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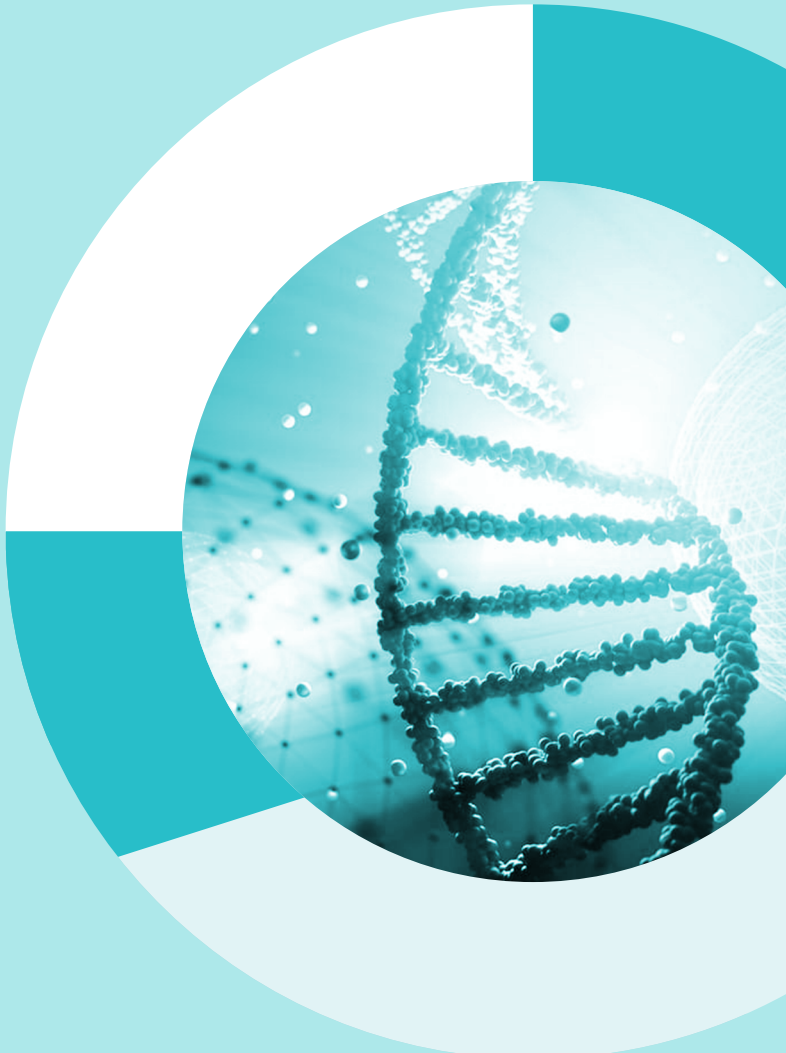

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REVIEW

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Extrapulmonary Comorbidities in COPD: A Bidirectional and Multimorbid Perspective

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Abstract

Chronic obstructive pulmonary disease (COPD) remains among the leading causes of death. The systemic characteristics of COPD are becoming clearer every day, and the importance of comorbidities is increasingly well understood. Comorbidities are one of the main causes of mortality in COPD. Although there are specific guidelines for each disease, there is limited literature explaining the effects of comorbidities on one another. Non-respiratory comorbidities associated with mortality in COPD include cardiovascular disease (CVD), metabolic, musculoskeletal, and psychiatric disorders. Both COPD and these comorbid conditions share a common aetiology and are associated with complex clinical and therapeutic interactions. CVD and metabolic comorbidities are more symptomatic and can be easily recognised by patients and physicians. However, osteoporosis, anxiety, and depression are often asymptomatic, despite the fact that these are more common than other comorbidities. Current data suggest that diagnosis and treatment of comorbidities improve prognosis in COPD. Instead of focusing solely on COPD treatment, we should adopt a holistic approach to patient care. This narrative review aims to summarise the bidirectional relationships between COPD and its most common fatal extrapulmonary comorbidities. Another aim of the study is to compile a substantial body of scattered information to provide clinicians with an overview.

Keywords: COPD, cardiovasculary diseases, diabetes mellitus, osteoporosis, depression

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a non-homogeneous pulmonary disease characterized by chronic symptoms (dyspnea, cough, sputum, and/or exacerbations) due to abnormalities of the airways and/or alveoli that cause persistent, often progressive, airflow obstruction.[1] However, COPD cannot be defined in a single sentence. Even though new evidence has emerged, COPD requires further research from aetiology to classification and from diagnosis to treatment. COPD is a disease, or more accurately a syndrome, that cannot be explained by a single mechanism and involves numerous gene-environment interactions and many confounding factors, such as comorbidities.

COPD, the third-most common cause of mortality, carries a high comorbidity burden owing to shared aetiological factors and pathophysiological characteristics.[2] Comorbidities are important in COPD because they increase the risk of exacerbation and mortality.[3] The majority of mild-to-moderate COPD patients die from non-respiratory causes.[4]

Today, COPD is recognised as a multimorbid disease. Comorbidities, which are increasingly emphasised in GOLD reports, are important because they share a common aetiology with COPD and contribute to difficulties in disease management. An understanding of comorbidities requires a multifaceted perspective. Just as we consider the impact of comorbidities on COPD, specialists in other fields also consider

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the impact of COPD as a comorbidity. Since comorbid conditions may interact and influence each other, including their diagnosis and treatment processes, a holistic assessment is important.

Both pulmonary and extrapulmonary comorbidities contribute to mortality in COPD. This article is a narrative review based on current evidence from observational studies, randomized trials, and international guidelines. The objective of this article is to review the reciprocal effects of COPD and extrapulmonary comorbidities.

Multimorbidity

The coexistence of multiple chronic conditions is referred to as multimorbidity. When examining the concept of comorbidity, the diagnostic and treatment methods for each disease are discussed. However, when discussing multimorbidity, it is necessary to consider the connections among comorbid conditions. The patient's age, genetic features, and sociocultural background should also be included among these intersecting sets (Figure 1).

The interactions between different morbidities and COPD are complex and can make their effects difficult to predict. In reality, the effect of multimorbidity on an individual's risk profile may be considerably greater than the simple aggregation of the individual effects anticipated from the identified conditions.

In GOLD 2026, the 4M model was introduced as part of the COPD management framework.[1] This model, which consists of mentation, mobility, morbidities, and medications, was initially developed for elderly populations without infectious conditions. Based on the 4M approach, GOLD 2026 offers a detailed assessment of multimorbidity in COPD[1] and organizes COPD-related conditions into five clusters. These include a mental cluster, a respiratory cluster, a multiple-organ loss-of-tissue cluster, a metabolic disease cluster, and a cardiovascular cluster, each encompassing a range of relevant comorbidities. As the present article specifically addresses extrapulmonary comorbidities associated with mortality in COPD, a condensed version of this framework is illustrated in Figure 1.

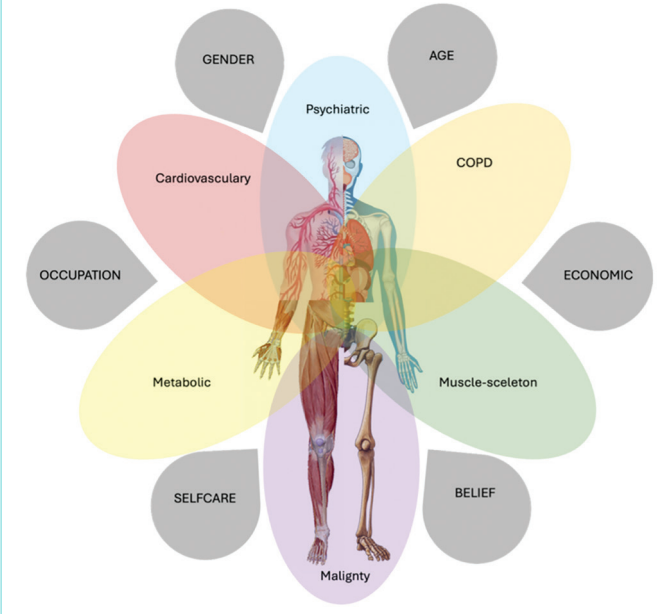

Figure 1. Multimorbid effect on human body. (Modified from reference 3).

COPD: Chronic obstructive pulmonary disease.

Today, multimorbidity is a global health priority, and the number of multimorbid individuals is increasing as the world's population ages.[5] The concepts of multimorbidity and frailty are often used together. Frailty is a clinical syndrome arising from multiple underlying factors and characterized by reduced strength, endurance, and physiological function that predispose individuals to increased dependency and an elevated risk of death.[6] It has been argued that multimorbidity and frailty lead to disability, and that their intersection may itself constitute disability.[6] Large-scale studies have shown that COPD is frequently part of multimorbidity.[7,8]

According to United Kingdom (UK) Biobank data, which includes nearly half a million participants, the five most common long-term conditions associated with frailty are multiple sclerosis, chronic fatigue syndrome, diabetes mellitus, COPD, and connective tissue diseases.[7] A multimorbidity study based on data from half a million people in China identified four multimorbidity patterns. The cardiometabolic and respiratory multimorbidity groups experienced a more fatal trajectory.[7] The gastrointestinal and rheumatological/mental multimorbidity groups were found to have lower mortality.[7] According to Divo and Celli,[9] mortality rises as the number of comorbid conditions increases among COPD patients. The main extrapulmonary comorbidities associated with mortality in COPD are cardiac, metabolic, musculoskeletal, and psychiatric.[3]

Chronic Obstructive Pulmonary Disease and Cardiovascular Comorbidities

Cardiovascular disease (CVD) includes acute myocardial infarction (AMI), coronary artery disease, hypertension, atrial fibrillation (AF), and heart failure (HF). CVD has been reported in 20-60% of patients with COPD.[10]

Processes such as smoking, air pollution, and ageing are common aetiological factors in both COPD and CVD. The pathophysiological relationship between COPD and CVD is explained in two ways (Figure 2). The first is a spillover mechanism. In COPD, local inflammation may spill over from the lungs into the systemic circulation and contribute to CVD.[10,11] A second in COPD mechanism is that emphysema, airway obstruction, small airway disease, epithelial dysfunction, remodelling, and hypoxia cause systemic inflammation. C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-8, and fibrinogen, which are responsible for this inflammation, reach the cardiovascular system via the systemic circulation and trigger local inflammation there. However, a causal relationship between COPD and comorbidities may also operate in the opposite direction: for example, atherosclerosis, arterial calcification, endothelial dysfunction, and tissue hypoxia in CVD may lead to the release of inflammatory cytokines similar to those released in the lungs. These may reach the lungs via the systemic circulation and contribute to the development and/or progression of COPD.[10]

Chronic obstructive pulmonary disease and acute myocardial infarction

The prevalence of AMI in COPD patients is 3.5 times higher than that in those without COPD,[12] and the prevalence of COPD in people with AMI varies between 7% and 30%.[13] In patients with COPD, comorbid conditions such as hypertension, diabetes mellitus, and hyperlipidaemia are more

common than in the general population and increase the risk of AMI. In addition, matrix metalloproteinases in COPD may trigger an AMI by rupturing plaques. Furthermore, elevated coagulation factor levels and increased platelet activation in COPD lead to coronary thrombosis and increase the risk of AMI.[13]

Diagnosing AMI in COPD is not always straightforward. Both AMI and COPD may present with similar clinical symptoms, such as dyspnoea and chest tightness. The frequency of typical chest pain in AMI patients with COPD is lower than in those without COPD, which may delay the diagnosis of AMI in patients with COPD and potentially increase myocardial damage or delay reperfusion strategies.[13] Clinicians should exercise caution in this regard. Making a differential diagnosis swiftly and correctly may prevent unnecessary tests and inappropriate treatment. One of the most commonly used biomarkers for differential diagnosis is cardiac troponin.

Almost 40% of stable COPD patients have elevated cardiac troponin levels compared with the general population.[1] Because cardiac troponin levels can be elevated in infectious COPD exacerbations, their utility in diagnosing AMI in this setting is limited. The mechanism underlying troponin elevation during COPD exacerbations is unclear; further research is needed.[14,15] The current GOLD 2026 recommendation is to measure troponin levels in patients with COPD who present with unexplained dyspnoea, chest tightness or chest pain, and worsening exercise capacity.[1]

The medical treatment of AMI in patients with COPD should be the same as in patients without COPD.[8] In AMI, percutaneous intervention performed at the appropriate time for the correct indication saves lives. However, it has been reported that patients with COPD presenting with non-ST-segment elevation myocardial infarction undergo fewer percutaneous interventions than patients without COPD, despite being at higher risk.[15,16] Possible reasons include

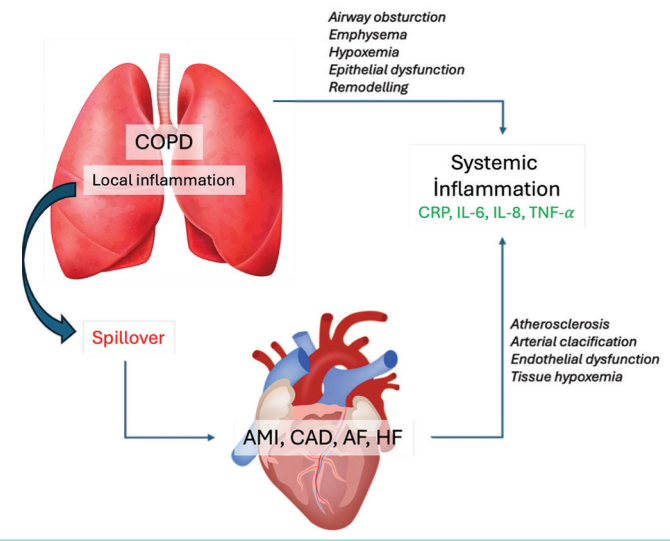

Figure 2. Local and systemic effects of COPD and CVD (This figure was created using the information in references 10 and 11).

COPD: Chronic obstructive pulmonary disease, CVD: Cardiovascular disease, CRP: C-reactive protein, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, AMI: Acute myocardial infarction, CAD: Coronary artery disease, AF: Atrial fibrillation, HF: Heart failure.

the older age of COPD patients and their multimorbidities, which may influence decisions regarding suitability for more aggressive interventions.[16]

Chronic obstructive pulmonary disease and atrial fibrillation

AF is the most common cardiac arrhythmia. It is described as a "growing epidemic" because 43 million people worldwide have AF. COPD, affecting 392 million people, is a growing epidemic.[2]

Various mechanisms have been proposed to explain AF in COPD. COPD is characterised by hyperinflation, ventilation/perfusion mismatch, and airway obstruction. These structural and functional alterations may trigger AF by causing sympathetic nervous system activation, vascular remodelling, and local cardiac conduction disturbances. In a study that analysed over 21 million hospitalised COPD patients, the prevalence of AF was 22.1%, and mortality was 5.7 times higher in patients with AF.[17] In a smaller study of patients with AF, the prevalence of COPD was 11.5%, and mortality was higher among patients with both AF and COPD.[18] In a prospective observational study of 4,000 AF patients in Asia, the risk of all-cause mortality was 3.9-fold higher among those with COPD.[19]

In COPD, AF frequently presents with increased dyspnoea. Despite electrocardiogram (ECG) being the primary diagnostic method, Holter monitoring is recommended, especially in patients with intermittent dizziness.[1] The mainstays of AF treatment are anticoagulants and rhythm-control agents. The potential adverse effects of rhythm controllers on COPD are discussed in the following sections.

Chronic obstructive pulmonary disease and heart failure

COPD and HF largely share common aetiologies. In both conditions, progressive dyspnoea is a major symptom and can complicate differential diagnosis and disease management. Some symptoms may be helpful in differential diagnosis and indicative of illnesses, but are not definitive.[20] Cough is mainly a symptom associated with COPD, but in HF it may be related to pulmonary congestion or to the use of angiotensin-converting enzyme (ACE) inhibitors. Although night-time symptoms are more common in HF, 20% of patients with severe COPD also experience them. Particular caution is therefore required for elderly patients with a history of smoking.[20]

COPD and HF share common pathophysiological mechanisms. In COPD, congestion develops due to increased airway obstruction, increased intrathoracic pressure, and a fall in left ventricular function. This reduces cardiac output. On the other hand, increased volume overload and/or myocardial dysfunction in HF lead to increased airway obstruction and worsening of COPD symptoms, and may cause hypoxaemia. In addition, both diseases involve sympathetic overdrive, which increases pulmonary vascular resistance by activating the renin-angiotensin-aldosterone system.[1,20]

The prevalence of COPD in HF, confirmed by pulmonary function testing, is between 25% and 50%.[20] However, the prevalence of COPD based solely on patient self-report, without spirometry, has been reported to be around 10%.[20] A recent study from Sweden found that the prevalence of COPD among patients with HF was 13%.[21] In the same study, the highest prevalence of COPD (16%) was found in HF with preserved ejection fraction (HFpEF). In HF with mildly reduced EF, the prevalence of COPD was 12%, and it was 11% in HF with reduced EF. Although the prevalence of HF confirmed by echocardiography in COPD

is 5-20%, nearly half of patients with COPD have elevated pro-brain natriuretic peptide (pro-BNP) levels, suggesting myocardial damage.[20]

The coexistence of COPD and HF is associated with greater mortality than that observed with COPD alone.[22] A 15% higher mortality rate was observed among patients with COPD and concomitant HF, independent of EF.[21] In a study of patients with HFpEF, the prevalence of COPD was found to be 14%, and COPD was associated with increased hospitalisation and mortality.[23]

GOLD 2026 recommends the measurement of natriuretic peptides in patients with heart failure, followed by ECHO if natriuretic peptides are abnormal.[1]

For clinicians, probably the most important issue is the differential diagnosis between COPD and heart failure. The absence of left HF on ECHO does not exclude HF. It should be borne in mind that HFpEF is common in these patients, and it can worsen symptoms and increase hospital admissions and mortality. Examinations and management strategies should be organised according to current guidelines.

Therapeutic interactions in chronic obstructive pulmonary disease and cardiovascular disease

One of the most controversial issues in the co-occurrence of COPD and CVD is treatment: cardiac drugs can have adverse effects on the lungs, while pulmonary drugs may adversely affect the heart. The most commonly used medication groups in CVD are beta-blockers, ACE inhibitors, angiotensin receptor blockers, and diuretics. There is particular concern regarding pulmonary side effects associated with beta-blockers. It is believed that beta-blockers, which have been proven to decrease mortality in CVD, may worsen COPD, while bronchodilators may trigger arrhythmias.

The first meta-analysis, showing that beta blockers do not adversely affect lung function, was published 22 years ago; however, there is still hesitation today in prescribing beta blockers to patients with COPD.[24] An increasing number of studies have shown that the bronchoconstrictor effect of B1-cardioselective beta-blockers is less than that of B2-selective ones.[25] A review of 23 observational studies and 14 randomized controlled trials (RCTs) concluded that beta-blockers can be used safely in patients with COPD with respect to adverse effects, exacerbation risk, and mortality.[26]

In terms of cardiac side effects of antimuscarinic bronchodilators, the 4-year Understanding Potential Long-term Impacts on Function with Tiotropium study provided reassurance about the use of tiotropium,[27] and the Aclidinium Safety and Cardiovascular Endpoints Trial study found that, after a three-year follow-up period, patients using aclidinium had a cardiac side-effect profile similar to that of patients receiving placebo.[28] In contrast, concerns regarding beta-2 agonists persist due in part to conflicting study results. The SUMMIT study, which examined 16,485 COPD patients with existing CVD or at high risk of it, compared placebo, long-acting beta-2 agonist (LABA), and inhaled corticosteroid (ICS) groups.[29] LABA was not found to affect cardiovascular outcomes (AMI, mortality, or AF). In contrast, a recently published cohort study involving 180,367 patients from the UK Clinical Practice Research Datalink reported that initiation of short-acting beta-agonist (SABA), LABA, or ICS plus LABA therapy in patients with COPD was associated with major adverse cardiac events (MACE).[30] A meta-analysis published in 2019 that examined 43 RCTs concluded that long-acting bronchodilators did not cause major cardiac adverse effects in stable COPD, but that LABA increased the risk of HF.[31] However, a more recent publication, including 74,974 asthma patients, 46,907 COPD patients, and 27,047 patients with asthma-COPD overlap, reported a low risk of arrhythmia, hypertension, heart failure, and cerebrovascular diseases among COPD patients using LABA.[32] In summary, although findings regarding MACE associated with beta-agonists are conflicting and it has been emphasised that LABAs should be used cautiously in patients with COPD and HF, the overall findings indicate that LABAs are safe to use in COPD. When interpreting these studies, researchers should pay attention to the methodology. The clinician's own experience is also important in the choice of treatment.

Ensfentrine is a novel inhaled treatment option introduced to relieve dyspnoea in patients with stable COPD. It is a phosphodiesterase-3 and -4 inhibitor.[1] There is no evidence that it causes adverse cardiovascular events. Ensifentrine is currently only available in the US.[1]

Due to shared aetiological and pathophysiological characteristics, CVD may be common among patients with COPD. If there is unexplained worsening of symptoms in a COPD patient, CVDs should be investigated, and vice versa. These patients should be carefully monitored for adverse drug effects and drug-drug interactions. The GOLD 2026 report recommends annual ECG and pro-BNP screening for patients with COPD. Although it is necessary to act in accordance with current guidelines, we need further research on this subject.

Chronic Obstructive Pulmonary Disease and Diabetes Mellitus

COPD and DM are among the most prevalent chronic diseases worldwide. The prevalence of DM in COPD has been reported to be between 20% and 30%.[1,33] Smoking is a common aetiological factor, and both systemic oxidative stress (associated with increased fibrinogen, CRP, IL-1, IL-6, and TNF-α) and chronic inflammation occur in COPD and DM.[33,34] However, the relationship between them is not well understood. Various mechanisms have been proposed. Smoking leads to oxidative stress, systemic inflammation and insulin resistance. Pro-inflammatory biomarkers such as CRP, TNF-α, and IL-6, which are released during systemic inflammation, promote COPD progression and disrupt insulin signalling. Oxidative stress impairs glucose uptake in skeletal muscle by causing mitochondrial dysfunction and thereby increases insulin resistance.[33] In COPD, recurrent acute hypoxic episodes activate the sympathetic nervous system, leading to increased catecholamine release and insulin resistance. Chronic hypoxia reduces skeletal muscle insulin sensitivity by causing permanent mitochondrial dysfunction and decreased adenosine triphosphate production.[33]

In COPD, physical inactivity caused by chronic dyspnoea and fatigue contributes significantly to insulin resistance, muscle atrophy, and metabolic dysfunction.[34] Because skeletal muscle plays a significant role in glucose homeostasis, the ability to regulate blood sugar levels is impaired. A sedentary lifestyle worsens systemic inflammation and impairs mitochondrial function. These factors increase the risk of metabolic complications in patients with COPD and type 2 DM.[33] Additionally, it has been suggested that the autonomic neuropathy caused by diabetes may reduce airway calibre by inducing parasympathetic dysfunction, impairing the respiratory response to hypoxia, increasing the risk of lung infection by causing mucociliary dysfunction, and reducing the strength of the diaphragm and other respiratory muscles.[35]

The most reliable methods for DM diagnosis are measurements of fasting glucose and glycated hemoglobin A1c (HbA1c). It is recommended that fasting glucose and HbA1c levels be monitored every 3-5 years in COPD patients in GOLD 2026.

Therapeutic interactions in chronic obstructive pulmonary disease and diabetes mellitus

When examining the relationship between two diseases, researchers must consider the negative impact of treatments on those diseases.

• Chronic obstructive pulmonary disease medications in diabetes mellitus

Although bronchodilators form the basis of COPD treatment, ICSs are used particularly in eosinophilic COPD, and systemic corticosteroids (SCSs) are used during COPD exacerbations.[1] Adherence to COPD guidelines is lower than expected, resulting in higher-than-anticipated use of inhaled steroids.

SCSs such as prednisone increase glucose production by promoting hepatic gluconeogenesis and reduce peripheral glucose uptake, thereby impairing insulin sensitivity.[36] This effect is dose-dependent: as the dose increases, the risks of hyperglycaemia, weight gain, and visceral fat accumulation increase. This also means that each dose of prednisone administered during an exacerbation increases the patient's risk of developing diabetes.

ICS also impairs glycaemic control, although less commonly than SCS; this impairment is dose-dependent.[33] A meta-analysis concluded that the risk of DM or impaired glucose control increased by 34% in patients using >1,000 µg/day of fluticasone or its equivalent.[37] Although corticosteroids are the main group of medications expected to have an adverse effect on DM in COPD, beta-agonists may affect glucose metabolism.[33] Both LABAs and SABAs may increase insulin resistance and hepatic glucose production through activation of beta-adrenergic receptors.[33,38] It is recommended that the effects of these medications be regularly monitored, particularly in COPD patients with DM and impaired glucose regulation. However, no adverse effect of antimuscarinics on glucose metabolism has been demonstrated. Considering these metabolic outcomes, it has been reported that LAMAs may be suitable as the first-choice for stable COPD to mitigate harmful metabolic effects.[33]

• Diabetes mellitus medications in chronic obstructive pulmonary disease

Metformin, which is the main medication option for type 2 DM, has been found to exhibit anti-inflammatory properties that can help in COPD by reducing systemic inflammation and oxidative stress.[39] Although metformin has been shown to improve lung function and reduce COPD exacerbations,[40,41] further well-designed research is needed in this area.

Semaglutide and liraglutide, glucagon-like peptide-1 receptor agonists, have anti-inflammatory effects that can be beneficial in the lungs.[33,41,42] Furthermore, it may help reduce breathing difficulties in obese individuals with COPD by facilitating weight loss.[33,41,42]

Thiazolidinediones, a class of antidiabetic drugs, are associated with fluid retention in patients with COPD and may worsen pulmonary congestion and exacerbate respiratory symptoms.[33,43] Therefore, its use is not recommended in DM patients with COPD.

Sodium-glucose cotransporter-2 inhibitors confer cardiovascular and renal protection but require careful monitoring in patients with COPD because of potential risks, such as electrolyte imbalance and dehydration, which can adversely affect the respiratory system.[33,43]

DM may develop in patients with COPD because of both shared aetiological factors and medication use. Increased risk of infection and autonomic neuropathy caused by DM can have a negative impact on COPD prognosis. GOLD 2026 recommended that systemic steroids be used for less than 6 days because of the risk of glucose imbalance.[1]

Chronic Obstructive Pulmonary Disease, Osteoposis and Fracture

Osteoporosis is one of the most commonly reported comorbidities in COPD; its prevalence has been reported to be between 38% and 58%, while osteopenia prevalence has been reported as 18-65% and increases with COPD severity, reaching approximately 84% in patients with severe COPD.[44]

The key risk factors for osteoporosis include nutritional deficiencies, reduced physical activity, cigarette smoking, weight loss, air pollution, and SCS use (Figure 3), which are all associated with COPD.[45] In addition, vitamin D deficiency, hypoxia, hypercapnia, and forced expiratory volume in 1 second (FEV_1) loss contribute to the development of osteoporosis in COPD.[40] Smoking is a major risk factor for osteoporosis by increasing osteoclastic activity and osteoblastic apoptosis. There is also evidence that nicotine disrupts bone structure by inhibiting oestrogen synthesis.[46] FEV_1 is the respiratory function parameter most closely associated with osteoporosis. The lower the FEV_1, the higher the risk of osteoporosis. This condition has been associated with the severity of COPD.[47,48]

The most controversial issue regarding the coexistence of COPD and osteoporosis is the use of CS. The risk of fractures increases significantly with the use of SCS.[47] The relationship between chronic glucocorticoid

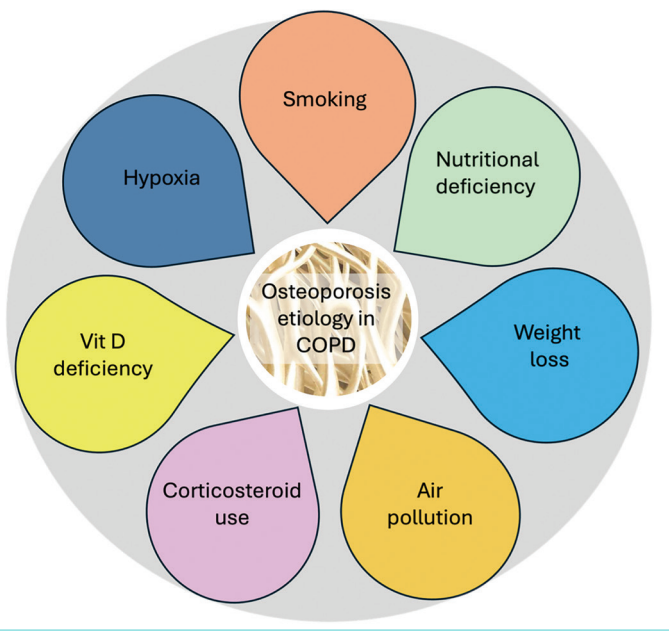

Figure 3. Osteoporosis etiology in COPD (This diagram was created using references numbered 40, 45 and 47).

COPD: Chronic obstructive pulmonary disease.

(GC) use and osteoporosis is well known. The prevalence of long-term oral GC treatment-related osteoporosis or fractures is approximately 30-50%, indicating that GC treatment is the most common cause of osteoporosis.[49] Bone loss occurs rapidly during the first few months of GC treatment. The risk of fracture increases within the first 3-6 months of treatment. This risk remains high as long as GC treatment continues. Discontinuing GC treatment rapidly reduces the risk of fractures,[50,51] but the risk may not return to baseline levels. Although maintenance treatment with SCS in COPD is not routine, it is frequently prescribed during exacerbations, and the cumulative dose is important. Because high cumulative doses of extaernal GCs cause increasing fracture risk.[49] SCS use in COPD patients with frequent exacerbations may accelerate the development of osteoporosis. No safe dose of CS with respect to osteoporosis development exists. The risk of fracture doubles in the 30 days following a course of SCS[52] and reaches a maximum level at 3-6 months after initiating SCS use.[49] GOLD recommends a course of SCS lasting fewer than 6 days for the treatment of COPD exacerbations.[1]

In a comprehensive meta-analysis involving 17,513 patients, ICS was found to be associated with a significant increase in fracture risk (OR: 1.21).[53] However, a systematic review that examined 26 RCTs involving 61,380 participants concluded that ICS use did not increase the incidence of fractures or osteoporosis in COPD patients.[54] Perhaps greater reliance should be placed on randomised controlled trials rather than observational studies because ICS-containing treatment (including triple therapy) is more likely to be given to more severe patients, who are also at increased risk of osteoporosis due to COPD.

Bone fractures are the most significant clinical outcomes of osteoporosis. In COPD, in addition to a procoagulant tendency, immobilisation due to fractures further increases the risk of venous thromboembolism and thus contributes to mortality. The most common type of fracture in osteoporosis is the vertebral compression fracture (VCF), which not only causes back pain but also contributes to increased symptoms because vital capacity is reduced by 9% with each vertebral fracture.[55] As VCF increases, kyphosis develops, and when it (the kyphotic angle) exceeds 55 degrees, spirometric deterioration is most pronounced. VCFs are rarely diagnosed because 60-70% are asymptomatic. In patients with VCFs associated with osteoporosis, the risk of a subsequent vertebral fracture increases at least fivefold, while the risk of hip fracture increases threefold.[56]

GOLD 2026 states that chest computed tomography scans can be used as a preliminary assessment tool for low bone mineral density to identify individuals requiring further investigation for osteoporosis.[1]

Osteoporosis is a common clinical manifestation in COPD, associated with numerous factors ranging from aetiology to treatment, and contributes to increased mortality. It is particularly important to avoid systemic steroids in patients with COPD because of the increased risk of osteoporosis. A healthy skeletal system is a mainstay of mobility, and mobility is one of the five key components of COPD management. It is recommended that osteoporosis screening with a DXA scan be performed every 3-5 years in patients with COPD, according to GOLD 2026.[1]

Chronic Obstructive Pulmonary Disease, Anxiety and Depression

Anxiety and depression are prevalent, yet frequently underdiagnosed, comorbidities in COPD.[57] These conditions do not directly affect lung function, but they can have numerous adverse effects on symptom perception and treatment compliance.[57] The prevalence of anxiety in stable COPD patients receiving outpatient treatment is reported to range from 13% to 46%, and to reach up to 55% in hospitalised patients.[58] As COPD severity increases, the prevalence of anxiety also increases; nearly 75% of patients with end-stage COPD experience anxiety.[57] The prevalence of depression in stable COPD ranges from 27% to 40%, increasing to 86% during exacerbations.[57]

Various aetiological factors may be implicated in anxiety and depression in COPD. Systemic inflammation in COPD is also believed to affect neurobiological mechanisms through proinflammatory mediators such as IL-6 and CRP.[57] Another aetiological factor associated with both COPD and depression is smoking. In addition, the frequent co-occurrence of other chronic conditions such as CVD, DM, and muscle weakness with COPD further increases susceptibility to anxiety and depression.[59]

Anxiety and depression are frequently observed together in COPD patients.[57] As these patients' perceptions of symptoms are worse, their ability to cope with their illness is lower and their risk of hospitalisation is higher.[49] Both of them significantly affect COPD, leading to worsening respiratory symptoms, frequent exacerbations, hospital admissions, reduced quality of life, and increased risk of death.[59] Depression has been shown to be an independent risk factor for readmission within 30 days following a COPD exacerbation.[60] Similarly, depression has been identified as a factor that increases the risk of hospitalisation within one year after discharge for COPD exacerbations[61] and has been reported to increase mortality risk in COPD by 40%.[62]

Treatment Options of Anxiety and Depression in Chronic Obstructive Pulmonary Disease

The most commonly used medications in the treatment of anxiety and depression are mirtazapine, serotonin reuptake inhibitors (SSRIs), serotonin noradrenaline reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), benzodiazepines, and opioids.[57,58] It has been reported that the majority of COPD patients are diagnosed with anxiety and/or depressive disorders, but only 31% receive treatment for these conditions.[63,64]

The effect of mirtazapine, a tetracyclic antidepressant, on reducing dyspnoea in COPD has been investigated. A phase-3 RCT found that it did not reduce dyspnoea; rather, it caused significant adverse reactions.[65] Similarly, a study involving 31,253 patients with COPD found that SSRIs, SNRIs, and TCAs were associated with an increased risk of pneumonia and COPD exacerbations, and that this risk decreased after discontinuation of antidepressants.[59] Several mechanisms have been proposed to explain the increased risk of COPD exacerbations and pneumonia associated with antidepressant use. These include sedation; nausea and vomiting; suppression of T-cell activity; decreased serotonin uptake; dry mouth and other anticholinergic effects; increased sensitivity to carbon dioxide in chemoreceptors; bidirectional systemic inflammation; and changes in the microbiota.[66]

One of the groups of drugs used for psychiatric problems in COPD is benzodiazepines. They are prescribed for insomnia, anxiety, and chronic dyspnoea caused by COPD.[64] The effects of benzodiazepines on the respiratory system include decreased minute ventilation, hypoxaemia, hypercapnia, and reduced respiratory muscle endurance and strength. COPD patients who use benzodiazepines have an increased risk of exacerbation and death.[66] The GOLD strategy document currently states that "there is no evidence that anxiety and depression should be treated

differently in the presence of COPD."[1] However, this situation stems from a lack of evidence.

Opioid use has been advocated as a treatment option for COPD patients with refractory dyspnoea. However, adverse respiratory outcomes pose a potential concern associated with opioid medications.[67] In a cohort of 130,979 elderly patients with COPD, the group that had started opioid treatment experienced more respiratory events and higher mortality.[68]

Another drug, gabapentin, has been frequently used in recent years, particularly in the treatment of neuropathic pain. Another large cohort study has shown that gabapentin was associated with an increased risk of exacerbations and death in patients with COPD.[69] In summary, care should be taken in the selection and use of antidepressants in COPD; pharmacological approaches should be implemented only after ensuring that non-pharmacological approaches have been fully applied.

A more proactive multidisciplinary approach is required to provide psychological support in addition to pharmacological methods, pulmonary rehabilitation, cognitive behavioural therapy, and group therapy, all of which have been shown to be beneficial.[59] In addition, a recently published systematic review and meta-analysis has concluded that tele-based interventions appear to be effective in reducing anxiety and depression in patients with COPD.[59]

Anxiety and depression in COPD are two cardinal clinical conditions associated with mortality and frequently remain undiagnosed. It isrecommended that the Patient Health Questionnaire-2 depression scale be used annually and the Generalised Anxiety Disorder-2 anxiety scale be used every 3-5 years as screening tools for depression and anxiety in people with COPD in GOLD 2026.[1] Treatment decisions should be made according to current guidelines.

CONCLUSION

The multifactorial nature of COPD, its shared aetiological and pathophysiological characteristics with comorbidities, and the need for specific clinical and therapeutic treatments and their possible interactions necessitate a thorough examination of comorbidities (Figure 4). The prevalence, key mechanisms, and management implications of extrapulmonary comorbidities associated with mortality in COPD are shown in Table 1. Symptoms associated with certain comorbid conditions may remain silent or be attributed to COPD. Cardiac and metabolic comorbidities are more easily recognisable by both patients and physicians, but physicians should be vigilant in identifying musculoskeletal and psychiatric conditions. All the extrapulmonary comorbidities discussed here may be related to increased mortality in COPD, so investigating and intervening in these comorbidities before symptom onset may improve prognosis in COPD.

Table 1. The features of mortality related extrapulmonary comorbidities in COPD

	Epidemiology	Key mechanism	Management implications
Acute MI	The prevalance of COPD in AMI varies between 7-30%.[13] AMI in patients with COPD is 3.5 times higher than in patients without COPD.[12]	Other comorbidities that increase MI risk (DM, HPL), MMPs, increased coagulation factors, and platelet activation are key factors for AMI in COPD.[13] Myocardial damage during AMI exacerbates COPD symptoms.	Cardiac troponin measrument (in unexplained worsening symptoms). Treatment should be administered according to current guidelines; standard AMI treatment should be applied.
Atrial fibrilliation	Prevalence of AF among hospitalized COPD patients is 22.1%.[17] Prevalence of COPD among AF patients is 11.5%.[18] Mortality rate is 3.9-5.7 times higher in co-occurence with COPD and AF.[17-19]	COPD-related structural and functional alterations may trigger sympathetic nervous system activation, vascular remodelling, and local cardiac conduction disturbances.[17] It resulted in AF. In AF, pulmonary congestion may be triggered by tachycardia, while in COPD, dyspnoea increases.	Unexplained increase in dyspnoea, intermittent dizziness: ECG and/or Holter monitoring.[1] Cardioselective beta-blockers are recommended for AF patients with COPD. Beta-agonists should be used with caution in COPD patients with AF.
Heart failure	The prevalence of COPD confirmed by spirometry in patients with HF ranges from 25% to 50%.[20] HF confirmed by ECHO in COPD is between 5-20%.[20]	Smoking and ageing are common etiologic factors. Increased intrathoracic pressure in COPD may cause reduced cardiac output, increased sympathetic activity, and RAAS activation. It results in HF.[20] Increased volume overload and/or myocardial dysfunction in HF can lead to increased airway obstruction, worsening COPD symptoms, and hypoxaemia.[20]	Natriuretic peptides are essential. If it is abnormal, it should be followed by ECHO. In COPD, both HFpEF and HFrEF are common. HF and COPD treatment should be administered in accordance with guidelines.
DM	The prevalance of DM in COPD is almost 30%.[33]	Smoking, chronic inflammation, and systemic oxidative stress - reflected by CRP, TNF-α, IL-1, IL-6, and fibrinogen - are common.	Fasting glucose and HbA1c measurements are essential. Systemic steroid use should be minimized in COPD. In DM, Blood sugar regulation should be ensured to prevent symptoms and exacerbations of COPD.
Osteoporosis and fracture	Osteoporosis in COPD is between 38-58%.[44] Osteopenia prevalance is between 18-65%.[44]	The key risk factors for osteoporosis in COPD are nutritional deficiencies, reduced physical activity, cigarette smoking, weight loss, air pollution, systemic corticosteroid use, vitamin D deficiency, hypoxia and hypercapnia, and decline in FEV_1.[44,45]	The dose and duration of systemic steroids used in COPD exacerbations should be kept to a minimum. The cumulative effect of systemic steroids may accelerate the development of osteoporosis. Loss of vertebral density may be observed on a thoracic CT scan. DEXA is the gold standard for diagnosis.

Table 1. Continued

	Epidemiology	Key mechanism	Management implications
Anxiety and depression	Anxiety in COPD is between 13-55%, rising during 55%.[57,58] Depression affects 27-40% of patients with COPD, rising to 86% during exacerbations.[57]	Smoking, systemic inflammation, co-occurrence of other chronic comorbidities are major common related factors.[57,59]	PHQ depression scale annually and GAD anxiety scale every 3-5 years use as a screening tool.[1]

COPD: Chronic obstructive pulmonary disease, AMI; Acute mycodar infarction, DM: Diabetes mellitus, HPL: Hyperlipidemia, MMPs: Matrix metalloproteniases, AF: Atrial fibrillation, RAAS: Renin-angiotensin-aldosterone system, ECHO: Echocardiography, HF: Heart failure, HFpEF: Heart failure preserved ejection fraction, HFrEF: Heart failure reduced ejection fraction, HbA1c: Glycated hemoglobin A1c, FEV_1: Forced expiratory volume in 1 second, DEXA: Dual-energy X-ray absorptiometry, PHQ: Patient Health Questionnaire-2 depression scale, GAD: Generalised Anxiety Disorder-2, CRP: C-reactive protein, IL: Interleukin, ECG: Electrocardiogram, CT: Computed tomography.

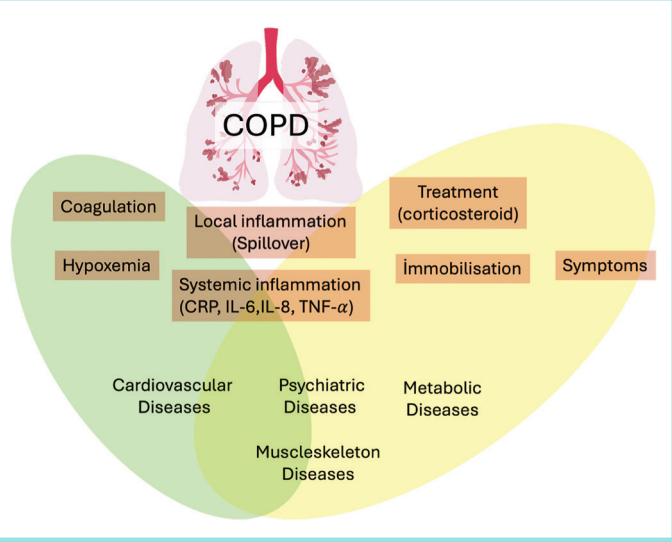

Figure 4. The relationship between inflammatory and clinical characteristics in COPD and extrapulmonary comorbidities (This figure was created using information from references numbered 3, 10, 11, 33, 34, 45, 46, 57, and 59).

COPD: Chronic obstructive pulmonary disease, CRP: C-reactive protein, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha.

MAIN POINTS

- Chronic obstructive pulmonary disease (COPD), the third leading cause of death among chronic diseases worldwide, is considered a multisystem disease because it affects multiple organ systems via systemic inflammation and hypoxia.
- The multifactorial nature of COPD, which shares common aetiological and pathophysiological characteristics with its comorbidities, coupled with the need for specific clinical and therapeutic treatments and the consideration of their possible interactions, necessitates a thorough examination of comorbidities.
- The main extrapulmonary comorbidities associated with mortality in COPD are cardiac, metabolic, musculoskeletal, and psychiatric.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.B., P.J., N.K., Concept: A.B., P.J., N.K., Design: A.B., P.J., N.K., Data Collection and/or Processing: A.B., N.K., Analysis and/or Interpretation: A.B., P.J., N.K., Literature Search: A.B., P.J., Writing: A.B., P.J.

DISCLOSURES

Conflict of Interest: One author of this article, Ayşe Baha, is a member of the Editorial Board of the Cyprus Journal of Medical Sciences. However, she did not involved in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other author declared no conflict of interest.

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RESEARCH ARTICLE

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Mandibular Fractures Between 2014 and 2024: A 10-Year Retrospective Study

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Abstract

BACKGROUND/AIMS: Mandibular fractures are a common type of maxillofacial fracture. Clinical symptoms of mandibular fractures include pain, swelling, difficulty chewing, and malocclusion. Collecting long-term epidemiological data on mandibular fractures can provide essential information for developing and evaluating preventive measures to reduce the incidence of these facial injuries.

MATERIALS AND METHODS: Ethical approval for the study was obtained prior to data collection. Patient information was retrieved from hospital records, radiological images, official reports, and the institutional information management system. Data from a single tertiary care center were reviewed, including demographic characteristics, fracture sites, and treatment methods for mandibular fractures in 185 patients treated between February 2014 and February 2024. Only patients with complete clinical and radiographic documentation were included, and the data were independently assessed by two researchers to ensure accuracy and consistency.

RESULTS: Among the 185 patients examined, fractures were most frequently observed in the 20-29 age group. Assault was identified as the leading cause of mandibular fractures in men, while falls were the most common cause in women. The incidence of fractures increased during the summer months. The mean number of fractures per person was 1.34. The region most frequently associated with mandibular fractures was the angle of the mandible.

CONCLUSION: Mandibular fractures commonly occur in young males. Properly planned treatments for these fractures yield high success rates. The findings of this study provide insights to surgeons, physicians, and health policymakers on addressing mandibular fractures.

Keywords: Etiology, mandibular fractures, retrospective studies

INTRODUCTION

The mandible, which is U-shaped, is the largest bone in the facial skeleton.[1] It is the only facial bone capable of movement through a joint and is particularly prone to trauma due to its protruding structure. Its functions include speech, mastication, and swallowing, all of which can be disrupted by mandibular fractures.

Mandibular fractures account for between 18.0% and 72.9% of all facial fractures.[2] This wide range is attributable to regional, demographic, and etiological differences among studies. Mandibular fractures occur after blunt or penetrating trauma. In addition, weakness in the mandible caused by tooth extraction, tumor, or cyst can cause pathological fractures. Although the etiologies are difficult to classify, the most common causes of fractures are assaults, falls, sports injuries, motor vehicle accidents (MVAs), and pathological conditions. Among these, the factors most responsible for fracture formation are MVAs, assaults, and falls.[3-6]

Across demographic studies, men consistently sustain mandibular fractures more frequently than women.[6-8] The most affected age group

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is typically between 21 and 34 years of age.[3,7,9] The epidemiology of facial fractures varies greatly across countries and may be explained by differences in economic and social conditions, local behavioral patterns, and legislation.

This study is a retrospective analysis of all treated mandibular fractures from 2014 to 2024. The aim of the study is to determine the frequency of mandibular fractures in males and females, identify the most commonly affected age groups, analyze the injury mechanisms, and report the treatment methods applied. The findings may contribute to the current understanding of mandibular fractures, inform clinical practice and research in this field, and serve as a reference for future studies. Additionally, they may help develop evidence-based guidelines and policies for the prevention, diagnosis, and treatment of these injuries.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this study was obtained from the Gazi University Rectorate Ethics Committee (approval number: 08, date: 18.04.2023).

Sample Size and Characteristics

In this retrospective study, 185 patients who presented to Gazi University Faculty of Dentistry Hospital with mandibular fractures between February 2014 and February 2024 were analyzed. Data were obtained from patient records, self-reports, radiological images and reports, and the hospital information management system, Metasoft (Metasoft Computer Information Processing Services Co. Ltd., Eskişehir, Türkiye). The analyzed data included patient age, gender, date of trauma, fracture location, and treatment methods.

Patients were included if they were diagnosed with isolated mandibular fractures between 2014 and 2024, had complete clinical and radiographic records, and had at least two months of post-treatment follow-up with available control radiographs. Patients presented with concomitant panfacial fractures, pathological fractures (e.g., cysts, tumors, osteomyelitis), secondary fractures following surgical interventions, or radiological image artifacts that affected diagnostic quality. Cases with incomplete medical records or missing radiographic data were also excluded.

Collected Data

Age data were categorized by decade. Fracture etiologies were classified into five categories: assaults, falls, sports injuries, MVAs, and other causes. Seasonal and monthly distributions were also analyzed. Fracture locations were classified as corpus, angulus, ramus, symphysis, parasymphysis, condyle, and coronoid based on patients' radiographic images. This anatomical classification was derived from the localization section of the Dingman and Natvig[10] mandibular fracture classification. This classification was preferred because it is common and easy to use. In addition to fracture locations, the number of fractures was considered because some cases involved multiple fractures. Treatment methods were documented using radiological images, patient files, and self-reports.

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 27 (SPSS Inc., Chicago, IL, USA). Descriptive statistics (frequencies and percentages) were presented for the variables. The differences in frequency distributions between categorical variables were examined using the chi-square test. According to the assumptions of the chi-square test, when more than 20% of the expected cell frequencies were less than 5, the Fisher-Freeman-Halton test was applied instead. A significance level of $\alpha=0.05$ was adopted for all statistical analyses.

RESULTS

Age and Gender

Among the 185 patients included in the study, 135 were male (72.97%), and 50 were female (27.02%), with a male-to-female ratio of 2.7:1. Patients were grouped into seven age categories: 0-9, 10-19, 20-29, 30-39, 40-49, 50-59, and 60 and above. The 20-29 age group was the most affected, accounting for 44.3% of cases (Figure 1).

Seasonal and Monthly Distribution

Mandibular fractures recorded between February 2014 and February 2024 showed a peak incidence in September. Fracture cases were higher during the summer months (Figure 2).

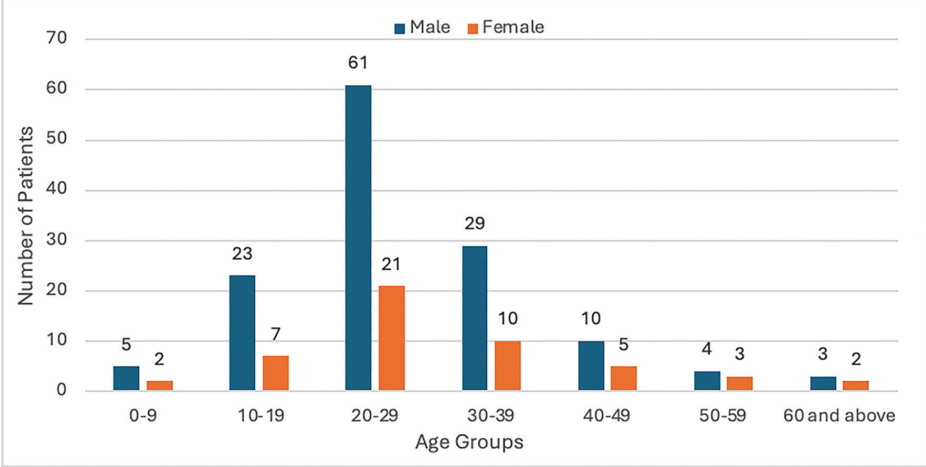

Figure 1. Age and sex distribution.

Etiology

Mandibular fractures were categorized into five main etiological groups: assault, falls, MVAs, sports injuries, and other causes. Assault was the leading cause of mandibular fractures (40.0%), followed by falls (32.4%) and MVAs (21.1%). Assault-related fractures were significantly more frequent among males (χ^2=9.250, p=0.002). The predominant etiology among females was falls. The "other causes" group included one case caused by an earthquake and one caused by an animal attack (Table 1).

Fracture Frequency and Location

A total of 249 fractures were detected in 185 patients, ranging from one to three fractures per patient. The mean number of fractures per patient was 1.34. Among the cases, 124 (67.02%) had a single fracture, 58 (31.35%) had two fractures, and 3 (1.62%) had three fractures in different locations.

The mandibular angle was the most common site (25.3%), followed by the condyle (22.9%) and the symphysis (22.5%) (Figure 3).

Angle fractures were significantly more frequent in assault-related cases (p<0.001), whereas condylar fractures were more common in non-assault cases (p<0.001) (Table 2). Condylar fractures were significantly more frequent among patients with falls (p<0.001), whereas angle fractures were significantly less common in this group (p<0.001) (Table 3). Angle fractures were significantly less frequent in MVA-related patients (p=0.017), whereas symphysis fractures were significantly more common (p=0.042). No statistically significant differences were found for MVA-related patients with condylar, body, ramus, or coronoid fractures (p>0.05), although condylar (p=0.086) and body (p=0.066) fractures approached significance (Table 4).

Among the 58 cases with two fracture lines, the most common combination was fractures of the angle and body (n=13). The angle region showed the strongest association with multiple fractures (Table 5).

Additionally, fractures were observed in three anatomical regions among three cases. Two cases involved bilateral condylar and symphyseal fractures, while the third had fractures of the symphysis, body, and condyle.

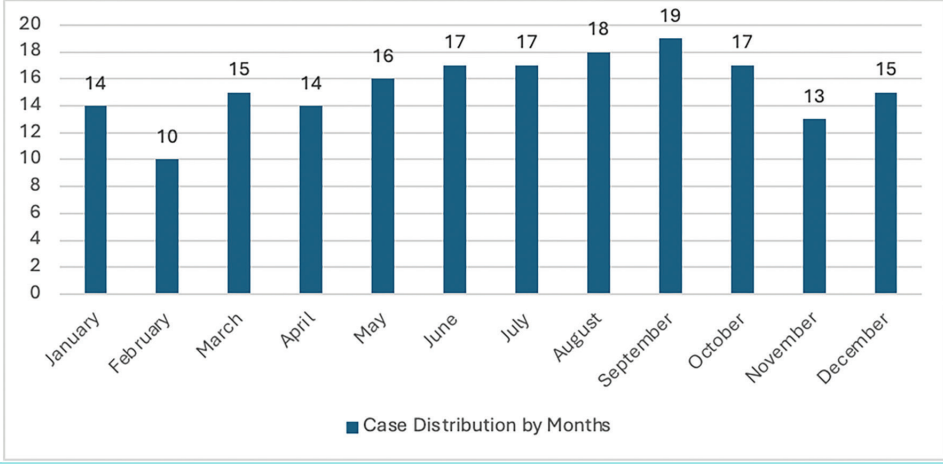

Figure 2. Monthly distribution of mandibular fractures.

Table 1. Distribution of mandibular fracture etiologies by gender

Fracture etiology		Gender									Test statistics	p
		Male			Female			Total				
		n	Row n %	Column n %	n	Row n %	Column n %	n	Row n %	Column n %		
Assault	No	72	64.9	53.3	39	35.1	78.0	111	100.0	60.0	χ^2=9.250	0.002*
	Yes	63	85.1	46.7	11	14.9	22.0	74	100.0	40.0		
Falls	No	95	76.0	70.4	30	24.0	60.0	125	100.0	67.6	χ^2=1.791	0.181
	Yes	40	66.7	29.6	20	33.3	40.0	60	100.0	32.4		
MVA	No	111	76.0	82.2	35	24.0	70.0	146	100.0	78.9	χ^2=3.276	0.070
	Yes	24	61.5	17.8	15	38.5	30.0	39	100.0	21.1		
Sports	No	128	73.1	94.8	47	26.9	94.0	175	100.0	94.6	-	1.000
	Yes	7	70.0	5.2	3	30.0	6.0	10	100.0	5.4		
Other	No	134	73.2	99.3	49	26.8	98.0	183	100.0	98.9	-	0.469
	Yes	1	50.0	0.7	1	50.0	2.0	2	100.0	1.1		

*p<0.05, χ^2: Chi-square test statistic.
MVA: Motor vehicle accident.

Treatment Methods

Among the 185 patients analyzed, 9 (4.9%) were managed conservatively, without surgical intervention. These patients had functional limitations, were placed on a soft diet, and underwent regular clinical and radiographic follow-up.

Closed reduction with intermaxillary fixation (IMF) was applied in 29 patients (15.7%). Among these, 11 were treated with arch bars, 15 with IMF screws, and 3 with orthodontic brackets.

A total of 65 patients (35.1%) underwent open reduction with adjunctive IMF. In these cases, IMF was applied perioperatively to support occlusal stabilization, followed by fixation with miniplates. Among these patients, 36 had IMF removed after miniplate fixation, whereas the remaining 29 had IMF maintained postoperatively alongside miniplate fixation. These patients predominantly presented with multiple fracture sites, requiring both open and closed methods to achieve stable occlusion and fracture healing.

Finally, open reduction and internal fixation (ORIF) was performed on 82 patients (44.3%), constituting the most frequently used treatment modality. Among these, 69 cases were fixed with miniplates, 3 with three-dimensional plates, 1 with lag screws, and 9 with reconstruction plates (Table 6).

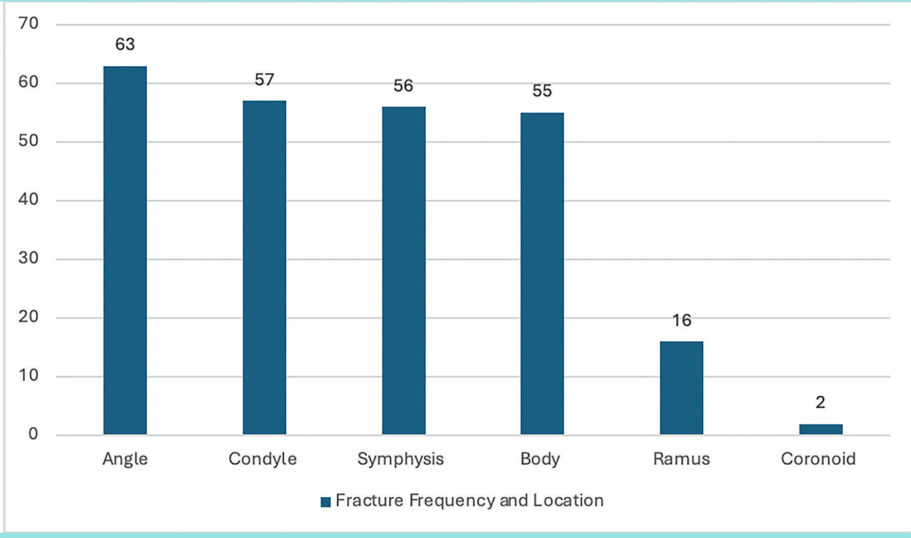

Figure 3. Distribution of cases according to fracture localization.

Table 2. Frequencies of fracture locations in assault-related cases

Fracture localization		Fracture etiology assault									Test statistics	p
		No			Yes			Total				
		n	Row n %	Column n %	n	Row n %	Column n %	n	Row n %	Column n %		
Angle	No	93	76.2	83.8	29	23.8	39.2	122	100.0	65.9	χ^2=39.318	<0.001*
	Yes	18	28.6	16.2	45	71.4	60.8	63	100.0	34.1		
Condyle	0	59	44.7	53.2	73	55.3	98.6	132	100.0	71.4	53.658	<0.001*
	1	48	98.0	43.2	1	2.0	1.4	49	100.0	26.5		
	2	4	100.0	3.6	0	0.0	0.0	4	100.0	2.2		
Symphysis	No	77	59.7	69.4	52	40.3	70.3	129	100.0	69.7	χ^2=0.017	0.896
	Yes	34	60.7	30.6	22	39.3	29.7	56	100.0	30.3		
Body	0	79	59.8	71.2	53	40.2	71.6	132	100.0	71.4	0.992	0.712
	1	30	58.8	27.0	21	41.2	28.4	51	100.0	27.6		
	2	2	100.0	1.8	0	0.0	0.0	2	100.0	1.1		
Ramus	No	101	59.8	91.0	68	40.2	91.9	169	100.0	91.4	χ^2=0.046	0.831
	Yes	10	62.5	9.0	6	37.5	8.1	16	100.0	8.6		
Coronoid	No	109	59.6	98.2	74	40.4	100.0	183	100.0	98.9	-	0.517
	Yes	2	100.0	1.8	0	0.0	0.0	2	100.0	1.1		

*p<0.05, χ^2: Chi-square test statistic.

Table 3. Frequencies of fracture locations in fall-related cases

Fracture localization		Fracture etiology falls									Test statistics	p
		No			Yes			Total				
		n	Row n %	Column n %	n	Row n %	Column n %	n	Row n %	Column n %		
Angle	No	69	56.6	55.2	53	43.4	88.3	122	100.0	65.9	χ^2=19.818	<0.001*
	Yes	56	88.9	44.8	7	11.1	11.7	63	100.0	34.1		
Condyle	0	105	79.5	84.0	27	20.5	45.0	132	100.0	71.4	29.427	<0.001*
	1	18	36.7	14.4	31	63.3	51.7	49	100.0	26.5		
	2	2	50.0	1.6	2	50.0	3.3	4	100.0	2.2		
Symphysis	No	85	65.9	68.0	44	34.1	73.3	129	100.0	69.7	χ^2=0.546	0.460
	Yes	40	71.4	32.0	16	28.6	26.7	56	100.0	30.3		
Body	0	86	65.2	68.8	46	34.8	76.7	132	100.0	71.4	1.451	0.447
	1	37	72.5	29.6	14	27.5	23.3	51	100.0	27.6		
	2	2	100.0	1.6	0	0.0	0.0	2	100.0	1.1		
Ramus	No	115	68.0	92.0	54	32.0	90.0	169	100.0	91.4	χ^2=0.205	0.651
	Yes	10	62.5	8.0	6	37.5	10.0	16	100.0	8.6		
Coronoid	No	124	67.8	99.2	59	32.2	98.3	183	100.0	98.9	-	0.545
	Yes	1	50.0	0.8	1	50.0	1.7	2	100.0	1.1		

*p<0.05, χ^2: Chi-square test statistic.

Table 4. Frequencies of fracture locations in motor vehicle accident cases

Fracture localization		Fracture etiology MVA									Test statistics	p
		No			Yes			Total				
		n	Row n %	Column n %	n	Row n %	Column n %	n	Row n %	Column n %		
Angle	No	90	73.8	61.6	32	26.2	82.1	122	100.0	65.9	χ^2=5.708	0.017*
	Yes	56	88.9	38.4	7	11.1	17.9	63	100.0	34.1		
Condyle	0	109	82.6	74.7	23	17.4	59.0	132	100.0	71.4	4.878	0.086
	1	35	71.4	24.0	14	28.6	35.9	49	100.0	26.5		
	2	2	50.0	1.4	2	50.0	5.1	4	100.0	2.2		
Symphysis	No	107	82.9	73.3	22	17.1	56.4	129	100.0	69.7	χ^2=4.154	0.042*
	Yes	39	69.6	26.7	17	30.4	43.6	56	100.0	30.3		
Body	0	106	80.3	72.6	26	19.7	66.7	132	100.0	71.4	5.636	0.066
	1	40	78.4	27.4	11	21.6	28.2	51	100.0	27.6		
	2	0	0.0	0.0	2	100.0	5.1	2	100.0	1.1		
Ramus	No	133	78.7	91.1	36	21.3	92.3	169	100.0	91.4	-	1.000
	Yes	13	81.3	8.9	3	18.8	7.7	16	100.0	8.6		
Coronoid	No	145	79.2	99.3	38	20.8	97.4	183	100.0	98.9	-	0.378
	Yes	1	50.0	0.7	1	50.0	2.6	2	100.0	1.1		

*p<0.05, χ^2: Chi-square test statistic.
MVA: Motor vehicle accident.

Table 5. Patients with two fracture lines	
Fracture site	**Patient**
Angle & condyle	4
Angle & symphysis	11
Angle & body	13
Angle & ramus	2
Condyle & symphysis	10
Condyle & body	5
Condyle & ramus	1
Symphysis & body	6
Symphysis & ramus	2
Bilateral body	2
Bilateral condyle	2

Table 6. Distribution of treatment methods among patients		
Treatment	**Patient**	**Percentage (%)**
Conservative management	**9**	**4.86**
Closed reduction	**29**	**15.68**
- IMF with arch bars	11	5.95
- IMF with IMF screws	15	8.11
- IMF using orthodontic brackets	3	1.62
Open reduction with adjunctive IMF	**65**	**35.13**
- IMF removed after miniplate placement	36	19.46
- Miniplate and IMF applied together	29	15.68
Open reduction and internal fixation	**82**	**44.32**
- Fixation with miniplates	69	37.30
- Fixation with 3D plates	3	1.62
- Fixation with lag screws	1	0.54
- Fixation with reconstruction plates	9	4.86
IMF: Intermaxillary fixation, 3D: Three-dimensional.		

DISCUSSION

The mandible is one of the most frequently fractured bones in the facial skeleton.[11] These fractures can result from trauma, pathological destruction caused by cysts and tumors, or surgical interventions such as tooth extractions. Researchers analyze case series of mandibular fractures, examining demographic, clinical, and radiographic data to identify patterns, trends, and factors associated with these injuries. Retrospective data on mandibular fractures are critical for developing effective prevention strategies and accurate trauma assessment protocols. The absence of recent studies on mandibular fractures at our clinic motivated us to re-examine our data.

Our study primarily focused on the demographic distribution of mandibular fractures. Factors such as age and gender were evaluated for their effects on fracture incidence. Of the 185 patients we treated, 135 were male and 50 were female, with a male-to-female ratio of 2.7:1. The higher incidence of fractures among males is consistent with findings in the literature.[3,9,12]

When patients were evaluated by age, fractures were most frequently observed in the second and third decades of life. This result is highly consistent with the literature.[3,12-14] The higher physical activity levels of this age group may explain their increased susceptibility to trauma. The findings suggest that mandibular fractures are more common among young males.

Mandibular fractures were observed most frequently in the summer.[15] Although it was hypothesized that severe winter conditions, such as heavy snow and ice, would increase the incidence of mandibular fractures in our region by causing slips, falls, and traffic accidents, our results indicated a higher incidence during the summer. Similarly, a previous study conducted at our faculty found an increase in fractures during the summer months.[16] This can be explained by increased physical activity during the summer. In our study, fractures were most common in September and least common in February.

Jin et al.[17] reported that among 2,076 patients, males aged 10 to 39 years were the most numerous. Patient numbers were significantly higher mid-month. Contrary to the findings in the literature, the relationship between fracture patterns and seasonality was examined in our study, and no seasonal differences were found.[17]

Silva et al.[18] reported that there was no significant seasonal difference in mandibular fractures in a study conducted in Brazil. They suggested that this might be due to the smaller seasonal differences in warm-temperate climates.[18]

When examining the etiology of fractures, assault was identified as the most common cause (n=74). Mandibular fractures are frequently associated with assaults and MVAs.[3,4,19,20] Falls were the second most common cause, accounting for 60 cases. Among women, falls were the most common etiological factor. Afrooz et al.[3] reported, that, in the United States, women were more affected by MVAs than men. Increased safety measures in motor vehicles have been found to reduce the incidence of facial injuries.[3,21]

Many studies report varying incidence rates for the most commonly fractured regions of the mandible, such as the symphysis, condyle, angle, and body.[3,9] In our study, the mandibular angle was the most frequently fractured region, followed by the condyle and the body of the mandible. When analyzing the relationship between the anatomical location of the fracture and the etiology, angle fractures were most frequently associated with assaults and sports injuries, while condylar fractures resulted from falls or MVAs.[3,19] Fractures of the mandible generally occur due to direct trauma, whereas fractures of the condylar region are typically due to indirect trauma.

Among the 185 patients we examined, 124 (67.02%) had a single fracture line in the mandible, 58 (31.35%) had two fracture lines, and 3 (1.62%) had three fracture lines. The literature suggests that the likelihood of observing single fractures was higher in our study.[11,22] This could be because our clinic is not the primary center where these trauma patients initially present; instead, patients often seek treatment at our clinic after their emergency care is completed, while more complex cases are treated at other centers.

The timing of treatment initiation for mandibular fractures is relatively important. Published studies have shown no difference in complication rates between starting treatment within 72 hours and starting treatment after 72 hours.[23-25] Although the timing of treatment is important, proper treatment planning is generally given priority. Diagnosing fractures can be challenging since mandibular fractures often occur

alongside other injuries. Delayed mandibular fractures can complicate reduction because of fibrin deposition at the fracture line. It should be remembered that every mandibular fracture is unique. Therefore, the timing of treatment should be individualized for each patient.

Treatment of mandibular fractures may involve various methods depending on the type, location, and severity of the fracture, as well as the patient's general health. In our series, 9 patients (4.9%) were managed conservatively, 29 (15.7%) underwent closed reduction, and most required open techniques. Hassanein[26] reported a conservative treatment rate of 4.38% in a study of 1,371 patients, which is consistent with our findings. A particularly noteworthy finding was that 65 patients (35.1%) who underwent open reduction with adjunctive IMF. Among these, 36 patients had IMF removed after miniplate fixation, whereas in the remaining 29 patients, IMF was maintained postoperatively in combination with miniplate fixation. In these patients, IMF was sometimes applied before surgery and in other cases after surgery, reflecting the need for individualized strategies in complex fracture patterns. Such cases were predominantly those involving multiple fracture sites, including condylar fractures combined with another mandibular fracture, in which integrating both open and closed principles was beneficial. This observation is consistent with the current literature, which indicates that tooth-bearing fractures are generally stabilized by ORIF, whereas condylar fractures may be managed with closed techniques, depending on displacement, loss of ramus height, and occlusal stability.[27] Finally, 82 patients (44.3%) underwent ORIF alone, consistent with recent reports highlighting this method as the preferred approach in contemporary mandibular trauma management.[14,15,26,27] Different treatment methods have their respective advantages and disadvantages, and the literature does not provide evidence that any single method is universally superior.

Study Limitations

This study is based on a retrospective analysis and is subject to certain data limitations. For example, our study focused only on data from a specific time period. Future studies with a larger dataset and a longer time period may address these limitations.

CONCLUSION

Mandibular fractures primarily affect young adult males; the leading causes vary by gender, commonly assaults and falls. The angle of the mandible was the most frequently fractured site, while condylar fractures were more often associated with falls and MVAs. Fracture incidence peaked during the summer months, reflecting increased outdoor physical activity; open reduction with internal fixation remained the most reliable treatment method.

From a preventive perspective, public health strategies focusing on violence reduction, particularly among young males, may help decrease the burden of mandibular trauma.

In clinical practice, we recommend that these patients be managed in specialized trauma centers through a multidisciplinary approach led by maxillofacial surgeons. Centralization of care not only ensures standardized, evidence-based management but also facilitates the collection of high-quality data, which will strengthen future research and contribute to the development of precise, evidence-based guidelines.

MAIN POINTS

- Mandibular fractures are most common among young males. The male-to-female ratio was 2.7:1.
- The most affected age group consisted of patients in their 20s, followed by those in their 30s and those in their teens.
- The etiological factors for mandibular fractures were assaults, falls, motor vehicle accidents, and sports injuries.
- On average, 1.34 fractures occurred per person, and the most frequently fractured site was the angle.
- The most commonly used treatment method is miniplate fixation.

ETHICS

Ethics Committee Approval: Ethical approval for this study was obtained from the Gazi University Rectorate Ethics Committee (approval number: 08, date: 18.04.2023).

Informed Consent: This study was designed as a retrospective analysis based on anonymized patient records. Due to the retrospective nature of the study, informed consent was not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.T., S.K., A.Y., Concept: Ö.T., S.K., Design: Ö.T., S.K., Data Collection and/or Processing: Ö.T., Analysis and/or Interpretation: Ö.T., Literature Search: Ö.T., S.K., A.Y., Writing: Ö.T., A.Y.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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Spermiogram Parameter Alterations in Acute Liver-Damaged Rats Following Lavandula Angustifolia Oil and Medical Ozone Therapy

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Abstract

BACKGROUND/AIMS: In animal experiments, carbon tetrachloride (CCl4) is commonly used to produce hepatotoxicity because it induces oxidative stress. Increased production of reactive oxygen species, resulting in oxidative stress, has detrimental effects on several cellular components. Lavandula angustifolia oil has been recognized as an indirect antioxidant that boosts the activity and expression of antioxidant enzymes. Medical ozone therapy, which uses appropriate concentrations of ozone, is being investigated for its potential to treat and prevent hepatotoxicity and other conditions. The effects of medical ozone, lavender, and the combined effects of both therapies on sperm morphology and count in hepatotoxic rats were examined in this study.

MATERIALS AND METHODS: CCl4 was used to cause acute hepatotoxicity in Wistar rats and sperm cells were obtained from - extracted epididymal structure. Then, the Spermac Kit (FertiPro Spermac Stain) was used to analyze sperm morphology after medical ozone, lavender, and combined treatment.

RESULTS: Adding lavender to the treatment increased sperm count ($p<0.001$), irrespective of medical ozone. However, the sperm count is unaffected when lavender and medical ozone are combined ($p>0.05$). However, The total number of defects in the head, neck, and tail varies among the three groups' sperm morphologies.

CONCLUSION: Only the rats who received Lavandula angustifolia oil treatment showed an increase in spermatozoa, suggesting that medical ozone may counteract Lavandula's effects. Therefore, this study is crucial to understanding the relationship between medical ozone and lavender oil.

Keywords: Carbon tetrachloride, Lavandula angustifolia oil, medical ozone, semen analysis, sperm quantification, morphology of sperm, hepatotoxicity

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INTRODUCTION

Hepatocellular toxicity, a significant medical concern, often results from exposure to various xenobiotics and can lead to severe liver damage.[1] In recent years, therapeutic strategies have focused on the antioxidant and anti-inflammatory properties of medicinal plant derivatives to mitigate such damage by scavenging reactive oxygen species (ROS) and enhancing endogenous antioxidant defenses.[2] In animal research carbon tetrachloride (CCl_4) is commonly used to induce hepatotoxicity by generating oxidative stress within the organism. An increase in ROS causes oxidative stress, which negatively affects cell metabolism and can result in a variety of diseases.[3] Prevention and treatment of oxidative stress remain significant challenges in modern medicine. One well-known dangerous chemical that causes poisoning, especially in the liver, is CCl_4. The enzyme cytochrome P450 2E1(CYP2E1) is responsible for the biotransformation of CCl_4, which produces free radicals such as chloroform and hexachloroethane. The trichloromethylperoxy moiety reacts with nucleic acids, proteins, and lipids covalently attached to biological macromolecules. Protein production in liver cells is mediated by ribosomes, which stop translating due to ribonucleic acid hypomethylation. Therefore, oxidative stress in the organism impairs liver enzyme function.[4] When the production and removal of ROS are out of equilibrium, oxidative stress occurs. Important biological constituents, such as proteins, lipids, and nucleic acids, are adversely affected by increased ROS production.[5] Many diseases, such as cancer and cardiovascular conditions, are influenced by oxidative stress in their onset and progression.[4] Essential oils have been used since ancient times because of their multiple benefits and medicinal properties.[5] With the increase in use of essential oils throughout the late twentieth and twenty-first centuries, and an increase in its use at the outset, this field became known as aroma science therapy.[6]

Lavender oil, derived from Lavandula angustifolia, is a potently perfumed oil obtained from a shrub-like plant of the family Lamiaceae. Lavender oil is used as a sedative in pain management, and as an analgesic, anti-inflammatory, antifungal, and antispasmodic agent; it is also used to lower blood pressure. It also aids wound healing and reduces anxiety.[7] Moreover, Lavandula angustifolia has calming effects on the central nervous system; is anticonvulsant, spasmolytic, antioxidant, and antimicrobial; and inhibits mast cell degranulation.[8] It has been established that lavender oil acts as an indirect antioxidant by enhancing the production and activity of antioxidant enzymes, hence raising the natural several studies have shown that lavender oil exhibits antioxidant properties by increasing the production and activity of antioxidant enzymes.[9,10] Lavandula angustifolia oil has demonstrated significant hepatoprotective effects, primarily by reducing oxidative stress and inflammation.[1]

Medical ozone therapy is the controlled administration of ozone gas at appropriate dosages, despite ozone being widely acknowledged as detrimental. Researchers have identified a promising strategy for disease prevention and therapy. The observation that ozone decreases oxidative stress suggests its potential application in preventing and treating hepatotoxicity. The medical benefits of ozone therapy require small quantities and a delicate balance among variables, including the number of antioxidants that react with ozone.[11] Medical ozone therapy is being investigated for its potential benefits in the treatment of various disorders. Moreover, Valacchi and Bocci[12] showed that applying ozonated water to skin wounds accelerated wound healing in rats.

Male infertility significantly contributes to infertility in couples, with impaired sperm motility being a primary factor.[13,14] Current medical and surgical interventions often fail to reverse compromised semen parameters, underscoring the need for alternative therapeutic strategies. This highlights the critical importance of exploring novel pharmacological agents and natural compounds that can enhance sperm quality parameters.[14] Essential oils, derived from various plants, have demonstrated potential to improve sperm parameters, including motility and vitality, and offer a promising avenue for further investigation.[13,15] Specifically, studies have shown that certain essential oils, such as those from oregano and sage, can positively influence sperm physiology by improving motility and vitality.[13,15,16] Among these, Lavandula angustifolia oil has garnered attention for its antioxidant and antimicrobial properties, which may mitigate the deleterious effects of ROS on spermatozoa during preservation. The protective effect of lavender oil extends to improving various spermatological parameters during cryopreservation, thereby enhancing the overall quality of frozen-thawed semen. Given its potential, further exploration into the specific mechanisms by which Lavandula angustifolia oil enhances sperm viability and motility, especially under cryogenic conditions, is warranted. Furthermore, medical ozone, known for its potent antimicrobial and oxidative properties, is a promising candidate for mitigating bacterial contamination and enhancing sperm quality during semen preservation.[17]

This study aims to investigate the individual effects of Lavandula angustifolia oil and of medical ozone on key sperm quality parameters, including viability, motility, and the acrosome reaction, under controlled experimental conditions. This investigation will also examine the chemical characterization of the essential oils to better understand their bioactive components and the mechanisms by which they affect sperm morpho-functional parameters. The purpose of this research was to examine the effects of medical ozone and lavender, alone and in combination, on the sperm count and morphology in hepatotoxic rats. Notwithstanding these drawbacks, medical ozone treatment remains important; hence, the uniqueness of this study is demonstrated by the lack of research combining medical ozone therapy with lavender oil rather than applying it to essential oils or "ozonated oils". This study aimed to examine whether the morphology and quantity of sperm in hepatotoxic rats were influenced by lavender oil, medical ozone therapy, or their combined (synergistic) application. This is among the first studies to demonstrate an association between medical ozone therapy and lavender oil in the literature.

MATERIALS AND METHODS

Study Design and Ethical Approval

Acute hepatotoxicity Model of Wistar Rats: Male Wistar rats weighing 250-500 g and 8-12 weeks of age were chosen randomly for the study. The 4 main experimental groups were: control, Lavandula angustifolia oil, medical ozone, and combined treatment (Lavandula angustifolia oil and medical ozone); each group included 7 randomly assigned animals. This research was authorized by the Maltepe University Animal Experiments Local Ethics Committee (approval no: 2023.06.03, date: 29.06.2023; informed consent: as procedures were carried out on animals, patient approval was not obtained). The Maltepe University Experimental Animals Research Unit bred and cared for all the rats, and the study employed a prospective design.

Hepatotoxicity in rats was induced by intraperitoneal injection of 0.2 mL/kg of CCl_4 daily for 10 days.[4] CCl_4-induced liver damage is shown in Figure 1. Following CCl_4 administration, 400 mg/kg Lavandula angustifolia oil was threatened by gavage every other day for 14 days to Lavandula angustifolia oil group.[18] The medical ozone group received intraperitoneal injections of medical ozone (1 mg/kg) for seven cycles.[11] In the final experimental group (combined therapy), animals received 400 mg/kg of Lavandula angustifolia oil by gavage for 14 days and 1 mg/kg of medical ozone by intraperitoneal injection on seven occasions, every other day. On days when medical ozone was not administered, a sham procedure was performed to induce comparable stress in rats (Table 1). Finally, 100 mg/kg of ketamine was used to euthanize the animals, and their epididymides were removed from the testicular tissue in all groups at the conclusion of the treatment regimen.

Sperm Collection: The extracted epididymal tissue was ruptured with an insulin needle in phosphate-buffered saline and incubated for ten minutes in a 37 ºC incubator. Following this procedure, samples were obtained from the incubators, diluted 1:10, and used to prepare smears that were then fixed.

Sperm Count: A light microscope with a 20X objective and a Makler camera were used to count sperm in the 1 mL sample pellet. The sperm count was taken to determine the spermatozoa concentration (10^6 sperm/mL).

Sperm Morphology: Spermac Kit (FertiPro Spermac Stain) was used to stain the smear preparations following fixation. The stained samples were examined under a light microscope. According to the Rat Sperm Morphological Evaluation Guide H.L. Science.(2000), at least two hundred sperm were counted and examined for head, neck, and tail abnormalities.[19,20]

Statistical Analysis

The software packages IBM SPSS Statistics 26.0 and GraphPad Prism 8 (Dotmatics) were used for all statistical evaluations. For sperm counts, Kolmogorov-Smirnov and Shapiro-Wilk normality distribution tests were performed, and the Tukey HSD test was used to compare groups. The data was provided as the mean ± standard deviation. Statistical significance was defined as $p<0.05$. The differences between groups were displayed in figures using the symbols *for $p<0.01$, **for $p<0.05$, and ***for $p<0.001$. The sperm morphology parameters (head, neck, and tail) were analyzed using two-way analysis of variance and were presented as mean values (upper limit; lower limit). Multiple comparisons of sperm morphology were performed using the Tukey HSD TEST.

Table 1. The chart illustrates the induction of acute liver damage with CCl4, followed by therapy with Lavandula angustifolia oil, medical ozone, and combination therapies

Days	Procedure applied
1-10	CCl_4 treatment
11	Lavandula angustifolia oil + medical ozone/medical ozone
12	Lavandula angustifolia oil
13	Lavandula angustifolia oil + medical ozone/medical ozone
14	Lavandula angustifolia oil
15	Lavandula angustifolia oil + medical ozone/medical ozone
16	Lavandula angustifolia oil
17	Lavandula angustifolia oil + medical ozone/medical ozone
18	Lavandula angustifolia oil
19	Lavandula angustifolia oil + medical ozone/medical ozone
20	Lavandula angustifolia oil
21	Lavandula angustifolia oil + medical ozone/medical ozone
22	Lavandula angustifolia oil
23	Lavandula angustifolia oil + medical ozone/medical ozone
24	Lavandula angustifolia oil

CCl_4: Carbon tetrachloride.

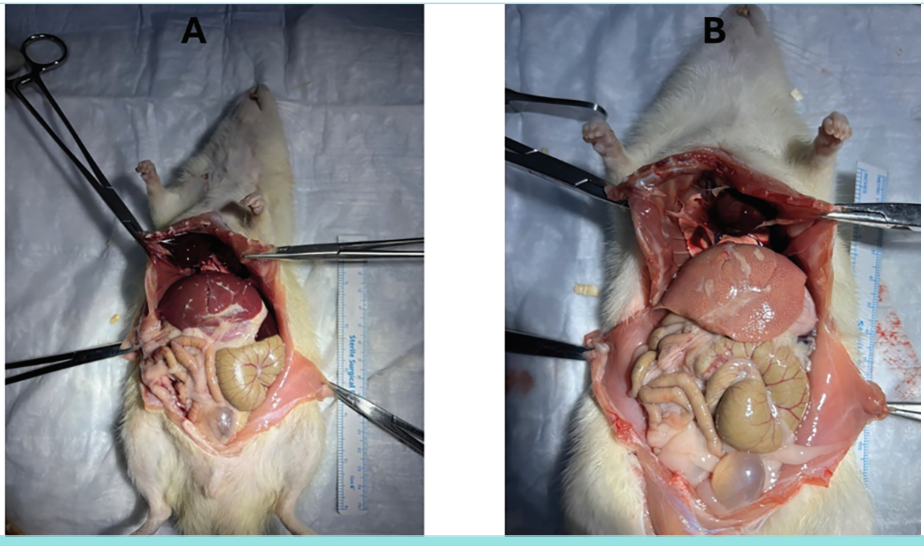

Figure 1. The impact of CCl4 treatment on liver after 10 days A) Healthy liver B) Acute toxic liver.
CCl4: Carbon tetrachloride.

RESULTS

Sperm Morphologies After Treatments

When examining sperm morphology for abnormalities, the three groups differ in the total numbers of head, neck, and tail abnormalities. Anomalies of the head, neck, and tail in the experimental groups were presented as mean (upper limit-lower limit). A statistically significant difference was observed between Lavender angustrofolia oil-treated (12; 8.198-17.234) and medical ozone-treated (14.5; 10.289-20.049) groups ($p<0.001$) for sperm head anomalies. Furthermore, for neck anomalies, there was a statistically significant difference between the Lavender angustrofolia oil-treated group (68; 61.248-74.073) and the medical ozone-treated group (65; 58.163-71.271) ($p<0.001$). Moreover, statistically significant differences in sperm tail anomalies were observed between groups treated with Lavender angustifolia oil (20; 15.045-26.086) and those treated with medical ozone (20.5; 15.486-26.626) ($p<0.001$). Nevertheless, there was no discernible difference between the control group (16.5; 11.997-22.266/54; 47.082-60.767/29.5; 23.614-36.159 respectively) and the Lavandula angustifolia oil group, the medical ozone group, and the combination group (10; 6.567-14.941/60.5; 53.588-67,016/29.5; 23.614-36.159 respectively; $p>0.05$; Figure 2A, B, and C).

Sperm Counts After Treatments

The findings indicate that Lavender angustrofolia oil (24.25±4.683), when incorporated into the treatment regimen for rats with liver impairment, both in combination (21.88±8.692) and medical ozone (22.88±7.936) significantly elevated sperm count compared to the control group (17±3.586; $p<0.001$). However, compared with the control group, the combination of lavender and medical ozone did not affect sperm count ($p>0.05$; Figure 3).

DISCUSSION

The available conventional therapies for drug-induced liver diseases are frequently insufficient and carry substantial adverse effects, necessitating the exploration of novel, efficacious therapeutic agents.[21] In this context, natural compounds (e.g., from Lavandula angustifolia oil) and therapeutic gases (e.g., medical ozone) present promising avenues for investigation due to their documented biological activities.[22] The liver plays a crucial role in metabolism, making it the primary target of toxicity induced by a range of drugs, xenobiotics, and oxidative stress.[23] Many medicinal plants are known to protect against CCl_4-induced liver injury in animal models, demonstrating their potential as hepatoprotective agents.[24] Essential oils, extracted from aromatic plants, are increasingly recognized for their medicinal potential, particularly their antioxidant and antigenotoxic properties, which are crucial for mitigating hepatocellular damage.[25] Lavandula angustifolia oil, in particular, has demonstrated significant antioxidant activity that can counteract oxidative stress implicated in various forms of hepatic injury.[26,27] Its constituents have been shown to modulate antioxidant enzymes and xenobiotic-metabolizing pathways, offering a multi-faceted approach to ameliorating liver damage.[28] The presence of compounds such as 1,8-cineole in Lavandula angustifolia oil has been shown to restore liver cell function and integrity, consequently reducing the leakage of serum aminotransferases and enhancing antioxidant enzyme activity.[1] Additionally, the essential oil of Lavandula angustifolia

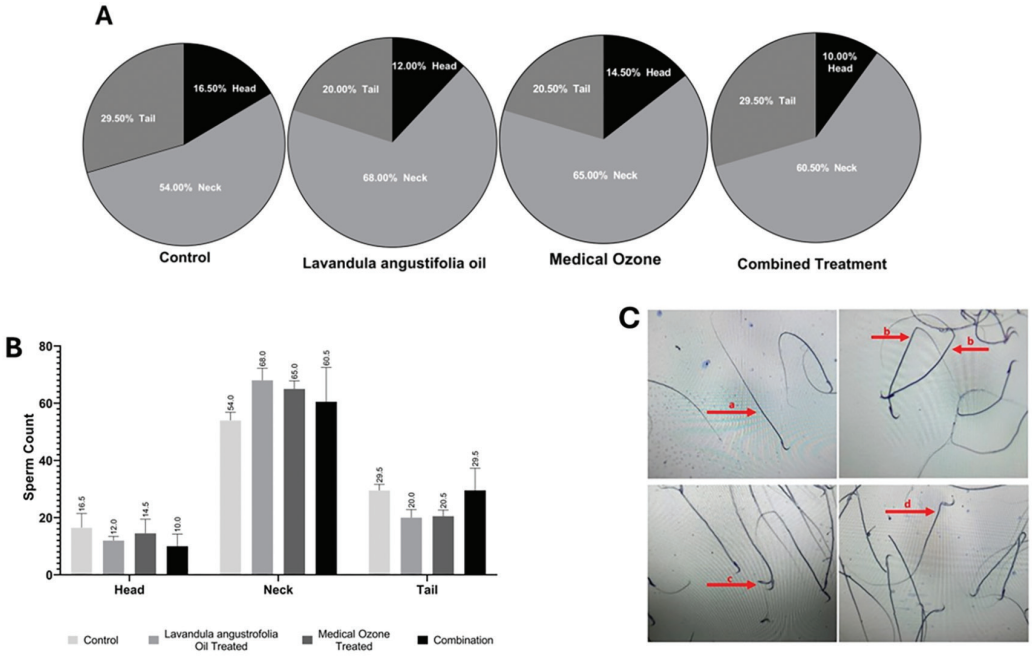

Figure 2. The impacts of Lavandula angustifolia oil, medical ozone and combined treatment (Lavandula angustifolia oil and medical ozone) in the sperm samples of CCl4 induced hepatoxic rats. A. Sperm morphology abnormalities (head, neck, and tail) between groups. B. Anomalies of the head, neck, and tail are observed across groups. C. Rat sperm morphologies: normal morphology (a), bent tail (b), double head (c), and flattened head (d). The control group did not differ from the other groups ($p>0.05$).

CCl4: Carbon tetrachloride.

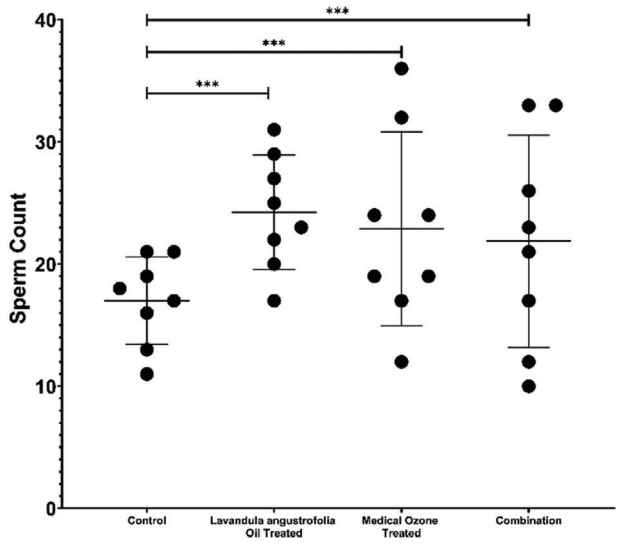

Figure 3. The sperm count was affected by the treatments, and the control group and the lavender-treated group. Sperm count differed among treatments; the control and lavender-treated groups differed ($p<0.001$).

has demonstrated anti-inflammatory properties, significantly reducing levels of interleukins interleukin (IL)-1 and IL-8, and of nuclear factor kappaB, which are key mediators in inflammatory hepatocellular injury.[29]

The therapeutic application of medical ozone has emerged as a complementary strategy to mitigate hepatocellular injury because of its immunomodulatory and oxidative preconditioning effects.[30] Medical ozone, through its controlled oxidative stress, can induce adaptive responses that upregulate endogenous antioxidant enzymes and modulate inflammatory pathways, thereby conferring hepatoprotection.[31] This preconditioning effect, often termed ozonated autohemotherapy, can activate the nuclear factor erythroid 2-related factor 2 pathway, leading to enhanced synthesis of antioxidant enzymes, such as heme oxygenase-1 and glutathione reductase. The transient and controlled oxidative stimulus provided by medical ozone also promotes immune modulation and improves microcirculation, both of which are crucial for attenuating hepatic I/R injury and enhancing tissue repair.[28] Lavandula angustifolia oil ameliorates oxidative stress by increasing glutathione levels and reducing concentrations of ROS, malondialdehyde (MDA), and oxidized glutathione.[1,26] Medical ozone acts indirectly by triggering an Nrf2/Keap1/ARE-mediated antioxidant response, which upregulates transcription of genes encoding various antioxidant and detoxification enzymes, including heme oxygenase-1, NADH:quinone oxidoreductase-1, and glutathione-synthesizing enzymes.[32,33] This indirect activation by ozone, distinct from direct antioxidant supplementation such as intravenous glutathione, involves the generation of ozonides and hydrogen peroxide (H_2O_2) as signaling molecules.[34] These reactive species subsequently interact with biomolecules, leading to the activation of adaptive cellular defense mechanisms.[35] This controlled induction of mild oxidative stress by ozone is believed to enhance the overall antioxidant capacity of the cell, preparing it to better withstand subsequent, more severe oxidative insults.[36]

The synergistic interplay between the direct antioxidant properties of Lavandula angustifolia oil and the redox bioregulatory effects of medical ozone could therefore offer a comprehensive strategy for protecting hepatocytes against various toxic insults by simultaneously neutralizing free radicals and bolstering intrinsic cellular defenses.[26,37] Linalool, a key component of Lavandula angustifolia oil, activates the Nrf2 signaling pathway, which is crucial for reducing ROS during inflammatory processes and thereby enhancing the cellular antioxidant defense system.[38] This activation of Nrf2 by linalool contributes to the overall antioxidant and anti-inflammatory effects observed with Lavandula angustifolia oil thereby protecting against cellular damage.[37] Similarly, medical ozone, through its hormetic action, specifically targets the Nrf2-Keap1-ARE system via ROS-mediated signaling, thereby inducing a potent antioxidant and pro-survival response within hepatocytes.[39] This induction of the Nrf2 pathway by both Lavandula angustifolia oil components and medical ozone - albeit through potentially distinct initial mechanisms - ultimately converges to enhance cellular detoxification and antioxidant capacities and to safeguard hepatocellular integrity.[40] Because activation of Nrf2 is a key mechanism of both Lavandula angustifolia oil components and medical ozone, their combined application may synergistically upregulate antioxidant and detoxification pathways, enhancing protection against hepatocellular insults. Specifically, the regulation of Nrf2 by ozone, a relationship first described in the 21st century, highlights its role in modulating antioxidant mechanisms, like the effects observed with low-dose ozone and Nrf2 activation.[41]

In the present research, we adopted a liver model mediated by CCl_4, which is one of the most widely used xenobiotics to cause tissue damage mediated by lipid peroxidation in animal model studies. This study aimed to investigate how lavender, medical ozone, and their synergistic combination affected sperm count and morphology in rats with hepatotoxicity. Initially, we demonstrated that Lavandula angustifolia oil without medical ozone enhanced sperm count in rats with hepatic impairment when administered to treat organ damage. The use of Lavandula angustifolia oil during treatment results in an increase in sperm count; this effect is reversible. The inactivation of Lavandula angustifolia oil raises the possibility that, when combined

with medical ozone, the oil's effect may be suppressed. Souli et al.[42] evaluated the protective effects of Rosmarinus officinalis essential oil (ROEO) and Lavandula stoechas essential oil (LSEO) in preventing reproductive impairment and oxidative stress in male diabetic rats induced by alloxan exposure. They discovered that ROEO and LSEO, in addition to preventing alloxan-induced increases in body weight, relative reproductive organ weights, testosterone levels, and loss of sperm quality, had a substantial antioxidant capacity. Conversely, they demonstrated that the administration of alloxan was associated with an oxidative stress status measured by elevated levels of H_2O_2 and MDA, as well as decreased levels of sulfhydryl group content and antioxidant enzyme activities of the testis, epididymis, and sperm include catalase, SOD, and glutathione peroxidase. Notably, in rats with alloxan-induced diabetes, ROEO and LSEO therapy substantially prevented oxidative damage to the male reproductive organ systems.[43] In the literature, studies report hepatoprotective and renoprotective effects of Lavender essential oils against malathion-induced oxidative stress in young male mice, showing that LSEO protect the rat liver and kidney, an effect due in part to their antioxidant properties. However, Tuşat et al.,[20] in an experimental torsion model, investigated the effect of ozone therapy on testicular damage resulting from I/R injury. They found that the ozone-treated rats had lower histopathological tissue levels, serum ischemia-altered albumin, total oxidant status, and oxidative stress index than the torsion/detorsion group.[20] Research indicates that medical ozone therapy may have beneficial effects on histopathological and biochemical findings in the reproductive systems of rats.

Study Limitations

Despite these strengths, there are a few limitations that should be noted. Sperm quality was mainly assessed by concentration and morphology, while motility and viability were not evaluated. Due to the length of rat sperm tails, motility is difficult to assess; however, including these parameters could provide a more complete understanding of treatment effects. Secondly, the study did not evaluate additional parameters such as histopathological examination at testicular and hepatic levels or inflammatory pathways. Consequently, the current findings serve as preliminary data in this regard; further studies with larger sample sizes are needed to elucidate the potential biological basis of the significant interaction observed between medical ozone and lavender oil treatments.

CONCLUSION

The literature contains a limited number of articles on medical ozone therapy; therefore, the novelty of this study is indicated by the lack of studies that combine medical ozone therapy with Lavandula oil, as opposed to applying medical ozone therapy to essential oils, or "ozonated oils". This investigation will critically evaluate the efficacy and safety profiles of "Lavandula angustifolia" oil and medical ozone in preclinical models of liver injury, thereby contributing to the development of novel therapeutic strategies. Although the sperm counts of rats treated with medical ozone alone and those treated with Lavandula angustifolia oil plus medical ozone did not differ significantly, our results showed that only rats treated with Lavandula angustifolia oil alone showed an increase in sperm counts, indicating that medical ozone may counteract the effects of Lavandula angustifolia oil. Our research is among the earliest to demonstrate a connection between medicinal ozone and lavender oil in the literature. Further research is required to determine the effectiveness of medical ozone treatment and lavender oil on spermiogram parameters in rats with liver impairment.

MAIN POINTS

- Lavender oil increased sperm count in rats with carbon tetrachlorideinduced liver injury.
- Medical ozone therapy alone did not improve sperm count.
- Combined lavender oil and ozone showed no synergistic benefit.
- Ozone treatment may counteract lavender's positive effect on spermiogram parameters.
- Sperm morphology was not significantly altered by combined therapy.

ETHICS

Ethics Committee Approval: This research was authorized by the Maltepe University Animal Experiments Local Ethics Committee (approval no: 2023.06.03, date: 29.06.2023).

Informed Consent: As procedures were carried out on animals, patient approval was not obtained

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.Ö.K., Ç.Ö., İ.Z.T., E.A., N.A., Concept: N.Ö.K., Ç.Ö., Design: N.Ö.K., Ç.Ö., Data Collection and/or Processing: N.Ö.K., Ç.Ö., İ.Z.T., E.A., N.A., Analysis and/or Interpretation: N.Ö.K., Ç.Ö., İ.Z.T., E.A., N.A., E.Ç., Literature Search: N.Ö.K., Ç.Ö., E.Ç., Writing: N.Ö.K., Ç.Ö., İ.Z.T.

DISCLOSURES

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RESEARCH ARTICLE

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Thirst Distress in Patients with Heart Failure

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Abstract

BACKGROUND/AIMS: This study aimed to determine the prevalence of thirst distress and its predictors in patients with heart failure (HF).

MATERIALS AND METHODS: The study was conducted between April 1, 2024, and July 15, 2025, and included 281 patients hospitalized in the cardiology wards of a training and research hospital in İstanbul, Türkiye.

RESULTS: Thirst distress was severe in 26.69% of patients, high in 31.67%, moderate in 30.25%, mild in 8.19%, and absent in 3.2%. The mean thirst distress scale score was 26.15±8.07, and the mean visual analog scale (VAS) thirst severity score was 5.53±2.30. Thirst was experienced almost daily by 34.16% of patients and several days per week by 28.47% of patients. 40.21% of patients reported feeling thirsty for one hour or less. The mean thirst distress scores were 29.48±7.35 for the New York Heart Association (NYHA) IV, 27.38±7.25 for NYHA III, 24.52±8.21 for NYHA II, and 22.11±10.29 for NYHA I patients. A strong positive correlation was observed between thirst-distress scores and VAS. Multiple regression analysis identified longer duration since HF diagnosis, the presence of diabetes and hypertension, and NYHA class III-IV as statistically significant positive predictors of thirst distress. These variables explained 10.2% of the total variance in thirst distress (adjusted R^2=0.102).

CONCLUSION: Thirst distress is highly prevalent in patients with HF. Longer duration since HF diagnosis, comorbid diabetes and hypertension, and higher NYHA class were predictors of thirst distress.

Keywords: Heart failure, thirst, prevalence, risk factors

INTRODUCTION

Chronic heart failure (CHF) is a progressive disease associated with high morbidity and mortality, and it impairs quality of life. Despite advances in care and treatment, the prognosis of heart failure (HF) remains poor.[1,2] An estimated 64.3 million people worldwide live with HF.[3] In Europe, the incidence of HF has been reported to be approximately 5 per 1,000 adults and 3 per 1,000 across all age groups. The prevalence of HF is 1-2% among adults. Since studies generally include only diagnosed cases of HF, the true prevalence is likely higher.[2] According to the Heart Failure Prevalence and Predictors in Türkiye study conducted in our country, the absolute prevalence of HF was 2.9%.[4]

Patients with HF experience numerous symptoms, such as dyspnea, fatigue, and exercise intolerance. However, they may also experience other symptoms, including thirst.[5-7] Thirst is a distressing and common symptom in patients with HF, as demonstrated in numerous studies. It is defined as a sensation that generates a strong desire to drink and can cause significant distress in patients with HF.[7] Several factors contribute to thirst in CHF. The first mechanism is the prolonged activation of the renin-angiotensin-aldosterone system (RAAS) in the pathophysiology of HF, which stimulates the thirst center via hormonal activation.[8] In HF, reduced cardiac output and decreased effective arterial blood volume lead to diminished renal perfusion, which in turn stimulates the release of renin from the juxtaglomerular cells of the kidneys. This process initiates a cascade of reactions resulting in increased production of angiotensin II and aldosterone. Angiotensin II plays a central role in the regulation of thirst by directly activating thirst centers in the hypothalamus. In addition, angiotensin II promotes sodium retention and vasoconstriction, thereby exacerbating fluid imbalance. Aldosterone increases renal sodium and water reabsorption, which may paradoxically worsen congestion while sustaining neurohormonal activation. The persistent activation of the RAAS in HF leads to continuous stimulation

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of central thirst mechanisms, contributing to increased thirst perception and heightened thirst-related distress in affected patients.[9] Second, patients with HF often receive high-dose diuretic therapy to manage fluid retention. This treatment can cause dry mouth and body fluid loss, which further intensifies thirst. Third, fluid restriction, recommended to prevent fluid overload, can also increase the patient's perceived thirst.[8,10,11] The patient's psychological state (anxiety, depression) can contribute to thirst.[8,12] Thirst can lead to dry mouth, which negatively affects the patient's oral health. Additionally, it may cause dysphagia and pose challenges for patients who use dentures, thereby affecting activities of daily living.[13]

In patients with HF, thirst is not merely a subjective discomfort but is closely associated with significant clinical outcomes. In particular, an increased perception of thirst in patients receiving fluid restriction and diuretic therapy has been reported to adversely affect adherence to treatment and medications.[10] Patients experiencing severe thirst have difficulty adhering to fluid restriction, and such nonadherence may lead to volume overload and subsequent worsening of symptoms. Furthermore, thirst distress has been shown to reduce patients' quality of life by diminishing physical comfort, disrupting sleep patterns, and limiting activities of daily living.[8,12] Inadequate identification and management of thirst increase overall symptom burden and complicate disease self-management, which, in turn, are associated with higher rates of emergency department visits and hospital readmissions. In this context, thirst should be considered an important yet often overlooked symptom in HF that may indirectly influence clinical prognosis.[14,15]

A study conducted in China found that patients with HF experienced intense thirst. The study identified associations between thirst and omeprazole use, renal failure, and coronary heart disease, as well as higher New York Heart Association (NYHA) class and low ambient humidity.[13] A study conducted in Spain found that 47% of patients experienced thirst.[6] A study conducted in Sweden, the Netherlands, and Japan reported that 33% of patients with HF experienced moderate thirst.[7] Studies have found that thirst is most often associated with high-dose loop diuretics[7] and fluid restriction.[16,17] Thirst may be perceived differently in various countries, depending on climate, diet, and cultural habits.[6]

Although thirst is frequently experienced by patients with HF, it is often overlooked in clinical practice, despite the fact that it has significant effects on patient comfort, treatment adherence, and activities of daily living. While the literature includes studies addressing the presence of thirst, research evaluating the prevalence and clinical significance of thirst distress remains limited. The lack of sufficient data on this issue in our country demonstrates the need for this study. This study aims to determine the prevalence of thirst distress among patients with HF and increase awareness of symptom management. In this context, the findings are expected to contribute to the early identification and effective management of thirst distress in clinical practice, and to underscore the importance of holistic symptom assessment in nursing care. Furthermore, the results are expected to inform the development of nursing interventions, including oral care, strategies to manage thirst, patient education, and counseling. This study aimed to determine the prevalence and predictors of thirst distress in patients with HF.

Research Questions

What is the level of thirst distress in patients with heart failure?

Which variables predict thirst distress in patients with heart failure?

MATERIALS AND METHODS

Study Objective

This descriptive and analytical cross-sectional study was conducted to determine the prevalence of thirst distress and to identify its predictors in patients with HF.

Study Setting and Period

The study was conducted between April 1, 2024, and July 15, 2025, in the cardiology wards of a training and research hospital in İstanbul, Türkiye.

Study Sample

The sample size was calculated using G*Power 3.1.9.7.[18] The calculation was performed using F tests (linear multiple regression: fixed model, R^2 deviation from zero). An effect size of $f^2=0.15$,[19,20] $\alpha=0.05$, power =0.95, and 3 predictors were used, resulting in a required sample size of 119. However, the study aimed to include the largest possible sample. Post-hoc power analysis indicated that, with an effect size of $f^2=0.07$, $\alpha=0.05$, 3 predictors, and a total sample of 281, the statistical power was 0.972. The sample size was considered sufficient. Patients were selected using convenience sampling. Only those who agreed to participate during the data collection process were included.

Inclusion Criteria

- Patients who voluntarily agreed to participate in the study and who signed the informed consent form were included.
- No visual or hearing impairments.
- Age over 18 years.
- At least literate (able to read and write but without completion of formal primary education).
- Diagnosed with HF for a minimum of six months.

Exclusion Criteria

- Patients who withdrew from the study voluntarily during the study period.
- Health conditions that would prevent the patient from answering the questions (e.g., dyspnea, pain).
- Patients receiving hemodialysis.

Data Collection Method and Instruments

The data collection instruments used were the Patient Information Form, the Thirst Severity Form, and the thirst distress scale. Data were collected face-to-face by the researchers in the patients' rooms, taking approximately 20 minutes per patient.

Patient Information Form

The patient information form was developed based on a literature review[7,13,21-23] and consists of 23 questions. It includes items on the patient's demographics (age, height, sex, smoking , alcohol use, medications, comorbidities) and on thirst-related factors (e.g., frequency of thirst episodes per month, times of day when thirst is most intense).

Thirst Severity: a single item in which the patient rates the intensity of their thirst on a visual analog scale (VAS) from 0 to 10.[24]

Thirst Distress Scale in Patients with Heart Failure: This scale was developed by Waldréus et al.[25] to measure thirst distress in patients with HF. It has a single subscale comprising eight items. The scale's internal consistency, as measured by Cronbach's alpha, was reported to be 0.90. It is a 5-point Likert-type scale designed to assess patients' thirst experiences over the past two to three days. Total scores range from 0 to 40, with 0-8 indicating no thirst distress, 9-16 indicating mild distress, 17-24 indicating moderate distress, 25-32 indicating high distress, and 33-40 indicating severe thirst distress.[25] The Turkish adaptation of the scale was conducted by Yakar et al.[26] The scale's Cronbach's alpha was found to be 0.90, indicating that it is reliable for use in Türkiye.[26] In this study, the Cronbach's α for the scale was found to be 0.911.

Ethical Considerations

Verbal and written informed consent was obtained from all participants. Ethical approval was granted by the Marmara University Non-Interventional Clinical Research Ethics Committee (approval number: 04, date; 25.01.2024). Institutional permission was obtained from the İstanbul Provincial Health Directorate (number: E-15916306-604.01-241397249/decision no: 2024/06, date: 28.03.2024). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

Data were analyzed using statistical software. Frequencies and percentages were used to describe participants' characteristics, while means and standard deviations were used to examine the scales. Skewness and Kurtosis values were assessed to determine whether the variables were normally distributed. Variables with Skewness and Kurtosis values between ±1.5 and ±2.0 were considered to be normally distributed.[27] The variables were found to be normally distributed, and parametric methods were used for data analysis. Relationships between two variables were examined using the Pearson correlation. Multiple linear regression analysis (enter model) was conducted to determine the effects of certain variables on thirst distress. Variables included in the regression models were selected based on factors known, clinically and theoretically, to be associated with thirst distress in patients with HF, consistent with the existing literature.[6,7,13,15,23] In addition, variables significantly associated with thirst distress in univariable analyses were included in the multivariable models.

RESULTS

Of the patients, 67.62% were male, 70.11% were married, and 58.36% had completed primary or middle school. A total of 75.09% were not employed, and 90.39% lived with their families. Active smoking was reported by 15.3% of patients. Nearly half (49.82%) had an income lower than their expenses, and 54.80% could perform activities of daily living independently. Fluid restriction was applied in 91.1% of patients, salt restriction was applied in 94.66% of patients, and 7.12% of patients had a diagnosis of depression. Comorbidities included hypertension in 64.77% of patients, coronary artery disease in 56.58% of patients, and diabetes in 48.04% of patients. Medication use included furosemide (95.73%) and beta-blockers (83.99%). According to the NYHA classification, 48.75% of patients were classified as NYHA II and 32.38% as NYHA III. The mean age of patients was 66.67±13.74 years, the mean duration since HF diagnosis was 5.23±4.85 years, the mean hospitalization frequency within one year was 2.36±1.91 times, and the mean length of hospital stay was 6.75±8.57 days (Table 1).

Among the patients, 26.69% reported severe thirst distress, 31.67% reported high thirst distress, 30.25% reported moderate thirst distress, 8.19% reported mild thirst distress, and 3.2% reported no thirst distress. The mean thirst distress scale score was 26.15±8.07. The mean VAS thirst severity score was 5.53±2.30. A significant positive correlation was found between thirst distress and thirst severity measured by the VAS (Table 2).

Among the patients, 34.16% experienced thirst almost daily, and 28.47% experienced it several times per week. Thirst lasted one hour or less in 40.21% of patients. The mean thirst distress scale scores were 29.48±7.35 for NYHA IV, 27.38±7.25 for NYHA III, 24.52±8.21 for NYHA II, and 22.11±10.29 for NYHA I patients (Figure 1).

In 45.55% of patients, the timing of thirst during the day was irregular. Patients who experienced thirst daily had higher thirst distress scores (Figure 2).

Table 1. Descriptive characteristics of patients (n=281)

Variables		n	%
Gender	Female	190	67.62
	Male	91	32.38
Marital status	Married	197	70.11
	Single	84	29.89
Educational status	Literate	57	20.28
	Primary-middle school	164	58.36
	High school/university	60	21.36
Working status	Unemployed	211	75.09
	Employed	70	24.91
Who live with	With family	254	90.39
	Alone	27	9.61
Smoking status	No	238	84.7
	Yes	43	15.3
Economic situation	Income less than expenses	140	49.82
	Income expense balanced	121	43.06
	Income more than expenses	20	7.12
Daily living activities	Can do it alone	154	54.80
	Can do it with help	101	35.95
	Cannot	26	9.25
Fluid restriction	Yes	256	91.1
	No	25	8.9
Salt restriction	Yes	266	94.66
	No	15	5.34

Table 1. Continued

Variables		n	%
Depression	No	261	92.88
	Yes	20	7.12
Comorbidities*	Hypertension	182	64.77
	Coronary artery disease	159	56.58
	Diabetes mellitus	135	48.04
	Atrial fibrillation	53	18.86
	Chronic kidney disease	37	13.17
	Chronic obstructive pulmonary disease	26	9.25
	Serebrovascular accident	16	5.69
	Cancer	3	1.07
Medications*	Furosemide	269	95.73
	Beta-blocker	236	83.99
	Gastroprotective	220	78.29
	SGLT2	163	58.01
	Spironolactone	128	45.55
	Statin	97	34.52
	ACEI	95	33.81
NYHA	I	9	3.20
	II	137	48.75
	III	91	32.38
	IV	44	15.66
Age mean ± SD (min-max)	66.67±13.74 (22-96)		
Duration of heart failure diagnosis mean ± SD (min-max)	5.23±4.85 (1-30)		
Frequency of hospitalization within a year mean ± SD (min-max)	2.36±1.91 (0-20)		
Days of hospitalization mean ± SD (min-max)	6.75±8.57 (1-70)		

*Multiple answers were selected.
SD: Standard deviation, NYHA: New York Heart Association, min-max: Minumum-maximum.

In Model 1, multiple linear regression analysis was conducted to examine the effects of time since HF diagnosis and the presence of diabetes and hypertension on thirst distress. The model was statistically significant ($F(3,277)=7.599$, $p<0.001$). These variables explained 6.6% of the total variance in thirst distress (Adjusted $R^2=0.066$). Longer duration since HF diagnosis, the presence of diabetes, and the presence of hypertension were identified as statistically significant positive predictors of thirst distress (Table 3).

In Model 2, multiple linear regression was used to examine the effects of the duration since HF diagnosis, diabetes, hypertension, and NYHA classification on thirst distress. The model was statistically significant ($F(6,274)=6.274$, $p<0.001$). These variables explained 10.2% of the total variance in thirst distress (Adjusted $R^2=0.102$). A longer duration since HF diagnosis, the presence of diabetes and hypertension, and NYHA class III or IV were identified as statistically significant positive predictors of thirst distress (Table 3).

In Model 3, the effects of furosemide use, salt and fluid restriction, and the presence of depression on thirst distress were not statistically significant($F(4,276)=1.914$, $p=0.108$).

DISCUSSION

In this study, the mean thirst distress score was high. A total of 88.61% of patients experienced moderate or severe thirst (Table 2). In a study by Younes et al.,[23] among patients with HF, the mean thirst distress score was at a moderate level, and 68% of patients reported moderate or severe distress. In the study by van der Wal et al.,[10] one-quarter of the patients reported experiencing severe thirst. In a study conducted in China, thirst distress was found to be moderate, with 75% of patients experiencing moderate-to-severe thirst.[13] A study conducted in Spain found that approximately half of patients with HF experienced thirst.[6] In a study conducted in Sweden, the Netherlands, and Japan, thirst distress was found to be moderate. No significant differences in thirst were observed among the three countries.[7] In another study, two-thirds of patients experienced moderate-to-severe thirst distress.[16] Our findings align with previous studies, confirming that thirst is common among patients with HF and therefore represents an important symptom that warrants careful consideration.

In this study, the mean VAS thirst severity score was 5.53±2.30 (on a 0-10 scale). In the study by Younes et al.,[23] the mean VAS score in patients with HF was 3.81±2.17 (on a 0-10 scale). In another study, the VAS score was reported as 47±22 (0-100 mm).[13] In a study including three countries, the VAS score in patients with HF was 53±15.[7] These studies indicate that VAS thirst severity scores are moderate in patients with HF. Additionally, the VAS is a simple and practical tool for routinely assessing thirst.

In this study, most patients experienced thirst almost daily or several times per week (Figure 1). For the majority of participants, the sensation of thirst lasted for one hour or less and the timing of peak thirst was irregular (Figure 2). In the study by Gong et al.,[13] most patients reported experiencing thirst several times per week or per month. Thirst typically lasted one hour or less, with the highest intensity occurring in the morning and afternoon.[13] In the study by Younes et al.,[23] nearly half of the patients reported experiencing thirst several times per week, one-third reported it several times per month, and most reported experiencing it in the morning. About 28% of patients indicated that a thirst episode lasted several hours.[23] In our study and in others, most patients reported thirst several times per week, typically lasting approximately one hour. These findings indicate that patients with HF frequently experience thirst throughout the day.

In this study, longer duration since HF diagnosis, presence of diabetes and hypertension, and NYHA class III or IV were identified as predictors of thirst distress. The use of furosemide, the restriction of salt and fluids, and the presence of depression were not statistically significant predictors of thirst distress (Table 3). In the study by Younes et al.,[23] salt restriction, use of statins, antidepressants, and any RAAS blocker were identified as predictors of thirst. van der Wal et al.[10] found a relationship between the use of high-dose diuretics, salt intake, and thirst intensity. In another study, factors associated with thirst included

Table 2. Patients' thirst distress levels (n=281)		
Variable	**n**	**%**
Thirst distress		
No	9	3.20
Mild	23	8.19
Moderate	85	30.25
High	89	31.67
Severe	75	26.69
Thirst distress mean ± SD (min-max)	26.15±8.07 (8-40)	
VAS mean ± SD (min-max)	5.53±2.30 (0-10)	
	VAS	
Thirst distress score	r	0.807
	p	**<0.001**

r: Pearson correlation, VAS: Visual analog scale, SD: Standard deviation, min-max: Minumum-maximum.

omeprazole use, renal failure, coronary heart disease, higher NYHA class, and low ambient humidity.[13] In the study by Eng et al.,[6] predictors of thirst were identified as lower use of angiotensin receptor blockers (ARBs), diuretic use >40 mg/day, depression, male sex, and worse NYHA class. In a systematic review, demographic characteristics, disease severity, psycho-environmental factors, medications, fluid restriction, and homeostasis were identified as risk factors for thirst. Conversely, increased fluid intake, sodium restriction, and the use of ARBs were identified as protective factors against thirst.[28] Several studies have found that fluid restriction is associated with both the severity of thirst and thirst distress.[7,16] The studies described above have identified numerous predictors of thirst. However, high NYHA class and fluid restriction are among the most commonly reported predictors. In our study, NYHA class was also found to be an important predictor. Non-pharmacological interventions can be applied to prevent thirst in patients with a high NYHA class who are on fluid restriction. These include implementing standardized oral care protocols, controlling the use of ice chips, chewing sugar-free gum, using oral moisturizers, and providing individualized education regarding fluid restriction and personalized fluid distribution. Systematic application of these interventions by nurses may help reduce perceived thirst and associated distress, particularly among patients with HF who are subject to fluid restriction. Although the model is statistically significant in this study, the relatively low adjusted R^2 indicates that thirst distress is multifactorial and complex, and cannot be fully explained by the variables included in this model alone. To achieve a more comprehensive understanding of thirst distress, environmental, psychosocial, and behavioral factors should be examined in greater detail in future studies.

In this study, diabetes mellitus and hypertension were identified as positive predictors of thirst distress. Diabetes and hypertension are important comorbid conditions that may contribute to increased thirst perception in patients with HF. In diabetes, particularly in cases of poor glycemic control, elevated blood glucose levels increase plasma osmolality, thereby stimulating hypothalamic osmoreceptors and triggering thirst. In addition, hyperglycemia-induced glucosuria leads to osmotic diuresis and increased urine output, resulting in intravascular volume depletion and further increase in thirst. Diabetes-associated xerostomia and reduced salivary secretion may also enhance thirst perception by decreasing oral moisture.[29,30]

Hypertension, on the other hand, may increase thirst indirectly through related pathophysiological processes and treatment-related factors. The widespread use of diuretic therapy, in particular, may induce mild hypovolemia and electrolyte disturbances that trigger thirst. Moreover,

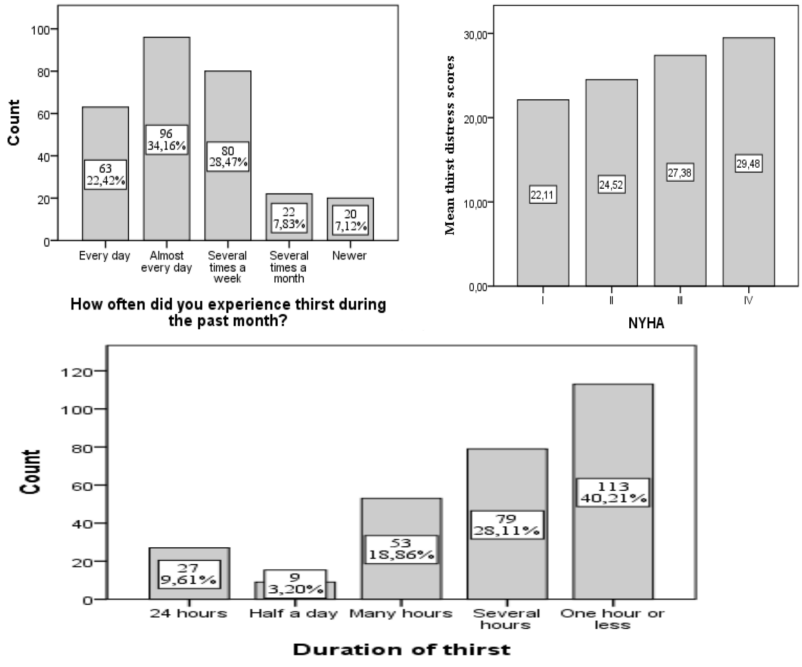

Figure 1. Frequency, duration of thirst, and levels of thirst distress according to NYHA classification among patients (n=281).
NYHA: New York Heart Association.

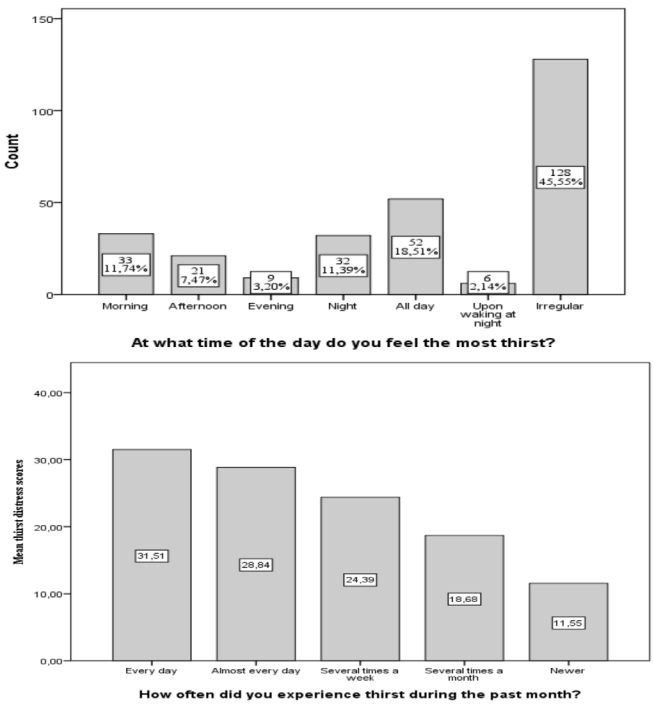

Figure 2. Timing and frequency of thirst experience among patients (n=281).

Table 3. Effects of selected variables on thirst distress							
Variable	**B**	**SE**	**β**	**t**	**p**	**95% CI**	**VIF**
Model 1							
(Constant)	22.04	0.98		22.47	<0.001	20.11-23.97	
Heart failure diagnosis time	0.23	0.10	0.14	2.43	**0.016**	0.04-0.42	1.01
Diabetes	2.28	0.95	0.14	2.42	**0.016**	0.42-4.15	1.03
Hypertension	2.75	0.99	0.16	2.78	**0.006**	0.80-4.70	1.04
F(3,277)=7,599, p<0.001, R^2=0.076, Adjusted R^2=0.066							
Model 2							
(Constant)	17.76	2.83		6.27	<0.001	12.18-23.34	
Heart failure diagnosis time	0.16	0.10	0.10	1.58	**0.115**	-0.04-0.37	1.20
Diabetes	2.23	0.93	0.14	2.40	**0.017**	0.40-4.06	1.03
Hypertension	2.60	0.97	0.15	2.68	**0.008**	0.69-4.52	1.04
NYHA II	3.49	2.71	0.22	1.29	0.199	-1.85-8.82	1.20
NYHA III	5.96	2.71	0.35	2.20	**0.029**	0.62-11.30	1.20
NYHA IV	7.35	2.80	0.33	2.62	**0.009**	1.83-12.86	1.20
F(6,274)=6,274, p<0.001, R^2=0.121, Adjusted R^2=0.102							
Model 3							
(Intercept)	21.02	3.07	0.00	6.85	<0.001	14.98-27.05	
Furosemide	2.31	2.45	0.06	0.94	0.346	-2.51-7.13	1.07
Salt restriction	-0.83	2.24	-0.02	-0.37	0.711	-5.25-3.59	1.11
Fluid restriction	4.09	1.83	0.14	2.24	0.026	0.50-7.69	1.18
Depression	-0.34	1.87	-0.01	-0.18	0.856	-4.01-3.33	1.01
F(4,276)=1,914, p=0.108, R^2=0.0270, Adjusted R^2=0.0129							

Dependent Variable: Thirst distress score, B: Unstandardized coefficient, SE: Standard error,
β: Standardized regression coefficient, VIF: Variance inflation factor, CI: Confidence interval, NYHA: New York Heart Association.

alterations in sodium balance and activation of the RAAS system influence central thirst mechanisms, with angiotensin II playing a key role in amplifying thirst responses. In patients with HF who also have hypertension, the convergence of these mechanisms may lead to a greater burden of thirst distress.[9] Therefore, diabetes and hypertension should be considered important clinical determinants in assessing and managing thirst distress among patients with HF.

Study Limitations

A limitation of this study is that the exact amounts of sodium and fluid intake were not measured. The dose of furosemide was not assessed, and patients' laboratory values, blood pressure, and pulse were not examined. Environmental temperature and humidity were not considered in this study. Future studies could evaluate patients' self-care behaviors. Our study included only hospitalized patients, which may limit the generalizability of the findings to all patients with HF. The relationship between neuroendocrine hormones and thirst could be explored in future research to better understand the mechanisms underlying HF.

This study has several notable strengths. First, it provides up-to-date data on the prevalence and severity of thirst distress in hospitalized patients with HF, an under-recognized yet clinically important symptom. Second, the use of both the thirst distress scale and the visual analog scale enabled a comprehensive assessment of thirst by capturing subjective distress and perceived severity; the strong correlation between these measures supports the robustness of the findings. Third, the inclusion of a relatively large sample from a tertiary training and research hospital enhances the reliability of the results and reflects real-world clinical practice. Fourth, this study is among the few to simultaneously examine clinical predictors, including the duration of HF, comorbid diabetes mellitus and hypertension, and NYHA functional class, thereby providing novel evidence to identify high-risk patient groups who may benefit from targeted symptom management strategies. To the best of our knowledge, this is the first study in Türkiye to examine the relationship between HF and thirst distress.

CONCLUSION

In this study, patients with HF experienced high thirst distress and moderate thirst severity. Longer duration since HF diagnosis, the presence of diabetes and hypertension, and NYHA class III or IV were identified as predictors of thirst distress. This study demonstrates the clinical importance of thirst as a symptom.

Thirst distress can negatively affect not only patients' quality of life, but also their adherence to treatment and their prognosis. It should not be overlooked during clinical assessments. Patients with comorbidities such as diabetes and hypertension were found to be at higher risk of experiencing thirst, and therefore should be monitored more closely. Because thirst distress is pronounced in patients with NYHA class III-IV, specific intervention protocols should be developed for this group. Non-pharmacological approaches to managing thirst, such as oral moisturizing techniques, ice chips, chewing gum, and ice sprays, can be incorporated into patient care.

Nurses' and other healthcare professionals' knowledge and awareness regarding the assessment and management of thirst distress should be enhanced. Structured education should be provided to patients and their caregivers on the purpose of fluid restriction, strategies for evenly distributing fluid intake throughout the day, and coping methods for reducing thirst. In addition, it is recommended that nursing education programs strengthen their content related to the assessment and management of thirst distress.

The relatively low proportion of variance explained by the regression models in this study suggests that thirst distress has a multidimensional and complex nature. Future studies should examine environmental, psychosocial, and behavioral factors, as well as cultural characteristics and patient experiences, in greater detail. Furthermore, planning randomized controlled trials and other interventional studies to evaluate the effectiveness of nursing interventions aimed at reducing thirst distress would contribute to the development of evidence-based clinical practice.

Nurses should integrate the assessment of thirst distress into routine patient evaluations, particularly for individuals subject to fluid restriction or with chronic conditions associated with thirst. This assessment should not be limited to the intensity of thirst alone; it should encompass multiple dimensions, including the patient's level of discomfort, the frequency of thirst throughout the day, and its impact on sleep and daily functioning. The use of valid and reliable thirst assessment instruments may facilitate the early identification of patients at risk and support the development of individualized care plans. In addition, factors such as oral dryness, medication use, environmental conditions, psychosocial stressors, and patients' knowledge and beliefs regarding fluid intake should be systematically evaluated.

Throughout the care process, nurses should reassess thirst distress at regular intervals, monitor the effectiveness of implemented interventions, and observe patients' adherence to fluid restriction regimens. Continuous clinical follow-up enables the early recognition of patient difficulties, treatment nonadherence, or deterioration in quality of life, thereby allowing care plans to be adjusted according to individual needs. Structured education for patients and their caregivers constitutes a fundamental nursing intervention in the management of thirst distress. Nurses should clearly explain the purpose of fluid restriction and its significance in disease management. Educational interventions should be individualized, culturally sensitive, and designed to enhance patients' self-management skills.

MAIN POINTS

- Thirst distress was highly prevalent among patients with heart failure (HF), with more than 88% experiencing at least moderate levels .
- The mean thirst-distress score was high, whereas the mean thirst-severity (visual analog scale) score was moderate, indicating a substantial symptom burden.
- A strong positive correlation was identified between thirst distress and thirst severity.
- Longer duration since diagnosis of HF, diabetes mellitus, hypertension, and New York Heart Association class III-IV were significant predictors of thirst distress.

- Thirst should be recognized as a significant and underappreciated symptom in HF management, with important implications for patient comfort and quality of life.

ETHICS

Ethics Committee Approval: Ethical approval was granted by the Marmara University Non-Interventional Clinical Research Ethics Committee (approval number: 04, date; 25.01.2024).

Informed Consent: Written informed consent was obtained from the patient who participated in this study.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: G.Ç., S.O., Concept: G.Ç., S.O., Design: G.Ç., S.O., Data Collection and/or Processing: G.Ç., Analysis and/or Interpretation: G.Ç., Literature Search: G.Ç., S.O., Writing: G.Ç., S.O.

DISCLOSURES

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RESEARCH ARTICLE

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Anal Fissure Research Across Four Decades: Global Trends and Emerging Directions

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Abstract

BACKGROUND/AIMS: Anal fissure is a common and painful anorectal disorder that substantially impairs quality of life. While therapeutic strategies have evolved from pharmacological sphincter relaxation and botulinum toxin injections to sphincter-preserving surgical and regenerative approaches, the global research landscape has not previously been assessed through bibliometric methods.

MATERIALS AND METHODS: A bibliometric analysis was conducted using the Web of Science Core Collection, covering the period 1980-2025. Publications were retrieved with the keywords "anal fissure*," "fissure-in-ano," "fissura ani," and "anorectal fissure*". Only articles and reviews were included. Network visualization was performed with VOSviewer, geographical mapping with Tableau, and statistical analyses with SPSS. Linear regression was applied to predict future publication trends.

RESULTS: A total of 1,504 records were identified, of which 1,241 were included (articles and reviews). The corpus accrued 23,277 citations across 8,572 publications, with an h-index of 71. Annual publications increased steadily, with a projected 78 papers in 2040 (R^2=0.864, $p<0.001$). The United States, Italy, and the United Kingdom were the most productive countries, while Türkiye, India, Pakistan, and Egypt demonstrated emerging but peripheral contributions. Collaboration mapping revealed five international clusters, with the United States acting as a global hub. The New England Journal of Medicine and Diseases of the Colon & Rectum were among the most cited journals. Keyword analysis showed a thematic transition from pharmacological therapies (glyceryl trinitrate, diltiazem, nifedipine) to botulinum toxin and sphincter-preserving surgery, with a recent emphasis on wound healing, recurrence, quality of life, and systematic reviews.

CONCLUSION: This first bibliometric analysis of anal fissure research highlights a sustained increase in global output and a shift toward patient-centered outcomes and minimally invasive strategies. While high-income countries dominate, emerging economies are contributing more actively. Future research should prioritize multicenter randomized trials, long-term quality-of-life and cost-effectiveness studies, and mechanistic investigations of ischemia, microbiota, and systemic factors in fissure chronicity.

Keywords: Anal fissure, benign anorectal disorders, bibliometrics, citation analysis, general surgery

INTRODUCTION

An anal fissure is a common and painful anorectal disorder characterized by a longitudinal tear in the anoderm, most commonly located at the posterior midline of the anal canal.[1,2] It presents with sharp anal pain, bleeding, and sphincter spasm, leading to a significant reduction in patients' quality of life.[2,3] Although acute fissures often heal spontaneously with conservative measures, chronic anal fissures are maintained by internal anal sphincter hypertonia and local ischemia, making spontaneous healing unlikely and necessitating medical or surgical intervention.[1,4,5]

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The lifetime prevalence of anal fissure is estimated at 7-8% in the general population; it affects both men and women and often occurs in young, otherwise healthy adults.[4] Despite its benign nature, chronic anal fissure can severely affect bowel habits, induce fear of defecation, and perpetuate constipation, creating a vicious cycle of pain and recurrence.[6] Furthermore, fissures may occasionally be associated with systemic diseases such as Crohn's disease, or with obstetric trauma, adding complexity to management.[6,7]

Therapeutic strategies have evolved considerably over the last three decades.[2,3] Lateral internal sphincterotomy (LIS) has long been considered the gold standard, achieving healing rates exceeding 90%, but it carries a risk of permanent fecal incontinence in a subset of patients.[8,9] To avoid this complication, non-surgical alternatives have been investigated extensively.[2] Randomized controlled trials demonstrated that topical nitric oxide donors, such as glyceryl trinitrate, reduce anal resting pressure and achieve healing in up to two-thirds of patients, though recurrence and adverse effects, such as headache, limit their long-term utility.[10-13] Calcium channel blockers, such as diltiazem and nifedipine, offer similar efficacy with fewer side effects and are preferred in recent guidelines.[6,13]

The advent of botulinum toxin injection into the internal sphincter represented a significant milestone.[14] Seminal studies in the late 1990's reported healing rates exceeding 80% with minimal risk of incontinence[15] and subsequent long-term follow-up studies have confirmed durable healing in carefully selected patients, especially in those without long-standing constipation.[16] Recent research has explored sphincter-preserving surgical options such as fissurectomy with or without anoplasty, demonstrating promising results with high healing rates and minimal impairment of continence.[17] At the same time, novel approaches including regenerative therapies (platelet-rich plasma, adipose-derived stem cells), topical methylene blue formulations, and snake venom-derived peptides highlight the dynamic and innovative research environment surrounding anal fissure management.[1,18]

Beyond treatment efficacy, research has increasingly focused on patient-centered outcomes such as recurrence, long-term continence, and quality of life.[8,17] Studies indicate that, while LIS achieves the highest cure rates, even mild incontinence significantly affects patient satisfaction and underscores the growing need for individualized treatment algorithms.[2,8] Psychological and lifestyle factors, such as obsessive-compulsive tendencies, smoking, and diet, have also been linked to fissure chronicity, emphasizing the multifactorial nature of the disease.[19]

Despite the abundance of clinical trials, narrative reviews, and evolving therapeutic strategies, no comprehensive bibliometric analysis of the anal fissure literature has been published to date.[5,20] Bibliometric methods allow for the systematic evaluation of global research output, citation impact, and international collaboration, providing insights into the historical trajectory and emerging trends of a field.[21,22] By applying bibliometric and statistical techniques to the global literature on anal fissure from 1980 to 2025, the present study aims to identify the most influential articles, journals, authors, institutions, and countries, to evaluate collaborative networks, and to uncover historical and emerging research hotspots. We aimed not only to contextualize the current state of anal fissure research but also to provide direction for future investigations and clinical practice.

MATERIALS AND METHODS

Formal approval from an ethics committee was not required, as this was a bibliometric analysis. The study was carried out in accordance with the ethical principles of the World Medical Association's Declaration of Helsinki.

Data were collected exclusively from the Web of Science (WoS) Core Collection database provided by Clarivate Analytics. WoS was selected because it offers standardized citation indexing, comprehensive reference metadata, and robust tools for citation and co-citation analyses, which are essential for mapping intellectual structures and collaboration networks in bibliometric research. Although databases such as Scopus and PubMed contain relevant biomedical literature, these databases were excluded from the analysis because PubMed does not provide citation linkage data suitable for co-citation analysis, and Scopus employs different citation coverage and indexing criteria that may introduce heterogeneity in longitudinal citation-based analyses. To ensure methodological consistency, reproducibility, and comparability with prior bibliometric studies, a single well-established citation database was used.

Search Details

Relevant studies were identified by searching the topic field using the keywords "anal fissure*," "fissure-in-ano," "fissure ani," and "anorectal fissure*". All records meeting these criteria in the title or abstract were included. The search covered the period from 1980 to 2025, and data were accessed on August 27, 2025. For reproducibility, the search strategy was as follows: [TS = ("anal fissure*" OR "fissure-in-ano" OR "fissura ani" OR "anorectal fissure*")], with the timespan 1980-2025, across the WoS Core Collection [science citation index-expanded, social sciences citation index, arts & humanities citation index, conference proceedings citation index-science (CPCI-S), CPCI-social science & humanities (CPCI-SSH), book citation index-science (BKCI-S), BKCI-SSH, emerging sources citation index] indexes. No language filters were used in the analysis.

Statistical Analysis

Bibliometric network visualization was conducted using VOSviewer (version 1.6.20, Leiden University, Center for Science and Technology Studies). In VOSviewer, synonymous keywords, spelling errors, and country names were standardized using the thesaurus option. For keyword analysis, the "all keywords" option was selected, and full counting was used in the main analyses. A world map illustrating the distribution of publications was generated using Tableau Software for Windows (version 2019.4.1, Tableau Software Inc., Seattle, WA). Statistical analyses were performed using IBM SPSS Statistics for Windows (version 27; IBM Corp., Armonk, NY). The Shapiro-Wilk test was employed to assess the normality of data distribution. A linear regression analysis was conducted to estimate overall publication trends and to explore future trajectories. The R^2 value was used to assess the model's performance in predicting publication trends. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 1504 publications on anal fissure were identified between January 1980 and August 2025. Of these publications, 1077 (71.61%) were articles, 164 (10.90%) were reviews, 109 (7.25%) were proceedings papers, 89 (5.92%) were meeting abstracts, and 65 (4.32%) were other

types of publications (editorial materials, letters, etc.). To strengthen the homogeneity of the analysis, only articles and reviews were included (1241 publications in total). Online-first articles were included in the analysis, but early-access articles without peer review were excluded (Figure 1).

The majority of the publications were in English (1125, 90.65%); 51 (4.11%) were in German, 19 (1.53%) were in French, 17 (1.37%) were in Spanish, 14 (1.13%) were in Russian, and 15 (1.21%) were in other languages. In total, anal fissure-related publications were cited,[23] 277 times by 8,572 publications, of which 7,600 citations were not self-citations. The mean citation rate per article was 18.76, while 237 of the 1241 papers (19.1%) had not been cited at all. Overall, the field demonstrated an h-index of 71.

Active Research Areas and Future Trend Analysis

The leading subject areas contributing to anal fissure research were identified as Surgery (616 publications, 49.64%), Gastroenterology (452 publications, 36.42%), and Internal Medicine (215 publications, 17.32%). Since many articles are indexed under more than one category, the total proportion exceeds 100%. Annual publication trends are illustrated in Figure 2, which also presents the results of a linear regression model used to project future output. The model demonstrated a strong fit with the observed data (R^2=0.864, p<0.001), explaining 86.4% of the variance. Based on this analysis, the projected numbers of publications were 58 in 2025 [95% confidence interval (CI): 43-73], 64 in 2030 (95% CI: 49-80), and 78 in 2040 (95% CI: 62-94). At the time of analysis, 35 of the 58 publications projected for 2025 had already been released.

Active Countries

Seventy-nine countries contributed to research on anal fissure. The twelve countries with the most publications on anal fissure were the United States (US) of America (193, 15.55%), Italy (134, 10.80%), England (128, 10.31%), India (94, 7.57%), Germany (71, 5.72%), Türkiye (67, 5.39%), Pakistan (64, 5.16%), Spain (48, 3.87%), Australia (39, 3.14%), Egypt (37, 2.98%), France (37, 2.98%), and Iran (37, 2.98). A total of 39 countries had 5 or more publications on anal fissure, while 6 had not collaborated with any other country. Total link strength scores of 33 nations that contributed at least 5 articles about anal fissure, had a minimum link strength of 1, and had international collaboration among their authors were measured and mapped (Figure 3). The analysis revealed five distinct clusters (Figure 4): Cluster 1: Germany, Canada, Sweden, Greece, Spain, Switzerland, Austria, Belgium, Portugal; Cluster 2: Italy, Israel, India, France, Bulgaria, Russia, Singapore, South Korea; Cluster 3: England, Pakistan, Egypt, Saudi Arabia, Scotland, Poland; Cluster 4: United States of America, Netherlands, Mexico, Brazil, Taiwan, China; Cluster 5: Australia, Türkiye, Ireland, Iran.

Active Authors and Institutions

A total of 4,573 authors contributed to research on anal fissure in publications indexed in WoS. The authors that contributed to the field with 10 or more publications were Brisinda G (23, 1.85%), Maria G (21, 1.69%), Scholefield JH (14, 1.13%), Albanese A (1.05%), Bentivoglio AR (13, 1.05%), Di Vita G (12, 0.97%), Gallo G (12, 0.97%), Altomare DF (11, 0.89%), Gupta PJ (11, 0.89%) and Jones OM (10, 0.81). A total of 1849 institutions contributed to the knowledge base on anal fissure, and the Egyptian Knowledge Bank (EKB) emerged as the leading contributor among them. The top ten institutions were EKB (37, 2.98%), Catholic University of the Sacred Heart (30, 2.42%), IRCCS Policlinico Gemelli (30, 2.42%), Sapienza University of Rome (23, 1.85%), Cleveland Clinic Foundation (19, 1.53%), Imperial College London (18, 1.45%), Mansoura University (15, 1.21%), University of Nottingham (15, 1.21%), University of Palermo (15, 1.21%), and University of London (14, 1.13%). After the removal of platform aggregators (EKB) from institutional rankings, the 10th-ranked institution by publications was the University of Oxford (14, 1.13%).

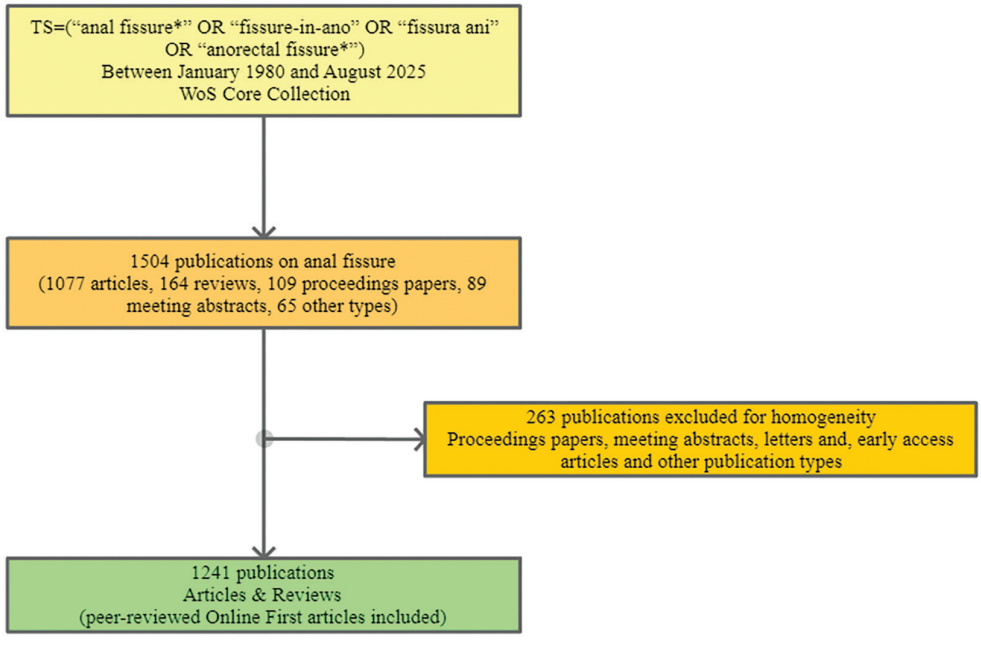

Figure 1. Flowchart diagram of publication selection.

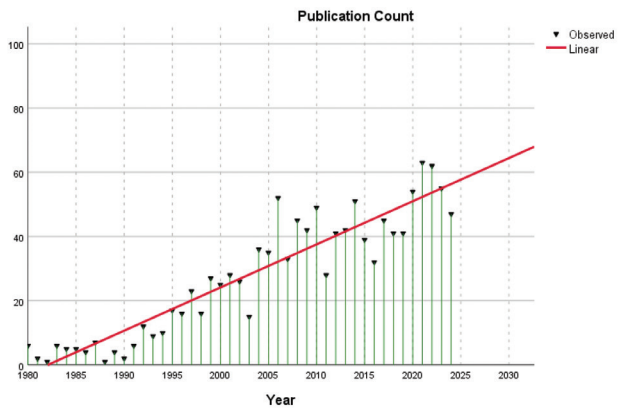

Figure 2. Distribution of anal fissure publications by year and projection of articles over the coming years using the linear model.

Active Journals

A total of 421 journals contributed to the field of anal fissure; the three journals contributing the most were Diseases of the Colon & Rectum (10.15%), International Journal of Colorectal Disease (3.55%), and Colorectal Disease (3.30%). The Top 11 journals are listed in Table 1, with each journal's total citation count and the average citation count per article.

Citation and Co-Citation Analysis

Of the 1,241 articles published between 1980 and 2025, the 10 most-cited papers on anal fissure are shown in Table 2. The study titled "A comparison of injections of botulinum toxin and topical nitroglycerin ointment for the treatment of chronic anal fissure" by Brisinda et al.,[23] published in New England Journal of Medicine in 1999, was the most cited article on anal fissure. The publications most frequently co-cited with other studies, serving as knowledge-base articles, were: Brisinda et al.[23] (link strength (LS): 286), Maria et al.[15] (LS: 276), Lund and Scholefield[11] (LS: 247), Schouten et al.[24] (LS: 220), and Lund and Scholefield[2] (NC: 183).[24]

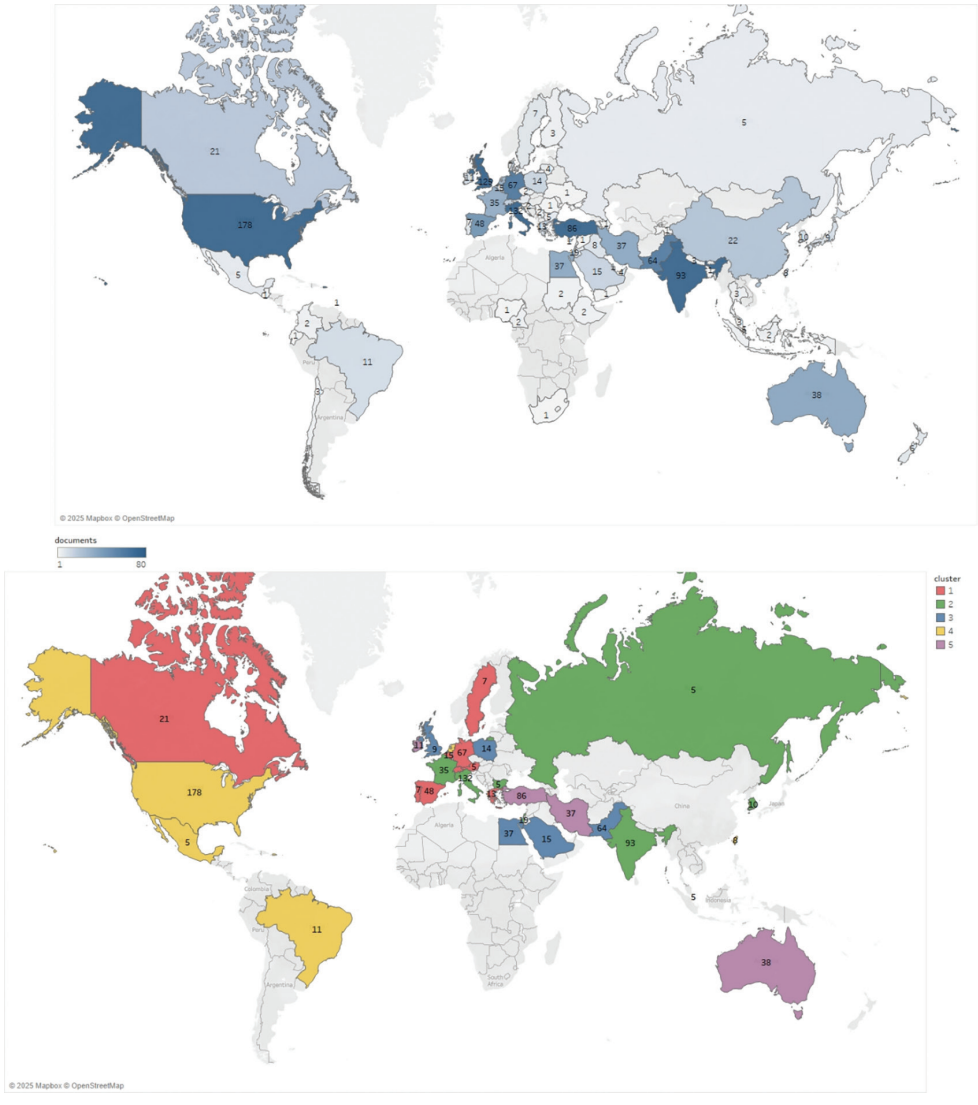

Figure 3. Global distribution of publications on anal fissure and color chart according to collaboration clusters.

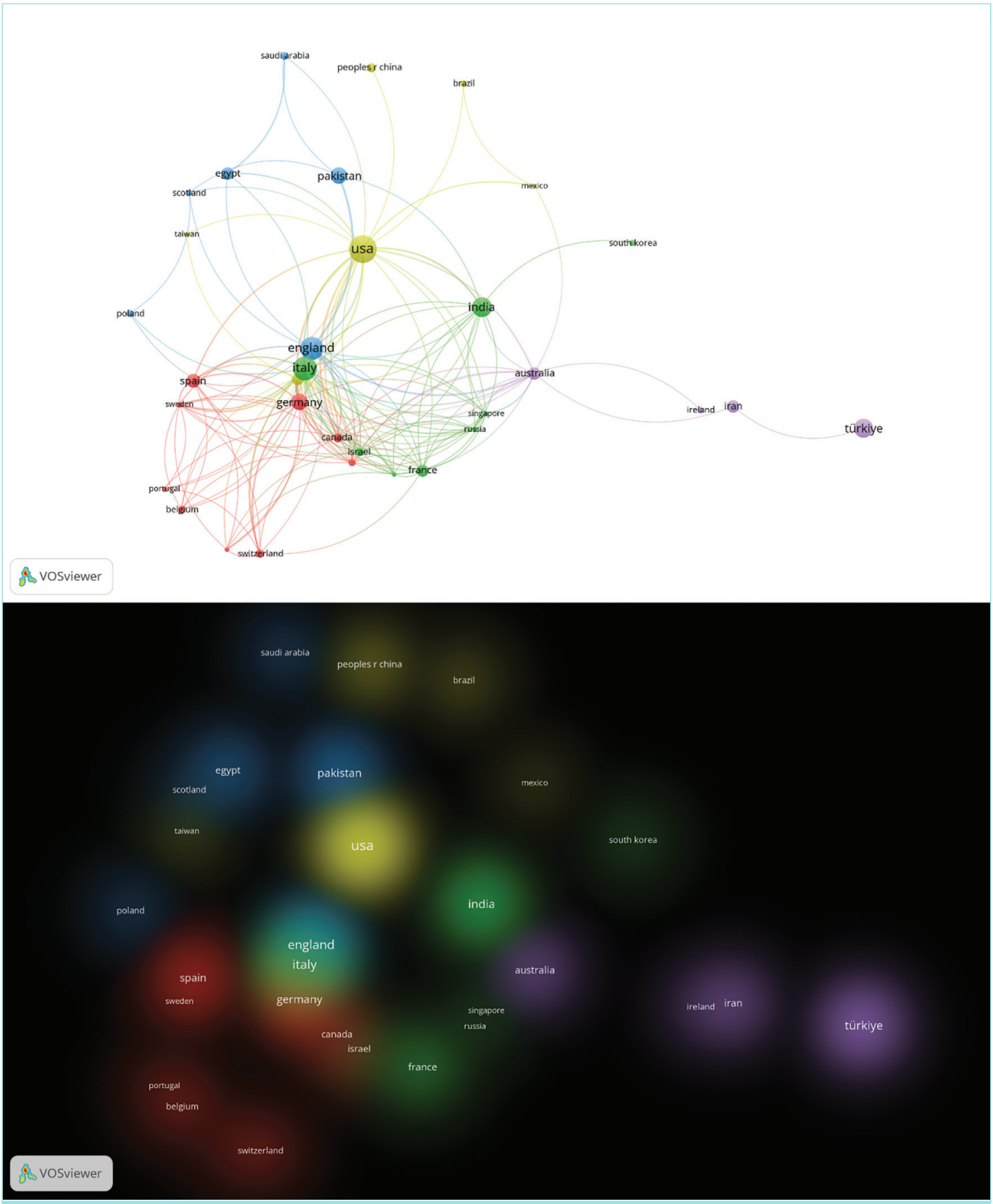

Figure 4. Network visualization map of cluster analysis and density map on worldwide cooperation on anal fissure.

Table 1. The top 11 most active journals with articles on anal fissure

No	Journal	TP	TC	ACPY	TSpIF
1	Diseases of the Colon & Rectum	**126**	**6033**	**260,99**	2,07
2	British Journal of Surgery	25	**1704**	61,65	2,47
3	International Journal of Colorectal Disease	**44**	**1006**	**68,22**	1,55
3	Colorectal Disease	**41**	915	**76,42**	1,86
4	New England Journal of Medicine	3	735	26,6	**8,87**
5	Gut	6	501	19,09	3,18
6	Techniques in Coloproctology	35	466	48,9	1,40
7	American Journal of Gastroenterology	7	455	41,06	**5,87**
8	Alimentary Pharmacology & Therapeutics	9	359	16,46	1,83
9	World Journal of Surgery	13	328	21,04	1,62
10	Journal of Gastrointestinal Surgery	15	276	15,79	1,05
11	Lancet	1	258	8,9	**8,90**

TP: Total publications, TC: Total citations, ACPY: Average citations per year, SpIF: Topic Specific Impact Factor.

Table 2. The top 10 most cited articles on anal fissure according to total citations

Title	Authors journal	PY	TC	ACpY
A comparison of injections of botulinum toxin and topical nitroglycerin ointment for the treatment of chronic anal fissure	Brisinda et al.[23] New England Journal of Medicine	1999	269	9,96
A randomised, prospective, double-blind, placebo-controlled trial of glyceryl trinitrate ointment in treatment of anal fissure	Lund et al.[11] The Lancet	1997	258	8,90
A comparison of botulinum toxin and saline for the treatment of chronic anal fissure	Maria et al.[15] New England Journal of Medicine	1998	240	8,57
Botulinum toxin type A injections: adverse events reported to the US food and drug administration in therapeutic and cosmetic cases	Coté et al.[25] Journal of the American Academy of Dermatology	2005	233	11,10
Intolerance of cow's milk and chronic constipation in children	Iacono et al.[26] New England Journal of Medicine	1998	226	8,07
Safety of botulinum toxin type A: a systematic review and meta-analysis	Naumann et al.[27] Current Medical Research and Opinion	2004	214	9,73
Anal cancer ıncidence - genital warts, anal-fissure or fistula, hemorrhoids, and smoking	Holly et al.[28] JNCI Journal of the National Cancer Institute	1989	212	5,73
ACG Clinical Guideline: management of benign anorectal disorders	Wald et al.[21] American Journal of Gastroenterology	2014	200	16,67
Long-term results of lateral internal sphincterotomy for chronic anal fissure with particular reference to incidence of fecal incontinence	Nyam and Pemberton[9] Diseases of the Colon & Rectum	1999	192	7,11
Aetiology and treatment of anal fissure	Lund and Scholefield[2] British Journal of Surgery	1996	191	6,37

PY: Publication year, TC: Total citations, ACpY: Average citations per year.

Trending Topics

Across all publications on anal fissure, 1,738 keywords were used. Figure 5 shows visualizations of 117 keywords that occurred in at least ten publications, displayed as cluster networks, trend analyses, and citation visualizations. The ten most frequently used keywords excluding anal fissure and fissure in ano, were botulinum toxin, chronic anal fissure, sphincterotomy, LIS, hemorrhoids, glyceryl trinitrate, diltiazem, fissurectomy, fecal incontinence, and constipation. Among treatment-related keywords, LIS (75 occurrences), botulinum toxin (119 occurrences), and fissurectomy (46 occurrences) were the most prominent, indicating both established and emerging approaches. Pharmacological agents such as glyceryl trinitrate (57 occurrences), diltiazem (49 occurrences), and nifedipine (24 occurrences) also appeared frequently. Reconstructive techniques, including anoplasty (14 occurrences), and terms related to wound repair, such as wound healing (19 occurrences), were observed to have later average publication years (2018 onwards). Symptom- and outcome-related keywords were also notable, with anal pain (19 occurrences), constipation (35 occurrences), recurrence (24 occurrences), and quality of life (18 occurrences) reflecting patient-centered aspects of research. Keywords such as meta-analysis and rectal bleeding showed relatively high citation averages, while botulinum toxin and anoplasty demonstrated more recent upward trends.

DISCUSSION

To our knowledge, this bibliometric analysis provides the first comprehensive overview of global research activity on anal fissure. By

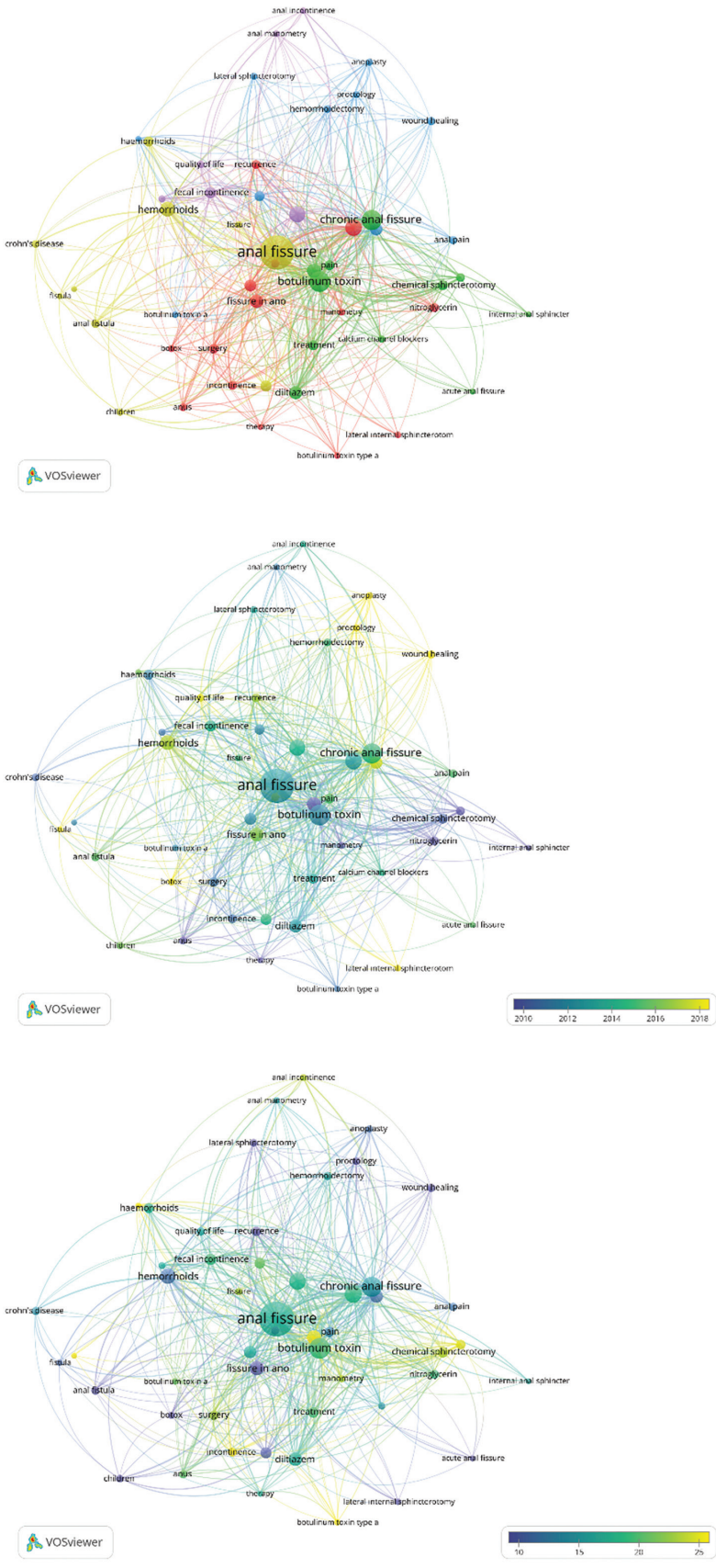

Figure 5. Keyword cluster analysis, keyword trend, and citation network visualization map of anal fissure.

relying on the WoS Core Collection, which offers standardized citation indexing and longitudinal coverage, the analysis enables consistent mapping of publication trends and collaboration patterns over time. By systematically evaluating 1,241 articles and reviews published between 1980 and 2025, we identified influential authors, institutions, and journals; mapped international collaboration networks; and characterized the thematic evolution of research in this field.

Our results demonstrate a steady growth of publications over the last four decades, with the annual output projected to reach approximately 78 articles by 2040. The rising trajectory underscores an enduring clinical and academic interest in anal fissure, reflecting both the high prevalence of the condition and the persistent challenges in achieving optimal management.[4,5,20] While most research originated in high-income countries such as the US, Italy, and the United Kingdom, significant contributions were also observed from Türkiye, India, Pakistan, and Egypt, reflecting increasing scholarly activity in emerging economies. Notably, the EKB was identified as the leading institution; however, this finding largely reflects WoSindexing practices, in which publications from multiple Egyptian universities and hospitals (e.g., Cairo University, Ain Shams University, Assiut University) are aggregated under the EKB label. This approach inflates the apparent contribution of the platform while obscuring institution-level outputs. Accordingly, the prominence of the EKB should be interpreted as a database-related artifact rather than a true institutional concentration.

Our co-citation analysis confirmed that seminal randomized trials of medical and surgical therapies-particularly the works of Brisinda et al.,[23] Maria et al.,[15] Lund and Schouten et al.[24] -constitute the intellectual foundation of the field.[2] The most cited article was the 1999 trial by Brisinda et al.,[23] published in the New England Journal of Medicine, which established botulinum toxin as a minimally invasive alternative to surgical sphincterotomy and nitroglycerin injections. Consistently, Diseases of the Colon & Rectum, the International Journal of Colorectal Disease, and Colorectal Disease emerged as the most prolific specialty journals, whereas high-impact generalist journals such as NEJM and The Lancet published fewer landmark studies that shaped clinical practice.[8,11,23] This pattern illustrates the dual dissemination of anal fissure research: detailed methodological and surgical advances appear primarily in subspecialty outlets, while paradigm-shifting trials achieve broader visibility in general medical journals.

Collaboration mapping revealed five distinct international clusters. The Americas-Asia Power Cluster, led by the US and including China, Taiwan, Brazil, Mexico, and the Netherlands, may reflect the US's role as a global hub with extensive research capacity and transcontinental collaborations. The UK-Middle East Nexus Cluster, centered in England, with partners including Egypt, Pakistan, Saudi Arabia, and Poland, could be influenced by historical academic ties and fellowship training pathways. The Mediterranean-Asia Bridge Cluster, anchored by Italy and France, and extending to India, Israel, Singapore, and South Korea, may be explained by the influence of Mediterranean surgical schools and by established exchange programs with Asian centers. The Western and Central Europe Core Cluster, comprising Germany, Spain, Switzerland, Belgium, Austria, among others, may reflect cohesion fostered by EU-funded research networks and geographic proximity. Finally, the Emerging Peripheral Cluster, including Türkiye, Iran, Australia, and Ireland, may suggest growing research contributions from these regions but limited integration into established hubs, possibly due to funding disparities, geographic distance, or language barriers. Taken together, these patterns may indicate that international collaboration in anal fissure research is shaped not only by scientific priorities but also by historical, cultural, and structural factors.

From a regional perspective, the collaboration map also offers insight into the positioning of countries with substantial clinical engagement but variable international visibility. As researchers from Türkiye, we find it striking that our country is placed in a peripheral cluster on the collaboration map, despite the high prevalence of anal fissure in the population and the active contributions of Turkish surgeons to its treatment and study.[16,19] We expected Türkiye to align more closely with the Western & Central Europe Core, given its geographical proximity, strong surgical tradition and contributions to the area of anal fissure (5th country in publication count). This peripheral positioning, however, may be explained by several factors. Many Turkish studies have historically been published in regional journals with limited indexing in international databases, which reduces their visibility and citation linkages. Cross-citations with global centers remain weak, as domestic research is more frequently referenced within local networks than by international counterparts. Opportunities for large-scale collaborative trials with major European or US institutions have also been limited, restricting Türkiye's integration into established hubs. In addition, structural barriers such as funding constraints may have reduced participation in high-profile projects. Finally, much of Türkiye's publication growth has occurred only in the past decade, after dominant bibliometric networks had already formed, a factor that may have further delayed Türkiye's central integration. Taken together, these considerations suggest that Türkiye's peripheral placement reflects structural, historical, and visibility-related factors rather than a lack of clinical engagement.

Keyword analysis illustrated the shifting focus in anal fissure research. In earlier decades, emphasis was placed on pharmacological sphincter relaxation using agents such as glyceryl trinitrate, diltiazem, and nifedipine, reflecting the search for non-surgical alternatives to sphincterotomy.[3,11] Subsequently, botulinum toxin emerged as a major research hotspot, supported by randomized trials demonstrating efficacy with minimal risk of incontinence.[15,23] More recently, sphincter-preserving surgical options have gained attention, often in response to concerns regarding postoperative continence impairment associated with LIS.[17] Additionally, newer topics, including wound healing, quality of life, and recurrence, reflect a broader shift toward patient-centered outcomes.[3,19,29] The increasing use of terms such as meta-analysis and systematic review indicates growing efforts to synthesize evidence and to guide evidence-based practice.[20,21,27]

Taken together, these findings mirror the clinical trajectory of anal fissure management: from the dominance of LIS to the rise of pharmacological therapies to the ongoing refinement of minimally invasive and reconstructive techniques.[8,11,17] At the same time, the literature reveals persistent controversies, including the optimal balance between efficacy and continence preservation, the durability of botulinum toxin compared with surgery, and the role of novel biologic and regenerative therapies.[8,15,23,30] Our analysis suggests that future research should prioritize multicenter randomized trials comparing sphincter-preserving surgery with established treatments; long-term studies of quality-of-life outcomes and economic evaluations; and mechanistic studies addressing the role of ischemia, microbiota, and systemic factors in fissure chronicity.

Study Limitations

This study has limitations inherent in bibliometric analyses that should be considered when interpreting the findings. First, data were obtained exclusively from the WoS Core Collection, which provides standardized citation indexing and comprehensive reference metadata but may exclude relevant publications indexed only in other databases such as Scopus or PubMed. Second, citation-based indicators were primarily derived from raw citation counts, although average publication year and normalized citation measures were considered, cumulative citation effects may still favor older publications, which is a recognized characteristic of bibliometric datasets. Third, publication trends were summarized using linear regression to describe long-term growth patterns. In bibliometric research, such trend analyses are exploratory in nature and are not intended to capture complex nonlinear dynamics or to serve as validated predictive models; therefore, extrapolated projections should be interpreted cautiously. Fourth, co-authorship and country collaboration networks were constructed as visual representations of structural relationships in the literature. Quantitative cluster validation metrics are not intrinsic to visualization-driven bibliometric mapping and were not central to the analytical framework of this study. While bibliometric approaches effectively quantify research productivity and influence, they cannot fully assess the methodological quality or the evidentiary strength of individual studies.

CONCLUSION

This bibliometric analysis of 1,241 articles and reviews on anal fissure published between 1980 and 2025 demonstrated a steady growth in publications and anticipates continued growth in the coming decades. The US, Italy, and the United Kingdom were the leading contributors, with the US acting as the primary global hub and the United Kingdom and Italy serving as regional bridges; countries such as Türkiye, India, Pakistan, and Egypt showed emerging but peripheral roles. Influential works were concentrated in high-impact journals, such as the New England Journal of Medicine, and specialty outlets including Diseases of the Colon & Rectum and Colorectal Disease; Brisinda et al.'s 1999 randomized trial was the most cited article. Keyword analysis revealed a thematic evolution from pharmacological therapies toward botulinum toxin injections, fissurectomy, and anoplasty, alongside an increasing emphasis on recurrence, quality of life, and patient-centered outcomes. These findings provide a comprehensive overview of the intellectual structure, collaboration patterns, and emerging research directions in anal fissure research, offering a framework to guide future investigations.

MAIN POINTS

- This study provides the first comprehensive bibliometric analysis of anal fissure research spanning 1980-2025, including 1,241 articles and reviews indexed in the Web of Science Core Collection.
- Publication output has steadily increased over four decades, with projections suggesting continued growth toward 2040, which indicates sustained global academic interest in anal fissure management. The United States, Italy, and the United Kingdom emerged as leading contributors, with distinct international collaboration clusters shaping the intellectual structure of the field.
- Citation and co-citation analyses identified seminal randomized trials on botulinum toxin and pharmacologic sphincter relaxation as the foundational knowledge base of contemporary management strategies.
- Analysis of keyword trends demonstrated a thematic evolution from pharmacological therapies (glyceryl trinitrate, diltiazem, nifedipine) to botulinum toxin, sphincter-preserving surgery, wound healing, recurrence, and quality-of-life outcomes.
- Despite growing global output, research remains concentrated in high-income countries, highlighting the need for broader international collaboration and multicenter randomized trials focusing on long-term outcomes and patient-centered measures.

ETHICS

Ethics Committee Approval: Not required because the study analyzed publicly available bibliographic data and did not involve human participants or identifiable patient information.

Informed Consent: Not applicable.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.B.T., V.B.T., Concept: M.B.T., V.B.T., Design: M.B.T., V.B.T., Data Collection and/or Processing: M.B.T., V.B.T., Analysis and/or Interpretation: M.B.T., V.B.T., Literature Search: M.B.T., V.B.T., Writing: M.B.T., V.B.T.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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Comparative Diagnostic Utility of Systemic Inflammatory Indices Across Hyperthyroidism Subgroups

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Abstract

BACKGROUND/AIMS: In this retrospective study, we aimed to examine the diagnostic performance of systemic inflammation response index (SIRI), systemic immune inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) parameters in terms of subgroups in hyperthyroidism cases.

MATERIALS AND METHODS: A total of 191 patient files were included in the study; these patients had applied to the Internal Medicine Department of Fatih Sultan Mehmet Hospital and the Internal Medicine and Endocrinology Department of Florence Nightingale Ataşehir Hospital were diagnosed with hyperthyroidism between January 2023 and June 2023. We evaluated age, gender, chronic drug usage, comorbidity, free T3, T4, anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin antibody (anti-TG), thyrotropin receptor antibodies (TRAb), neutrophil, lymphocyte, platelet (PLT), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), NLR, PLR, SIRI, and SII parameters of the patients.

RESULTS: Seventy-four patients (89.2%) had hashitoxicosis (HT), 28 patients (33.7%) had Graves' disease (GD), 16 patients (19.3%) had subacute thyroiditis (SAT), and 32 patients (38.6%) had overlapping entries in their files. Only the free T4 level was significantly correlated with hyperthyroidism ($r=0.288$, $p<0.01$). Correlations between hyperthyroidism and age, gender, chronic drug usage, comorbidity, free T3 and free T4, anti-TPO, anti-TG, TRAb, neutrophils, lymphocytes, PLT, CRP, ESR, NLR, PLR, SIRI, and SII were not statistically significant ($p>0.05$). The diagnostic performance of NLR, PLR, SIRI, and SII for hyperthyroidism was not statistically significant ($p>0.05$). Graves was significantly correlated with monocytes ($r=0.240$, $p<0.05$) and with SIRI ($r=0.221$, $p<0.05$). SAT was significantly correlated with CRP ($r=0.574$, $p<0.01$) and ESR ($r=0.626$, $p<0.01$). Overlap was significantly correlated with CRP ($r=0.409$, $p<0.01$) and with ESR ($r=0.246$, $p<0.01$). SIRI had a diagnostic performance of 63.5% for Graves ($p<0.05$). The diagnostic performance of all other blood parameters for HT, GD, SAT, and overlap groups was not significant ($p>0.05$). The area under the curve (AUC) for SIRI in GD was 0.635. At a SIRI cut-off value of 3.70, the sensitivity was 78.6% and the specificity was 52.7%.

CONCLUSION: Although blood parameters lack sufficient diagnostic performance to distinguish hyperthyroidism subtypes, SIRI may have diagnostic performance specific to GD. Despite the low diagnostic performance, it would be beneficial to examine the diagnostic value of SIRI for GD in larger sample sizes and multicenter studies. SIRI should be interpreted as an adjunct biomarker rather than a diagnostic test.

Keywords: Hyperthyroidism, blood parameters, NLR, PLR, SIRI, SII

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INTRODUCTION

Hyperthyroidism is a disease characterized by elevated triiodothyronine (T3) and free thyroxine (FT4) concentrations and suppressed thyroid-stimulating hormone (TSH) levels. Low TSH, even with normal T3 and FT4 levels, is defined as subclinical hyperthyroidism and is seen in 0.7% to 1.4% of the population.[1] Graves' disease (GD) is the most common cause of hyperthyroidism; other common causes include drug side effects, toxic multinodular goiter, thyroiditis, and toxic adenomas.[2] Furthermore, iodine intake is the most significant risk factor; smoking, advanced age, ethnicity, genetic predisposition, immune inhibitors, and endocrine disruptors are other risk factors.[3] Hypothalamic and pituitary lesions, ectopic production of thyroid-stimulating proteins by neoplasms outside the thyroid, exogenous drug reactions, and mechanical destructive pathologies are also cited as causes and risk factors in the literature.[4] The clinical presentation often includes arrhythmias (including tachycardia), weight loss, sweating, tremors, anxiety, and palpitations.[5] Common treatments include antithyroid drugs such as propylthiouracil and methimazole, thyroidectomy, or radioactive iodine ablation.[6]

Chronic autoimmune thyroiditis, one of the most common causes of chronic hypothyroidism, has been associated with a transient hyperthyroid phase, termed hashitoxicosis (HT).[7] HT has been reported in 4.2% of cases of unclassified hyperthyroidism.[8] HT has been reported to progress to Hashimoto's thyroiditis.[9] GD, one of the most common causes of hyperthyroidism, has been reported to trigger hyperactivity of the adrenergic nervous system, which is associated with cognitive dysfunction, ophthalmopathy, and goiter.[10] Subacute thyroiditis (SAT), on the other hand, is an inflammatory disease of the thyroid; its clinical course and pathogenesis are still poorly understood.[11] Treatment of hyperthyroidism requires accurate diagnosis and effective management.[12-14] Laboratory and clinical parameters, along with the physician's physical examination and experience, play an important role in diagnosing hyperthyroidism.[15] However, distinctive indicators are still needed in this regard. Although studies have examined the diagnostic and predictive value of hyperthyroidism, studies addressing blood parameters and systemic immune-inflammation indices are lacking. The inflammatory nature of the SAT type and recent, limited studies examining the role of blood parameters in mortality in autoimmune types[16] suggest that these parameters may have diagnostic utility for predicting hyperthyroidism. Therefore, this study aimed to examine the diagnostic performance of the systemic inflammation response index (SIRI), the systemic immune inflammation index (SII), the neutrophil-to-lymphocyte ratio (NLR), and the platelet-to-lymphocyte ratio (PLR) across subgroups of hyperthyroid patients. This study is the first in the literature to evaluate the diagnostic performance of the SIRI, the SII, the NLR, and the PLR across subgroups of hyperthyroidism cases at the multivariate level.

MATERIALS AND METHODS

Research Model

The study had a retrospective, descriptive screening design to identify patients' blood parameters and systemic immune-inflammatory indices. The correlational screening model analyzed the diagnostic performance of blood parameters for hyperthyroidism.

Patients

A total of 191 patient files from the Internal Medicine Department of Fatih Sultan Mehmet Hospital and the Internal Medicine and Endocrinology Departments of Florence Nightingale Ataşehir Hospital were diagnosed with hyperthyroidism between January and June 2023. Based on the study closest to ours by Wang et al.,[16] an effect size of 0.5 (95% confidence interval), a critical t value of 1.6802300 (calculated using G*Power 3.1.9.2), and a minimum sample size of 45 were obtained. This number was significantly exceeded in the study, with 191 patients included.

The inclusion criteria were as follows:

- Over 18 years of age
- Diagnosed with hyperthyroidism
- With complete data in their file
- No non-steroidal anti-inflammatory drugs or steroids have been started
- Not taking steroids or chemotherapy for rheumatological, oncological, or other reasons
- No active infection

Exclusion criteria were as follows:

- Under 18 years of age
- With incomplete data
- Using steroids or anti-inflammatory drugs
- Using steroids or chemotherapy for rheumatological, oncological, or other reasons

TSH levels below 0.4 mU/L are considered indicative of hyperthyroidism. HT was defined as elevated anti-thyroid peroxidase (anti-TPO) (≥5.61 IU/mL) or anti-thyroglobulin antibody (anti-TG) (≥4.11 IU/mL) levels. GD was identified by thyrotropin receptor antibodies (TRAb) ≥1.7 IU/L, and SAT was diagnosed when CRP ≥5 mg/L or erythrocyte sedimentation rate (ESR) ≥20 mm/h. Cases fulfilling more than one criterion were designated as overlapping phenotypes, including the HT+GH, HT+SA, and HT+GH+SA subgroups.

NLR = Neutrophil count/lymphocyte count ratio;

PLR = Platelet (PLT) count/lymphocyte count ratio;

SII = (PLT count × neutrophil count)/lymphocyte count ratio.

The following parameters of the patients were evaluated: age, gender, chronic drug use, comorbidities, free T3 and free T4, anti-TPO, anti-TG, TRAb, neutrophil and lymphocyte counts, PLT, CRP, ESR, NLR, PLR, SIRI, and SII.

The research algorithm initially involved defining and analyzing the dataset. Subsequently, a correlational screening analysis was performed on biomarker values of blood parameters and their ratios.

In examining the relationships between patients' blood parameters, ratios and logit-model analysis were used to minimize deflator effects arising from different units. This aimed to minimize unit conversion errors and systematic errors.

Ethical Approval

Ethical approval was obtained from Demiroğlu Science University Clinical Research Ethical Committee (approval number: 44140529/34423, date: 05.12.2023). Patient consent was not obtained because the study was

retrospective. The Declaration of Helsinki and good clinical practice procedures were followed in the study.

Statistical Analysis

Patients' nominal and ordinal parameters were described using frequencies, while measurement parameters were described using means and standard deviations. The Kolmogorov-Smirnov test was used to assess whether the measurement parameters conformed to a standard normal distribution. An Independent Samples t-test was used to compare parameters with a normal distribution, and the Mann-Whitney U test was used to compare parameters that did not conform to a normal distribution. Fisher's exact test was used to analyze differences between nominal and ordinal variables. Since the hyperthyroidism (yes/no) and Graves (yes/no) parameters are dummy variables, Spearman's rho correlation analysis was used. Due to linearization deviations,[17,18] Spearman's rho correlation analysis was used in the relational screening analysis. Receiver operating characteristic (ROC) analysis was performed to assess diagnostic performance. All analyses were performed using SPSS 25.0 for Windows with 95% confidence intervals and a significance level of 0.05. Since our study relies on relationship analysis and does not include between-group comparisons, we did not use Bonferroni or FDR corrections.

RESULTS

74 patients (89.2%) had HT, 28 (33.7%) had GD, 16 (19.3%) had SAT, and 32 (38.6%) had overlap, among patients with values recorded in their files. The mean age of patients without hyperthyroidism was 59.67±13.49 years, and the mean age of patients with hyperthyroidism was 56.78±13.77 years. Females comprised 93.3% of patients without hyperthyroidism and 83.8% of patients with hyperthyroidism. Differences in age, gender, chronic drug usage, comorbidity, free T3 and T4, anti-TPO, anti-TG, TRAb, neutrophil, lymphocyte, PLT, CRP, ESR, NLR, PLR, SIRI, and SII between hyperthyroidism subtypes were not significant ($p>0.05$) (Table 1).

Spearman's rho correlation analysis showed that only the Free T4 level was significantly correlated with hyperthyroidism ($r=0.288$, $p<0.01$). No statistically significant correlations were found between hyperthyroidism and age, gender, chronic drug usage, comorbidity, free T3, free T4, anti-TPO, anti-TG, TRAb, neutrophil, lymphocyte, PLT, CRP, ESR, NLR, PLR, SIRI, and SII ($p>0.05$; Table 2).

Spearman's rho correlation analysis showed that Graves' correlated significantly with monocytes ($r=0.240$, $p<0.05$) and SIRI ($r=0.221$, $p<0.05$). SAT was significantly correlated with CRP ($r=0.574$; $p<0.01$) and ESR ($r=0.626$; $p<0.01$). Overlapping was significantly correlated with CRP ($r=0.409$, $p<0.01$) and with ESR ($r=0.246$, $p<0.01$) (Table 3).

ROC analysis showed that SIRI had a diagnostic performance of 63.5% for GD ($p<0.05$). Diagnostic performance of all other blood parameters in HT, GD, SAT, and overlap groups was not significant ($p>0.05$) (Table 4).

The mean SIRI in the Graves group was higher, while the range of SIRI in Graves' patients was lower than in patients without Graves' (Figure 1).

Area under the curve (AUC) was 0.635 for SIRI for Graves. For a SIRI cut-off level of 3.70, sensitivity was 78.6% and specificity was 52.7% (Figure 2).

DISCUSSION

This study examined the diagnostic performance of the SIRI, SII, NLR, and PLR across subgroups of patients with hyperthyroidism. The files of 191 patients were analyzed. The results showed that the SIRI parameter demonstrated statistically significant diagnostic performance for GD.

Hyperthyroidism is a disease affecting between 0.2% and 1.4% of the general population worldwide1, with risk factors ranging from thyroid lesions and other thyroid diseases to general risk factors and demographics.[2,3] Because the clinical presentation often includes features seen in common diseases, such as arrhythmia, weight loss, sweating, and anxiety,[5] additional biomarkers or diagnostic criteria are needed to facilitate differential diagnosis.

HT occurs in approximately 4.2% of unclassified hyperthyroidism cases;[7] GD is among the most common causes;[8] SAT is a lesser-known inflammatory disease.[11] The diverse characteristics of these subtypes and etiologies, along with the differences in treatment approaches, highlight the importance of differential diagnosis and diagnostic performance in achieving successful treatment and preventing disease progression. However, studies on this topic are limited, and more biomarkers and improved diagnostic performance are needed for clinical applications. Although studies on this topic are limited, they highlight the importance of these findings. Among these, Wang et al.[16] reported that SIRI and SII parameters are statistically significant and useful biomarkers in patients with autoimmune thyroiditis. In our study, the diagnostic performance of SIRI for GD was also significant. In another study, Wang et al.[19] reported that the SIRI and SII parameters have significant diagnostic performance for thyroid cancer. He et al.[20] examined the predictive value of blood parameters for SAT and reported that the NLR, PLR, MLR, SII, and SIRI indices were higher in the SAT group. In this study, however, differences in ESR and thyroid peroxidase antibody were not significant among SAT patients. In this respect, the findings are consistent with our study.

Zhai et al.[21] examined the relationship between thyroid function and SIRI and reported that inflammation may trigger the development and progression of thyroid disorders, suggesting potential diagnostic utility. However, diagnostic performance has not been directly studied; the relationship between inflammation and thyroid progression has been demonstrated by correlation. Piticchio et al.[22] analyzed the inflammatory profile in an athyreotic population with a history of hypothyroidism and reported that the median values of blood inflammatory markers may be predictive of thyroid cancer, particularly among obese individuals. Munteanu et al.[23] examined the role of indices derived from the CBC parameters in the evaluation of Hashimoto's thyroiditis in children. In their study, the NLR was reported as a diagnostic index for Hashimoto's thyroiditis in pediatric patients. Soyer et al.[24] examined the predictive value of blood parameters for SAT and GD and reported that these parameters had significant power to discriminate between the two diseases. Our study also yielded similar results, with SIRI demonstrating significant diagnostic performance for GD. Although recent studies have examined the diagnostic performance of blood parameters in hyperthyroidism, few have comprehensively evaluated their ability to discriminate among subtypes. Therefore, our results suggest that SIRI may have significant diagnostic performance for GD.

Study Limitations

The most significant limitation of the study is the limited literature on the diagnostic performance for hyperthyroidism, which reduces the number of studies available for comparison with our results. Although this places the study among pioneering work, further multicenter studies are needed in this area with larger sample sizes.

Another significant limitation of the study is its retrospective nature. Therefore, the parameters used in the study were based on data compiled from patient files that represented the majority of patients. However, our results suggest that further prospective studies, particularly in patients with GD, would be beneficial. The relatively small subgroup sizes may have limited the detection of additional predictive markers. Although

Table 1. Baseline characteristics and clinical parameters of patient groups with difference analysis results

	Hyperthyroidism		p-value
	No (n=15; 18.1%)	Yes (n=68; 81.9%)	
Age, years, mean ± SD Median (min-max)	59.67±13.49 59.00 (41.00-79.00)	56.78±13.77 59.00 (21.00-81.00)	0.463[a]
Gender, n (%)			
Female	14 (93.3)	57 (83.8)	0.313[b]
Male	1 (6.7)	11 (16.2)	
Chronic drug usage, n (%)	11 (73.3)	55 (80.9)	0.366[b]
Comorbidity, n (%)	7 (46.7)	32 (47.1)	0.603[b]
TSH, mUI/mL	1.33±0.94 0.95 (0.42-3.25)	0.12±0.10 0.11 (0.01-0.38)	0.000[c]
Free T3, ng/dL	2.91±0.42 2.94 (1.96-3.58)	3.44±1.10 3.11 (2.05-6.83)	0.189[c]
Free T4, ng/dL	1.22±0.28 1.18 (0.57-1.94)	1.61±0.66 1.48 (0.53-4.79)	0.009[c]
Anti-TPO, IU/mL	32.47±40.14 16.00 (0.68-139.00)	90.26±193.25 12.40 (0.48-986.00)	0.896[c]
Anti-TG, IU/mL	48.34±63.42 20.00 (0.90-190.00)	100.03±261.55 15.50 (0.90-1731.00)	0.896[c]
TRAb, IU/L	5.92±10.62 0.80 (0.28-31.53)	4.10±6.25 1.07 (0.10-22.80)	0.703[c]
Neutrophil, µL	4.21±0.88 4.30 (3.00-6.30)	4.35±1.47 3.90 (1.70-10.40)	0.999[c]
Lymphocyte, µL	2.27±0.66 2.10 (1.50-4.10)	2.33±0.67 2.30 (0.70-4.40)	0.420[c]
Monocyte, µL	0.47±0.12 0.50 (0.30-0.80)	0.47±0.14 0.49 (0.20-0.80)	0.885[c]
PLT, µL	255.33±55.09 252.00 (175.00-359.00)	276.57±60.49 265.00 (113.00-456.00)	0.227[c]
CRP, mg/L	3.23±3.25 1.61 (0.60-12.00)	4.83±14.73 1.96 (0.09-116.34)	0.657[c]
ESR, mm/hour	14.33±10.85 13.00 (2.00-37.00)	13.97±8.37 13.50 (2.00-44.00)	0.896[c]
NLR	1.94±0.53 1.74 (1.41-3.07)	2.07±1.06 1.80 (0.69-5.60)	0.749[c]
PLR	118.46±33.20 125.91 (66.55-170.95)	128.94±50.35 122.80 (44.32-380.00)	0.628[c]
SIRI	5.09±4.58 3.28 (1.89-20.66)	5.02±3.52 4.20 (0.86-20.80)	0.767[c]
SII	489.75±147.06 504.06 (303.95-775.10)	576.39±341.75 471.42 (163.85-1976.00)	0.705[c]

[a]Independent Samples t-test, [b]Fisher's exact test, [c]Mann-Whitney U test.
SD: Standard deviation, TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase, Anti-TG: Anti-thyroglobulin antibody, TRAb: Thyrotropin receptor antibodies, PLT: Platelet, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SIRI: Systemic inflammation response index, SII: Systemic inflammation index.

the power analysis performed on our sample of patients indicates that the sample size is sufficient, a larger sample is needed to improve the generalizability of the results. However, we were able to achieve this number at a single center within this time period. Therefore, it would be beneficial to conduct the study in a multicenter setting with a larger sample size. SAT is defined solely by CRP or ESR levels. Clinical findings (neck pain, fever), ultrasound, and RAI uptake criteria are not included. This situation may create misclassification bias. This deficiency may affect the results and must be evaluated in further research.

Table 2. Spearman's rho correlation analysis results between hyperthyroidism and baseline and clinical parameters

Hyperthyroidism	r	p
Age	-0.069	0.533
Gender	0.104	0.349
Drug usage	0.072	0.518
Comorbidity	0.003	0.978
Free T3	0.145	0.191
Free T4	0.288**	0.008
Anti-TPO	0.014	0.897
Anti-TG	-0.014	0.897
TRAb	0.043	0.706
Neutrophil	0.000	1.000
Lymphocyte	0.089	0.424
Monocyte	-0.016	0.886
PLT	0.133	0.229
CRP	-0.049	0.660
ESR	0.014	0.897
NLR	-0.035	0.751
PLR	0.054	0.630
SIRI	0.033	0.769
SII	0.042	0.707

**p<0.01.
TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase, Anti-TG: Antithyroglobulin antibody, TRAb: Thyrotropin receptor antibodies, PLT: Platelet, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SIRI: Systemic inflammation response index, SII: Systemic inflammation index.

The Study's Contribution to the Literature and Clinical Practice

The study's most significant contribution to the literature is demonstrating that blood parameters may have discriminatory diagnostic performance across hyperthyroidism subgroups, highlighting the need for further research in this area. The study results also demonstrate the potential diagnostic value of the SIRI parameter for GD.

This study demonstrates the clinical benefit of monitoring the SIRI parameter in patients with GD. Although the AUC values obtained from ROC analysis of SIRI in GD are modest, they are statistically significant. Therefore, while higher AUC values are needed to allow generalization, the results demonstrate the clinical benefit of monitoring SIRI values at the extremes in GD.

CONCLUSION

Although blood parameters lack sufficient diagnostic performance to distinguish hyperthyroidism subtypes, SIRI may have specific diagnostic performance for GD. Although SIRI has low diagnostic performance, larger sample sizes and multicenter studies would be beneficial for examining the predictive and diagnostic value of SIRI for GD. This would allow expanded control and scope of research with more cofounders and could contribute to both the literature and clinical practice of thyroid disorders, including hyperthyroidism. SIRI should be interpreted as an adjunct biomarker rather than a diagnostic test. SIRI showed a statistically significant but clinically modest association with GD.

Table 3. Spearman's rho correlation analysis results for relationship between hyperthyroidism sub groups and blood parameters

	Hashitoxicosis (n=74; 89.2%)		Graves (n=28; 33.7%)		Subacute thyroiditis (n=16; 19.3%)		Overlap (n=32; 38.6%)	
	r	p	r	p	r	p	r	p
Neutrophil	-0.001	0.994	0.078	0.485	0.154	0.165	0.101	0.362
Lymphocyte	0.002	0.988	0.171	0.122	-0.126	0.257	0.045	0.690
Monocyte	-0.156	0.158	**0.240***	0.029	-0.007	0.953	0.067	0.550
PLT	0.034	0.760	-0.104	0.351	-0.004	0.968	-0.035	0.756
CRP	-0.039	0.727	0.143	0.199	**0.574****	0.000	**0.409****	0.000
ESR	-0.087	0.436	-0.084	0.452	**0.626****	0.000	**0.246***	0.025
NLR	-0.024	0.828	-0.068	0.544	0.183	0.098	0.021	0.853
PLR	-0.102	0.359	-0.158	0.154	0.166	0.134	-0.026	0.813
SIRI	-0.063	0.571	**0.221***	0.045	-0.022	0.846	0.075	0.501
SII	-0.036	0.749	-0.035	0.756	0.194	0.079	0.078	0.483

*p<0.05, **p<0.01.
PLT: Platelet, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SIRI: Systemic inflammation response index, SII: Systemic inflammation index.

Table 4. ROC analysis results for diagnostic performance of blood parameters on hyperthyroidism subtypes

Test result variable (s)	Area	Std. error	p	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Hashitoxicosis					
NLR	0.477	0.102	0.826	0.277	0.678
PLR	0.405	0.118	0.356	0.174	0.637
SIRI	0.441	0.109	0.568	0.228	0.655
SII	0.467	0.099	0.747	0.273	0.661
Graves					
NLR	0.459	0.068	0.541	0.325	0.593
PLR	0.404	0.066	0.153	0.274	0.533
SIRI	**0.635**	**0.065**	**0.046**	**0.508**	**0.762**
SII	0.479	0.067	0.754	0.347	0.610
Subacute thyroiditis					
NLR	0.634	0.083	0.098	0.470	0.797
PLR	0.621	0.087	0.133	0.450	0.792
SIRI	0.484	0.089	0.844	0.310	0.658
SII	0.642	0.076	0.079	0.492	0.791
Overlap					
NLR	0.512	0.068	0.852	0.380	0.645
PLR	0.484	0.066	0.811	0.355	0.613
SIRI	0.544	0.066	0.498	0.416	0.673
SII	0.546	0.065	0.480	0.419	0.674

NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SIRI: Systemic inflammation response index, SII: Systemic inflammation index, ROC: Receiver operating characteristic.

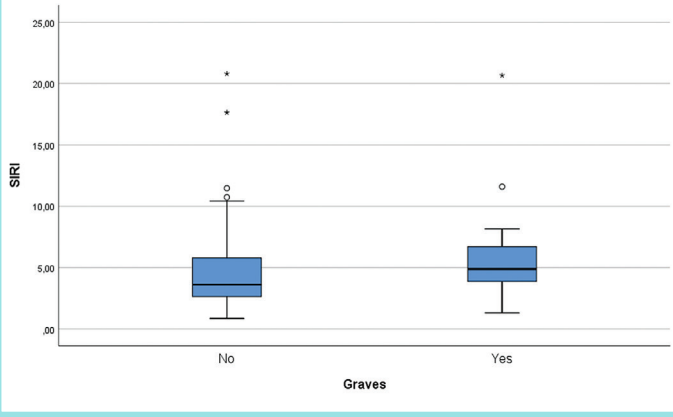

Figure 1. SIRI means and changes according to Graves' groups.
SIRI: Systemic inflammation response index.

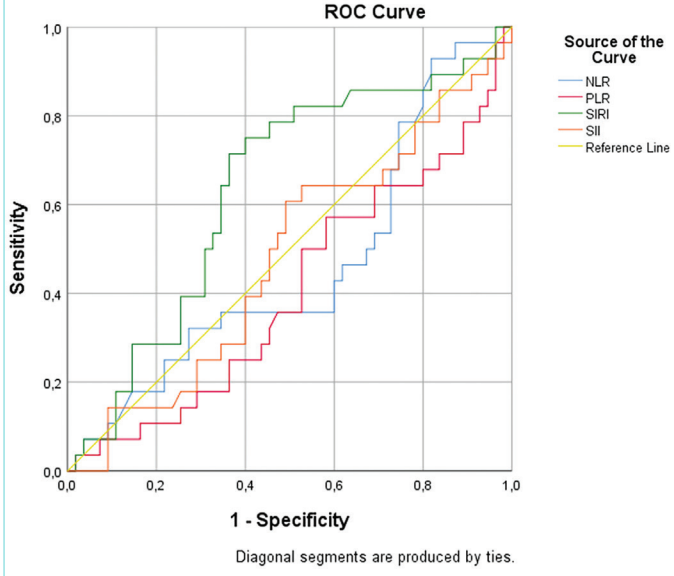

Figure 2. ROC analysis results for NLR, PLR, SIRI and SII.
ROC: Receiver operating characteristic, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SIRI: Systemic inflammation response index, SII: Systemic inflammation index.

MAIN POINTS

- Hyperthyroidism is a disease affecting between 0.2% and 1.4% of the general population worldwide.
- Biomarkers or diagnostic criteria are needed for a differential diagnosis.
- Blood parameters may have discriminatory diagnostic performance across subgroups of hyperthyroidism.
- Systemic inflammation response index may have specific diagnostic performance for Graves' disease.

ETHICS

Ethics Committee Approval: Ethical approval was obtained from Demiroğlu Science University Clinical Research Ethical Committee (approval number: 44140529/34423, date: 05.12.2023).

Informed Consent: Patient consent was not obtained because the study was retrospective.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.E., A.G., Concept: M.E., A.G., K.D., Design: M.E., Data Collection and/or Processing: M.E., A.G., Analysis and/or Interpretation: K.D., Literature Search: K.D., Writing: M.E., A.G., K.D.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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The Impact of Diagnostic Hysteroscopy on Treatment Outcomes in Patients with Recurrent IVF Failure

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Abstract

BACKGROUND/AIMS: To assess whether diagnostic hysteroscopy, performed alone or in combination with endometrial scratching, influences reproductive outcomes in women with recurrent implantation failure (RIF).

MATERIALS AND METHODS: This retrospective cohort study included women with unexplained infertility who experienced recurrent in vitro fertilization failures despite transfer of good-quality embryos. All patients had normal uterine imaging prior to enrollment. Diagnostic hysteroscopy was performed in all cases; and patients were divided into two groups according to whether endometrial scratching was also performed. Baseline demographic characteristics, stimulation parameters, embryological outcomes, and pregnancy results were compared between groups.

RESULTS: Baseline clinical and hormonal profiles were comparable between the two groups. While luteinizing hormone levels on the trigger day were modestly higher in the scratching group, other stimulation characteristics and embryological outcomes did not differ significantly ($p>0.05$). Although the scratching group had higher biochemical, clinical, and live-birth rates than the non-scratching group, the difference in overall pregnancy outcomes was not statistically significant ($p=0.172$).

CONCLUSION: In this selected cohort of women with RIF, endometrial scratching performed during diagnostic hysteroscopy did not significantly improve pregnancy or live-birth rates. These findings suggest that the benefit of endometrial scratching may be limited and highly dependent on patient selection. Larger, prospective, randomized studies that incorporate molecular markers of endometrial receptivity are needed to identify subgroups that may benefit from this intervention.

Keywords: Diagnostic hysteroscopy, endometrial scratching, recurrent implantation failure

INTRODUCTION

Infertility is a common and significant health problem in modern societies, adversely affecting couples' quality of life. Although advances in assisted reproductive technologies have provided effective treatment options for many infertile couples, cumulative pregnancy rates following in vitro fertilization/embryo transfer (IVF/ET) cycles remain approximately 30% even in the most experienced centers, highlighting the ongoing need to improve treatment success rates.[1] Moreover, recurrent IVF/ET failures are associated with increased psychological burden - including anxiety and depression, impaired social functioning, and loss of work productivity - and substantially increase healthcare expenditures, creating a considerable economic burden at the societal level.[2]

A standardized definition of recurrent implantation failure (RIF) has not yet been established in the literature. While some investigators describe RIF as the absence of pregnancy following three successive IVF/ET cycles

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with good-quality embryos, others apply a less stringent definition based on failure to achieve pregnancy after at least two consecutive IVF/ET attempts involving high-quality embryos.[3] This variability in diagnostic criteria likely contributes to the divergent and sometimes conflicting results reported among studies. Moreover, much of the existing RIF research has concentrated on identifying uterine structural abnormalities, potentially at the expense of consistent consideration of other critical factors, including embryo quality and ET strategies.

Beyond structural uterine abnormalities, increasing attention has been directed toward functional and molecular aspects of endometrial receptivity. Successful implantation may still be compromised by disrupted embryo-endometrium signaling, even when the uterine cavity appears morphologically normal and high-quality embryos are transferred. Endometrial receptivity is a complex, tightly regulated process involving hormonal signaling, immune modulation, angiogenesis, and expression of implantation-related genes and cytokines during the "window of implantation".[4] Subtle endometrial alterations that are undetectable by routine imaging modalities may therefore play a critical role in RIF.

Within this framework, diagnostic hysteroscopy provides a direct assessment of the uterine cavity and may reveal subtle abnormalities not detected by ultrasonography, whereas endometrial scratching has been suggested as a strategy to influence endometrial receptivity by inducing localized inflammatory and regenerative responses through controlled mechanical injury.[5,6] However, evidence regarding the clinical benefit of these interventions remains conflicting, particularly in patients with RIF, highlighting the need for further well-defined studies focusing on carefully selected populations. This study aimed to evaluate the effects of diagnostic hysteroscopy and endometrial scratching on treatment outcomes in patients with recurrent IVF failure.

MATERIALS AND METHODS

Ethical Permission

Ethical approval was obtained from the University of Health Sciences Türkiye, Zeynep Kamil Women and Children Diseases Training and Research Hospital Non-Interventional Research Ethics Committee (approval number: 62, date: 25.09.2024). The study was conducted in compliance with the Declaration of Helsinki and with the institutional regulations. Written consent from patients was waived due to the retrospective nature of the study.

This retrospective cohort study comprised patients aged 18-45 years who were diagnosed with unexplained infertility at the Assisted Reproduction Unit of University of Health Sciences Türkiye Zeynep Kamil Women and Children Disease Training and Research Hospital between 2019 and 2024. Patients who underwent diagnostic hysteroscopy after at least two failed IVF attempts and who had no apparent uterine anomaly on transvaginal ultrasonography were enrolled; patients with a history of systemic disease and/or use of chronic medications, and those in whom an uterine anomaly was detected during hysteroscopy, were excluded.

Demographic and clinical information, including age, duration of infertility, and baseline hormonal parameters, was retrieved from the hospital's electronic database. Data on IVF cycle characteristics and outcomes within six months following diagnostic hysteroscopy were extracted from individual patient records. Recorded variables included the type and cumulative total dose of gonadotropins, ovarian stimulation duration, peak estradiol level and final endometrial thickness, total number of oocytes, the number and proportion of mature oocytes and of fertilized oocytes, total number of embryos, the number and grade of embryos transferred, transfer strategy (fresh or frozen), and subsequent pregnancy outcomes.

In Vitro Fertilization Steps

Controlled ovarian hyperstimulation was started following transvaginal ultrasonographic evaluation of ovarian follicles at early follicular phase, using follitropin alpha and/or human menopausal gonadotropin. The starting gonadotropin dose was individualized based on patient age, hormonal profile, and antral follicle count. Once the leading follicle reached a diameter of ≥12 mm, daily administration of a 0.25-mg gonadotropin-releasing hormone (GnRH) antagonist (cetrorelix) was initiated and continued until the day of ovulation triggering. Follicular development and serum estradiol concentrations were assessed on alternate days. When at least three follicles measured ≥17-18 mm in diameter, oocytes were triggered using either 250 µg recombinant human chorionic gonadotropin (hCG) or 0.2 mg GnRH agonist (triptorelin acetate). After 35 hours, oocyte retrieval was carried out transvaginally under ultrasound guidance.

Intracytoplasmic sperm injection (ICSI) was performed after denudation by an experienced embryologist. Fertilization was assessed 18-24 hours after ICSI, and embryo quality was evaluated according to the Gardner-Schoolcraft system.[7] The freeze-all approach was chosen for patients with a continued risk of ovarian hyperstimulation syndrome, insufficient luteal phase support, unfavorable endometrial conditions, or when ET was scheduled for a subsequent cycle. For analytical consistency, only the first frozen-thawed ET cycle was included in the study.

Luteal phase support was standardized with vaginal micronized progesterone at a total daily dose of 600 mg, initiated on the day of oocyte pickup, for patients undergoing fresh embryo transfer. For frozen-thawed cycles, a hormone-replacement protocol was used for endometrial preparation. A six-mg daily dose of oral estradiol was administered; after ultrasonographic evaluation on cycle day 12, progesterone supplementation was initiated with 600 mg vaginal and 25 mg subcutaneous progesterone.

Pregnancy was initially assessed 10-12 days after ET; serum β-hCG values ≥40 IU/L were considered indicative of biochemical pregnancy. At 6-7 weeks of gestation, the presence of fetal cardiac activity on ultrasonography was considered diagnostic of clinical pregnancy.

Hysteroscopy Procedure

Hysteroscopy was performed in the luteal phase of the cycle. Under sedo-analgesia, after antiseptic preparation of the perineum and vagina with povidone-iodine, the cervix was grasped with a tenaculum and dilated using cervical dilators. Diagnostic hysteroscopy was then performed to visualize the cervical canal, endometrial cavity, and both tubal ostia using a rigid hysteroscope with a 4-mm diameter and a 30° optical system (Olympus, Japan). Normal saline (0.9%) was used as the uterine distension medium. In 19 patients, endometrial scratching was performed with 3-mm hysteroscopic scissors selected to enable controlled and targeted endometrial injury under direct hysteroscopic visualization. The intervention was applied to the posterior-fundal region of the endometrium, chosen for its close proximity to the physiological implantation site, as supported by previous studies.[8]

After the procedure, all patients were observed for at least 6 hours and discharged with prophylactic antibiotic therapy (doxycycline 100 mg, 2x1 for 5 days).

Statistical Analysis

All statistical evaluations were performed using SPSS version 23 (Statistical Package for the Social Sciences). Quantitative variables were reported as mean ± standard deviation or as median with corresponding minimum and maximum values, depending on their distribution, whereas qualitative variables were presented as counts and percentages. The distribution of continuous data was assessed using the Shapiro-Wilk test. For intergroup comparisons, the Independent Samples t-test was applied to variables that were normally distributed, while the Mann-Whitney U test was used for variables that did not meet the assumption of normality. Comparisons of categorical variables were carried out using the chi-square test or Fisher's exact test, when appropriate. A two-tailed p-value below 0.05 was considered statistically significant, and analyses were performed at the 95% confidence level.

RESULTS

Among the 200 patients initially eligible for inclusion, 87 were excluded following hysteroscopic identification of endometrial polyps or intrauterine adhesions, and a further 73 were excluded because of inadequate embryo development or cancellation of ET after hysteroscopy. Consequently, in the main analysis, 40 patients were evaluated: 19 underwent diagnostic hysteroscopy combined with endometrial scratching, while 21 underwent diagnostic hysteroscopy alone. The baseline characteristics and hormonal profiles of the groups are summarized in Table 1.

No significant differences were observed between groups in age, infertility duration, or number of previous IVF attempts (p>0.05). Baseline hormonal profiles were also largely similar. Serum hormone concentrations were comparable between patients who underwent endometrial scratching and those who did not (p>0.05). In contrast, luteinizing hormone levels were higher in the endometrial scratching group than in the diagnostic hysteroscopy-only group (6.37±4.00 vs. 3.87±1.96 mIU/mL, p=0.038).

Table 2 presents the cycle characteristics and outcomes for the groups. No statistically significant differences were identified with respect to stimulation length, cumulative gonadotropin dose, or endometrial thickness (p>0.05). Although peak estradiol concentrations were numerically higher in the endometrial scratching group (2050.89±1487.03 pg/mL vs. 1210.52±809.75 pg/mL), this difference was not statistically significant (p=0.060). Endometrial thickness was comparable between groups (9.54±2.66 vs. 9.17±2.02 mm, p=0.413).

Likewise, embryological outcomes-including total number of oocytes, number and proportion of mature (metaphase II) oocytes, number of fertilized oocytes and fertilization rate, total number of embryos generated, number of embryos transferred, and embryo grade at transfer-did not differ significantly between the two groups (p>0.05).

As shown in Table 3, overall pregnancy outcomes were similar between the groups (p=0.172). Higher rates of biochemical pregnancy (36.85% vs. 23.81%), clinical pregnancy (36.85% vs. 14.28%), and live birth (31.57% vs. 14.28%) were observed in the endometrial scratching group compared with the non-scratching group.

DISCUSSION

Diagnostic hysteroscopy and endometrial scratching were evaluated in a strictly defined RIF population characterized by homogeneous baseline characteristics, including age, ovarian reserve parameters, oocyte maturity, and embryo quality. By limiting inclusion to patients without sonographically detectable uterine pathology and with comparable embryological parameters, we aimed to reduce confounding factors and better isolate the potential effect of endometrial intervention itself. Our findings suggest that although hysteroscopy and endometrial scratching are biologically plausible strategies, their clinical impact may be modest when applied to an already-selected cohort of patients with RIF. This observation aligns with growing evidence that RIF is a multifactorial condition in which endometrial receptivity, embryo competence, immunologic balance, and molecular signaling pathways interact in complex ways, making it unlikely that a single intervention can improve outcomes in all patients.[9,10]

Table 1. Baseline characteristics and hormonal profiles of the groups

Variables	Dx H/S + scratching n=19 Mean ± SD (min-max)	Dx H/S n=21 Mean ± SD (min-max)	p*
Age (years)	33.47±5.25 (25-42)	34.14±5.1 (26-45)	0.685
Infertility duration (months)	47.95±27.32 (19-108)	60.71±39.36 (18-168)	0.237
Number of IVF attempts	3.26±0.56 (3-5)	3.48±0.87 (3-6)	0.509
FSH (mIU/mL)	7.62±4.04 (2.77-21.4)	6.7±1.68 (4-9.8)	0.560
LH (mIU/mL)	6.37±4 (2-16.1)	3.87±1.96 (0.1-8.7)	0.038
E2 (pg/mL)	46.02±15.92 (22-82)	48.47±29.35 (14-148)	0.675
PRL (ng/mL)	22.77±12.15 (1.5-57.1)	20.69±12.42 (5-54.8)	0.350
TSH (mIU/L)	2.48±1.04 (0.68-4.3)	2.25±1.13 (0.7-5.4)	0.521
AMH (ng/mL)	2.59±2.29 (0.23-8.2)	3.16±3.83 (0.2-17.1)	0.860

*p<0.05.
Dx H/S: Diagnostic hysteroscopy, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, PRL: Prolactine, TSH: Tyroid-stimulating hormone, AMH: Anti-Müllerian hormone, SD: Standard deviation, min-max: Minimum-maximum.

Table 2. Cycle characteristics and outcomes of the groups

	Dx H/S + scratch Mean ± SD (min-max)	Dx H/S Mean ± SD (min-max)	p*
Duration of stimulation (days)	9.32±2.11 (6-13)	9.95±2.27 (7-15)	0.449
Total gonadotropin dose (IU)	2005.26±1139.71 (1050-5850)	2498.1±1363.36 (1200-6750)	0.095
E2 on trigger day (pg/mL)	2050.89±1487.03 (428-5178)	1210.52±809.75 (276-3000)	0.06
ET on trigger day (mm)	9.54±2.66 (4.5-13.7)	9.17±2.02 (4-14.5)	0.413
Total oocyte number	11±7.23 (1-27)	8.86±6.14 (1-25)	0.283
MII oocyte number	7.74±5.43 (1-24)	6.43±4.63 (1-19)	0.384
MII oocyte rate (%)	73.6±19.31 (50-100)	74.68±19.78 (33-100)	0.615
Fertilized oocyte number	5.74±3.6 (1-14)	4.48±3.53 (1-14)	0.129
Fertilization rate (%)	80.27±19.33 (44.44-100)	69.24±27.65 (1-100)	0.248
Number of obtained embryo	3.68±1.8 (1-7)	3±1.82 (1-8)	0.155
Transferred embryo number	1.42±0.51 (1-2)	1.52±0.6 (1-3)	0.641
Transferred embryo grade	1.30±0.47 (1-2)	1.26±0.45 (1-2)	0.746

*p<0.05.
Dx H/S: Diagnostic hysteroscopy, E2: Estradiol, ET: Endometrial thickness, MII: Metaphase II, SD: Standard deviation, min-max: Minimum-maximum.

Table 3. Pregnancy outcomes of the groups

	Dx H/S + scratch n=19		Dx H/S n=21		
	n	%	n	%	p*
No pregnancy	12	63.15	16	76.19	0.172
Biochemical pregnancy	7	36.85	5	23.81	
Clinical pregnancy	7	36.85	3	14.28	
Live birth	6	31.57	3	14.28	
Total	19	100.00	21	100.00	

*p<0.05.
Dx H/S: Diagnostic hysteroscopy.

An increasing number of studies have examined whether diagnostic hysteroscopy and endometrial injury can enhance reproductive outcomes in patients diagnosed with RIF. Demirol and Gurgan[11] reported a significant increase in pregnancy rates when hysteroscopy was performed prior to IVF-ICSI, even among patients with normal hysterosalpingography findings, suggesting that undetected intrauterine abnormalities or hysteroscopy-related endometrial disruption may improve implantation. Consistent findings were later reported by Tomaževič et al.[1] and Kilic et al.,[12] who observed improved pregnancy and live-birth outcomes, particularly when hysteroscopy was performed before the first IVF-ICSI attempt. The more favorable results reported in these studies, compared with the present findings, may be explained by differences in patient selection, because those cohorts largely consisted of treatment-naïve individuals rather than women with established RIF.

The therapeutic relevance of endometrial scratching remains a matter of debate, as findings reported in the literature have been inconsistent. Although current studies have proposed that localized endometrial injury may facilitate implantation by triggering inflammatory pathways and increasing the expression of cytokines and growth factors,[5,6] other investigations have not demonstrated a meaningful improvement in reproductive outcomes.[13] Notably, the multicenter, randomized SCRaTCH trial did not report an increase in live birth rates associated with endometrial scratching; however, only a limited subset of the study population met the criteria for RIF.[14] In addition, Baum et al.[15] reported no apparent benefit from the procedure, though the conclusions were constrained by the small sample size of their cohort.

Conversely, investigations specifically targeting women with RIF have yielded more encouraging results. Seval et al.[16] reported significantly increased biochemical and clinical pregnancy rates following endometrial scratching in patients with RIF. Similarly, Demirol and Gurgan[11] observed a marked improvement in reproductive outcomes after hysteroscopic endometrial injury in a clearly defined RIF cohort, despite comparable stimulation protocols and embryo quality between the intervention and control groups. These observations lend support to the concept that endometrial injury may confer benefit predominantly in carefully selected patients with RIF. Nevertheless, more recent systematic reviews and meta-analyses indicate that routine hysteroscopy prior to IVF does not uniformly enhance live birth rates in women without suspected intrauterine pathology, including those with prior implantation failure.[17,18] Variability in study design, patient selection, timing of hysteroscopy, and the inclusion of treatment-naïve versus RIF populations may contribute to the inconsistent results reported across studies.

Several biological pathways have been hypothesized to explain the benefit of endometrial injury, including the initiation of a localized inflammatory cascade, increased release of cytokines and growth factors, activation of implantation-associated genes such as HOXA10 and HOXA11, stimulation of endometrial regeneration, and enhancement of uterine microcirculation. Collectively, these processes may facilitate improved endometrial receptivity and more effective embryo-endometrium communication.[19]

The mechanistic rationale for endometrial scratching is largely grounded in the concept that controlled mechanical disruption of the endometrium provokes a transient inflammatory response. This response is characterized by immune cell recruitment, upregulation of cytokines, chemokines, and growth factors, and increased expression of implantation-related markers, including HOXA10, HOXA11, leukemia inhibitory factor, and integrins.[20,21] Such molecular and cellular alterations are thought to enhance endometrial-embryonic synchrony and support decidualization. However, accumulating evidence indicates

that these effects are not uniform across all patient populations. In women with an otherwise normal endometrial environment, injury-induced inflammation may offer limited benefit or potentially disrupt endometrial homeostasis. In contrast, patients with RIF may harbor subtle defects in endometrial receptivity or remodeling that render them more responsive to targeted endometrial interventions.[22,23] This biological and clinical heterogeneity likely underlies the inconsistent outcomes reported in clinical studies and meta-analyses, underscoring the critical role of appropriate patient selection when considering endometrial scratching or diagnostic hysteroscopy as adjunctive therapeutic strategies.

Study Limitations

The principal strength of this study lies in the inclusion of a highly selected patient population, limited to women with normal uterine imaging and who developed good-quality embryos, thereby reducing potential confounding related to underlying uterine pathology or embryonic competence. Consequently, our results align with evidence suggesting that any potential benefit of endometrial injury may be more evident in carefully defined populations with RIF.

Nonetheless, it should be acknowledged that the relatively modest sample size may have limited the statistical power of the study and increased the risk of a type II error, which may partly explain the absence of statistically significant differences in some reproductive outcomes. Additional limitations include loss to follow-up, exclusion of patients who required operative hysteroscopy despite normal preprocedural imaging, and cycle cancellations due to inadequate embryo development.

CONCLUSION

In conclusion, this study evaluated the impact of diagnostic hysteroscopy with or without endometrial scratching in a well-defined cohort of women with RIF who exhibited comparable baseline clinical characteristics and embryological outcomes. Although pregnancy rates were numerically higher in the endometrial scratching group, these differences did not reach statistical significance, likely due to the limited sample size resulting from strict inclusion criteria designed to ensure cohort homogeneity. The interventions were found to be safe and biologically plausible; however, their clinical benefit could not be conclusively demonstrated within this underpowered, highly selected population.

Given the heterogeneous and multifactorial nature of implantation failure and endometrial receptivity, larger, adequately powered, prospective, randomized studies that focus on carefully phenotyped RIF populations are warranted. Incorporation of molecular and immunological markers of endometrial function may help identify subgroups of patients who could benefit from hysteroscopic evaluation or endometrial injury, thereby supporting more personalized treatment strategies for patients with recurrent IVF failure.

MAIN POINTS

- In a selected cohort of women with recurrent implantation failure and normal uterine imaging, endometrial scratching performed during diagnostic hysteroscopy did not result in a statistically significant improvement in pregnancy or live birth rates.
- Although biochemical pregnancy, clinical pregnancy, and live birth rates were numerically higher in the endometrial scratching group, these differences did not reach statistical significance.
- No significant differences were observed between the groups with respect to cycle characteristics, embryological parameters, or embryo quality.
- While diagnostic hysteroscopy and endometrial scratching are biologically plausible and safe interventions, their clinical benefit appears to be highly dependent on careful patient selection.
- Larger, adequately powered prospective randomized studies incorporating molecular and immunological markers of endometrial receptivity are needed to identify patient subgroups that may benefit from endometrial scratching.

ETHICS

Ethics Committee Approval: Ethical approval was obtained from the University of Health Sciences Türkiye, Zeynep Kamil Women and Children Diseases Training and Research Hospital Non-Interventional Research Ethics Committee (approval number: 62, date: 25.09.2024).

Informed Consent: Written consent from patients was waived due to the retrospective nature of the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.B.Y., Concept: G.A., M.B.Y., Design: G.A., M.B.Y., Data Collection and/or Processing: G.A., Analysis and/or Interpretation: M.B.Y., Literature Search: G.A., Writing: G.A., M.B.Y.

DISCLOSURES

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RESEARCH ARTICLE

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Anthropometric Characteristics of Turkish Cypriots and the Association Between Obesity and Comorbidities: A Cross-Sectional Study

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Abstract

BACKGROUND/AIMS: Obesity has become a serious public health problem in several countries, including North Cyprus. The aim of this study was to determine the anthropometric measurements of Turkish Cypriots and evaluate the association between obesity and comorbidities.

MATERIALS AND METHODS: Blood pressure, weight, height, and waist circumference (WC) measurements were obtained by the same physician during home visits to the participants. Socio-demographic characteristics, smoking and alcohol consumption habits, physical activity levels, dietary patterns, and previously diagnosed conditions, including type 2 diabetes, dyslipidemia, coronary heart disease (CHD), and hypertension (HT), were assessed.

RESULTS: The mean body mass index (BMI) was 26.69±5.65 kg/m² in females and 28.32±4.71 kg/m² in males. Among participants aged 18-39, the mean BMI was 25.05±6.19 kg/m² in females and 27.63±4.62 kg/m² in males. The overall prevalence of obesity was 27.4%, with rates of 24.9% among females and 30.1% among males. The prevalence of excess weight was 64.3% overall, occurring in 55.3% of females and 74.4% of males. Abdominal obesity was present in 47.4% of participants, affecting 45.5% of females and 49.5% of males. An increase in both the prevalence and the number of cases of HT, dyslipidemia, CHD, and type 2 diabetes mellitus was observed in parallel with increases in BMI and WC.

CONCLUSION: The prevalence of both general and abdominal obesity is high in North Cyprus. As BMI and WC increased, both the prevalence of comorbidities and their number increased.

Keywords: Body mass index, waist circumference, obesity, comorbidities

INTRODUCTION

Obesity is a chronic disease characterized by excessive accumulation of adipose tissue, which can reduce quality of life and is associated with comorbidities such as type 2 diabetes mellitus (T2DM), dyslipidemia, cardiovascular disease (CVD), hypertension (HT), and several types of cancer.[1,2] The prevalence of excess weight varies significantly across countries due to differences in lifestyle and dietary patterns. Factors such as the type and amount of food consumed, beverage consumption, sleep habits, alcohol consumption, medication use, level of physical activity, living conditions, and genetic predisposition are associated with excess weight. According to the World Health Organisation (WHO), adults are classified as overweight when body mass index (BMI) is 25.0-29.9 kg/m², and as obese when BMI is equal to or greater than 30.0 kg/m².[3]

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The prevalence of obesity and obesity-related diseases is increasing worldwide.[4] According to the WHO, one-third of children in Europe are obese or overweight. In the European region, the prevalence of obesity was 23% and that of excess weight was 59%.[5] Projections indicate that by 2030, the prevalence of adult obesity in the United States will reach 48.9%, while severe obesity is expected to rise to 24.2%.[6] Worldwide, adolescent obesity has quadrupled since 1990. Being overweight in childhood and adolescence is associated with an increased risk and earlier onset of some chronic diseases, such as T2DM, dyslipidemia, and HT.[2]

The risk of cardiometabolic multimorbidity increases progressively with rising BMI, ranging from ranging from approximately a twofold increase among participants who are overweight to over tenfold risk among those with severe obesity, compared to participants with a healthy BMI.[7] Participants who developed obesity before the age of 50 had a higher risk of complex multimorbidity compared with those who became obese at older ages.[4] Maintaining a stable, healthy weight and achieving weight loss in early adulthood and midlife are important for preserving quality of life during the aging process. Compared with participants who maintained a healthy weight, those with persistent obesity exhibited the highest risk of developing obesity-related diseases later in life.[8] This study aimed to assess the anthropometric measurements of Turkish Cypriots and to examine the associations between obesity and HT, T2DM, dyslipidemia, and coronary heart disease (CHD).

MATERIALS AND METHODS

Participants and Study Design

This cross-sectional study was conducted between January 21, 2025, and March 25, 2025. Ethical approval for this research was obtained from the Cyprus Science University Ethics Committee (approval number: 2025/01.012, date: 21.01.2025). Participants in this study were Turkish Cypriots residing in North Cyprus, aged 18-83 years. Participants were randomly selected from six towns (n=339) and 27 villages (n=311), taking into account the towns' population distribution. All participants were verbally informed by the physician about the aims and procedures of the study, and verbal informed consent was obtained. Participants who were pregnant or lactating, or who had a malignancy, severe cardiac failure, severe hepatic failure, or severe renal insufficiency, were excluded from the study during data collection. Participants were asked ten survey questions regarding their age, gender, dietary habits, alcohol consumption and its frequency, smoking status, engagement in physical exercise and its frequency, as well as whether they had been diagnosed with T2DM, HT, dyslipidemia, or CHD.

Antropometric Measurements

The waist circumference (WC) was measured using a non-elastic measuring tape at the midpoint between the last palpable rib and the iliac crest. The participants' height was measured without shoes using a stadiometer, and their weight was measured without shoes while wearing light clothing using a calibrated Omron BF511 weighing scale. Participants' weight, WC, and height were measured by the physician, and BMI was subsequently calculated based on these measurements.

Blood Pressure Measurement

After at least five minutes of seated rest, each participant's blood pressure (BP) was measured twice by a physician using a calibrated automated Omron sphygmomanometer, and the mean of the two readings was used as the recorded BP value.

Definitions

All participants were classified according to WC and BMI. Based on WC, optimal WC was defined as <80 cm for females and <94 cm for males; suboptimal WC was defined as 80-87 cm for females and 94-101 cm for males; abdominal (central) obesity was defined as WC ≥88 cm for females and ≥102 cm for males. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Adults were classified as underweight if their BMI was under 18.5 kg/m^2, healthy (normal) weight if their BMI ranged from 18.5 to 24.99 kg/m^2, overweight (but not obese) if their BMI ranged from 25.0 to 29.99 kg/m^2 and obese if their BMI was equal to or greater than 30.0 kg/m^2. Excess weight was defined as a BMI equal to or greater than 25.0 kg/m^2.[3] HT was defined as a systolic BP (SBP) equal to or exceeding 140 mmHg, a diastolic BP (DBP) equal to or exceeding 90 mmHg, or the presence of both.[9]

Statistical Analysis

Categorical data were summarized as frequencies (n) and percentages (%), while continuous variables were expressed as mean ± standard deviation, along with minimum and maximum values. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. For normally distributed data, the Independent Samples t-test was used to compare two groups, whereas one-way analysis of variance was applied for comparisons involving more than two groups. When significant differences were detected, post-hoc Tukey's tests were performed to identify the source of the differences. Statistical significance was set at $p<0.05$. All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Within the study population, 53.1% (n=345) of participants were female and 46.9% (n=305) were male. In terms of age distribution, 34.6% (n=225) were aged 18-39 years, 36.5% (n=237) were aged 40-59 years, and 28.9% (n=188) were aged 60-83 years. Among female participants, 33.6% were aged 18-39, 35.4% were aged 40-59, and 31.0% were aged 60-83, whereas among male participants, 35.7% were aged 18-39, 37.7% were aged 40-59, and 26.6% were aged 60-83. The mean age of all participants was 48.14±16.13 years, with mean ages of 47.95±16.44 years for males and 48.31±15.87 years for females. The association between age group and gender was not statistically significant ($p>0.05$; Table 1).

65.7% of the participants had a diet primarily based on animal products. This rate was 59.7% among females and 72.5% among males. Consumption of animal-based diets was significantly more prevalent among males than among females ($p<0.05$). 69.8% of the participants were non-smokers. 35.7% of male participants and 25.2% of female participants were smokers. When stratified by gender, the prevalence of non-smokers was significantly higher than that of current smokers ($p=0.002$). 49.4% of the participants did not consume alcohol. 65.8% of females and 30.8% of males did not consume alcohol. 6.1% of females and 21.3% of males consumed at least two shots (70 mL) of alcohol on at least two days per week. The frequency of alcohol consumption among females decreased gradually, and females consumed statistically significantly less alcohol than males ($p<0.05$). 71.7% of participants, 72.4% of males, and 71.0% of females reported no physical exercise. 29.0% of females and 27.6% of males were engaged in physical activity. There was a statistically significant relationship between physical activity level and gender ($p<0.05$) (Table 2).

A healthy weight was observed in 35.7% of the participants, while 36.9% were classified as overweight and 27.4% as obese. With increasing age, the prevalence of healthy weight decreased, whereas the prevalence of overweight and obesity increased significantly ($p<0.05$). Specifically, the prevalence of overweight was 31.6% amoung participants aged 18-39 years, 36.7% among those aged 40-59 years, and 41.5% among those aged 60-83 years. Obesity prevalence was 22.2%, 24.9%, and 36.7% in the respective age groups.

Overall, 64.3% of participants were classified as having excess weight. Among females, the prevalences of obesity, overweight, and excess weight were 24.9%, 30.4%, and 55.3%, respectively. Among males, the corresponding prevalences were 30.1% for obesity, 44.3% for overweight, and 74.4% for excess weight. Male participants had a significantly higher BMI than female participants ($p<0.05$).

The overall prevalence of abdominal obesity was 47.4%. Age-specific prevalence rates were 29.3% in the 18-39 age group, 48.1% in the 40-59 age group, and 68.1% in the 60-83 age group. A significant inverse association was observed between age and optimal WC, and abdominal obesity increased significantly with advancing age ($p<0.05$). In contrast, no statistically significant association was found between WC and gender ($p>0.05$). The prevalence of abdominal obesity was 49.5% among males and 45.5% among females (Table 3).

The mean height of female participants was 159.80±6.45 cm. When participants were stratified by age group, mean heights were 162.04±6.11 cm (18-39 years), 161.30±5.86 cm (40-59 years), and 155.66±5.50 cm (60-83 years). The mean height of male participants was 174.35±7.79 cm. Corresponding mean heights were 176.65±6.94 cm in the 18-39 age group, 174.59±7.50 cm in the 40-59 age group, and 170.93±8.13 cm in the 60-79 age group. A statistically significant difference in height was observed between genders: males were taller than females ($p<0.05$). The difference between age groups was statistically significant ($p<0.05$). Post-hoc analyses indicated significant differences in height between the 18-39 and 40-59 age groups, as well as between the 40-59 and 60-83 age groups. Height decreased progressively with age ($p<0.05$ for all comparisons). The mean body weight of female participants was 68.05±14.71 kg, whereas that of male participants was 85.91±15.45 kg. Male participants had significantly higher values than female participants ($p<0.05$). Mean BMI and WC increased with age in both female and male participants. The overall mean BMI among female participants was 26.69±5.65 kg/m². When stratified by age group, mean BMI values were 25.05±6.19 kg/m² for those aged 18-39

Table 1. Demographic characteristics of participants

Age groups	Female (n=345, 53.1%)		Male (n=305, 46.9%)		Total		Statistics
	n	%	n	%	n	%	χ^2; p
18-39	116	33.6	109	35.7	225	34.6	1.565; 0.457[1]
40-59	122	35.4	115	37.7	237	36.5	
60-83	107	31.0	81	26.6	188	28.9	
Total	345	110.0	305	100.0	650	100.0	
	Mean ± SD	**Min-max**	**Mean ± SD**	**Min-max**	**Mean ± SD**	**Min-max**	
Age	48.31±15.87	18-82	47.95±16.44	18-83	48.14±16.13	18-83	

[1]Pearson's chi-square test (χ^2).
SD: Standard deviation, Min-max: Minumum-maximum.

Table 2. Lifestyle of participants

		Female (n=345, 53.1%)	Male (n=305, 46.9%)	Total	Statistics
		n (%)	n (%)	n (%)	χ^2; p
Food consumption	**Plant-based diet**	139 (40.3)	84 (27.5)	223 (34.3)	**0.000**[2]*
	Animal-based diet	206 (59.7)	221 (72.5)	427 (65.7)	
Smoking habits	**Current smoker**	87 (25.2)	109 (35.7)	196 (30.2)	**0.002**[2]*
	Non-smoker	258 (74.8)	196 (64.3)	454 (69.8)	
Alcohol consumption	**Never**	227 (65.8)	94 (30.8)	321 (49.4)	85.360; **0.000**[1]*
	Once a week or less	97 (28.1)	146 (47.9)	243 (37.4)	
	More than 2 shots (70 mL) at least 2 days a week	21 (6.1)	65 (21.3)	86 (13.2)	
Physical activity	**Walking, jogging, swimming or cycling for 30-45 minutes 2-3 days a week**	55 (16.0)	21 (6.9)	76 (11.7)	17.050; **0.000**[1]*
	30-45 minutes of brisk walking, running, swimming, or cycling at least 4-5 days a week	45 (13.0)	63 (20.7)	108 (16.6)	
	Not doing any physical activity	245 (71.0)	221 (72.4)	466 (71.7)	
	Total	345 (100.0)	305 (100.0)	65 (100.0)	

[1]Pearson's chi-square test (χ^2), [2]Fisher's exact test; *p<0.05.

years, 26.45±5.43 kg/m² for those aged 40-59 years, and 28.84±4.66 kg/m² for those aged 60-83 years. The overall mean BMI among male participants was 28.32±4.71 kg/m². Corresponding mean BMI values were 27.63±4.62 kg/m² in the 18-39 age group, 28.59±4.88 kg/m² in the 40-59 age group, and 28.74±4.39 kg/m² in the 60-83 age group. A statistically significant difference in mean BMI was observed between genders ($p<0.05$). Mean BMI was lower in females than in males. In addition, a statistically significant difference in BMI was observed across age groups ($p<0.05$). Post-hoc analysis indicated that mean BMI differed significantly between the 18-39 and 40-59 age groups, between the 18-39 and 60-83 age groups, and between the 40-59 and 60-83 age groups ($p<0.05$ for all comparisons). The mean WC among female participants was 86.73±13.84 cm. When stratified by age group, the mean WC values were 80.05±14.62 cm for those aged 18-39 years, 86.89±11.80 cm for those aged 40-59 years, and 93.79±11.49 cm for those aged 60-83 years. The mean WC among male participants was 102.03±12.38 cm. Corresponding mean WC values were 97.55±12.49 cm in the 18-39 age group, 103.53±11.71 cm in the 40-59 age group, and 105.95±11.40 cm in the 60-83 age group. Mean WC differed significantly between sexes and across age groups ($p<0.05$), with males exhibiting higher WC than females. In addition, mean WC increased significantly with advancing age (Table 4).

HT was identified in 15.5% of participants with a healthy weight compared with 59.6% of those with obesity. Dyslipidemia was present in 11.2% of participants with a healthy weight and 41.0% of those with obesity. T2DM was observed in 5.6% of participants with a healthy weight and 20.2% of those with obesity, while CHD was identified in 3.4% of participants with a healthy weight and 7.9% of those with obesity. HT was detected in 13.0% of participants with optimal WC in 56.2% of those with abdominal obesity. Dyslipidemia was found in 8.7% of participants with optimal WC and 39.3% of those with abdominal obesity. T2DM was present in 3.8% of participants with optimal WC and 19.2% of those with abdominal obesity. CHD was observed in 1.9% of participants with optimal WC and in 6.8% of those with abdominal obesity (Table 5).

In the study, the mean BMI of participants without any of the following conditions (HT, dyslipidemia, CHD, or T2DM) was 25.63±4.57 kg/m². The mean BMI increased progressively with the number of coexisting conditions: 29.42±5.60 kg/m² in participants with one condition, 29.75±4.92 kg/m² in participants with two conditions, and 30.29±4.78 kg/m² in participants with three or more conditions. A statistically significant difference in mean BMI was observed across these groups ($p<0.05$). Post-hoc analyses showed that participants with no comorbidities differed significantly from those with one, two, or three comorbidities ($p<0.05$ for all comparisons), whereas no statistically significant differences were observed among those with one to three comorbidities. Among female participants, the mean BMI was 24.64±4.83 kg/m² in those without comorbidities, 29.07±5.90 kg/m² in those with one comorbidity, 29.15±5.10 kg/m² in those with two comorbidities, and 30.82±4.84 kg/m² in those with three comorbidities. Mean BMI increased progressively with comorbidity burden, and these differences were statistically significant ($p<0.05$). Post-hoc comparisons indicated that females without comorbidities had significantly lower

Table 3. Antropometric characherictics of the participants according to their age groups and gender

		BMI (kg/m²)			Total
		Healthy weight	Overweight	General obesity	
		n (%)	n (%)	n (%)	n (%)
Age groups	18-39	104 (46.2)	71 (31.6)	50 (22.2)	225 (100.0)
	40-59	87 (36.7)	91 (38.4)	59 (24.9)	237 (100.0)
	60-83	41 (21.8)	78 (41.5)	69 (36.7)	188 (100.0)
	Total	232 (35.7)	240 (36.9)	178 (27.4)	650 (100.0)
Statistics χ^2; p		28.854; **0.000**[1]*			
Gender	Female	154 (44.6)	105 (30.4)	86 (24.9)	345 (100.0)
	Male	78 (25.6)	135 (44.3)	92 (30.1)	305 (100.0)
	Total	232 (35.7)	240 (36.9)	178 (27.4)	650 (100.0)
Statistics χ^2; p		26.488; **0.000**[1]*			
		WC (cm)			Total
		Optimal	Suboptimal	Abdominal obesity	
		n (%)	n (%)	n (%)	n (%)
Age groups	18-39	116 (51.6)	43 (19.1)	66 (29.3)	225 (100.0)
	40-59	66 (27.8)	57 (24.1)	114 (48.1)	237 (100.0)
	60-83	26 (13.8)	34 (18.1)	128 (68.1)	188 (100.0)
	Total	208 (32)	134 (20.6)	308 (47.4)	650 (100.0)
Statistics χ^2; p		82.249; **0.000**[1]*			
Gender	Female	124 (35.9)	64 (18.6)	157 (45.5)	345 (100.0)
	Male	84 (27.5)	70 (23.0)	151 (49.5)	305 (100.0)
	Total	208 (32)	134 (20.6)	308 (47.4)	650 (100.0)
Statistics χ^2; p		5.638; 0.060[1]			

[1]Pearson's chi-square test (χ^2), [2]Fisher's exact test; *p<0.05.
BMI: Body mass index, WC: Waist circumference.

mean BMI values than those with one, two, or three comorbidities ($p<0.05$ for all comparisons). Among male participants, the mean BMI was 26.86±3.90 kg/m² in those without any comorbidities, 29.79±5.29 kg/m² in those with one comorbidity, 30.47±4.68 kg/m² in those with two comorbidities, and 29.85±4.75 kg/m² in those with three or more comorbidities. Differences among the three groups were statistically significant ($p<0.05$). According to the results of the post-hoc analysis, participants without comorbidities had significantly lower mean BMI than those with one, two, or three comorbidities ($p<0.05$). Overall, an increase in mean BMI was associated with a greater number of comorbidities in both female and male participants, and this relationship was statistically significant ($p<0.05$) (Table 6).

In the study, the mean WC of participants without any of the following comorbidities: HT, dyslipidemia, CHD, or T2DM, was 88.14±13.97 cm. Among participants with one of these conditions, the mean WC was 99.70±14.73 cm; among those with two comorbidities, it was 100.94±12.68 cm; and among participants with at least three comorbidities, the mean WC increased to 103.95±11.64 cm. The differences in mean WC across these groups were statistically significant ($p<0.05$). Post-hoc comparisons indicated that the mean WC of participants without comorbidities was significantly lower than that of participants with one, two, or three comorbidities ($p<0.05$ for all comparisons). Among female participants, the mean WC was 81.03±12.31 cm in those without comorbidities, 92.83±12.92 cm in those with one comorbidity, 94.65±11.39 cm in those with two comorbidities, and 98.16±9.49 cm in those with three comorbidities. A progressive increase in mean WC was observed with increasing comorbidity burden; these differences were statistically significant

Table 4. Mean height, mean weight, mean BMI and mean WC of the participants according to their age groups, and gender

	Gender	Age groups								Statistics F; p[1]
		18-39		40-59		60-83		Total		
		Mean ± SD	Min-max	Mean ± SD	Min-max	Mean ± SD	Min-max	Mean ± SD	Min-Max	
Height (cm)	Female	162.04±6.11	148-179	161.30±5.86	144-178	155.66±5.50	143-169	159.80±6.45	143-179	
	Male	176.65±6.94	157-192	174.59±7.50	145-192	170.93±8.13	148-187	174.35±7.79	145-192	
	Total	169.12±9.79[a]	148-192	167.75±9.43[a]	144-192	162.24±10.15[b]	143-187	166.63±10.16	143-192	**0.000[1]***
Statistics p[2]								**0.000[2]***		
Weight (kg)	Female	66.07±17.90	39.5-148	68.30±13.66	44.5-109	69.90±11.58	45-106	68.05±14.71	39.5-148	
	Male	85.98±14.96	55.5-127	87.24±16.28	61-160	83.92±14.88	56-132	85.91±15.45	55.5-160	
	Total	75.72±19.28	39.5-148	77.49±17.71	44.5-160	75.94±14.80	45-132	76.43±17.50	39.5-160	0.500[1]
Statistics p[2]								**0.000[2]***		
BMI (kg/m²)	Female	25.05±6.19	15.2-50	26.45±5.43	16-42.5	28.84±4.66	18-43	26.69±5.65	15.2-50	
	Male	27.63±4.62	18.2-40.3	28.59±4.88	21-52	28.74±4.39	18.7-43.7	28.32±4.71	18.2-52	
	Total	26.30±5.63[a]	15.2-50	27.49±5.27[b]	16-52	28.80±4.53[c]	18-43.7	27.45±5.29	15.2-52	**0.000[1]***
Statistics p[2]								**0.000[2]***		
WC (cm)	Female	80.05±14.62	50-132	86.89±11.80	66-123	93.79±11.49	62-123	86.73±13.84	50-132	
	Male	97.55±12.49	72-132	103.53±11.71	84-150	105.95±11.40	83-136	102.03±12.38	72-150	
	Total	88.53±16.18[a]	50-132	94.96±14.39[b]	66-150	99.03±12.92[c]	62-136	93.91±15.22	50-150	**0.000[1]***
Statistics p[2]								**0.000[2]***		

[1]One-way ANOVA [Tukey's multiple comparisons test, (mean ± SD)], [2]Independent Samples t test; *p<0.05.
Groups labeled with the same letter (a-c) were not significantly different from one another.
p[1]: Difference among three groups in age groups.
p[2]: Difference between two groups in gender.
BMI: Body mass index, WC: Waist circumference, SD: Standard deviation, Min-max: Minumum-maximum, ANOVA: Analysis of variance.

Table 5. Prevalence of HT, dislipidemia, CHD, and T2DM

		HT		Dyslipidemia		T2DM		CHD	
		n	%	n	%	%	%	n	%
BMI (kg/m²)	Healthy weight	36	15.5	26	11.2	13	5.6	8	3.4
	Overweight	90	37.5	69	28.8	30	12.5	7	2.9
	Obesity	106	59.6	73	41.0	36	20.2	14	7.9
	Total	232	35.7	168	25.8	79	12.2	29	4.5
WC (cm)	Optimal WC	27	13.0	18	8.7	8	3.8	4	1.9
	Sub optimal WC	32	23.9	29	21.6	12	9.0	4	3.0
	Abdominal obesity	173	56.2	121	39.3	59	19.2	21	6.8
	Total	232	35.7	168	25.8	79	12.2	29	4.5

HT: Hypertension, CHD: Coronary heart disease, T2DM: Type 2 diabetes mellitus, BMI: Body mass index, WC: Waist circumference.

Table 6. Association between comorbidity and mean BMI and WC by gender

		Mean BMI (kg/m²)					
		Gender					
		Female		Male		Total	
		Mean ± SD	Min-max	Mean ± SD	Min-max	Mean ± SD	Min-max
Comorbidity	No	24.64±4.83[a]	15.2-44	26.86±3.90[a]	18.2-40.3	25.63±4.57[a]	15.20-44
	1 comorbidity	29.07±5.90[b]	17-50	29.79±5.29[b]	18.7-52	29.42±5.60[b]	17-52
	2 comorbidities	29.15±5.10[b]	17.5-40	30.47±4.68[b]	23.1-40.5	29.75±4.92[b]	17.5-40.5
	At least 3 comorbidities	30.82±4.84[b]	21.2-43	29.85±4.75[b]	23-43.7	30.29±4.78[b]	21.2-43.7
	Total	26.69±5.65	15.2-50	28.32±4.71	18.2-52	27.45±5.29	15.2-52
Statistics	F; p	**25.651; 0.000**[1]*		**12.434; 0.000**[1]*		**11.510; 0.000**[1]*	
		Mean WC (cm)					
		Gender					
		Female		Male		Total	
		Mean ± SD	Min-max	Mean ± SD	Min-max	Mean ± SD	Min-max
Comorbidity	No	81.03±12.31[a]	50-123	96.90±10.51[a]	72-130	88.14±13.97[a]	50-130
	1 comorbidity	92.83±12.92[b]	62-132	107.04±12.94[b]	83-150	99.70±14.73[b]	62-150
	2 comorbidities	94.65±11.39[b]	66-120	108.35±9.88[b]	92-136	100.94±12.68[b]	66-136
	At least 3 comorbidities	98.16±9.49[b]	78-116	108.77±11.16[b]	89-132	103.95±11.64[b]	78-132
	Total	86.73±13.84	50-132	102.03±12.38	72-150	93.91±15.22	50-150
Statistics	F; p	**35.584; 0.000**[1]*		**24.326; 0.000**[1]*		**48.088; 0.000**[1]*	

[1]One-way ANOVA [Tukey's multiple comparisons test, (mean ± SD)]; *p<0.05.
Groups labeled with the same letter (a-b) were not significantly different from one another.
BMI: Body mass index, WC: Waist circumference, SD: Standard deviation, ANOVA: Analysis of variance.

(p<0.05). Post-hoc analyses further demonstrated that females without comorbidities had significantly lower mean WC than those with one, two, or three comorbidities (p<0.05 for all comparisons). Among male participants, the mean WC was 96.90±10.51 cm in those without comorbidities, 107.04±12.94 cm in those with one comorbidity, 108.35±9.88 cm in those with two comorbidities, and 108.77±11.16 cm in those with three comorbidities. The differences among the groups were statistically significant (p<0.05). Post-hoc comparisons showed that males without comorbidities had significantly lower mean WC values compared with those with one or more comorbidities (p<0.05 for all comparisons; Table 6).

DISCUSSION

In many countries, height and weight data are based on self-reported measurements. It is well established that self-reported height tends to be overestimated, whereas self-reported weight is often underestimated compared with objectively measured values.[10] In the present study, participants' height, weight, and WC were measured by the same physician using a standardized scale and a measuring tape to ensure consistency and minimize measurement bias. The mean height of female participants was 159.8 cm, while the mean height of the 18-39-year age group was 162 cm. According to data from Worlddata.info, mean female height among individuals aged 18-25 varies between countries. Cross-national differences in height have been well documented and are influenced by genetic, nutritional, and environmental factors. Detailed country-specific height data are publicly available, and are therefore not presented in this study.[11]

In our study, the mean height of male participants was 174.3 cm, whereas the mean height among those aged 18-39 years was 176.6 cm. According to data from Worlddata.info for the 18-25 age group, the average male height varies considerably across countries. These international differences in male stature have been widely reported in the literature and are influenced by genetic, nutritional, and environmental factors. Therefore, detailed country-specific height data are not presented in this study but are referenced elsewhere.[11]

In our study, the mean BMI of females residing in Northern Cyprus was 26.6 kg/m². Among those aged 18-39 years, the mean BMI was 25.0 kg/m². In Southern Cyprus, the closest neighboring region, the reported mean BMI for females was 26 kg/m².[12] According to data from Worlddata.info, mean BMI values among females aged 18-25 years show substantial variation across countries. These cross-national differences in BMI have been well documented and are influenced by lifestyle, socio-economic, and environmental factors. Therefore, detailed country-specific BMI data are not presented in this study; instead, they are provided in the cited references.[11]

In our study, mean BMI was 28.3 kg/m² among males and 27.6 kg/m² among those aged 18-39 years. In Southern Cyprus, the closest neighboring region, the reported mean BMI for males was 27.7 kg/m².[12] According to data from Worlddata.info, mean BMI values among males aged 18-25 years vary widely across countries. These international differences in BMI have been widely reported and are influenced by lifestyle, nutritional, and socio-economic factors. Therefore, detailed country-specific BMI data are not presented in this study; instead, they are referenced.[11]

According to the WHO obesity report 2022, approximately 16% of adults aged 18 years and older were classified as obese worldwide. However, obesity prevalence varies substantially across regions, with higher rates reported particularly in the Eastern Mediterranean and American regions. In our study, the overall prevalence of obesity was 27.4%, which is considerably higher than the global average reported by the WHO. This finding is consistent with data from countries in the same geographical and cultural region, including South Cyprus, where the prevalence of obesity has been reported as 23.6%. These results suggest that North Cyprus follows a regional pattern of elevated obesity prevalence, underscoring the growing public health burden of obesity.[13]

In our study, the prevalence of obesity was 30.1% in males and 24.9% in females. In our country, this difference may be partly attributed to higher alcohol consumption and a more animal-based diet among males than among females. When evaluated by gender, obesity prevalence varies across countries, with higher rates observed among males in some countries and among females in others. For example, higher obesity prevalence among males has been reported in countries such as Spain, Germany, Slovakia, Poland, Greece, Croatia, Hungary, Malta, and Cyprus. In contrast, higher prevalence among females has been documented in countries, including Nigeria, Ukraine, Iran, Türkiye, Libya, Iraq, Saudi Arabia, Egypt, Brazil, Kenya, Indonesia, and Ethiopia.[13] In our study, the overall prevalence of excess weight was 64.3%, with a prevalence of 55.3% amoung females and 74.4% amoung males. According to the WHO obesity report 2022, the prevalence of excess weight in the European region was 59%, affecting 54% of females and 63% of males.[5]

The prevalence of overweight and obesity increased with age. According to the WHO obesity report 2022, excess weight in the European Union was least prevalent among participants aged 16-24 years (20.3%), while the highest prevalence was observed among those aged 65-74 years (63.6%).[5] In a French study, the prevalence of obesity and excess weight increased progressively across age groups, with obesity and excess weight reported as 9.2% and 23.2%, respectively, in the 18-24 age group; 13.8% and 35.2% in the 25-34 age group; 16.7% and 44.0% in the 35-44 age group; 18.4% and 50.7% in the 45-54 age group; 19.2% and 57.2% in the 55-64 age group; and 19.9% and 57.3% among participants aged 65 years and older.[14] Similarly, in our study, both obesity and excess weight increased with age: in the 18-39 age group, the prevalence of obesity was 22.2% and excess weight was 53.8%; in the 40-59 age group, obesity prevalence was 24.9% and excess weight was 63.3%; and in the 60-79 age group, obesity was 36.7% and excess weight was 78.2%.

Bodyweight is influenced by poor nutritional choices, overeating, genetic predisposition, cultural factors, and metabolism. Obesity prevalence varies considerably across countries due to differences in lifestyle and dietary patterns. Factors such as the type and quantity of food consumed, alcohol and sugary-sweetened beverage intake, physical activity level, genetics, sleep habits, medication use, and living environment are all associated with excess body weight. In our study, 72.5% of male participants and 59.7% of female participants predominantly consumed an animal-based diet. Alcohol consumption and obesity are among the leading risk factors for chronic liver disease and liver cancer,[15] whereas tobacco use is a major lifestyle-related risk factor for premature mortality.[8] In this study, 21.3% of male participants and 6.1% of female participants reported consuming at least two servings of alcohol on at least two days per week. Additionally, 35.7% of males and 25.2% of females were identified as smokers. Physical activity is recommended for both the prevention and treatment of obesity and is associated with a reduced risk of CVD, cancer, and all-cause mortality. Moderate to high levels of physical activity are associated with a 21% lower risk of all-cause mortality and a 24% lower risk of CVD-related mortality among adults with obesity.[16] In our study, 72.4% of male and 71.0% of female participants reported not engaging in any form of physical exercise.

Observational studies have shown that obesity is associated with a reduction of 3 to 8 years in disease-free life expectancy.[17] Excess body weight is associated with an increased risk of cardiometabolic diseases (CMDs), and this association cannot be fully explained by shared genetic factors or early-life environmental influences. However, positive lifestyle habits may attenuate the risk of CMDs associated with elevated BMI.[18] Even in the absence of current obesity, a history of excess body weight has been associated with an increased risk of CMDs, with SBP and triglyceride levels mediating this relationship. Maintaining a stable and healthy body weight and achieving weight reduction during early adulthood and midlife play a crucial role in promoting better quality of life as individuals age. Compared with participants who remained at a healthy weight, those who were persistently obese exhibited the highest risk of developing obesity-related diseases in later life. Moreover, transitions between non-obese and obese states, in either direction, were associated with an increased risk of long-term health disorders.[8]

The risk of T2DM increases with rising body weight. A study involving more than 21,000 adult participants from the National Health and Nutrition Examination Survey (NHANES) reported that the prevalence of T2DM increased from 8% among participants with normal weight to 43% among those with morbid obesity.[19] In our study, T2DM was observed in 5.6% of participants with a healthy weight and in 20.2% of those with obesity. Additionally, T2DM was present in 3.8% of participants with optimal WC, compared with 9.0% and 19.2% among those with abdominal obesity.

Findings from the Framingham Heart Study indicated that obesity accounted for 78% of cases of primary HT in males and 65% in females.[20] Across adult populations, an approximately linear relationship has been observed between BMI and BP, and weight loss has been shown to reduce BP in most participants with HT.[21] A weight reduction of 5.1 kg has been associated with decreases of 4.44 mmHg in SBP and 3.57 mmHg in DBP.[22] Conversely, weight gain increases the risk of primary HT; an increase of 1.7 kg/m^2 in BMI has been associated with a 1-mmHg rise in SBP.[23] In our study, HT was identified in 15.5% of participants with a healthy weight, 37.5% of those who were overweight, and 59.6% of those with obesity.

Obesity adversely affects lipid metabolism. It is associated with increased levels of low-density lipoprotein (LDL) cholesterol, very-LDL cholesterol, triglycerides, and decreased levels of high-density lipoprotein cholesterol.[24] In our study, dyslipidemia was identified in 11.2% of participants with a healthy weight and in 41.0% of those with obesity. Obese participants have a 1.6-fold higher risk of developing CHD than those of healthy weight.[25] In a study conducted in South Korea, Choi et al.[26] reported that a reduction in BMI was associated with decreased risk of CHD among young adults. Additionally, a 4 kg/m^2 increase in BMI has been shown to correspond to a 26% increase in the odds of CHD.[27] Data from the NHANES, encompassing mortality records for 2.3 million American adults, demonstrated that obesity was significantly associated with increased risk of death from CHD and

other cardiovascular conditions.[28] Although BMI may influence CHD risk indirectly through intermediate factors such as HT, dyslipidemia, and T2DM, current evidence indicates that obesity is an independent risk factor for CHD.[27] In our study, CHD was observed in 3.4% of participants with a healthy weight and in 7.9% of those with obesity. Furthermore, overweight and obesity are associated with substantial reductions in life expectancy. Increasing BMI is associated with a progressively higher risk of coexistence of multiple cardiometabolic conditions, ranging from approximately a twofold increase among overweight participants to more than a tenfold increase among those with severe obesity, relative to participants with normal BMI.[7]

In our study, increases in both BMI and WC were linked with a higher prevalence of, and a greater number of, obesity-related comorbidities. The prevalence of HT, T2DM, CHD, and dyslipidemia was higher among participants with excess body weight than among those with healthy weight. The mean BMI was 25.66 kg/m² among participants without HT, T2DM, CHD, or dyslipidemia; 29.38 kg/m² among those with one comorbidity; 29.77 kg/m² among those with two comorbidities; and 30.2 kg/m² among those with three or more comorbidities. These findings are consistent with and support previously published studies.[29-31]

Study Limitations

In our study, diagnoses of dyslipidemia, CHD, and T2DM were assessed based on participants' self-reported information, whereas HT was assessed separately. In addition, weight status was determined from measurements obtained at assessment; therefore, participants who had previously been obese but were of healthy weight at that assessment were classified in the healthy-weight group. Consequently, some participants in the healthy-weight group who were diagnosed with obesity-related diseases may previously have been overweight.

CONCLUSION

This study demonstrated a high prevalence of both general and abdominal obesity among adults in North Cyprus. Approximately one in two adults (47.4%) had abdominal obesity, and nearly one in four (27.4%) had general obesity. While the associations between age, gender, and BMI are well established in the literature, our findings highlight the strong relationship of increasing BMI and WC with both the prevalence and the number of obesity-related comorbidities. These results emphasize that excess weight substantially increases the risk of obesity-related health conditions, including CVD, while weight reduction may reduce the risk of cardiometabolic disorders and all-cause mortality.

MAIN POINTS

- The prevalence of obesity was 27.4% (24.9% in females and 30.1% in males).
- The prevalence of excess weight was 64.3% (55.3% among females and 74.4% among males).
- The prevalence of abdominal obesity was 47.4% (45.5% in females and 49.5% in males).
- Higher body mass index and waist circumference values were associated with increased prevalence of type 2 diabetes mellitus, coronary heart disease, hypertension, and dyslipidemia.

ETHICS

Ethics Committee Approval: Ethical approval for this research was obtained from the Cyprus Science University Ethics Committee (approval number: 2025/01.012, date: 21.01.2025).

Informed Consent: Patient consent was obtained.

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Footnotes

Authorship Contributions

Concept: E.B., Design: E.B., Data Collection and/or Processing: E.B., G.Ö., Analysis and/or Interpretation: E.B., S.A., G.Ö., Literature Search: E.B., S.A., Writing: E.B., S.A., G.Ö.

DISCLOSURES

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RESEARCH ARTICLE

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Histological, Biochemical and Genetic Investigation of Liver Aging in Rats

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Abstract

BACKGROUND/AIMS: Aging is a chronic, complex and irreversible process that leads to functional deterioration in cells. Structural and functional changes occur in the liver during aging. This study aimed to determine age-related changes in rat liver tissue using histological, biochemical, and gene-expression analyses.

MATERIALS AND METHODS: Wistar albino male rats were used in the study; two groups were formed: a young group (10 weeks) and an aged group (18 months). Blood and tissue samples were collected from the subjects while they were under general anesthesia. Liver enzymes were measured in blood samples. Gene expression levels of interleukin-6 (IL-6), nuclear factor kappa B (NF-kB), mammalian target of rapamycin (mTOR) protein complex, and caspase-3 (Cas-3) in liver tissues were evaluated by real-time polymerase chain reaction following total ribonucleic acid isolation and complementary DNA synthesis. Histological scoring was performed on tissues from the groups. Tissues were evaluated with hematoxylin-eosin, Masson's trichrome, periodic acid-Schiff, and Ki-67 proliferation index stains.

RESULTS: Biochemically, significant increases in cholesterol, triglycerides, alanine transaminase, and alkaline phosphatase were observed in the aged group. Light microscopic examination revealed an increased number of Ki-67-positive and binucleated hepatocytes, vacuolization, sinusoidal congestion, bile-duct proliferation, and increased collagen in the aged group. While *Cas-3* and *mTOR* gene expression increased significantly in the aged group, no differences were observed in IL-6 and NF-kB levels.

CONCLUSION: Histological, apoptotic, and autophagic changes have been observed during physiological aging, and they are known to influence liver function.

Keywords: Aging, liver, Cas-3, mTOR, IL-6, NF-kB

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INTRODUCTION

Cellular senescence is a stress response that links degenerative and hyperplastic pathologies. Aging occurs in diverse cell types throughout the body, and these cells secrete numerous biologically active factors that cause physiological and pathological effects.[1-4] It is an important risk factor for the development of both acute and chronic liver disease, causing changes in all hepatic cell types, altering their phenotype and function, and worsening disease prognosis. Accordingly, increasing attention has been directed toward developing new therapeutic strategies to prevent or ameliorate age-related hepatic disorders.[5] The aging liver shows many structural and functional changes: its size decreases by approximately 20-40%; endothelial fenestration decreases; and hepatic blood flow and bile acid secretion by hepatocytes decrease. Decreased numbers of smooth endoplasmic reticulum and mitochondria in hepatocytes, together with high triglyceride levels, trigger the formation of non-alcoholic fatty liver disease.

There is an age-related increase in hepatocyte size and the appearance of polyploid (binucleated) cells. In humans, polyploid cells constitute 6%-15% of hepatocytes in adults aged 20 years, and 25%-42% of hepatocytes in adults aged 80 years. In this context, polyploidy is considered a sign of cellular aging and stress.[6]

Reduced autophagic activity in hepatocytes increases lipid accumulation and promotes hepatic steatosis.[7] Therefore, activating and regulating autophagy may be a promising strategy to prevent age-related hepatic steatosis by reducing lipid accumulation.[7,8] Other factors observed in liver aging include apoptosis due to oxidative stress, increased caspase activity, decreased antioxidant capacity, and mitochondrial dysfunction.[9,10] Activation of a significant number of inflammatory cells (neutrophils and macrophages) and increased inflammatory cytokine levels lead to inflammation and liver fibrosis.[11,12]

The aim of the present study was to determine the differences in liver tissue at the histological, biochemical, and molecular levels between young and aged rats. Simultaneously, determination of apoptotic caspase-3 (Cas-3), proliferation-associated Ki-67, autophagy-associated mammalian target of rapamycin (mTOR), and inflammation-associated interleukin-6 (IL-6) and *nuclear factor kappa B (NF-kB)* gene expression will be important in defining biological activities involved in the development of age-related changes in the liver.

MATERIALS AND METHODS

In this research, a total of 12 male Albino Wistar rats in total with an average weight of 235-740 gr were provided from Experimental Animals Research Center. The protocols are based on animal experiments approved by the Near East University Experimental Animals and Research Centre Ethics Committee of Research and Application Center (approval number: 2024/175, date: 18.04.2024).

All rats were housed in conventional cages and maintained under the same environmental and nutritional conditions (22±10 °C), with ad libitum access to water and food. Regarding age, rats were divided into two groups: a young group (n=6) consisting of 10-week-old rats, and an aged group (n=6) consisting of 18-month-old rats.

The body weights of the rats were recorded at the beginning and end of the experiment. Ketamine/xylazine anesthesia was administered; intracardiac blood was collected; the liver was removed and weighed. All liver tissue samples were taken from the right lobe.

Histological Analyses

Liver tissues were fixed in 10% neutral formaldehyde solution for histological preparations. Tissues dehydrated in a graded alcohol series were cleared and embedded in paraffin. Thin sections (4-5 µm) were prepared from paraffin blocks and stained with hematoxylin-eosin (Merck, USA), Masson's trichrome (Bio-Optica, Milan, Italy), and periodic acid-Schiff (PAS) (Bio-Optica, Milan, Italy) for microscopic examination. Ki-67 immunohistochemical staining was performed to evaluate proliferation in hepatocytes. Histological scoring of liver tissue from each rat in each group was performed for inflammation, necrosis, fibrosis, and bile duct proliferation in five areas at ×40 objective magnification. Changes were evaluated as 0 (no change), 1 (mild change, <25%), 2 (moderate change, 25-50%), and 3 (severe change, >50%).[13] Similarly, binucleated hepatocytes and Ki-67-positive hepatocytes were counted in 10 fields per preparation.

Ki-67 Immunohistochemical Analysis

Formaldehyde-fixed tissue was embedded in paraffin for immunohistochemical examination. Sections were deparaffinized in xylene and passed through a descending series of alcohols. Antigen retrieval was performed in citrate buffer (pH 6.0) in a microwave oven at 700 W for 15 minutes. Sections were allowed to cool to room temperature for 30 minutes and were washed twice in phosphate-buffered saline (PBS) for 5 minutes. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 7 minutes. The washed samples were incubated in Ultra V Block for 8 minutes. Blocking solution was removed from the sections, which were then incubated overnight at 4 °C with the primary antibody. Ki-67 was evaluated using Rabbit Polyclonal Antibody, Ready-to-Use (Cat. #RB-9043-R7, Thermo Scientific, USA). After washing the sections in PBS, the secondary antibody was applied for 20 minutes. The sections were washed in PBS twice for 5 minutes each and then incubated with streptavidin-peroxidase for 20 minutes. Sections washed with PBS were incubated with the 3,3'-diaminobenzidine chromogen. Counterstaining with hematoxylin was applied, and after washing, the preparations were mounted.

Biochemical Analyses

The enzyme activities of aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) as well as the levels of cholesterol and triglycerides were measured enzymatically on the Abbott Architect c400 system (Abbott Laboratories, IL, USA).

Genetic Analysis

Liver tissues taken for messenger RNA (mRNA) expression analysis of selected genes were stored in dry Eppendorf tubes at -20 °C until nucleic acid isolation. Trizol reagent (Hibrizol, Hibrigen, İstanbul, Türkiye) was used for ribonucleic acid (RNA) isolation. For each sample, 500 ng of RNA was used to synthesize complementary DNA (cDNA) using the ABM first-strand cDNA synthesis kit, according to the manufacturer's instructions. Reference mRNA sequences for the rat *NF-kB*, *IL-6*, *Casp3*, and *mTOR* genes, and for the housekeeping gene *glyceraldehyde 3-phosphate dehydrogenase (GAPDH)*, obtained from the National Center for Biotechnology Information Nucleotide Database, were used to design gene-specific primers. After primer optimization, the expression levels of NF-kB, IL-6, Casp3, mTOR, and GAPDH were evaluated using AMPIGENE® qPCR Green Mix Lo-ROX (ENZO Life Sciences Inc., Lausen, Switzerland) and gene-specific primers on the *Rotor*-Gene Q Plex real-time polymerase chain reaction system (Qiagen, Hilden, Germany). cDNA was diluted tenfold for use as template, with each sample

analysed in triplicate. Normalization of Ct values was carried out using the reference gene, and the comparative ΔΔCt method was applied to evaluate relative gene expression changes between the two groups.

Statistical Analysis

The results were statistically evaluated using the GraphPad Prism 8.3.1 program. Differences among the groups were analyzed using one-way ANOVA. A significance threshold of $p \leq 0.05$ was used, and standard errors were reported as ± (value not specified).

RESULTS

Body and Liver Weights

The change in body weight differed significantly between the aged and young groups, with the 18-month-old group having more than twice the body weight of the 10-week-old group ($p<0.05$). No significant difference was observed in liver weights between young and old groups.

Biochemical Results

Changes in lipid profile and liver enzyme levels, which are indicators of metabolic and physiological alterations that occur with aging, were observed between the two groups. In the elderly group, cholesterol ($p<0.001$), triglycerides ($p<0.05$), and the liver enzymes ALT ($p<0.01$) and ALP ($p<0.05$) were significantly higher than in the young group. Changes in AST enzyme levels, on the other hand, were not statistically significant (Figure 1).

Histological Results

Age-related changes in the frequency of binucleated hepatocytes were observed in the study groups (Figures 2a and 2b). Binucleated hepatocytes were counted at 40x magnification. While the number was 4.967±2.178 in the 10-week group, it increased to 8.433±3.734 in the 18-month group. This increase was statistically significant ($p<0.0001$) (Figure 2c). The number of Ki-67-positive hepatocytes was higher in the young group than in the aged group ($p<0.01$) (Figures 3a-c).

In histological scoring, a significant increase in congestion, vacuolization, fibrosis, and bile duct proliferation was observed in the aged group compared with the young group, whereas mononuclear cell levels did not differ significantly between groups (Figure 4). In young rats, portal areas, hepatic cords, sinusoids, and Kupffer cell distribution showed normal histological structure (Figure 5a). Increased vacuolization was detected in hepatocytes of the aged group and attributed to lipid accumulation (Figure 5b). While bile duct proliferation occurred in small numbers in the portal areas of the young group (Figure 5c), the aged group showed widespread proliferation and an increase in mononuclear cells in those areas (Figure 5d).

Compared with the young group (Figure 6a), the amount of collagen around the portal area and vena centralis was increased in the aged group (Figure 6b); prominent collagen strands were observed around the sinusoids (Figure 6c); and fibrotic bridging between the portal areas was detected (Figure 6d). PAS staining for glycogen indicated that the staining intensity in hepatocytes of the elderly group was low or absent because of vacuolization (data not shown).

Gene Expression Results

Changes in mRNA expression levels of the genes IL-6, mTOR, Cas-3, and NF-kB were examined in the liver tissue of young and old rats (Figure 7). IL-6 and NF-kB expression levels increased with age; however, no statistically significant differences were observed between the two groups. On the other hand, mTOR levels have been shown to increase significantly with age.

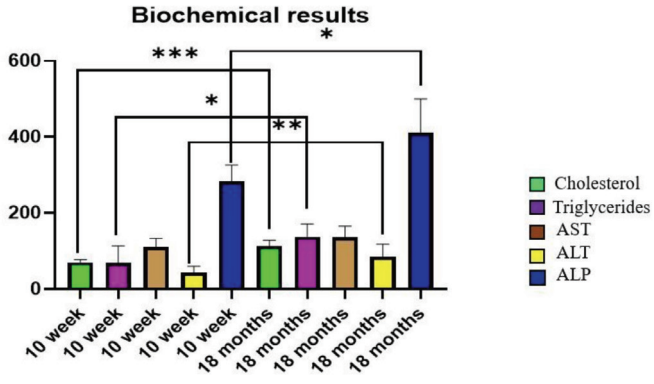

Figure 1. Comparison of biochemical enzymes in groups. *($p<0.05$), **($p<0.01$) and ***($p<0.001$).

AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase.

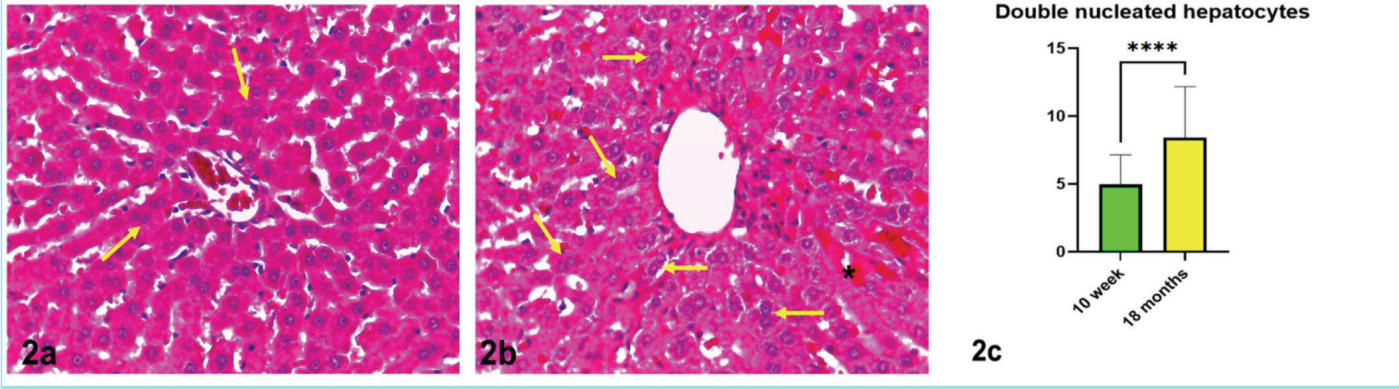

Figure 2. Binucleated hepatocytes (arrows) are present in both the young (2a) and aged (2b) groups; the statistical graph is shown in (2c, **** $p<0.0001$), irregularity in hepatocyte cell cords and congestion in sinusoids (*) are observed in the aged group. Hematoxylin-eosin, x40.

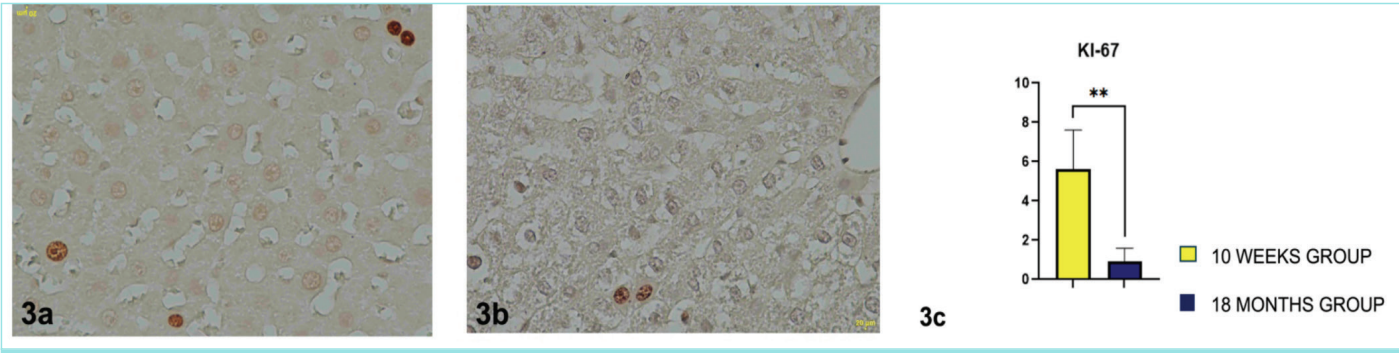

Figure 3. Ki-67 immunohistochemical staining in hepatocytes in young (3a) and aged groups (3b) and its statistical distribution (3c), x40. **(p<0.01).

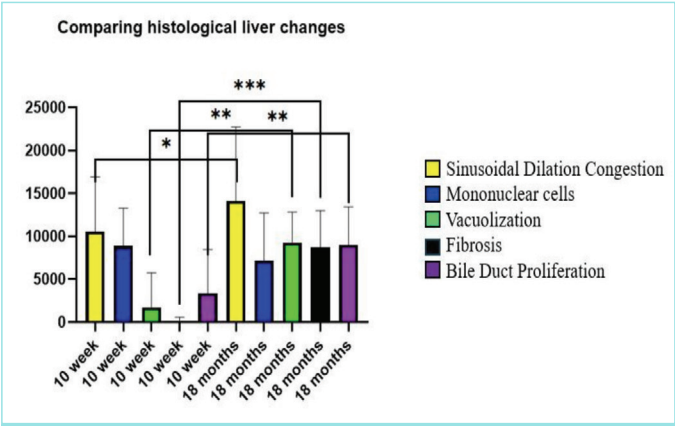

Figure 4. Quantitative histological scoring values in rats of different age groups.*(p<0.05), **(p<0.01) and ***(p<0.001).

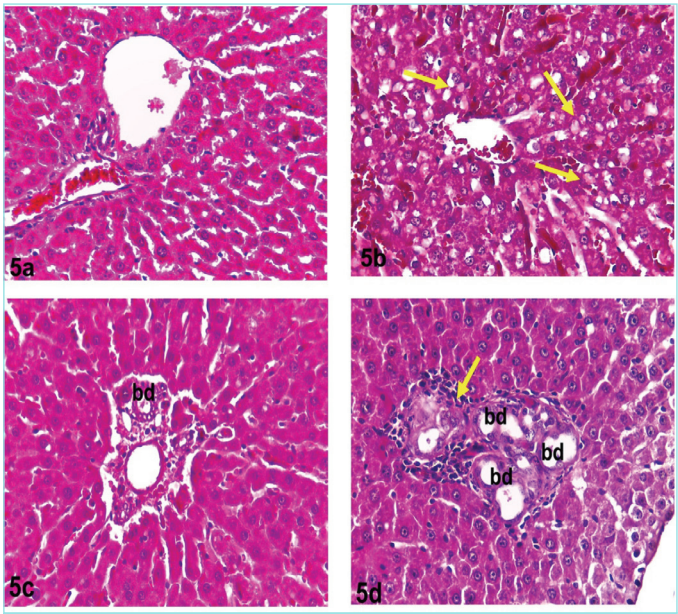

Figure 5. Light microscopic images of young and aged rat liver tissue. While normally structured hepatocytes, sinusoids, the portal area are observed in the livers of young rats (5a), widespread vacuolization (arrow) is observed in hepatocytes of the aged group (5b). Bile duct (bd) proliferation is markedly increased in the aged group compared with the young group. Mononuclear cells (arrow) (5c, 5d). Hematoxylin-eosin, x40.

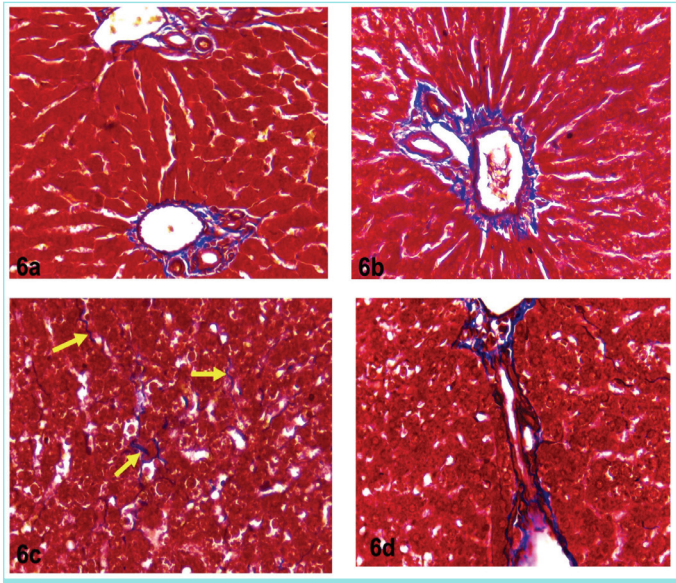

Figure 6. In contrast to the normal distribution of collagen fibers within the portal areas in the young group (6a), the aged group exhibits a significant increase in collagen in the portal areas (6b) and around the sinusoids (arrows) (6c), together with the formation of connective tissue bridges between the portal areas (6d). Masson trichrome stain, x40.

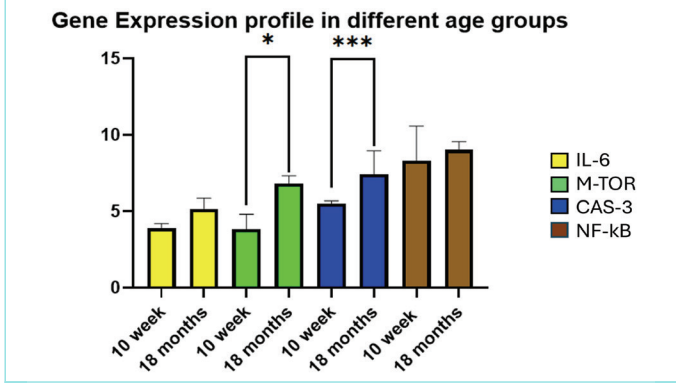

Figure 7. Gene expression levels in groups. *(p<0.05), and ***(p<0.001).

IL-6: Interleukin-6, NF-kB: Nuclear factor kappa B, mTOR: Mammalian target of rapamycin, Cas-3: Caspase-3.

In the older group, mTOR levels were nearly twofold higher than in the 10-week group ($p<0.05$). This result demonstrates the potential role of mTOR in regulating autophagy and aging processes. An age-related increase in Cas-3 expression was also observed. It was significantly increased in the 18-month group ($p<0.001$). These results further emphasize the role of Cas-3 in the aging process and reflect an increase in apoptotic activity.

DISCUSSION

This study examined changes in histological features and in the expression of biochemical and molecular markers associated with aging in rat liver tissue. Aging generally leads to decreased liver size and weight, and to alterations in biochemical enzyme activity. Although subtle signs of cellular aging were observed in the liver tissues of young adult rats, these changes were offset by the liver's regenerative capacity. In aged rats, both senescence and decreased regenerative capacity result in a prolonged or absent regenerative process.[14,15] Elevated AST and ALT levels have been reported in older adults in previous studies.[16,17]

Although a decrease in liver weight was observed in the aged group compared with the young group in this study, the difference was not statistically significant. Biochemically, increases in ALP and ALT levels were significant, but not in AST levels. Significant increases in cholesterol and triglyceride levels were also observed in the aged group.

It is known that aging hepatocytes generally lose some of their regenerative capacity.[6] Extracellular matrix components are altered, leading to fibrosis.[18] This results in impaired blood flow and altered hepatocyte function. The distribution and function of nonparenchymal cell populations, such as liver Kupffer cells and stellate cells, change during the aging process.[15,19] When Kupffer cells are insufficient to clear pathogens and debris, activated stellate cells cause fibrosis. This study identified fibrosis in the elderly group, with increased collagen fibers in the portal areas and around the sinusoids and central vein.

Another change that can occur in the liver with age is bile duct proliferation.[20] Increased bile duct proliferation in portal areas was assessed for its association with increased ALP.

Furthermore, aging causes increases in inflammatory molecules such as IL-6 and NF-kB, and alterations in immune cell function, ultimately leading to increased susceptibility to infections and diseases.[21,22] Although expression levels of IL-6 and NF-kB were increased in the elderly group, these differences were not statistically significant. Light microscopic examination confirmed this finding; inflammatory cell populations were not widespread.

Polyploidy increases in aged hepatocytes. This change appears to be a compensatory mechanism to cope with the decline in cell division capacity in aging tissues.[23] In our study, the number of binucleated hepatocytes was significantly higher in the aged group than in the younger group. Furthermore, a significantly greater number of hepatocyte nuclei stained positive for Ki-67 in the aged group. Increased hepatocyte proliferation and polyploidy are adaptive mechanisms that help maintain liver function despite age-related decline.

Cas-3-controlled apoptosis in the liver is important for cellular homeostasis and prevents the proliferation of harmful cells that lead to liver disease. During aging, some degenerating hepatocytes undergo apoptosis.[9,10,24] Studies have shown an increase in Cas-3 expression and activity during aging. The increased *Cas-3* gene expression in the liver of our elderly experimental group is consistent with this information.

Autophagy, on the other hand, is a cellular defense mechanism that removes and recycles damaged organelles and proteins, maintaining homeostasis and cellular function. Fibrosis, along with increased steatosis and inflammation, has been associated with impaired hepatic autophagy.[25] Therefore, autophagy is considered a strategy for restoring age-related hepatic changes and for highlighting the complex relationships between aging, dietary habits, and environmental factors in liver histology.[8] Autophagy is active in young rats and underlies the high functional activity and cellular resilience of the liver.[26] Autophagic activity decreases with aging. This decrease is associated with the onset of mild cellular dysfunction, as the liver begins to show signs of accumulation of oxidative stress and of lipid peroxidation products.[27] The autophagic process is regulated by mTOR kinase.[28] The mTOR signaling pathway is a key genetic factor in liver aging and plays a crucial role in cellular growth and metabolism. Therefore, inhibition of mTOR potently extends lifespan and significantly delays signs of pathological aging in rat livers. This inhibition leads to reduced fibrosis and improved cellular function.[29-31] This study found increased *mTOR* gene expression in liver tissue in the elderly group, in contrast to other studies. These results demonstrate that during aging, cells continue to attempt to slow the aging process and protect themselves.

Study Limitations

Disadvantages of the studyincluded a small number of subjects in the groups, the inability to define fibrosis morphometrically and biochemically, and the inability to perform immunohistochemical staining of genetically expressed molecules in the tissue.

CONCLUSION

Liver aging is a complex process, and some events remain unexplained. Changes in liver structure and function among individuals of the same age, and between women and men, vary depending on factors such as living conditions, nutrition, and genetic background. The differences between the results obtained in our study - such as high mTOR levels, increased Ki-67 related to mitotic changes, low AST enzyme levels, no significant change in liver weight, and absence of inflammation - and those reported in other studies can be explained by individual differences. The mechanisms and molecules that contribute to the aging process require more detailed investigation. Delaying orpreventing age-related functional disorders and chronic diseases will be the primary focus of the studies.

MAIN POINTS

- Aging caused significant increases in cholesterol, triglyceride, alanine transaminase, and alkaline phosphatase levels, indicating altered liver metabolism.
- The aged group showed vacuolization, sinusoidal congestion, bile duct proliferation, collagen accumulation, and more Ki-67-positive and binucleated hepatocytes, reflecting structural and regenerative changes.
- *mTOR* and *Cas-3* gene expressions were upregulated in aged livers, suggesting enhanced autophagic and apoptotic activity.

- Interleukin-6 and nuclear factor kappa B levels did not differ significantly between groups, indicating no prominent inflammatory response.
- Physiological aging leads to histological, apoptotic, and autophagic changes that impair liver function, with variations likely influenced by genetic and environmental factors.

ETHICS

Ethics Committee Approval: The protocols are based on animal experiments approved by the Near East University Experimental Animals and Research Centre Ethics Committee of Research and Application Center (approval number: 2024/175, date: 18.04.2024).

Informed Consent: There is no informed consent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.A.H., G.K., M.T., Concept: A.K., Design: Y.A.H., Data Collection and/or Processing: Y.A.H., G.K., M.T., Analysis and/or Interpretation: Y.A.H., G.T., G.Ö., O.E., T.F., A.K., Literature Search: Y.A.H., G.Ö., A.K., Writing: Y.A.H., G.Ö., A.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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Beneficial Effects of Rosuvastatin and L-Arginine on High Glucose-Induced Oxidative Stress in Human Umbilical Vein Endothelial Cells

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Abstract

BACKGROUND/AIMS: The study sought to examine the impact of rosuvastatin and L-arginine, alone or in combination, on hyperglycemia-caused oxidative stress in human umbilical vein endothelial cells (HUVECs).

MATERIALS AND METHODS: HUVECs were divided into five groups: (1) control, (2) hyperglycemia, (3) hyperglycemia + rosuvastatin, (4) hyperglycemia + L-arginine, and (5) hyperglycemia + L-arginine + rosuvastatin. The incubation period was 24 hours for all study groups. Cytotoxicity assays were performed for L-arginine and rosuvastatin. After incubation with glucose, L-arginine, and rosuvastatin, malondialdehyde (MDA) level, an oxidative stress marker, and catalase activity were measured.

RESULTS: In cytotoxicity tests, the highest non-cytotoxic concentration of L-arginine and rosuvastatin was 12.5 µM. Under hyperglycemic conditions, catalase activity was significantly decreased in HUVECs (p=0.0007 versus control). L-arginine alone (p=0.0016 vs. control) and L-arginine combined with rosuvastatin (p=0.0099 vs. control) resulted in a partial increase in catalase activity. Hyperglycemia caused a significant elevation in MDA levels in HUVECs (p=0.0054 vs. control). L-arginine induced a partial reduction in MDA levels (p=0.03 vs. control), whereas the combination of rosuvastatin and L-arginine restored the altered MDA levels.

CONCLUSION: These results indicate that combined incubation with rosuvastatin and L-arginine is more effective in reducing hyperglycemia-induced oxidative stress than administration of either agent alone.

Keywords: Hyperglycemia, HUVEC, L-arginine, rosuvastatin, oxidative stress

INTRODUCTION

Diabetes is one of the most widespread diseases worldwide, significantly contributing to mortality, morbidity, and healthcare costs.[1] By 2045, the global number of people with diabetes is expected to reach approximately 693 million.[2] Diabetic complications, which include microvascular and macrovascular manifestations, are the leading causes of mortality and disability among patients with diabetes.[3]

Oxidative stress is an imbalanced redox state indicated by overproduction and accumulation of reactive oxygen species (ROS) and by impaired antioxidant mechanisms in cells or tissues.[4] Oxidative stress is a primary component of the progression of diabetic vascular disease and is strongly associated with endothelial dysfunction.[5] ROS production promotes vascular dysfunction by scavenging nitric oxide (NO) and via various direct or indirect mechanisms identified in diabetic cardiovascular disorders.[6]

L-arginine, a semi-essential amino acid present in dietary proteins,[7] is essential for the synthesis of NO, a significant vasodilator beneficial to the cardiovascular system.[8] L-arginine has been shown to reduce

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oxidative stress and superoxide radical levels in the liver and brain of diabetic rats, and in the aorta of hypercholesterolemic rabbits.[9,10] Furthermore, L-arginine supplementation ameliorated oxidative stress by reducing malondialdehyde (MDA) levels and elevating the levels of superoxide dismutase, glutathione peroxidase, and catalase in diabetic rats.[11]

Rosuvastatin is a fully synthetic lipid-lowering drug that inhibits 3-hydroxy-3-methylglutaryl-CoA reductase in cholesterol biosynthesis. Rosuvastatin is a water-soluble statin with a shorter onset of action and greater clinical efficacy.[12] Previous studies have shown that rosuvastatin improves endothelial function by reducing inflammatory responses and exerting antioxidant effects in diabetic patients.[13,14] Furthermore, the incubation with rosuvastatin reduced apoptosis and oxidative stress as well as migration in human umbilical vein endothelial cells (HUVECs) under a high concentration of glucose.[15,16]

The objective of the study was to examine the potential antioxidant efficacy of L-arginine and rosuvastatin, alone or when co-incubated in HUVECs under hyperglycemic conditions.

MATERIALS AND METHODS

The current study was performed only using commercial cell lines and *in vitro* experimental techniques. No human subjects or experimental animals were used. Consequently, the ethical committee approval and informed consent were not needed for the present study.

Chemicals

MDA, thiobarbituric acid (TBA), rosuvastatin, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), L-arginine, and glucose were obtained from Sigma-Aldrich (Mannheim, Germany). The HUVEC cell line was obtained from the American Type Culture Collection (Manassas, VA). All materials used for cell culture [fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), penicillin-streptomycin, and L-glutamine] were obtained from Biowest (Riverside, MO).

Cell Lines and Experimental Groups

HUVEC, an endothelial cell line isolated from the umbilical cord vein, was used in the study. This cell line is commonly utilized in cardiovascular disease research. The cells were cultured in DMEM/F12 medium with 10% FBS, 1% L-glutamine, and 1% penicillin-streptomycin and incubated at 37 °C in 5% CO_2.

1. **For the control group, only DMEM/F12 medium was used.**
2. **Hyperglycemia group:** HUVECs exposed to 35 mM glucose for 24 h. The glucose concentration in DMEM/F12 was adjusted to 35 mM.
3. **Hyperglycemia + rosuvastatin group:** HUVECs exposed to IC_{30} dose (12.5 µM) of rosuvastatin and glucose (35 mM) for 24 h.
4. **Hyperglycemia + L-arginine group:** HUVECs exposed to IC_{30} dose (12.5 µM) of L-arginine and glucose (35 mM) for 24 h.
5. **Hyperglycemia + L-arginine + rosuvastatin group:** HUVECs were exposed to L-arginine (12.5 µM), rosuvastatin (12.5 µM), and glucose (35 mM) for 24 h.

All IC30 doses were determined by cytotoxicity assays.

Cytotoxicity

The MTT assay is commonly used to assess cell viability, proliferation, and cytotoxicity in many cell types.[17] The cells were incubated for 24 h in a medium containing glucose (0-50 mM), rosuvastatin (0-100 µM), and L-arginine (0-50 µM) in 96-well plates. Following the incubation, 100 µl of MTT solution was added to each well, and the plate was subsequently incubated for 3 hours. After removing the MTT solution, formazan crystals were dissolved in 150 µL of DMSO and mixed for 5 minutes in each well. The spectrophotometric measurement of the color intensity of this solution at 570 nm. The viability of cells in the control group was set to 100% based on their absorbance, and viability in the other groups was expressed as a percentage relative to the control.

Catalase Activity

Catalase activity in the study groups was measured by the dichromate method.[18] The procedure involves reducing dichromate dissolved in acetic acid to chromic acetate using hydrogen peroxide. The concentration of hydrogen peroxide is directly proportional to the concentration of chromic acetate generated in the process. The generated chromic acetate was quantified colorimetrically at 570 nm. The catalase activity in the study groups was quantified in kU/mg protein.

Lipid Peroxidation

MDA levels were assessed as markers of lipid peroxidation in all experimental groups.[19] The procedure involves quantifying the pink product formed by the reaction of MDA, a degradation product of lipid peroxides, with TBA, measured at 532 nm. MDA levels in the study groups were expressed in µM/mg protein.

Measurement of Protein Levels

The method is based on the principle that, under alkaline conditions, divalent copper ions react with peptide bonds in the presence of tartrate to form a complex and are reduced to monovalent copper ions. The complex is then reduced with Folin phenol reagent, and the resulting blue-violet color is measured spectrophotometrically at 540 nm to determine the protein level.[20] Protein concentrations were calculated using a standard curve and expressed as mg/mL.

Statistical Analysis

All data were analyzed by ANOVA, followed by Bonferroni's post hoc test, using GraphPad Prism 10 software (San Diego, CA). Results are expressed as mean ± standard deviation; $p<0.05$ is considered statistically significant.

RESULTS

The Viability of Human Umbilical Vein Endothelial Cells

HUVECs were exposed for 24 hours to glucose (0-50 mM), L-arginine (0-50 µM), and rosuvastatin (0-100 µM), and effects on cell viability were assessed by the MTT assay. Cell viability was expressed as a percentage relative to control cells.

The concentration of glucose causing 30% inhibition of cell viability (IC30) was determined to be 34.48 mM (Figure 1A and Table 1).

In Figure 1B, the highest dose of L-arginine that did not cause cytotoxicity was 12.5 µM. This dose was used in the *in vitro* experiments (Figure 1B).

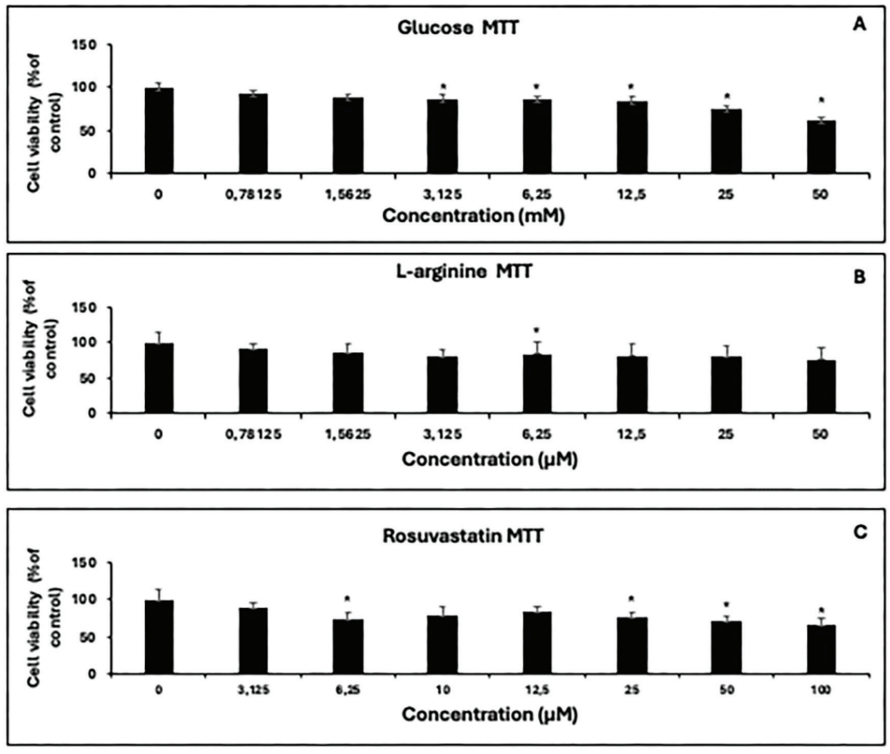

Figure 1. Cell viability at different glucose concentrations (A), different L-arginine concentrations (B), and different rosuvastatin concentrations (C) (*p<0.001 represents a statistically significant difference between the control group and each treatment dosage. Values are given as mean ± SD) (ANOVA, Bonferroni post-hoc).

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, SD: Standard deviation, ANOVA: Analysis of variance.

Table 1. Inhibitory concentrations for glucose

	IC30 (mM)	IC20 (mM)
Glucose	34,48	18,90

The effects of rosuvastatin on the viability of HUVECs are shown in Figure 1C. Based on cytotoxicity tests, the highest dose of rosuvastatin that did not cause cytotoxicity or induce cell proliferation was 12.5 µM.

Catalase Activity

The catalase activity in all groups is shown in Figure 2 and Table 2. The hyperglycemia group (HG) group exhibited the lowest catalase activity (43%) compared with controls (p=0.0007). Incubation with rosuvastatin did not further increase this reduction [p=0.0005 vs. control; p=0.017 vs. hyperglycemia + L-arginine + rosuvastatin group (HG-R+A)]. The alteration showed a partial increase in the hyperglycemia + L-arginine group (HG-A) (p=0.0016 vs. control) and HG-R+A (p=0.0099 vs. control; p=0.035 vs. HG) groups (Figure 2, Table 2 and Supplementary Table 1).

Malondialdehyde Levels

The levels of MDA in HUVECs are displayed in Table 2 and Figure 3. The levels of MDA in the HG group were increased by 48.09% compared with controls (p=0.0054). Rosuvastatin did not reduce MDA levels (p=0.0075 vs. control). L-arginine partially decreased the increased MDA levels (p=0.03 vs. control). Combined treatment with rosuvastatin and

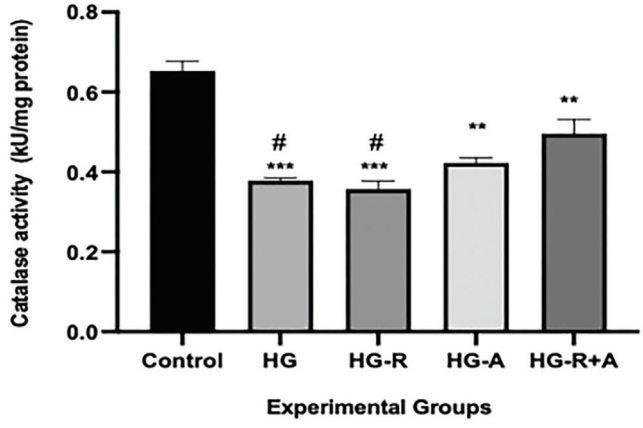

Figure 2. Catalase activity in all groups. All values are displayed as mean ± SD. **p<0.01, ***p<0.001 vs. control; #p<0.05 vs. HG-R+A. (ANOVA, Bonferroni post-hoc).

HG: Hyperglycemia group, HG-R: Hyperglycemia + rosuvastatin group, HG-A: Hyperglycemia + L-arginine group, HG-R+A: Hyperglycemia + L-arginine + rosuvastatin group, SD: Standard deviation, ANOVA: Analysis of variance.

L-arginine reversed changes in MDA levels (p=0.038 vs. HG; Figure 3, Table 2 and Supplementary Table 2).

Table 2. Catalase activity and MDA levels in HUVEC

	Control	HG	HG-R	HG-A	HG-R+A	ANOVA p-values
Catalase activity (kU/mg protein)	0.65±0.02	0.37±0.01***	0.35±0.02***	0.42±0.01**	0.50±0.03**#	0.0003
MDA levels (µM/mg protein)	10.09±0.89	14.95±0.13**	14.62±0.11**	13.38±0.47*	11.80±0.92#	0.0024

*p<0.05, **p<0.01, ***p<0.001 vs. control; #p<0.05 vs. HG. All values are presented as mean ± SD. (ANOVA, Bonferroni post-hoc).
HG: Hyperglycemia group, HG-R: Hyperglycemia-rosuvastatin group, HG-A: Hyperglycemia-L-arginine group, HG-R+A: Hyperglycemia-L-arginine + rosuvastatin group, SD: Standard deviation, MDA: Malondialdehyde, ANOVA: Analysis of variance.

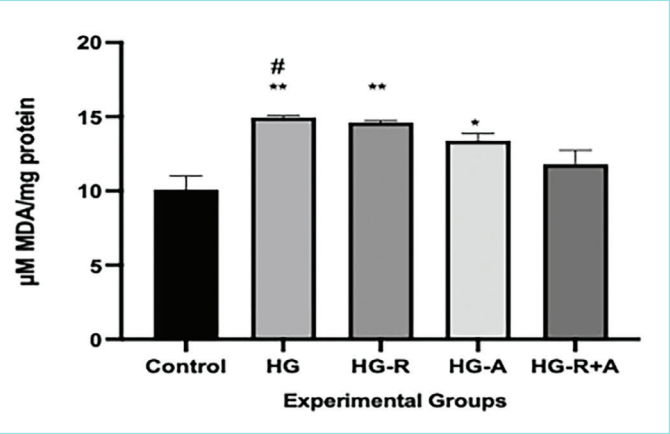

Figure 3. MDA levels in all groups. All values are displayed as mean ± SD. *p<0.05, **p<0.01 vs. control; #p<0.05 vs. HG-R+A. (ANOVA, Bonferroni post-hoc).

MDA: Malondialdehyde, HG: Hyperglycemia group, HG-R: Hyperglycemia + rosuvastatin group, HG-A: Hyperglycemia + L-arginine group, HG-R+A: Hyperglycemia + L-arginine + rosuvastatin group, SD: Standard deviation, ANOVA: Analysis of variance.

DISCUSSION

The current study has demonstrated that combined incubation of HUVEC with rosuvastatin and L-arginine is likely to be more effective at reducing hyperglycemia-induced oxidative stress than rosuvastatin or L-arginine alone.

High blood glucose contributes significantly to endothelial dysfunction in diabetes mellitus.[21] Recent data reveal that increased glucose levels significantly affect endothelial cells, thereby contributing to the clinical complications of type 2 diabetes mellitus. In the current study, HUVECs were incubated in high glucose (35 mM) for 24 hours to model diabetes mellitus *in vitro*. High-glucose-induced oxidative stress in HUVECs was consistent with earlier studies.[22-24]

According to the results of cytotoxicity tests, the highest doses of rosuvastatin and L-arginine that did not cause cytotoxicity or induce cell proliferation were 12.5 µM. In a previous study, rosuvastatin at concentrations of 0.01, 0.1, and 1 µM did not induce apoptosis in HUVECs incubated with oxidized low-density lipoprotein.[25,26] Furthermore, rosuvastatin at 10 µM improved endothelial integrity during incubation with 5-hydroxycholesterol.[27] In addition, L-arginine at a concentration of 12.5 µM did not cause cytotoxicity in HUVECs. Direct data on the cytotoxicity of 12.5 µM L-arginine in HUVECs are not explicitly detailed in the search results. Additionally, Scalera et al.[28] showed that L-arginine alleviated cytotoxicity at millimolar concentrations. Lower micromolar concentrations, such as 12.5 µM, are unlikely to be cytotoxic.

In the current study, HUVECs exposed to high glucose concentrations showed a considerable elevation in MDA levels and a decrease in catalase activity. Similarly, previous studies indicate that hyperglycemia increases oxidative stress in HUVECs, which implies increased MDA levels and decreased catalase activity.[29-32] Furthermore, incubation with rosuvastatin or L-arginine alone partially reversed the alterations, whereas combined administration produced the most significant improvement. In addition, previous data showed that rosuvastatin exerted partially protective antioxidant effects in renal tissue of diabetic rats.[33] Also, rosuvastatin decreased MDA levels and enhanced catalase activity, thereby mitigating oxidative stress and improving oxidizedlowdensity lipoprotein-induced endothelial dysfunction in HUVECs.[26] Furthermore, in studies of alloxan-induced diabetic rats, L-arginine treatment lowered MDA levels and increased catalase activity in gastrointestinal tissues and blood, suggesting protective antioxidant effects against diabetic oxidative stress.[11,34]

Study Limitations

The current study has some limitations. First, measurements of oxidative stress were limited to MDA and catalase activity. Although these are well-established indicators of lipid peroxidation and antioxidant response, inclusion of additional markers such as intracellular ROS production, superoxide dismutase, glutathione peroxidase, and NO-related parameters may provide enhanced mechanistic insight. Second, protein or gene expression levels of oxidative stress markers could not be analyzed; therefore, the involvement of pathways, such as endothelial NO synthase and antioxidant signaling, is inferred rather than directly demonstrated. Furthermore, the findings were obtained from experiments using a single endothelial cell line (HUVECs) at a single time point with only a 24-hour exposure. Despite these limitations, the study provides initial evidence supporting the observed effects; future studies incorporating broader oxidative stress profiling, molecular analyses, and additional models are warranted.

CONCLUSION

These findings indicate that combined incubation with rosuvastatin and L-arginine is more effective than either agent alone in reversing hyperglycemia-induced oxidative stress. Further studies are necessary to evaluate the therapeutic effects of the combination in preventing diabetic complications in preclinical models.

MAIN POINTS

- High glucose exposure increases the levels of malondialdehyde and decreases catalase activity in in human umbilical vein endothelial cells (HUVECs).
- The highest non-cytotoxic concentration for both rosuvastatin and L-arginine in HUVECs was 12.5 µM, ensuring safety and effectiveness at this dose *in vitro*.
- Combined incubation with rosuvastatin and L-arginine significantly reduces hyperglycemia-induced oxidative stress in HUVECs more effectively than either treatment alone.

ETHICS

Ethics Committee Approval: The author declare that the materials and methods used in the current study do not need approval from an ethics committee or special legal permission.The study was not conducted on humans or experimental animals.

Informed Consent: The study did not include human participants or samples. Informed consent was not applicable.

DISCLOSURES

Financial Disclosure: The author declared that this study received no financial support.

Supplementary Material: https://d2v96fxpocvxx.cloudfront.net/58770459-5a06-4076-a747-5b73e24cd7c0/content-images/04cfcfca-74dd-4e46-96e7-81e1d688343e.pdf

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