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The Moldovan Association for Biosafety and Biosecurity (MDBBA) is a scientific and practical, instructive and educational, non-governmental, apolitical and non-profit professional organization, founded in 2017.

The main objective of the association is the development of good practices and culture in the field of biosafety and biosecurity and the promotion of knowledge within professional and research-innovation groups.

BIOSAFETY includes security principles, technologies and rules to be followed to prevent unintended exposure to pathogens and toxins or their accidental release/ leakage.

"Protection of personnel, population from unintended exposure to pathogens/ biohazardous material".

BIOSECURITY includes a wide spectrum of measures (biosecurity policies, regulatory regime, scientific and technical measures) applied in an organized framework, necessary to minimize risks (prevention of actions, terrorist attacks by the intentional release of pathogens or toxins as well as loss, their theft or misuse).

"Protection and prevention of theft, intentional misuse of pathologies/biohazardous material".

RISK MANAGEMENT is a decision-making process in which the results of risk assessment (the process of estimating workplace hazards) are integrated with economic, technical, social and political principles to generate strategies for risk reduction.

One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems.

It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent.

While health, food, water, energy and environment are all wider topics with sector-specific concerns, the collaboration across sectors and disciplines contributes to protect health, address health challenges such as the emergence of infectious diseases, antimicrobial resistance, and food safety and promote the health and integrity of our ecosystems.

By linking humans, animals and the environment, One Health can help to address the full spectrum of disease control – from prevention to detection, preparedness, response and management – and contribute to global health security.

Strengthening Global Health Through the One Health Approach: Challenges, Collaboration, and the Role of Information

Prof. **Mircea-Ioan POPA**, M.D., Ph.D., M.P.H.
"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
Faculty of Medicine, Department of Clinical Medicine II
Microbiology II Discipline, "Cantacuzino" National Military Medical Institute for Research and Development, Bucharest, Romania, Bucharest, Romania

In a world where challenges are growing in both number and complexity, every effort and contribution made by a dedicated team in the shared fight to prevent and control them is truly commendable.

Today, more than ever, we must remain vigilant about the role of diverse pathogens, ranging from prions to large parasitic organisms, in affecting human, animal, and environmental health. Our focus must center on a concept that has repeatedly proven its relevance and necessity, encapsulated in just two words: One Health.

Around 50 to 60 years ago, there was a widespread belief that infectious diseases would soon be a thing of the past, thanks to the discovery of antibiotics and the development of vaccines. However, particularly after 1998, the world was forced to acknowledge that this optimism was premature. Even now, the fight against infectious diseases must continue, with renewed focus and collaboration.

While public health authorities in Europe and across the globe work tirelessly to implement proven strategies and innovate new methods for prevention and treatment, the role of information and data sharing remains absolutely vital.

The medical journal One Health & Risk Management plays an active and essential role in this mission. Through the articles it publishes, its comprehensive approach to the One Health paradigm, and its commitment to fostering interdisciplinary collaboration, the journal continues to support and strengthen our collective efforts, which are essential to building a healthier, more resilient world.

Prof. Mircea-Ioan Popa, M.D., Ph.D., M.P.H.

SYNTHESIS ARTICLE – ARTICLES DE SYNTHÈSE

HELICOBACTER PYLORI – RISK FACTOR FOR GASTRIC CANCER

Adriana BOTEZATU

Department of Internal Medicine, Discipline of geriatrics and occupational medicine, *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

Corresponding author: Adriana Botezatu, e-mail: adriana.botezatu@usmf.md

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ABSTRACT

Introduction Over the last 7-8 decades, a global average annual percentage reduction of 2.1% in gastric cancer has been reported, partly due to the effective eradication and decreased prevalence of *Helicobacter pylori* infection, particularly among younger age cohorts, along with improved dietary habits and cancer screening.

Material and methods Publications were selected from the *PubMed, Hinari, SpringerLink,* and *Google Search* databases using the keyword "*Helicobacter pylori*" in various combinations with the terms "gastric cancer" and "carcinogenesis" to maximize the search yield. In the final bibliography, 36 representative articles were included for the purposes of this synthesis.

Results Inflammation is the most important and frequent factor in *Helicobacter pylori*-induced carcinogenesis. Chronic inflammation induces cancer by increasing the production of reactive oxygen species leading to oxidative stress, apoptosis of epithelial cells with a compensatory proliferative response of the remaining cells, and a higher risk of mutations in proliferating epithelial cells. In addition, *Helicobacter pylori* impairs DNA repair, causing epigenetic alterations in gastric epithelial cells.

Conclusions The pathogenesis of gastric cancer includes a sequence of events starting with *Helicobacter pylori*-induced chronic superficial gastritis, progressing to chronic atrophic gastritis, gastric intestinal metaplasia, gastric epithelial dysplasia, and ultimately gastric cancer.

Keywords Risk factors, *Helicobacter pylori*, gastric cancer, epidemiology, virulence, inflammation.

HELICOBACTER PYLORI – FACTOR DE RISC AL CANCERULUI GASTRIC

Introducere În ultimele 7-8 decenii a fost raportată o reducere procentuală medie anuală globală a cancerului gastric de 2,1%, parțial din cauza eradicării eficiente și reducerii prevalenței infecției cu *Helicobacter pylori*, îndeosebi în cohortele de vârstă mai tânără, obiceiurilor alimentare mai bune și screening-ului cancerului.

Material și metode Publicațiile au fost selectate din bazele de date *PubMed, Hinari, SpringerLink* și *Google Search* utilizând cuvintele-cheie: „*Helicobacter pylori*" folosit în diferite combinații cu cuvintele „cancer gastric" și „carcinogeneză" pentru a maximiza randamentul căutării. În bibliografia finală a lucrării au fost incluse 36 de articole reprezentative pentru scopul acestui articol de sinteză.

Rezultate Inflamația este cel mai important și frecvent factor în procesul carcinogenezei induse de *Helicobacter pylori*. Inflamația cronică induce cancer prin creșterea producției de specii reactive de oxigen care duc la stres oxidativ, apoptoza celulelor epiteliale cu un răspuns proliferativ compensator al celulelor rămase și creșterea riscului de mutații în celulelor epiteliale în proliferare. În plus, Helicobacter pylori afectează repararea ADN-ului cu alterări epigenetice în celulele epiteliale gastrice.

Concluzii Patogenia cancerului gastric include o secvență de evenimente care începe cu gastrita cronică superficială, indusă de *Helicobacter pylori*, progresând spre gastrită cronică atrofică, metaplazie intestinală gastrică, displazia epiteliului gastric și cancer gastric.

Cuvinte-cheie Factori de risc, *Helicobacter pylori*, cancer gastric, epidemiologie, virulență, inflamație.

INTRODUCTION

Over the past 7-8 decades, a global average annual percentage reduction of 2.1% in gastric cancer (GC) has been reported (1). This positive trend is partly attributed to the effective eradication and decreased prevalence of *Helicobacter pylori* (HP) infection, especially among younger age groups, as well as the adoption of healthier dietary habits and regular cancer screening. Nevertheless, GC remains one of the most widespread types of malignancies worldwide, with marked global variability, accounting for 5.5% of all new cancer cases. Moreover, according to recent statistics, GC is the fourth most lethal cancer globally, responsible for 7.7% of total cancer deaths. The highest incidence rates are found in East Asia, while lower incidence rates are observed in North America, Northern Europe, and Africa. The incidence of GC is approximately twice as high in men compared to women, and in recent years, increasing rates have been reported among younger patients (under 50 years of age) (2, 3, 4, 5).

According to GLOBOCAN 2018 data, the cumulative lifetime risk (up to the age of 74) of GC incidence worldwide was 1.87% among men and 0.79% among women, with higher rates in the Western Pacific region (1, 3, 6). According to study results, the vast majority of GC cases (approximately 75% of all new GC cases and about 80–90% of new non-cardia GC cases) are associated with HP infection, and more than 70% of the total number of GC cases occur in developing countries (7).

These changes in the epidemiology of GC justify further research and cancer control measures, with primary and secondary prevention as the main goal, given the poor prognosis in many parts of the world (5). Preventing HP infection and ensuring its timely eradication (before the development of extensive atrophic changes) are the most effective strategies for preventing the development of precancerous gastric lesions and for the primary prevention of GC. An attractive proposal for reducing the incidence of the disease is to identify individuals at high risk who may benefit from screening, as well as from prophylactic and therapeutic measures aimed at preventing the onset of malignancy (8, 9, 10).

In this context, the aim of the article is to develop a narrative synthesis of contemporary studies in order to review current concepts regarding *Helicobacter pylori* infection as a risk factor for gastric cancer, with a view to establishing strategies for the prevention of this disease. We will highlight the main mechanisms through which HP can lead to GC: indirectly, through inflammatory processes, and directly, through its action on gastric epithelial cells.

MATERIAL AND METHODS

To achieve the stated objective, an initial comprehensive search of scientific publications was carried out, using the *PubMed, Hinari (Health Internet Work Access to Research Initiative), SpringerLink, National Center for Biotechnology Information*, and Medline databases. A combination of Boolean operators and keywords was used to identify rel-

evant studies. The article selection criteria included contemporary data on HP infection as a risk factor for GC, based on the following keywords: *"Helicobacter pylori,"* "risk factors," "cancer," "gastric cancer," and "carcinogenesis." To maximize the search yield, the following Boolean operators were applied:

- *"Helicobacter pylori"* OR "risk factors" AND "cancer"
- *"Helicobacter pylori"* OR "risk factors" AND "gastric cancer"
- *"Helicobacter pylori"* OR "risk factors" AND "carcinogenesis."

For the advanced selection of bibliographic sources, the following filters were applied: full-text articles, articles in English, and articles published between 2000 and 2024. After a preliminary analysis of the titles, original articles, editorials, narrative reviews, systematic reviews, and meta- analyses containing relevant information and contemporary concepts regarding HP infection as a risk factor for GC were selected. Additionally, the reference lists of the identified sources were searched to highlight further relevant publications that were not found during the initial database search.

A total of 36 studies were analyzed, including 4 retrospective cohort studies, 1 prospective, randomized, placebo-controlled study, 1 prospective observational cohort study, 29 narrative literature reviews, and 1 systematic review and meta-analysis (Table 1).

In order to minimize the risk of systematic errors (bias) in the study, we conducted thorough database searches to identify the maximum number of publications relevant to the study objective, evaluated only studies that met validity criteria, and applied strict exclusion criteria for articles.

Studies were included if they met the following criteria:

1. Comparison of the regional prevalence of HP infection with the incidence of GC in order to examine the association between these diseases.
2. Evaluation of the presence and effects of HP in healthy individuals, in patients with precancerous gastric lesions, and in those with GC.
3. Association of HP infection with the occurrence of GC, based on the detection of anti-HP antibodies in serum or the detection of the bacteria in biopsy specimens or surgical samples from patients with GC.
4. The effect of HP eradication on GC incidence.

Table 1. Analysis of articles on *Helicobacter pylori* infection as a risk factor for gastric cancer.

No.	Authors, Year, Country	Type	Population	Main Findings
1.	Scheiman J. et al., 1999, USA	Systematic literature review	-	The mechanisms of HP-induced carcinogenesis are independent. HP induces chronic inflammation and oxidative stress, which can damage DNA and promote carcinogenesis.
2.	Alexander G. et al., 2000, USA	Systematic literature review	-	HP is classified as a Group 1 carcinogen. The association between HP and GC is based on epidemiological studies, the detection of anti-HP antibodies in serum, or the detection of the bacterium in biopsy or surgical specimens from GC patients.
3.	Axon A., 2002, UK	Systematic literature review	-	HP increases the relative risk of developing GC sixfold. HP eradication can prevent GC incidence.
4.	Correa P., 2003, USA	Systematic literature review	-	Treatment of HP infection is recommended for infected individuals because of the high risk of GC.
5.	Matysiak-Budnik T. et al., 2006, France	Systematic literature review	-	The association between HP and GC is supported by experimental data showing HP's ability to induce GC in animals and by interventional studies demonstrating that HP eradication can reduce the risk of GC and prevent the development of precancerous gastric lesions in humans and experimental animals. Mechanisms through which chronic inflammation leads to epithelial and precancerous lesions include induction of oxidative stress, disruption of the proliferation/apoptosis balance in epithelial cells, and cytokine secretion.
6.	Fuccio L. et al., 2007, Italy	Systematic literature review	-	Epidemiological studies have demonstrated a clear causal link between HP and GC. HP eradication reduces the incidence of GC in patients without precancerous gastric lesions. HP eradication is also reasonable for individuals with precancerous gastric lesions and a family history of GC.

No.	Authors, Year, Country	Type	Population	Main Findings
7.	Herrera V. et al., 2009, USA	Systematic literature review	-	The proportion of GC in the population that would not occur in the absence of HP has been estimated at 75%. HP is responsible for up to 5.5% of all GC worldwide. HP-induced inflammation promotes cancer by increasing the production of free radicals, increasing apoptotic and necrotic epithelial resistance, inducing cell death, and promoting cell proliferation. HP interacts directly with epithelial cells, modulating proteins and activating genes.
8.	Pandey R. et al., 2010, India	Systematic literature review	-	There is a direct association between HP infection and GC. HP strains that are CagA-positive and VacA-positive present a much higher risk of developing GC.
9.	Polk D. et al., 2010, USA	Systematic literature review	-	HP infection is the most important known risk factor for GC, with an attributable risk of approximately 75%. HP eradication significantly reduces the risk of developing GC in infected individuals without premalignant lesions, confirming the organism's role in the early stages of gastric carcinogenesis.
10.	Wroblewski L. et al, 2010, USA	Systematic literature review	-	A potential factor contributing to the inflammation–carcinoma sequence is the generation of oxidative stress. Oxidative DNA damage induced by HP infection has been well documented in gastric tissues. The CagA oncoprotein is one of the most significant determinants of HP virulence, contributing to gastric carcinogenesis.
11.	IARC, 2013, France	Systematic literature review (expert opinion)	-	HP is classified as a Group 1 carcinogen. Approximately 89% of non- cardia GC cases and 78% of all GC cases are attributed to chronic HP infection. HP treatment reduces GC incidence by 30–40% and also decreases the incidence of metachronous GC.

No.	Authors, Year, Country	Type	Population	Main Findings
12.	Ishaq S. et al., 2015, UK	Systematic literature review	-	Infecția cu HP predispune indivizii la adenocarcinom gastric. Efectele oncogene ale HP pot apărea printr-o varietate de mecanisme, inclusiv efectele inflamatorii indirecte ale HP asupra mucoasei gastrice și efectele epigenetice directe ale HP asupra celulelor gastrice. Two important bacterial virulence factors of HP are CagA and VacA. Controversies remain regarding the impact of HP eradication on preventing the progression of gastric lesions and the possibility of regression of atrophic gastritis.
13.	Mégraud F. et al., 2015, France	Systematic literature review	-	In addition to long-term inflammation, the HP oncoprotein CagA is involved in the carcinogenic process. HP can induce GC indirectly through inflammatory processes and directly by acting on gastric epithelial cells via the CagA protein.
14.	Loor A. et al., 2016, Romania	Systematic literature review	-	HP infection is clearly correlated with gastric carcinogenesis. HP is estimated to be responsible for 5.5% of all cancer cases and more than 60% of GC cases.
15.	Moss S., 2016, USA	Systematic literature review	-	The majority of non-cardia GC cases are attributable to HP infection. Screening and HP eradication in areas with a high GC prevalence reduce the risk of GC by half.
16.	Talebi Bezmin Abadi A., 2016, Iran	Systematic literature review	-	Certain HP strains increase GC risk to different degrees. CagA, secreted by the bacteria and responsible for inducing high levels of chronic inflammation, is the main factor increasing mutagenesis rates, oxidative stress, and the activation of error-repair pathways, leading to gastric carcinogenesis. HP eradication reduces bacterial effects relevant to GC.
17.	Seta T. et al., 2017, Japan	Systematic literature review and meta-analysis	-	HP infection is strongly associated with the occurrence of GC. The efficacy of HP eradication therapy in suppressing primary GC occurrence was significant.

No.	Authors, Year, Country	Type	Population	Main Findings
18.	Ari A. et al. 2018, Turkey	Retrospective cohort study	60 GC patients	HP is one of the etiological factors of GC.
19.	Díaz P. et al., 2018, Chile	Systematic literature review	-	HP infection is the main risk factor associated with the development of GC. HP promotes chronic inflammation and oxidative stress, leading to primary tissue damage.
20.	Mentis A. et al., 2019, Swizerland	Systematic literature review	-	HP infection is a critical risk factor for GC, contributing to approximately 75% of all GC cases. The CagA protein plays a role in GC in adults.
21.	Choi I. et al., 2020, South Korea	Prospective, randomized, double-blind, placebo-controlled study	1,676 HP-positive patients (first-degree relatives of GC patients): 832 received eradication therapy; 844 received placebo	Over a median follow-up period of 9.2 years, GC developed in 1.2% of patients in the treatment group and 2.7% in the placebo group ($p = 0.03$). HP eradication reduced GC incidence, with effects observed even among individuals with a first-degree family history of GC.
22.	Liou J. et al., 2020, Taiwan	Systematic literature review	-	At the individual level, HP eradication reduces GC risk in asymptomatic subjects and is recommended. In vulnerable cohorts (first-degree relatives of GC patients), a screening and treatment strategy is also beneficial. HP eradication in patients with early GC after curative endoscopic resection reduces the risk of metachronous cancer. At the population level, screening and treatment for HP infection is the most cost-effective strategy among young adults in regions with high GC incidence and is recommended preferably before the development of chronic atrophic gastritis and intestinal metaplasia.
23.	White J. et al., 2020, UK	Systematic literature review	-	GC develops through a gradual progression from normal mucosa to adenocarcinoma, most commonly triggered by HP infection. HP eradication reduces subsequent GC risk. This benefit is not consistently maintained in patients with gastric intestinal metaplasia or dysplasia.

No.	Authors, Year, Country	Type	Population	Main Findings
24.	Adeeb AT., 2021, Iraq	Systematic literature review	-	HP is the strongest risk factor for GC. Treatment of this bacterium can prevent GC development.
25.	Piscione M. et al., 2021, Italy	Systematic literature review	-	GC, particularly antral cancer, is linked to HP infection. The progression of HP infection through chronic active gastritis has been demonstrated. The CagA virulence factor, inflammation, and oxidative stress are essential in the development of HP-induced carcinogenesis.
26.	Joshi S. et al., 2021, USA	Systematic literature review	-	HP infection is a major risk factor for GC.
27.	Matysiak-Budnik T., 2021, France	Systematic literature review	-	HP eradication is always beneficial, but to achieve the greatest effect, it should be performed in young adults.
28.	Senchukova M. et al., 2021, Russian Federation	Prospective observational cohort study	109 GC patients	HP is involved not only in the initiation and development but also in the progression of GC.
29.	Yan L. et al., 2022, Sweden	Prospective, randomized, placebo-controlled study	1,630 asymptomatic HP-infected individuals: 817 received standard triple therapy; 813 received placebo	Over 26.5 years of follow-up, 21 participants (2.57%) in the treatment arm and 35 (4.31%) in the placebo arm were diagnosed with gastric cancer. HP eradication may provide long-term protection against GC in high-risk populations, particularly in infected individuals without precancerous gastric lesions.
30.	Kesharwani A. et al., 2023, India	Systematic literature review	-	HP is the main factor responsible for GC pathogenesis. The coexistence of a genetically susceptible host, a virulent bacterial strain, and a sensitized gastric environment can contribute to cancer development. The most important pathogenic components of HP virulence are the CagA and VacA cytotoxins. The mechanisms through which HP leads to GC development include chronic inflammation, DNA damage, suppression of the host immune system, anti- apoptotic activity, production of oxidative stress contributing to chronic inflammation, mutation accumulation, and cancer progression.

No.	Authors, Year, Country	Type	Population	Main Findings
31.	Reyes V., 2023, USA	Systematic literature review (or narrative literature review)	-	HP is the primary risk factor involved in the development of GC. HP infection induces chronic inflammation that affects the gastric epithelium, leading to DNA damage and the promotion of precancerous lesions. CagA oncoprotein is one of the most important determinants of Helicobacter pylori virulence in host cells.
32.	Salvatori S. et al., 2023, Italy	Systematic literature review	-	HP is one of the main risk factors for GC. The carcinogenic mechanisms associated with HP are based on the onset of chronic inflammation (oxidative stress) and on specific bacterial virulence factors (CagA, VacA), which can damage the DNA of gastric epithelial cells and promote genomic instability.
33.	Usui U. et al., 2023, Japan	Retrospective cohort study	1,433 GC patients and 5,997 control individuals	Patients with HP infection had a higher cumulative risk of GC than non-carriers of the infection.
34.	Kouroumalis E. et al., 2024, Greece	Systematic literature review	-	HP strains producing the CagA and VacA cytotoxins are more dangerous in initiating GC. HP eradication should be reserved for specific patient groups, such as first-degree relatives of GC patients.
35.	Yoo H. et al., 2024, South Korea	National retrospective cohort study	69,722 patients with endoscopically treated gastric dysplasia; 49.5% received HP eradication therapy	HP eradication after endoscopic resection of gastric dysplasia was associated with a reduced risk of both primary and metachronous GC.
36.	Zhao Z. et al., 2024, China	Retrospective cohort study	1,293 GC patients after radical gastrectomy (125 with anti-HP treatment; 1,168 without)	Patients receiving anti-HP treatment had a significant advantage in terms of overall survival and disease-free survival compared to those without HP treatment.

The information from the publications included in the bibliography was collected, classified, evaluated, and synthesized, highlighting the main aspects of the contemporary understanding of *Helicobacter pylori* infection as a risk factor for gastric cancer. Particular attention was paid to the type of study (retrospective, cohort, prospective, cross-sectional, case-control), the number of patients included in each study, and the detection methods used.

When necessary, additional information sources were consulted to clarify certain concepts. Duplicate publications, articles that did not correspond to the purpose of the study, and those that were not available for full-text viewing were excluded from the list of publications generated by the search engine.

RESULTS

Following the processing of information identified from the *PubMed, Hinari, SpringerLink, National Center for Biotechnology Information*, and *Medline* databases according to the search criteria, a total of 246 full-text articles addressing the role of HP infection in the development of GC were found. After a detailed analysis of eligibility, titles, and abstracts, 60 duplicate articles and those without full- text versions were excluded, as well as 95 articles for not meeting the inclusion criteria and 55 articles for irrelevant details and/or insufficient methodology. Ultimately, 36 publications considered representative of the materials published on this topic were selected and included in the final bibliography (Fig. 1). The search and selection of publications were carried out by the author.

Definition: *Helicobacter pylori* is a Gram-negative, spiral-shaped, flagellated bacterium that selectively colonizes the semi-permeable gastric mucus gel layer covering the apical surface of the gastric epithelium (4). Approximately 20% of the bacteria attach to gastric epithelial cells, thereby evading the immune response. Researchers have identified HP in the cytoplasm of epithelial cells, in intercellular spaces, in the lamina propria of the gastric mucosa, and in the lumen of small vessels (11, 12).

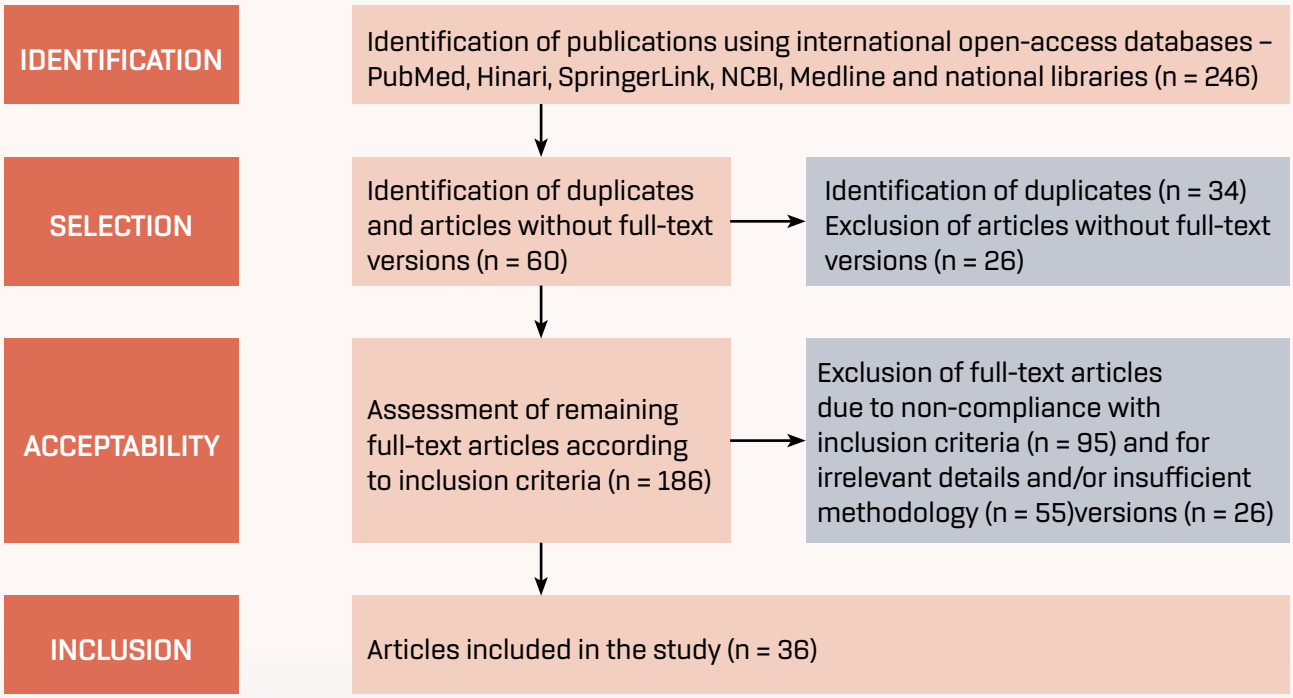

Figure 1. Literature search algorithm.

HP causes the most common bacterial infection worldwide. HP has developed mechanisms that enable it to colonize the highly acidic gastric environment by metabolizing urea into ammonia through urease, which is abundantly produced by the bacterium. This process creates a local microenvironment with a neutral pH. In most cases, the infection is asymptomatic and is universally associated with chronic or acute inflammation and, at times, with other types of gastric lesions, including gastric ulcers, mucosa-associated lymphoid tissue, chronic atrophic gastritis, and gastric intestinal metaplasia. Antimicrobial therapy leads to the regression of inflammation over time (13, 14, 15, 16, 17, 18, 19, 20).

HP differs from other bacteria through a set of properties that enable it to colonize the gastric mucosa and persist for long periods under conditions that are unfavorable for other microorganisms. These include: (1) the ability to produce a special enzyme – urease; (2) the synthesis of lytic enzymes that cause the depolymerization and dissolution of gastric mucus, which consists mainly of mucin; (3) the bacterium's mobility, ensured by the presence of 5-6 flagella; (4) the high adhesiveness of the bacteria to the epithelial cells of the gastric mucosa; (5) the production of various exotoxins (VacA, CagA, and others); (6) the instability of the HP genome; (7) the presence of the bacterium in both vegetative and biofilm forms; and (8) the ability to persist intracellularly and translocate beyond the gastric mucosa (11).

Epidemiology

HP is usually acquired in childhood, most commonly before the age of 10, leading to an infection that persists throughout life. Currently, an average of 50% of the world's population is infected with HP. Transmission occurs largely from person to person via the fecal-oral or gastric-oral route within families, particularly in settings with poor sanitation and hygiene. However, the bacterium is commensal and harmless for the vast majority of the infected population (1, 3, 6, 13, 15, 21).

The prevalence of infection varies worldwide depending on the geographical distribution and the socioeconomic status of the population. This indicator is higher in developing countries (up to 80%) and significantly lower in economically developed countries (up to 30%) (1, 8, 13, 15, 17, 19, 21). However, there is a discrepancy between the prevalence of HP infection and GC rates in certain populations from Africa, India, and coastal areas of Latin America. These populations have high infection rates but low GC rates, a phenomenon largely unexplained and referred to as the "African enigma." This variation may possibly be explained by a combination of the following factors: age at acquisition of infection, HP strain type, host genetic profile, dietary habits, and environmental factors (8, 17, 19, 20, 22, 23).

Diagnosis

HP infection can be defined through non-invasive methods (serological – detection of serum HP-IgG using enzyme-linked immunosorbent assay (ELISA) and immunoblotting, urea breath test, detection of HP antigen in stool samples) and invasive methods (histological examination of gastric biopsy specimens collected endoscopically and rapid urease testing) (2, 21).

Epidemiological relationships between GC and HP

The major breakthrough in GC research came with the pioneering discovery of the HP bacterium by Barry Marshall and Robin Warren in 1983, the scientists who were the first to identify the relationship between HP infection in gastric tissue and gastritis and peptic ulcer disease (2, 6, 13, 14, 21, 23, 24). Since its initial description, HP has inspired intensive investigations aimed at better understanding its interactions with the human host at the cellular and molecular levels, as well as its pathogenic potential. Interest in HP as an etiological factor for gastric cancer arose following observations of a causal correlation between infection and the neoplastic process. The first studies examining the association between HP and GC compared the regional prevalence of HP infection with GC incidence. Subsequently, numerous epidemiological studies, systematic literature reviews, meta-analyses of case–control studies, and experimental models have confirmed the association between GC and HP infection. This close relationship has been observed between HP and both intestinal-type GC and diffuse-type GC (4, 10, 13, 20, 21, 23). It is generally accepted that the risk of GC is highest among patients in whom primary colonization with HP causes acute and then chronic inflammation (22).

Following the discovery of HP in the early 1980s, more than 1,000 studies have been conducted on the association between the bacterium and GC, including observational studies (ecological, case–control, and cohort), clinical studies on HP eradication, pathological studies, and experimental studies using animal models. The results largely confirmed the relationship between infection and malignancy. The most convincing observational evidence of the association between HP infection and GC comes from longitudinal cohort studies (13, 24, 25). Epidemiological data have shown that HP infection is associated with a 3- to 6-fold increased risk of developing non-cardiac GC, with GC progression, and with poorer long-term outcomes (11, 18, 19, 20, 21, 24, 26). Many studies have confirmed the association between HP infection and the occurrence of GC based on the detection of anti-HP antibodies in serum or the detection of the bacteria in biopsy specimens or surgical samples from GC patients. The results of surgical specimen studies increased HP positivity rates to as high as 90% (21). In addition, the prevalence of infection was statistically significantly higher among patients with the intestinal type (80-90%) compared to those with the diffuse type (30.0-31.8%) of GC. These results demonstrate a direct association between HP infection and the intestinal type of gastric carcinoma (19, 23, 24).

Among individuals infected with HP, approximately 10% develop peptic ulcers, 1-3% develop gastric adenocarcinoma, and 0.1% develop mucosa-associated lymphoid tissue (MALT) lymphoma (1, 6, 15, 23, 27, 28). A family history of GC in a first-degree relative is associated with a two- to threefold increased risk of GC (29).

Based on strong evidence supporting the etiological role of HP infection in GC, in 1994 the World Health Organization classified HP as a class I human carcinogen (a definite cause of human GC) (17, 18, 22).

At the population level, the “screen and treat” strategy for HP is the most cost-effective among young adults in regions with a high inci-

dence of GC and is recommended preferably before the development of chronic atrophic gastritis and gastric intestinal metaplasia (6, 21). A pilot study of mass screening and HP eradication in a population from an area with highly endemic HP infection and a high incidence of GC reported very promising initial results. Over a relatively short period of time, the authors observed a 78.7% reduction in HP infection, a 77.2% reduction in the incidence of gastric atrophy, a 25% reduction in GC incidence, and a 67.4% reduction in peptic ulcer incidence (24). Among HP-infected individuals with a family history of GC in first-degree relatives, eradication therapy reduced the risk of GC occurrence (29). A recent prospective, randomized, placebo-controlled study published in 2022, with 26.5 years of follow-up, along with other studies and meta-analyses, has provided strong evidence that HP eradication therapy may offer long-term protection against GC in high-risk populations, particularly in healthy, asymptomatic infected individuals without advanced gastric lesions at baseline (10, 12, 26, 30). However, HP eradication in patients with advanced preneoplastic gastric lesions does not prevent the development of GC, and endoscopic surveillance must always be performed (10).

Because HP eradication requires a large number of subjects and many years of follow-up, researchers have used GC precursors (multifocal atrophic gastritis and gastric intestinal metaplasia) as surrogates for measuring the effects of HP eradication on the malignant process. Overall, these studies suggest regression of preneoplastic gastric lesions, although the change is slow and not universally observed. Thus, the studies conducted support an unequivocal role for HP infection in the development of GC and indicate that eradication of the infection may be an effective means of preventing both primary and metachronous GC (10, 13, 14, 15, 30, 31, 32).

Thus, HP infection is responsible for 5.5% of all cancer cases and approximately 60% of GC cases, making this infection the primary cause of GC worldwide and the second leading defined cause of malignancy after smoking (10, 13, 14).

DISCUSSIONS

Pathogenetic mechanisms of HP-induced carcinogenesis

In general, GC is the consequence of a multifactorial process involving host responses, specific host–microbe interactions, bacterial virulence, diet, and other environmental factors. Although the mechanisms of HP-induced carcinogenesis have not been fully elucidated, inflammation is considered the most important and frequent factor in this process. Chronic inflammation is thought to induce cancer by increasing the production of reactive oxygen species, leading to oxidative stress, apoptosis of epithelial cells with a compensatory proliferative response of the remaining cells, and an increased risk of mutations in proliferating epithelial cells. In addition, HP affects DNA repair both in vivo and in vitro, causing epigenetic alterations in gastric epithelial cells. All these adaptive responses enhance cell survival and proliferation, leading to the acquisition of malignant characteris-

tics that enable the progression of precancerous gastric lesions, invasion, and metastasis (3, 12, 13, 15, 18, 25, 33, 34).

The importance of inflammation as a risk factor is confirmed by three complementary observations: (1) bacterial strains that induce the greatest inflammation have the strongest associations with malignancy; (2) host proinflammatory cytokine polymorphisms increase cancer risk; and (3) nonsteroidal anti-inflammatory agents reduce cancer risk (13, 24).

Other mechanisms in addition to inflammation have been identified in HP-related gastric carcinogenesis. HP interacts directly with epithelial cells, leading to protein modulation and gene activation, and damages parietal cells, thereby altering the maturation process of epithelial stem cell lineages (4, 13).

There are two important mechanisms through which HP can ultimately lead to intestinal-type GC:

1. *Indirect mechanisms through inflammatory processes*. Intestinal-type gastric adenocarcinoma represents the final stage of a long precancerous process known as Correa's cascade of multistep gastric carcinogenesis. The pathogenesis of GC involves a sequence of events that begins with superficial (non-atrophic) chronic gastritis induced by HP, progressing to chronic atrophic gastritis (initially limited to the gastric corpus or antrum, and later becoming multifocal), gastric intestinal metaplasia (initially "complete" and then "incomplete"), gastric epithelial dysplasia (initially low-grade and later high-grade), and finally GC. Progression to gastric epithelial dysplasia and GC is thought to involve processes that no longer require the presence of HP (7, 24, 25, 33, 34, 35).

   Persistent HP infection is maintained through a variety of mechanisms: HP can protect itself from toxic substances such as reactive oxygen species, induce macrophage apoptosis, and increase the expression of proinflammatory factors. Aberrant DNA methylation in gastric epithelial cells occurs in parallel with the HP-associated inflammatory response (8).

2. *Direct mechanisms of HP* on gastric epithelial cells through the toxic action of virulence factors. Mutations in cell cycle regulatory genes, deficiencies in DNA repair mechanisms, loss of cellular adhesive properties, and epigenetic changes can alter cellular behavior, leading to cellular autonomy and malignant transformation (1, 8, 16, 22, 25, 27, 28, 34).

   Two widely studied virulence factors are the CagA and VacA cytotoxins, which play a crucial role in the pathogenicity of HP infection. These virulent strains contribute to gastric carcinogenesis through their immunosuppressive activities, promotion of bacterial survival, and maintenance of gastric inflammation, and are associated with precancerous gastric lesions and progression to a malignant phenotype (4, 12, 19, 20, 25, 28).

   Urease, which is abundantly secreted by HP, influences the host response beyond its enzymatic action by activating monocytes and polymorphonuclear leukocytes, leading to inflammation and epitheli-

al damage. Adhesins (BabA, SabA) are bacterial surface proteins that contribute to the attachment of the bacterium to host cells. HP adhesion to the gastric epithelium is a key step in the colonization of the gastric mucosa (28).

Thus, research suggests that the oncogenic effects of HP infection may occur through a variety of mechanisms, including the indirect inflammatory effects of HP on the gastric mucosa and the direct epigenetic effects of HP on epithelial cells. During HP infection, a combination of environmental and genetic factors plays a crucial role in the progression of gastric disease and the development of GC (8, 34, 36). However, it should be noted that despite the large number of studies dedicated to HP, it is still unclear whether the infection is involved only in the initiation of the gastric tumor process or whether it can also affect tumor progression mechanisms (11).

Cofactors in carcinogenesis

Although half of the world's population is infected with HP, only a minority of individuals (an estimated 1-3%) are exposed over their lifetime to progression toward GC. When only middle-aged adults are considered, the risk of cancer becomes more substantial. For example, in prospective studies conducted in Asia, between 3% and 6% of HP-infected subjects developed GC within a decade (12, 13, 28).

It is currently accepted that bacterial virulence factors of the HP strain are the most important in GC development. However, as in other infections, there are environmental and host genetic factors that predispose to and interact with certain pathogenic strain factors, such as the CagA cytotoxin. HP may harbor pathogenic factors such as cytotoxins and a pathogenicity island (cag) that encodes the secretion of the bacterial oncoprotein CagA, which is involved in the carcinogenic process in addition to the inflammation it generates. Studies have found that approximately 60% of HP bacterial strains possess the CagA cytotoxin (3, 15, 17, 21, 25, 28, 33).

There is evidence that HP may not be the only microbe responsible for the development of GC, as this bacterium diminishes as atrophy progresses and practically disappears in areas of tumor tissue. HP does not colonize areas of cancer, intestinal metaplasia, or atrophy, and there is evidence that as advanced gastric disease develops, the bacterium may disappear from the stomach. These findings may indicate that HP infection is not essential at all stages of GC development and that the mechanism may differ from the progression of chronic atrophic gastritis and gastric intestinal metaplasia (1). Oral microbiota is also involved through various mechanisms, such as anti-apoptotic activity, immune system suppression, initiation of chronic inflammation, and the development of mutations (16).

HP infection alone is not sufficient for the development of GC. The bacterium has a synergistic relationship with host factors, primarily immune and reparative responses, and environmental factors, which amplify the risk of subsequent neoplastic transformation. After bacterial genetics, the most important factor influencing carcinogenesis is likely host genetics. Single nucleotide polymorphisms in interleu-

kin genes (IL-1β, IL-1-RN2, IL-8, IL-10, TNF-α) are genetic susceptibility factors associated with individual or familial susceptibility to HP-mediated carcinogenesis (3, 8, 13, 15, 19, 33, 34). Individuals with the IL-1β-31C gene and the IL-1 receptor antagonist gene (IL-1-RN2) are more likely to develop HP-induced gastric atrophy, gastric cancer, or hypochlorhydria (16, 18, 19, 25).

The risk factors associated with the development of precancerous gastric lesions are practically similar to those associated with GC. Environmental factors such as iron deficiency or dietary habits (consumption of salt-preserved foods and N-nitroso compounds, and a diet low in micronutrients from fresh fruits and vegetables), smoking, and alcohol consumption damage the gastric mucosa, facilitate HP infection and persistence, and increase susceptibility to tumorigenesis. In addition, bacterial factors interacting with environmental factors further increase the risk of GC (13, 17, 18, 24, 27, 33).

CONCLUSIONS

1. *Helicobacter pylori* infection is found on average in 50% of the population across all regions of the world. The prevalence of this infection varies considerably worldwide depending on the geographical distribution and socioeconomic status of the population – higher rates are observed in developing countries (up to 80%) compared to economically developed countries (up to 30%).

2. *Helicobacter pylori* is a heterogeneous species that can harbor virulence factors, the most important being urease, adhesins (BabA and SabA), and cytotoxins (CagA and VacA), which play a crucial role in the pathogenicity of the infection and are involved in the carcinogenic process. These virulent strains contribute to gastric carcinogenesis through their immunosuppressive activities, promotion of bacterial survival, and maintenance of gastric inflammation, and are associated with precancerous gastric lesions and progression to a malignant phenotype.

3. The etiology of gastric cancer is complex and multifactorial, involving environmental and host-related factors, as well as genetic and epigenetic changes. *Helicobacter pylori* infection requires multiple known mechanisms (and likely others yet to be discovered) to induce the onset and progression of gastric cancer, making it the most important risk factor in the pathogenesis of this malignancy.

4. Inflammation is the most important and frequent factor in the process of *Helicobacter pylori*-induced carcinogenesis. Chronic inflammation induces cancer by increasing the production of reactive oxygen species, which leads to oxidative stress, apoptosis of epithelial cells with a compensatory proliferative response of the remaining cells, and an increased risk of mutations in proliferating epithelial cells. In addition, *Helicobacter pylori* affects DNA repair, causing epigenetic alterations in gastric epithelial cells. All these adaptive responses enhance cell survival and proliferation, leading to the acquisition of malignant characteristics that enable the progression of precancerous gastric lesions, invasion, and metastasis.

5. The pathogenesis of gastric cancer involves a sequence of events that begins with superficial (non-atrophic) chronic gastritis induced by *Helicobacter pylori*, progressing to chronic atrophic gastritis (initially limited to the gastric corpus or antrum, and later becoming multifocal), gastric intestinal metaplasia (initially "complete" and then "incomplete"), gastric epithelial dysplasia (initially low-grade and later high-grade), and finally gastric cancer.
6. *Helicobacter pylori* eradication therapy can provide long-term protection against gastric cancer in high-risk populations, particularly in healthy, asymptomatic infected individuals without advanced gastric lesions at baseline. Eradication of *Helicobacter pylori* in patients with advanced preneoplastic gastric lesions does not prevent the development of gastric cancer, and endoscopic surveillance must always be performed.

CONFLICT OF INTEREST

There is no conflict of interest.

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EFFECT OF MICROWAVE-ASSISTED TREATMENT ON THE COMPOSITION OF SPRAY-DRIED *PORPHYRIDIUM CRUENTUM* EXTRACT

Ludmila RUDI, Ana VALUȚA, Tatiana CHIRIAC, Svetlana DJUR

Institute of Microbiology and Biotechnology, Technical University of Moldova, Chisinau, Republic of Moldova

Corresponding author: Ludmila Rudi, e-mail: ludmila.rudi@imb.utm.md

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ABSTRACT

Introduction Microalgae are a valuable source of natural products with bioactive properties, with applications in biomedicine, the pharmaceutical industry, nutrition, and cosmetics. Among them, the red microalga *Porphyridium cruentum* is notable for its high content of biomolecules with antioxidant, anti-inflammatory, and immunomodulatory effects. Efficient extraction and preservation methods are essential to optimize the use of these biomolecules.

Material and methods Biomass from *Porphyridium cruentum* was treated with microwaves (MW) at 180 W, 300 W, and 450 W for 10, 20, and 30 seconds, followed by aqueous extraction at 80 °C. The resulting extracts were spray-dried into powders at 100 °C. Both aqueous extracts and powders were analyzed for their composition (proteins, carbohydrates, phenolic compounds) and antioxidant activity.

Results Moderate microwave treatment (180 W for 20–30 seconds) enhanced the extraction of proteins and carbohydrates while preserving high antioxidant activity. The resulting powders retained up to 90.96% of proteins, 95.81% of carbohydrates, and 74.91% of phenolic compounds, with only minimal losses in antioxidant activity after six months of storage.

Conclusions These findings demonstrate that microwave treatment of *Porphyridium cruentum* biomass, followed by aqueous extraction and spray drying, is an effective strategy for obtaining and preserving microalgal bioactive compounds. This approach supports their potential applications in the pharmaceutical, nutraceutical, and cosmetic fields.

Keywords *Porphyridium cruentum*, microwave-assisted pretreatment, bioactive composition, spray drying, powder stability.

EFECTUL TRATAMENTULUI ASISTAT DE MICROUNDE ASUPRA COMPOZIȚIEI EXTRACTULUI DE *PORPHYRIDIUM CRUENTUM* USCAT PRIN PULVERIZARE

Introducere Microalgele reprezintă o sursă valoroasă de produse naturale cu proprietăți bioactive, având aplicații în biomedicină, industria farmaceutică, nutriție și cosmetică. Microalga roșie *Porphyridium cruentum* se remarcă prin conținutul său bogat în biomolecule cu efecte antioxidante, antiinflamatoare și imunomodulatoare. Pentru valorificarea optimă a acestor biomolecule, este esențială aplicarea unor metode eficiente de extracție și conservare.

Material și metode Biomasa de *Porphyridium cruentum* a fost tratată cu microunde (MW) la 180W, 300W și 450W pe durata a 10, 20 si 30 sec, apoi supusă extracției hidrice la 80°C. Pulberile au fost obținute prin atomizare la 100°C. În extractele hidrice si in pulberile obținute s-a evaluat compoziția bioactivă (proteine, carbohidrați, compuși fenolici) și activitatea antioxidantă (teste ABTS și DPPH).

Rezultate Tratamentul moderat cu microunde (180W timp de 20–30 sec) a favorizat extracția proteinelor și carbohidraților, menținând o activitate antioxidantă ridicată. Pulberile obținute au păstrat până la 90.96% din proteine, 95.81% din carbohidrați și 74.91% din compușii fenolici, cu pierderi minore ale activității antioxidante, după șase luni de păstrare.

Concluzii Rezultatele cercetării demonstrează că tratamentul biomasei de *Porphyridium cruentum* cu microunde, urmat de extracția hidrică și uscarea extractului prin pulverizare, reprezintă o strategie eficientă pentru obținerea și conservarea compușilor bioactivi microalgali, facilitând utilizarea acestora în domeniul farmaceutic, nutraceutic și cosmetic.

Cuvinte-cheie *Porphyridium cruentum*, tratare asistată de microunde, compoziția bioactivă, uscare prin atomizare, stabilitatea pulberii.

INTRODUCTION

Microalgae are a valuable source of natural products, characterized by their diverse bioactive composition. They have important applications across multiple fields, including biomedicine, the pharmaceutical industry, nutrition, and cosmetics (1). In addition, they contribute to sustainable and cost-effective solutions in health, food, and environmental protection (2–4).

The red microalga *Porphyridium cruentum* is a significant source of biologically active compounds with notable properties, including sulfated polysaccharides, polyunsaturated fatty acids, and functional proteins (5–7). The sulfated polysaccharides of *Porphyridium cruentum* exhibit antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory effects. They stimulate the immune system and show antiviral activity by inhibiting viral replication and protecting host cells. Due to these properties, they are being investigated as complementary therapies for infections and autoimmune diseases (8). Polyunsaturated fatty acids, such as arachidonic acid and eicosapentaenoic acid, are essential for cardiovascular health and lipid metabolism, which makes them valuable in nutraceuticals and functional foods (9). The proteins of *Porphyridium cruentum* provide essential amino acids and bioactive peptides with antioxidant and immunostimulatory roles. These contribute to cellular health and may be applied in protein supplements with nutritional and therapeutic benefits.

Porphyridium cruentum is highly valued in the cosmetic industry for its exopolysaccharides, which provide hydration, antioxidant protection, and promote skin regeneration. Its proteins and polyunsaturated fatty acids further support skin elasticity and exert anti-aging effects, making them key ingredients in creams and skin care formulations (10).

Optimizing the use of *P. cruentum* bioactive compounds requires advanced biomass processing and extraction techniques. Among these, microwave-assisted extraction is particularly effective, as it achieves high yields while preserving the structural integrity of active molecules (11). Spray drying has also proven to be an efficient method for stabilizing and preserving extracts. By incorporating bioactive compounds into a dry matrix, it minimizes the risk of oxidation and degradation (12). This approach improves stability, extends shelf life, and facilitates both long-term storage and international distribution (13). In addition to protecting active molecules, these techniques enhance bioavailability, enabling straightforward integration into pharmaceutical, food, and cosmetic products. Modern processing and extraction technologies are therefore essential for converting microalgal bioactives into stable, valuable, and widely applicable industrial ingredients.

The purpose of this study is to evaluate the effect of microwave-assisted treatment of *P. cruentum* biomass on the bioactive composition of aqueous extracts and to assess their stability following spray drying.

MATERIAL AND METHODS

The study used the red microalga *Porphyridium cruentum* strain CNMN-AR-01, which is deposited in the National Collection of Non-Pathogenic Microorganisms at the Institute of Microbiology and Biotechnology, Technical University of Moldova. Biomass was produced by cultivating the microalga in Brody mineral medium. Cultivation took place in 1000 mL Erlenmeyer flasks containing 500 mL of medium under the following conditions: a temperature of 25–28°C, a pH of 6.8–7.2, and continuous illumination at 56 µmol

photons m^{-2} s^{-1}. After a 14-day cultivation cycle, the biomass was separated from the medium by centrifugation at 4000 rpm for 7 min (NÜVE NF-800, Ankara, Turkey). Excess salts were removed by washing with a 2.0% ammonium acetate solution. The biomass was then standardized to a concentration of 10 mg/mL for subsequent treatment and extraction.

Three types of raw material were prepared: native biomass (NBM) and frozen/thawed biomass (FTBM), both used as controls, and microwave-treated biomass, used as the experimental variant. For FTBM, biomass was frozen at -20 °C (Snaigė AB, Alytus, Lithuania) and thawed at room temperature. This procedure was repeated six times, after which phycobiliproteins were removed by centrifugation at 4000 rpm for 7 min. For the experimental microwave treatment, 20 mL aliquots of standardized biomass (10 mg/mL) were irradiated in a Samsung microwave device (2450 MHz, Seoul, South Korea). The aliquots were exposed at power levels of 180W and 300W for 10, 20, and 30 seconds each. At 450W, exposure times were 10 and 20 seconds. Following microwave exposure, the biomass was centrifuged to separate the phycobiliproteins.

Bioactive compounds were extracted from control and experimental biomass samples with purified water at a 1:40 (g/mL) ratio. The mixture was homogenized and incubated at 80°C for 60 minutes in a water bath (GFL 1023, Burgwedel, Germany). After cooling to room temperature, the mixture was centrifuged at 4000 rpm for 7 minutes to separate the solid fraction. The supernatant, representing the crude aqueous extract, was collected, filtered to remove residual particles, and stored at -20°C.

Biochemical assays were performed using a UV-Vis spectrophotometer (80T, PG Instruments Ltd., Lutterworth, UK). The protein content of the microalgae biomass was determined by a modified Lowry method, and carbohydrate content was quantified using the anthrone reagent. Phenolic compounds were assessed with a modified Folin-Ciocalteu method. All results are expressed as g/100 g dry weight (DW). Antioxidant activity was evaluated using the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays (14), with results expressed as the percentage of radical inhibition per 10 mg DW. All reagents were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany).

Spray drying was performed using a UnoPex laboratory-scale spray dryer (Izmir, Turkey) with an integrated air compressor. The process was conducted at a constant inlet temperature of 100°C with extract solutions prepared at a 12% solid concentration. The resulting powders were collected and analyzed to evaluate the retention of biochemical components relative to the initial extracts.

To monitor stability, the spray-dried powders were stored in hermetically sealed, dark glass containers with silica gel stoppers at 4°C for 6 months to prevent light exposure and moisture absorption. For biochemical analysis, the powders were rehydrated in deionized water (1:10 m/v), homogenized for 30 minutes at room temperature, and centrifuged at 10,000 rpm for 5 minutes.

Statistical analyses were performed in three settings: (i) microwave-treated biomass extracts compared with controls (native biomass, NBM, and freeze–thawed biomass, FTBM); (ii) spray-dried powders compared with their corresponding aqueous extracts to assess compound retention; and (iii) powders stored for 1, 3, and 6 months compared with baseline values to evaluate biochemical stability over time. Results are reported as Mean ± Standard Deviation (SD). The percentages of protein, carbohydrates, and phenols, calculated on a dry weight, extract, or powder basis, were ana-

lyzed. Comparisons were made using Student's *t*-test. Statistical significance was defined as $p < 0.05$ at the 95% confidence interval (CI). All analyses were conducted in Microsoft Excel.

RESULTS

Figure 1 presents a detailed analysis of the percentage composition of proteins, carbohydrates, and phenolic compounds in aqueous extracts of *Porphyridium cruentum* biomass, depending on the processing method (microwave treatment of native biomass or repeated freeze–thaw cycles).

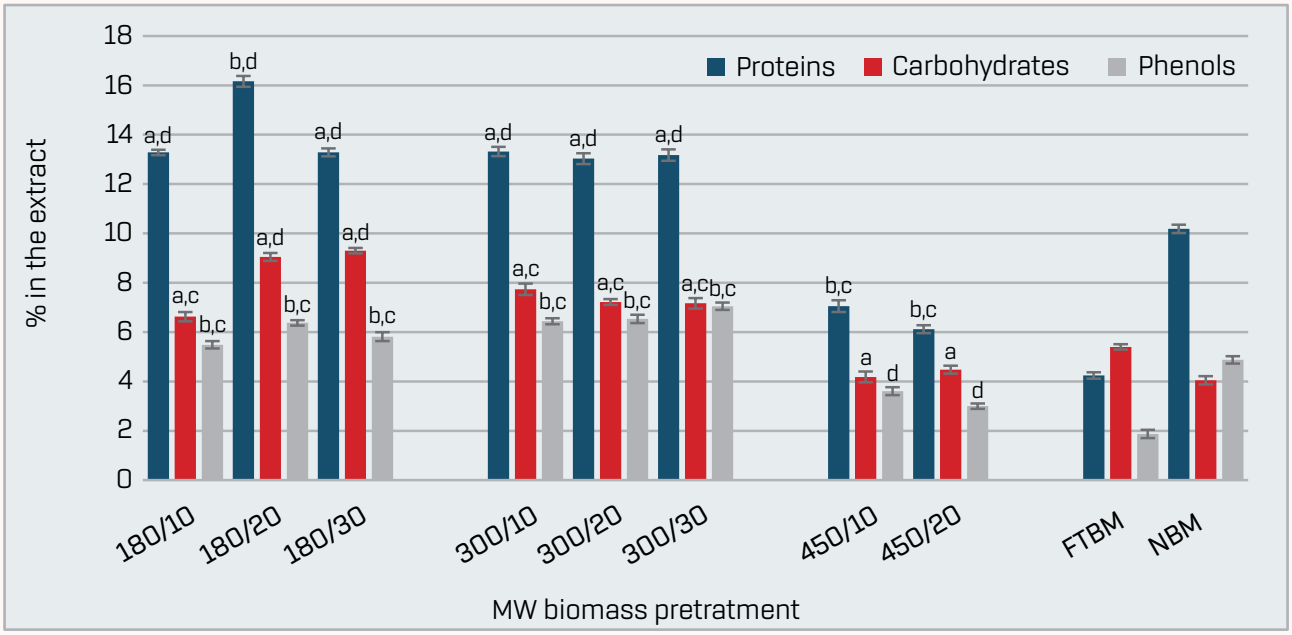

Figure 1. Composition of the aqueous extract obtained from *Porphyridium cruentum* biomass treated with MW (W/sec). Control: NBM - native biomass and FTBM-freeze-thaw biomass;

a, b: significantly different from NBM at p < 0.01 and p < 0.001, respectively;

c, d: significantly different from FTBM at p < 0.01 and p < 0.001, respectively.

The highest protein content (16.16%) was obtained from biomass treated with microwaves at 180 W for 20 seconds, corresponding to a 36.9% increase ($p < 0.001$) compared with untreated biomass and a 3.8-fold increase ($p < 0.001$) compared with biomass subjected to repeated freezing/thawing (10.19% and 4.25%, respectively). Extracts from biomass treated with microwaves at 300 W contained protein levels similar to those observed at 180 W with exposure times of 10 and 30 seconds. These values were 22.82% higher ($p < 0.01$) than in untreated biomass and 3.1-fold higher ($p < 0.001$) than after repeated freezing/thawing. In contrast, treatment of *Porphyridium* biomass at 450 W significantly reduced protein content, which was 44.38% lower ($p < 0.01$) than in untreated biomass.

The highest carbohydrate levels were obtained in extracts from biomass treated at 180 W for 20 or 30 seconds, ranging from 9.05% to 9.30%. These values were 2.2–2.3 times higher than in untreated biomass ($p<0.001$) and 1.7 times higher than in frozen/thawed biomass ($p<0.001$). At 300 W, microwave exposure time had no significant influence, and carbohydrate levels remained between 7.17% and 7.74%. In contrast, treatment at 450 W significantly reduced carbohydrate content, yielding values similar to those observed in untreated or frozen/thawed biomass.

The highest phenolic concentration was achieved in extracts from biomass treated at 300 W for 30 seconds, reaching 7.05%. In untreated biomass, the phenolic content was 4.88%, while frozen/thawed biomass contained significantly less ($p<0.001$), at 1.88%. Treatment at 180 W resulted in values between 5.48% and 6.38%, indicating effective extraction. However, treatment at 450 W markedly reduced phenolic levels, with a maximum of only 3.61%.

Figure 2 presents the antioxidant activity (ABTS and DPPH assays) of aqueous extracts from *Porphyridium* biomass treated with microwave-assisted technology. This treatment significantly increases the extracts' capacity to neutralize free radicals, suggesting that process parameters may affect antioxidant efficiency.

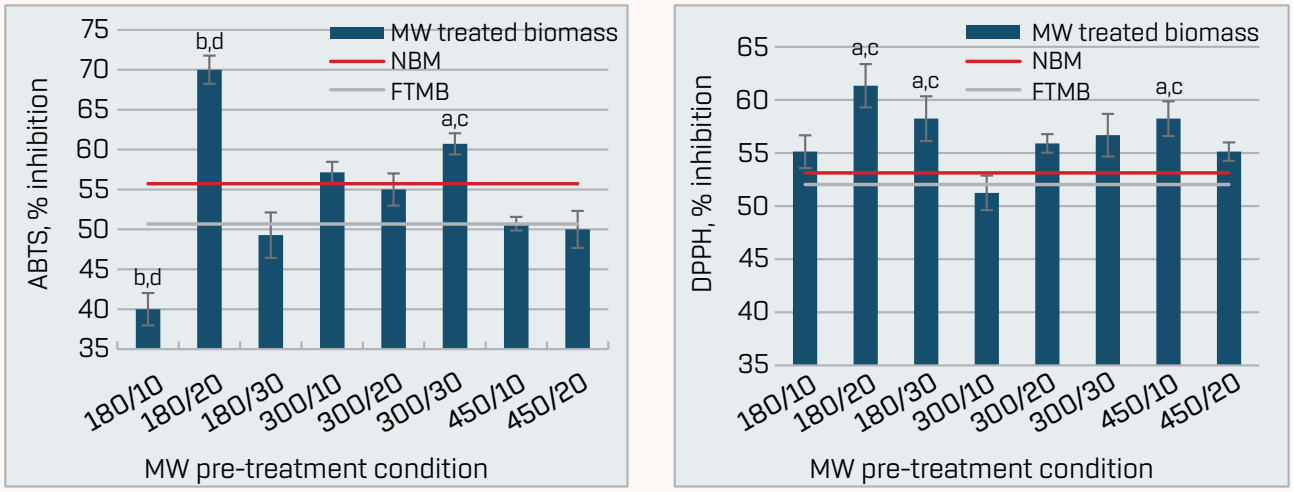

Figure 2. Antioxidant activity of the aqueous extract obtained from *Porphyridium cruentum* biomass treated with microwaves (W/sec). Control: NBM - native biomass and FTBM - freeze-thaw biomass.

a, b: significantly different from NBM at $p < 0.01$ and $p < 0.001$, respectively;

c, d: significantly different from FTBM at $p < 0.01$ and $p < 0.001$, respectively.

The highest antioxidant activity, as determined by the ABTS assay, was observed in extracts from biomass treated at 180 W for 20 seconds, showing 70.0% inhibition. Treatment at 300 W for 10 and 30 seconds resulted in ABTS inhibition levels of 57.1% and 60.7%, respectively. The lowest antioxidant activity, 40.0% inhibition, was obtained in extracts from biomass treated at 180 W for 10 seconds.

The DPPH assay revealed maximum antioxidant activity of 61.3% inhibition in extracts from biomass treated at 180 W for 20 seconds. The lowest activity, 51.2% inhibition, was recorded in extracts from biomass treated at 300 W for 10 seconds.

Extracts from native biomass demonstrated inhibition levels of 50.7% for ABTS and 52.0% for DPPH. In comparison, extracts from frozen/thawed biomass exhibited 55.7% ABTS inhibition and 53.6% DPPH inhibition.

Table 1 summarizes the protein, carbohydrate, phenolic content, and antioxidant activity of the spray-dried powder in comparison with the aqueous extracts obtained from *Porphyridium cruentum* biomass processed by different techniques.

Table 1. Composition of the spray-dried powder in comparison to the aqueous extracts from *Porphyridium cruentum* biomass, treated using various techniques.

Biomass treatment variant	Bioactive compound	Compound/activity in the extract	Compound/activity in powder	Recovering after drying
MW-180W/20 sec	Proteins (g/100g DW)	16.16±0.22	14.38±0.30*	88.99
	Carbohydrates (g/100 DW)	9.05±0.16	8.73±0.80	96.46
	Phenols (g/100g DW)	6.38±0.11	5.37±0.31**	84.17
	ABTS (% Inhibition)	70.00±3.2	66.52±3.4	95.03
	DPPH (% Inhibition)	61.33±4.1	54.55±2.5	88.95
MW-180W/30 sec	Proteins (g/100g DW)	13.28±0.16	12.08±0.80*	90.96
	Carbohydrates (g/100g DW)	9.30±0.11	8.91±0.84	95.81
	Phenols (g/100g DW)	5.82±0.18	4.36±0.22*	74.91
	ABTS (% Inhibition)	49.30±4.2	46.13±3.3	93.57
	DPPH (% Inhibition)	58.23±1.5	48.33±2.4*	82.99
MW-300W/30 sec	Proteins (g/100g DW)	13.17±0.23	11.58±0.52*	87.93
	Carbohydrates (g/100g DW)	7.17±0.21	6.82±0.85	95.12
	Phenols (g/100g DW)	7.05±0.15	5.54±0.21**	78.58
	ABTS (% Inhibition)	60.70±3.8	56.08±2.5*	92.39
	DPPH (% Inhibition)	56.60±3.1	50.77±2.8	89.70
NBM	Proteins (g/100g DW)	10.19±0.17	8.66±0.63*	84.99
	Carbohydrates (g/100g DW)	4.05±0.17	3.93±1.14	97.04
	Phenols (g/100g DW)	4.88±0.15	4.52±0.33	92.62
	ABTS (% Inhibition)	50.68 ±3.8	45.83±3.1	90.43
	DPPH (% Inhibition)	52.02±4.1	46.15±2.6	88.72
FTBM	Proteins (g/100g DW)	4.25±0.13	3.55±0.18*	83.53
	Carbohydrates (g/100g DW)	5.40±0.11	5.13±0.84	95.00
	Phenols (g/100g DW)	1.87±0.17	1,47±0.22*	78.61
	ABTS (% Inhibition)	55.71±2.5	50.11±3.3*	89.58
	DPPH (% Inhibition)	53.12±3.4	48.66±4.1	91.60

Data are presented as Mean ± (SD) of three independent experiments. The asterisks indicate statistically significant differences (*p<0.05; **p<0.01) between spray-dried powders and their corresponding aqueous extracts (Student's t-test). MW: microwave-treated biomass; NBM: native biomass; FTBM: freeze-thaw biomass.

Following the spray-drying process, carbohydrates exhibited the lowest losses, ranging from 3.54% to 5.00%. In contrast, proteins showed significantly higher losses, between 9.03% (p<0.05) and 16.47% (p<0.05). In extracts obtained from biomass subjected to microwave treatment, protein losses were comparatively lower, ranging from 11.01% (p<0.05) to 12.07% (p<0.05). Phenolic compounds were the most affected, with losses in powders ranging from 15.83% (p<0.05) to 25.09% (p<0.01). Antioxidant capacity was also reduced: DPPH radical reduction decreased by 8.40%–17.01% (p<0.05), while ABTS radical reduction declined by 4.94%–10.05% (p<0.05).

Notably, proteins in powders from biomass treated with microwaves at 180W for 20 and 30 seconds retained 88.99% and 90.96% of their bioactive components, corresponding to losses of 11.01% (p<0.05) and 9.04% (p<0.05), respectively. The ABTS radical inhibition capacity of these powders decreased only moderately, by 4.97% and 6.43%, indicating good stability of the antioxidant compounds during spray drying. In contrast, losses were more pronounced in powders from extracts of biomass treated at 300W for 30 seconds. Here, the phenolic content decreased by 21.42% (p<0.01), and the ABTS inhibition capacity decreased by 7.61% (p<0.05). The powder from the native biomass best preserved carbohydrates, retaining 97.04% of the content found in the

aqueous extract prior to spray drying, which was a minimal loss of 2.96%. However, this powder showed a 15.01% ($p<0.05$) reduction in protein content. The highest losses were observed in the powder derived from the aqueous extract of frozen/thawed biomass, with phenolic compounds decreasing by 21.39% ($p<0.05$) and proteins by 16.47% ($p<0.05$).

The powders exhibiting the highest biological value were selected for a stability study over 1, 3, and 6 months. These included powders from biomass extracts treated with microwave-assisted technology at 180 W for 20 and 30 seconds and at 300 W for 30 seconds. Powders from native and frozen/thawed biomass extracts served as reference samples. Table 2 presents the results of the stability study, showing the biochemical composition and antioxidant activity of the powders derived from aqueous extracts of *Porphyridium cruentum* biomass treated using different techniques over a storage period of 1, 3, and 6 months.

Table 2. Stability of the powder from *Porphyridium cruentum* biomass during 1, 3, and 6 months of storage.

Biomass treatment variant	Bioactive compound	Compound/ activity in the initial powder	Weight of the compound after storage, % of initial content			Compound/ activity in powder
			1 month	3 months	6 months	
MW-180W/20 sec	Proteins (g/100g DW)	14.38±0.30	98.33	97.50	96.11	13.82±0.16
	Carbohydrates (g/100g DW)	8.73±0.80	94.16	93.13	91.87	8.02±0.18
	Phenols (g/100g DW)	5.37±0.31	98.14	97.21	93.67	5.03±0.31
	ABTS (% Inhibition)	66.52±3.4	98.38	97.75	97.11	64.60±2.6
	DPPH (% Inhibition)	54.55±2.5	98.92	98.00	98.45	54.05±3.2
MW-180W/30 sec	Proteins (g/100g DW)	12.08±0.80	98.25	98.34	96.52	11.46±0.11
	Carbohydrates (g/100g DW)	8.91±0.84	97.87	95.96	94.61	8.43±0.22
	Phenols (g/100g DW)	4.36±0.22	93.12	91.97	86.47	3.77±0.27
	ABTS (% Inhibition)	46.13±3.3	98.29	97.79	96.60	44.56±2.6
	DPPH (% Inhibition)	48.33±2.4	98.40	99.03	97.74	47.24±4.1
MW-300W/30 sec	Proteins (g/100g DW)	11.58±0.52	97.06	95.94	93.61	10.84±0.21
	Carbohydrates (g/100g DW)	6.82±0.85	97.51	95.75	92.82	6.33±0.17
	Phenols (g/100g DW)	5.54±0.21	96.03	91.34	82.49	4.57±0.44
	ABTS (% Inhibition)	56.08±2.5	97.86	96.50	95.44	53.52±5.2
	DPPH (% Inhibition)	50.77±2.8	97.85	97.45	96.44	48.96±3.1
NBM	Proteins (g/100g DW)	8.66±0.63	97.23	96.30	94.92	8.22±0.17
	Carbohydrates (g/100g DW)	3.93±1.14	97.96	94.40	91.09	3.58±0.15
	Phenols (g/100g DW)	4.52±0.33	95.35	88.72	73.45	3.32±0.22
	ABTS (% Inhibition)	45.83±3.1	95.51	94.85	94.55	43.33±3.6
	DPPH (% Inhibition)	46.15±2.6	97.83	97.53	95.67	44.15±4.1
FTBM	Proteins (g/100g DW)	3.85±0.18	88.73	86.20	98.03	3.48±0.18
	Carbohydrates (g/100g DW)	5.13±0.84	98.64	94.74	94.74	4.86±0.21
	Phenols (g/100g DW)	1,47±0.22	97.96	76.87	76.87	1.13±0.22
	ABTS (% Inhibition)	50.11±3.3	98.12	97.29	97.29	48.75±3.4
	DPPH (% Inhibition)	48.66±4.1	94.57	93.88	93.88	45.68±4.2

Data are presented as Mean ± (SD). MW- microwave-treated biomass; NBM - native biomass; FTBM - freeze-thaw biomass.

The highest protein stability after 6 months of storage was observed in powders obtained from biomass extracts treated using microwave-assisted technology at 180W for 20 seconds (96.11% stability) and 180W for 30 seconds (96.52% stability).

Carbohydrate stability remained high (>91%) over 6 months in all microwave-treated samples. The highest stability was in the untreated biomass powder, at 97.96% after 1 month and 91.09% after 6 months.

Antioxidant activity was largely preserved, remaining >95% in most variants after 6 months. In contrast, powders from biomass treated at 300W for 30 seconds and from frozen/thawed biomass exhibited significant losses of bioactive compounds. This was attributed primarily to a reduction in phenolic compounds, which declined by 17.51% to 23.16% ($p<0.05$) during storage.

DISCUSSIONS

Microwave-assisted technology is an efficient and environmentally friendly method for releasing valuable components from microalgal biomass, including lipids, pigments, carbohydrates, vitamins, and proteins, either individually or as part of bioactive extract complexes (15, 16).

In this study, the intensity and duration of microwave treatment significantly influenced the biochemical composition of the aqueous extracts. Treating *Porphyridium* biomass at a lower power with a moderate exposure time (e.g., 180W for 20 sec) increased the protein and carbohydrate content compared to untreated or repeatedly frozen/thawed biomass. In contrast, more aggressive treatments (450W for 10 sec and 450W for 20 sec) substantially reduced these compounds, suggesting potential thermal degradation. Previous studies support the efficacy of microwave treatment for this purpose. For instance, one study demonstrated its efficiency in extracting proteins from *Chlorella vulgaris* using 600W for 20 seconds (17). Similarly, microwave-assisted extraction has been used to obtain carbohydrates from *Scenedesmus* sp., with the highest yield achieved at 1075W under direct acid extraction conditions for 22 minutes (18).

The analysis of phenolic content revealed that these compounds varied significantly in aqueous extracts depending on the treatment, with the maximum values observed in biomass treated at 300W for 30 seconds. This suggests that microwave-assisted extraction enhances the release of these metabolites. A similar finding was reported in a study of *Chlorella vulgaris* biomass, where a 300W treatment for 14 minutes was optimal for extracting phenolic compounds into ethanol (19). Therefore, microwave-assisted technology can optimize the yield of bioactive compounds, depending on the extraction method and intended application.

Antioxidant assays (ABTS and DPPH) indicated that biomass treated at 180W/20 sec exhibited the highest inhibition, suggesting this condition optimally releases antioxidant compounds into aqueous extracts. Conversely, more intense microwave treatments (450W/10 sec and 450W/20 sec) reduced antioxidant activity, likely due to the degradation of active metabolites. This is consistent with reports that optimized microwave-assisted extraction enhances antioxidant activity in microalgae; for example, a power of 380W yielded a significant DPPH inhibition of 17.58% in *Chlorella vulgaris* (20). Thus, moderate microwave treatment of *Porphyridium cruentum* biomass produced extracts with superior antioxidant activity compared to those from native (untreated) or repeatedly frozen/thawed biomass. These results confirm that the strategic selection of microwave power and exposure time is crucial for enhancing the bioactive potential of the aqueous extracts.

Spray drying effectively transforms microalgal biomass into fine, easily dispersible powders, making it a popular technique for various applications (3). In a study on *Scenedesmus acuminatus*, the loss of pigments during processing was influenced by the inlet and outlet air temperatures, as well as the

suspension's solid content. Despite this, the method did not significantly alter the content of proteins, carbohydrates, or lipids, demonstrating its overall effectiveness for preserving these key bioactive compounds (12).

In this study, the atomization of aqueous extracts from *Porphyridium cruentum* biomass caused only minimal losses in bioactive compounds, with maximum reductions of 11% for proteins, 5% for carbohydrates, and 39% for phenolic compounds. These losses were influenced by the biomass pretreatment. Extracts from repeatedly frozen-thawed, native, and 450W microwave-treated biomass showed the greatest degradation. The best results were obtained with powders from aqueous extracts of biomass treated with 180W microwaves for 20 and 30 seconds. These powders provided a balance between protein preservation (losses of 9.04–11.01%) and carbohydrate retention (losses of 2.96–4.19%), alongside minor losses in antioxidant activity (4.97–7.61%). For comparison, one study demonstrated that the bioactive stability of *Chlamydomonas reinhardtii* powder containing a recombinant protein was highly dependent on storage temperature. The protein was relatively stable at -80°C, losing approximately 38% over 27 months. Degradation was more pronounced at +4°C, with a 50% loss, and severe at room temperature, where a 92% loss was recorded, indicating this condition is unsuitable for long-term storage (21).

Therefore, an efficient strategy for preserving bioactive compounds from the red microalga *Porphyridium cruentum* is to use moderate-intensity microwave-assisted technology, followed by extraction and spray drying. This approach enhances the stability of the final product, and optimizing the process parameters can ensure both bioactive efficacy and long-term stability.

CONCLUSIONS

1. Microwave-assisted treatment significantly alters the extraction process and biochemical composition of aqueous extracts from the red microalga *Porphyridium cruentum*. A moderate regimen of 180W for 20–30 seconds increases the yield of proteins and carbohydrates, while a more intense treatment at 450W leads to their degradation.
2. Treating *Porphyridium cruentum* biomass with microwaves at 300W for 30 seconds enhances the extraction of phenolic compounds. Antioxidant activity is also influenced by the pretreatment parameters, peaking in extracts derived from biomass treated at 180W for 20 seconds.
3. Spray drying effectively preserved proteins and carbohydrates, with minimal losses of 9.04–11.01% and 2.96–4.19%, respectively. Phenolic compounds, however, were the most vulnerable, with losses of 15.83–25.09%.
4. After six months of storage, the powders retained a stable bioactive composition, with only a minimal reduction in antioxidant activity (4.97–7.61%). These results confirm that spray drying is an efficient method for preserving bioactive compounds from the red microalga *Porphyridium cruentum.*

CONFLICT OF INTEREST

The authors deny any conflict of interest in the publication of this material.

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Ludmila RUDI, WoS Researcher ID: AAY-3219-2020, SCOPUS ID: 55681134100

Tatiana CHIRIAC, WoS Researcher ID: AIB-8864-2022, SCOPUS ID: 38861074900

Svetlana DJUR, SCOPUS ID: 57164884800

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THE PARTICULARITIES OF ACUTE KIDNEY INJURY IN CRITICALLY ILL PATIENTS WITH COVID-19

Felicia BULAI, Pavel BANOV, Emil CEBAN

Departament of urology and surgical nephrology, *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

Corresponding author: Felicia Bulai, e-mail: felicia.bulai@gmail.com

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ABSTRACT

Introduction Acute kidney injury (AKI) is a common complication in critically ill patients with COVID-19, associated with increased mortality. This study aimed to evaluate the incidence of acute kidney injury in COVID-19 patients hospitalized in intensive care units, identify risk factors for its development, and determine the impact of AKI on mortality and clinical outcomes.

Material and methods A retrospective cohort study was conducted and included patients with confirmed COVID-19 infection, admitted to the Republican Clinical Hospital "Timofei Moșneaga" between June 01, 2020, and August 31, 2020. The association between potential risk factors and AKI was assessed using relative risk (RR) with 95% confidence intervals (CI).

Results Of the 81 patients included, 20 (24.69%) developed AKI. Significant risk factors associated with AKI included mechanical ventilation (RR = 7.96; 95% CI: 2.86–22.15, p < 0.001) and vasopressor therapy (RR = 4.12; 95% CI: 1.43–11.86, p = 0.009). The mortality rate was significantly higher in patients with AKI compared to those without AKI (90% vs. 36.06%, RR = 2.49; 95% CI: 1.85–3.36, p < 0.001).

Conclusions Acute kidney injury is a severe complication in critically ill COVID-19 patients and significantly increases mortality among patients hospitalized in intensive care units. Identifying modifiable risk factors could help optimize management strategies and improve survival rates.

Keywords Acute kidney injury, COVID-19, risk factors, intensive care units, mortality.

PARTICULARITĂȚILE LEZIUNII RENALE ACUTE LA PACIENȚII ÎN STARE CRITICĂ CU INFECȚIA COVID-19

Introducere Leziunea renală acută (LRA) este o complicație frecventă la pacienții cu COVID-19, fiind asociată cu o mortalitate crescută. În acest studiu, am avut ca obiectiv evaluarea incidenței leziunii renale acute la pacienții cu COVID-19 internați în unitățile de terapie intensivă (UTI), identificarea factorilor de risc în dezvoltarea acesteia și determinarea impactului asupra mortalității și rezultatelor clinice.

Material și metode A fost realizat un studiu retrospectiv de cohortă, care a inclus pacienți cu infecția COVID-19, internați în Spitalul Clinic Republican „Timofei Moșneaga" în perioada 01 iunie 2020-31 august 2020. Asocierea dintre factorii de risc și LRA a fost evaluată utilizând Relative Risk (RR) cu intervale de încredere (CI) de 95%.

Rezultate Dintre cei 81 de pacienți incluși, 20 (24,69%) au dezvoltat LRA. Factorii de risc semnificativi asociați cu LRA au inclus ventilația mecanică (RR = 7,96; 95% CI: 2,86–22,15, p < 0,001) și terapia vasopresoare (RR = 4,12; 95% CI: 1,43–11,86, p = 0,009). Rata mortalității a fost semnificativ mai mare la pacienții cu LRA comparativ cu cei fără (90% vs. 36,06%, RR = 2,49; 95% CI: 1,85–3,36, p < 0.001).

Concluzii Leziunea renală acută este o complicație severă la pacienții cu COVID-19 și crește semnificativ mortalitatea în rândul celor spitalizați în UTI. Identificarea factorilor de risc modificabili ar putea contribui la optimizarea strategiilor de conduită și la îmbunătățirea ratei de supraviețuire.

Cuvinte-cheie Leziune renală acută, COVID-19, factori de risc, unități de terapie intensivă, mortalitate.

INTRODUCTION

Coronaviruses are a large family of viruses that cause diseases ranging from the common cold to more severe illnesses, such as Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome. COVID-19 is a new disease first identified in 2019 (1). Infection with the novel coronavirus (COVID-19) is a pandemic infection caused by the SARS-CoV-2 virus (2). On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a global pandemic (3).

Clinically, the features of COVID-19 range from asymptomatic disease to acute respiratory distress syndrome (ARDS) and multi-organ dysfunction. The most common clinical features include cough, fever, headache, sore throat, fatigue and dyspnea (4). Although the respiratory system is the major target of COVID-19 (5), the disease can also have repercussions on other organs, including the kidneys (6). Renal involvement in COVID-19 infection is common (7).

Although initial reports from China suggested relatively low rates of renal involvement, subsequent reports from Europe and the USA indicate much higher rates of acute kidney injury, particularly in the intensive care unit (ICU) setting (8), with an incidence ranging from 0.5% to 80% (9). Patients with COVID-19 have increased need for renal replacement therapy, ICU admission and mechanical ventilation (10). Current research suggests that mortality among hospitalized patients with renal impairment is higher compared to those without renal impairment (7, 8, 10).

During the COVID-19 pandemic, our institution observed a notable increase in COVID-19 hospitalizations. At the same time, we noticed an alarming number of COVID-19 patients who developed acute kidney injury.

This study aimed to evaluate the incidence of acute kidney injury in COVID-19 patients hospitalized in intensive care units, identify risk factors for its development, and determine the impact of AKI on mortality and clinical outcomes.

MATERIAL AND METHODS

Study setting

The study was approved by the institutional ethics committee, and authorization was obtained to access patient records and archives.

A retrospective cohort study was conducted, including 81 patients with confirmed COVID-19 hospitalized in the intensive care units at the Republican Clinical Hospital “Timofei Moșneaga” between June 1, 2020, and August 31, 2020.

Study population

The inclusion criteria for the study were: patients with COVID-19, diagnosed by real-time PCR technique and admitted to the Republican Clinical Hospital “Timofei Moșneaga” during the study period. Exclusion criteria were patients aged < 18 or >80 years, chronic kidney disease stage IV-V, patients transfer to other institutions and monitoring for less than 48 h, or insufficient clinical data for evaluation.

Patients were followed from hospital admission until discharge or death. AKI was considered the primary exposure, while mortality, ICU length of stay, and need for renal replacement therapy were the main outcomes of inter-

est. Patients who did not develop AKI were defined as group I (No AKI) and patients who developed AKI were defined as group II (With AKI). The study design is illustrated in Figure 1.

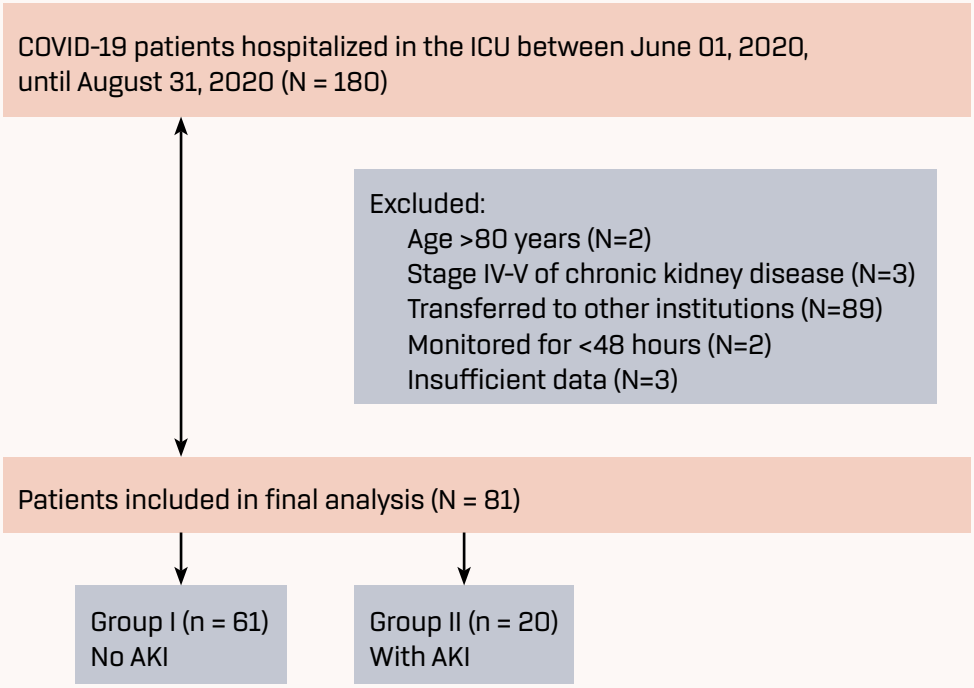

Figure 1. Study design.

Staging of acute kidney injury was performed by serum creatinine level, according to the Kidney Disease Improving Global Outcomes (KDIGO) classification. Thus, an increase in serum creatinine (SCr) values by 1.5-1.9 times from baseline was classified as AKI stage I, an increase in SCr by 2-2.9 times from baseline was classified as AKI stage II, and an increase in SCr by 3 times from baseline or a decrease in GFR to 35 ml/min/1.73 m^2 was classified as AKI stage III.

Data collection and research tools

Data were extracted from the electronic patient records and compiled into an electronic database. The following data were collected: patients' demographics, duration of hospitalization, mode of discharge, comorbidities (cardiovascular disease, cerebrovascular disease, chronic kidney disease, diabetes, body mass index), treatments administered and the impact of aggressive factors on renal function during intensive care unit admission (mechanical ventilation, renal replacement therapy, vasoactive therapy, antiviral and nephrotoxic medication.

Data analysis

Continuous variables were analyzed using the Student's t-test, while categorical variables were assessed using the chi-square test or Fisher's exact test, as appropriate. Relative risk (RR) with 95% confidence intervals (CI) was calculated to evaluate associations between AKI and potential risk factors. Statistical significance was defined as $p < 0.05$.

RESULTS

Patient Characteristics

The age of the study participants ranged from 29 to 77 years, with a mean of 58.8 ± 10.6 years. The age distribution is shown in Figure 2.

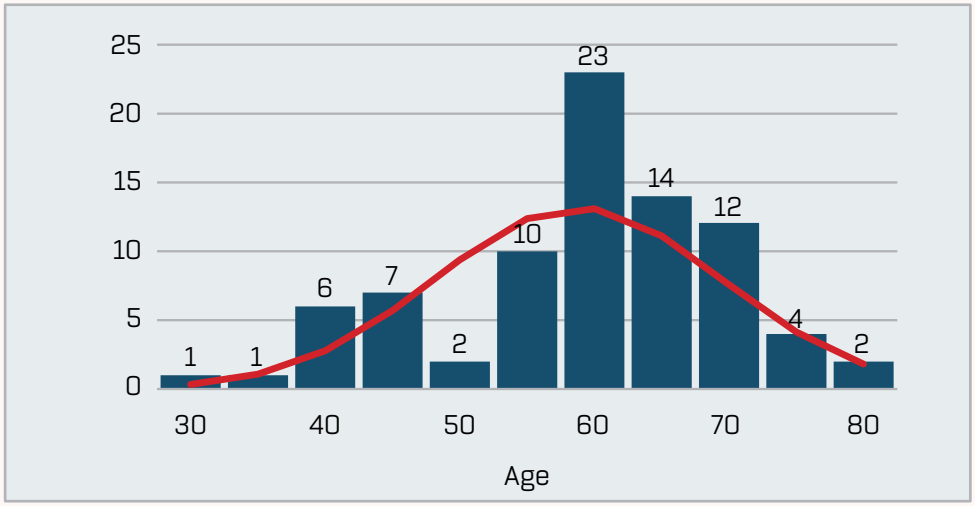

Figure 2. Distribution of subjects according to age.

Thus, Figure 2 shows that most of the subjects included in the study were aged between 60-70 years, indicating that elderly patients develop severe forms of COVID-19, requiring follow-up and treatment in intensive care units.

Of the 81 patients included in the study, 49 (60.49%) were men and 32 (39.50%) were women. Various studies have reported that the rate of COVID-19 infection is relatively similar in both males and females, but the mortality rate is higher (1.5 to 2.5 times) in males. Studies have suggested that sex-based differences in hormonal and immune response may be responsible for the higher mortality in men. Similarly, sexual dimorphism is also common in acute kidney injury. In AKI, males had 2.19 times higher rate of developing AKI than females (11). In our study, also the mortality rate among the subjects included in the study was higher in males (72.2% vs. 27.8%).

The most common comorbidity was cardiovascular disease (81.48%), followed by obesity (48.14%), diabetes (38.27%), cerebrovascular disease (20.98%), enterocolitis (20.98%) and chronic kidney disease (CKD) (2.46%) (fig. 3).

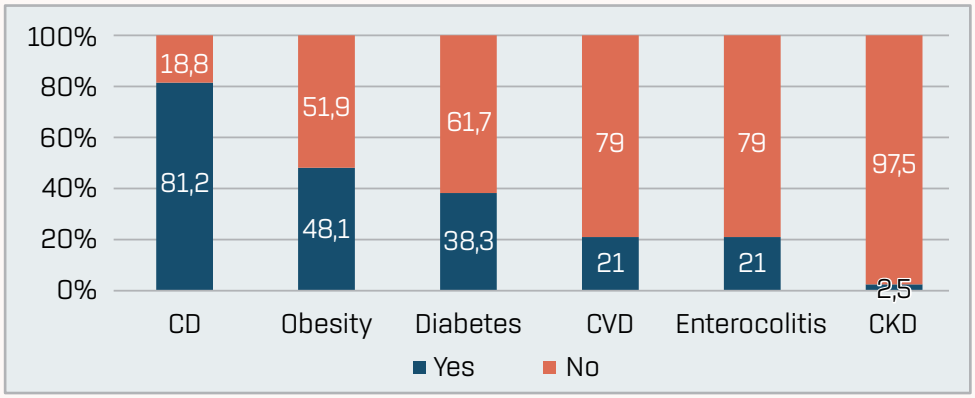

Figure 3. Comorbidities of the patients, %.

Legend: CD – cardiac disease, CVD – cerebrovascular disease; CKD – Chronic Kidney Disease.

Treatments

Out of all patients, 85.18% received nephrotoxic medication, 59.25% were intubated, 41.97% received antiviral medication, 34.56% vasopressor treatment and 30.86% diuretics. Renal replacement therapy required 4 patients (20%).

Clinical characteristics and treatments administered to patients during hospitalization in intensive care units are shown in Table 1.

Table 1. Clinical characteristics and treatment of COVID-19 patients.

Characteristics	All patients, n = 81	Patients with AKI, n = 20	Patients without AKI, n = 61	P value
Demographic Data				
Age, mean (range)	58.8 (29-77)	64.7 (55-72)	56.85 (29-77)	<0,001
Male sex, n (%)	49 (60.49%)	14 (70%)	35 (57.37%)	0.32
Duration of hospitalization, days (range)	18.89 (5-46)	18.71 (7-46)	18.03 (5-45)	0.78
Chronic Diseases, n (%)				
Cardiovascular diseases	66 (81.48%)	18 (90%)	48 (78.68%)	0.25
Chronic kidney disease	2 (2.46%)	1 (5%)	1 (1.63%)	0.42
Cerebrovascular diseases	17 (20.98%)	3 (15%)	14 (22.95%)	0.55
Diabetes	31 (38.27%)	9 (45%)	22 (36.06%)	0.45
Obesity	39 (48.14%)	11 (55%)	28 (4.9%)	0.47
Enterocolitis	17 (20.98%)	4 (20%)	13 (21.31%)	1.00
Treatments, n (%)				
Mechanical ventilation	48 (59.25%)	19 (95%)	29 (47.54%)	<0.001
Renal replacement therapy	4 (20%)	4 (20%)	-	-
Diuretics	25 (30,86%)	16 (80%)	9 (14.75%)	<0.001
Vasopressor treatment	28 (34.56%)	13 (65%)	15 (24.59%)	<0.001
Nephrotoxic medication	68 (85.18%)	17 (85%)	45 (73.77%)	0.38
Antiviral treatment	34 (41.97%)	14 (70%)	20 (32.78%)	0.002

Note: Statistical significance was assessed between groups (patients with AKI and patients without AKI) using the two-tailed Student's t-test for continuous variables (e.g., age, duration of hospitalization) and the chi-square test or Fisher's exact test for categorical variables, depending on the expected frequencies. Statistically significant differences (p < 0.05) are indicated in bold. AKI – acute kidney injury.

Acute kidney injury and risk factors

Of the 81 patients, 20 patients (24.69%) had acute kidney injury, of which 5 developed stage I AKI (25%), 7 (35%) developed stage II and 8 (40%) stage III, according to the KDIGO classification (fig. 4). Among the patients with AKI and available urine analysis, 65% had proteinuria, and 45% had hematuria. In a prospective cohort study that included 701 hospitalized patients infected with COVID-19, the prevalence of proteinuria and hematuria on hospital admission was 44 and 27%, respectively (12).

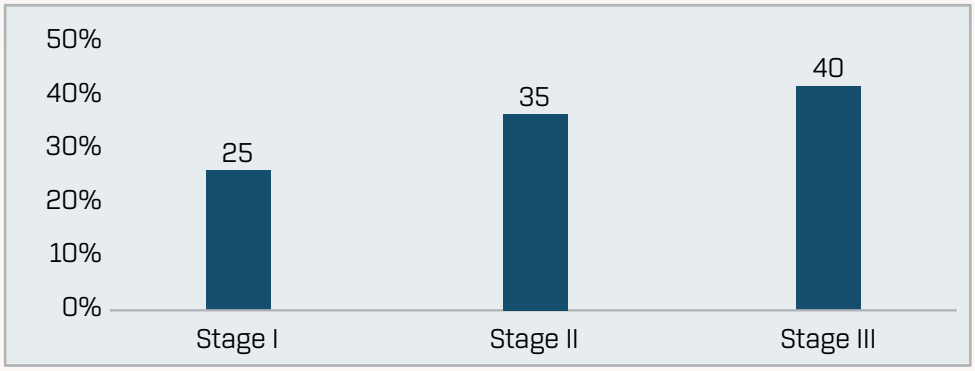

Figure 4. Staging of acute kidney injury, %.

Significant risk factors for the development of acute kidney injury during hospitalization were mechanical respiration and vasopressor therapy (tab. 2).

Table 2. Risk Factors for Acute Kidney Injury in patients with COVID-19.

Risk factor	Relative Risk (RR)	95% CI	p-value
Male sex	1.37	0.67–2.81	0.49
Mechanical ventilation	7.96	2.86–22.15	**<0.001**
Vasopressor therapy	4.12	1.43–11.86	**0.009**
Obesity	1.24	0.57–2.70	0.58
Diabetes mellitus	1.25	0.56–2.78	0.58

Note: *Relative Risk (RR) with 95% confidence intervals (CI) was calculated to assess the association between risk factors and the development of acute kidney injury (AKI). Statistically significant differences (p < 0.05) are indicated in bold.*

Male patients had a 1.37 times higher risk of developing AKI compared to female patients; however, this association was not statistically significant (RR = 1.37; 95% CI: 0.67–2.81, p = 0.49). Patients requiring mechanical ventilation had a 7.96 times higher risk of developing AKI and a statistically significant association was observed (RR = 7.96; 95% CI: 2.86–22.15, p < 0.001). The use of vasopressors was associated with a 4.12 times higher risk of AKI, which was statistically significant (RR = 4.12; 95% CI: 1.43–11.86, p = 0.009). Obesity was not significantly associated with an increased risk of AKI (RR = 1.24; 95% CI: 0.57–2.70, p = 0.58). Diabetes mellitus was not significantly associated with AKI in this cohort (RR = 1.25; 95% CI: 0.56–2.78, p = 0.58).

Complications and Mortality

The most common complication during hospitalization was disseminated intravascular coagulation (DIC) syndrome (33.3%), followed by secondary infections (30.9%), liver injury (29.6%), sepsis (19.8%), septic shock (14.8%), and cardiac injury (11.1%) (tab. 3).

Table 3. Complications and outcomes of patients with COVID-19.

Variable	All patients, N = 81	Patients with AKI, N = 20	Patients without AKI, N = 61	RR	95% CI	p-value
Complications, n (%)						
Sepsis	16 (19.75%)	11 (55%)	5 (8.19%)	6.71	2.60–17.30	**<0.001**
Septic shock	12 (14.81%)	10 (50%)	2 (3.27%)	15.29	3.60–64.90	**<0.001**
DIC	27 (33.33%)	7 (35%)	20 (32.78%)	1.07	0.55–2.08	0.84
Cardiac injury	9 (11.11%)	6 (30%)	3 (3.7%)	8.11	2.25–29.20	**<0.001**
Liver injury	24 (29.62%)	12 (60%)	12 (19.67%)	3.05	1.75–5.32	**<0.001**
Secondary infections	25 (30.86%)	11 (55%)	14 (22.95%)	2.40	1.40–4.10	**<0.01**
Discharge type, n (%)						
In-hospital death	40 (49.38%)	18 (90%)	22 (36.06%)	2.49	1.85–3.36	**<0.001**
Discharged	41 (50.61%)	2 (10%)	39 (63.93%)	0.16	0.04–0.60	**<0.01**

Note: DIC – disseminated intravascular coagulation.

The relative risk (RR) of developing complications was significantly higher in patients with AKI compared to those without AKI. Patients with AKI had a 3.05 times higher risk of liver injury compared to those without AKI (60% vs. 19.67%, RR = 3.05; 95% CI: 1.75–5.32, $p < 0.001$). The risk of cardiac injury was 8.11 times higher in patients with AKI (30% vs. 3.7%, RR = 8.11; 95% CI: 2.25–29.20, $p < 0.001$). Patients with AKI had a 2.40 times higher risk of developing secondary infections (55% vs. 22.95%, RR = 2.40; 95% CI: 1.40–4.10, $p < 0.01$). The risk of sepsis was 6.71 times higher in patients with AKI (55% vs. 8.19%, RR = 6.71; 95% CI: 2.60–17.30, $p < 0.001$). Patients with AKI had a 15.29 times higher risk of septic shock compared to those without AKI (50% vs. 3.27%, RR = 15.29; 95% CI: 3.60–64.90, $p < 0.001$).

The mortality rate was significantly higher in patients with AKI compared to those without AKI (fig. 5). Patients with AKI had a 2.49 times higher risk of in-hospital mortality (90% vs. 36.06%, RR = 2.49; 95% CI: 1.85–3.36, $p < 0.001$). The mortality rate also increased with the severity of AKI, as classified by the KDIGO staging system. Among AKI patients, mortality was highest in stages II-III ($p<0.05$) (fig. 6). For stage I AKI, the mortality rate was 60% (RR = 1.66; 95% CI: 1.10–2.50, $p = 0.02$). For stage II AKI, the mortality rate was 85.71% (RR = 2.38; 95% CI: 1.65–3.42, $p < 0.001$). For stage III AKI, the mortality rate was 100% (RR = 2.77; 95% CI: 2.10–3.65, $p < 0.001$).

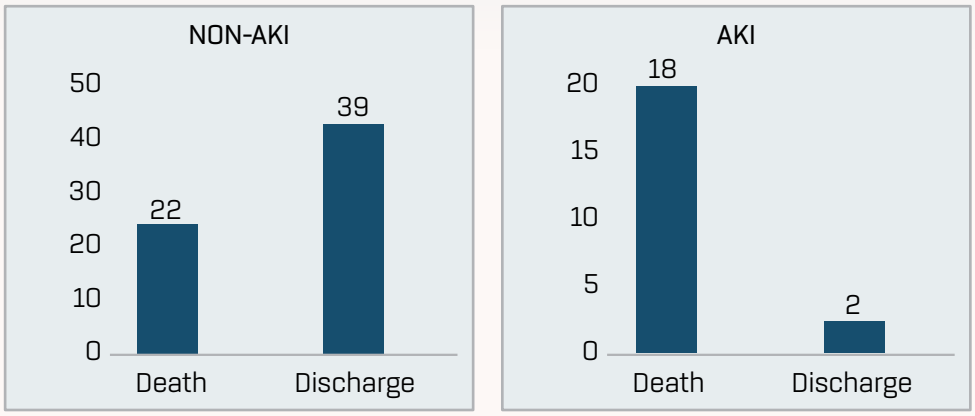

Figure 5. Patient mortality rate, n.

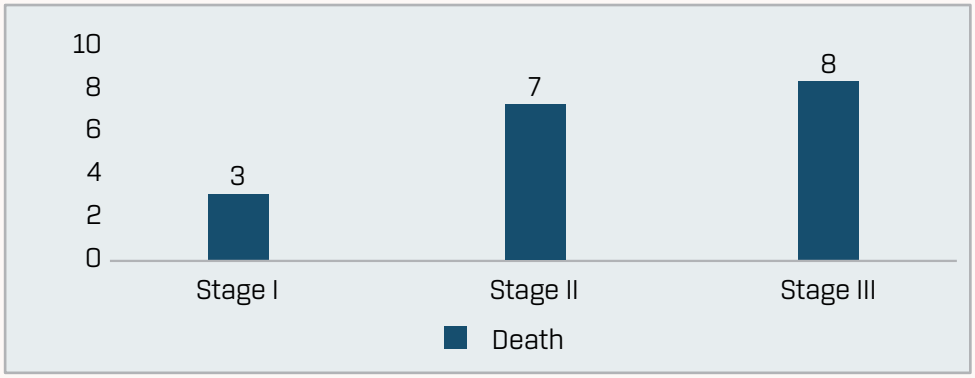

Figure 6. Mortality rate by AKI staging, n.

DISCUSSIONS

This study has several limitations, including its retrospective design and relatively small sample size, which may limit the generalizability of the findings.

According to previous studies, risk factors associated with the development of acute kidney injury include: advanced age, multiple comorbidities (particularly diabetes, hypertension, and cardiovascular disease), high body mass index, non-O blood group, and African American race (13). Chronic kidney disease (CKD) is a well-recognized risk factor for AKI in hospitalized patients (8). AKI occurred nearly three times more often in patients with pre-existing kidney disease (11.4% vs. 4%) (13) and has been identified as the most relevant risk factor for AKI requiring renal replacement therapy in critically ill COVID-19 patients (8). Higher rates of AKI have consistently been documented in critically ill populations (13).

Additional factors associated with an increased risk of AKI on admission include leukocytosis, leukopenia, elevated inflammatory markers (ferritin, C-reactive protein [CRP], D-dimer), severity of COVID-19, viremia, respiratory status, hypovolemia, rhabdomyolysis, medications (e.g., NSAIDs), and non-respiratory involvement such as diarrhea. Risk factors for AKI developing during hospitalization include the use of vasopressors, mechanical ventilation, and fluid overload or hypovolemia (12). Nephrotoxic medications may also contribute, particularly antibiotics, antiviral therapy, and traditional medicines (14).

The pathogenesis of kidney injury in COVID-19 is multifactorial (6). Both direct and indirect mechanisms have been implicated (7), such as hemo-

dynamic disturbances, endothelial dysfunction, hypercoagulation, altered microcirculation, nephrotoxic exposure, the impact of invasive mechanical ventilation (15), local and systemic inflammatory response, and renin-angiotensin-aldosterone system (RAAS) imbalance (6). However, there is emerging evidence that additional infection-specific factors also play an important role (15).

Our findings align with previous studies showing that AKI is a significant predictor of mortality in critically ill COVID-19 patients. Mechanical ventilation and vasopressor therapy were major contributors to AKI development.

CONCLUSIONS

1. Acute kidney injury is a serious complication in critically ill COVID-19 patients and is strongly associated with increased mortality.
2. Early identification of high-risk patients, particularly those requiring mechanical ventilation and vasopressor therapy, is crucial for timely intervention.
3. This study emphasizes the importance of continuous monitoring and personalized treatment strategies to reduce the risk of AKI and improve outcomes in hospitalized COVID-19 patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The study was approved by the Research Ethics Committee of the State University of Medicine and Pharmacy “Nicolae Testemitanu” (Decision no. 76, of 14.11.2022).

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MEDICAL SCIENCES

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COMORBIDITY BURDEN IN PATIENTS WITH PSORIATIC ARTHRITIS

Lucia DUTCA[1], Eugeniu RUSSU[1,2], Liliana GROPPA[1]

[1]*Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
[2]*Timofei Moșneaga* Republican Clinical Hospital, Chisinau, Republic of Moldova

Corresponding author: Lucia Dutca, e-mail: lucia.dutca@usmf.md

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ABSTRACT

Introduction Psoriatic arthritis (PsA) is a chronic inflammatory disease affecting both the skin and musculoskeletal system, characterized by significant clinical heterogeneity and a substantial comorbidity burden. PsA is associated with increased cardiovascular, metabolic, and autoimmune conditions, yet regional data remain scarce. This study aims to assess the prevalence and impact of comorbidities in PsA patients compared to those with psoriasis alone (PsO).

Material and methods A prospective cohort study with retrospective components was conducted between 2017 and 2019, including 184 patients: 92 with PsA and 92 with PsO. Clinical, laboratory, and imaging data were analyzed. The prevalence of comorbidities was compared between groups using statistical tests, and logistic regression was applied to identify independent predictors.

Results Comorbidities were significantly more frequent in PsA (77.2%) than PsO (48.9%) ($p<0.05$). The prevalence of hypertension (38% vs. 19.6%), osteoarthritis (39.1% vs. 19.6%), type 2 diabetes (8.7% vs. 4.3%), and obesity (25% vs. 13%) was markedly higher in PsA. Increased rates of cardiovascular risk factors, metabolic syndrome, and autoimmune thyroiditis were also identified. This comorbidity burden may reflect a systemic inflammation, thus emphasizing the need for early intervention.

Conclusions PsA is a systemic disease with a substantial comorbid burden. A multidisciplinary approach integrating rheumatology, cardiology, and endocrinology is crucial to optimizing patient outcomes. Early recognition and proactive management of comorbid conditions are essential to mitigate long-term disease complications.

Keywords Psoriatic arthritis, comorbidities, cardiovascular disease, metabolic syndrome.

POVARA COMORBIDITĂȚILOR ÎN ARTRITA PSORIAZICĂ

Introducere Artrita psoriazică (APs) este o boală inflamatorie cronică care afectează pielea și sistemul musculo-scheletal, având o mare variabilitate clinică și un impact crescut al comorbidităților. APs este asociată cu afecțiuni cardiovasculare, metabolice și autoimune, însă datele regionale în cazul dat sunt limitate. Acest studiu evaluează prevalența și impactul comorbidităților la pacienții cu APs, comparativ cu cei cu psoriazis fără artrită (PsO).

Material și metode Au fost analizate date clinice, de laborator și imagistice. Studiu de cohortă prospectiv, având componente retrospective, realizat între 2017-2019, a inclus 184 de pacienți: 92 cu APs și 92 cu PsO. Prevalența comorbidităților a fost comparată între grupuri, utilizând teste statistice și regresia logistică.

Rezultate În cadrul studiului s-a constatat că, comorbiditățile au fost mai frecvente la APs (77,2%) decât la PsO (48,9%) ($p<0,05$); iar hipertensiunea (38% vs. 19,6%), osteoartrita (39,1% vs. 19,6%), diabetul zaharat tip 2 (8,7% vs. 4,3%) și obezitatea (25% vs. 13%) au fost semnificativ mai ridicate la APs, comparativ cu PsO. Factorii de risc cardiovasculari, metabolici și tiroidita autoimună au fost mai prevalenți, iar inflamația sistemică contribuie, probabil, la această povară a comorbidităților, impunând intervenții precoce.

Concluzii APs este o afecțiune sistemică cu un impact crescut al comorbidităților. Abordarea multidisciplinară, cu aspecte din reumatologie, cardiologie și endocrinologie, este esențială pentru optimizarea rezultatelor pacienților. Recunoașterea timpurie și gestionarea proactivă a comorbidităților sunt necesare pentru a preveni complicațiile pe termen lung.

Cuvinte-cheie Artrita psoriazică, comorbidități, boli cardiovasculare, sindromul metabolic.

INTRODUCTION

Psoriatic arthritis (PsA) is a complex inflammatory disease that affects both the skin and the musculoskeletal system, presenting significant clinical heterogeneity (1). The interplay between immune dysregulation, genetic predisposition, and environmental factors contributes to its pathogenesis, yet many aspects of the disease remain poorly understood (2). Epidemiological studies indicate that PsA affects approximately 6–39% of psoriasis (PsO) patients, with prevalence varying based on geographic and demographic factors (1). European data from the EuroPSO study suggest an association between PsO and arthritis in up to 30% of cases, while US studies report a lower prevalence of 11% (1,2). This variability highlights the need for region-specific research to elucidate the burden of PsA among different populations.

The chronic and progressive nature of PsA results in significant joint damage and disability, contributing to a higher morbidity and mortality rate (3). Structural joint damage, including erosive and deforming changes, occurs in 40–60% of patients, often leading to irreversible functional impairment (4). Moreover, the inflammatory burden in PsA extends beyond the musculoskeletal system, contributing to an increased prevalence of cardiovascular disease, metabolic syndrome, type 2 diabetes, obesity, osteoporosis, and mental health disorders such as depression and anxiety (5). The presence of these comorbidities not only worsens disease progression but also complicates therapeutic decision-making and reduces the overall quality of life of affected individuals (6).

The systemic inflammation characteristic of PsA plays a crucial role in driving metabolic and cardiovascular complications (7). Recent studies suggest that psoriasis as an independent risk factor for cardiovascular disease, with PsA patients exhibiting an even higher prevalence of hypertension, atherosclerosis, and insulin resistance (8). Compared to patients with rheumatoid arthritis or the general population, PsA patients tend to have a higher body mass index (BMI) and an increased prevalence of metabolic syndrome (9). The underlying immunological mechanisms linking PsA with these systemic conditions are still under investigation, though the role of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-17 (IL-17), and interleukin-23 (IL-23) in mediating both joint and systemic inflammation is increasingly recognized (10).

Despite advancements in understanding the immunopathogenesis of PsO and PsA, significant knowledge gaps remain regarding the differential impact of comorbidities on disease severity and progression. Current studies suggest that PsA patients have a higher prevalence of neurological, hepatic, and gastrointestinal comorbidities compared to those with PsO alone. However, the exact mechanisms underlying these associations remain unclear, highlighting the need for further research into the inflammatory pathways involved (11, 12).

In Moldova, the prevalence and impact of comorbidities in patients with PsA have not been systematically assessed compared to those with PsO alone. Given the growing recognition of PsA as a multisystemic disease, there is an urgent need to evaluate its comorbid burden in local populations. A comprehensive understanding of the interplay between systemic inflammation and comorbid conditions will allow for improved disease stratification and the development of targeted, multidisciplinary management approaches.

Patients with PsA are hypothesized to exhibit a significantly higher prevalence and a broader range of comorbidities than those with PsO, with these comorbidities being closely associated with systemic inflammation and disease severity.

This study aims to bridge this knowledge gap by investigating the impact of comorbidities on clinical severity, inflammatory markers, and quality of life in PsA patients. The analysis will provide insights into how systemic inflammation, disease activity, and functional impairment correlate with the presence and severity of comorbid conditions, ultimately guiding the development of personalized therapeutic strategies and optimizing long-term patient outcomes.

MATERIAL AND METHODS

This study was a prospective cohort analysis with retrospective components, conducted between 2017 and 2019 at the Rheumatology and Arthrology Departments of MPHI Republican Clinical Hospital "Timofei Moșneaga" and MPHI Municipal Clinical Hospital "Sfânta Treime," as well as at the Republican Dermatovenerology Dispensary from Chișinău. The study included 184 patients, divided into two equal groups:

1. Psoriatic Arthritis Group (APs, n=92) – patients diagnosed with psoriatic arthritis (PsA) according to the CASPAR criteria (2006).
2. Psoriasis-only Group (PsO, n=92) – patients with confirmed PsO vulgaris, without clinical or imaging evidence of arthritis.

Informed consent was obtained from all participants prior to their inclusion in the study.

Eligibility for inclusion required an age between 18 and 65 years and a confirmed diagnosis of either psoriatic arthritis (PsA) or psoriasis (PsO) without arthritis. Exclusion criteria included the presence of other inflammatory or autoimmune rheumatic diseases, severe cardiovascular, hepatic, or renal comorbidities preceding the onset of PsA, and age outside the predefined range due to the higher incidence of age-related metabolic and degenerative disorders.

The PsA group had a mean age of 42.9±9.6 years (range 22–60), with 45.7% being male and 54.3% female. The median duration of PsA was 7 years (IQR 2–11.8), while PsO had been present for a median of 11 years (IQR 7–25.8). The severity of cutaneous involvement, as assessed by the PASI score, had a median value of 3.8 (IQR 1.2–9.6). Nail involvement was documented in 28.3% of cases. Metabolic parameters were characterized by a prevalence of obesity (BMI >30 kg/m^2) of 25%. Anthropometric measurements revealed median waist and hip circumferences of 95 cm (IQR 82.8–104) and 102.5 cm (IQR 95.3–109.8), respectively. A positive family history of PsO was reported in 33.7% of patients. Axial involvement was documented in 32.6% of patients, with sacroiliitis in 30% and spondylitis in 25%.

The PsO-only group had a comparable mean age of 43.2±9.3 years (range 21–60), with 47.8% male and 52.2% female. The median duration of PsO was 11 years (IQR 7–24.5), with a median PASI score of 3.7 (IQR 1.1–9.2). Obesity was present in 13% of patients, with median waist and hip circumferences of 93 cm (IQR 81.5–102) and 101 cm (IQR 94–108), respectively. A positive family history of PsO was recorded in 28.2% of cases. Unlike the PsA group, none of these patients exhibited axial involvement or clinical arthritis. Comorbidities were identified in 48.9% of the cohort, with hypertension and osteoarthritis being the most prevalent, each occurring in 19.6% of cases.

Clinical assessment included the evaluation of joint and entheseal involvement, with tender and swollen joint counts being documented. Inflammatory

markers such as high-sensitivity C-reactive protein (hs-CRP) and erythrocyte sedimentation rate (ESR) were measured. Metabolic parameters, including fasting glucose, lipid profile, and HbA1c, were analyzed. Cardiovascular status was assessed through blood pressure measurements, electrocardiography (ECG), and echocardiography in selected cases.

Comorbidities were evaluated based on patient history, medical records, and laboratory findings. The frequency and distribution of hypertension, diabetes mellitus, cardiovascular disease, metabolic syndrome, and other relevant conditions were compared between the two groups. Functional status was assessed using validated instruments measuring joint mobility, pain, and quality of life.

Statistical analyses included descriptive methods for summarizing data, with continuous variables reported as mean ± standard deviation or median (IQR), and categorical data as percentages. Continuous variables were compared using the Mann-Whitney U test, whereas categorical data were analyzed with the chi-square test. To identify independent predictors of comorbidities in PsA patients, logistic regression models were employed. This approach enabled a comprehensive assessment of the comorbidity burden and its impact on disease severity through a direct comparison of PsA and PsO-only patient cohorts.

RESULTS

Comorbid conditions are frequently encountered in both psoriatic arthritis (PsA) and cutaneous psoriasis (PsO), though their prevalence and type vary significantly between these two groups. This comparative study analyzed comorbidity profiles in two cohorts of 92 patients each, highlighting differences in disease burden and estimating an incidence and prevalence approximately 1.5–2 times lower in the PsO group. The presence of comorbidities was markedly more frequent in the PsA cohort, affecting 77.2% of patients. Among these, hypertension (38.0%) and osteoarthritis (39.1%) were the most commonly identified conditions.

Comorbidity structure in patients with PsO

Among the 92 PsO patients, comorbidities were significantly less frequent, with 48.9% (45 patients) having at least one comorbidity and 32.6% (30 patients) presenting multiple comorbid conditions. Musculoskeletal and connective tissue disorders included osteoarthritis (OA) in 19.6% (18 patients) and gout in only 1.1% (1 patient). Cardiovascular comorbidities were less frequent, with hypertension (HTN) diagnosed in 19.6% (18 patients), chronic heart failure (CHF) in 3.3% (3 patients), angina pectoris in 2.2% (2 patients), and post-infarction cardiosclerosis in 1.1% (1 patient). Gastrointestinal diseases were less common in PsO than in PsA, with upper gastrointestinal disorders and hepatic pathology both observed in 7.6% (7 patients). Type 2 diabetes mellitus (T2DM) was diagnosed in 4.3% (4 patients), while thyroid disorders affected 6.5% (6 patients), including autoimmune thyroiditis in 3.3% (3 patients). Obesity (BMI >30) was present in 13% (12 patients). Uveitis was rare, occurring in only 1.1% (1 patient), and Crohn's disease was absent.

Comorbidity structure in patients with PsA

In contrast, PsA patients exhibited a significantly higher prevalence of comorbidities ($p<0.01$) compared with PsO patients, with notable differences in cardiovascular, musculoskeletal, and endocrine disorders. Systemic inflammation in PsA Is likely to increase the risk of comorbid conditions, emphasizing the need for comprehensive medical monitoring in this population.

Table 1 summarizes the comparative distribution of comorbidities between PsA and PsO patients. The overall comorbidity prevalence in PsA was 77.2%, compared with 48.9% in PsO, showing a prevalence ratio of 1.58. This suggests that PsA patients have a 58% higher incidence of comorbidities than PsO patients.

Table 1. Prevalence of Comorbidities in PsA and PsO Patients.

Comorbidity	PsA (n=92)	PsO (n=92)	Prevalence Ratio (PsA/PsO)	p-value
Total comorbidities	77.2%	48.9%	1.58	<0.05
Osteoarthritis	39.1%	19.6%	1.99	<0.05
Gout	3.3%	1.1%	3.00	<0.01
Hypertension	38%	19.6%	1.94	<0.05
Chronic heart failure	6.5%	3.3%	1.97	<0.05
Type 2 diabetes mellitus	8.7%	4.3%	2.02	<0.01
Autoimmune thyroiditis	5.4%	3.3%	1.64	<0.05
Obesity	25%	13%	1.92	<0.05

Comorbidity burden and clinical implications

Osteoarthritis was diagnosed in 39.1% of PsA patients compared with 19.6% of PsO patients (prevalence ratio: 1.99), indicating that PsA patients were nearly twice as likely to develop OA. Chronic inflammation in PsA likely contributes to joint degeneration and increased susceptibility to OA. Similarly, gout was three times more common in PsA (3.3%) than in PsO (1.1%) ($p<0.01$), reflecting a greater predisposition to metabolic dysregulation and hyperuricemia in PsA.

Hypertension was significantly more prevalent in PsA (38%) than in PsO (19.6%), with a prevalence ratio of 1.94. Chronic inflammation in PsA is a known contributor to endothelial dysfunction and vascular stiffness, exacerbating the risk of hypertension. The prevalence of chronic heart failure was also higher, nearly doubling from 3.3% in PsO to 6.5% in PsA (prevalence ratio: 1.97). This discrepancy may be explained by the concomitant effects of systemic inflammation and cardiovascular risk factors.

Endocrine and metabolic disorders were also found to be more prevalent among patients with PsA. Type 2 diabetes mellitus was diagnosed in 8.7% of PsA patients versus 4.3% of PsO patients (prevalence ratio: 2.02). This increased risk is likely attributable to chronic inflammation and associated insulin resistance. Autoimmune thyroiditis affected 5.4% of PsA patients and 3.3% of PsO patients, with a prevalence ratio of 1.64, suggesting a stronger predisposition for autoimmune disorders in PsA.

Obesity was significantly more common in PsA (25%) compared with PsO (13%) (prevalence ratio: 1.92). The relationship between chronic inflammation and increased adiposity is well-documented, as inflammatory cytokines such as TNF-α and IL-6 promote metabolic dysregulation and fat accumulation, which in turn exacerbate systemic inflammation.

Graphical representation of comorbidity distribution

Figure 1 illustrates the proportional burden of comorbidities in PsA relative to PsO, with a prevalence ratio scale highlighting the increased risk in PsA.

- A ratio of 1.0 indicates an equivalent prevalence in both groups.
- Ratios greater than 1 indicate a higher prevalence in PsA, demonstrating the disproportionate comorbidity burden in this group.
- The highest prevalence ratio (3.00) was observed for gout, emphasizing the strong metabolic component of PsA.

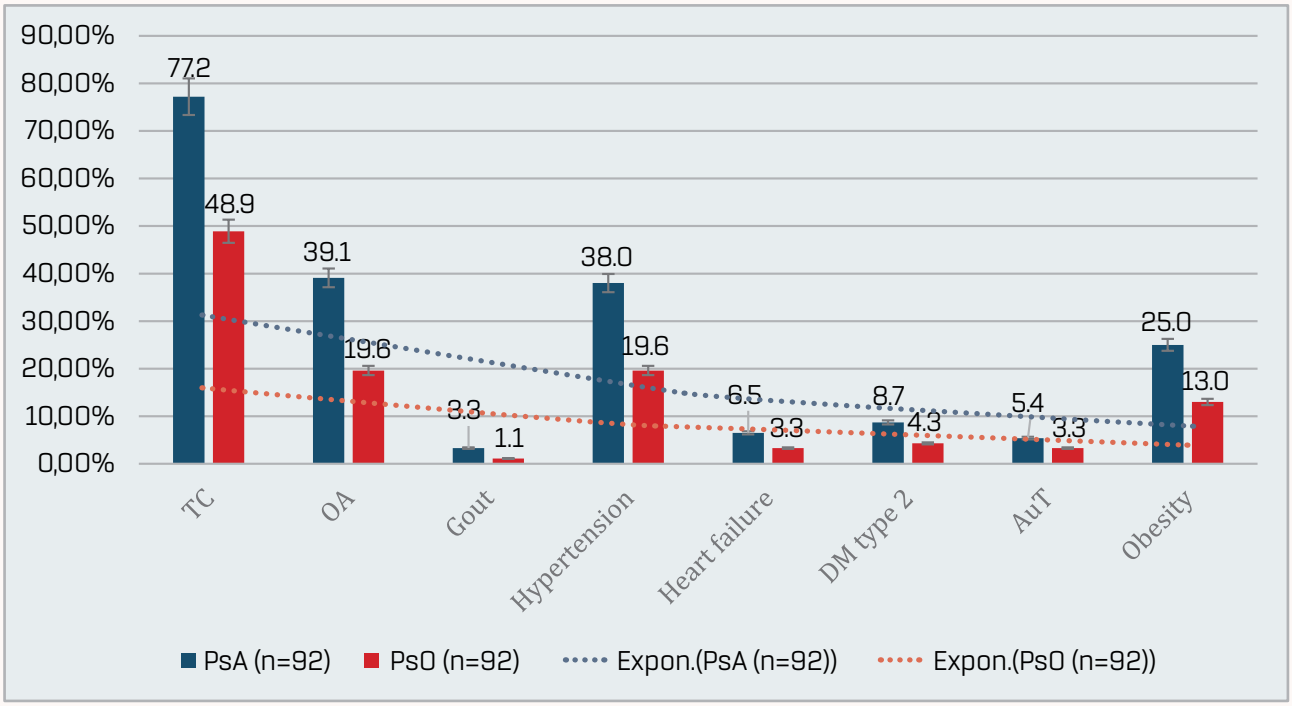

Figure 1. Relative burden of comorbidities in PsA vs. PsO patients, %.

Note: A radar plot comparing the prevalence ratios of comorbidities in PsA relative to PsO. TC – total comorbidities; OA – osteoarthritis; DM – diabetes mellitus; AuT – autoimmune thyroiditis

Clinical Implications and Multidisciplinary Management

These findings emphasize the significantly higher burden of comorbidities in PsA compared with PsO, particularly in cardiovascular, metabolic, and autoimmune conditions. The chronic inflammatory state in PsA likely contributes to these associations, necessitating a multidisciplinary approach for comprehensive patient management.

- Cardiovascular risk: Hypertension, heart failure, and metabolic syndrome require early screening and intervention.
- Musculoskeletal impact: Higher prevalence of OA and gout suggests an increased need for musculoskeletal monitoring and joint protection strategies.
- Metabolic and endocrine considerations: Given the higher rates of T2DM and obesity, lifestyle modifications and metabolic risk management should be integrated into PsA treatment plans.

DISCUSSIONS

The findings of this study emphasize the significantly higher burden of comorbidities in PsA compared with PsO-only patients, supporting the concept that PsA is a systemic inflammatory disease with multi-organ involvement (13). The results indicate that PsA patients are at an increased risk of developing cardiovascular, metabolic, musculoskeletal, and autoimmune conditions, highlighting the importance of early screening and comprehensive management strategies (10, 13).

The prevalence of comorbidities was markedly higher in the PsA cohort (77.2%) compared with the PsO group (48.9%), with a relative risk increase of 58%. Among the most prevalent conditions, hypertension was diagnosed in 38% of PsA patients, nearly twice the prevalence observed in PsO patients (19.6%). The chronic inflammatory state associated with PsA is a well-recognized contributor to endothelial dysfunction, arterial stiffness, and atherosclerosis, increasing the risk of cardiovascular complications (14). These findings align with previous reports that suggest a direct association between systemic inflammation and an increased cardiovascular disease risk in PsA patients.

Osteoarthritis (OA) was another significant comorbidity, affecting 39.1% of PsA patients compared with 19.6% of those with PsO (prevalence ratio: 1.99). The higher prevalence of OA in PsA patients may be attributed to the chronic inflammatory environment, which accelerates joint degeneration. Similarly, gout was three times more common in PsA patients (3.3%) compared with PsO patients (1.1%), suggesting a metabolic component contributing to hyperuricemia and crystal deposition (3, 15).

Endocrine and metabolic disturbances were also more prevalent among PsA patients, with type 2 diabetes mellitus (T2DM) diagnosed in 8.7% of cases compared with 4.3% in PsO patients (15). The relative risk of diabetes was twice as high in PsA, likely due to the combined effects of systemic inflammation, insulin resistance, and metabolic syndrome. These findings underscore the importance of integrating metabolic monitoring into PsA patient management to prevent long-term complications. Furthermore, obesity (BMI >30) was identified in 25% of PsA patients, significantly higher than the 13% observed in PsO patients. The interplay between adipose tissue and inflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), is known to exacerbate both disease severity and metabolic dysfunction in PsA.

Autoimmune thyroiditis was another notable comorbidity, with a prevalence of 5.4% in PsA patients compared with 3.3% in PsO patients (prevalence ratio: 1.64). This association may reflect a shared underlying immunopathogenic mechanism, as PsA is increasingly recognized as an autoimmune disease with complex genetic and immunological factors contributing to its pathogenesis (15, 16). Additionally, chronic heart failure (CHF) was diagnosed in 6.5% of PsA patients compared with 3.3% of PsO patients, further reinforcing the need for cardiovascular risk stratification in this population.

The higher prevalence of comorbidities in PsA has critical implications for clinical practice. Given that systemic inflammation plays a central role in the pathogenesis of both PsA and its associated comorbidities, a multidisciplinary approach to patient management is essential (6, 8, 12). Cardiovascular risk assessment should be integrated into routine PsA care, accompanied by regular monitoring of blood pressure, lipid profiles, and glucose metabolism. Lifestyle modifications, including dietary interventions and physical activity, should be encouraged to mitigate metabolic risks. Rheumatologists should also collaborate closely with endocrinologists, cardiologists, and dermatologists to optimize patient outcomes (15).

Another crucial consideration is the impact of comorbidities on treatment decisions (10, 12, 16). The presence of metabolic syndrome, diabetes, and cardiovascular disease may influence the choice of disease-modifying antirheumatic drugs (DMARDs) and biologic therapies. For example, TNF-α inhibitors have been associated with both beneficial and adverse effects on cardiovascular health, necessitating individualized treatment strategies (10). Similarly, newer therapeutic agents targeting the IL-17 and IL-23 pathways may offer additional benefits in controlling both joint and systemic inflammation.

While this study provides valuable insights into the comorbidity burden in PsA patients, however, several limitations should be considered (17, 18, 19). As the study was conducted within a single geographic region, its findings may not be generalized to other populations. Additionally, the study design was observational, which prevents establishing causal relationships between PsA and comorbid conditions. Future longitudinal studies with larger sample sizes are needed to further elucidate the mechanisms driving comorbidity development in PsA patients and to assess the long-term impact of systemic inflammation on multi-organ health.

This study further supports the concept of PsA as a systemic disease with a high prevalence of comorbid conditions, particularly in the cardiovascular, metabolic, and autoimmune domains. The findings highlight the necessity of a comprehensive, patient-centered approach that includes early screening, risk stratification, and multidisciplinary care to optimize disease management and improve patient outcomes. Further research is required to develop targeted interventions that can effectively mitigate the long-term consequences of comorbidities in PsA patients.

CONCLUSIONS

1. This study confirms that psoriatic arthritis is a systemic inflammatory disease with a substantially higher comorbidity burden compared to psoriasis-only. Cardiovascular conditions (especially hypertension), metabolic disorders (type 2 diabetes, obesity), musculoskeletal complications (osteoarthritis, gout), and autoimmune thyroiditis were significantly more frequent in psoriatic arthritis.
2. These findings emphasize the need for routine screening, early recognition, and proactive multidisciplinary management integrating rheumatology, cardiology, and endocrinology. Tailored therapeutic strategies and lifestyle interventions are crucial to reducing long-term complications and improving patient outcomes.

CONFLICT OF INTEREST

The authors of the article deny the existence of any conflict of interest in the publication of this material.

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ETHICAL APPROVAL

The study was approved by the Research Ethics Committee of the State University of Medicine and Pharmacy "Nicolae Testemițanu" (Decision no. 82 of 19.06.2018).

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Liliana GROPPA, SCOPUS ID: 57214966323

MEDICAL SCIENCES

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ASSESSMENT OF NEUROLOGISTS' KNOWLEDGE, ATTITUDES, AND PRACTICES REGARDING THE IMPACT OF HEAT STRESS DURING HEATWAVES ON PATIENTS WITH NEUROLOGICAL DISORDERS: DEVELOPMENT AND VALIDATION OF THE SURVEY

Ioana CALIGA[1], Cătălina CROITORU[1], Elena CIOBANU[1], Oxana GROSU[2], Ala OVERCENCO[3]

[1]*Nicolae Testemițanu* State University of Medicine and Pharmacy, the Republic of Moldova
[2]*Diomid Gherman* Institute of Neurology and Neurosurgery, the Republic of Moldova
[3]National Agency for Public Health, the Republic of Moldova

Corresponding author: Ioana Caliga, e-mail: caligaioana@gmail.com

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ABSTRACT

Introduction In the context of accelerated climate change and the rising frequency of heatwaves, patients with neurological disorders represent a high-risk group. Heat stress can adversely affect their health, necessitating appropriate adaptations in medical practice. This study aimed to develop and validate a KAP (Knowledge, Attitudes, Practices) questionnaire to assess neurologists' perceptions and approaches regarding the impact of heat stress during heatwaves on patients with neurological disorders.

Material and methods The study included a literature review phase to establish the theoretical foundation, followed by a practical phase for questionnaire development, content validation through expert consultation, and pre-testing on a sample of 104 neurologists. The clarity and relevance of the items, as well as the instrument's internal consistency, were evaluated (Cronbach's alpha coefficient = 0,82).

Results An original 35-item questionnaire was developed and organized into three thematic sections. The pilot study confirmed the instrument's clarity and applicability, and preliminary psychometric analysis demonstrated satisfactory internal consistency.

Conclusions The questionnaire is a valid tool, adapted to the climatic and professional context of the Republic of Moldova. Applying it to larger samples will enable more in-depth psychometric analysis and support the development of adaptive medical strategies for addressing heat stress.

Keywords Questionnaire validation; knowledge, attitudes, and practices; heat stress impact; neurologists; neurological disorders; heatwave.

EVALUAREA CUNOȘTINȚELOR, ATITUDINILOR ȘI PRACTICILOR MEDICILOR NEUROLOGI VIZÂND IMPACTUL STRESULUI TERMIC PE TIMP DE CANICULĂ ȘI CONDUITA PACIENȚILOR CU AFECȚIUNI NEUROLOGICE: STUDIU PILOT

Introducere În contextul schimbărilor climatice accelerate și al intensificării frecvenței valurilor de căldură, pacienții cu afecțiuni neurologice reprezintă o categorie de risc sporit. Expunerea la stres termic poate agrava starea lor de sănătate, ceea ce impune o ajustare adecvată a practicilor medicale. Scopul cercetării a constat în elaborarea și validarea unui chestionar de tip KAP (cunoștințe, atitudini, practici) destinat evaluării percepției și conduitei medicilor neurologi față de impactul stresului termic în timpul episoadelor de canicula asupra pacienților cu patologii neurologice.

Material și metode Studiul a fost structurat în două etape.: o etapă de analiză documentară pentru fundamentarea teoretică și o etapă practică de dezvoltare a chestionarului, urmată de validarea conținutului prin consultarea experților și pretestare pe un eșantion de 104 de medici neurologi. S-a evaluat claritatea itemilor, relevanța și consistența internă a instrumentului (coeficientul Cronbach α = 0,82).

Rezultate A fost elaborat un chestionar original cu 35 de itemi, structurat în trei compartimente tematice. Studiul pilot a confirmat claritatea și aplicabilitatea instrumentului, iar analiza preliminară a evidențiat o consistență internă satisfăcătoare.

Concluzii Chestionarul este un instrument valid, adaptat contextului climatic și profesional din Republica Moldova. Aplicarea sa pe eșantioane extinse va permite aprofundarea analizei psihometrice și va contribui la dezvoltarea unor strategii medicale adaptative în fața stresului termic.

Cuvinte-cheie Validarea chestionarului,cunoștințe atitudini și practici, impactul stresului termic, medici neurologici, afecțiuni neurologice, val de căldură.

INTRODUCTION

Heat stress is an underrecognized pathway through which climate change impacts human health, representing a major threat to human well-being. Each 1 °C increase in ambient temperature triggers a range of pathophysiological effects that can worsen pre-existing conditions and, in severe cases, lead to premature death (1).

Although the international community is striving to limit the rise in global average temperature to below 1.5 °C by 2100, irreversible environmental changes have already occurred, and as the planet continues to warm, these changes will persist and intensify (2).

The progressive increase in temperature, coupled with elevated levels of pollution, has a profound impact on population health, leading to a rise in both the frequency and severity of various neurological disorders (3). Extreme air temperatures can compromise the brain's resilience mechanisms, thereby exacerbating existing conditions or increasing susceptibility to neurological diseases (4). High ambient temperatures can cause disturbances in the nervous system, potentially resulting in strokes or even death. While climate change is not a direct cause of neurodegenerative diseases, it can aggravate their symptoms (3).

Patients with multiple sclerosis are particularly sensitive to elevated ambient temperatures, as heat is known to exacerbate symptoms and disrupt body temperature regulation (5).

The relationship between ambient temperature and the frequency and severity of stroke events remains inconclusive. Some studies suggest that certain stroke types are more likely to occur in extreme heat or cold, while others report seasonal variations in stroke incidence (1, 3).

Changes in ambient temperature have been shown to influence stroke-related mortality. In four Korean cities, data from 1992 to 2007 indicated that each 1 °C rise in mean ambient temperature was associated with an average 4% increase in stroke-related mortality (3).

A 1 °C increase in ambient temperature has been linked to a 23.1% rise in hospital admissions for Alzheimer's disease. Although there is no direct evidence that high ambient temperatures contribute to the development of Parkinson's disease, a study analyzing data from Spain (2001–2009) found that for each 1 °C increase above the threshold of 34 °C, hospital admissions for Parkinson's disease rose by 11.47%, while the mortality rate increased by 12.11% (3).

Scientific literature also indicates that climate change contributes to increased rates of depression, anxiety, substance abuse, and suicide (3, 6).

Individuals with chronic neurological disorders are particularly vulnerable to climate change and often have a reduced capacity to manage their illnesses. Neurological disorders rank among the most burdensome diseases, and regardless of whether climate change has direct effects on them, the reduced resilience of this population to environmental change requires specialists to remain aware of potential impacts and to take appropriate measures to address them (7).

The effects of climate change are pervasive. In 2021, a collective statement published simultaneously in over 220 medical journals worldwide raised serious concerns about the health and biodiversity impacts of heat stress, urging immediate action and calling on neurologists to educate themselves about climate change (2, 8). The accumulation of new knowledge, research on the effects of global warming, and the implementation of responses addressing neurological diseases are now integral responsibilities for those caring for neurological patients (9, 10).

The aim of this study was to develop and validate a questionnaire assessing neurologists' knowledge, attitudes, and practices regarding the impact of heat stress during heatwaves on patients with neurological disorders.

MATERIAL AND METHODS

In 2023, a study was conducted with the aim of developing and validating a KAP-type (Knowledge, Attitudes, Practices) questionnaire. The instrument was designed to assess neurologists' knowledge, attitudes, and practices regarding climate change, global warming, and the impact of heat stress during heatwaves on patients with neurological disorders.

The methodological approach followed the three-phase model proposed by Boateng et al. (2018), as outlined below:

The first phase – *Item Development*

Step 1: *Conceptualization.* A literature review was conducted to establish the conceptual framework for the questionnaire and to define the key dimensions of the KAP model in the context of heat-related health risks.

Step 2: *Theoretical Analysis.* Relevant scientific articles, existing instruments, and theoretical models were analyzed to determine the scope and structure of the questionnaire and ensure content representativeness.

A literature search was conducted using the open-access international database **PubMed**, social networking platform **ResearchGate,** and reference manager and academic social network **Mendeley** employing relevant search terms such as: *"questionnaire validation," "KAP survey," "instrument development," "heat stress," "neurological disorders," "heatwave," "neurological patients," "attitudes," "practices," "neurologists."* To highlight research conducted in the Republic of Moldova, the largest Open Access electronic library in the country – National Bibliometric Instrument (IBN) and the repository of the *Nicolae Testemițanu* State University of Medicine and Pharmacy were consulted, resulting in the selection of three articles. To enhance the relevance of the results and narrow the search scope, a Boolean operator AND, search field tags Title/Abstract [ti.ab] were applied.

A total of 25 relevant scientific sources were analyzed, which explicitly addressed:

- the current relevance of the studied problem;
- the importance of raising awareness about climate change;
- the standard stages of questionnaire development (including item formulation, section structuring, and content validation);
- the methods used for testing validity and reliability (e.g., internal consistency, factor analysis, test–retest);
- the application of KAP instruments in specific medical contexts, including neurology, public health, and climate-related risks.

Step 3: *Item generation.* Based on the theoretical analysis, an initial pool of items was developed and organized into three thematic sections: Knowledge, Attitudes, and Practices.

The second phase – *Scale Development and Refinement*

Step 4: *Content validation through expert review.* The preliminary version of the questionnaire was evaluated by four multidisciplinary experts (neurology, public health, environmental climatology, and sociology) for clarity, relevance, and comprehensiveness. Their feedback guided the revision and refinement of the items.

In parallel with this step, the study protocol, including the developed questionnaire, was evaluated and approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, which issued positive opinion no. 1 on 26 May 2023.

Step 5: *Pre-test (cognitive interviewing).* A pilot study was conducted with a sample of 104 neurologists to evaluate the clarity and feasibility of the questionnaire. Based on participants' feedback, several items were reworded, redundant questions were removed, and the overall structure was refined to improve flow and comprehension.

Step 6: *Assessment of internal consistency.* As part of the instrument's refinement, internal consistency was assessed using Cronbach's alpha coefficient. A value of ≥ 0.70 was considered acceptable for reliability, in line with the specialized literature (11, 12). Internal consistency was evaluated separately for each of the three sections of the questionnaire (Knowledge, Attitudes, and Practices).

Table 1 presents the standardized interpretation of Cronbach's alpha values in relation to the instrument's level of internal consistency and reliability (13).

Table 1. Showing internal consistency value and significance (13).

	Cronbach's α value	Internal consistency/Reliability test
1	$\alpha \geq 0.9$	Excellent (high-stakes testing)
2	$0.7 \leq \alpha \geq 0.9$	Good (low-stakes testing)
3	$0.6 \leq \alpha < 0.7$	Acceptable
4	$0.5 \leq \alpha < 0.6$	Poor
5	$\alpha < 0.5$	Unacceptable

The *Cronbach's alpha* coefficient, used to evaluate the internal consistency of the questionnaire items, was calculated with licensed IBM SPSS Statistics software, version 27.0 (Software: **IBM Corp.** IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.; 2020).

The third phase – *Exploratory Factor Analysis*

The first step in conducting the Exploratory Factor Analysis (EFA) was to test data adequacy. To verify the suitability of the dataset for EFA, two standard statistical tests were applied: the Kaiser-Meyer-Olkin (KMO) index and Bartlett's test of sphericity.

The KMO index assesses the proportion of common variance among variables and determines whether factor analysis is statistically justified. Values close to 1 indicate a high degree of adequacy, whereas values below 0.60 are considered inadequate for EFA (14).

Bartlett's test evaluates the null hypothesis that the correlation matrix is an identity matrix, in which case factor analysis would not be appropriate. A significant result ($p < 0.05$) rejects the null hypothesis and confirms the suitability of the data for EFA (15).

For these tests, only the Likert scale items from the three questionnaire sections (Knowledge, Attitudes, and Practices) were included.

The dataset was analyzed using the Principal Component Analysis (PCA) extraction method to identify the latent structure of the questionnaire. Calculations were performed with IBM SPSS Statistics version 27 and Python (pandas, scikit-learn, and seaborn libraries).

For the interpretation of the factorial structure, the loadings of each item on the extracted factors were analyzed, with absolute loadings of ≥ 0.40 considered indicative of relevance for the corresponding factor.

Processing of dichotomous and multiple-choice questions

Dichotomous (Yes/No) and multiple-choice questions from the KAP questionnaire administered to neurologists were analyzed separately, given the nominal nature of these variables. Such items are not suitable for EFA, as it is not possible to apply Pearson correlation-based analysis between continuous variables.

Questions in this format were selected, and for each item absolute frequencies (number of responses) and relative frequencies (percentage of respondents) were calculated. For multiple-choice questions, each option was treated as a binary variable (selected/not selected) (16).

Data processing was performed using Microsoft Excel and Python (pandas and NumPy libraries). Descriptive analysis was applied in accordance with KAP study methodology for categorical variables.

RESULTS

Questionnaire development

The study began with an extensive literature review to identify existing instruments for assessing the knowledge, attitudes, and practices of healthcare professionals, particularly neurologists, in relation to heat stress during heatwaves. The search confirmed that no standardized, validated, and publicly available questionnaire aligned with the specific objectives of this study.

To address this gap, the first phase involved developing an original item pool, informed by the literature and adapted to the professional and climatic context of neurologists in the Republic of Moldova. This step corresponds to the "item development" stage (Phase 1) as described by Boateng et al. (2018), and laid the foundation for subsequent validation and refinement of the instrument.

This preparatory stage led to the identification of several thematic categories that structured the questionnaire. Specifically, the literature analysis highlighted key domains such as neurologists' awareness of heat-related health risks, clinical practices during heatwaves, attitudes toward climate adaptation, and patient guidance strategies. These domains formed the basis of the item pool, ensuring both clinical relevance and alignment with the KAP model.

Based on the preliminary analyses, the instrument entitled "Assessment of Neurologists' Knowledge, Attitudes, and Practices Regarding the Impact of Heat Stress During Heatwaves and the Behavior of Patients with Neurological Disorders" was developed.

The construction process followed six defined phases (steps), each generating concrete outcomes that contributed to the final structure of the questionnaire:

Step 1 established clearly articulated research objectives and hypotheses directly aligned with the overarching aim of the study.

Step 2 led to the formulation of precise research questions to operationalize those objectives.

Step 3 delineated the key informational categories required to guide item development.

Step 4 produced a draft questionnaire comprising 36 items, distributed across five structured sections aligned with the KAP model.

Step 5 generated qualitative feedback from a panel of four multidisciplinary experts (neurology, hygiene, climatology, and sociology), which led to revisions in item clarity, language, and logical flow.

Step 6 yielded internal consistency values (Cronbach's alpha) indicating good reliability of the instrument (see details below).

In developing the questionnaire, several key requirements for question formulation were observed, including:

- avoiding ambiguous wording,
- preventing double-barrelled questions,
- excluding leading questions or those containing implicit assumptions.

The questionnaire was subsequently reviewed and revised for linguistic, grammatical, and logical coherence, with ambiguities eliminated. Throughout the process, the principle of neutral and clear wording was maintained.

The initial version of the questionnaire included the three core components typical for KAP instruments: 1) neurologists' general knowledge of global warming and its impact on individuals with neurological disorders; 2) neurologists' attitudes toward heat stress associated with global warming and its influence on patients' neurological health; 3) practices applied by neurologists in supervising patients with neurological disorders during heatwaves.

In total, the questionnaire comprised 36 items in a mixed format (closed- and open-ended questions) and served as the basis for the subsequent pilot survey. It included five open-ended questions (items 9a and 9b from the *Knowledge* section, and items 34a, 35, and 36 from the *Practice* section). These were designed to capture nuanced perspectives, personal experiences, or examples not addressed by closed-ended items. Open-ended responses were excluded from the calculation of Cronbach's alpha, as internal consistency applies only to standardized response scales. Instead, they were analyzed qualitatively to supplement quantitative findings, identify emerging themes, and inform potential revisions to the instrument. Their role was exploratory and supportive rather than psychometric.

The questionnaire items employed different assessment formats depending on the KAP domain. In the *Knowledge* section, most questions were multiple-choice (5 items) or dichotomous ("Yes/No") (4 items), along with one item using a Likert-type 4-point ordinal scale (*Quite a lot / A lot / A little / Not at all*) to assess factual understanding. The *Attitudes* section included 9 dichotomous ("Yes/No") items and 4 Likert-type items: three on a 4-point ordinal scale (*Quite a lot / A lot / A little / Not at all*) and one on a scale ranging from *Very useful* to

Useless (*Very useful / Useful / Neutral / Useless*). The *Practices* section comprised 7 dichotomous ("Yes/No") items and 6 multiple-choice items.

Both the theoretical and practical construction of the instrument was informed by and adapted from international methodological recommendations, particularly those related to KAP model-based tool development (17, 18), sequential phases of item generation and pre-testing (19, 20), expert content validation (17–21), and the assessment of internal consistency using Cronbach's alpha (19, 20).

Pilot study for questionnaire validation (pre-testing)

The developed questionnaire underwent a pre-testing phase to assess its clarity, comprehensibility, and content validity. This phase served as a pilot validation study, allowing for the refinement and adjustment of the instrument prior to its application in the main sample.

Pre-testing was conducted on a sample of 104 neurologists, selected through convenience sampling. Participants completed the questionnaire in paper format during face-to-face, interviewer-assisted sessions. This approach enabled the collection of quantitative data as well as valuable qualitative feedback, both of which were essential for improving the instrument.

Participants were invited to share their impressions of the wording and overall structure of the questionnaire by responding to three open-ended evaluation questions:

1. Which questions did you find unclear or difficult to understand?
2. Which questions were easy to understand?
3. What suggestions do you have for improving the wording of the items and the structure of the questionnaire?

By responding to these questions, participants provided constructive feedback that contributed to the improvement of the final version of the questionnaire. Most suggestions focused on rewording technical items to reduce ambiguity, standardizing response formats, and reorganizing questions to improve logical flow within their respective sections.

As part of the validation process, one question was excluded, four were revised, and two questions were transferred from one section to another.

All 20 completed questionnaires were validated for analysis, with no exclusions, as they fully met the criteria for completeness and consistency. The responses were verified and entered into an electronic database for subsequent analysis.

Preliminary evaluation

A preliminary analysis was conducted on the responses collected during the pre-testing phase. Its objective was to assess the content validity of the instrument and to verify its internal consistency.

1. **Content Validity.** The evaluation panel consisted of four experts from relevant fields (neurology, hygiene, climatology, and medical sociology). They assessed the items for clarity and lack of ambiguity, relevance to the targeted theoretical constructs, and suitability to the professional context of neurologists.

Qualitative feedback obtained from respondents during the pilot study was also taken into account. Based on their observations, several items were revised, and the questionnaire was restructured to improve logical clarity and terminology.

2. **Internal consistency** (reliability). To estimate internal consistency, Cronbach's alpha coefficient was calculated separately for each of the three main sections, Knowledge, Attitudes, and Practices, which comprised the final version of the questionnaire.

   Although the sample size of 104 respondents was limited for robust statistical analysis, the Cronbach's alpha values obtained exceeded the minimum acceptable threshold of 0.70, indicating a satisfactory level of reliability for the instrument (tab. 2).

Table 2. Cronbach α coefficients.

Sections	Number of items	α Cronbach
Knowledge	10	0.82
Attitudes	12	0.84
Practices	14	0.79
Total	**36**	**0.82**

Thus, all values exceeded the minimum acceptable threshold of 0.70, indicating good internal consistency of the instrument and a satisfactory degree of item homogeneity within each section.

Factor analysis

As described in the *Materials and Methods* section, the first step of the exploratory factor analysis involved testing data adequacy using the Kaiser-Meyer-Olkin (KMO) index and Bartlett's test of sphericity. The KMO index indicated a moderate to good level of adequacy, and Bartlett's test was statistically significant ($\chi^2 = 899.98$; $p < 0.001$), confirming that the correlation matrix differed from the identity matrix and justifying the application of EFA (tab. 3).

Table 3. Results of the adequacy tests for exploratory factor analysis.

Indicator	Value	Interpretation
KMO Index	0.674	Moderate to good adequacy; justifies the application of EFA
Bartlett's Test (Chi-Square)	899.98	High value, statistically significant
Bartlett's p-value	< 0.001	Statistically significant; rejects the null hypothesis (identity matrix excluded)

Note: *Only Likert-scale items were included in the adequacy tests.*

To identify the latent dimensions, principal component analysis (PCA) was conducted separately for each of the three KAP questionnaire domains – *Knowledge*, *Attitudes*, and *Practices* – including only Likert-scale items, in accordance with international methodological guidelines.

According to Kaiser's criterion (eigenvalues > 1), principal component analysis (PCA) identified three main factors in the "Knowledge" domain, which together explained 70.9% of the total response variance.

Factor 1: General knowledge about climate change – items addressing the impact of global warming on neurological health and the concept of heat stress.

Factor 2: Access to information and clinical protocols – items concerning the availability of guidelines, official strategies, and information sources for physicians.

Factor 3: Knowledge of non-pharmacological interventions and preventive measures – items focused on practical methods to prevent heat stress.

All items demonstrated significant loadings (tab. 4).

The EFA conducted for the questions in the "Attitudes" domain highlighted three main factors, together explaining 76.3% of the total variance. The factor structure was interpreted as follows:

Factor 1: Perceived risk to the population – physicians' beliefs about the impact of heat stress on both healthy and vulnerable individuals, as well as the importance of climate education.

Factor 2: Support for organizational measures and policies – attitudes regarding the need for guidelines/protocols and interdisciplinary collaboration.

Factor 3: Confidence in preventive measures – perceived usefulness of avoiding exposure and implementing preventive interventions.

This structure highlights neurologists' clear orientation toward protecting vulnerable patients and supporting organizational and educational interventions (tab. 4).

For the "Practices" domain, the identified factors accounted for 74.5% of the total variance. The extracted factors were:

Factor 1: Patient education and professional involvement – items concerning patient information, the use of educational materials, and participation in specialized training.

Factor 2: Thermal comfort and institutional infrastructure – perceptions of summer working conditions and their impact on clinical activity.

Factor 3: Personal behavior and sources of information – the personal application of recommendations and the methods by which physicians stay informed about heatwaves.

The analysis suggests a practical approach centered on physicians' educational responsibilities and adaptation to both institutional and personal contexts (tab. 4).

This result indicates good internal consistency of the instrument and supports construct validity.

Table 4. Results of Exploratory Factor Analysis (EFA) by KAP domains and percentage of variance explained.

Factors	Explained Variance (%)	Interpretation
Knowledge Domain		
Factor 1	72.8	General knowledge about climate change
Factor 2	69.2	Access to information and clinical protocols
Factor 3	70.8	Knowledge of non-pharmacological interventions and preventive measures
Attitudes Domain		
Factor 1	72.8	Perceived risk to the population
Factor 2	74.7	Support for organizational measures and policies
Factor 3	81.4	Confidence in preventive measures
Practices Domain		
Factor 1	70.3	Patient education and professional involvement
Factor 2	78.3	Thermal comfort and institutional infrastructure
Factor 3	74.5	Personal behavior and sources of information

Correlations between the first factor scores from each domain (Knowledge, Attitudes, Practices) revealed a moderate positive association between *Knowledge* and *Practices*, suggesting that higher knowledge levels are linked to better-adapted practices for heat stress management. Correlations with *Attitudes* were weaker, indicating a potential gap between beliefs and actual behaviors, a phenomenon frequently reported in KAP studies (fig. 1).

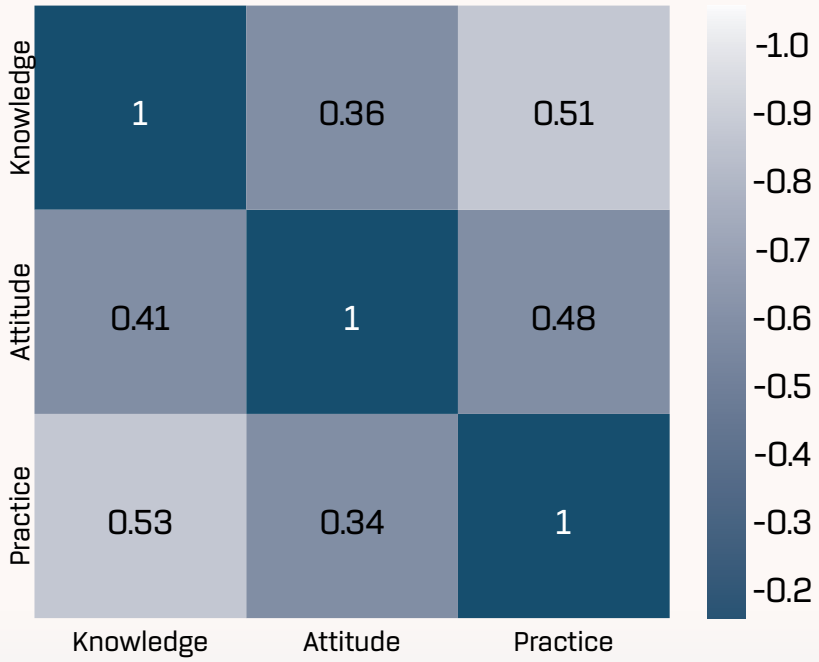

Figure 1. Correlations between KAP factor scores.

The questions analyzed in the EFA addressed medical infrastructure, professional education, sources of information about heatwaves, and the availability of informational materials within institutions. The data reflect real challenges in current medical practice and highlight the need for institutional interventions to support neurologists in adapting to the risks associated with heat stress.

Structure of the final questionnaire

The questionnaire begins with an introductory note to respondents, outlining the purpose and usefulness of the study, assurances of data confidentiality, and brief instructions for completing the form. To ensure consistent understanding of the items, a definition of the term "heatwave" was also provided, as it appears in several subsequent questions.

The final instrument was organized into three sections, corresponding to the three KAP model dimensions, and included a total of 35 items.

The first section, entitled *Global Warming and Its Impact on People with Neurological Disorders*, contains 9 questions addressing events that may contribute to global warming, the main effects of global warming, respondents' understanding of the term *"heat stress"* associated with global warming, and general information about the treatment of neurological patients affected by heat stress. It also covers physicians' knowledge of the existence of national clinical guidelines or protocols addressing the impact of global warming on patients with neurological disorders, non-pharmacological techniques or treatments for preventing the impact of global warming on neurological health, the existence of medications that may mitigate the effects of heat stress on neurological patients, whether strategies approved by competent authorities exist to reduce the risk of heat stress among neurological patients, and whether respondents consider the information they currently possess to be sufficient regarding upcoming heatwaves, behavior during heat events, assistance to be provided to patients, and preventive measures related to heat stress (tab. 5).

The next section, entitled *The Heat Stress Problem Associated with Global Warming and Its Impact on the Health of Neurological Patients (as Perceived by Physicians)*, includes 12 questions focusing on neurologists' attitudes. These address their beliefs about the current existence of global warming, their views on environmental news, whether heat stress affects the healthy population in the Republic of Moldova, and whether vulnerable groups, particularly those with certain pathologies, including neurological disorders, are impacted. The section also explores physicians' opinions on whether the population should be educated about climate change and heat stress, whether patients with neurological disorders should avoid exposure to high temperatures, and how useful preventive measures are for mitigating the effects of heat stress. Further questions examine whether managing neurological patients during heatwaves is a shared responsibility across medical specialties, whether time should be allocated during consultations to inform patients about the impact of heatwaves, and whether guidance should be provided to patients on how to behave during such events. Finally, the section assesses physicians' views on the need for a national guideline for managing neurological patients affected by heatwaves in the Republic of Moldova and their evaluation of working conditions in terms of thermal comfort during the warm season (tab. 5).

The third section of the questionnaire, entitled *Surveillance of Patients with Neurological Disorders During Heatwaves, Activity Conditions, and Climate*

Education, includes 14 questions and focuses on the practices of neurologists in patient management, as well as their personal working conditions and education. The questions address physicians' clinical activity during consultations with patients whose neurological disorders are aggravated by high air temperatures, the most frequent symptoms reported by such patients, the challenges encountered in managing them during heatwaves, the non-pharmacological techniques or treatments recommended, and whether physicians provide information to patients about health risks related to heatwaves. To better understand working conditions, neurologists were asked whether high temperatures had affected their professional activity during summer in the past five years, whether their consulting rooms were equipped with air conditioning, and whether their institutions provided access to a drinking water dispenser (cooler). Another set of questions explored how physicians receive information about heatwaves, including how they learn about upcoming events and appropriate patient care recommendations, whether healthcare institutions provide guides, brochures, leaflets, or other educational materials for patients on the effects of and behavior during heatwaves, whether they have received continuing medical education or training related to patient behavior during heatwaves, what type of information on health and heatwaves they feel they need to better support their work with patients, and through which channels such information should ideally be communicated. Finally, physicians were asked how the general population could best gain access to information about appropriate behavior during heatwaves, from the perspective of healthcare providers (tab. 5).

The questionnaire concluded with a professional identification item, asking respondents to indicate their number of years of experience in the field of neurology. Finally, participants were presented with a thank-you message acknowledging their contribution to the study.

Table 5. Final structure of the KAP questionnaire applied to neurologists.

Thematic compartment	Number of items	Type of questions
Knowledge about the phenomenon of global warming and its impact on patients with neurological disorders	9	8 closed, 1 open
Attitudes towards heat stress and its influence on the health of neurological patients	12	12 closed
Practices regarding patient supervision, working conditions and climate education	14	12 closed, 2 open
Total	**35**	**32 closed, 3 open**

DISCUSSION

Against the backdrop of increasingly frequent and intense heatwaves, and growing evidence of the vulnerability of patients with neurological diseases to heat stress, the role of neurologists has become essential. However, the scientific literature on the preparedness of medical professionals for such challenges remains limited, and existing questionnaires are either designed for the general public (7) or targeted at other medical specialties (22).

The development and validation of a KAP (Knowledge, Attitudes, Practices) questionnaire specifically targeting neurologists in the context of heat stress during heatwaves represents an innovative initiative, given the absence of

standardized instruments tailored to this field. The development process followed methodological steps recommended in the literature (18, 20, 21), including literature review, expert consultation, pre-testing, and preliminary evaluation.

The information extracted from the selected bibliographic sources formed the basis for the theoretical and structural design of the proposed questionnaire, enabling a rigorous approach to developing a valid and reliable instrument tailored to the professional context of neurologists in the face of climate change and heat stress.

Regarding content validity, the involvement of a multidisciplinary panel of experts ensured a comprehensive evaluation of item clarity, relevance, and coherence, in line with previous recommendations for the development of KAP instruments in healthcare. Pre-testing the questionnaire on a sample of 20 neurologists allowed for the identification and correction of potential ambiguities, thereby enhancing its clarity and feasibility. Its modular structure (36 items grouped into three thematic sections) ensured multidimensional coverage of the research domain.

The evaluation of internal consistency, performed by calculating Cronbach's alpha for each of the three main sections (Knowledge, Attitudes, Practices), yielded values exceeding the minimum acceptable threshold of 0.70, within the range considered satisfactory for behavioral research instruments (11, 12, 13). This finding supports the conclusion that the included items are coherent and relevant to the targeted constructs. These results are consistent with previous studies that reported similar Cronbach's alpha values in the validation of KAP-type questionnaires (11, 17, 18, 21, 22).

Therefore, the instrument developed in this study addresses an urgent need for applied research in the field of climate and health and may contribute to the development of educational interventions, public policies, and professional standards.

The questionnaire development methodology adhered to international guidelines on the creation of standardized tools for psychosocial evaluation in healthcare (10, 11). The steps followed – literature review, item generation, expert review, pre-testing, and internal consistency evaluation – are supported by multiple recent sources on KAP questionnaire validation in various domains (10, 17, 19, 21, 23, 24, 25, 26), including the medical field in the Republic of Moldova.

Feedback from experts in four domains (neurology, hygiene, climatology, and medical sociology) strengthened content validity, while the involvement of neurologists in the pre-testing phase enabled item adjustment and clarification, consistent with other methodological approaches described in the literature (17, 18, 25).

Nevertheless, studies focused on neurology and climate remain scarce, which makes the proposed instrument a novel contribution to interdisciplinary research in the field of climate-related health.

Study limitations

Moreover, the questionnaire was administered in the Republic of Moldova, which may limit the generalizability of the results to other geographical and cultural contexts. However, the flexible structure of the instrument allows its adaptation to other categories of healthcare professionals or regions with only minor adjustments.

Practical implications and future directions

The results suggest that the proposed instrument may serve as a foundation for:

- conducting large-scale national surveys;
- identifying training gaps among neurologists;
- supporting the development of educational interventions for managing vulnerable patients under heat stress conditions;
- contributing to the creation of climate-sensitive clinical guidelines.

In the next phase, it is recommended that the questionnaire be applied to a larger sample and that exploratory factor analysis be conducted to confirm the instrument's latent structure and validate the proposed dimensions.

CONCLUSIONS

1. An original KAP-type questionnaire was developed in accordance with methodological standards and adapted to the professional context of neurologists in the Republic of Moldova, in light of current climate-related risks.
2. Pre-testing confirmed the clarity of items, the relevance of content, and the feasibility of implementation in real-world clinical settings.
3. Findings from the development, pre-testing, and preliminary validation phases demonstrated that the instrument adequately captures the key constructs of the study – neurologists' knowledge, attitudes, and practices regarding heat stress during heatwaves. The questionnaire is coherent, relevant, and demonstrates satisfactory internal reliability.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (approval no. 1, May 26, 2023).

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Ioana CALIGA, WoS Researcher ID: IRZ-4551-2023
Cătălina CROITORU, WoS Researcher ID: AAB-4330-2019, SCOPUS ID: 58142857000
Elena CIOBANU, WoS Researcher ID: P-2844-2018, SCOPUS ID: 58142967700
Oxana GROSU, WoS Researcher ID: AAF-1589-2019, SCOPUS ID: 57309254400
Ala OVERCENCO, WoS Researcher ID: 1QV-5018-2023, SCOPUS ID: 36545158500

MEDICAL SCIENCES

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IN VITRO STUDY OF COPPER COORDINATION COMPOUNDS WITH THIOSEMICARBAZONE ACTION ON ANTIOXIDANT ENZYMES

Valeriana PANTEA, Ecaterina PAVLOVSCHI, Silvia STRATULAT, Olga TAGADIUC, Valentin GUDUMAC

Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

Coresponding author: Ecaterina Pavlovschi, e-mail: ecaterina.pavlovschi@usmf.md

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ABSTRACT

Introduction Thiosemicarbazones represent a class of organic compounds with significant pharmacological potential, known for their antitumor, antimicrobial, and antiviral activities. Recently, researchers have increasingly focused on how these compounds influence cellular redox balance, particularly through modulation of antioxidant system activity. Thus, the study aimed to evaluate the antioxidant properties of selected thiosemicarbazones via *in vitro* experiments.

Material and methods The research was performed on peripheral blood samples collected from 10 clinically healthy individuals. The compounds were tested at two concentrations (10.0 µmol/L and 1.0 µmol/L) to assess their impact on the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT).

Results The results revealed that certain thiosemicarbazones can impact the activity of SOD, and CAT in particular manner, thereby affecting cellular capacity to neutralize reactive oxygen species.

Conclusions Due to their ability to stimulate antioxidant responses, thiosemicarbazones emerge as promising platforms for the development of targeted therapeutic agents, particularly in cancer and degenerative disease treatment. In the current context of pursuing low-side-effect therapies that maintain cellular homeostasis, investigating the influence of thiosemicarbazones on the antioxidant system is a highly innovative research direction.

Keywords Copper coordination compounds with thiosemicarbazones, the supernatant, antioxidant enzymes.

EVALUAREA *IN VITRO* A ACȚIUNII COMPUȘILOR DE COORDONARE AI CUPRULUI CU TIOSEMICARBAZONE ASUPRA ENZIMELOR ANTIOXIDANTE

Introducere Tiosemicarbazonele reprezintă o clasă de compuși organici cu potențial farmacologic semnificativ, recunoscuți pentru activitățile lor antitumorale, antimicrobiene și antivirale. În ultimul timp, cercetătorii au acordat o atenție sporită modului în care acești compuși influențează echilibrul redox celular, în special prin modularea activității sistemului antioxidant. Astfel, studiul și-a propus evaluarea proprietăților antioxidante ale unor tiosemicarbazone selectate prin experimente *in vitro*.

Material și metode Cercetarea s-a desfășurat pe probe de sânge periferic, recoltate de la 10 indivizi clinic sănătoși. Compușii au fost testați la două concentrații (10,0 µmol/L și 1,0 µmol/L), în vederea evaluării impactului acestora asupra enzimelor antioxidante superoxid dismutaza (SOD) și catalaza (CAT).

Rezultate Rezultatele au evidențiat că anumite tiosemicarbazone pot influența în mod specific activitatea SOD și a CAT, afectând astfel capacitatea celulară de neutralizare a speciilor reactive de oxigen.

Concluzii Prin capacitatea lor de a stimula răspunsuri antioxidante, tiosemicarbazonele se impun ca platforme promițătoare pentru dezvoltarea de agenți terapeutici – țintă, în special în tratamentul cancerului și al bolilor degenerative. În contextul actual, al terapiei cu efecte adverse reduse, ce mențin homeostazia celulară, investigarea influenței tiosemicarbazonelor asupra sistemului antioxidant reprezintă o direcție de cercetare inovatoare.

Cuvinte-cheie Compuși de coordonare ai cuprului cu tiosemicarbazone, supernatant, enzime antioxidante.

INTRODUCTION

Reactive oxygen species (ROS) are essential regulators of normal cellular functions. However, their dysregulation is associated with the onset of various disorders, including multifactorial diseases. Compared to healthy cells, malignant cells exhibit higher levels of ROS due to an intensified metabolism. While elevated ROS levels can promote tumor development, they also represent a vulnerability in cancer cells. Exposure to additional oxidative stress (OS) makes these cells more susceptible to cell death, thereby providing an opportunity for selective therapeutic strategies (1).

OS results from an imbalance between ROS production and the antioxidant defense mechanisms that neutralize them. This disruption of redox homeostasis can profoundly damage vital cellular structures such as proteins, lipids, and genetic material, leading to systemic consequences and an increased risk of mutations. Such ROS-induced effects are thought to contribute to aging and may play a role in the initiation of cancer (2).

The accumulation of ROS, along with reactive nitrogen species (RNS) from both endogenous and exogenous sources, contributes to OS – a hallmark of many cancer cell types – characterized by redox imbalance and disrupted cellular signaling pathways. This redox imbalance is more pronounced in tumor cells than in normal cells and may contribute to oncogenic activation (3).

The superoxide anion, generated through metabolic processes or by the activation of oxygen under physical irradiation, is recognized as a primary type of ROS. This anion can interact with other molecules to generate secondary ROS, either directly or more commonly *via* enzyme- or metal-catalyzed mechanisms (4). Although the superoxide radical does not directly react with polypeptides, carbohydrates, or nucleic acids, and its role in lipid peroxidation remains unclear, it is primarily eliminated through dismutation. In this process, two superoxide molecules are converted into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), catalyzed by the enzyme superoxide dismutase (SOD). The resulting hydrogen peroxide is further broken down into water and oxygen by catalase (CAT) or peroxidase, thereby completing the detoxification of free radicals (5).

SOD is crucial in this elimination process, catalyzing the dismutation of superoxide radicals and working synergistically with CAT and peroxidase to maintain redox balance (6). These antioxidant enzymes are vital for cellular protection against ROS, including those oxidative processes implicated in chronic diseases such as cancer, cardiovascular, and neurodegenerative conditions. Recent studies emphasize the role of SOD in converting superoxide anion within various cellular compartments, a process vital for redox homeostasis and intracellular signaling. SOD also protects nitric oxide from oxidative inactivation, thereby preventing peroxynitrite formation and supporting endothelial and mitochondrial function (7).

Understanding the biological control mechanisms and metabolic processes across molecular, cellular, tissue, and systemic levels remains one of the major challenges in modern medicine, particularly in unraveling the pathogenesis of cancer. At the same time, there is growing interest in developing novel drugs and alternative in vitro methods for toxicological assessment that eliminate the use of animals, driven by increasing ethical considerations.

Copper(II) coordination compounds have attracted significant attention due to the redox properties and biological compatibility of copper ions, which give rise to a broad spectrum of biological activities. The pharmacological

efficacy of these metal-based compounds can be improved by modifying the ligand type and donor atoms. Copper(II) coordination compounds have demonstrated promising antitumor activity and significant therapeutic potential in the treatment of microbial infections, tuberculosis, malaria, fungal diseases, and inflammation (8).

The anticancer potential of the copper(II) coordination compounds is primarily attributed to their ability to induce intracellular ROS accumulation, which in turn activates cellular antioxidant defense mechanisms in response to OS. These findings support the exploration of ROS-inducing copper(II) coordination compounds as potential antiproliferative agents in cancer chemotherapy (10-13).

Control of OS is critical in both tumor development and response to anticancer treatments. Multiple carcinogenesis-related signaling pathways directly or indirectly influence ROS metabolism. The redox balance in cancer cells differs significantly from that seen in normal cells. Metabolic and signaling alterations result in enhanced ROS levels, often counterbalanced by an upregulated antioxidant system. This dual role of ROS, as both a barrier to and a driver of tumor progression, has important implications for therapeutic strategies targeting ROS modulation (1).

In this context, investigating the biochemical impact of such compounds proves highly relevant. Copper(II) coordination compounds represent a promising direction for the development of new effective treatments for multifactorial disorders, including tumors, chronic inflammatory diseases, autoimmune pathologies, especially through their modulation of antioxidant mechanisms.

The aim of this study was to assess the *in vitro* antioxidant properties of thiosemicarbazones with significant biological potential, using blood samples obtained from clinically healthy individuals.

MATERIAL AND METHODS

Study Design and Setting

This was an experimental *ex vivo/in vitro* study designed to investigate the response of the antioxidant system to copper(II) coordination compounds with various thiosemicarbazones and their derivatives. The compounds were synthesized at the Advanced Materials Research Laboratory in Biopharmaceutics, Moldova State University (Tab. 1). All biochemical assays were performed using a Synergy H1 Hybrid Microplate Reader (BioTek Instruments, USA). Incubations were carried out in 24-well culture plates at 37 °C, 3.5 % CO_2 for 48 h.

Table 1. Newly Studied Copper(II) Coordination Compounds with Thiosemicarbazones and Their Derivatives (9).

No.	Cod	Chemical name of the substance
1	Control	0.1 mL of 0.9% saline solution + Dulbecco's modified eagle medium (DMEM)
2	DOXO	Doxorubicin
3	CMA-18	Chloro-{1-(1,2-benzothiazol-3-yl)-2-[1-(pyridin-2-yl)ethylidene]diazanido} copper
4	CMD-8	Chloro-{4-ethyl-2-[phenyl(pyridin-2-yl)methylidene]hydrazine-1-carbothioamido} copper
5	MG-22	Di-Chloro-{N'-(4-methoxyphenyl)-N,N-dimethylcarbamimidothioato} copper
6	CMC-34	Chloro-{N'-[phenyl(pyridin-2-yl)methylidene]-N-pyridin-2-ylcarbamohydrazonothioato} copper
7	CMJ-33	Chloro-{4-(3-methoxyphenyl)-2-[1-(pyridin-2-yl)ethylidene]hydrazine-1-carbothioamido} copper
8	CMT-67	Nitrato-{N-phenyl-N'-(pyridin-2-ylmethylidene)carbamohydrazonothioato} copper
9	CMG-41	Nitrato-{N'-[phenyl(pyridin-2-yl)methylidene]-N-prop-2-en-1-ylcarbamohydrazonothioato} copper
10	TIA-123	Di-Chloro-{N'-[phenyl(pyridin-2-yl)methylidene]-N-prop-2-en-1-ylcarbamohydrazonothioato} copper
11	TIA-160	Acetato-{2-({[(methylsulfanhyl)(prop-2-en-1-lamino)ethylidene]hydrazinylidene} methyl)enolato}copper

Note: *The chemical structures of the compounds are available in bibliographic reference no. 9.*

Study Population and Ethics

The study protocol was reviewed and approved by the Research Ethics Committee of "Nicolae Testemițanu" State University of Medicine and Pharmacy (Approval no. 5, Ref. no. 38, June 20, 2024).

Participants were enrolled only after signing written informed consent forms.

Control and Reference Groups

- Negative control (baseline): 0.1 mL of 0.9 % NaCl solution added to DMEM.
- Reference drug: Doxorubicin (DOXO) at final concentrations of 10.0 µmol/L and 1.0 µmol/L.

Investigational Compounds

Newly synthesized copper(II) coordination compounds tested at final concentrations of 10.0 µmol/L and 1.0 µmol/L, diluted in 0.1 mL of 0.9 % saline. Each dilution was tested in duplicate.

Table 1 lists the full names and codes of the compounds (CMA-18, CMD-8, MG-22, CMC-34, CMJ-33, CMT-67, CMG-41, TIA-123, TIA-160). Chemical structures are available in bibliographic reference 9.

Data Collection and Research Tools

Sample Collection and Processing

Blood samples were drawn in the morning, under fasting conditions, *via* venipuncture of the cubital vein (5 mL/each subject). The blood was transferred into flasks containing 20 mL of Dulbecco's modified eagle medium (DMEM) with heparin (2.5 IU/mL), gentamicin (100 µg/mL), and L-glutamine (0.6 mg/mL).

For the evaluation of the antioxidant system response to the copper(II) coordination compounds, 0.9 mL of this mixture was pipetted into each well of a 24-well culture plate. As a control (baseline values) 0.1 mL of 0.9% NaCl solution was added in parallel to 4 wells. The remaining wells received the tested compounds, diluted in 0.1 mL of physiological saline (final concentrations – 10.0 µmol/L and 1.0 µmol/L). All dilutions were tested in duplicate. The reference drug, Doxorubicin (DOXO), was added to the wells to the same final concentration (10.0 µmol/L and 1.0 µmol/L), previously being diluted with 0.9% NaCl solution.

The plates were incubated at 37°C, 48 hours, with 3.5% CO_2. After incubation, the contents of each well were transferred into 2.0 mL Eppendorf tubes and centrifuged for 5 minutes at 3000 rpm. The supernatants were stored at –40°C until analysis.

Outcome Measures

In the supernatant, the activity of SOD and CAT was assessed by spectrophotometry. SOD activity was determined according to the method described by Matyushin B.N. et al., and CAT activity according to the method of Korolyuk M.A. et al. (15, 16). SOD activity was expressed in conventional units (c.u.). One unit of SOD activity was defined as the amount of enzyme required to achieve 50% inhibition of the nitro blue tetrazolium reduction reaction. Enzyme activity was normalized to 1 mL of serum. CAT activity was expressed in micromoles of degraded H_2O_2 per liter of serum (µmol/L). All biochemical assays were carried out using methods adapted for Synergy H1 Hybrid Microplate Reader (BioTek Instruments, USA).

Data Analysis

The statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 23 (SPSS Inc., Chicago, IL, USA). After checking the distribution and dispersion of the data, intergroup differences in the analyzed biochemical parameters were evaluated using one-way analysis of variance (ANOVA), followed by the Games-Howell post-hoc test for multiple comparisons. Statistical significance was defined as $p < 0.05$. Results are presented as median values with interquartile ranges (IQR).

RESULTS

The reference drug doxorubicin, tested *in vitro*, demonstrated a marked increase in SOD activity at 10.0 µmol/L by 61%, $p < 0.001$, and at 1.0 µmol/L by 27%, $p < 0.001$ compared to the control group, whereas CAT activity was significantly elevated only at 10.0 µmol/L, by 23% ($p < 0.001$).

Analyzing the research results of the impact of the copper(II) coordination compounds with thiosemicarbazone *in vitro* on SOD, a statistically significant increase was recorded for the compound CMA-18 at both concentrations (of 24% to 88%, $p < 0.001$). In comparison, the CMD-8 showed an increase at the concentration of 10.0 µmol/L by 48%, $p < 0.001$, while at the concentration of 1.0 µmol/L it increased by 17%, $p > 0.05$. The MG-22 at the concentration of 10.0 µmol/L increased by 47%, $p < 0.001$, and at the concentration of 1.0 µmol/L, an increase of 10%, $p > 0.05$, was observed compared to the control group (Tab. 2).

CMC-34, CMJ-33, and CMT-67 copper(II) coordination compounds with thiosemicarbazones *in vitro* at a concentration of 10.0 µmol/L significantly increase SOD by 41–80%, $p < 0.001$. Concurrently, at 1.0 µmol/L CMC-34 showed an increase of 18%, $p>0.05$, while CMJ-33 induced a decrease of 17%, $p>0.05$

and CMT-67 – a statistically significant decrease of 30%, p<0.001 compared to the control group.

The *in vitro* influence of the CMG-41, TIA-123 and TIA-160 copper(II) coordination compounds with thiosemicarbazones on SOD activity revealed a statistically significant increase for all compounds at concentration of 10.0 µmol/L (from 34% to 65%, p < 0.001). For CMG-41 at 1.0 µmol/L, a significant decrease was observed (24%, p < 0.05), while TIA-123 and TIA-160 induced an increase by 9% to 24%, p < 0.01.

Table 2. The Influence of Copper(II) Coordination Compounds with Thiosemicarbazones and Their Derivatives *in vitro* on Activity of the Antioxidant Enzymes.

Study Groups	SOD		CAT	
	u/c	% vs. control	µmol/L	% vs. control
Control	47.07 ; IQR 5.65	100%	14.20 ; IQR 1.29	100%
DOXO – 10 µmol/L	75.61 ; IQR 5.65 ***	161%	17.53 ; IQR 1.46 ***	123%
DOXO – 1 µmol/L	59.74 ; IQR 2.73 ***	127%	14.80 ; IQR 0.89	104%
CMA-18 – 10 µmol/L	88.53 ; IQR 1.62 ***	188%	15.08 ; IQR 0.36	106%
CMA-18 – 1 µmol/L	58.48 ; IQR 1.55 **	124%	15.05 ; IQR 0.73	106%
CMD-8 – 10 µmol/L	69.58 ; IQR 2.00 ***	148%	15.83 ; IQR 1.23 *	111%
CMD-8 – 1 µmol/L	54.88 ; IQR 5.57	117%	14.64 ; IQR 0.73	103%
MG-22 – 10 µmol/L	69.32 ; IQR 5.63 ***	147%	17.20 ; IQR 1.79 **	121%
MG-22 – 1 µmol/L	52.03 ; IQR 4.87	110%	15.10 ; IQR 2.49	106%
CMC-34 – 10 µmol/L	84.80 ; IQR 2.38 ***	180%	16.08 ; IQR 1.38 *	113%
CMC-34 – 1 µmol/L	55.92 ; IQR 10.34	118%	15.15 ; IQR 1.54	107%
CMJ-33 – 10 µmol/L	71.32 ; IQR 2.80 ***	151%	15.53 ; IQR 3.14	109%
CMJ-33 – 1 µmol/L	43.82 ; IQR 1.07	93%	14.00 ; IQR 0.39	99%
CMT-67 – 10 µmol/L	66.65 ; IQR 1.57 ***	141%	14.60 ; IQR 0.73	103%
CMT-67 – 1 µmol/L	32.95 ; IQR 2.10 ***	70%	13.85 ; 0.37	97%
CMG-41 – 10 µmol/L	77.72 ; IQR 1.60 ***	165%	16.18 ; 2.11 *	114%
CMG-41 – 1 µmol/L	40.50 ; IQR 1.98 *	86%	14.45 ; 0.77	102%
TIA-123 – 10 µmol/L	62.92 ; IQR 5.27 ***	134%	14.05 ; 0.86	99%
TIA-123-1 µmol/L	51.37 ; IQR 2.89 **	109%	13.98 ; 0.98	98%
TIA-160-10 µmol/L	68.33 ; IQR 5.22 ***	145%	13.98 ; 0.41	98%
TIA-160-1 µmol/L	58.43 ; IQR 4.49 **	124%	13,85 ; 0.41	97%

Note: *Statistical significance compared to the control group: * – p < 0.05; ** – p < 0.01; *** – p < 0.001. SOD – Superoxide Dismutase; CAT – Catalase;*

CAT activity was increased by CMA-18 by 6%, $p > 0.05$ at concentrations of 10.0 µmol/L and 1.0 µmol/L. At the same time, the compound CMD-8 at the concentration of 10.0 µmol/L showed an increase of 11%, $p < 0.05$, and at 1.0 µmol/L, a rise of 3%, $p > 0.05$ was noted. The coordination compound MG-22 indicated an increase at the concentration of 10.0 µmol/L by 21%, $p < 0.01$, while at 1.0 µmol/L, an increase of 6%, $p > 0.05$ was observed compared to the control group.

Analysis of the impact of the copper(II) coordination compounds on CAT highlighted CMC-34 at 10.0 µmol/L with an increase of 13%, $p < 0.05$, and at 1.0 µmol/L – of 7%, $p>0.05$. For the compounds CMJ-33 and CMT-67, a non-significant increase was observed at 10.0 µmol/L by 3% to 9%, $p > 0.05$, while at 1.0 µmol/L, no changes were noted compared to the control group (Tab. 2).

CAT activity showed a statistically significant increase of 14% with CMG-41 at 10.0 µmol/L ($p < 0.05$) compared to the control. In contrast, the other compounds caused non-significant decreases ranging from 1% to 3% ($p > 0.05$) relative to the control group (Tab. 2).

DISCUSSIONS

In this study, the activity of the antioxidant system was analyzed in the supernatant derived from peripheral blood exposed to copper(II) coordination compounds with thiosemicarbazones. Copper(II) coordination compounds with thiosemicarbazones have attracted significant interest among chemists and biologists due to their wide range of pharmacological effects. These compounds have demonstrated highly effective antitumor properties across various cancer types, including leukemia, pancreatic cancer, breast cancer, lung cancer, cervical cancer, prostate cancer, and bladder cancer (9,12,14).

To enhance their biological activity, several copper(II) coordination compounds with thiosemicarbazones series have been synthesized with modifications targeting the heteroaromatic system. Antineoplastic activity increased significantly when the carbonyl group of the side chain was attached at the α-position to the nitrogen atom in the ring, while attachment at the β- or γ-position rendered the compounds inactive (17). The tested copper(II) coordination compounds with thiosemicarbazones showed selective effects on antioxidant system indices *in vitro*, which may contribute to their strong antiproliferative and cytotoxic effects on tumor cells while sparing healthy cells. Elucidating their molecular mechanisms expands the theoretical understanding of these compounds and offers new prospects for developing effective drugs.

The antioxidant mechanisms include inhibition or scavenging of reactive species, metal reduction and chelation, and inhibition of oxidative enzymes (17). Although endogenous antioxidants play a vital role in protecting the body against OS, the intake of exogenous antioxidants through diet is considered to provide significant additional health benefits, contributing to the reduction of risk for chronic diseases. The role of antioxidants in maintaining health has been extensively studied over the past decades, particularly in the context of the free radical theory, which suggests that oxidative cellular damage is a central mechanism in the pathogenesis of chronic diseases and aging. This theory has been rigorously investigated through observational, clinical, and biochemical studies, which have highlighted the role of oxidative stress in numerous pathologies, including cardiovascular, neurodegenerative diseases, and cancer (18).

Although experimental *in vitro* studies using supplementation with isolated bioactive antioxidants have not consistently demonstrated significant benefits, scientific literature suggests that foods rich in natural antioxidants may positively contribute to health maintenance. This disparity in results may reflect the complexity of interactions between nutrients and the human physiological environment, emphasizing that the protective effects of antioxidants are more evident in the context of a balanced diet than through isolated supplement intake (19). Antioxidants have garnered increasing interest due to their protective roles in food and pharmaceutical products against oxidative degradation, as well as in the body against pathophysiological processes mediated by oxidative stress (18).

In this study, functional biomarkers within the antioxidant system were identified, quantified, and selected to assess OS levels in the supernatant derived from *in vitro* exposure of peripheral blood from healthy donors to locally administered copper(II) coordination compounds with thiosemicarbazones. These biomarkers can be used to determine the efficacy of new local pharmaceutical formulations.

Recent studies have increasingly provided evidence supporting the fundamental importance of copper in the formation and function of several enzymes and proteins, such as Cu/Zn SOD and cytochrome C oxidase. These molecules are involved in processes such as the neutralization of superoxide radicals, tissue respiration, energy metabolism, and DNA synthesis (20). Copper(II) coordination compounds have proven to be promising antitumor therapeutic agents, acting through multiple mechanisms (21). Aiming to protect the body from certain harmful prooxidants, this study evaluated a complex system of enzymatic and non-enzymatic antioxidants. The evaluation included the measurement of SOD activity, total antioxidant capacity, and CAT activity (22).

Catalase plays a crucial role in the decomposition of hydrogen peroxide into water and oxygen, thereby contributing to the efficient progression of cellular processes. H_2O_2 has been identified as a central redox metabolite, actively involved in detection, signaling, and regulation of redox homeostasis and has been recognized as the principal redox mediator in these fundamental cellular mechanisms. H_2O_2 is acknowledged as one of the leading non-transcriptional signaling molecules, alongside Ca^{2+} and ATP. As a signaling molecule, H_2O_2 diffuses through cells and tissues to initiate immediate cellular responses, such as changes in cell morphology, initiation of proliferation, and recruitment of immune cells. It is now clear that H_2O_2 plays fundamental regulatory roles in metabolic processes, going beyond its traditional function as a marker of oxidative stress or cellular damage and serving as a key regulator of metabolic homeostasis (23).

The function attributed to catalase is critical for protecting cells against oxidative damage caused by H_2O_2. Hydrogen peroxide is not only toxic due to its capacity to form other ROS, such as the hydroxyl radical *via* the Fenton reaction, but it also acts as a secondary messenger, being involved in multiple physiological and pathophysiological processes (24).

CONCLUSIONS

1. Copper(II) coordination compounds with thiosemicarbazones represent promising agents in biomedical research due to their redox properties and their antitumor, antimicrobial, and antioxidant activities. Regarding the antioxidant system, these compounds exhibit a complex action, influencing the balance between the production of ROS and the cellular antioxidant defense.
2. *In vitro* experimental studies have demonstrated that certain thiosemicarbazones modulate the activity of key antioxidant enzymes, such as SOD and CAT, thereby influencing the cellular capacity to neutralize ROS. These interactions suggest therapeutic potential in reducing oxidative stress involved in various degenerative and inflammatory conditions, including multifactorial diseases.
3. Such findings provide a valuable perspective on how copper(II) coordination compounds with thiosemicarbazones can modulate cellular antioxidant responses and open new directions for the development of treatment strategies based on targeted antioxidant activity. They also support the exploration of controlled oxidative stress as a therapeutic mechanism in the design of future therapeutic approaches.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The study protocol was approved by the Research Ethics Committee of the "Nicolae Testemițanu" State University of Medicine and Pharmacy of the Republic of Moldova (approval no. 5, ref. no. 38, on June 20, 2024). Participants were included in the study only after signing informed consent forms.

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Valeriana PANTEA, SCOPUS ID: 57211133811
Ecaterina PAVLOVSCHI, SCOPUS ID: 57225980274
Olga TAGADIUC, SCOPUS ID: 57212761286
Valentin GUDUMAC, SCOPUS ID: 57204126291

The *One Health* concept

Globally, the One Health concept is a worldwide strategy to expand interdisciplinary collaborations and communications in all aspects related to the health care of humans, domestic animals or wildlife, which can no longer be approached separately, but only jointly.

One Health addresses not only human and animal disease concerns, but also issues related to lifestyle, diet, exercise, the impact of different types of human-animal relationships, and environmental exposures that can affect both populations. In order to achieve the expected effects, it is also necessary to educate the population to make them aware of the risk factors and benefits of prevention, as well as communication and understanding between patients and healthcare providers.

HUMAN HEALTH

The WHO defined health in 1946 as “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity”, with the later addition of “the capacity to lead a socially and economically productive life”.

ANIMAL HEALTH

The OIE defines animal welfare in 2008: an animal is in good condition if it is healthy, enjoys comfort, is well fed, is safe, is able to display its innate (natural) behavior and does not suffer from unpleasant conditions such as pain, fear and stress.

PLANT AND ENVIROMENTAL HEALTH

Environmental health refers to those aspects of human health that include the quality of life determined by physical, biological, socio-economic and psycho-social factors in the environment. The interrelationships of people with the environment concern medicine, when an ecological system is in a state of equilibrium, the health of the population prevails.


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