent reviewers screening articles, with disagreements resolved through discussion or third-party consultation, followed by detailed data extraction on study characteristics, intervention details, and outcomes.

Ten studies were identified that met all inclusion criteria. This systematic review highlights that moderate–intensity continuous training and high-intensity interval training have positive acute effects on glycemic control in individuals with T2DM.

These findings suggest that both modalities are effective non-pharmacological strategies for optimizing glycemic control in patients T2DM.

Keywords: exercise, fitness, glucose, diabetes mellitus

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INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is projected to rise dramatically, with an estimated 642 million individuals affected by 2040, which will present significant social and economic challenges (1) Furthermore, despite pharmacological treatments, previous research (2) indicates that a majority of T2DM individuals continue to experience prolonged periods of hyperglycemia throughout the day. Consequently, these patients remain at elevated risk for diabetes-related complications and cardiovascular disease due to persistent hyperglycemia and elevated postprandial glucose levels. Thus, the development of effective and accessible interventions is essential for mitigating diabetes-related complications and improving the life expectancy of individuals with T2DM.

Exercise is one of the most effective non-pharmacological methods for the prevention and treatment of T2DM, due to its benefits on long-term glycemic control and other clinically significant cardiometabolic parameters, such as body composition, cardiorespiratory fitness, and blood pressure. However, previous studies (3, 4) have predominantly utilized glycated hemoglobin (HbA1C) as the primary indicator of glycemic control. HbA1C is a marker used to assess long-term glycemic regulation, with evidence suggesting that a minimum of 12 weeks is required to observe significant improvements. Nevertheless, substantial variability in findings has been observed when evaluating this parameter in relation to sex, ethnicity, or health status (5). Moreover, HbA1C does not provide information on acute glucose fluctuations associated with stress, immediate dietary intake, or exercise (6). Conversely, evidence (7) suggests that acute daily exercise interventions may have a more pronounced effect on enhancing long-term glycemic control compared to the chronic adaptations achieved through various exercise modalities. Given that other parameters of glycemic control, such as 24-h mean glucose levels, postprandial glucose and time spent in hypergly-cemia (%) are associated with an increased risk of additional complications (8, 9) and subsequent alterations in HbA1C; it is imperative to analyze how acute exercise influences these markers.

Nevertheless, there remains the ongoing debate regarding which exercise modality is most effective and well-tolerated for individuals with T2DM. A single session of resistance training (RT) (10) and moderate-intensity continuous training (MICT) (11) have been shown to improve glucose uptake, with effects persisting for up to 48 hours post-exercise (12). Moreover, previous research (13–15) indicates that glycemic control and glucose utili-zation are more closely associated with exercise intensity, challenging the traditional view that stadard exercise regimens serve as the “gold standard” for optimizing glycemic outcomes. Thus, a com-prehensive understanding of these acute exercise effects may provide valuable insights for optimizing exercise prescriptions aimed at reducing cardiometabolic risk and improving overall glycemic stability in patients with T2DM. Therefore, the aim of this systematic review was to examine the effects of acute exercise on continuous glucose monitoring (CGM) outcomes in T2DM, with a primary focus on 24-h mean glucose levels, postprandial glucose levels, and time spent in hyperglycemia.

METHODS

Data source and search strategy

Two major electronic databases, Web of Science and MEDLINE (accessed via PubMed), were searched from 2014 to Sep 20, 2024. The search was limited to the past ten years to capture the most recent advancements in the field of acute effects of different exercise modalities on glycemic control in T2DM patients. The key terms selected for the search strategy were associated with exercise, T2DM, and CGM. The search included the following keywords: „type 2 diabetes”, “diabetes mellitus type 2” “T2DM”, “glycemic control”, “glycemia”, “blood glucose”, “continuous glucose monitoring system”, “CGM”, “24-h mean glucose levels”, “ acute effects” “exercise”, “MICT”, “HIIT”, “resistance training”, “hyperglycemia”, “time spent in hyperglycemia”, “postprandial glucose”, and “postprandial glycemia”. These terms were utilized to capture relevant literature on glycemic regulation and exercise interventions in T2DM patients, focusing on studies that monitor glucose levels through CGM technology.

Eligibility criteria

Two different authors (A.L and N.T.) independently assessed the eligibility criteria of the selected studies. The following inclusion criteria were based on the PICO strategy (P–participants, I–intervention, C–comparison, O–outcome): (1) Adult patients (18-65) diagnosed with T2DM free of complications and any other major health issue or disease

(e.g., cardiovascular diseases, cancer); (2) We considered both randomized and non-randomized studies published in peer-reviewed journals with a Journal Citation Reports Index; studies published in English; (3) Studies with a duration of ≤ 2 weeks were included if they investigated the acute effects of different exercise modalities (RT, MICT, HIIT) specified by frequency, intensity, type, and duration; (4) Comparison group including no exercise condition or different exercise modality; 5) Outcome measures included at least one CGM glycemic control parameter (24-h mean glucose, postprandial glucose levels and/or time spent in hyperglicemia).

Studies that met any of the following criteria were excluded: studies including athletes, recreationally active population or clinical populations diagnosed with any other chronic disease (i.e., cardiovascular diseases, cancer), long-term (> 2 weeks) investigating the effects of different exercise modalities on glycemic control; studies lacking defined exercise intensity, and ineligible publication types (e.g., reviews, editorials, letters, commentaries, unpublished studies, guidelines, cross-sectional or case reports studies) (Table 1).

Table 1. *Eligibility criteria*

	Inclusion criteria	Exclusion criteria
Population	1. Patients with T2DM aged 18-65.	1. Healthy population; 2. Professional and recreational athletes; 3. Patients with T2DM bellow 18 years old and above 65 years old.
Intervention	1. Exercise interventions (RT, MICT, HIIT) that lasted ≤ 2 weeks.	MICT, HIIT, SIT, structured training programs; Acute studies.
Comparator	Control group and/or different exercise modality.	
Outcome	Glycemic control (24-h mean glucose, postprandial glucose levels and time spent in hyperglicemia).	No data on at least for one outcome

Data collection and extraction process

An EndNote library was created for data collection (Clarivate Analytics, New York, NY, USA). The extracted data from the included were: (1) study (first author's surname and year of publication); (2) sample size (male/female); (3) groups; (4) characteristics of the study sample, i.e., age (expressed as mean and standard deviation or range), diabetes du-ration (expressed as mean and standard deviation or range), medication; (5) study design; (6) intervention characteristics (acitivity description, intensity and type of activity; (7) outcomes: glycemic control (i.e., 24–h mean glucose, postprandial glucose levels, time spent in hyperglicemia (%). When data were graphically presented, we extracted data using WebPlotDigitizer online software. Data collection and extraction were independently double-checked by two authors (A.L. and N.T.).

RESULTS

Study selection

The initial database search identified a total of 4,730 articles. After the removal of 1,760 duplicates and the exclusion of 2,685 studies deemed irrelevant based on their titles and abstracts, 285 studies remained for further evaluation. Upon thorough screening of titles and abstracts, an additional 218 studies were excluded, resulting in 67 studies that pro-gressed to full-text assessment. During the detailed review phase, 57 studies were excluded for the following reasons: ineligible study sample (n = 19), ineligible eligible study design (n = 11) and ineligible intervention (n = 27). The selection process is depicted in Figure 1. Finally, 10 studies were included in the final systematic review and meta-analysis (Figure 1).

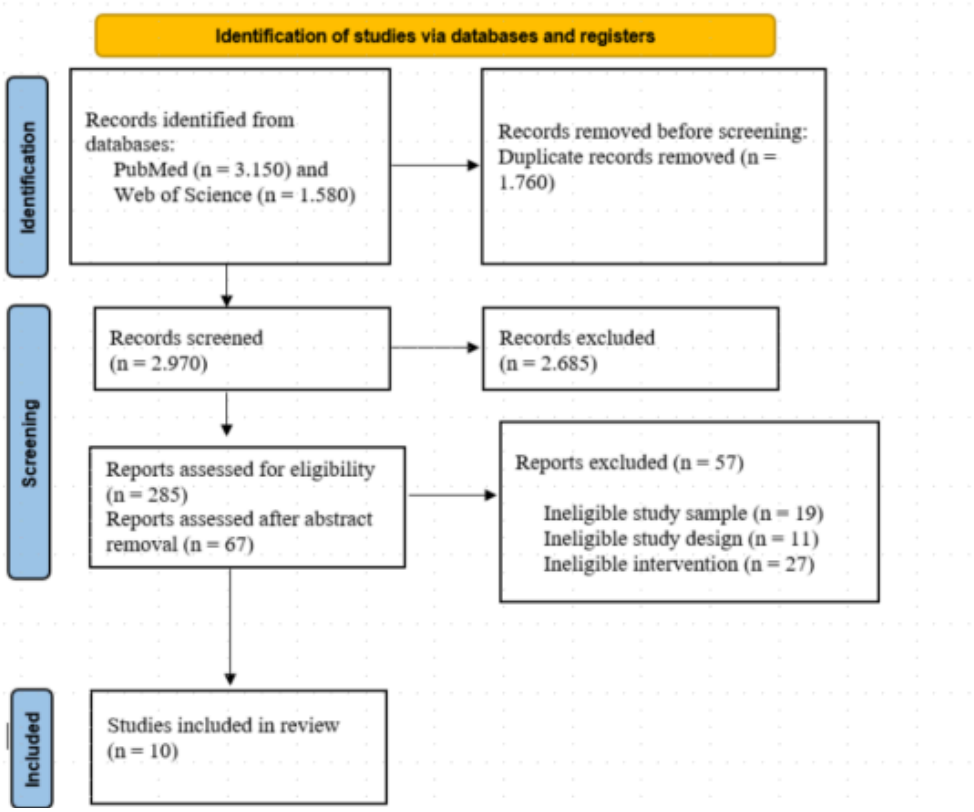

Figure 1. *The updated guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flowchart of search and eligible study selection*

Sample characteristics

Total sample included in this systematic re-view consisted of 194 participants with T2DM. Among the total number of participants, 62 were female. The number of participants ranged from seven (16) to 73 participants (17). All participants were on hypoglycemic oral therapy, while in three studies (18-20), it was reported that participants were also undergoing insulin therapy (Table 2).

Intervention characteristics

All experimental interventions were designed to investigate the acute effects of aerobic training modalities, specifically MICT and HIIT on glycemic control. The predominant forms of exercise included cycle ergometry and treadmill running. A variety of parameters were utilized to quantify exercise intensity (e.g., Wmax, HRmax, HRR) resulting in challenges when defining a specific intensity range. The highest intensity recorded was "all-out" (21) and 95% HRmax (22) (Table 3).

Table 2. *Characteristics of included studies and participants*

Study	Sample size (M/F)	Groups (n)	Characteristics of the study sample		
			Age (y)	Diabetes duration (y)	Medication
Gillen et al. (16)	7	HIIT CONT	62 ± 3	< 1	Oral medication
Van Dijk (18)	30 (30)	MICT CONT	60. ± 6	8.7 ± 7.5	Insulin therapy and oral medication
Terada et al. (23)	10 (8/2)	$HIIT_{fast}$ $HIIT_{fed}$ $MICT_{fast}$ $MICTf_{ed}$ CONT	40 – 75	NA	Oral medication
Erickson et al. (24)	8 (5/3)	NA	60 ± 10.7	NA	Oral medication
Li et al. (25)	29 (22/7)	NA	51 ± 11.2	5.7 ± 6.0	Oral medication
Metcalfe et al. (21)	11 (M)	HIIT (11) REHIT (11) MICT (11) CONT (11)	52 ±6	4 ±3	Oral medication
Rees et al. (17)	73 (33/40)	NA	63.5 ± 9.1	9.5 ± 6.0	Oral medication
Iida et al. (19)	11 (7/4)	MICT (11) CONT (11)	63.9 ± 15.4	NA	Insulin therapy and oral medication
Zhang et al. (20)	15 (9/6)	WG (15) JG (15) CONT (15)	54.7 ± 5.8	5.3 ± 4.4	Insulin therapy and oral medication
Marcotte-Chénard et al. (22)	14 (NA)	$HIIT_{4x4}$ (14) $HIIT_{10x1}$ (14) CONT (14)	69.9 ± 4.3	10.2 ± 6.4	Oral medication

Legend: HIIT — high-intensity interval training; MICT — moderate continuous training; CONT — control group; HIIT fast — high-intensity interval training in fasted state; HIIT fed — HIIT in fed state; REHIIT – reduced — exertion high intensity interval training; WG — walking group; JG — jogging group; NA — non applicable; HIIT 4x4 — high volume high intensity interval training; HIIT 10x1 — low volume high-intensity interval training

Outcomes

Mean 24–h mean glucose levels were reported in eight studies (16–19, 21–24). Moreover, outcome measures for glycemic control were obtained for postprandial glucose levels from seven studies (16, 17, 19, 20, 23–25) and time spent in from four studies (16, 18, 21, 22) (Table 3).

DISCUSSION

The main finding of the systematic review was that different exercise modalities have an acute impact on improving overall glycemic control in patients with T2DM by reducing 24–h mean glucose levels, decreasing postprandial glucose levels, and lowering the time spent in hyperglycemia.

24–h mean glucose levels

Our review revealed that various exercise modalities positively affect the reduction of 24–hour glucose levels. Our findings are consistent with previous meta-analyses (26, 27) which reported significant reductions in 24–hour mean glucose levels by 5 mmol/L (26) and 8 mmol/L (27) following exercise, respectively. Moreover, the analysis suggests that HIIT may be more effective in reducing glycemic concentrations compared to MICT. Some authors (18, 28) attribute this effect to the relationship between 24–h glucose levels and HbA1C, indicating that moderate-intensity activities might have a greater impact on long-term glycemic control. Additionally, 24–h glucose concentrations are influenced by various factors such as sex (27) and baseline glycemic control (18, 27). It has been emphasized

Table 3. *Characteristics of exercise interventions*

Study	Study design	Characteristics of the experimental interventions		Outcomes		
		Intervention characteristics	Type	Mean 24–h glucose	PPG	Time spent in hyperglycemia (%)
Gillen et al (16)	Acute crossover	HIIT – 10 x 60 s (85% HR_{max}) CONT – No exercise	Cycling/cycle ergometer	↔	↓*	↓*
Van Diijk et al. (18)	Acute crossover	MICT – 2 x 30 min (40 W_{max}) CONT – no exercise	Cycling/cycle ergometer	↓ (MICT) ↔ (CONT)		↓* (MICT) ↔ (CONT)
Terada et al (23)	Acute crossover	HIIT – 15 x 3 (100% VO_{2peak}) MICT – 45 min (55% VO_{2peak}) CONT – no exercise	Walking/ treadmill	↓* ($HIIT_{fast}$) ↓* ($HIIT_{fed}$) ↔ ($MICT_{fast}$) ↔ ($MICT_{feed}$) ↔ (CONT) ($HIIT_{fast}$ vs. $HIIT_{feed}$) ↓* ($HIIT_{fast}$)	↓* ($HIIT_{fast}$) ↓* ($HIIT_{fed}$) ↓* ($MICT_{fast}$) ↓* ($MICT_{feed}$) ↔ (CONT) ($HIIT_{fast}$ vs. $HIIT_{feed}$ vs $MICT_{fast}$ vs. $MICT_{feed}$ ↓* ($HIIT_{fast}$)	NA
Erickson et al. (24)	Acute crossover	3 x 10 min (50% VO_{2peak})	Walking/ treadmill	↓*	↓*	NA
Li et al. (25)	Acute crossover	MICT – 20 min (40% HRR) CONT – no exercise	Walking/ treadmill	NA	↓* (MICT) ↔ (CONT)	NA
Metcalfe et al. (21)	Acute crossover	HIIT – 10 x 60 s (85% W_{max}) REHIIT - 10 min with 2 all-out sprints MICT - 30 min at an intensity equivalent to 50% of W_{max} CONT – no exercise	Cycling	↔ (HIIT) REHIT ↓* (MICT) ↔ (CONT)	NA	HIIT ↓* REHIT ↓* MICT ↓* CONT ↔
Rees et al. (17)	Acute crossover	MICT – 50 min at 5 km/h CONT – no exercise	Walking/ treadmill	↓* (MICT) ↔ (CONT)	↔ (MICT) ↔ (CONT)	NA
Iida et al. (19)	Acute crossover	MICT – 3 x (15 min) (40% HR_{max}) CONT – no exercise	Walking/ treadmill	↓* (MICT) ↔ (CONT)	↓* (MICT) (↔) CONT	NA
Zhang et al. (21)	Acute crossover	WG – 2 km at 4-4.5 km/h JG – 2 km at 5-6 km/h CONT – no exercise	Walking, jogging/ treadmill	NA	↓* (WG) ↓* (JG) ↔ (CONT) ↓* (WG vs. JG) (JG)	NA
Marcotte-Chénard et al. (22)	Acute crossover	$HIIT_{4x4}$ – 4 x 4 min (90% HR_{max}) $HIIT_{10x1}$ – 10 x 1 min (90% HR_{max}) CONT – no exercise	Cycling/cycle ergometer	↔ ($HIIT_{4x4}$) ↔ ($HIIT_{10x1}$) ↔(CONT)		↔ ($HIIT_{4x4}$) ↓* ($HIIT_{10x1}$) ↔ (CONT)

Legend: HIIT – high-intensity interval training; MICT – moderate continuous training; CONT – control group; HIITfast – high-intensity interval training in fasted state; HIITfed – HIIT in fed state; REHIIT – reduced – exertion high intensity interval training; WG – walking group; JG – jogging group; HRmax – maximal heart rate; Wmax – maximum power output; VO2peak - peak oxygen uptake; HIIT4x4 — high volume high intensity interval training; HIIT10x1 — low volume high-intensity interval training; HRR – heart rate reserve; PPG – postprandial glucose levels; ⬆*- significant improvement; ⬇*- significant reduction; ↔ - unchanged; NA – non-applicable

that sex is a significant predictor, with greater improvements typically observed in male participants (27). This difference may be explained by physiological mechanisms, such as increased insulin sensitivity (29) and post-exercise glucose metabolism (30) in males compared to females. Finally, when considering individual studies that measured 24–h mean glucose levels and included both male and female participants, general improvements were observed across genders. Thus, although several individual studies in this review have reported overall improvements in 24-h mean glucose levels across both male and female participants, none have specifically stratified these outcomes by sex or thoroughly examined sex-specific physiological responses. This lack of targeted analysis creates a gap in the literature, hindering a comprehensive understanding of the underlying mechanisms that may account for sex-specific differences in the response to exercise. Finally, participants with higher baseline glucose values tend to show more substantial improvements in 24-h mean glucose levels, while participants with well-controlled conditions may experience minimal or no benefit from exercise training (18). Postprandial glucose levels constitute an independent risk factor for cardiovascular disease (31). Notably, impaired postprandial glucose regulation has been linked to elevated oxidative stress (32), upregulated expression of proinflammatory markers (33), and endothelial dysfunction (8). Therefore, our review demonstrated a reduction in postprandial glucose following exercise interventions when compared to the control group in individuals with T2DM. These findings suggest that exercise, irrespective of modality, is an effective strategy for improving postprandial glycemic control in T2DM patients. Despite a limited number of individual studies exploring the effects of exercise on postprandial glucose in this population, our results are consistent with previous research (26) that indicates the beneficial impact of exercise on postprandial glucose regulation. However, postprandial glucose levels are influenced by numerous factors, with timing being one of the most crucial. It has been demonstrated that engaging in an exercise session following a meal is superior in reducing postprandial glucose levels compared to exercising before a meal. Another significant factor is the duration of the activity. Finally, postprandial glucose levels largely depend on the type of the activity performed. Specifically, it was demonstrated that aerobic exercises such as walking, running, or cycling lead to more pronounced changes in this parameter due to the predominant engagement of lower body musculature. This suggests that activating the upper body muscles offers little to no benefit in terms of improving postprandial glucose levels (34).

Time spent in hyperglycemia is associated with increased risk of all-cause and cardiovascular diseases (CVD's) mortality among patients with T2D (35). Previous research (36, 37) has highlighted that despite pharmacological therapy, a significant proportion of individuals with T2DM spend considerable time in hyperglycemia, underscoring the need for additional non-pharmacological strategies to improve glycemic control. The analysis of a limited number of studies (n = 4) demonstrated that exercise interventions are effective in reducing the time spent in hyperglycemia, with HIIT showing a greater impact compared to other modalities. This finding is clinically significant since there are concerns that HIIT may initially exacerbate hyperglycemia due to the secretion of counter-regulatory hormones. However, this transient hyperglycemic response is a normal physiological reaction to high-intensity exercise and is typically followed by the stabilization and gradual decline of blood glucose levels shortly after the HIIT session. Moreover, this transient response to HIIT may help prevent the occurrence of hypoglycemia which is frequently observed following MICT (38) and represents one of the primary perceived barriers to exercise among individuals with T2DM (39). Thus, despite initial concerns, HIIT may offer a safer alternative for managing glycemic fluctuations in individuals with T2DM. This systematic review is not without limitations. Firstly, the limited number of studies evaluating the impact of different exercise modalities restricts the generalizability of findings to the broader population with T2DM. Moreover, the lack of studies examining the potential role of resistance training as

effective training as effective further limits the scope of this review, as resistance training is known to independently influence long-term glycemic control and other cardiometabolic outcomes. Another notable limitation is the presence of heterogeneity in the experimental protocols such as variations in exercise type, intensity, duration, and methods of intensity quantification which complicates the direct com-parison and synthesis of results.

In conclusion, both MICT and HIIT demonstrated acute reductions in mean 24-hour glycemic concentrations, postprandial glucose excursions, and the percentage of time spent in hyperglycemia. These findings suggest that both modalities are effective non-pharmacological strategies for optimizing glycemic control in patients T2DM. Future research should focus on examining additional indices of glycemic control, including glycemic variability and time-in-range (TIR). Moreover, studies should explore the underlying physiological mechanisms contributing to these acute responses, particularly insulin sensitivity and β-cell function, to better understand the role of exercise in the comprehensive management of T2DM.

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Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

REVIEW ARTICLE

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A META-ANALYTIC REVIEW OF THE RELATIONSHIP BETWEEN GENERALIZED ANXIETY DISORDER AND EMOTIONAL DYSREGULATION

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This meta-analytic study was conducted with the aim of quantitative integrating the findings obtained in individual studies that were concerned with determining the relationship between generalized anxiety disorder (GAD) and emotional dysregulation in studies conducted on non-clinical sample and adult population.
The studies included in the meta-analysis are quantitative correlational studies in English, published in scientific journals in the last twenty years and whose methodological features correspond to the context of this analysis. The average weighted correlation, expressed by the Pearson correlation coefficient, is .497 and can be characterized as moderate.
The obtained results are in line with the expectations and results of other researchers. The obtained results indicate a high heterogeneity and the study is discussed with suggestions for researchers in this field in the direction of continuing research on the relationship between the variables that are the subject of research.
Empirical evidence testifies to the fact that problems of emotional regulation occur not only in persons with GAD, but also in panic disorder, social phobia and depression, which opens the door to the investigation of potential mediating relationships or covariates that influence the development of psychopathological symptomatology.

Keywords: emotional regulation, generalized anxiety disorder, meta-analysis

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INTRODUCTION

Generalized anxiety disorder (GAD) is characterized by intense anxiety and worry about a number of events or activities. The main symptom reported with this disorder is worry, which is difficult to control. This concern is associated with physical symptoms such as muscle tension, fatigue, irritability, sleep problems, and restlessness, which are associated with a significant reduction in function-ality in daily life, and they last for at least six months (1). When compared to other anxiety disorders, it is interesting that GAD differs from others in that there is no clearly defined stimulus or situation that causes not characterized by expressed avoidance behaviors (2). Research that studied the content of care in people with this diagnosis found that GAD individuals usually do not have a specific issue they worry about, although these topics are often not in the focus of the observer, i.e., the person seems to worry for no particular reason. A large number of people suffering from this problem predict the catastrophic outcomes of some future events that certainly have a low probability of happening (2).

As there are certain disagreements in understanding the etiology and differentiating GAD from others in this group, various theories are present with the aim of making a more subtle distinc-tion and enabling a better understanding. Mennin (3) is the first representative of a group of theories in the field of emotional regulation, which, as its starting point, takes the idea that worry is a cognitive strategy to avoid emotions and other unpleasant content. Menin assumes that the key to understanding that problem should be sought in emotional regulation, and he finds the starting point for this idea in the explanation that, at its core, the problem involves the need to avoid, where one also avoids one's own emotions. Emotional regulation was defined by this author as a set of abilities that concern the way a person expresses and reacts to one's own emotions (3).

Empirical research has largely supported this conceptualization. In particular, Menin and colleagues found that individuals meeting the criteria for this disorder tend to experience emotions more intensely and face greater difficulties in identifying and describing their own emotions, which are often evaluated negatively (4). In one experimental study, it was established that these deficits in persons diagnosed with GAD do not only relate to the regulation of emotions such as fear and anxiety but also involve the emotion of sadness, suggesting the existence of a general emotional-regulatory deficit (5). It was also found that, regardless of their current emotional state, individuals diagnosed with GAD demonstrated reduced awareness of emotions and the ability to accept them compared to a control group (5). These data and research are mostly recent, but there is a base of results that do not support this theory. For example, during the daily monitoring of emotions in people with GAD, it was confirmed that they indeed experience more intense emotions, but that they do not have a reduced ability to recognize them or that they rely only on a narrow range of strategies to modulate their emotional experience (6). Such data already question previous results and point to the need for further research. This is not an isolated case of conflicting findings.

For example, in a study that used independent assessors and not subjective evaluation of emotions, it was not found that people with the diagnosis of GAD lag behind the control group in understanding and identifying emotions (7). Roemer and Orsilova (8) also made a relevant and similar observation in their research. They suggest that a central problem in individuals diagnosed with GAD is a tendency to negatively judge their own intrapsychic experiences. This includes emotions, which is why they try to avoid them, either behaviorally or cognitively. Therefore, a negative assessment of one's own emotional experiences leads to the inability of a person to experience an emotion, accept it, or understand the current feelings. One of the first studies was conducted by Roemer and his associates, the author of the theory. In a study he conducted both on the student and clinical sample (9), he obtained data that did not support the theory. While in the student sample a positive connection was established between the tendency to avoid experience, negative evaluation of emotions (e.g., the presence of fear of emotions), and the degree of expression of GAD symptoms, in the clinical sample, no expected connections were found.

This study examines the relationship between emotional dysregulation and generalized anxiety disorder in a non-clinical population and synthesizes the available empirical data in this area. Everything shown above indicates that the study results do not indicate the existence of agreement among researchers when it comes to the connection between emotional regulation and generalized anxiety disorder, and these are also the conclusions of individual researchers in this field (10). The importance of this research question lies in the fact that, by reviewing the above studies, although the results imply that emotional regulation

and generalized anxiety disorder are negatively related, it is not possible to unequivocally make conclusion about the intensity of this relationship. The review of the literature revealed particular differences depending on which population of respondents was included; therefore, it was decided to focus on a non-clinical population. The results of this meta-analytic study are aimed at providing answers on the basis of which certain implications could be given both for further psychological practice and for further research in this area. Considering that the field is relatively in its infancy and that, despite the large number of works, little is still known, we believe that the theoretical goal of the research has been brought into focus for a reason.

METHODS

Operationalization of variables

Emotional dysregulation is operationalized through the score on the questionnaire that measures the ability of emotional regulation; that is, in this case, dysregulation was measured, which indicates a low-developed ability. Therefore, higher scores indicate low emotional regulation, while low scores indicate the absence of behaviors typical of people with regulation problems but do not necessarily indicate a high capacity for regulation. Only those measures that are in accordance with the theory of emotional regulation, whose questionnaires have shown good psychometric characteristics and are often used, are considered valid.

Generalized anxiety disorder: this variable was operationalized through a variable that indicates the overall experience of anxiety and feeling of worry, where higher scores indicate a more pronounced feeling of anxiety and lower scores indicate the absence of that unpleasant feeling. When choosing the measures, both the variables identified by the authors as adequate for assessing generalized anxiety disorder and other scales with satisfactory psychometric properties that directly relate to the investigated phenomenon were considered appropriate for use.

Sample

In this meta-analytic study, we included studies that were conducted on a non-clinical population. The study also included those where it was possible to clearly separate a subsample of respondents from the clinical and non-clinical population. The criteria that the mentioned additional studies should have fulfilled were the following:

1. The research had to be published in a scientific journal with an impact factor;
2. The journal in which the research was published was in English;
3. The study was published after 2000;
4. Variables were operationalized in a clear way in the study;
5. There were significant correlation coefficients between variables.

Literature search

The literature search was conducted through available internet sources for accessing scientific publications that do not require special permissions and are used in the scientific community: Google Scholar, ResearchGate, and ScienceDirect, during November and December 2020. The criteria entered for the search were: generalized anxiety disorder, anxiety disorders, anxiety, emotional regulation, emotional dysregulation, and dysregulation. The search was narrowed only to papers published after 2000, and papers that did not have the full text available were not included (Figure 1).

The effect size included in the analysis is the Pearson's correlation coefficient, so there was no need to convert the measures additionally. A sample size was used to weight the effect size. The meta-analysis procedure includes studies whose subjects probably do not come from the same population, primarily because the subjects in the study differ in terms of country of origin, age, education, and potentially other covariates. Therefore, it is assumed that there is not one but a distribution of true effects, which indicates that a random effects model would be appropriate for computing the overall effect size measure. However, the random effects model tends to overestimate the error variance (11), and bearing in mind that the random effects model can be reduced to a fixed model if the variance between studies approaches zero (12), both types of overall effect size analysis will be compared, as the number of studies we include in the final analysis is not that large. For the purposes of this analysis, the program comprehensive meta-analysis was used to calculate metastatistics and deviation measures.

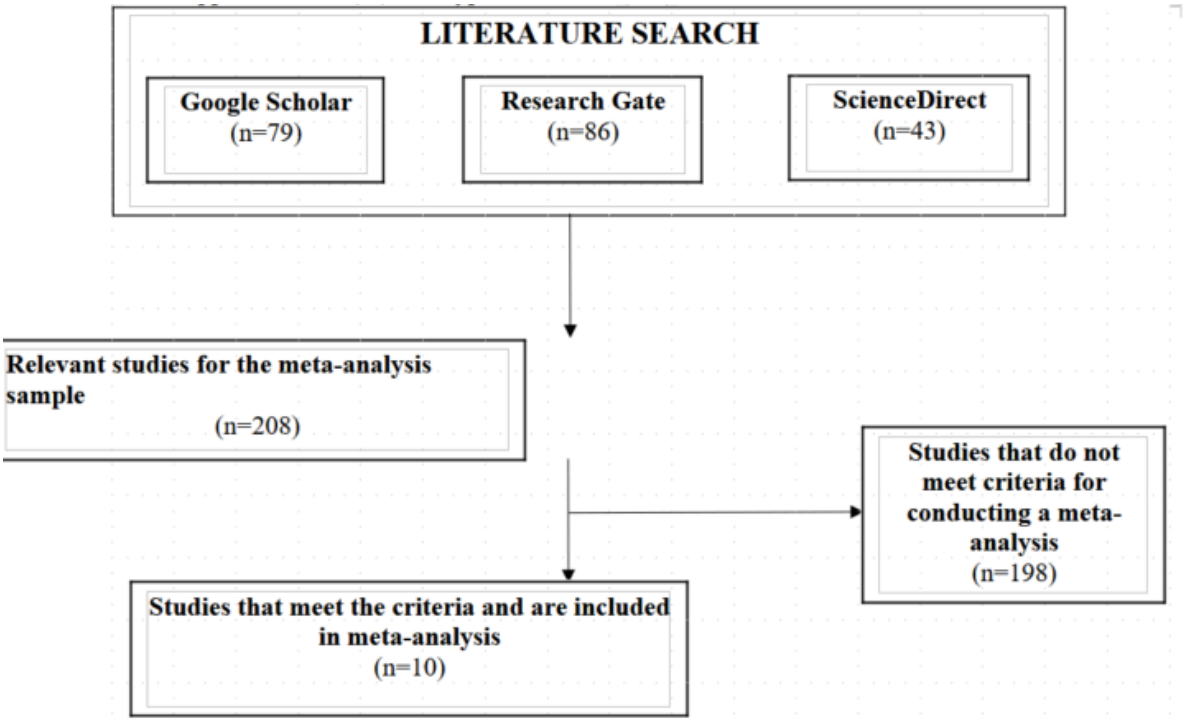

Figure 1. *Presentation of the flow of the search and selection of literature for the analysis*

An overview of the 10 studies included in the final analysis is provided in Table 1. As mentioned earlier, Pearson's correlation coefficient was used as a measure of effect size, and only one measure from each study was included.

We see that there are certain differences in the studies when it comes to the operationalization of the constructs but in the case of the variable related to generalized anxiety. However, for the measure to be accepted, the authors had to offer an explanation as to why it was considered equivalent. Considering the rigorous selection criteria of the journals that will be included in the study, this procedure is justified, in the author's opinion.

Table 2 shows the results of the conducted meta-analysis for the fixed and variable effect models, together with the average weighted correlation.

The obtained metastatistics, that is, the average weighted correlation coefficient, points to the existence of a connection between low emotional dysregulation and generalized anxiety disorder. We can interpret the obtained statistic as a correlation of medium intensity.

The evaluation of heterogeneity in this meta-analysis was performed by determining the significance indicator of heterogeneity (via the Q statistic) and the percentage of total variability that can be attributed to heterogeneity (via the I2 statistic). Based on the value of the Q statistic and its statistical significance, we can reject the hypothesis that there is a fixed effect. Furthermore, we can see that more than 90% of the total variance can be attributed to heterogeneity, i.e., variances between individual studies (I2 = 90,450), which can be interpreted as very high heterogeneity (13).

Table 1. *Studies included in the final analysis*

No.	Study	Sample	Sample size	GAP	Emotional disregulation	r
1	Roemer L, Lee JK, Salters-Pedneault K, Erisman SM, Orsillo SM, Mennin DS. Behav Ther.	General population	411	The Generalized Anxiety Disorder Questionnaire-IV (GAD-Q-IV; Newman et al., 2002)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.55
2	Tull MT, Stipelman BA, Salters-Pedneault K, Gratz KL. J Anxiety Disord.	Students	410	The Generalized Anxiety Disorder Questionnaire-IV (GAD-Q-IV; Newman et al., 2002)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.41
3	Ouellet C, Langlois F, Provencher MD, Gosselin P. Eur Rev Appl Psychol.	General population	204	The Generalized Anxiety Disorder Questionnaire-IV (GAD-Q-IV; Newman et al., 2002)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.61
4	Marganska A, Gallagher M, Miranda R. Am J Orthopsychiatry.	Students	284	The Generalized Anxiety Disorder Questionnaire-IV (GAD-Q-IV; Newman et al., 2002)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.41
5	Gratz KL, Roemer L. J Psychopathol Behav Assess.	Adolescents	210	The Screen for Child Anxiety Related Emotional Disorders (Birmaher et al., 1997)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.42
6	Soenke M, Hahn KS, Tull MT, Gratz KL. Cogn Ther Res.	Students	396	The Generalized Anxiety Disorder Questionnaire-IV (GAD-Q-IV; Newman et al., 2002)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.47
7	Suveg C, Morelen D, Brewer GA, Thomassin K. J Anxiety Disord.	Students	676	Symptom Checklist-90-Revised (SCL-90R; Derogatis, 1994)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.53
8	Mennin DS, Holaway RM, Fresco DM, Moore MT, Heimberg RG. Behav Ther.	Students	869	The Generalized Anxiety Disorder Questionnaire-IV (GAD-Q-IV; Newman et al., 2002)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.27
9	Nielsen SKK, Lønfeldt N, Wolitzky-Taylor KB, Hageman I, Vangkilde S, Daniel SIF. J Affect Disord.	General population	147	Beck Anxiety Inventory (BAI; Beck et al., 1988)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.73
10	Salters-Pedneault K, Roemer L, Tull MT, Rucker L, Mennin DS. Cogn Ther Res.	Students	325	Penn State Worry Questionnaire (PSWQ; Meyer et al., 1990)	The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	.51

Table 2. *Weighted average correlation between generalized anxiety and emotional dysregulation*

Model	Effect size				Test of null		Heterogeneity			
	N	*r̂*	UL	LL	*Z*	*p*	*Q*	*df*	*p*	I^2
Fixed	10	.460	.435	.484	31.049	.000	94.238	9	.000	90.450
Random	10	.497	.414	.571	10.229	.000				

N – number of studies included in the analysis; r̂ – weighted average coefficient; UL – upper limit; LL – lower limit; I^2 – percentage of total variability that can be attributed to heterogeneity

Drawer effect

The drawer effect refers to the bias in studies that are included in a meta-analysis relative to studies that are not included. It is hypothesized that this could have an impact on the size of the effect obtained, and additional analysis will be conducted to examine such possibilities.

As can be seen in Figure 2, the studies included in the meta-analysis are shown as circles. They are expected to be evenly distributed around the vertical axis. As can be seen from the attached graph, the studies are evenly distributed around the vertical axis, so there can be no doubt that there is a bias in the selection of studies for analysis.

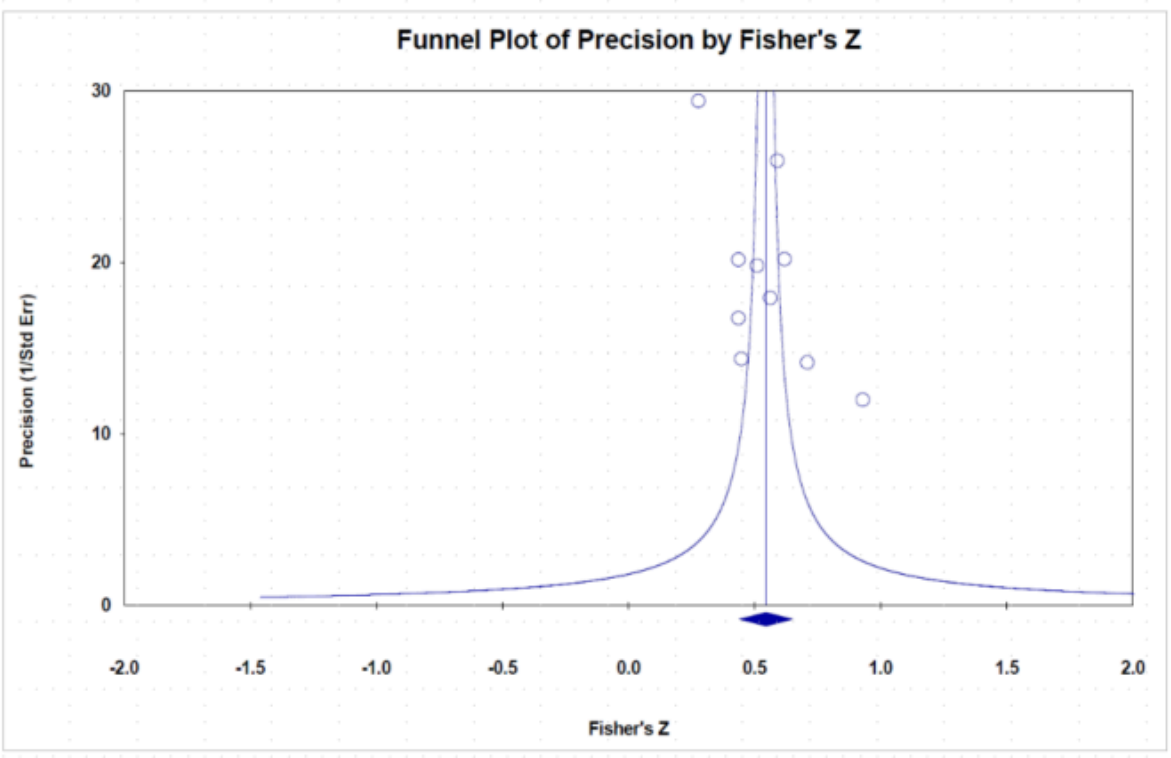

Figure 2. *Display of the drawer effect for the random effects model*

DISCUSSION

The main goal of the study was to integrate the data obtained in earlier studies in order to determine whether there is a connection between generalized anxiety disorder and poor ability to regulate emotions, presented here as emotional dysregulation.

The research idea came from an extensive review of the literature that is available to researchers, especially in the past ten years since this topic has really been in the focus of researchers. On the other hand, the very broad material failed to cover all important topics and approach the problem in depth. Primarily, we come across data indicating that the results of studies do not indicate the existence of agreement among researchers when it comes to the connection between emotional regulation and generalized anxiety disorder, according to Mihić (10). Some of the reasons are related to the specificity of the population in which the research is conducted. Another specificity of the subject of the study is the sample and population of adults that are in focus, given that it was assumed that emotional regulation does not have to play the same role in this segment of the population as it does in earlier developmental stages related to early adolescence or childhood, and also differs from its role in later stages, including older adulthood and old age (14–17).

The results of this study confirmed the expectations set by this research and the findings of previous studies (14, 18–22). The obtained metastatistics, i.e., the average weighted Pearson's correlation coefficient, point to the connection between low emotional dysregulation and generalized anxiety disorder. The obtained correlation of .497 indicates a medium intensity of connection, which is also just a confirmation of what other studies have found. Namely, in the research included in the analysis, the range went from low to approximately high, which is why the information obtained in this study is not surprising.

The analyses showed that about 90% of the total variance can be attributed to heterogeneity, i.e., variances between individual studies. This result not only confirms the justification of using random or variable effects' models but also gives us important methodological guidelines. Namely, Sanchez-Meca et al. (13) state that in a situation where the I2 statistic is of moderate or high intensity, there is a meaningful basis for additional examination of the relationship between the constructs, i.e., examination of the influence of moderator variables, which can explain heterogeneity. In our case, the heterogeneity is very high, and it unequivocally indicates the existence of space for further research. It is believed that other parameters should be included in this relationship to begin with, especially those concerning the methodological value of the conducted study.

The instruments used to operationalize the constructs, especially the one used to approach the concept of emotional regulation, justify the obtained conclusions. It is very important that, when operationalizing the construct, the same measure was used in each study, especially considering the excellent psychometric characteristics of the scale. When it comes to generalized anxiety disorder, the deviation in measures is justified in the methodological part of the study, and in addition to the subjective assessment of the adequacy of the authors of this paper, we also relied on the reputation of the journal. Thus, only studies whose quality met the expected standards of this paper were included in the analysis. The methodological value of this study is further enhanced by the fact that the selection bias analysis carried out showed that there is no drawer effect. One of the basic qualities for evaluating a study is the way in which papers are chosen and the criteria according to which they are selected. In addition, in order to have a better insight, the impact factor of the journal in which it was published is listed for each paper, which should additionally testify to the quality of the study itself in addition to the confirmed effect.

This study has a greater value when considering the context in which it was created and the implications it offers. Those implications are primarily of a theoretical nature, and the importance of the research is greater in this regard. There is no doubt that practitioners in the field of mental health and clinical psychology have been dealing with this topic for a long time and that they see emotional regulation as an ability essential for understanding the etiology of psychopathological manifestations. However, it is important to comment on the specificity of the connection between emotional regulation difficulties and the diagnosis of GAD. A decent body of empirical evidence testifies to the fact that problems of emotional regulation (difficulties in describing and understanding emotions, as well as the appearance of fear of intense emotions) do not occur only in these persons, but there are reports of similar problems in panic patients as well.

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Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

INVESTIGATING FACTORS INFLUENCING CLINICAL PREGNANCY RATES IN HORMONE REPLACEMENT THERAPY FROZEN-THAWED EMBRYO TRANSFER CYCLES: A CROSS-SECTIONAL STUDY

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Approximately 50% of embryo transfer cycles are performed as frozen embryo transfer (FET) cycles; however, research on the factors influencing pregnancy rates in these cycles is limited in northern Iran. The aim of this study was to identify the factors influencing the clinical pregnancy rate in hormone replacement therapy (HRT) FET cycles among infertile women.

This descriptive-analytical observational study analyzed HRT FET cycles of 429 infertile couples whose embryos were obtained by microinjection at two in vitro fertilization (IVF) centers in Sari, northern Iran, from April 2015 to March 2019. Data were analyzed using SPSS software, version 22, with a significance level set at $p < 0.05$.

The mean ± SD age of women and men was 32 ± 2.52 and 36 ± 1.62 years, respectively. The mean ± SD age of women at the time of oocyte collection was 31.06 ± 5.3 years. Among the 429 patients, 171 cases (39.9%) achieved chemical pregnancy and 156 cases (36.3%) achieved clinical pregnancy. Multivariate regression analysis revealed significant differences between the clinically pregnant and non-pregnant groups ($p < 0.05$) in factors such as the woman's age at oocyte retrieval, duration of infertility, occupation, body mass index (BMI), developmental stage of the transferred embryo, type of catheter used for transfer, and embryo grade.

The study concluded that younger maternal age at oocyte retrieval, shorter duration of infertility, optimal BMI, higher embryo grade, and appropriate selection of transfer techniques are key determinants of achieving clinical pregnancy in HRT-FET cycles. These findings can guide specialists in optimizing FET protocols to improve pregnancy outcomes.

Keywords: frozen, embryo transfer, pregnancy, infertility, in vitro fertilization

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INTRODUCTION

In vitro fertilization (IVF) has revolutionized reproductive medicine and brought hope to couples facing infertility. Among the various techniques used in assisted reproductive technology, frozen-thawed embryo transfer (FET) has gained prominence due to its potential benefits, including the flexibility in transfer timing and reduced risk of ovarian hyperstimulation syndrome. Despite advances in cryopreservation techniques, clinical pregnancy rates following FET cycles exhibit significant variability (1, 2).

Numerous factors are thought to influence the success of FET, including embryo quality, endometrial receptivity, timing of transfer, and patient characteristics such as age and hormonal profile. In addition, clinical factors such as the ovarian stimulation protocol used, the experience of the medical team, and laboratory conditions can also play critical role. The interaction of these elements can create complex scenarios that challenge our understanding of the optimal conditions for successful implantation and subsequent pregnancy. Moreover, the increased use of preimplantation genetic testing has introduced additional dimensions to the evaluation of embryo viability and selection, adding layers to the decision-making process in FET cycles (3–5).

Investigating factors that influence clinical pregnancy rates in FET cycles is particularly important given the impact of demographic factors on reproductive health. Variations in age, ethnicity, and body mass index (BMI) can significantly influence fertility treatments and outcomes (6).

Although some studies have been conducted in Iran (7, 8), research specifically addressing factors that influence clinical pregnancy rates in FET cycles remains limited. Furthermore, global studies on this topic have yielded inconclusive results, highlighting the need for more focused research to clarify these relationships. Understanding the influences of associated factors on FET outcomes may lead to the development of targeted strategies that address barriers to successful pregnancy (9–11). This knowledge is essential for improving clinical practice and ensuring equitable access to reproductive technologies for all individuals seeking assistance with infertility.

By examining how these factors interact with clinical variables, this study aims to provide insights that can improve patient care and optimize clinical protocols in different populations. Therefore, this study aims to identify and analyze the specific fac-tors that influence clinical pregnancy rates in frozen-thawed embryo transfer cycles in IVF.

METHODS

This descriptive-analytical study focused on frozen-thawed embryo cycles resulting from microinjection in couples referred to the IVF Center of Imam Khomeini Hospital and the Private Mother IVF Center in Sari, Northern Iran, between 2015 and 2020. The study was approved by the Ethics Committee of Mazandaran University of Medical Sciences (code: IR MAZUMS.IMAMHOSPITAL. REC.1399.042) and adhered to the tenets of the Declaration of Helsinki. Eligible couples were selected by availability sampling, and written informed consent was obtained from all participants to ensure confidentiality and permission to use the data for research purposes.

Inclusion criteria consisted of patients undergoing a frozen embryo transfer cycle with at least one grade A or B embryo designated for transfer and who also consented to participate in the study. In addition, only patients undergoing hormone replacement therapy (HRT) cycles were included in this study. Exclusion criteria included participants in donation or surrogacy cycles, individuals with uterine abnormalities or severe endometriosis, the presence of hydrosalpinx detected by hysterosalpingography or ultrasound, a history of difficult uterine embryo transfers, azoospermia requiring testicular sperm extraction (TESE), and individuals with uncontrolled endocrine disorders such as diabetes, hypothyroidism, or hyperthyroidism.

A total of 30 variables related to individual patient factors, treatment cycle characteristics, and embryo factors were evaluated. These variables included the woman's age at the time of embryo transfer and oocyte retrieval, the spouse's age, both partners' occupations, smoking and alcohol consumption habits, duration of infertility, type of infertility (primary or secondary), cause of infertility, reason for embryo freezing (extra embryos, risk of ovarian hyperstimulation syndrome, or inappropriate endometrium), endometrial thickness on the day of embryo transfer, and grade of transferred embryos (A, B, or C), time interval between embryo thawing and transfer, developmental stage of the transferred embryo (cleavage, morula, or blastocyst), embryo grading before freezing, grading after thawing, endometrial pattern on the day of transfer (triple-line, hyperechoic, or isoechoic), type of catheter used for

transfer (with or without obturator), presence of blood at the catheter tip after transfer, ease of transfer (easy, forced, difficult, requiring tenaculum or anesthesia), performance of hysteroscopy or laparoscopy prior to the transfer cycle, season of transfer, number of previous failed cycles, use of oral contraceptives prior to the transfer cycle, treatment protocol during the ovarian stimulation cycle (agonist or antagonist), suppression with or without GnRH (gonadotropin-releasing hormone) agonist in the transfer cycle, body mass index (BMI), timing of the transfer within the cycle, and duration of embryo freezing. In addition, we recorded the outcomes of clinical and chemical pregnancy rates, early and late spontaneous abortion rates, ectopic pregnancy rates, and multiple pregnancy rate.

Endometrial preparation with hormonal drugs followed a specific protocol. All patients were referred to the clinic on the second or third day of their menstrual cycle for a vaginal ultrasound to evaluate the uterus and ovaries. If the ultrasound results were normal, patients were prescribed estradiol valerate tablets (2 mg estradiol tablets manufactured by Aburaihan Pharmaceutical Company, Iran), starting on the third day of the menstrual cycle, with a daily dosage ranging from 2 to 4 mg. Periodic vaginal ultrasounds were performed to measure endometrial thickness, and the estradiol dose was adjusted as needed. The maximum prescribed dose of estradiol tablets was 8 mg per day. Progesterone supplementation began when the endometrial thickness reached 8 mm, with patients receiving either vaginal progesterone suppositories (Fertigest® 400 mg, Aburaihan Pharmaceutical Company, Iran) every 12 hours at 400 mg or daily intramuscular injections of 100 mg progesterone (50 mg vial, Iran Hormon Pharmaceutical Company) for 3 to 5 days prior to embryo transfer. Estradiol supplementation was continued along with progesterone supplementation until the day of the embryo transfer.

The timing of embryo thawing and transfer was planned based on the age of the frozen embryo and the decision to transfer the embryo at the blastocyst stage or another stage of development. In some cases, patients were prescribed oral contraceptive pills for one month prior to starting estradiol treatment to synchronize cycles or in cases where the endometrium required suppression. In addition, a daily subcutaneous injection of a GnRH agonist (Sinafact 5 mg vial, Sinagen Pharmaceuticals, buserelin) at a dose of 0.5 mg in the middle of the luteal cycle was used to suppress the hypothalamic-pituitary axis and increase endometrial receptivity. The GnRH agonist dose was reduced to 0.25 mg per day with the onset of menses and continued until progesterone treatment was initiated.

The frozen embryos were created by microinjection of oocytes obtained during the ovarian stimulation cycle, using sperm obtained from the spouses' ejaculations. These embryos were frozen between days 3 and 5 after microinjection, from the 8-cell stage to the blastocyst stage. Embryos were graded based on blastomere morphology and cytoplasmic fragmentation, both before freezing and after thawing. Cleavage stage embryos were graded as follows: grade A indicated the absence of fragmentation with 6–8 equally sized blastomeres; grade B had fragmentation of less than 25% with blastomeres that may or may not be equally sized; grade C had fragmentation between 25–50% or blastomeres that were not equally sized; and grade D had fragmentation greater than 50%. Blastocyst grading followed the Gardner scoring system, with grade D embryos typically not frozen (12, 13). Embryos were frozen using Kitazato embryo vitrification media freezing kits from Tokyo, Japan.

Prior to transfer, the embryos were thawed and placed in embryo culture medium (Life Global, single-step media, CooperSurgical, US) using Kitazato embryo thawing kits. Embryos with less than 50% fragmentation after thawing were considered suitable for transfer, which was performed under abdominal ultrasound guidance by one or two fertility specialists. Two types of catheters were used for embryo transfer: one without an obturator (CCD catheter, Paris, France) and one with an obturator, provided by either a CCD catheter (TDT SET, Paris, France) or a Wallace obturator (Sure-Pro Ultra, PEB623, CooperSurgical, Denmark). The report documented the method of embryo transfer, classified it as difficult or easy, and noted the use of tenaculum or anesthesia, along with any bleeding from the catheter after transfer. After the embryo transfer, patients rested in the supine position for approximately 30 minutes before being discharged. The administration of estradiol tablets and vaginal or injectable progesterone continued until two weeks after embryo transfer, at which time serum β-HCG levels were measured. A serum β-HCG level greater than10 milliunits per milliliter indicated a positive chemical pregnancy and prompted reassessment two days later. If the pregnancy progressed, estradiol and progesterone therapy was continued until 10 weeks' gestation, with clinical pregnancy confirmed by ultrasound observation of the gestational sac and fetal pole with heartbeat at 7 weeks' gestation (14).

Statistical analysis

Data were analyzed using SPSS software, version 22, and the Kolmogorov-Smirnov test was used to test the assumption of normality. Frequencies and percentages were used to describe qualitative variables, and means and standard deviations were used for quantitative variables. The sample size for this study was determined to be 429 infertile couples based on a similar study, considering a clinical pregnancy rate of 29.2%, a significance level of 0.05, a test power of 0.80, and an effect size of 6.15%, selected by convenience sampling.

Chi-square tests, Fisher's exact tests, and independent samples t-tests were used to compare demographic and clinical data between clinically pregnant and non-pregnant women undergoing frozen embryo transfer (FET) cycles. For variables with non-normal distributions, the non-parametric Mann-Whitney test was used to compare the two groups. To investigate the effect of different factors on clinical pregnancy, individual variables were first entered into a simple logistic regression model (crude). Those with a p-value < 0.2 were then entered into a multiple logistic regression model (adjusted). Effective demographic and clinical variables affecting the success of frozen-thawed embryo transfer cycles were identified based on a significance level of $p < 0.05$, and the results were expressed as odds ratios (OR) with a 95% confidence interval. The validity of the regression models was confirmed using the Hosmer-Lemeshow test and the omnibus test. A significance level of $p > 0.05$ was considered for all tests.

RESULTS

In this study, 429 frozen embryo transfer (FET) cycles involving 429 patients were analyzed. The mean age of the women at the time of transfer was 32 ± 2.52 years, while the mean age of their spouses was 36 ± 1.62 years. The mean age of the women at the time of oocyte retrieval was 31.06 ± 5.3 years. Among the participants, 171 cases (39.9%) resulted in chemical pregnancies, and 156 cases (36.3%) resulted in clinical pregnancies. In the group of women with clinical pregnancies, there were 127 singleton pregnancies, 28 twin pregnancies, and one triplet pregnancy. The mean body mass index (BMI) of the women was 25.06 ± 4 kg/m^2. Of the women, 58.7% were homemakers, and 11% were employed. Demographic characteristics and treatment cycle outcomes for the pregnant and non-pregnant groups are detailed in Tables 1 and 2. The results show significant differences in certain variables between the pregnant and non-pregnant groups, including the woman's age at oocyte retrieval, BMI, duration of infertility, occupation, embryo grade before freezing and after thawing, quality and developmental stage of the transferred embryo, type of catheter used for transfer, and ease of transfer ($p < 0.05$). Other variables did not reach statistical significance (Table 1).

When looking at embryo quality and women's age, the study found that clinical pregnancy rates were higher in women under 37 years of age who received two A-A or A-B embryos compared to those who received two B-B embryos. In addition, pregnancies were more successful in women over 37 years of age when two A-A embryos were transferred (Figure 1A).

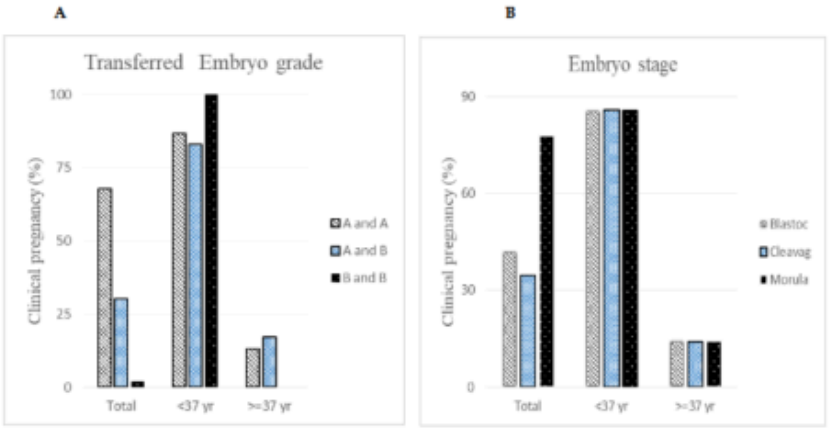

Figure 1. *A. Clinical pregnancy (%) for each embryo grade in females aged under 37 and > = 37, B. Clinical pregnancy (%) for each embryo stage in females aged under 37 and > = 37*

Table 1. *Demographic and clinical characteristics in two groups of pregnant and non-pregnant women in frozen-thawed embryo transfer cycles*

Variables	Clinical pregnancy		Total (n = 429)	P-value
	No (n = 273)	Yes (n =156)		
Female age on transfer day				
< 37 years	219 (80.2%)	134 (85.9%)	353 (82.3%)	0.138
≥ 37 years	54 (19.8%)	22 (14.1%)	76 (17.7%)	
Spouse's age				
< 37 years	153 (56%)	96 (61.5%)	249 (58.0%)	0.268
≥ 37 years	120 (44%)	60 (38.5%)	180 (42.0%)	
Female age on oocyte pick-up day				
< 37 years	233 (85.3%)	146 (93.6%)	379 (88.3%)	0.010
≥ 37 years	40 (14.7%)	10 (6.4%)	50 (11.7%)	
Female occupation				
Employed	23 (8.4%)	24 (15.4%)	47 (11.0%)	0.020
Housekeeper	170 (62.3%)	82 (52.6%)	252 (58.7%)	
Self-employment	8 (2.9%)	11 (7.1%)	19 (4.4%)	
Other	72 (26.4%)	39 (25.0%)	111(25.9%)	
Spouse's occupation				
Employed	62 (22.7%)	44 (28.2%)	106 (24.7%)	0.439
Self-employment	105 (38.5%)	57 (36.5%)	162 (37.8%)	
Other	106 (38.8%)	55 (35.3%)	161 (37.5%)	
Cigarette smoking				
No	252 (92.3%)	138 (88.5%)	390 (90.9%)	0.183
Yes	21 (7.7%)	18 (11.5%)	39 (9.1%)	
Alcohol				
No	263 (96.3%)	151 (96.8%)	414 (96.5%)	0.804
Yes	10 (3.7%)	5 (3.2%)	15 (3.5%)	
Infertility duration				
< 5 years	147 (53.8%)	101 (64.7%)	248 (57.8%)	0.028
≥ 5 years	126 (46.2%)	55 (35.3%)	181 (42.2%)	
Infertility type				
Primary	172 (63.0%)	93 (59.6%)	265 (61.8%)	0.487
Secondary	101 (37.0%)	63 (40.4%)	164 (38.2%)	
Infertility cause				
Anovulation	39 (14.3%)	16 (10.3%)	55 (12.8%)	0.555
Endometriosis	21 (7.7%)	11 (7.1%)	32 (7.5%)	
Male factor	112 (41.0%)	72 (46.2%)	184 (42.9%)	
Multiple female	10 (3.7%)	4 (2.6%)	14 (3.3%)	
Both male and female	53 (19.4%)	30 (19.2%)	83 (19.3%)	
Tubal factor	37 (13.6%)	20 (12.8%)	57 (13.3%)	
Unexplained	1 (0.4%)	3 (1.9%)	4 (0.9%)	
Embryo grade before freezing				
A - A	139 (50.9%)	106 (67.9%)	245 (57.1%)	0.001
A - B	115 (42.1%)	47 (30.1%)	162 (37.8%)	
B - B	19 (7.0%)	3 (1.9%)	22 (5.1%)	
Embryo grade after thawing				

A - A	139 (50.9%)	106 (67.9%)	245 (57.1%)	0.001
A - B	115 (42.1%)	47 (30.1%)	162 (37.8%)	
B - B	19 (7.0%)	3 (1.9%)	22 (5.1%)	
Embryo grade on transfer day				
A - A	138 (50.5%)	107 (68.6%)	245 (57.1%)	0.001
A - B	116 (42.5%)	46 (29.5%)	162 (37.8%)	
B - B	19 (7.0%)	3 (1.9%)	22 (5.1%)	
Interval between thawing and transfer				
0 day	73 (26.7%)	32 (20.5%)	105 (24.5%)	0.352
1 day	197 (72.2%)	122 (78.2%)	319 (74.4%)	
2 day	3 (1.1%)	2 (1.3%)	5 (1.2%)	
Endometrial line thickness on transfer day				
< 9 mm	17 (6.2%)	9 (5.8%)	26 (6.1%)	0.848
≥ 9 mm	256 (93.8%)	147 (94.2%)	403 (93.9%)	
BMI (mean ± SD)	25.17±3.44	25.96±4.02	25.45±3.68	0.031*
Protocol for ovarian stimulation cycles				
Agonist	23 (8.4%)	11 (7.1%)	34 (7.9%)	0.380
Antagonist	241 (88.3%)	143 (91.7%)	384 (89.5%)	
None	9 (3.3%)	2 (1.3%)	11 (2.6%)	
Protocol for suppression in transfer cycles				
With Agonist	88 (32.2%)	46 (29.5%)	134 (31.2%)	0.555
Without Agonist	185 (67.8%)	110 (70.5%)	295 (68.8%)	
Embryo growth stage on transfer day				
Blastocyst	29 (10.6%)	21 (13.5%)	50 (11.7%)	0.020
Cleavage	242 (88.6%)	128 (82.1%)	370 (86.2%)	
Morula	2 (0.7%)	7 (4.5%)	9 (2.1%)	
History of laparoscopy-hysteroscopy				
No	133 (48.7%)	75 (48.1%)	208 (48.5%)	0.898
Yes	140 (51.3%)	81 (51.9%)	221 (51.5%)	
Season on transfer cycles				
Autumn	64 (23.4%)	36 (23.1%)	100 (23.3%)	0.339
Spring	64 (23.4%)	39 (25.0%)	103 (24.0%)	
Summer	79 (28.9%)	34 (21.8%)	113 (26.3%)	
Winter	66 (24.2%)	47 (30.1%)	113 (26.3%)	
Prior transfer cycles—number				
0	165 (60.4%)	88 (56.4%)	253 (59.0%)	0.577
1	64 (23.4%)	46 (29.5%)	110 (25.6%)	
2	27 (9.9%)	13 (8.3%)	40 (9.3%)	
≥ 3	17 (6.2%)	9 (5.8%)	26 (6.1%)	
Pre-treatment use of contraceptive pills				
No	11 (2.5%)	12 (2.7%)	23 (5.3%)	0.132
Yes	271 (63.1%)	135 (31.4%)	406 (94.7%)	
Cause of freezing				
Endometrial insufficiency	1 (0.4%)	0 (0.0%)	1 (0.2%)	0.313
OHSS risk	0 (0.0%)	1 (0.6%)	1 (0.2%)	
Surplus Embryos	272 (99.6%)	155 (99.4%)	427 (99.5%)	
Endometrial pattern				
Hyperechoic	2 (0.7%)	0 (0.0%)	2 (0.5%)	0.556
Isoechoic	3 (1.1%)	2 (1.3%)	5 (1.2%)	
Trilaminar	268 (98.2%)	154 (98.7%)	422 (98.4%)	
The day of transfer in the cycle (mean± SD)	17.89±1.56	17.83±1.20	17.79±1.37	0.722*
Embryo catheter type				

With obturator	142 (52.0%)	103 (66.0%)	245 (57.1%)	0.005
Without obturator	131 (48.0%)	53 (34.0%)	184 (42.9%)	
Bloody catheter after transfer				
No	268 (98.2%)	152 (97.4%)	420 (97.9%)	0.611
Yes	5 (1.8%)	4 (2.6%)	9 (2.1%)	
Freeze duration by months (mean± SD)	6.65±8.40	7.04±10.33	6.80±9.14	0.675*
Ease of doing transfer				
Easy under anesthesia	3 (1.1%)	0 (0.0%)	3 (0.7%)	0.033
Easy (without instrument)	249 (91.2%)	147 (94.2%)	396 (92.3%)	
Enforcement	0 (0.0%)	2 (1.3%)	2 (0.5%)	
Difficult and need anesthesia	21 (7.7%)	6 (3.8%)	27 (6.3%)	
Difficult and need to use tenaculum	0 (0.0%)	1 (0.6%)	1 (0.2%)	

Chi-square *Independent sample t-tests

Table 2. *The result of frozen-thawed embryo transfer cycles in patients*

Variables	Results	
B-HCG	Positive	171 (39.9%)
	Negative	258 (60.1%)
Clinical pregnancy (embryo sac number)	(1)	127 (81.4%)
	(2)	28 (17.9%)
	(3)	1 (0.6%)
Abortion	Early	15 (8.7%)
	Late	9 (5.2%)
EP	0	
Multiple pregnancy	29 (18.5%)	

* Frequency; B-HCG: Beta-Human Chorionic Gonadotropin; EP: Ectopic pregnancy

Regarding embryo development stage and women's age, morula-stage embryo transfers resulted in higher clinical pregnancy rates compared to blastocyst-stage and cleavage-stage transfers. However, morula-stage embryo transfers were performed in only 2.1% of all FET cycles (nine out of 429 patients). The small sample size of morula-stage transfers limits the ability to draw definitive conclusions about their efficacy compared to other embryo stages (Figure 1B).

Table 3 shows the relationship between factors affecting clinical pregnancy rates and the results of the multiple logistic regression model.

The adjusted model shows that patients under 37 years of age at oocyte retrieval had nearly four times the odds of achieving clinical pregnancy compared with those over 37 years of age. Conversely, patients with infertility of less than five years had 1.66 times the odds of clinical pregnancy compared to those with infertility of more than five years. In addition, the analysis revealed that homemakers had a 0.69 lower chance of clinical pregnancy compared to employed individuals.

Table 3. *The results of logistic regression test in predicting success in frozen-thawed embryo transfer cycles in patients (based on the occurrence of clinical pregnancy)*

Variable	Crude		Adjusted	
	OR (95%CI)	P-value	OR (95%CI)	P-value
Female age on transfer day (reference: ≥ 37)				
< 37 years	1.51 (0.85, 2.58)	0.140	0.74 (0.32, 1.75)	0.490
Female age on oocyte pick-up day (reference: ≥ 37years)				
< 37	2.51 (1.22, 3.28)	0.013	4.17 (1.41, 12.81)	0.011
Infertility duration (reference: ≥ 5)				
< 5 year	1.57 (1.05, 2.36)	0.028	1.66 (1.06, 2.61)	0.026
Cigarette smoking (reference: yes)				
No	0.64 (0.33, 1.24)	0.185	0.64 (0.30, 1.33)	0.225
Female occupation (reference: employed)				
Housekeeper	0.46 (0.25, 0.87)	0.016	0.31 (0.15, 0.63)	0.001
Self-employment	1.32 (0.45, 3.86)	0.615	0.79 (0.25, 2.59)	0.698
Other	0.52 (0.26, 1.04)	0.063	0.38 (0.18, 0.81)	0.012
Transferred embryo grade (ref: A - A)				
A - B	0.51 (0.33, 0.78)	0.002	0.54 (0.33, 0.84)	0.007
B - B	0.20 (0.06, 0.71)	0.012	0.26 (0.06, 0.81)	0.037
BMI	1.06 (1.01, 1.12)	0.032	1.06 (1.01, 1.13)	0.044
Embryo stage (ref: morula)				
Blastocyst	0.21 (0.04, 1.10)	0.064	0.14 (0.02, 0.78)	0.039
Cleavage	0.15 (0.03, 0.74)	0.020	0.15 (0.02, 0.77)	0.038

Moreover, patients with A-A grade embryos had approximately 0.46 higher odds of clinical pregnancy than those with A-B grade embryos, while patients with B-B grade embryos had approximately 0.74 lower odds of clinical pregnancy than those with A-A grade embryos. The results showed that for each unit increase in BMI, the chance of clinical pregnancy increased by 6%. The odds of clinical pregnancy for blastocyst embryos were 0.14 lower than for morula stage embryos, and the odds of cleavage stage embryos were 0.15 lower than for morula stage embryos. In addition, the odds of pregnancy were 1.67 times higher for catheters with an obturator compared to those without (Table 3, Figure 1).

DISCUSSION

This aim of this study was to identify the factors influencing the clinical pregnancy rate of FET cycles in infertile women referred to infertility centres between 2015 and 2020. The analysis revealed significant factors influencing the success of FET cycles, distinguishing between pregnancy and non-pregnancy groups. Key variables included the woman's age at egg retrieval, duration of infertility, occupation, body mass index (BMI), developmental stage of the transferred embryo, type of catheter used, and embryo grade. These variables have been identified as influential in the success or failure of FET cycles.

A woman's age is an important determinant of pregnancy success in fresh transfer cycles, as declining oocyte quality in women over 35 years of age can affect success rates (15, 16). However, in this study, neither the age of the woman at transfer nor the age of the man correlated with clinical pregnancy outcomes. Notably, women under 37 years of age at oocyte retrieval had improved odds of achieving a successful clinical pregnancy. This is consistent with the results of another study, which showed that age did not influence the outcome of cryopreservation in women younger than 35 years (17). Moreover, research has demonstrated comparable pregnancy rates in FET cycles between women under and over 40 years of age when high quality embryos are used, underscoring the importance of a woman's age at egg retrieval as a critical determinant of cycle success (8). Thus, the age at oocyte retrieval may be more important than the age at transfer in predicting the outcome of a transfer cycle.

Our results also showed a significant relationship between morula stage embryo transfer and clinical pregnancy rates.

Previous research has highlighted blastocyst embryo transfer as a primary pre-dictor of live birth rates, with lower abortion rates associated with grade A blastocyst embryos compared to grade C (18, 19). In our study, the odds of clinical pregnancy were 0.14 lower for blastocyst embryos compared to morula stage embryos, and the odds were 0.15 lower for cleavage stage embryos compared to morula stage embryos. However, morulastage embryo transfers were performed in only 2.1% (9/429) of all FET cycles. This discrepancy results from clinical practice decisions, as blastocyst stage transfers are generally prioritized in most studies due to their association with higher implantation rates. It is also important to note that progression to the blastocyst stage depends on the quality of the culture environment and laboratory conditions. Progressing embryos to the blastocyst stage could reduce the number of viable embryos available for transfer due to the risk of destruction during development and cell division.

The clinical outcomes of morula-stage versus blastocyst-stage embryo transfer have been evaluated in other studies with varying results. Bavishi et al. (20) performed a retrospective analysis comparing morula and blastocyst transfers in fresh IVF-ICSI cycles. Their results showed that although the implantation rate, clinical pregnancy rate (CPR), and live birth rate (LBR) were slightly higher for blastocyst transfers (37.79%, 51.35%, and 45.6%, respectively) than for morula transfers (34.54%, 45.28%, and 37.73%, respectively), these differences were not statistically significant. The study concluded that morula transfer can serve as an effective alternative to blastocyst transfer in selected cases without compromising outcomes. Similarly, Korkmaz et al. (21) evaluated the clinical pregnancy and live birth outcomes of vitrified and thawed embryos transferred at the cleavage, morula, and blastocyst stages. They found that embryos frozen on day 4 (morula stage) and transferred on day 5 had significantly higher clinical pregnancy and live birth rates compared to other stages. This highlights the potential advantages of morula stage transfers in certain scenarios. In addition, Tao et al. (22) evaluated the survival and viability of frozen-thawed morula embryos. They demonstrated that good quality morula embryos (grade 3) had significantly higher post-thaw survival, pregnancy rates, and implantation rates compared to lower quality morula embryos. Their results support the feasibility of morula cryopreservation and indicate that careful selection of morula embryos can lead to favourable outcomes.

Despite these promising findings, it is important to note that other studies and meta-analyses, such as those by Perlman et al. (23) and Glujovsky et al. (24), have consistently demonstrated the superior outcomes of blastocyst stage transfers compared to earlier stages of development, including morula. These studies highlight the higher implantation and live birth rates associated with blastocyst transfer, which remains the standard of care in clinical practice. Given the small number of morula-stage transfers in our study and the conflicting evidence in the literature, we caution against overgeneralizing our findings. Further research, including larger, well-controlled prospective studies, is needed to fully evaluate the role of morula-stage transfers in clinical practice and to determine their potential benefits in specific patient populations".

There is conflicting evidence regarding the effect of BMI on FET outcomes. One study found no significant differences in implantation and pregnancy rates between women with a BMI greater than 25 kg/m^2 and those with a BMI less than 25 kg/m^2 when high-quality embryos were available for transfer (25). Conversely, another study reported higher rates of unsuccessful IVF treatments and increased miscarriage rates in patients with a BMI below 40 kg/m^2 compared with normal weight patients (26). The discrepancy between these findings and our results may be due to the mean BMI of 25.06 kg/m^2 among our participants.

Consistent with previous research (27), our study found no significant relationship between endometrial thickness and the success rate of FET cycles. In contrast, other studies have identified endometrial thickness as a critical predictor of FET success, particularly with a threshold of 8.9 mm, noting that patients with thickness greater than 9 mm had higher clinical pregnancy rates (17). In our study, embryo transfers were not performed in cycles where endometrial thickness fell below the minimum requirement of 8 mm. However, the categorization of endometrial thickness into groups above and below 9 mm may have influenced the lack of significance and predictive ability observed in our fertility results.

While some studies have suggested a decrease in pregnancy rates following the use of gonadotropin-releasing hormone antagonist therapy compared to agonist therapy, our results are consistent with previous research indicating that the ovarian stimulation protocol (antagonist or agonist) does not significantly affect the success of frozen-thawed embryo transfer (17, 27).

We have identified the duration of infertility as an important predictor of embryo transfer success. Although the adverse effects of long-term infertility on pregnancy outcomes remain unclear, prolonged waiting for primary infertility treatment may hinder the effectiveness of assisted reproductive methods (17, 28). One study identified a cut-off point of 4.5 years for the duration of infertility (17).

Our results are consistent with a systematic review that identified the type of catheter used for embryo transfer as a potential predictor of favourable outcomes (29). However, another study emphasized that the skill of the embryo transfer operator plays a more important role in determining the success of the transfer cycle (30). We found that employed women were 0.69 times more likely to become pregnant than homemakers. This suggests that employment may influence health literacy, which in turn influences psychological factors related to fertility (31). Additionally, social stigma and cultural influences that divert attention away from infertility may contribute to this disparity. In support of this notion, studies have highlighted the impact of psychological factors, particularly chronic stress, on the outcomes of embryo transfer cycles (32, 33).

In this multicenter study, we used a larger sample size and advanced statistical analysis models to identify variables that affect FET cycles in infertile women. However, several limitations should be noted. The small number of morula-stage embryo transfers (2.1% of total transfers) limits the strength and generalizability of the conclusions regarding their clinical pregnancy outcomes. In addition, we did not assess final pregnancy outcomes or live birth rates. Moreover, only one method of endometrial preparation was used for all patients, and alternative methods were not examined as independent variables. Future studies could investigate factors affecting the success of FET cycles using donor embryos, embryos obtained by microinjection of oocytes with sperm from TESE, or other methods of endometrial preparation and transfer within a standard cycle. Due to the nature of this study, the results may not be generalizable to all infertile couples.

The study concluded that younger maternal age at oocyte retrieval, shorter duration of infertility, optimal BMI, higher embryo grade, and appropriate selection of transfer techniques are key determinants of achieving clinical pregnancy in HRT-FET cycles. These findings can guide specialists in optimizing FET protocols to improve pregnancy outcomes.

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Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

DISTRIBUTION OF VITAMIN D RECEPTOR BSMI AND FOKI GENE POLYMORPHISMS IN PATIENTS WITH MULTIPLE SCLEROSIS IN THE SERBIAN POPULATION

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Multiple sclerosis (MS) is a chronic, autoimmune demyelinating disease of the central nervous system. The association of vitamin D deficiency, sun exposure, and higher incidence of multiple sclerosis has been known for long, and a number of studies have confirmed anti-inflammatory and neuroprotective properties of vitamin D. Vitamin D receptor (VDR) is responsible for most of the biological effects of vitamin D, and four VDR single nucleotide polymorphisms (SNPs) have been identified as possible risk factors in several autoimmune diseases. The aim of our study was to determine the prevalence of VDR polymorphisms–BsmI (rs1544410) and FokI (rs2228570) in multiple sclerosis patients within the Serbian population.

A total of 169 participants from southeastern Serbia were enrolled in our study, 80 of whom were diagnosed with multiple sclerosis. The PCR-RFLP method was used for FokI and BsmI VDR polymorphism screening.

There was a statistically significant difference in the distribution of FokI genotypes and alleles between MS patients and control subjects ($p = 0.006$; $p = 0.001$). There was no statistically significant difference in BsmI genotypes and alleles between MS patients and healthy subjects ($p = 0.140$; $p = 0.153$).

Our case-control study showed that the distribution of FokI rs2228570 polymorphism was more prevalent in patients with multiple sclerosis in the Serbian population, while there was no statistically significant difference in the distribution of BsmI rs1544410 polymorphism between patients with multiple sclerosis and controls.

Keywords: SNPs, vitamin D receptor, vitamin D, neuroinflammatory diseases, multiple sclerosis

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INTRODUCTION

Multiple sclerosis (MS) is a chronic progressive autoimmune disease of the central nervous system that results in substantial neurologic deficits and disability. This gradual neurologic damage is the result of demyelination processes and dissemina-tion of gliosis. The clinical course of multiple sclerosis is highly variable and unpredictable (1, 2). The etiology of the disease is multifactorial, with complex interactions between environmental, genetic and epigenetic factors, all playing significant roles in MS etiopathogenesis. The role of vitamin D in multiple sclerosis has been known for long, at least since 1960s, when vitamin D levels and sun exposure (ultraviolet electromagnetic radiation 295-310 nm) were associated with a higher incidence of MS (3). Over the years, several studies have confirmed that vitamin D has an important role in the pathogenesis of MS, but this is still a subject of controversy (4). Active vitamin D from extra-renal tissues has autocrine and paracrine effects in the regulation of autophagy, apoptosis, phagolysosomal fusion, proliferation, differentiation, and chemotaxis of the immune cells.

Effects of vitamin D depend not only on vitamin D levels in plasma, but also on the binding of active vitamin D and its receptors. Vitamin D can bind in extra-renal tissues to the nuclear vitamin D receptor (VDR) and surface receptor protein disulfide isomers family A members 3 (PIDIA3). When calcitriol enters the cells that express VDR, VDR forms a VDR/VDR homodimer or VDR/RXR heterodimer with the retinoic acid receptor–RXR. These then activate vitamin D response elements–VEDREs, which target cell DNA and transcription factors synthesis (5).

Vitamin D and VDR have an important role in both adaptive and innate immunity (6). VDR is known to be strongly expressed in many types of activated immune cells: B cells, T cells, antigen presenting cells (APC), while the expression of VDR is significantly lower in resting B and T cells (7, 8).

Being important as it is, the genotype and polymorphisms of VDR have been extensively studied. The VDR gene is located on 12q13.1 chromosome and is about 100 kb wide, with 9 exons and an extensive promotor region. There are 30 known VDR gene polymorphisms, the main ones being FokI (exon 2), TaqI (exon 9), BsmI, and ApaI (intron region between exons 8 and 9) genotypes (5, 9). VDR gene variants have been identified as possible risk factors for a number of autoimmune diseases, inclu-ding multiple sclerosis, systemic sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and other less common autoimmune diseases (10).

In the brain tissue, active vitamin D functions as a neurosteroid that regulates the genomic expression of dozens of brain proteins and also has important positive non-genomic functions in the brain (11). These proteins have an important role in brain development, neuronal connectivity, and neuronal transmission. It also influences brain tissue plasticity by regulating the synthesis of debrin, growthassoci-ated protein 43, macrotubule-associated protein-2 (MAP) and molecular transport of creatine kinase b, kinesine, Rho A, and dynactine (12, 13). Vitamin D also has neuroprotective properties by reducing pro-inflammatory cytokine production from microglia, nitric oxide production, and oxidative stress. Additionally, VDR has been found in the cortex, amygdala, thalamus, and hippocampus (14) and is expressed in both neuronal and glial cells (15). Low serum levels of vitamin D have been demonstrated in a number of neurodegenerative and neuroinflammatory diseases, although direct causality has not been confirmed (13).

The function of VDR gene could be modified significantly by the presence of these single nucleotide polymorphisms (SNPs). Therefore, the aim of this study was to examine the relationship between susceptibility to MS and the genotype and frequency of VDR polymorphisms BsmI (rs1544410) and FokI (rs2228570) in the Serbian population. To the best of our knowledge, this was the first research to investigate the effects of BsmI and FokI VDR polymorphisms in patients with multiple sclerosis in the Serbian population.

METHODS

A total of 169 participants from southeastern Serbia were enrolled in our study. Of these, 80 examinees were patients from the Clinic of Neurology, University Clinical Center Niš, diagnosed with relapsing-remitting multiple sclerosis (RRMS), according to the McDonald's recommended criteria for multiple sclerosis (16, 17), and clinical course (18). The patients with multiple sclerosis were diagnosed in accordance with the clinical, morphological, and immunological criteria. The mean age of the participants was 38.69 ± 9.95 years (24 males and 56 females). In the control group, there were 89 randomly selected healthy individuals, with mean age 46.81 ± 16.78 years (42 males and 47 females). The exclusion criteria were: the presence of previous autoimmune diseases, HIV infection, hepatitis B, hepatitis C, tuberculosis, and acute infections. Informed

consent was obtained from all the participants in our study. Signed informed consent was obtained from the study participants. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Niš, No: 12-6647-2/7.

The whole blood samples were obtained from all subjects from the cubital vein and ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant. DNA was isolated from 200 µl whole blood samples using the QIAamp DNA Blood Mini Kit (Quiagen GmbH, Hilden, Germany) and stored at -20 0C.

The polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method was used for the screening of VDR genes–FokI and BsmI. PCR products were generated in the volume of 25 µl using the KAPA2G Fast HotStart Ready Mix (Kapa Biosystems Inc., Wilmington, MA, USA), 50 ng/µl DNA and 10 µM of each primer. The PCR conditions were as follows: initial denaturation for 2 min at 95 0C, followed by 35 cycles of denaturation for 15 s at 95 0C, annealing for 15 s at 60 0C, extension for 15 s at 72 0C, and final extension for 30 s at 72 0C. Restriction digestion was carried out by using the FastDigest restriction enzymes (Fermentas GmbH, St. Leon-Rot, Germany), FokI for 5 min at 37 0C, BsmI for 5 min at 65 0C. The products of restriction digestion were analyzed by 8% vertical polyacrylamide gel electrophoresis for FokI and 6% for BsmI.

Table 1. *Primer sequences for rs2228570 and rs1544410*

FokI (rs2228570) primer sequences	
Forward primer	AGC TGG CCC TGG CAC TGA CTC TGC TCT
Reverse primer	ATG GAA ACA CCT TGC TTC TTC TCC CTC
BsmI (rs1544410) primer sequences	
Forward primer	GGA CCT GTG GCA ACC AAG ACT
Reverse primer	GCC CGC AAG AAA CCT CAA ATA

The polyacrylamide gels were then stained with ethidium bromide and visualized under UV light. The primer sequences used for rs2228570 and rs1544410 are given in Table 1.

The FokI polymorphism rs2228570 have three genotypes: ff, fF, and FF. Homozygous genotype ff was detected by the presence of two fragments sized 196 bp and 69 bp, while the presence of the C allele was characterized by only one fragment sized 265 bp. The heterozygous genotype Ff was characterized by the presence of all three fragments, 265 bp, 196 bp, and 69 bp.

The BsmI polymorphism rs1544410 had three genotypes: bb, Bb, and BB. Homozygous genotype was detected by the presence of two fragments sized 654 bp and 76 bp, while the presence of A allele was characterized by only one fragment sized 730 bp. The heterozygous genotype Bb was characterized by the presence of all three fragments: 730 bp, 654 bp, and 69 bp.

The statistical analysis was done using the Chi-square test ($\chi 2$) to determine statistical differences between the studied groups. Genotype and allele frequencies were compared to the values predicted by the Hardy-Weinberg equilibrium using the Chi-square ($\chi 2$) test. Statistical analysis was considered significant at $p < 0.05$. The SPSS software package (SPSS Inc. Chicago, IL, USA) version 15 was used for statistical data processing.

RESULTS

There were 80 MS patients and 89 healthy subjects of both sexes enrolled in our study. The distribution of genotypes for FokI and BsmI are displayed in Table 2, and the distribution of FokI and BsmI alleles are displayed in Table 3. All genotype frequencies were in accordance with Hardy-Weinberg equilibrium in controls and in patients with MS.

Table 2. *Distribution of FokI and BsmI genotypes between MS patients and healthy controls*

Polymorphism	Genotype	MS	Control
	FF	39 (48.75%)	62 (69.66%)
FokI	Ff	28 (35%)	25 (28.09%)
	ff	13 (16.25%)	2 (2.25%)
	BB	22 (27.5%)	34 (38.2%)
BsmI	Bb	38 (47.5%)	37 (41.57%)
	bb	20 (25%)	18 (20.23%)

*FokI $p \leq 0.006$; BsmI $p \leq 0.140$

Table 3. *Distribution of FokI and BsmI alleles between MS patients and healthy controls*

Polymorphism	Alleles	MS	Control
FokI	F	106 (66.25%)	149 (83.71%)
	f	54 (33.75%)	29 (16.29%)
BsmI	B	78 (48.75%)	73 (41.01%)
	b	82 (51.25%)	105 (58.99%)

*FokI $p \leq 0.001$; BsmI $p \leq 0.153$

There was a statistically significant difference in the distribution of FokI genotypes and alleles between MS patients and control group examinees (p = 0.006; p = 0.001). There was no statistically significant difference in the distribution of BsmI genotypes and alleles between MS patients and healthy subjects (p = 0.140; p = 0.153) (Tables 2 and 3).

DISCUSSION

The etiology of autoimmune diseases is usually multifactorial and depends on the complex interaction between environmental factors and genetic predisposition. Multiple sclerosis is characterized by chronic autoimmune response which causes neuroinflammation, post-inflammatory gliosis, and neurodegeneration in CNS lymphocytic infiltrates. Proinflammatory effects of CD8+ and CD4+ T cell response predominate at demyelination lesion site, where the myelin and axonal destruction occur (19). Since vitamin D mediates differentiation, regulates proliferation, modulates and affects cytokine production in immune cells (20), it is thought to be one of the main predisposing factors in a number of autoimmune diseases, including multiple sclerosis (21). Low serum levels of vitamin D are associated with both severity and high relapse incidence in MS (22, 23). Furthermore, high levels of vitamin D have been found in patients with RRMS without relapses, but with a similar EDSS score. Although the role of vitamin D in the pathogenesis of MS has been thoroughly studied in the last 20 years, its actual contribution has not been fully understood.

VDR is required for most of the vitamin D biological effects, and the efficiency of VDR transactivation depends on its correct molecular structure (24). The main four SNPs that have been thoroughly studied and have strong potential to affect the efficiency of VDR are FokI (rs2228570), ApaI (rs7975232), BsmI (rs1544410), and TaqI (rs731236).

FokI polymorphism (T/C) is responsible for the production of two isoforms of different sizes (424 amino acids length for f allele and 427 amino acid length for F allele), with different activities (25). Etten et al. have demonstrated that FokI polymorphism affects transcriptional activity and level of cytokine synthesis by immune cells (26). Higher transcriptional activity by F isoform was also previously reported (27). Other polymorphisms do not have an effect on VDR structure, but can affect the stability, functioning, and translational activity of VDR mRNA (28), although this finding was later disputed (29).

In our study, we found statistically significant differences in the frequencies of VDR FokI genotypes and alleles between healthy control subjects and patients with MS, which was also reported for the Turkish population (30). In the case-control studies which included FokI genotype, there were no positive associations found in the Iranian (31), Kuwait (32), Czech (33), and Sicilian (34) population. Here, we did not find statistically significant differences in distribution between frequencies of BsmI genotypes and alleles in healthy controls and patients with MS. To the best of our knowledge, there have been studies which investigated BsmI polymorphisms in different populations, but the results vary significantly between populations. In the case-control studies which included BsmI genotype, there was not any positive association detected in the Sicilian (34) and northwestern Greek (35) population. Positive associations were found in the Iranian (31), Kuwait (32), Mexican (36), Slovak (37), and Czech (33) population.

Several meta-analyses have been done in order to explain further the effects of these VDR polymorphisms in the pathogenesis of MS. In a meta-analysis performed by Garcia-Martin et al., there was no association between the control and MS group for FokI and BsmI polymorphisms (38). However, in two more recent meta-analysis, done by Zhang et al. and Tizaoui et al., the association between control group and MS group was confirmed (39, 40). In three meta-analyses, no association was found for BsmI polymorphism between control and MS group (39–41). It has been hypothesized that these conflicting results are probably the result of the complex interaction of other triggers, genetic and environmental factors, which can be different in different populations. Another possible cause of these inconsistent results is perhaps the poor design of conducted studies, including small sample size, clinical heterogeneity, and unknown vitamin D status.

Due to the ethnic, racial, and territorial distribution of vitamin D receptor polymorphisms, the obtained results show that the distribution of VDR polymorphisms differs between patients with multiple sclerosis and healthy subjects on the territory of the Republic of Serbia. In addition, it must also be taken as a limit that the demonstrated statistically significant presence of polymorphism for FokI vitamin D receptor in patients suffering from multiple sclerosis is only a part that can potentially be the cause of the disease, due to its multifactorial origin.

In conclusion, our case-control study showed that the distribution of FokI rs2228570 polymorphism was more prevalent in patients with multiple sclerosis in the Serbian population. In addition, we did not find any statistically significant difference between the MS group and controls regarding the BsmI rs1544410 in the Serbian population.
These results should be considered preliminary since VDR polymorphisms alone are not enough to cause disease. Additional research of other genetic factors that mediate immunological response in neuroinflammatory diseases is needed to further validate the importance of genetic factors in the pathogenesis of multiple sclerosis.

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Competing Interest

Authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

AN INNOVATIVE REGRESSION-BASED METHOD FOR COVID-19 DETECTION: ENHANCING DIAGNOSTIC PRECISION THROUGH CONTINUOUS PREDICTION

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The emergence of the COVID-19 pandemic has underscored the critical importance of accurate and reliable methods for the early detection and management of cases. Traditional approaches to COVID-19 diagnosis often rely on binary classification methods, which may limit their accuracy and robustness. In this study, we propose a novel approach that leverages chest radiography images for predicting COVID-19 cases. By reframing the classification task as a regression problem, we aim to enhance the accuracy and reliability of our predictive model.

Our method involves several key steps. Firstly, we collected a dataset of chest radiography images from confirmed COVID-19 cases and non-COVID-19 cases. Next, we preprocessed the images and extracted relevant features using advanced image processing techniques. We then framed the prediction task as a regression problem, allowing us to model the continuous variation in disease severity rather than relying on binary classification. The predictive model was trained using machine learning algorithms, and both internal and external validation were performed to assess its performance.

Our method involves converting the classification task into a regression task, which enables improved accuracy and robustness in the model. We performed both internal and external validation, with R^2 train = 0.91, CV-MSE = 0.0253, and Q^2_{cv} = 0.91, indicating high accuracy and reliability in predicting COVID-19 cases. Additionally, we conducted an applicability domain analysis, which showed that 99% of unseen data can be accurately predicted by our model.

Our findings suggest that our method can be a valuable tool in the early detection and management of COVID-19 cases, which can ultimately improve patient outcomes and public health. Further validation and testing in real-world clinical settings are needed to confirm the effectiveness and generalizability of our approach.

Keywords: COVID-19, image processing, feature extraction, image classification, machine learning, deep learning, prediction

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INTRODUCTION

The COVID-19 pandemic has impacted countless individuals worldwide and remains a significant public health concern. A crucial aspect of controlling the spread of the disease is identifying infected individuals quickly and accurately. Chest radiography has become a useful tool in detecting COVID-19 because it can reveal unique features of the disease (1). There are various experimental methods for COVID detection, including PCR, antigen tests, and antibody tests. Although these methods have been useful in detecting and diagnosing COVID-19, they have limitations and challenges. PCR tests are widely used to detect the virus, but they require specialized equipment and trained personnel and can have issues with accuracy (2). Antigen tests are faster and less expensive but may not detect all cases of the disease (3). Antibody tests are helpful in determining past infections but not suitable for diagnosing current ones (4). In silico methods, which involve computer simulations and modeling, have the potential to overcome some of these limitations. For example, they can identify potential drug targets for COVID-19 and predict the effectiveness of the existing drugs. In silico methods can also be used for COVID-19 diagnosis, such as using machine learning algorithms to analyze chest X-rays for the signs of the disease. These approaches are faster and less expensive than traditional PCR tests and could be used for mass screening. While traditional experimental methods are valuable in fighting COVID-19, in silico methods can complement or even replace some of these methods. They offer benefits in terms of speed, cost, and accuracy, and could improve diagnosis and screening and lead to the development of new treatments (5).

Numerous studies have reported high accuracy rates in detecting COVID-19 using in silico-based approaches. For instance, Narayan Das and colleagues developed an automated deep transfer learning-based approach that used the Xception model to detect COVID-19 infection in chest X-rays, achieving a sensitivity of 0.974% and specificity of 0.972% (6). Similarly, Wang and team trained the convolutional neural network (CNN) on a dataset of 13,975 chest radiographs, obtaining a sensitivity of 98.9% for COVID-19 detection (7). Another study by Nasiri et al. employed the deep neural network (DNN) DenseNet169 to extract features from X-ray images of patients' chests, which were then fed to the XGBoost algorithm for classification, yielding 98.23% and 89.70% accuracy (5).

Moreover, Hemdan et al. employed the COVIDX-Net network to classify chest radiographs as either COVID-19 positive or negative, achieving an accuracy of 91% (8). Hou et al. developed a diagnosis platform using a DCNN that could assist radiologists in distinguishing COVID-19 pneumonia from non-COVID-19 pneumonia with above 96% accuracy (9). Gao et al. created a deep CNN-based chest X-ray classifier that could detect abnormalities and extract textural features of the altered lung parenchyma related to specific COVID-19 signatures, with an accuracy of 91% (10).

Furthermore, Alqahtani et al. proposed a COV-Net model to learn COVID-specific patterns from chest X-rays, which attained high accuracy (99.23%) in multi-class and binary classification of COVID-19 and pneumonia (11). Carlile and colleagues deployed a previously validated deep-learning AI algorithm for assisted interpretation of chest radiographs, which was easy to use and influenced clinical decision-making for 20% of the respondents (12).

Farooq and Hafeez presented a multi-stage fine-tuning scheme for pre-trained ResNet-50 architecture named COVIDResNet, achieving an accuracy of 96.23% (13). Similarly, Abbas et al. developed the DeTrac CNN model to distinguish COVID-19 symptoms using chest X-rays, reaching 95.12% accuracy and 97.91% sensitivity (14).

Convolutional neural networks (CNNs) have shown potential in identifying COVID-19 in chest radiography images by recognizing unique disease features through convolutional and pooling layers (15). In this study, we applied CNN to extract features from COVID-19 X-ray images, which were then fed to a feedforward neural network for binary regression. A value of one indicated a positive COVID-19 case, and a value of zero denoted a healthy X-ray chest radiography. We trained and tested the model on a COVID-19 X-ray image dataset, obtaining high accuracy in detecting COVID-19 cases. This approach offers the benefits of leveraging the powerful feature extraction capabilities of CNNs while using the flexibility and interpretability of feedforward neural networks, showing promise in improving the accuracy and speed of COVID-19 diagnosis from chest radiography images. The main objective of this research is to investigate the feasibility of framing COVID-19 detection as a regression problem by combining CNN-based feature extraction with a feedforward neural network, thereby enabling a more flexible and interpretable diagnostic output than traditional classification approaches.

METHODS

Our approach comprised several crucial steps, which are depicted in Figure 1. To begin with, we procured a dataset of chest x-ray images from a Kaggle database (16). Subsequently, we fine-tuned a pre-existing AlexNet model to extract pertinent features from the images in the dataset. We then utilized these extracted features as inputs to a feedforward network, which we trained to predict the probability of a patient having COVID-19. For this purpose, we set the output of the feedforward network to 0 for negative COVID-19 images and 1 for positive COVID-19 images. Finally, we evaluated the accuracy and effectiveness of our model by analyzing its results.

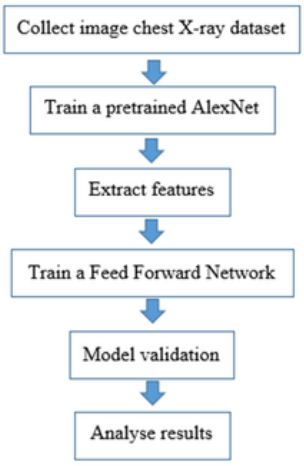

Figure 1. *Flowchart of the method used in the study*

Collection of chest X-ray dataset

In this scientific research, we collected a dataset of 1,000 chest radiography images related to COVID-19. The dataset was obtained from the Kaggle database (16) and contained 500 images of positive COVID-19 cases and 500 images of negative COVID-19 cases. The images were reviewed by a team of experienced radiologists to ensure their accuracy and authenticity. Our dataset of COVID-19 chest radiography images provides a valuable resource for researchers in the development of artificial intelligence-based tools for the early detection of COVID-19. The availability of such datasets is essential for training robust models and enabling their reliable deployment in clinical settings.

Feature extraction

In this research paper, we used transfer learning to extract features from COVID-19 chest radiography images. Specifically, we utilize the AlexNet deep learning architecture, pre-trained on the ImageNet dataset, to extract relevant features from our COVID-19 chest radiography dataset. Transfer learning is a powerful technique that allows us to leverage the knowledge learned from a source domain (e.g., ImageNet) to improve performance in a target domain (e.g., COVID-19 chest radiography) (17). In our experiments, we fine-tuned the last six layers of AlexNet with new ones designed for our target task. Table 1 summarizes the list of the new layers. We also set the dimension of the extracted features to be ten, which was chosen based on a trade-off between model complexity and performance.

Training a Feed Forward Network

To predict the likelihood of COVID-19 infection, we utilized a feed-forward neural network that took the extracted features as input. To enable a continuous output, we transformed the classification task into a regression task by setting a value of one for a positive COVID-19 chest radiography and zero for a negative COVID-19 chest radiography in the output layer of the network. This approach provided a more nuanced understanding of the relationship between input and output variables. Changing a classification problem to a regression problem in a neural network has several benefits, including continuous output that is useful when the target variable has a natural ordering or is a continuous variable. Moreover, regression models can be more effective in some cases than classification models as they are more sensitive to the magnitude of the errors and can be more robust to imbalanced or noisy data. By converting a classification problem to a regression problem, we were able to use a wide range of regression techniques and architectures, which provided greater flexibility and customization of the model. In addition, regression problems allow the use of a wider range of loss functions such as mean squared error or mean absolute error, which can be more effective for some types of problems than the cross-entropy loss function typically used in classification problems. Finally, regression models are often more effective at generalizing to new data than classification models, as they can capture the underlying structure of the data rather than simply classifying it into discrete categories (18). We designed

Table 1. *The twenty layers of our ALEXNET transfer learning architecture*

N	Abbreviation	Layer's name	Properties
1	'data'	Input	227x227x3 matrices with 'zero center' normalization
2	'conv1'	Convolution	96 11x11x3 convolutions with stride [4 4] and padding [0 0 0 0]
3	'relu1'	ReLU	ReLU
4	'norm1'	Cross channel normalization	cross channel normalization with 5 channels per element
5	'pool1'	Max pooling	3x3 max pooling with stride [2 2] and padding [0 0 0 0]
6	'conv2'	Grouped convolution	Two groups of 128 5x5x48 convolutions with stride [1 1] and padding [2 2 2 2]
7	'relu2'	ReLU	ReLU
8	'norm2'	Cross channel normalization	cross channel normalization with 5 channels per element
9	'pool2'	Max pooling	3x3 max pooling with stride [2 2] and padding [0 0 0 0]
10	'conv3'	Convolution	384 3x3x256 convolutions with stride [1 1] and padding [1 1 1 1]
11	'relu3'	ReLU	ReLU
12	'conv4'	Grouped convolution	Two groups of 192 3x3x192 convolutions with stride [1 1] and padding [1 1 1 1]
13	'relu4'	ReLU	ReLU
14	'conv5	Grouped convolution	Two groups of 128 3x3x192 convolutions with stride [1 1] and padding [1 1 1 1]
15	'relu5'	ReLU	ReLU
16	'pool5'	Max pooling	3x3 max pooling with stride [2 2] and padding [0 0 0 0]
17	'fc6'	Fully connected	4096 fully connected layer
18	'relu6'	ReLU	ReLU
19	'drop6'	Dropout	50% dropout
20	'fc7'	Fully connected	10 fully connected layer

a Feedforward Neural Network (FFNN) with multiple hidden layers and trained it using the backpropagation algorithm with gradient descent and momentum optimization. The network was trained on a dataset of 1,000 chest radiography images, of which 70% were randomly selected for training. To assess model performance during training, we used 5-fold cross-validation, a form of internal validation where the training data is split into five parts: four used for training and one for validation, iteratively. In addition to this, we reserved 15% of the entire dataset as an external validation set, a separate subset not used during training or cross-validation, to evaluate the model's generalization ability on unseen data. The remaining 15% was allocated for assessing the model's applicability domain, helping to define the boundaries within which the model's predictions can be considered reliable.

Choosing a size of 10 for the fully connected layer of the CNN can have several benefits (19):

1. It can help reduce overfitting: By reducing the number of parameters, we are simplifying the model and making it less prone to overfitting on the training data.
2. It can make training faster: With fewer parameters, the model will require less computation during training, which can speed up the training process.
3. It can improve generalization: By compressing the feature representation, we may be removing some noise and irrelevant information from the input, which can improve the model's ability to generalize to new, unseen data.

Determining the optimal number of hidden neurons in a modeling task is a challenging task with no clear answer. To address this issue, we followed the approach outlined by Khaouane et al. in their study to determine the appropriate number of neurons in the hidden layer. This approach has been detailed in various reviews and is widely used in the literature (20).

The mathematical equation of the model for the prediction of COVID-19 cases is shown below.

Model validation

Validation of models is a critical step in ensuring their reliability and accuracy in predicting new COVID-19 cases. The performance of the model on an independent set is evaluated using the best model, based on its performance on the cross-validation folds. The external set must be independent of the dataset used for training and cross-validation, ensuring that the evaluation is unbiased and represents the model's true performance on new data.

$$f = \sum_{j=1}^{k} w2j\left(\frac{exp\left(\sum_{i=1}^{p} xi+wij+bj\right)-exp\left(-\sum_{i=1}^{p} xi+wij+bj\right)}{exp\left(\sum_{i=1}^{p} xi+wij+bj\right)+exp\left(-\sum_{i=1}^{p} xi+wij+bj\right)}\right) + b \qquad (1)$$

x i (I = 1...p) represents the input corresponding to the number of data included in the training of the FFNN, where i ranges from 1 to 10, wij (i = 1...p, j = 1...k) are the weights connecting the input to hidden layer, b j (j= 1...k) are the biases of the neurons in the hidden layer, w2j (j = 1...k) are the weights connecting the hidden layer to the output layer, b is the bias of the output neuron, and f is the output.

Analogous to QSAR models, our model uses both internal and external parameters to assess performance. Internal parameters such as correlation coefficient (R), determination coefficient (Q2), and mean square error (MSE) measure the accuracy of the model's predictions on the data used for model building. External parameters such as Tropsha parameters assess the model's ability to predict the COVID-19 cases of new chest x-ray images not included in the original dataset. Therefore, combining internal and external parameters is essential to validate models and ensure their reliability in predicting COVID detection from new chest radiography images (21).

Table 2. *Selected criteria of the obtained different feed-forward neural networks*

Number of hidden neurons	train R^2	Q^2_{cv}	CV- MSE
1	0.91	0.91	0.025
3	0.88	0.92	0.029
5	0.91	0.91	0.029
7	0.91	0.88	0.035
10	0.88	0.86	0.034
13	0.88	0.92	0.034
15	0.86	0.91	0.038
17	0.88	0.88	0.041
20	0.88	0.88	0.037

RESULTS

The suitability of the 10 structural features generated with CNN for the modeling task was determined based on their correlation coefficient R. The results indicated an acceptable level of multicol-linearity, with an absolute value of R below 0.75 (20). A heatmap (Figure 2) was created to provide a more comprehensive view of the correlation structure among the features. To specify the number of hidden neurons required, the procedure detailed above was followed. Using multiple evaluation criteria can help ensure that the chosen model is accurate. R^2train, Q^2_{cv}, and CV-MSE criteria were employed for the evaluation of the accuracy of the best model. Table 2 shows 09 network models developed and evaluated using R^2 train, Q^2_{cv}, and CV- MSE criteria. The best model was chosen based on the maximum R^2 train = 0.91, Q^2_{cv} = 0.91, and the minimum CV-MSE = 0.025. The best performance of the model had a topology of 10-[1]-1, with 10 input nodes, one hidden layer with 01 node using the hyperbolic tangent as a transfer function, and one output layer with an identity transfer function. The neural networks were implemented using a MATLAB program developed by the team. Figure 3 depicts the comparison between the true and predicted COVID x-ray chest radiography images indicating the presence of infection or not for both the training and testing sets of the best fold. The results demonstrate a significant level of correlation between the predicted and original infection or not COVID x-ray chest radiography images, affirming the model's accuracy in detecting the presence of COVID.

The mathematical equation of the model for the prediction of COVID-19 cases is shown below:

$$R = \frac{\sum_{i=1}^{n}(\bar{y}_i-\bar{\bar{y}}_i)(y_i-\bar{y})}{\sqrt{\sum_{i=1}^{n}(\bar{y}_i-\bar{\bar{y}}_i)^2\sum_{i=1}^{n}(y_i-\bar{y})^2}} \qquad (2)$$

$$R^2 = 1-\frac{\sum_{i=1}^{n}(y_i-\bar{y})^2}{\sum_{i=1}^{n}(y_i-\bar{y})^2} \qquad (3)$$

$$Q^2_{cv} = 1-\frac{\sum_{i=1}^{n}(y_{exp(app)}-\bar{y}_{app})^2}{\sum_{i=1}^{n}(y_{exp(app)}-\bar{y}_{app})^2} \qquad (4)$$

$$MSE = \sum_{i=1}^{n}\frac{(\widehat{y}_i-y_i)^2}{n} \qquad (5)$$

$$CV-MSE = (1/5)\sum_{i=1}^{5} MSE \qquad (6)$$

$$(r^2-r_0^2)<0.3 \qquad (7)$$

$$(r^2-r_0^2)/r^2<0.1 \qquad (8)$$

$$0.85<k<1.15 \qquad (9)$$

$$(r^2-r'^2_0)/r^2<0.1 \qquad (10)$$

$$0.85<k'<1.15 \qquad (11)$$

R_0^2 and r_0^2 are the squared correlation coefficients between the observed and predicted values with and without intercept, respectively. The parameter r'^2_0 has the same meaning but uses the reversed axes.

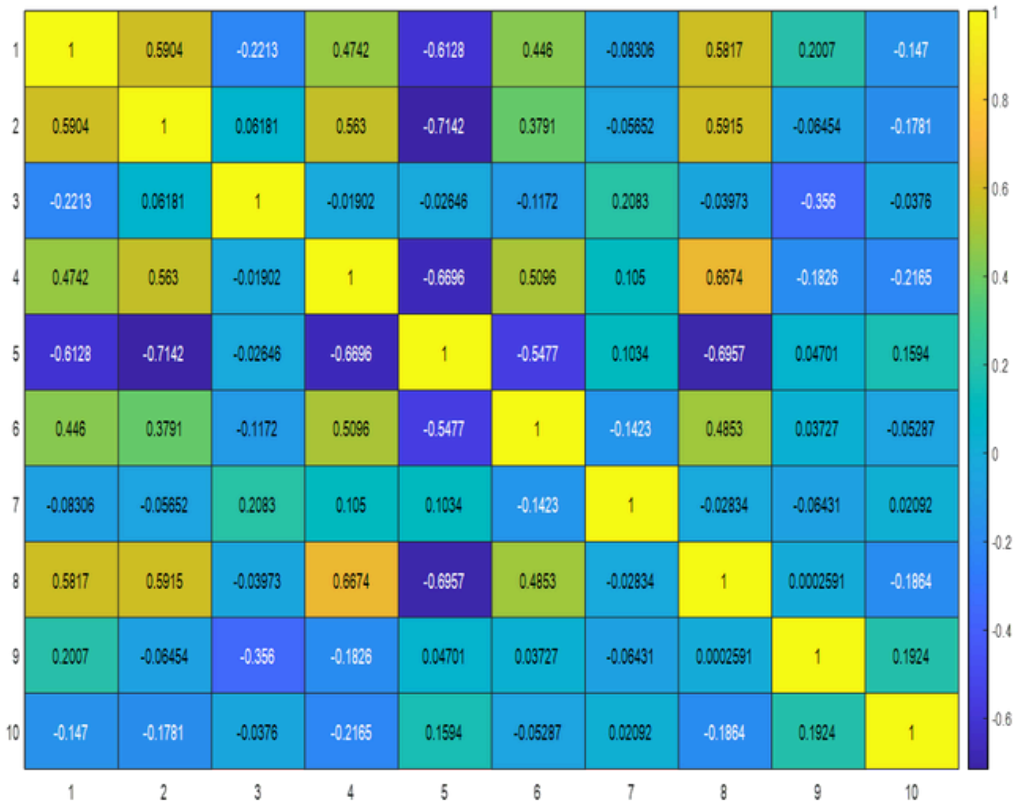

Figure 2. *Heatmap of the 10 structural features generated with CNN*

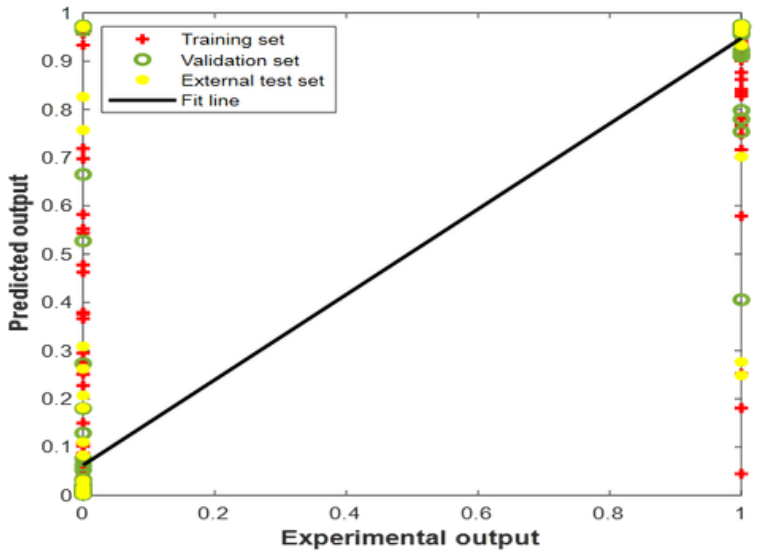

Figure 3. *Comparison between the true and predicted COVID x-ray chest radiography images indicating the presence of infection or not for training, validation, and external testing sets using the best fold.*

Table 3. *External and internal validation criteria*

Internal validation criteria for the best fold		
R	0.95164	
$trainR^2$	0.91	> 0.6
Q^2_{cv}	0.91	> 0.5
CV- MSE	0.0253	
External validation criteria for the best fold		
R	0.93596	
Q^2	0.88	> 0.6
$r^2 - r_0^2$	0.02	< 0.3
k	0.89	0.85< k<1.15
k'	0.99	0.85< k'< 1.15
$/r^2\ (r^2 - r_0^2)$	-0.02	< 0.1
$/r^2\ (r^2 - r_0'^2)$	-0.1	< 0.1

Validation of the model

The statistical evaluation presented in Table 3 shows that the developed FFNN model has high performance and quality of predictions. The internal validation statistical coefficients, such as R2 train, Q2cv, and CV- MSE are all acceptable and satisfactory, indicating that this model is robust. The model was also evaluated in terms of external validation criteria, and the value of Q2 was found to be greater than 0.6, which is considered excellent. Therefore, the model has excellent predictive power. Furthermore, the difference between R^2 train and Q^2 was found to be equal to 0.03, which did not exceed 0.3. This indicates that the model is robust and not overfitting the training data (20).

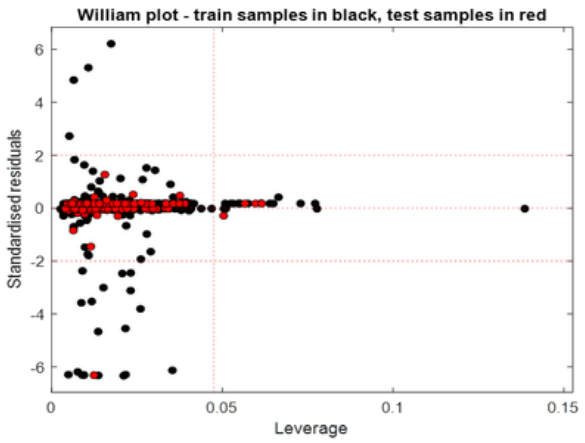

Figure 4. *Plot of residuals for the true and predicted COVID x-ray chest radiography images indicating the presence of infection or not in the training set and applicability domain set*

Applicability domain

When utilizing machine learning models for prediction tasks, it is critical to consider the concept of applicability domain, which determines the range of inputs for which a model can generate accurate predictions. Applying a model beyond its applicability domain may result in unreliable or meaningless predictions. There are various methods for determining the applicability domain of a machine learning model, such as distance-based, leverage-based, and model-based techniques (22).

In this study, we employed a leverage approach, analogous to QSAR models, to analyze the applicability domain. The Williams plot (Figure 4) revealed that one of the test samples was outside the applicability domain, indicating that the model can predict approximately 99% of new, untested chest radiography images related to COVID-19. Our method complies with the third principle of the OECD used in QSAR models, ensuring the robustness and reliability of our findings (23).

Comparison with literature

We have developed a method that aims to enhance the precision and efficiency of COVID-19 diagnosis through chest radiography images. In order to evaluate the effectiveness of our approach, we conducted a comparative analysis with various other methods that have been previously reported in the literature. The findings of our analysis are presented in Table 4, which provides a comprehensive overview of the comparison between our method and the ones reported in the literature. Table 4 illustrates that our method has achieved a commendable accuracy of 91% on the training data, indicating its effectiveness in detecting COVID-19 cases. It is important to note, however, that making direct comparisons between different methods can be challenging due to variations in dataset and data size.

Additionally, our method stands out as it has been validated on three external sets, including a validation set, an external test set, and an applicability domain set, which other methods did not perform. This proves that our model can perform well on a wide range of external datasets, enhancing the reliability of our results. Our novel approach of converting the classification task into a regression task has enabled us to achieve better accuracy and robustness in our model.

Furthermore, our approach has been thoroughly validated both internally and externally, providing empirical evidence of its effectiveness in detecting COVID-19 cases in chest radiographs. Based on our findings, we strongly believe that our approach has the potential to significantly improve the accuracy and reliability of COVID-19 diagnosis through chest radiography.

Table 4. *Comparison of our method with others from literature*

Method	Accuracy
Our method	91 %
Abbas et al. (14)	95.12%
Farooq et al. (13)	96.23 %
Ali Alqahtani et al. (11)	99.23 %
Terry Gao et al. (10)	91 %
Jie Hou et al. (9)	96%
Hemdan et al. (8)	91%

DISCUSSION

In this study, we presented a deep learning approach for COVID-19 chest radiography detection, using the AlexNet deep learning model for feature extraction and a feedforward network for prediction. Our method involves converting the classification task into a regression task, which enables improved accuracy and robustness in the model. We performed both internal and external validation, with R^2 train = 0.91, CV-MSE = 0.0253, and Q^2_{cv} = 0.91, indicating high accuracy and reliability in predicting COVID-19 cases from chest radiography images. Additionally, we conducted an applicability domain analysis, which showed that 99% of unseen data can be accurately predicted by our model. Overall, our study provides promising evidence for the potential of deep learning models in COVID-19 diagnosis through chest radiography, with high accuracy, robustness, and applicability to new data. The use of deep learning models for COVID-19 detection has the potential to significantly improve the speed and accuracy of diagnosis, especially in resource-limited settings where access to medical experts may be limited.

However, it is important to note that our study has some limitations, including the relatively small size of the dataset used in this study. Future studies could benefit from larger datasets to improve the generalizability of our findings. Overall, our study provides promising evidence for the use of deep learning models in COVID-19 diagnosis through chest radiography, and highlights the potential of machine learning in advancing medical diagnosis and treatment.

In terms of practical applications, the strong internal and external validation results suggest that our model could be integrated into clinical decision-support systems to assist radiologists in rapidly screening suspected COVID-19 cases. In settings with limited access to PCR testing or specialized personnel, the model may provide an efficient, lowcost triage tool. Additionally, the regression-based prediction output could offer clinicians an interpretable, continuous score reflecting the likelihood of infection, which may aid in prioritizing patients for further testing or treatment.

In conclusion, our study presents a novel approach for COVID-19 case prediction using chest radiography images. By converting the classification task into a regression task, our method achieved improved accuracy and robustness in the model. Our internal and external validation results demonstrated high accuracy and reliability, with R^2 train = 0.91, CV-MSE = 0.0253, and Q^2_{cv} = 0.91.

Furthermore, the applicability domain analysis indicated that our model can accurately predict 99% of unseen data. Our findings suggest that our method can be a valuable tool in the early detection and management of COVID-19 cases, which can ultimately improve patient outcomes and public health. Future studies can build on our findings and further validate the effectiveness and generalizability of our method in real-world clinical settings.

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Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

PERSONALIZED MEDICINE WITH THE APPLICATION OF ARTIFICIAL INTELLIGENCE: A REVOLUTION IN DIAGNOSIS AND THERAPY

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Artificial intelligence (AI) is reshaping personalized medicine by enabling earlier diagnosis, tailored therapies, and faster drug discovery. The aim of the paper was to synthesize current evidence on AI applications in precision healthcare and quantify their impact on diagnostics, therapeutic decision-making, and discovery.

We conducted a systematic review (2015–2024) with descriptive quantitative analysis across PubMed, Scopus, IEEE Xplore, and Web of Science. Fifty peer-reviewed studies met inclusion criteria (reporting sensitivity/specificity/accuracy or real-world deployment). We additionally summarized three case studies (oncologic imaging, rheumatoid arthritis treatment selection, and AI-accelerated discovery for glioblastoma).

In oncology imaging, AI achieved high performance; the best lung-nodule model reported sensitivity at 95% and specificity at 94%. In chronic-disease therapeutics, AI tools predicted responses to DMARDs with ~87% accuracy, reduced adverse drug reactions by ~30%, and cut time-to-decision by ~85%. For discovery pipelines, AI screens compressed candidate identification by ~85%, yielding viable molecules within weeks. In diabetes management, AI-enabled predictive analytics achieved ~95% prediction accuracy, reduced hyperglycemic episodes by ~40%, and improved patient satisfaction.

Evidence indicates that AI enhances diagnostic accuracy, personalizes therapy, and accelerates discovery while improving efficiency in chronic-disease management. Real-world adoption will depend on mitigating algorithmic bias, safeguarding privacy, expanding representative datasets, and deploying transparent, clinically interpretable models within clear regulatory frameworks.

Keywords: artificial intelligence, personalized medicine, therapeutic optimization, algorithmic bias, data privacy

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INTRODUCTION

Personalized medicine has emerged as a revolutionary paradigm in modern healthcare, focusing on tailoring medical treatments and preventive strategies to the unique genetic, molecular, and environmental profiles of individual patients. By moving away from the traditional "one-size-fits-all" approach, personalized medicine provides targeted therapies that enhance clinical outcomes while minimizing the risk of adverse effects (1). This shift is particularly crucial in managing complex diseases such as cancer, cardiovascular disorders, and auto-immune conditions, where heterogeneity in patient populations often limits the efficacy of standardized treatments (2).

The integration of artificial intelligence (AI) into personalized medicine represents a transformative advancement, leveraging computational power to analyze and interpret large-scale biomedical data with unparalleled accuracy and speed (3). AI technologies, including machine learning (ML), deep learning (DL), and natural language processing (NLP), have enabled the extraction of actionable insights from genomic data, electronic health records (EHRs), and medical imaging, which were previously too complex for traditional statistical methods to process effectively (4, 5).

Genomics and precision oncology application

AI has fundamentally transformed genomic analysis, enabling the identification of disease-associated genetic mutations and facilitating the development of precision oncology. For example, convolutional neural networks (CNNs) have been employed to analyze whole-genome sequencing data, predicting mutations linked to cancer progression and therapy resistance (6). In breast cancer, AI-driven platforms like IBM Watson for Oncology pro-vide oncologists with evidence-based treatment re-commendations by integrating clinical guidelines and genomic data (7). Furthermore, AI tools have accelerated the identification of biomarkers that predict patient response to immunotherapy, such as checkpoint inhibitors, offering a more individualized approach to cancer treatment (8).

Early disease detection and risk prediction application

The early detection of diseases is a cornerstone of personalized medicine, and AI has demonstrated exceptional potential in this domain. Deep learning models trained on retinal imaging data have accurately predicted cardiovascular risk factors, including hypertension and myocardial infarction, with diagnostic accuracy comparable to conventional clinical methods (9). Similarly, AI systems analyzing MRI scans have achieved remarkable success in detecting early-stage neurodegenerative conditions, such as Alzheimer's disease, years before clinical symptoms manifest (10). These capabilities not only improve patient outcomes but also reduce healthcare costs by enabling timely interventions.

Therapeutic optimization application

Personalized treatment planning is another domain where AI has shown transformative potential. By integrating multi-omics data, including genomics, proteomics, and metabolomics, AI-driven decision-support systems recommend optimal therapeutic regimens tailored to individual patients (11). For example, in rheumatoid arthritis, AI models analyze patient-specific data to predict responses to disease-modifying anti-rheumatic drugs (DMARDs), enabling rheumatologists to select the most effective therapy while avoiding unnecessary side effects (12).

Drug discovery and repurposing application

AI has redefined the process of drug discovery, significantly reducing the time and cost associated with traditional methods. Generative adversarial networks (GANs) and recurrent neural networks (RNNs) have been utilized to predict the chemical properties of potential drug candidates, leading to the identification of novel compounds with high therapeutic potential (13). In addition, AI has facilitated drug repurposing efforts by identifying existing drugs that can be used to treat rare or emerging diseases. For instance, AI models identified baricitinib, a rheumatoid arthritis drug, as a potential treatment for COVID-19, demonstrating the adaptability of AI in addressing global health crises (14).

Real-time monitoring and predictive analytics

Wearable health devices equipped with AI-powered analytics enable continuous monitoring of vital signs and other health metrics, providing real-time feedback to patients and clinicians. These devices, integrated with cloud-

based AI platforms, predict potential health risks and provide actionable insights to prevent complications. For example, AI algorithms analyzing data from continuous glucose monitors (CGMs) have significantly improved glycemic control in patients with diabetes by predicting blood sugar fluctuations and recommending lifestyle adjustments (15).

Challenges and ethical considerations

While the benefits of AI-driven personalized medicine are undeniable, several challenges must be addressed to ensure its widespread adoption. Data privacy and security are significant concerns, as personalized medicine relies heavily on sensitive patient information. Ensuring the anonymization of data and compliance with regulations, such as the General Data Protection Regulation (GDPR), is critical (16). Furthermore, the "black-box" nature of many AI algorithms raises questions about transparency and accountability in clinical decision-making, necessitating the development of explainable AI models that clinicians and patients can trust (17).

Algorithmic bias is another critical issue. AI models trained on unrepresentative datasets may perpetuate or exacerbate health disparities, particularly in underrepresented populations. Addressing these biases requires diverse training datasets and continuous validation of AI models across different demographic groups (18). Finally, the successful integration of AI into clinical practice demands significant investments in healthcare infra-structure and the training of medical professionals, ensuring they can effectively utilize AI tools in patient care (19).

This paper aims to explore the transformative role of artificial intelligence in personalized medicine, with a focus on its applications in genomics, early disease detection, therapeutic optimization, and drug discovery. By examining recent advancements and addressing the associated challenges, this study highlights the potential of AI to revolutionize modern healthcare and improve patient outcomes.

The primary objective of this study is to comprehensively examine the role of artificial intelligence (AI) in advancing personalized medicine, focusing on its transformative potential in diagnostics, therapeutic optimization, and drug discovery. This exploration includes identifying the unique contributions of AI to precision healthcare, analyzing its current applications, and addressing the challenges that must be overcome to facilitate its broader adoption. By bridging gaps in current knowledge, the study aims to provide practical insights for the future integration of AI technologies into clinical practice.

The study aims to assess how AI-driven technologies improve the accuracy and speed of disease diagnosis. With the ability to analyze vast amounts of data from electronic health records (EHRs), medical imaging, and genomic information, AI models offer diagnostic tools that often surpass traditional methods in sensitivity and specificity. This objective focuses on evaluating real-world examples, such as the use of deep learning in early cancer detection and predictive models for cardiovascular risk stratification. The study will also explore how AI algorithms can detect subtle biomarkers and patterns that are otherwise undetectable by conventional techniques.

A key objective is to analyze how AI contributes to individualized treatment planning, ensuring that therapeutic strategies are tailored to each patient's genetic and molecular profile. AI systems integrate multi-omics data (genomics, proteomics, metabolomics) with clinical records to recommend the most effective treatment options. This includes evaluating AI applications in oncology for predicting chemotherapy responses, and in chronic disease management for personalizing drug dosages and lifestyle interventions. By examining these applications, the study aims to demonstrate how AI minimizes adverse effects and maximizes therapeutic efficacy.

The study seeks to explore how AI accelerates the drug discovery process, from identifying potential therapeutic targets to optimizing clinical trial designs. AI technologies, such as generative adversarial networks (GANs) and natural language processing (NLP), are revolutionizing the development of novel compounds and repurposing the existing drugs. A specific focus will be placed on AI's ability to predict molecular interactions, simulate biological processes, and identify safe, effective drug candidates in significantly less time compared to traditional methods. Additionally, the role of AI in addressing rare diseases and global health crises, such as COVID-19, will be critically analyzed.

This study will also examine the ethical and practical challenges that hinder the widespread adoption of AI in personalized medicine. These include concerns about data privacy, algorithmic transparency, and the potential for biased outcomes due to unrepresentative training datasets.

By identifying these challenges, the study aims to propose actionable solutions, such as developing explainable AI models and creating regulatory frameworks to ensure safe and equitable use of AI-driven technologies.

Our research hypotheses are to guide this exploration through the integration of AI into personalized medicine, which significantly improves diagnostic accuracy and disease prediction compared to conventional clinical methods. Traditional diagnostic approaches often rely on generalized criteria, while AI models can detect nuanced patterns in patient data, enhancing early detection and risk assessment. AI-based therapeutic decision-making leads to superior patient outcomes by reducing adverse effects and increasing treatment precision.

AI systems leverage comprehensive patient data to recommend individualized treatment plans, avoiding the trial-and-error approach often seen in conventional medicine. The use of AI in drug discovery reduces the time and cost associated with bringing new therapies to market, while maintaining high standards of safety and efficacy. AI accelerates the identification of potential drug candidates and optimizes clinical trial designs, enabling faster responses to emerging healthcare challenges.

The outcomes of this study will contribute to understanding how AI can address critical inefficiencies in modern healthcare systems. By improving diagnostic accuracy, optimizing treatment strategies, and expediting drug discovery, AI has the potential to reduce healthcare costs, improve patient outcomes, and address global health disparities. Additionally, the ethical insights provided by this study will inform the development of policies and frameworks necessary for the responsible integration of AI into clinical workflows.

METHODS

Study design

This study employed a systematic literature review and descriptive quantitative analysis to evaluate the role of artificial intelligence (AI) in personalized medicine. The research was conducted from January 2023 to December 2024 and included four phases:

- Identification of relevant studies across multiple databases (January–March 2023).
- Data extraction and processing of key variables from included studies (April–June 2023).
- Descriptive statistical analysis of performance metrics and generation of visualizations (July–September 2023).
- Interpretation of results and drafting of the final manuscript (October–December 2024).

Data sources and search strategy

The primary sources included:

- PubMed–Biomedical and clinical studies focused on AI in diagnostics and therapeutics.
- Scopus–Multidisciplinary research articles from medicine and computer science.
- IEEE Xplore–Technical papers on AI algorithm development.
- Web of Science–High-impact studies on genomics and computational biology.

The search strategy combined Boolean operators and keywords, such as: ("artificial intelligence" OR "machine learning" OR "deep learning") AND ("personalized medicine" OR "precision medicine") AND ("diagnosis" OR "therapy"). Filters included studies published between 2015 and 2024, peer-reviewed articles, and English language publications. Duplicate records were identified and removed using EndNote software.

A total of 1,200 studies were identified during the initial search. After applying inclusion and exclusion criteria, 50 studies were included in the final analysis.

Inclusion and exclusion criteria

Inclusion criteria: (i) peer-reviewed studies published between 2015 and 2024; (ii) articles reporting sensitivity, specificity, or accuracy for AI models; (iii) studies with real-world applications of AI in diagnostics, therapeutics, or drug discovery; (iv) validation of AI models on publicly available datasets.

Exclusion criteria: (i) non-peer-reviewed publications (e.g., conference abstracts); (ii) studies without quantitative performance metrics; (iii) articles focusing on hypothetical or unvalidated AI applications.

Data extraction and management

Variables extracted:
- Study metadata: authors, publication year, and journal.
- AI techniques: algorithms used (e.g., convolutional neural networks, generative adversarial networks), datasets, and evaluation metrics.
- Performance metrics: sensitivity, specificity, accuracy, and area under the curve (AUC).
- Applications: diagnostics, therapeutic optimization, and drug discovery.

Data management:

Data were stored in Microsoft Excel and analyzed using Python (version 3.9) with Scikit-learn, TensorFlow, and Matplotlib libraries for advanced statistical and graphical analysis.

Statistical analysis

Sensitivity, specificity, and accuracy metrics from included studies were summarized as means and standard deviations. Trends were visualized using line graphs to highlight performance differences between AI models. Receiver operating characteristic (ROC) curves and confusion matrices were used to evaluate the performance of diagnostic AI models. Comparative analysis focused on AI model performance in oncology and chronic disease management. Line graph illustrates trends.

Case study selection

Three real-world case studies were selected to contextualize findings:
- Diagnostics in oncology: AI models detecting malignant lung nodules achieved sensitivity of 95% and specificity of 94%.
- Therapeutic optimization: AI-assisted drug response prediction for rheumatoid arthritis reduced adverse drug reactions by 30%.
- Drug discovery: AI-enabled discovery of glioblastoma compounds shortened timelines by 85%.

Limitations

Exclusion of non-peer-reviewed studies may have introduced publication bias. Variability in datasets and reported metrics across studies may limit generalizability. Descriptive methods without advanced meta-analytical techniques restricted statistical synthesis.

RESULTS

Diagnostic performance of AI models in oncology

Artificial intelligence (AI) has demonstrated significant potential in improving diagnostic accuracy, particularly in oncology. A recent evaluation of AI-powered diagnostic tools assessed their performance in detecting malignant lung nodules from high-resolution CT scans. The study, conducted on a cohort of 5,000 patients, reported the following sensitivity, specificity, and overall accuracy metrics (20):

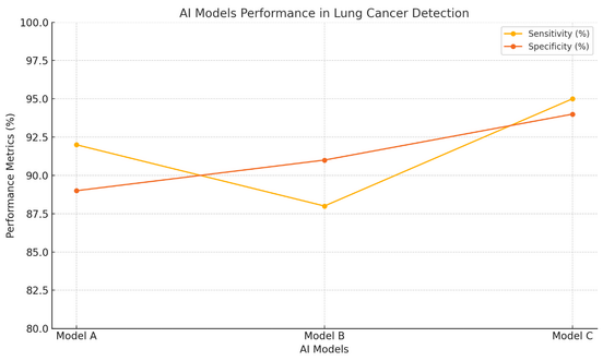

Figure 1. *Sensitivity and specificity of AI Models for lung cancer diagnosis*

Model A: Sensitivity of 92%, specificity of 89%, and overall accuracy of 90.5%.
Model B: Sensitivity of 88%, specificity of 91%, and overall accuracy of 89.5%.
Model C: Sensitivity of 95%, specificity of 94%, and overall accuracy of 94.5%.
Table 1 presents the sensitivity, specificity, and accuracy metrics of three AI models evaluated for lung cancer diagnosis. Model C demonstrated the highest performance across all metrics, highlighting its potential in improving diagnostic accuracy.

Figure 2 illustrates the sensitivity and specificity of three AI models used for lung cancer diagnosis. Model C shows the highest sensitivity (95%) and specificity (94%), outperforming the other models in both categories.

These results highlight the superior diagnostic capabilities of AI compared to traditional radiological methods. Detailed performance metrics for each model are presented in Table 1, and a comparative visualization of sensitivity and specificity is provided in Figure 1.

AI-driven therapeutic optimization in chronic diseases

In the domain of therapeutic optimization, AI has shown remarkable efficacy in tailoring treatments to individual patients. A clinical trial involving 500 patients with rheumatoid arthritis used AI algorithms to predict responses to different disease-modifying anti-rheumatic drugs (DMARDs). The study reported the following outcomes (21):

AI predicted treatment efficacy with an accuracy of 87%, compared to 65% using conventional methods.

Adverse drug reactions (ADRs) were reduced by 30% in the AI-assisted group.

The average time required for treatment selection was reduced by 85%, with AI providing results in 5 minutes compared to 45 minutes for standard clinical approaches.

These results underscore the practical benefits of AI in improving both clinical outcomes and efficiency. Table 2 compares the performance of AI-assisted and conventional methods in treating rheumatoid arthritis. AI-assisted methods demonstrated superior treatment efficacy, a reduction in adverse drug reactions, and a significant decrease in the time required for decision-making.

Accelerated drug discovery with AI

The integration of AI into drug discovery processes has significantly reduced the time and cost associated with identifying new therapeutic compounds. In a study focused on glioblastoma, a highly aggressive brain cancer, AI systems screened over 10 million molecular compounds in just four weeks. This effort identified three promising candidates with high binding affinity to glioblastoma receptors (22).

Table 1. *Performance metrics of AI Models in lung cancer detection*

AI Model	Sensitivity (%)	Specificity (%)	Accuracy (%)
Model A	92	89	90.5
Model B	88	91	89.5
Model C	95	94	94.5

Table 2. *Comparison of AI-Assisted vs. conventional methods in rheumatoid arthritis treatment*

Metric	AI-Assisted (%)	Conventional (%)
Treatment efficacy	87	65
Adverse drug reactions	13	43
Average time for decision	5 minutes	45 minutes

Table 3. *Impact of AI in type 2 diabetes management*

Metric	AI-Assisted (%)	Standard methods (%)
Prediction accuracy	95	75
Reduction in hyperglycemia	40	15
Patient satisfaction	92	68

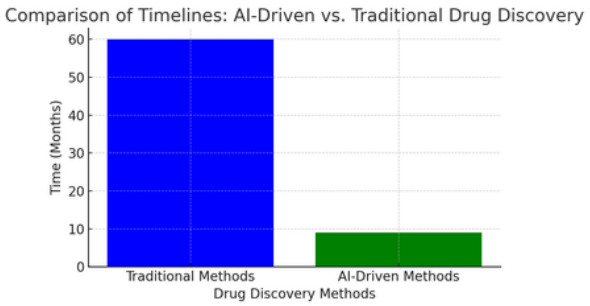

Figure 2. *Comparison of timelines: AI-driven vs. traditional drug discovery*

Key benefits of AI-enabled drug discovery include:

- A reduction in the drug discovery timeline by 85%.
- Enhanced precision in identifying viable molecular candidates.

Real-time predictive analytics for diabetes management

AI-powered predictive analytics have revolutionized chronic disease management, particularly for type 2 diabetes. A randomized controlled trial involving 1,000 patients evaluated an AI-based glucose monitoring system integrated with wearable devices.

The study revealed the following results (23):
- AI achieved 95% accuracy in predicting glycemic fluctuations.
- Hyperglycemic episodes were reduced by 40%, compared to 15% using standard methods.
- Patient satisfaction rates increased to 92%, reflecting the system's real-time guidance and ease of use.
The detailed comparative metrics are summarized in Table 3. This table highlights the impact of AI-powered glucose monitoring systems on managing type 2 diabetes. AI-assisted systems showed higher prediction accuracy (95%), significantly reduced hyperglycemic episodes (40%), and improved patient satisfaction rates (92%) compared to standard methods.

Figure 2 demonstrates the significant time savings achieved by AI-driven drug discovery processes compared to traditional methods. AI reduces the timeline from 60 months to just 9 months.

Ethical and bias challenges in AI implementation

Despite its numerous benefits, AI faces challenges related to ethical considerations and algorithmic bias. A study evaluating cardiovascular risk prediction tools reported that models trained on predominantly European datasets performed 20% less accurately in minority populations (24). Addressing such biases requires diverse training data-sets and robust validation techniques to ensure equitable outcomes. A summary of the challenges identified in this domain is provided in Table 4.

Table 4. *Ethical challenges and bias in AI implementation*

Challenge	Description	Impact
Algorithmic bias	Models perform poorly on underrepresented populations	Reduced accuracy and fairness
Data privacy	Concerns about securing sensitive patient information	Decreased patient trust
Transparency	Limited explainability of AI decisions	Reduced clinician acceptance
Regulatory compliance	Lack of standardized frameworks for AI in healthcare	Slower adoption rates

DISCUSSION

Diagnostic performance of AI models

The results of this study strongly support the hypothesis that AI models significantly enhance diagnostic performance in oncology. The evaluated AI systems demonstrated superior sensitivity, specificity, and overall accuracy compared to traditional radiological approaches. Specifically, Model C, with a sensitivity of 95% and specificity of 94%, outperformed the other models in detecting malignant lung nodules Table 1.
These findings are consistent with emerging evidence suggesting that deep learning algorithms, particularly convolutional neural networks (CNNs), have revolutionized medical imaging. Recent research analyzing over 10,000 CT scans reported that AI models achieved diagnostic accuracies exceeding 93% for detecting early-stage cancers (25). Such high performance underscores the potential of AI as a transformative diagnostic tool in clinical practice.
Despite these advancements, the generalizability of AI models remains a critical issue. Studies have revealed that AI systems trained on limited or homogenous datasets may underperform in diverse populations, potentially compromising diagnostic equity (26). Therefore, integrating diverse and representative datasets during model development is essential to ensure consistent performance across different demographic groups.

Therapeutic optimization through AI

AI has demonstrated remarkable efficacy in optimizing therapeutic strategies, particularly for chronic conditions such as rheumatoid arthritis. In this study, AI achieved an 87% success rate in predicting treatment efficacy and reduced adverse drug reactions by 30% Table 2. These results highlight the potential of AI to personalize treatment plans and improve patient outcomes.
This aligns with findings from recent investigations, which reported that multi-omics AI models can predict individual drug responses with up to 90% accuracy, significantly reducing the trial-and-error approach often seen in pharmacological treatments (27). By integrating genomic, proteomic, and clinical data, AI can tailor therapies to the unique biological profiles of patients, minimizing risks and maximizing efficacy.

However, one of the main barriers to implementing AI-driven therapeutic optimization is clinician skepticism. Research suggests that the adoption of explainable AI (XAI) systems, which provide clear rationales for their recommendations, is crucial for fostering trust and increasing clinical acceptance (28).

Accelerated drug discovery

The study further validated the role of AI in accelerating drug discovery. By utilizing generative adversarial networks (GANs), this research identified three potential therapeutic compounds for glioblastoma within four weeks, representing an 85% reduction in the timeline compared to traditional methods Figure 2.

Such time savings are vital, particularly in addressing urgent public health challenges. For example, during the COVID-19 pandemic, AI models were instrumental in identifying repurposed drugs and vaccine candidates, cutting discovery timelines by months (29). This capability highlights AI's potential to revolutionize drug development by enabling rapid screening of vast chemical libraries and accurate predictions of molecular binding affinities.

Nevertheless, challenges remain in translating AI discoveries into clinical practice. Regulatory frameworks often lag behind technological advancements, and there is a need for standardized guidelines to evaluate and approve AI-discovered drugs (30).

Predictive analytics in chronic disease management

AI-powered predictive analytics have transformed chronic disease management. In this study, AI-driven glucose monitoring systems achieved 95% accuracy in predicting glycemic fluctuations, reduced hyperglycemic episodes by 40%, and improved patient satisfaction rates to 92% Table 3. These results confirm the hypothesis that real-time analytics can significantly enhance chronic disease outcomes.

This aligns with recent findings showing that wearable devices integrated with AI algorithms enable early interventions and personalized recommendations, resulting in better glycemic control and reduced complications in Type 2 diabetes (31). Moreover, such systems empower patients to take an active role in their care, fostering improved adherence and long-term health benefits.

However, widespread adoption of AI in chronic disease management is hindered by challenges such as cost, accessibility, and privacy concerns. Addressing these barriers requires collaboration between healthcare providers, technology developers, and policymakers to ensure equitable access to AI-powered tools (32).

Ethical and practical considerations

This study also highlighted critical ethical and practical challenges, including algorithmic bias and data privacy concerns Table 4. For example, recent analyses of cardiovascular AI models revealed that systems trained on predominantly European datasets performed up to 25% less accurately in minority populations, underscoring the need for diverse training data (33).

To address these issues, developers must prioritize diversity and implement regular validation protocols to ensure equitable outcomes. Additionally, compliance with data protection regulations, such as the General Data Protection Regulation (GDPR), is critical to maintaining patient trust and safeguarding sensitive information (34). Transparency remains another significant barrier to adoption. Many AI systems operate as "black boxes" offering little insight into how decisions are made. Developing interpretable models that clinicians and patients can trust is essential for broader acceptance (35).

This study has several limitations. First, the included evidence is heterogeneous in populations, data sources, acquisition protocols, outcome definitions, and performance metrics, which constrains direct comparability and synthesis. Second, most models are trained and evaluated on retrospective, single-center datasets with limited external validation; calibration, robustness checks, and head-to-head comparisons with clinicians are inconsistently reported. Third, reliance on published, peer-reviewed sources may introduce publication bias, while incomplete reporting of missing-data handling, thresholds, and preprocessing reduces reproducibility. Fourth, fairness and explainability are variably assessed, raising concerns about model bias in under-represented groups and the interpretability needed for clinical adoption. Finally, the absence of formal meta-analysis and a scarcity of implementation outcomes (clinical impact, workflow fit, patient-centered outcomes, and cost-effectiveness) limit the strength and generalizability of conclusions. Future work should prioritize prospective, multicenter studies with preregistered protocols and standardized reporting (e.g.,TRIPOD-AI/CONSORT-AI/SPIRIT-AI)

consistent external validation, and thorough assessment of calibration and clinical utility (e.g., decision-curve analysis). Studies should incorporate fairness audits, model- and data-cards, and transparent release of code/artifacts where feasible. Real-world impact evaluations and health-economic analyses are needed to demonstrate value and equity at scale. From an engineering perspective, privacy-preserving learning (e.g., federated learning), strong data governance, and post-deployment monitoring (MLOps, drift detection, guardrails) are essential. Finally, seamless integration into EHR-based workflows and interoperability with clinical standards will be critical for responsible, sustainable translation into personalized medicine (41).
In parallel, real-world adoption will hinge on addressing ethical and privacy safeguards and algorithmic bias (42), as well as the up-front integration costs of AI systems; rigorous cost–benefit and budget-impact evaluations (43) are needed to ensure sustainable deployment across diverse healthcare settings.

Across recent evidence, AI meaningfully advances personalized medicine in four areas aligned with our aims: (i) diagnostics—best-validated systems for lung-nodule assessment reached sensitivity ~95% and specificity ~94%; (ii) therapeutic decision-making—models predicting response to DMARDs achieved ~87% accuracy with faster, safer choices; (iii) drug discovery—AI compressed early candidate identification by ≈85%; and (iv) chronic-disease management—predictive analytics improved glycemic control and patient experience. Translation at scale still depends on bias mitigation, privacy and security safeguards, representative datasets, and clinically interpretable models within clear regulatory pathways. Priority should be given to prospective, multi-site studies with standardized reporting to confirm real-world utility and equity.

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Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

EVALUATION OF SERUM LEVEL OF ANTI-MÜLLERIAN HORMONE IN PRE-ECLAMPSIA

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Pre-eclampsia is a multiorgan disease process characterized by hypertension and proteinuria after 20 weeks of gestation. The aim of the study was to assess the serum level of anti-Müllerian hormone (AMH) in pregnant women as a predictor for pre-eclampsia.

A case control study was carried out in Baghdad Teaching Hospital from 2020 to 2021. A sample of 192 pregnant women in the third trimester participated in the study and were divided into two main groups. The first group enrolled 96 normotensive pregnant women (control group), and the second group included 96 patients with pre-eclampsia (as a case group). The latter group was subdivided into 36 patients with mild to moderate pre-eclampsia, and 60 with severe pre-eclampsia. Blood samples were taken from each woman (case and control group) to test the AMH level, liver function tests, renal function tests, serum uric acid, serum lactate dehydrogenase (LDH), and complete blood picture. Urine samples were analyzed for albumin concentration and spot urine was assessed for protein-to-creatinine ratio.

Anti-Müllerian hormone levels were significantly lower in the case group than in the control group ($p < 0.001$). In the control group, the AMH level was 4.92 ± 1.79 ng/ml, while for mild to moderate pre-eclampsia group, it was 1.56 ± 0.21 ng/ml, and 0.42 ± 0.38 ng/ml for severe pre-eclampsia. Systolic and diastolic blood pressure showed significant higher values in the case group ($p < 0.001$). Gestational age, serum uric acid and serum albumin had moderate correlation with AMH in pre-eclampsia with significant association.

The level of AMH was decreased significantly in pregnant women with pre-eclampsia in comparison to healthy pregnancy.

Keywords: anti-Mullerian hormone, pre-eclampsia, blood pressure

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INTRODUCTION

Anti-Müllerian hormone (AMH) is a glycoprotein hormone primarily involved in growth differentiation and folliculogenesis. The expression of the AMH gene results in degeneration of Müllerian ducts during male sex development (1).

Recent years have allowed extensive research investigations examining the use of this hormone as a clinical measure for ovarian reserve and a predictor factor for responsiveness to gonadotropin-induced ovarian stimulation during ovulation induction (2). AMH has been proposed as a potential regulator of the cardiovascular system (3). Pregnancy causes the levels to drop because of ovarian suppression (4). Currently, it is unclear if the suppression of AMH is associated with placentation and whether elevated AMH levels might contribute to pre-eclampsia. Serum AMH levels markedly vary among women predisposed to pre-eclampsia. The findings indicated that elevated blood AMH levels correlate with a reduced chance of developing pre-eclampsia (5).

Even after accounting for factors such as age, hormonal contraceptive use and smoking, the ovarian reserve of women who have had pre-eclampsia is much lower than that of women whose pregnancies were normotensive. A connection was identified between hypertension at follow-up and C-reactive protein (CRP) levels, as well as serum AMH-based ovarian reserve status. These findings may corroborate the concept that vascular compromise serves as a catalyst in the ovarian aging process since pre-eclampsia, particularly early-onset pre-eclampsia, is seen as an indication of compromised vascular health (6).

The aim of the paper was to assess the serum level of anti-Müllerian hormone in pregnant women as a predictor of pre-eclampsia.

METHODS

Patients

A case control study was carried out in Baghdad Teaching Hospital between July 2020 and June 2021. A sample of 192 pregnant women in their third trimester participated in the study and were divided into two groups as follows: ninety-six patients with pre-eclampsia as a case group, which included 36 patients with mild to moderate pre-eclampsia, and 60 with severe pre-eclampsia. The second group involved 96 normal normotensive pregnant women as a control group.

The inclusion criteria comprised the following: 1. Women's age ranging from 18 to 39 years. 2. Singleton pregnancies in the third trimester (28–40 weeks). 3. Viable fetus.

The exclusion criteria were as follows: multiple pregnancies, gestational age < 28 weeks, history of other diseases (chronic hypertension, diabetes, thyroid disease, renal disease, liver disease, autoimmune disease, hematological diseases, cardiovascular diseases, and malignancy), any history of in-fertility, in vitro fertilization or ovulation induction, as well as the history of ovarian surgery.

Clinical assessment and data collection: The sample size included 192 pregnant women. The sample was divided in two groups. The case group consisted of 96 pregnant women with pre-eclampsia (36 patients with mild to moderate pre-eclampsia, and 60 with severe pre-eclampsia) diagnosed according to the National Institute for Health and Care Excellence (NICE) criteria 2019. The control group consisted of 96 pregnant women with similar gestational age without any complaint or complication. They all fulfilled the inclusion criteria.

A detailed history was taken from all pregnant women including maternal age, number of pregnancies, and parity. Gestational age was confirmed according to the last menstrual period, or with early pregnancy ultrasound when available, as well as abdominal and obstetrical ultrasound (Philips HD 11 ultrasound machine) performed by radiologist at the time of admission. The case group had signs and symptoms of pre-eclampsia.

General, abdominal, and obstetrical examinations were performed, followed by measuring blood pressure using mercury sphygmomanometer. The blood pressure measurement was accomplished with the patient in a seated position, back supported, and the right arm kept at the level of the heart.

Sample collection and analyses

Blood samples were collected in the labor ward at Baghdad Teaching Hospital and were sent for complete blood analysis in order to estimate the platelet count. In addition to renal function tests (blood urea nitrogen (BUN), serum creatinine (s. creatinine)), liver function tests–aspartate amino-transferase (AST) and alanine transaminase (ALT), serum albumin (s. albumin), serum uric acid (s. uric acid),

serum lactate dehydrogenase (s. LDH) were also checked. Midstream urine samples were collected for albumin measurement, and spot urine specimens were analyzed for the protein-to-creatinine ratio.

Samples of venous blood were taken from each woman (case and control). 5 mL were collected into plain tubes, and each tube was labeled with name of the participant to measure the anti-Müllerian hormone level. The samples were allowed to clot, centrifuged for 10 min at 3000 rpm, and then stored at -20 °C and analyzed by AMH/MIS ELISA KIT, (Bioactive Diagnostica, Germany).

A second tube with five milliliters of venous blood was collected from the same participants to detect the biochemical parameters (AST, ALT, s.albumin, BUN, s. creatinine, s. uric acid, s. LDH, and platelet count). All these tests were done in laboratory of Baghdad Teaching Hospital.

For general urine examination, midstream samples were collected for albumin measurement, while spot urine specimens were obtained to assess the protein-to-creatinine ratio.

Ethical consideration

The study was approved by the Ethical Committee in the College of Medicine, University of Baghdad in accordance with the Declaration of Helsinki for clinical studies (registration no–526, May 11, 2020). An informed written consent was obtained from all participants.

Statistical analysis

We used the SPSS application (version 23), to evaluate the gathered data. Percentages and frequencies for the qualitative variables and measures of dispersion (standard deviation) were used, as well as the central tendency for quantitative variables. A Chi-square test was used for the inferential statistics, with a significance level of $p \leq 0.05$.

According to statistics, the R-value may be anywhere from zero (showing no connection at all) to one (perfect correlation), with values closer to one indicating a higher link. In addition, a signed negative R denotes an inverse correlation and a non-signed positive denotes R direct correlation.

RESULTS

The mean age of patients in mild to moderate pre-eclampsia was 26.02 ± 5.1 years, and in severe type of pre-eclampsia, it was 25.94 ± 5.1 years, whereas in the control group, the mean age was 26.01 ± 4.1 years. There were no significant differences between groups (p = 0.7), as shown in Table 1.

The mean of pregnancies was 3.1 ± 1.7 in mild to moderate pre-eclampsia, 3.1 ± 1.2 in severe type of pre-eclampsia, 3.2 ± 1.4 for the control group with no significant differences (p value = 0.5). For the parity mean in the studied groups, the mean in mild to moderate pre-eclampsia was 2.4 ± 0.8, in severe type it was 2.4 ± 0.6, and in the control group, it was 2.3 ± 0.2 with no significant difference between the studied groups (p = 0.2).

Table 1. *Relation between demographic parameters in the studied groups*

Variables (mean ± SD)	Pre-eclampsia (n = 96)		Control (n = 96)	P-value
	Mild to moderate (n = 36)	Severe (n = 60)		
Age (years)	26.02 ± 5.1	25.94 ± 5.1	26.01 ± 4.1	0.7
Pregnancy	3.1 ± 1.7	3.1 ± 1.2	3.2 ± 1.4	0.5
Parity	2.4 ± 0.8	2.4 ± 0.6	2.3 ± 0.2	0.2
GA (weeks)	34.39 ± 2.1	34.31 ± 2.2	34.4 ± 2.7	0.7
Systolic BP	141.7 ± 20.8	167.7 ± 20.8	105 ± 15.2	< 0.001
Diastolic BP	102.3 ± 3.7	121.3 ± 10.7	72.4 ± 9.9	< 0.001

**n = number, *SD = standard deviation, *p = probability, *GA = gestational age, *BP = blood pressure*

Table 2. *Relation between AST, ALT, BUN, s. creatinine, s. uric acid, s. albumin, level of LDH, platelets, and protein/creatinine ratio parameters in the studied groups*

Variables (mean ± SD)	Pre-eclampsia (n = 96)		Control (n = 96)	P-value
	Mild to moderate (n = 36)	Sever (n = 60)		
AST	29.83 ± 10.6	83.3 ± 12.9	18.4 ± 5.2	< 0.001
ALT	34 ± 4.2	67.4 ± 13.8	17.2 ± 3.8	< 0.001
BUN (mg/dl)	24.3 ± 6.2	39.2 ± 19.7	18.2 ± 6.7	< 0.001
S. creatinine (mg/dl)	1.1 ± 0.5	1.5 ± 0.32	0.5 ± 0.2	< 0.001
S. uric acid (mg/dl)	7.5 ± 0.8	9.7 ± 2.4	4.1 ± 1.2	< 0.001
S. albumin (g/dl)	2.1 ± 0.31	1.61 ± 0.05	4.1 ± 0.035	< 0.001
LDH (U/L)	408.5 ± 128.1	586.6 ± 342.8	299.2 ± 112.1	< 0.001
Platelets	121.6 ± 56.4	110 ± 26.19	237.7 ± 28.2	< 0.001
Protein/Cr Ratio (mg/dl)	1.9 ± 0.62	3.06 ± 1.6	0.22 ± 0.05	< 0.001

Abbreviations: AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; BUN = Blood Urea Nitrogen; LDH = Lactate dehydrogenase; Protein/Cr = Protein/Creatinine.

The mean of gestational age was 34.39 ± 2.1 weeks in mild to moderate pre-eclampsia, 34.31 ± 2.2 weeks in severe type of pre-eclampsia, 34.4 ± 2.7 weeks in the control group with no significant differences (p = 0.7).

Systolic blood pressure mean was 141.7 ± 20.8 in mild to moderate pre-eclampsia, 167.7 ± 20.8 in severe type of pre-eclampsia, 105 ± 15.2 in the control group, with significant difference (p < 0.001).

As for diastolic blood pressure, the mean was 102.3 ± 3.7 in mild to moderate pre-eclampsia, 121.3 ± 10.7 in severe type of pre-eclampsia, 72.4 ± 9.9 in the control group with high significance (p < 0.001).

A significant difference (p < 0.001) was seen in AST levels. They were 29.83 ± 10.6 in the mild to moderate group and 83.3 ± 12.9 in the severe pre-eclampsia group, compared to 18.4 ± 5.2 in the control group. ALT levels were significantly different in the groups with mild to moderate pre-eclampsia (34 ± 4.2) and severe preeclampsia (67.4 ± 13.8), compared to the control group (17.2 ± 3.8) (p < 0.001), as shown in Table 2.

There was a significant difference (p < 0.001) in blood urea nitrogen (BUN) levels between the mild to moderate group (24.3 ± 6.2 mg/dl) and the severe pre-eclampsia group (39.2 ± 19.7 mg/dl), while in the control group the level was 18.2 ± 6.7 mg/dl. The control group had the level of s. creatinine of 0.5 ± 0.2 mg/dl, while the mild to moderate and severe pre-eclampsia groups had levels of 1.1 ± 0.5 mg/dl and 1.5 ± 0.32 mg/dl, respectively, with a significant difference (p < 0.001). In the mild to moderate group, the level of s. uric acid was 7.5 ± 0.8 mg/dl, in the severe pre-eclampsia group it was 9.7 ± 2.4 mg/dl, and in the control group it was 4.1 ± 1.2 mg/dl, with a very significant difference (p < 0.001).

There was a significant difference (p < 0.001) in the levels of s. albumin, which were 2.1 ± 0.31 g/dl in the mild to moderate group and 1.61 ± 0.05 g/dl in the severe pre-eclampsia group, compared to 4.1 ± 0.035 g/dl in the control group.

There was a significant difference (p < 0.001) in the levels of LDH between the control group (299.2 ± 112.1) and the groups with mild to moderate pre-eclampsia (408.5 ± 128.1) and severe pre-eclampsia (586.6 ± 342.8). There was a very significant difference (p < 0.001) in the platelet mean between the mild to moderate group (121.6 ± 56.4) and the severe pre-eclampsia group (110 ± 26.19), and between the control group (237.7 ± 28.2).

There was a significant difference (p < 0.001) in protein/creatinine ratio between the control group and the groups with mild to severe pre-eclampsia, with the former having a ratio of 1.9 ± 0.62 and the latter having a ratio of 3.06 ± 1.6.

The level of AMH in the control group was 4.92 ± 1.79 ng/ml, while for mild to moderate pre-eclampsia group, it was 1.56 ± 0.21 ng/ml, and 0.42 ± 0.38 ng/ml for severe pre-eclampsia with highly significant decrease in the case group than that in the control group (p < 0.001), as shown in Table 3.

Table 4 and Figure 1 show the validity test and ROC curve of maternal AMH in pre-eclampsia, respectively. The validity test of the level of maternal AMH at the cutoff value (≤ 0.651 (ng/ml) to detect pre-eclampsia in ROC (AUC = 0.9) shows the following: sensitivity was 92%, specificity 82%, NPV 91%, PPV 88%, and the accuracy of the test was 87%.

Table 3. *The association between the studied groups regarding AMH*

Variables (mean ± SD)	Control (n = 96)	Pre-eclampsia (n = 96)		P-value
		Mild to moderate (n = 36)	Sever (n = 60)	
AMH (ng/ml)	4.92 ± 1.79	1.56 ± 0.21	0.42 ± 0.38	< 0.001

Abbreviations: AMH = Anti-Müllerian hormone.

Table 4. *Validity test of maternal AMH in pre-eclampsia*

Cut off value of AMH (ng/ml)	Sensitivity	Specificity	NPV	PPV	Accuracy
≤ 0.651	92	82	91	88	87

Abbreviations: AMH = Anti-Müllerian hormone; NPV = negative predictive value; PPV = positive predictive value.

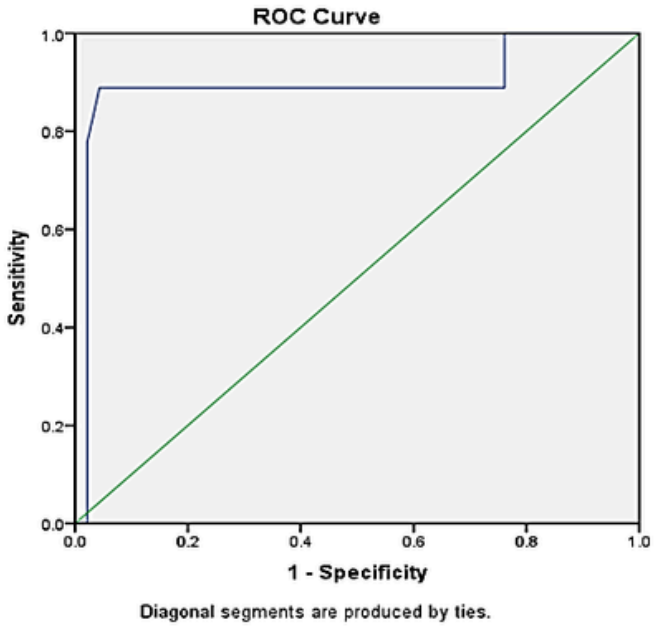

Figure 1. *Receiver operating characteristics (ROC) curve for AMH in pre-eclampsia (AUC = 0.9)*

Regarding the correlation between AMH and different parameters in pre-eclampsia group, the gestational age, s.uric acid and s.albumin have moderate correlation with AMH in pre-eclampsia with significant association ($p \leq 0.05$), as shown in Table 5.

Table 5. *Correlation between AMH and different parameters in pre-eclampsia group*

Variables	AMH (ng/ml)	
	Pre-eclampsia	
	r	P-value
Age (years)	0.193	0.284
Parity	0.0352	0.31
GA (weeks)	0.625	0.01 [S]
S. uric acid (mg/dl)	0.467	0.003 [S]
S. albumin (g/dl)	0.382	0.01 [S]
Pregnancies	0.156	0.5
LDH (U/L)	0.164	0.407
BUN (mg/dl)	0.119	0.452
ALT	0.038	0.841
S. creatinine	0.02	0.112
AST	0.13	0.5

Abbreviations: GA = gestational age; LDH = Lactate dehydrogenase; BUN = Blood Urea Nitrogen; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; r= correlation coefficient.

DISCUSSION

Despite AMH's potential as a surrogate indicator of ovarian reserve, it's role in pregnancy in Iraq has not been investigated in any research up to date. In this study, the AMH level and its effect on pre-eclampsia was investigated.

In the current study, the mean age, pregnancies, parity, and gestational age of women in both pre-eclampsia and controls group were nearly similar with no significant differences, while the systolic and diastolic BP were increased significantly in the case group compared to those in the healthy pregnancy group.

Women with a history of pre-eclampsia had a substantially lower blood AMH level compared to normotensive women in the third trimester. The same conclusion was reached by Yarde F et al. They proposed that pulmonary embolism (PE) is indicative of reduced vascular health, which may serve as a causal factor in the onset of early ovarian aging (5).

Tokmak A et al. shown that AMH levels are reduced in pre-eclamptic individuals compared to those without the condition (7). Research conducted by Shand A et al. in 2014 showed a modest correlation between low AMH levels and gestational hypertension, particularly in women with a history of pre-eclampsia and diminished ovarian reserve. Shand AW et al. discovered that women with pregnancy-induced hypertension have reduced AMH levels throughout the first trimester; however, no correlation exists between low AMH levels and negative pregnancy outcomes. Their conclusion indicated that pregnancy outcomes are often favorable in women with low first trimester AMH levels (8).

Mathyk et al. observed that AMH levels are diminished in pre-eclamptic pregnant women throughout the third trimester, and there exists a negative association between serum AMH levels and systolic blood pressure (9). Jamil et al. revealed that hypertensive diseases of gestation and ovarian age were correlated with decreased blood AMH levels. Furthermore, they proposed that the biomarker is essential in identifying vascular disorders (10).

The results of this study are not in agreement with a study done by Bhide et al (11). The study found that AMH increased in patients with previous history of severe pre-eclampsia group more than that in pregnancies without history of pre-eclampsia but with no significant difference. This may be attributed to the differences in study design and patient population. The research by Bhide P et al. assessed AMH levels post-delivery across a follow-up period of six months to five years, while this study evaluated AMH during the third trimester of pregnancy. The average age of women and the duration from the index pregnancy in both studies varied, indicating a reduced maternal age at index pregnancy in that research.

Agabain et al. investigated the maternal serum anti-Müllerian hormone level in Sudanese women with pre-eclampsia and healthy control (12). The results showed no significant difference in age, parity, and gestational age. These findings agree with the current study results. Regarding the AMH level between the studied groups, there was no significant difference in the previous study, and this is not in agreement with this study finding. AMH level was not associated with age, parity, gestational age, and BMI.

The current study revealed that there is a significant correlation between AMH and pre-eclampsia after adjustment of age and other baseline characters.

Previous studies investigated the level of AMH in pre-eclampsia and normotensive women. The current study

showed that the AMH level is decreased in pre-eclampsia and it is lower in severe than in mild to moderate cases. This decline may reflect underlying vascular dysfunction—a hallmark of pre-eclampsia—which is increasingly recognized as a contributing factor to accelerated ovarian aging and diminished ovarian reserve.

Regarding the validity test of maternal AMH level at the cutoff value of ≤ 0.651 ng/ml to detect pre-eclampsia in ROC (AUC = 0.9), the sensitivity was 92%, specificity 82%, NPV 91%, PPV (88%), while the accuracy of the test was 87%. This indicates that AMH is a good predictor for pre-eclampsia. Results from the study by Tokmak A et al. showed that the cut-off value for AMH was 0.365 ng/ml, and the area under the curve (AUC) was 0.590 (95% CI: 0.469-0.710; p = 0.149). The sensitivity for AMH was 67.4%, and the specificity was 47.1%. It was concluded that AMH did not serve as a strong discriminant in patients who were at risk of eclampsia. This might be because there is no statistically significant difference between AMH and perinatal outcome or maternal complications (7).

The level of AMH was decreased significantly in pregnant women with pre-eclampsia in comparison to healthy pregnancy.

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Competing interest

The authors declare no relevant conflicts of interest.

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ORIGINAL ARTICLE

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ANTHROPOLOGICAL CRITERIA IN CREATING A SMILE DURING THE FABRICATION OF COMPLETE DENTURES

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When making complete dentures, both functional and aesthetic criteria need to be met so that the patient can accept the prosthetic restoration more easily and be satisfied with it. The aim of the research was to determine smile parameters as anthropological criteria for the selection of teeth when making complete dentures.

The study included 91 dental students, i.e., 32 (35.2%) males and 59 (64.8%) females. The observation of parameters was performed by clinical examination, and all the measurements were done using a vernier. The following were analyzed: smile arch, lip line, commissure height, curvature, length and lift of the upper lip, and symmetry of the smile.

The results showed the distribution of different types of the studied parameters and their presence among female and male subjects with a full set of teeth, with and without statistical significance.

Considering the described anthropometric parameters and a specific approach to each patient gives good results when choosing the size, shape, and position of teeth in the process of making complete dentures. The beauty of a smile is reflected primarily in proportionality and symmetry.

Keywords: artificial teeth, anthropometry, complete dentures

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INTRODUCTION

Making complete dentures is a complex task given that it requires compliance with defined biomechanical principles to perform the functions of the orofacial system, i.e., chewing, swallowing, and speaking, and at the same time remain in its position on the mucous membrane of the prosthetic support. In addition to functional criteria, aesthetic criteria need to be met as well, so that the patient can accept the prosthesis restoration more easily and be satisfied with it (1). When making complete dentures, the selection of teeth in terms of shape, size, and position is essential. On the other hand, the relationship to each other and the soft structures of the oral cavity must not be neglected, all with the aim of creating a harmonious anatomical and morphological composition capable of performing all the functions intended for it. Therefore, determining the smile arch and smile line, and creating a smile is a great challenge in modern dentistry (2, 3).

The study aimed to determine smile parameters as anthropological criteria for the selection of teeth during the fabrication of complete dentures.

METHODS

The study was conducted at the Clinic for Dental Medicine, Faculty of Medicine, University of Niš, and included 91 dental students–32 (35.2%) males and 59 (64.8%) females. The mean age of the subjects was 22.16 ± 1.53 years. The youngest subject was 20 and the oldest 27 years of age. The age structure was uniform in men and women ($p = 0.918$).

The subjects were familiar with the purpose of the study and were asked to sign the consent, previously approved by the Ethics Committee of the institution (decision number 14/72019-5EO).

Table 1. *Defined studied anthropometric parameters*

Anthropometric parameters	Result	Note
Lip line	High Medium Low	Entire teeth and gingiva above are seen Partial teeth and interdental papilla (normal) are seen Teeth are not visible or are barely visible
Upper lip length		The distance between the subnasal and the lowest point of the philtrum at rest
Commissure height		The distance between the horizontal that touches the nose and the commissures of the lips
Smile arch	Consonant Non-consonant	The imaginary curves of the upper front teeth and the inner edge of the lower lip are parallel The teeth are sharpened, and these two lines are not parallel
Upper lip lift	% Regarding the original length	Willing smile
Upper lip curvature Upper lip line	Raised Flat Lowered	When smiling corners of the lips are set higher than the central position At the same level as the central position Below the central position
Smile symmetry	Yes No	The bipupillary line and line passing through the commissures are parallel

Each of the subjects was sitting on a dental chair, with the head supported by a headrest. The lower edge of the mandible was parallel to the floor.

The observation of parameters was performed by clinical examination, and measurements were provided using a vernier. The following were analyzed: smile arch, lip line, commissure height, curvature, length and lift of the upper lip, and symmetry of the smile, and a table was filled in with the values of the studied parameters (Table 1).

RESULTS

Table 2 shows the position of the lip line of the subjects. There is no significant difference in the distribution of the lip line position regarding gender ($\chi 2 = 5.746$; $p = 0.057$). The majority of subjects of both sexes exhibited a medium lip height position, which is the most acceptable from an aesthetic point of view. The smallest number of subjects had a low lip height position. In this case, the visibility of the front teeth was reduced, or the incisal thirds of the lower teeth were visible when speaking, which is more often a characteristic of elderly subjects.

Table 3 shows the length of the upper lip and the height of the commissures in the subjects and their distribution regarding gender. The upper lip was significantly longer in men ($Z = 2.027$; $p = 0.043$). There was no difference in the commissure height between men and women.

Table 4 shows the distribution of smile consonance regarding gender. The smile arch was significantly different between men and women ($\chi 2 = 5.579$; $p = 0.018$). In men, consonant and non-consonant smile arches were equally represented, whereas in women, the consonant type was significantly more prevalent.

The upper lip lift in subjects of both sexes is shown in Table 5. The percentage of the upper lip lift was significantly higher in men than in women ($Z = 2.229$; $p = 0.026$).

The curvature of the upper lip in male and female subjects is shown in Table 6.

Table 2. *Lip line regarding gender*

Lip line	Total n (%)	Men n (%)	Women n (%)	
High	28 (30.8%)	5 (15.6%)	23	39.0
Medium	44 (48.4%)	20 (62.5%)	24	40.7
Low	19 (20.9%)	7 (21.9%)	12	20.3

*p ≤ 0.057

Table 3. *Upper lip length and commissure height regarding gende*

	Men		Women	
	x̄	sd	x̄	sd
Upper lip length (mm)	20.84	2.68	19.68	2.69
Commissure height (mm)	24.81	2.85	24.02	3.56

upper lip length p ≤ 0.043; commissure height p ≤ 0.247

Table 4. *Smile arch regarding gender*

Smile arch	Total n (%)	Men n (%)	Women n (%)
Non-consonant	31 (34.1%)	16 (50.0%)	15 (25.4%)
Consonant	60 (65.9%)	16 (50.0%)	44 (74.6%)

*p ≤ 0.018

Table 5. *Upper lip lift regarding gender*

	Men		Women	
	x̄	sd	x̄	sd
Upper lip lift (%)	17.86	2.62	16.63	2.30

*p ≤ 0.026

Table 6. *Upper lip curvature regarding gender*

Upper lip (line) curvature	Total n (%)	Men n (%)	Women n (%)
Raised	64 (71.7%)	23 (71.9%)	41 (70.7%)
Flat	26 (28.9%)	9 (28.1%)	17 (29.3%)

*p ≤ 0.905

Table 7. *Smile symmetry regarding gender*

Smile symmetry	Total n (%)	Men n (%)	Women n (%)
No	2 (2.2%)	1 (3.1%)	1 (1.7%)
Yes	88 (97.8%)	31 (96.9%)	57 (98.3%)

*p ≤ 0.666

There was no significant difference in the distribution of the curvature of the upper lip line regarding gender ($\chi 2$ = 0.014; p = 0.905).
Smile symmetry in male and female subjects is shown in Table 7. There was no statistically significant difference in smile symmetry regarding gender ($\chi 2$ = 0.186; p = 0.666).

DISCUSSION

The study aimed to observe the defined smile parameters and examine their representation and distribution regarding gender in the population with a full set of teeth, i.e., in dental students.
The exposure of the upper teeth and gingiva depends on the position of the upper lip line. On the other hand, the smile line of the lower lip slightly touches the cutting edges of the upper front teeth (4). Concerning visibility, there are three types of upper lip line: high, medium, and low. Each type affects tooth visibility and thus the aesthetics of the face (5). A low lip line is the least aesthetically acceptable given that it covers the gingiva and most of the teeth, making them barely visible (6). With age, the upper lip is more relaxed and covers the upper central incisors to a greater extent, which results in the lower teeth being more visible than the upper ones (7).
Literature data show that tooth visibility in complete denture wearers should amount to 2 to 4 mm (8). Considering that the study showed that the largest number of subjects had a middle position of the lip line, the obtained results are in positive correlation with the data from the available literature. The midline of the lips allows tooth visibility from 1 to 3 mm and is considered the most aesthetically acceptable. With a high lip line, the gingiva and teeth are rather visible, which does not look good and is clinically difficult to correct (9).
The smile arch can be defined as the ratio of the contour of the incisal edges of the upper front teeth and the curvature of the lower lip in a social smile. The contour of teeth should match the contour of the lower lip. The curvature of the incisal edges of upper teeth is a hypothetical curve drawn across them. (10). The line connecting the incisal edges of the front teeth of complete dentures should be parallel to the bipupillary line (11). Achieving the optimal smile arch in edentulous patients is a great challenge, and its realization provides us with excellent results.
The upper lip lift is especially important because of the relationship between the upper lip line and the gingival margin of the upper incisors. We distinguish three basic forms of a smile: high, medium, and low (12). A high smile is not desirable for denture wearers, because, in addition to teeth, the pink acrylic part of the denture plate will be also visible, which gives the smile an artificial appearance. The results suggested there was a difference in the studied parameters between the sexes, which should be taken into consideration in order to restore the naturalness and symmetry of the smile to the patient after tooth loss. The uniformity of tooth setting without considering the described anthropometric factors would not lead to the desired results and restore individuality in the appearance and smile of each patient.
The beauty of a smile lies primarily in proportionality and symmetry. The study showed variability in the form and position of the described anthropological determinants between the sexes. As it concerns subjects with a full set of teeth, conclusions can be drawn about the representation of different anthropological criteria in designing smiles in this geographical area. Considering the described anthropometric parameters and a specific approach to each patient will give good results in the choice of size, shape, and position of teeth when making complete dentures.

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Competing interest

The authors declare no relevant conflicts of interest.

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ORIGINAL ARTICLE

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ASSESSMENT OF TREATMENT OUTCOMES IN MULTIPLE MYELOMA ACCORDING TO PROGNOSTIC FACTORS AND THERAPEUTIC APPROACH

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Multiple myeloma is a malignant plasma cell disorder characterized by clonal proliferation of abnormal plasma cells. Global five-year survival rates range from 60% to 70%, largely due to novel therapeutic strategies. In our country, conventional therapies remain standard, with monoclonal antibodies recently introduced for relapsed/refractory cases. This study aimed to assess treatment outcomes in relation to therapy type and prognostic factors.

A retrospective-prospective analysis was conducted on 200 patients with multiple myeloma. The relationship between treatment modality, disease biology, clinical status, and therapeutic response was evaluated, including progression-free survival (PFS), overall survival (OS), and protocol efficacy across prognostic subgroups.

Best treatment responses were achieved in patients with good performance status and low comorbidity, particularly those receiving first-line VTD (bortezomib, thalidomide, dexamethasone). Among patients under 65, the CTD (cyclophosphamide, thalidomide, dexamethasone) protocol showed the longest average OS. In second-line therapy, PAD (bortezomib, doxorubicin, dexamethasone) yielded the highest response rates and best survival outcomes. Elderly patients with high Charlson Comorbidity Index (CCI) benefited most from the MPT regimen (melphalan, prednisone, thalidomide). In contrast, Vel-Dex (bortezomib, dexamethasone) was linked to the highest progression rates.

Therapeutic outcome in myeloma strongly correlates with prognostic factors and treatment selection. Proper risk stratification enables personalized therapy and improves outcome.

Keywords: multiple myeloma, prognostic factors, risk stratification, therapeutic protocols, treatment outcome

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INTRODUCTION

Multiple myeloma is a malignant lymphoproliferative disorder, primarily characterized by the clonal proliferation and accumulation of pathologically altered plasma cells. The clinical manifestations result from the suppression of normal hematopoiesis in the bone marrow by plasma cells that produce a homogeneous monoclonal immunoglobulin, known as the M component. This disease is considered the prototype of neoplasms involving well-differentiated, mature, and typically slow-proliferating B lymphocytes–plasma cells (1). Clinically, multiple myeloma is defined by a characteristic pentad: anemia, detection of monoclonal (M) protein in serum and/or urine, skeletal lesions (mostly osteolytic), renal impairment, and hypercalcemia (2). After the diagnosis is established according to the criteria of the International Myeloma Working Group (IMWG), it is essential to assess the patient's prognostic profile. This patient-centered approach has significantly improved five-year survival rates over the past 15 years, now reaching 60%–70%, with curative potential observed in approximately 15% of cases. Prognostic factors influencing disease course and final outcome are based on the patient's clinical characteristics, the biological features of the disease, and nature of the therapeutic response, which are critical for defining a patient–tailored therapeutic approach (3).

Key clinical characteristics of patients are reflected through various comorbidity indices and patient age, while biological features are assessed using the Durie-Salmon classification, International Staging System (ISS), revised ISS (R-ISS), R2-ISS, and mSMART (Mayo Stratification for Myeloma and Risk-Adapted Therapy) classification (4). The depth and duration of therapeutic response significantly impact progression-free survival and overall survival. Achieving complete remission (CR) is considered one of the strongest prognostic markers (5).

The aim of this study was to evaluate the impact of different treatment modalities on therapeutic response, time to disease progression, and overall survival, with the identification of the most effective therapeutic combination in relation to patients' performance status and risk profile.

METHODS

This retrospective–prospective study included 200 patients with multiple myeloma, treated at the Clinic for Hematology, Allergology, and Clinical Immunology, University Clinical Center Nis, between 2016 and March 2023. The treatment outcomes of surviving patients are still being actively monitored. The diagnosis was established according to the criteria of the International Myeloma Working Group. The cohort comprised 105 men and 95 women, with a mean age of 62.49 ± 9.30 years(range 38–80 years). During the study, the following clinical and laboratory parameters were monitored: age, sex, performance status, clinical stage according to Durie-Salmon criteria, International Staging System (ISS), revised ISS for patients who underwent genetic analysis by FISH (Fluorescence In Situ Hybridization), Charlson Comorbidity Index, type of monoclonal (M) protein, serum albumin, B2-microglobulin, lactate dehydrogenase (LDH), serum calcium level, presence of osteolytic bone lesions, renal function, extramedullary disease, hemoglobin level, and platelet count. Treatment protocols were provided according to the Serbian Myeloma Group guidelines, following ESMO (European Society for Medical Oncology) and NCCN (National Comprehensive Cancer Network) recommendations. First-line regimens included VTD, CTD, VCD, PAD, TAD, MPV, MPT. Second-line regimens included PAD, Vel-Dexa, VTD, VCD, TCED, VTD-PACE, etc. (V/P–Velcade; T–thalidomide; D– dexamethasone; C–cyclophosphamide; A–Adriamycin; M–melphalan; P–prednisolone; E– etoposide). We assessed therapeutic response after first, second, and subsequent lines: complete response (CR), very good partial response (VGPR), partial response (PR), stable disease (SD), progression (PD) and relapse, as well as progression-free survival and overall survival in different prognostic groups. During the follow-up, 127 patients died and 73 were alive at study closure. Data are presented in the form of arithmetic mean and standard deviation, minimum and maximum values, and as absolute and relative frequencies. Comparisons of numerical variables between two groups were performed using the t-test or the Mann-Whitney test. Categorical variables were compared using the Chi-square test. Survival analysis was performed using the Kaplan-Meier survival curve and the log-rank test. The null hypothesis was tested at a significance level of $p < 0.05$. Statistical analyses were performed using the SPSS software package, version 16.0.

The study was approved by the Ethics Committee of the University Clinical Center Niš on June 19, 2024 (Approval No. 17351/9).

RESULTS

In the studied population, all patients diagnosed with multiple myeloma received first-line therapy (100.0%). Second-line treatment was administered to 52.0% of patients, third-line therapy to 24.0%, fourth-line therapy to 12.0%, and fifth-line therapy to 4.5% (Figure 1).

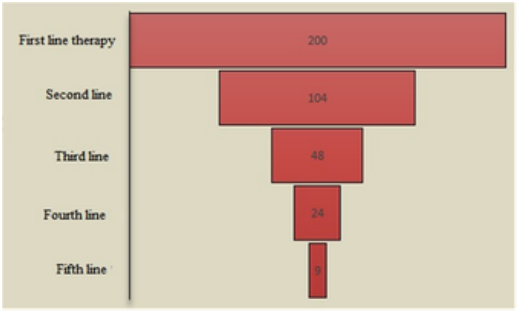

Figure 1. *Number of patients by treatment line*

The initiation and choice of therapeutic protocol were in accordance with the relevant clinical guidelines applicable at the time of treatment, which underwent changes over the study period (6). In the first-line therapy, the most frequently administered regimens were CTD (40.5%), followed by MPT (20.5%) and VTD (12.5%) (Figure 2). Among patients under the age of 65, the most commonly used therapeutic protocols were CTD (47.7%) and VTD (20.6%), whereas in older patients, MPT (37.6%) and CTD (32.3%) were predominantly used.

Following first-line therapy, complete remission (CR) was achieved in 2.1% of patients, very good partial remission (VGPR) in 48.9%, partial remission (PR) in 19.5%, stable disease (SD) in 8.4%, disease progression (PD) occurred in 18.9%, and in 2.1% of patients' treatment-related adverse effects prevented response assessment. No statistically significant difference in first-line response rates was observed between different age groups (p = 0.239) (Table 1).

Analysis of therapeutic response according to the first-line therapy showed that VGPR was achieved in 55.0% of patients treated with CTD and 50.0% of those treated with VTD. The highest rate of disease progression (53.3%) was observed in the group treated with Vel-Dex protocol as first-line therapy (Table 2). In second-line therapy, younger patients most frequently received PAD (33.9%), Vel-Dex (19.4%), and VTD (12.9%). Among patients over 65 years of age, Vel-Dex was the most frequently administred regimen (45.2%).

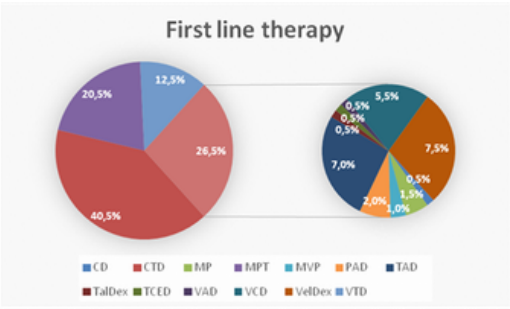

Figure 2. *First-line therapy in the studied population*

Table 1. *First-line therapy response by age group*

Response		Age (years)		Total
		≤65 years	>65 years	
CR	N	3	1	4
	%	2,9	1,1	2,1%
VGPR	N	46	47	93
	%	44,7	54,0	48,9%
PR	N	19	18	37
	%	18,4	20,7	19,5%
SD	N	12	4	16
	%	11,7	4,6	8,4%
PD	N	22	14	36
	%	21,4	16,1	18,9%
Tox eff	N	1	3	4
	%	1,0	3,4	2,1%

CR – complete remission; VGPR – very good partial remission; PR – partial remission; SD – stable disease; PD – disease progression; tox eff – toxic effects

When used as second–line therapy, PAD protocol was associated with the highest response rate (34.3% of patients achieved VGPR). In the studied population, 127 patients (63.5%) died. A statistically significant difference in mortality was observed across age groups (p = 0.014) and Charlson Comorbidity Index (CCI) categories (p < 0.001).

Sex, performance status (PS), clinical stage, ISS stage, and type of M-protein were not found to be statistically significant predictors of mortality. In our study population, the mean overall survival (OS) was 32.96 months (SE 1.92; 95% CI: 21.19–36.72 months), while the median overall survival was 36.00 months (range 12.00–48.00 months) (Figure 3). Overall survival significantly differed according to ISS stage (p=0.008). Median survival among patients with ISS stage I was 48.0 months (95% CI 36.1–59.9), for ISS stage II it was 30.0 months (95% CI 22.0–38.0), and for ISS stage III it was 24.0 months (95% CI 17.1–30.9) (Figure 4). In patients aged over 65 years, overall survival differed significantly according to Charlson Comorbidity Index (CCI) score (p = 0.012). Patients with CCI score 1 had a median overall survival of 48.5 months, compared to 29.5 months in those with a CCI score ≥ 2 (Figure 5).

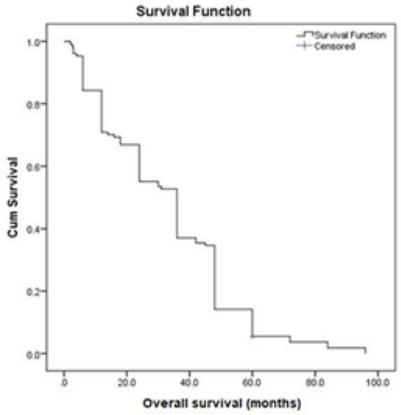

Figure 3. *Kaplan-Meier survival curve in the studied population*

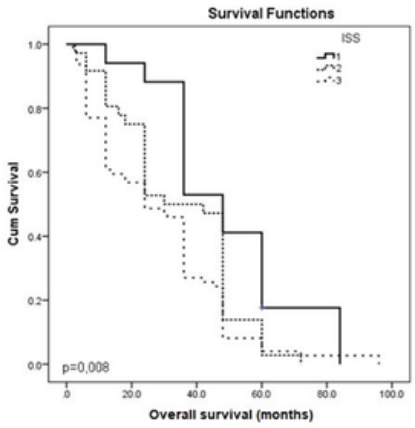

Figure 4. *Kaplan-Meier survival curve according to International Staging System (ISS) stage*

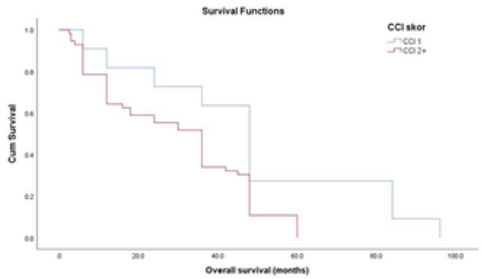

Figure 5. *Overall survival by Charlson Comorbidity Index (CCI) score in patients > 65 years*

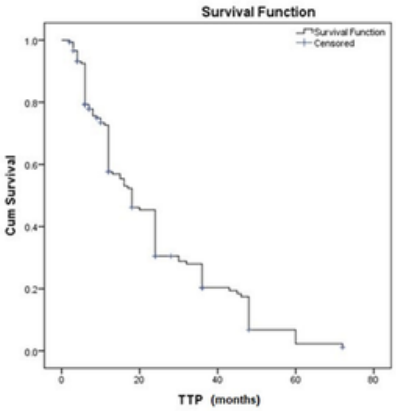

Figure 6. *Kaplan-Meier curve for time to progression (TTP) in the studied population*

Table 2. *Therapeutic response after first-line treatment*

		First line response						Total
		CR	VGPR	PR	SD	PD	Tox eff	
CD	Nbr	0	1	0	0	0	0	1
	%	0,0%	100,0%	0,0%	0,0%	0,0%	0,0%	100,0%
CTD	Nbr	0	44	16	9	9	2	80
	%	0,0%	55,0%	20,0%	11,3%	11,3%	2,5%	100,0%
MP	Nbr	0	2	1	0	0	0	3
	%	0,0%	66,7%	33,3%	0,0%	0,0%	0,0%	100,0%
MPT	Nbr	0	15	15	1	4	1	36
	%	0,0%	41,7%	41,7%	2,8%	11,1%	2,8%	100,0%
MVP	Nbr	0	1	0	0	0	0	1
	%	0,0%	100,0%	0,0%	0,0%	0,0%	0,0%	100,0%
PAD	Nbr	0	4	0	0	0	0	4
	%	0,0%	100,0%	0,0%	0,0%	0,0%	0,0%	100,0%
TAD	Nbr	0	5	2	1	5	1	14
	%	0,0%	35,7%	14,3%	7,1%	35,7%	7,1%	100,0%
TalDex	Nbr	0	0	0	0	1	0	1
	%	0,0%	0,0%	0,0%	0,0%	100,0%	0,0%	100,0%
TCED	Nbr	0	0	1	0	0	0	1
	%	0,0%	0,0%	100,0%	0,0%	0,0%	0,0%	100,0%
VAD	Nbr	0	1	0	0	0	0	1
	%	0,0%	100,0%	0,0%	0,0%	0,0%	0,0%	100,0%
VCD	Nbr	0	4	0	2	5	0	11
	%	0,0%	36,4%	0,0%	18,2%	45,5%	0,0%	100,0%
VelDex	Nbr	0	5	0	2	8	0	15
	%	0,0%	33,3%	0,0%	13,3%	53,3%	0,0%	100,0%
VTD	Nbr	4	11	2	1	4	0	22
	%	18,2%	50,0%	9,1%	4,5%	18,2%	0,0%	100,0%
Total	Nbr	4	93	37	16	36	4	190
	%	2,1%	48,9%	19,5%	8,4%	18,9%	2,1%	100,0%

C – cyclophosphamide; D – dexamethasone; T – thalidomide; M – melphalan; P – prednisolone; V,P – Velcade; A- Adriamycin; E- etoposide; CR – complete remission; VGPR – very good partial remission; PR - partial remission; SD – stable disease; PD – disease progression, tox eff – toxic effects

Overall survival (OS) differed significantly according to performance status (PS) ($p = 0.013$). The median OS for patients with PS 0–1 was 36.0 months (95% CI 32.0–40.0 months), whereas for those with PS ≥ 2 it was 31.0 months (95% CI, 19.7–42.4 months). In both younger and older patient subgroups, a statistically significant difference in OS was observed in relation to first-line therapy ($p = 0.034$ and $p = 0.004$, respectively). Among younger patients, the longest mean OS was achieved with CTD (40.0 months) and VTD (30.0 months), while the shortest was observed with VCD (16.0 months). Among older patients, the longest mean OS was achieved with MPT (36.5 months) and CTD (35.7 months), while the shortest was observed with VTD (4.5 months). Following second-line therapy, patients receiving PAD achieved the longest OS–48 months (95% CI, 44.76–51.24 months).

Time to progression (TTP) in the study population was 23.36 months (SE 1.55; 95% CI, 20.33–26.40), with a median TTP of 18.00 months (9.00–36.00 months) (Figure 6). Mean TTP did not vary significantly by sex, age, International Staging System (ISS) stage, Charlson Comorbidity Index (CCI) score, clinical stage, serum lactate dehydrogenase (LDH) > 460 U/L, or Eastern Cooperative Oncology Group (ECOG) performance status. A statistically significant difference in TTP was observed only concerning first-line therapy ($p < 0.001$). The longest median TTP was observed in patients treated with CTD (22.93 months), whereas the shortest was observed in those treated with Vel-Dex (9.22 months). Cox regression analysis identified statistically significant risk factors: prior autologous stem cell transplantation (HR 0.532, $p = 0.047$), CCI score 2+ (HR 1.298, $p = 0.041$), and elevated beta-2 microglobulin levels (HR 1.146, $p = 0.010$). In

the multivariate model, a failure to achieve VGPR after first-line therapy was the only statistically significant predictor (HR 3.162, p < 0.001). These patients had a 3.1-fold higher risk of mortality compared with the other patients.

DISCUSSION

Each year, more than 80.000 individuals worldwide are diagnosed with multiple myeloma, including approximately 520 cases in Serbia. In our unselected cohort of 200 patients, followed between 2016 and 2023, no substantial deviations in baseline characteristics were observed compared with those reported in the literature (7,8). The mean age at diagnosis was 62.5 years (range 38–80 years), which is notably younger than the mean age commonly reported (9, 10). The cohort comprised 105 men and 95 women. In this population, overall survival (OS) and median time to progression (TTP) did not differ statistically by sex, which is consistent with previously published findings (11).

Women more frequently presented with high-risk chromosomal aberrations; however, no significant differences in TTP or OS were observed compared with men (12, 13). Notably, women diagnosed with multiple myeloma before the age of 50 demonstrated a significantly poorer progression-free survival (PFS). It has been speculated that higher estrogen levels in younger women with multiple myeloma may contribute to an increased rate of disease progression, suggesting the potential consideration of anti-estrogen–based therapeutic strategies (14).

The Durie–Salmon staging system was used to determine the clinical stage of disease, serving as a parameter of tumor burden and classifying patients into three clinical stages, each subdivided into A and B categories according to renal function. In a 10-year prospective study of 109 patients with multiple myeloma, Spasov et al. reported no significant difference in OS between clinical stages II and III (15). However, patients with stage IIA disease had a longer mean survival compared with those in stage IIB (40 vs. 26 months), and patients in stage IIIA survived longer than those in stage IIIB (38 vs. 18 months). The authors concluded that patient survival depended more on the degree of renal impairment at diagnosis (A vs. B substages) than on tumor mass or clinical stage alone (15). In our cohort, median OS values were shorter than the averages reported in the literature: 34.8 months for CS I, 28.1 months for CS II, and 27 months for CS III.

Moreover, in the present study, the Durie–Salmon staging system did not demonstrate predictive value for time to progression, aligning with evidence that conventional clinical staging lacks sufficient prognostic accuracy and highlighting the necessity for incorporation of biological and additional prognostic factors. The mean overall survival (OS) in our cohort was 32.96 months, with a median OS of 36.00 months (range, 12.00–48.00 months). Published data demonstrate a progressive improvement in median OS over the decades: 22.4 months during 1980–1990, 37.4 months in 1991–2000, 61.8 months in 2001–2010, and 103.6 months from 2011 to 2020 (16).

This clearly reflects significant advancements in survival outcomes concomitant with the development of novel therapeutic modalities. The median overall survival of our patients, analyzed between 2016 and 2023, was 36 months, which corresponds to survival outcomes reported in the literature for patients treated between 1991 and 2000 (16). This finding is multifactorial. Therapeutic options used locally mirrored those applied worldwide, across the different time periods. Despite a mean patient age of 63.5 years, most treated patients were in their seventh or eighth decade, frequently burdened with multiple comorbidities. This influenced the choice of treatment regimens favoring reduced toxicity, albeit with potentially diminished efficacy. Survival outcomes in our population were significantly associated with ECOG performance status, International Staging System (ISS) stage, Charlson Comorbidity Index (CCI), beta-2 microglobulin levels, autologous stem cell transplantation (ASCT) status, first-line treatment protocol, and depth of response after both (first and second-line therapies). ECOG performance status 1 was predominant (36.0%), 53.0% of patients were classified as high-risk (ISS stage 3), and the majority of patients (44.5%) were at clinical stage IIIA. The most frequent M-protein subtype was IgG kappa (44.9%), and a CCI score 2 was most common (32.0%). Consistent with the existing literature, advanced age and comorbidities were linked to increased mortality risk in multiple myeloma (17). The impact of comorbidities was most pronounced during the first-year post-diagnosis in patients with CCI ≥ 3 and cardiovascular disease (18). Similarly, in our cohort, patients aged over 65 exhibited a statistically significant difference in survival stratified by CCI score. Since the mid-1990s, ASCT has been integrated into myeloma treatment and is associated with improved survival (19); however, ongoing research is required to optimize patient selection and outcomes.

The transplantation procedure must be evaluated holistically, considering patient age, disease burden, renal function, prior chemotherapy regimens, duration of disease before transplantation, stem cell dose, neutrophil engraftment kinetics, and psychosocial factors. Current evidence suggests that disease subtype, post-transplantation recovery rate, preserved renal function, and achieving complete remission prior to ASCT are the key determinants of transplant efficacy (20–22). In our center, 34 patients underwent ASCT. Median OS was significantly longer in transplanted patients (48.0 months) compared to non-transplanted patients (30.0 months). Beta-2 microglobulin remains the most robust and independent prognostic biomarker for survival in multiple myeloma, irrespective of renal function and clinical stage (23). Median OS was 48.0 months for ISS stage I, 30.0 months for ISS stage II, and 24.0 months for ISS stage III (beta-2 microglobulin > 5.5 g/L).

Within our cohort, FISH analysis was conducted in 34 patients. The limited number reflects the recent implementation of routine FISH testing, which commenced at the end of 2022. A subset of patients with double-hit myeloma exhibited rapid mortality following diagnosis, underscoring the significantly increased disease aggressiveness associated with the presence of concurrent high-risk mutations (24, 25).

First-line treatment outcomes

The majority of patients in our study received CTD as first-line therapy (40.5%), while 12.5% were treated with VTD. Treatment was administered in accordance with the current local clinical guidelines, and the lower number of patients receiving VTD protocol due to its delayed incorporation into practice. The CTD regimen as first-line therapy induced a VGPR in 55% of cases and a PR in 20%. It controlled disease symptoms, achieving stable disease in 11.3% of patients, while disease progression occurred in an equal percentage (11.3%). Overall survival (OS) was 38 months, and time to progression (TTP) was 22 months, representing the longest TTP observed in our cohort. We previously established a statistically significant difference in TTP depending on the first-line therapy (26-28), with the longest TTP observed in patients treated with CTD, and the shortest in those receiving Vel-Dex. Vasquez et al. reported that CTD therapy yielded a CR rate of 5% and VGPR of 32% (29). This protocol demonstrates good efficacy with tolerable toxicity, it is suitable for both transplant-eligible and transplant-ineligible patients, it is cost-effective, widely used in our country, and can be administered entirely orally. It can also be combined with novel agents, as described in the Cyklone study (cyclophosphamide, carfilzomib, thalidomide, and dexamethasone) (30), which showed high efficacy of this combination. Patients treated with the MPT protocol at our clinic achieved VGPR and PR in 41.7% of cases. This regimen rarely induced stable disease (2.8%) and led to progression in 11.1% of patients. The time to progression was 20 months, comparable to Hulín's study (31) (24 months) and Antonio Polumba's study (32) (21 months). In elderly patients, thalidomide is often omitted due to prior thrombotic events, resulting in the use of the MP protocol. Among patients treated with the VTD protocol, CR was achieved in 18.2%, VGPR in 50%, and progression in 18.2%. The TTP was 15 months. In the PETHEMA/GEM study (33), CR was achieved in 35% of patients treated with VTD, representing the highest complete remission rate attained with any pre-transplant regimen, even in patients with high-risk cytogenetic abnormalities. VGPR was achieved in 60%, and progression occurred in 13%. Median PFS was 56 months in patients with standard cytogenetic risk but significantly shorter (18 months) in those with high-risk abnormalities. In our cohort, VTD was the only protocol associated with CR according to International Myeloma Working Group criteria, achieved in 18.2% of patients. The highest progression rate (53.3%) was observed in patients treated with the Vel-Dex protocol. Vel-Dex induced VGPR in 33.3% and stable disease in 13.3% of patients.

When Vel-Dex induces a therapeutic response, its duration is typically short. Correspondingly, the shortest TTP in our cohort was 9 months for Vel-Dex, consistent with literature data from Huynh et al. (34) reporting TTP of 13.2 months and OS of 26.9 months. The average overall survival (OS) associated with the most commonly used first-line therapies was analyzed and found to differ significantly. Patients treated with CTD had the longest median OS of 38 months, while those receiving VMP had the shortest OS at 12.0 months. Patients on MPT achieved a median OS of 35.6 months, on VelDex 21.4 months, on VTD 17.2 months, and on VCD, the median OS was 17 months.

Second-line treatment outcomes

In our cohort, the highest overall response rate at first relapse was observed following the PAD regimen, with a

VGPR rate of 34.3% and a median overall survival (OS) of 48 months. Zhang et al. corroborated the efficacy of the PAD protocol in relapsed/refractory multiple myeloma (RRMM), demonstrating outcomes independent of conventional prognostic factors, particularly in patients exhibiting extramedullary disease. The triplet combination of bortezomib, doxorubicin, and dexamethasone reliably achieves at least a partial response (PR), even in patients with prior bortezomib exposure. This efficacy is attributed to the synergistic cytotoxic effects of bortezomib when combined with anthracyclines and alkylating agents, such as melphalan (35).

The survival of patients with multiple myeloma has markedly improved worldwide due to the availability of novel therapeutic options. Younger, transplant-eligible patients benefit from intensive regimens, including multi-drug combinations and autologous stem cell transplantation, while elderly, transplant-ineligible patients gain from agents with improved safety profiles. The introduction of anti-CD38 monoclonal antibodies (36), CAR-T cell therapies (37), bispecific antibodies (38), and antibody–drug conjugates (39) has expanded treatment possibilities, although these approaches are mostly reserved for relapsed/refractory cases. Additionally, novel therapeutic classes are expected to emerge (40); however, their clinical positioning and the development of MRD-guided treatment strategies have yet to be fully defined (41–43).

- The median overall survival (OS) of our entire patient cohort, as well as within individual disease stages, was shorter compared to internationally reported outcomes.
- The average survival of patients treated at our Clinic significantly differed based on performance status, ISS score, Charlson comorbidity index, β2-microglobulin levels, administration of ASCT regimen, first-line therapy protocol, therapeutic response after the first and second lines of treatment, identifying patients with poorer overall survival.
- The highest rate of complete remissions was observed in patients with good general condition and without significant comorbidities who were treated with the VTD protocol in the first line. The longest average survival was noted in younger patients treated with the CTD protocol.
- Elderly patients with a high Charlson comorbidity score benefited from MPT protocol, while the highest progression rate was associated with the Vel-Dex protocol.
- The combination of bortezomib, doxorubicin and dexamethasone (PAD) achieved the highest response rate and best survival outcomes in patients with multiple myeloma in the second-line treatment.
- Performing FISH analysis for every patient is essential. Patients with high-risk chromosomal aberrations or multiple concurrent high-risk genetic mutations (double or triple hit) require an individualized therapeutic approach, as they show statistically significant differences in mortality outcomes.

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Competing interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

CASE REPORT

POLATUZUMAB-VEDOTIN+BENDAMUSTIN+RITUXIMAB AS SALVAGE AND BRIDGING THERAPY IN RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA

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Although diffuse large B-cell lymphoma (DLBCL) represents a paradigm of highly curative disease with a complete remission (CR) rate of ≈ 60%–70% in an upfront setting, the remaining 30%–40% of patients present a relapse/refractory setting. These population are highly critical and amended for salvage treatment approach. Novel approaches and agents can overcome a problem in 20%–25%. Regarding this problem, polatuzumab vedotine represents one of the options. The use of this agent as bridging to further consolidation has been introduced from the real world experience with encouraging results.

We present a 54-year-old male patient diagnosed with primary gastric relapse/refractory (R/R) DLBCL who had been successfully treated by the introduction of antibody-drug conjugate polatuzumab-vedotine. After achieving complete response patient has been further consolidated with a high-dose chemotherapy followed by autologous graft.

Given the lack of availability of cellular therapies in developing countries, antibody-drug conjugate may be a plausible approach.

Keywords: polatuzumab-vedotine, bridging, relapse/refractory diffuse large B-cell lymphoma

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INTRODUCTION

Although diffuse large B-cell lymphoma (DLBCL) represents a paradigm of highly curative disease with a complete remission (CR) rate of ≈ 60%–70% in an upfront setting (1-3), the remaining 30%–40% of patients present a challenge. Recently, the treatment algorithm for relapse/refractory (R/R) DLBCL profoundly changed with the introduction of cellular therapies (CD19 CAR-T), particularly in patients suitable for this approach, relapsing early (≤ 12 months). Patients relapsing late (> 1 year) remained amenable for high-dose consolidation with an autologous stem cell transplantation (ASCT). Nevertheless, cumulatively, both approaches raised the cure rate by almost 20%–25% in relapse/refractory setting (4, 5). It should be added that a significant number of new compounds entered a regular use in R/R DLBCL: bispecific antibodies (glo-fitamab, epcoritamab) and others, such as polatuzumab-vedotin, tafasitamab, loncastuximab-tesirine as monoclonal agents and selinexor as the first in class nuclear export protein inhibitors. Notably, all of the novel agents have been primarily designated for transplant ineligible patients (6). Nevertheless, real world evidence data showed that all of the compounds emerged may be used as a bridging option to further high-dose consolidation in responding and fit patients by inclusion of chimeric antigen receptor (CAR-T-cell) therapy, allogeneic SCT or eventually by ASCT (7).

CASE REPORT

We present a 54-year-old male patient diagnosed with primary gastric R/R DLBCL. The disease started with typical gastrointestinal symptoms (nausea, dyspepsia, vomiting, weight loss). An endoscopic biopsy performed in May 2020 revealed a “bulky” unresectable tumor, initially marked as an anaplastic gastric carcinoma. As a result of misdiagnosis, the patient received three cisplatinum-based chemotherapy cycles. Magnetic resonance imaging showed progressive disease and radiological signs of highly suspected gastric lymphoma. A new biopsy was performed in August 2020, with a definitive diagnosis of DLBCL, not otherwise specified (NOS), non-germinal center B-like (non-GCB) with consequent immune profile: LCA+, CD20+, CD3+, bcl2+, bcl6-, CD10-, CyclinD1-, MUM1+, Ki 67 > 80%. The patient underwent R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) x 4, and R-COEP x 4 inductions (doxorubicin➔etoposide due to left ventricular ejection fraction decrease). A minimum of partial response (PR) was achieved, and the final 18-fluoro-deoxygenase positron emission tomography/computed tomography (FDG-PET/CT) scan assessment was planned. However, a massive relapse followed by gastric perforation occurred in less than a month upon the induction completion. After the palliative surgical treatment, and patient stabilization, ICE (ifosfamide, carboplatin, etoposide) salvage regimen was introduced with PR after three cycles. The molecular assessment showed no genetic alterations (double/triple hit rearrangements). Although PR was achieved, the patient was still transplant-ineligible and further treatment was continued with polatuzumab-vedotin + bendamustin + rituximab. After six cycles of treatment, a clinical complete response (CR) was achieved. The patient underwent high-dose consolidation and autologous stem cell transplantation (ASCT). Post-ASCT FDG-PET/CT showed metabolic CR in September 2022. Currently, the patient is on regular follow-up, with persistent two-year CR.

DISCUSSION

Polatuzumab-vedotin is an anti-CD79b targeted agent as antibody-drug conjugate with a monomethyl auristatin E (MMAE) payload recently approved for transplant ineligible R/R DLBCL patients after at least two prior therapies. The MMAE, as an immunotoxine, leads to a microtubule disruption which further drives to the G2/M-phase cell cycle arrest causing cell death. Polatuzumab-vedotin has been investigated as a single agent and in com-bination in relapse/refractory setting with other agents reaching an overall response rate (ORR) of > 50% (CR 16%–28.5%) (8). Toxicity profile was acceptable, mostly hematological (neutropenia grade 3/4 in ≈ 20% in phase 1 and 2 and low-grade peripheral neuropathy in 36% of patients (9-11). Real world data shows comparable activity with an ORR of 33%–60% (CR 14%–40%) without new safely concerns (12-16).
Polatuzumab-vedotin in combination with bendamustin and rituximab has been approved as salvage regimen in transplant-ineligible patients after two prior lines (6-8). The stronger benefit has been obtained in non-primary refractory patients and when this salvage was used in the earlier line (i.e. 2nd line). The retrospective real-world data showed that polatuzumab-vedotin regimens may be implemented as a successful bridging therapy to CAR-T cell therapy or allogeneic SCT in responding and fit patients (6). However, data on ASCT as a consolidation was obtained in

a very small cohort of patients based on real-world single-center experiences. Our patient received polatuzumab-vedotin with rituximab and bendamustin as a salvage in the 3rd line, achieving CR after six cycles. No serious adverse event was detected during treatment. Due to the aggressiveness of the disease, primary refractoriness, and a high risk of another consecutive relapse, we decided to use ASCT as a consolidation tool.

Although cellular therapies (i.e. CAR-T-cell) significantly changed the therapeutic landscape in the group of patients with R/R DLBCL relapsing early, they are still inaccessible in many countries given the high cost and lack of infrastructure. Therefore, antibody-drug conjugates, bispecific antibodies and agencies similar to the drugs we used historically, polatuzumab-vedotin remains a plausible treatment of choice in countries without cellular therapies, considering the fact the benefits do not differ significantly.

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Competing Interest

The authors declare no relevant conflicts of interest.

Statement of Ethics

Complete written informed consent was obtained from the involved patient for the publication of the study.

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AFMN BIOMEDICINE

CASE REPORT

CYSTIC DUCT WITH MEDIAL SPIRAL INSERTION

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The biliary system is well known for its anatomical variability. Precise imaging and evaluation of the cystic duct are essential for surgeons and interventional radiologists.

Herein we reported a rare case of cystic duct variation. Coronal oblique 3D magnetic resonance cholangiopancreatography (MRCP) revealed a posterior spiral course of the cystic duct, with a medial spiral insertion into the middle part of the extrahepatic bile duct. Intraoperative cholangiography confirmed this finding.

Specific anatomical variations might require modifications to the surgical approach. Understanding the cystic duct anatomy, variants, and disease processes aids in better diagnosis and interpretation of imaging results. MRCP is essential for providing key anatomical information and helps to reduce the risk of complications during percutaneous, endoscopic, and surgical procedures.

Keywords: cystic duct, anatomical variations, magnetic resonance cholangiopancreatography

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INTRODUCTION

The biliary system is well known for its anatomical variability which is usually unrecognized (1). With the growing frequency of laparoscopic cholecystectomy, hepatobiliary surgery, and transcholecystic biliary treatments, precise imaging and evaluation of the cystic duct are essential for surgeons and interventional radiologists (2). The biliary tract consists of intrahepatic and extrahepatic components. The cystic duct is roughly 2-4 cm long and 1-5 mm in diameter, connecting the neck of the gallbladder to the common hepatic duct (CHD) and forming the common bile duct (CBD). The cystic duct's point of insertion into the CHD varies. It typically enters the CHD from the right lateral aspect. It joins the CHD approximately halfway between the hepatic confluence and the ampulla of Vater (3).

CASE REPORT

Here, we report a rare case of cystic duct with medial spiral insertion to the mid part of the extrahepatic bile duct. A 60-year-old male was admitted to our department due to abdominal pain for seven days. The patient had a five-year history of recurrent visits to the hospital for right upper quadrant pain, nausea, and vomiting. Alcohol abuse was denied.

On admission he had normal vital signs. Physical examination demonstrated right upper quadrant tenderness without peritoneal signs with ne-gative clinical Murphy's sign. His blood work showed white blood cell count of 12,500/microliter, alanine aminotransferase of 218 U/L, aspartate aminotransferase of 159 U/L, alkaline phosphatase of 100 U/L, and total bilirubin of 1.4 mg/dL.

Ultrasound of the abdomen and pelvis revealed a dilated gallbladder with a thicker wall and with stones. The common bile duct measured 15 mm in diameter, and stones were visible within it. Furthermore, the ultrasonography revealed the dilatation of the intrahepatic bile ducts. Magnetic resonance cholangiopancreatography (MRCP) was conducted using 1.5 T MRI (Ingenia; Philips Healthcare, Best, the Netherlands) in the coronal, axial, and sagittal planes in accordance with the standard protocol. T2-weighted sequences in multi planar reconstruction were used for the evaluation. Respiratory-triggered T2 SPAIR axial and T2 coronal sequences with slice thickness of 5 mm, comprising the liver and region, were acquired. MRCP revealed a gallbladder with three small stones and a 16 mm-diameter CBD with four stones visible inside. Furthermore, coronal oblique 3D MRCP revealed a posterior spiral course of the cystic duct with medial insertion into the CHD (Figure 1).

Due to a large number of adhesions from previous operations, the laparoscopic approach was abandoned, and open cholecystectomy with bile duct exploration was indicated for symptomatic choledocholithiasis and acute-on-chronic cholecystitis.

Before the end of the operation, intraoperative cholangiogram was performed through the cystic duct, which showed the affirmative spiral course of cystic duct with medial insertion into the CHD, intact CBD, CHD, as well as right and left hepatic ducts without stones (Figure 2).

The patient's liver function tests returned to normal after surgery. On the third postoperative day after surgery, the drain was removed, and the patient was tolerating a diet. At his 2 and 4 week post-operative assessments in the outpatient clinic, there were no concerns regarding a bile leak or any problems. The pathology report showed acute-on-chronic cholecystitis.

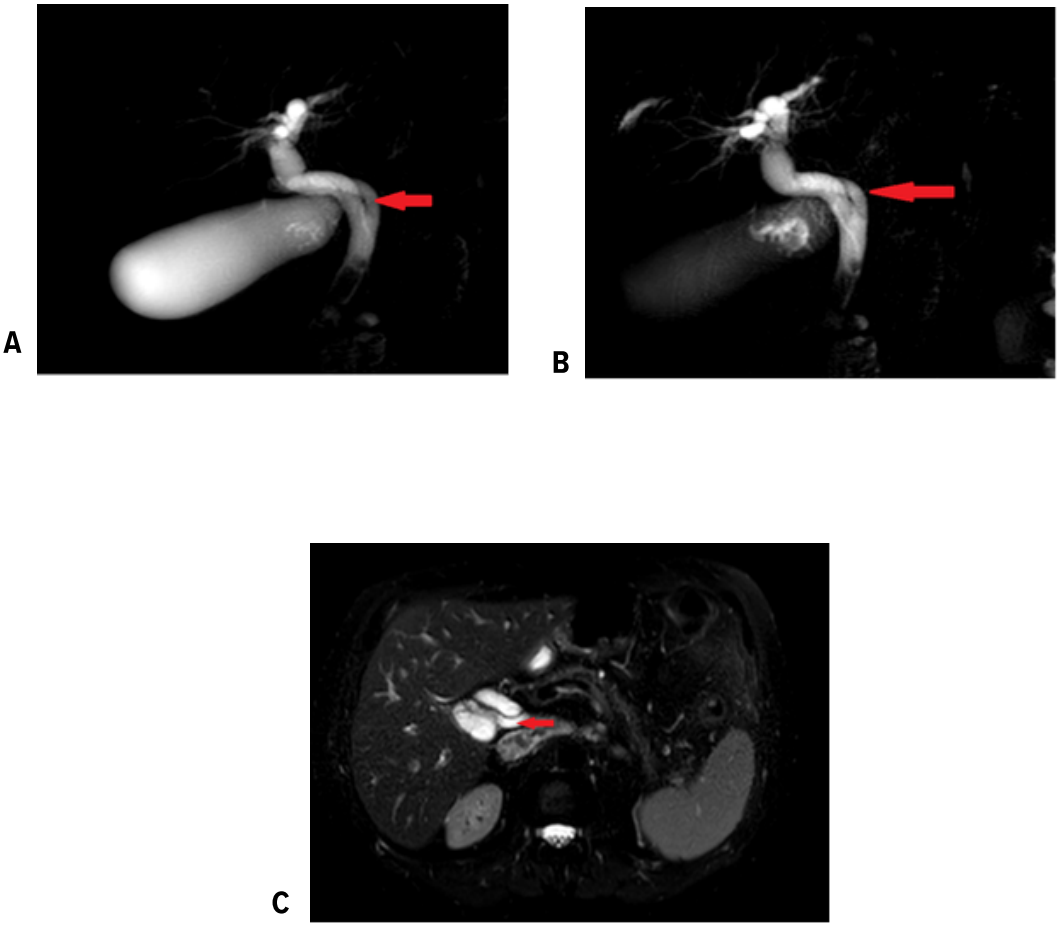

Figure 1. *Coronal oblique 3D MRCP (A and B) and T2w-SPAIR sequence in axial plane (C) show posterior spiral course of the cystic duct (red arrow) with medial insertion into the CHD*

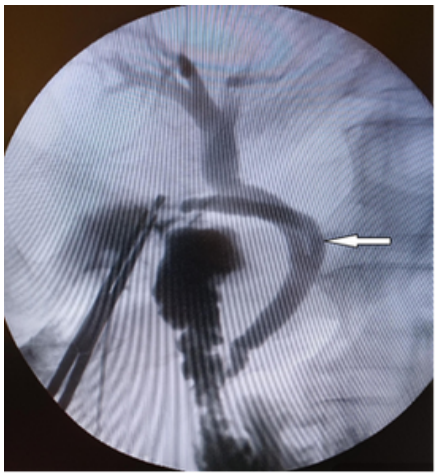

Figure 2. *Intraoperative cholangiogram shows spiral course of the cystic duct (white arrow) with medial insertion into the CHD*

Table 1. *Different cystic duct variations which are clinically more important*

I	low insertion of cystic duct
II	parallel course of cystic duct with CHD
III	anterior or posterior spiral course with medial insertion
IV	absent or short cystic duct (length < 5 mm)
V	aberrant drainage of cystic duct to right hepatic or left hepatic duct
VI	aberrant or accessory intrahepatic ducts draining into cystic duct
VII	double cystic duct

Abbreviation: CHD—the common hepatic duct

DISCUSSION

Ultrasonography (US), computed tomography (CT), endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiography (PTC), T-tube cholangiography, intraoperative cholangiography (IOC), magnetic resonance cholangiopancreatography (MRCP), and cholescintigraphy can all be used to evaluate the biliary system (4).

In one set of studies incorporating IOC, only 57% of cases yielded conclusive results, hence it is not frequently conducted (5). To address these limitations, MRCP is considered as the primary imaging modality. MRCP has a reported accuracy of 94.8% when compared to CT and US for detecting the anatomical variants. MRCP is crucial for providing essential data regarding cystic duct anatomy in cross-section and three-dimensional reconstruction images of the biliary tree, and it considerably improves the safety of laparoscopic cholecystectomy (6).

Different cystic duct variations are described in the literature depending on their length, course, and site of insertion with CHD. Sarawagi et al. (7) presented some variations which are clinically more important (Table 1). In their study, medial insertion was seen in 16% of cases, of which 4% were low medial insertions (7).

In conclusion, we reported a rare case of cystic duct with medial spiral insertion to the mid part of the extrahepatic bile duct. Specific anatomical variations might require modifications to the surgical approach. Understanding the cystic duct anatomy, variants, and disease processes aids in better diagnosis and interpretation of imaging results.

Acknowledgement

This study was not supported by any sponsor of funder.

Competing Interest

The authors declare no relevant conflicts of interest.

Statement of Ethics

Complete written informed consent was obtained from the involved patient for the publication of the study and accompanying images.

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AFMN BIOMEDICINE

CASE REPORT

CONCURRENT ISCHEMIC STROKES FROM OCCLUSION OF CAROTID AND VERTEBRAL ARTERIES FOLLOWING A WASP STING IN THE TONGUE

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There are no specific risk factors for stroke after bee or wasp stings; however, several researchers have cited multiple stings in the head and neck region as a significant factor in the occurrence of these serious complications. We present a case of a previously healthy man with multiple acute cerebral infarctions in the subcortical and borderline part of the cerebral media artery, resulting from total bilateral thrombosis of the internal carotid artery and right vertebral artery, triggered by a single axial puncture in the tongue, with complete neurological recovery.

We present the case of a male, aged 57 years, right-handed, with a history of arterial hypertension, hyperlipoproteinemia, smoking, and a positive family history of cardiovascular disease. He was hospitalized due to speech disorders, left-sided weakness, and altered behaviour. Magnetic resonance imaging of the brain showed multiple infarcts in the border and subcortical area of the cerebral media artery. Computed tomographic angiography of the blood vessels of the head and neck confirmed total bilateral occlusion of the carotid artery of the internal carotid artery and thrombosis of the right vertebral artery. Complete neurological recovery followed during hospitalization.

Non-specific clinical picture and neurological findings, characteristic of infarction in border zones, especially bilateral localization, can confuse the emergency physician, and massive thrombosis of blood vessels in the neck may be incorrectly predicted.

Keywords: wasp sting, stroke, border-zone infarction

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INTRODUCTION

It is known that doctors around the world who practice emergency medicine encounter a large number of Hymenoptera stings (1). These are predominantly local reactions lasting only a few hours (2), while neurological and vascular manifestations, including ischemic stroke, are very rare after wasp stings (3). In numerous reports, multiple wasp stings in the head and neck region are predominantly re-sponsible for acute stroke and neck blood vessel thrombosis (4, 5). Stroke after a wasp sting is the consequence of toxins containing vasoactive peptides, such as thromboxane, leukotriene, serotonin, and histamine (6, 7). According to the literature, cerebral infarctions following wasp stings are territorial (4, 5) and mainly localized in the vascular territory of the middle cerebral artery (MCA), whereas watershed infarctions are rare or only seldom described.

We present a previously healthy man who, after one wasp sting in the tongue, developed multiple cerebral infarctions, total bilateral occlusion of the internal carotid artery and the right vertebral artery, followed by complete neurological recovery.

CASE REPORT

The paper reports a 57-year-old right-handed male farmer with a history of arterial hypertension, hyperlipoproteinemia, smoking, and a positive family history of cardiovascular disease. He was admitted to the Department of Neurology for speech impairment, left-sided weakness, and altered behaviour. The patient reported the absence of previous illnesses and hospitalizations and allergic reactions to insect stings. The problems coincide with the wasp sting on the tongue, after which he felt severe, excruciating pain at the tongue tip, lips, and upper and lower jaws. Ten minutes after the wasp sting, the patient collapsed and was taken to the local infirmary 10 km away from the residence, where corticosteroids, antihistamines, and symptomatic therapy were administered. After being stabilized, the patient returned home.

The next day, the patient became drowsy, had no spatial awareness, collided with walls and doors while walking, did not recognize his family members, and could not perform simple actions. On the third day, he developed arm and leg weakness on the left side. He was drowsy most of the time.

Initially performed computed tomography of the brain (CTM) did not indicate pathological changes. Due to potentially significant neurological symptomatology, the patient was referred to the Neurology Clinic on the seventh day after the accident.

On admission, he was conscious, with complete amnesia, between the loss of consciousness and his return home from the local infirmary. The neurological findings included the signs of left-sided hemiparesis, 3/5 arm strength, and 4/5 leg strength, with hemihypoesthesia and partial right-sided hemianopia. His speech was fluent with verbal paraphasia, with good repetition ability, but with difficulty understanding. His blood pressure (BP) was 160/90 mmHg; findings across all organ systems were within normal limits.

Magnetic resonance imaging (MRI), including T1-weighted, T2-weighted, T2-FLAIR, and diffusion-weighted images, was performed on the sixth day after the sting. It showed multiple acute infarcts in the subcortical and border zones of vascularization of the right middle cerebral artery (MCA) and an-terior cerebral artery (ACA), as well as a cortical in-farct at the border between the left middle cerebral artery (MCA) and posterior cerebral artery (PCA) (Figure 1. a, b, c). Colour Doppler sonography (CDS) and computed tomographic angiography (CTA) of the neck and head vessels showed the complete bilateral occlusion of the carotid arteries and the right vertebral artery (Figure 2. a, b, c). An electro-cardiogram (ECG) was in sinus rhythm, and a two-dimensional echocardiogram was normal with pro-per left ventricular systolic function. The lipid profile was pathological (cholesterol 6.4 mmol/L, LDL cho-lesterol 4.1 mmol/L, HDL cholesterol 0.90 mmol/L, triglycerides 2.4 mmol/L), while the parameters of renal, hepatic, and thyroid functions, as well as the parameters of immune and coagulation status, were within the reference values. On the thirteenth day after the incident, the patient was fully neurologically recovered and discharged home. He was treated with antiedema treatment, aspirin, and low molecular weight heparin.

One month after complete neurological recovery, the patient was subsequently treated with dual antithrombotic therapy (acetylsalicylic acid–ASA 100 mg/day, clopidogrel 75 mg/day) and HMG-CoA reductase inhibitor (atorvastatin 40 mg/day) under the treatment protocol. At follow-up examinations over 12 months, our patient was neurologically stable.

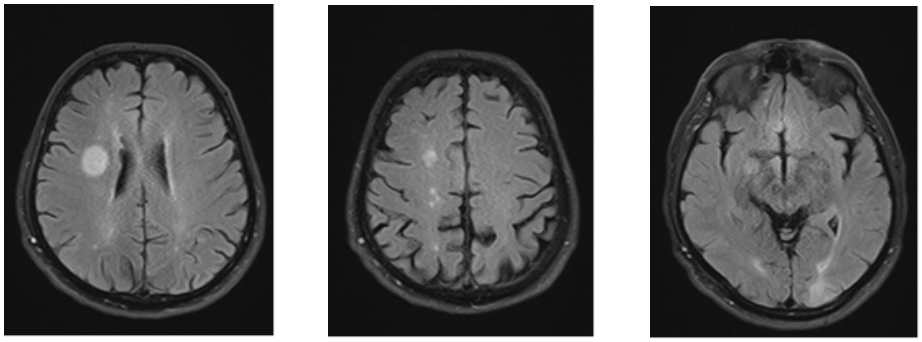

Figure 1. *Magnetic resonance imaging (MRI) T2 FLAIR image: (a) axial tomography–acute subcortical infarct on the right, T2 FLAIR image; (b) axial tomography–borderline multiple acute subcortical infarcts between the right MCA and ACA (rosary-like pattern), T2 FLAIR image; (c)–axial tomography–borderline cortical infarct between the left MCA and PCA*

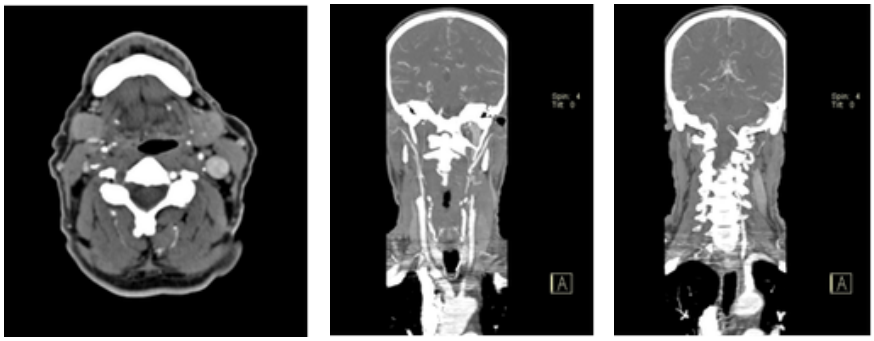

Figure 2. *Computed tomographic angiography (CTA)–axial projection: (a) bilateral internal carotid artery and right vertebral artery without contrast coronal MIP reconstruction (b) complete bilateral ICA occlusion, coronal MIP reconstruction; (c) right vertebral artery occlusion*

DISCUSSION

Hymenoptera bites are common during summer. The sting response can be local or systemic (2). Neurological complications such as cerebral infarction and thrombosis of blood vessels of the neck and head are very rare but often with significant complications (8), especially if a large number of wasps sting in a short time (4, 5).

Riggs and associates reported the case of a man who experienced multiple wasp stings on the shoulders and left half-face. Two days later, the left internal carotid artery occluded, and an ischemic stroke followed (8). The mechanism of ischemic stroke in this patient was supported by a neuropharmacological model emphasizing sympathetic sensitization of the terminal part of the internal carotid artery by stimulating the upper cervical ganglion, suggesting multiple wasp stings as a risk factor for the ipsilateral carotid artery thrombosis.

In our case, occlusive lesions of the blood vessels are far more extensive. Total and symmetrical thrombosis spreads to carotid arteries up to their intracranial terminal segment and the right vertebral artery in its extracranial and intracranial segment, as reported in the pathophysiological model (9).

The difference between our case and other case reports (10–13) in the first instance suggested multiple wasp stings as a risk factor for brain stroke and/or vascular spasm and thrombosis, while in our case, there was a single wasp sting. Multi-day drowsiness, focal neurological symptoms, intraluminal occlusion of vessels in the neck, and multiple acute brain infarcts in specific vascular distribution suggested the development of occlusion after the accident (Figure 1 and 2).

Payeman et al. (5) determined that the time interval between a Hymenoptera insect sting and stroke varied from 15 minutes to 4 days, with a median of 16 hours, while in our patient, it was between 12 hours and three days. Initially, the clinical picture indicated transcortical sensory aphasia and right-sided anopsia, as a result of a cortical infarction in the border zone of vascularization on the left between the

middle cerebral artery (MCA) and the posterior cerebral artery (PCA). A day later, a left-sided sensorimotor deficit appeared, caused by a subcortical infarction in the border zone of vascularization on the right between the middle cerebral artery (MCA) and the anterior cerebral artery (ACA). The findings of the affected areas in the systematic review are not consistent with our patients' vascular areas. Our case deals with the brain infarct of border vascular distribution, and, to our knowledge, this is the first case indicating that a wasp sting localized as described above can be an etiological factor in the genesis of a stroke.

In addition to the hemodynamic mechanism responsible for multiple brain infarctions in the border area, we must not ignore a possible additional impact of the vasospasm of peripheral branches in the middle cerebral artery. This is mainly related to the infarction lesion localized in the subcortical segment that alternatively supports the pathogenic mechanism proposed by Kulhari et al. (4).

Non-specific clinical picture and neurological findings that characterize patients with infarction of border zones, especially bilateral localizations, can confuse emergency physicians; therefore, massive thrombosis of blood vessels of the neck is overlooked.

Occlusion of the blood vessels of the neck, caused by a wasp sting in the region of the head or neck, imposes an urgent need for doctors who take care of these patients to conduct timely diagnostic processing, among others colour Doppler sonography of the blood vessels of the neck, especially in middle-aged and elderly population.

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Competing Interest

The authors declared no relevant conflicts of interest.

Statement of Ethics

Complete written informed consent was obtained from the involved patient for the publication of the study and accompanying images.

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ERRATUM

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TREATMENT OF BURN INJURIES IN CHILDREN (VOL. 42, NO. 2, P. 153–164)

Editorial Board of the Journal *Acta facultatis medicae Naissensis*

This erratum is published to address inaccuracies in the first affiliation of the author of the above-mentioned article. The first affiliation, as presented on the title page, the last page, and within the abstract in Serbian, was not cited consistently. In addition, the official name of the clinic changed shortly after the article's publication. The enumeration of affiliations was also inadvertently omitted.
We express our gratitude to the author for identifying these errors. The corrected title of the affiliation and the proper enumeration of affiliations are provided below.

Acta facultatis medicae Naissensis 2025; 42(2):153–164.

In the article entitled "Treatment of Burn Injuries in Children", authored by Vesna Marjanović, published in volume 42, issue 2, pages 153-164, the title of the author's affiliations were not consistently cited. Therefore, the full and correct first affiliation of the author should be:
Clinic of Anesthesiology, Reanimatology and Intensive Therapy, University Clinical Center Niš, Serbia
Secondly, the affiliations should be enumerated as follows:
[1]Clinic of Anesthesiology, Reanimatology and Intensive Therapy, University Clinical Center Niš, Serbia
[2]Department of Surgery and Anesthesiology with Reanimatology, University of Niš Faculty of Medicine, Niš, Serbia

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