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Abstract

Although the causes of depression include a wide range of conditions, from neurodegenerative to thyroid, they all have one common denominator, changes in the brain. Depression can be located in multiple brain regions simultaneously, which may be derived from a common molecular abnormality found in neurons in many brain regions. Abnormalities of receptors or impulse transmission could be the answer. In this work, a simple and accurate spectrophotometric method was developed for the analysis of escitalopram oxalate in various pharmaceutical formulations. In this method, absorbance was measured at 238 nm for escitalopram oxalate. Method validation parameters were studied. The method can be used for quality control of pharmaceutical forms containing escitalopram. Also, by the same method, the actual active substance concentrations compared to those listed on the package, the standard deviations and the relative standard deviations.

Keywords: validation of method, antidepressant drugs, spectrophotometric

Introduction

Depression can be translated as an imbalance at the level of certain neurotransmitters, the role of antidepressants being to increase the ratio of serotonin at the synaptic level. Depression can be due to totally unfortunate events that affect patients either at very young ages or those with major affective sensitivity, regardless of age. Serotonin also acts on other neurotransmitters, which activate brain areas responsible for mood and concentration. Major depressive disorder is a serious medical condition that is responsible for a considerable number of comorbidities. Despite decades of research, the neural basis for depression is still not fully understood. Evidence from neuroimaging, neuropsychiatry, and brain stimulation studies is explored to locate depression in the brain.

Also, the long-term effects of antidepressant drugs have not been studied. This paper presents a study on a pharmaceutical formulation that contains Escitalopram oxalates and which is presented in different commercial products. Certain general aspects that can generate depression are also presented. In order to apply an efficient treatment, the causes of this disease and the form of manifestation must be known.

The objective of the work is to carry out a study for the validation of a simple and precise spectrophotometric method for the analysis of Escitalopram oxalate.

Medical causes of depression

The medical and neurological causes of depression can be systematized in neurological, endocrinological, infectious and inflammatory causes and in various other causes such as cancer. Neuroimaging studies indicate that, despite the fact that many brain regions have been repeatedly implicated in the pathophysiology of depression, there is a notable absence of consistent findings to date. Recently, attention in neuroimaging has shifted from abnormalities at the regional level to those at the level of intraneuronal connections. Neuropsychiatric studies of Parkinson's disease and stroke provide clues to areas involved in depression. Similarly, stimulation of a variety of regions has been reported to be effective in treating depression. The nature of the distribution of depression needs to be thoroughly investigated, the primary and secondary affected areas need to be identified, and new models to explain the complexity of mental functions await exploration.

The most used antidepressants belong to the class of selective serotonin reuptake inhibitors, with two major indications: depressive episode and anxiety disorders. In the case of mild forms of depression, psychotherapy is recommended as a first step, and for moderate and severe forms, medication plus psychotherapy. Contrary to popular belief, antidepressants are not addictive. The effects appear gradually and set in after a few weeks of daily administration over a period of at least six months. It should be noted that antidepressants do not make you happy, they make you feel more in control of your own dark thoughts.

Anxiety disorders and depressive disorders are often underdiagnosed in contemporary medical practice due to their placement in the secondary, tertiary, or even further plane, often not being taken into account as a response to externalizing imbalance family chemist (Kaplan et. al, 2001). Everything we feel is due to connections that occur between neurons and chemical mediators. It was Mclean who introduced the concept that the brain is composed of three different assemblies, radically distinct in terms of their underlying chemistry, structure and, in evolutionary terms, the so-called triune brain (Maclean, 1985). Regional cortical imaging showed abnormalities in each subdivision to investigate the location of depression in the brain. A decreased metabolism at the level of the prefrontal cortex, especially at the dorsolateral and dorsoventral level within the prefrontal cortex is often found in major depressive disorder (Rigucci et. al, 2009), (Kimbrell et. Al, 2002). Deficient perfusion in these regions is associated with a reduction in problem-solving skills, a tendency to act on negative emotions, and suicidal behaviour (Desmyter et. al, 2011). This finding was successfully used in the formulation of a therapeutic strategy in which the dorsolateral prefrontal cortex was stimulated using transcranial magnetic stimulation (George, 2010). Decreased metabolism and blood circulation in this region in depression could be combated with antidepressant treatment (Mayberg et. al, 2000).

Structural imaging of the cortex has suggested a diminished volume of the frontal lobe present in depression, as well as a diminished volume of the orbitofrontal cortex (Kumar et. Al., 2000), (Schweitzer et. Al., 2001), (Bremner et. Al. 2002). The anterior cingulate cortex has become a subject of study in the psychopathology of depression because reductions in cingulate gyrus metabolism have been recorded in familial depression, and studies by Mayberg have described abnormalities in the cingulate and dorsal gyrus in depressive disorder (Critchley, 2004). This anterior cingulate cortex has been shown to be functionally divided into dorsal and ventral parts. The dorsal part is involved in cognitive aspects of emotion, including the association between emotional stimuli with negative valences, while the ventral part (gyrus) shows an extensive bilateral connection with limbic regions such as the cerebral amygdala and dorsomedial thalamus, as well as with the areas that control mood, the medial prefrontal cortex and the lateral and medial orbitofrontal cortex (Etkin et. al., 2006).

The influence of the ventral anterior cingulate cortex on the hypothalamus, which controls the endocrine system. The insular cortex and especially its anterior subdivision are involved in the expression of emotions such as disgust, self-esteem, self-evaluation of internal visceral state (Critchley et. al., 2004) and in response to taste and smell stimuli. In depression, insular cortex activity has been enhanced in response to disgust-inducing stimuli (Surguladze et. al., 2010), to negative images (Anand et. al., 2005), and insular volume is correlated with depression scores (Sprengelmeyer et. al., 2011). The main subcortical limbic regions implicated in depressive disorder are the amygdala, hippocampus, and dorsomedial thalamus. Both

structural and functional abnormalities in these areas have been found in depression. A reduced hippocampal volume (Schweitzer et. Al., 2001) has been noted in depressed patients. Patients who relapse with treatment have been shown to have higher hippocampal volumes before treatment, while patients with lower hippocampal volumes were more prone to relapse (Sprengelmeyer et. al., 2011).

The medication administered in the treatment of depression is very diverse and still insufficiently studied. The association of various diseases with depression complicates treatment schemes.

This paper presents studies on a drug presented as a commercial product under the name of Escitalopram. The objective of the work is to carry out a study for the validation of a simple and precise spectrophotometric method for the analysis of escitalopram oxalate.

Material and Method

Escitalopram is a selective serotonin reuptake inhibitor. It is the pure S enantiomer of the bicyclic racemic phthalate derivative of citalopram. The chemical formula is S-(+)-1-[3-(dimethylamino) propyl]-1-(p-fluorophenyl)-5-phthalancarbonitrile oxalate. Several analytical methods have been described for the quantitative determination of this substance, such as LC-MS for escitalopram (Singh et. al., 2004).

As equipment, a UV-VIS spectrophotometer, Shimadzu model 1601, which was set at a bandwidth of 1.8 nm and a wavelength with an accuracy of ± 0.5 nm, with a pair of cells of quartz having an optical wavelength of 10 mm. A digital balance HR 200 (Afcoset) and an ultrasonic bath SW 45 were also used. The standard stock solution was prepared by dissolving 10 mg of escitalopram oxalate in 100 mL of reference solvent to obtain a stock solution with a concentration of 100 μg/mL of the drug. The standard stock solution was diluted with reference solvent, obtaining solutions with concentrations of 2-20 μ_g/mL. They were scanned in UV and the calibration curve was made taking into account absorbance and concentrations.

To prepare the stock solution, ten 20 mg escitalopram oxalate tablets and ten 10 mg tablets from three different other brands were triturated to a fine powder. The powder equivalent to one 20 mg and one 10 mg tablet was transferred into a separate 100 mL container. They were then dissolved in the reference solvent by sonication for 20 minutes. The final solution was diluted with reference solvent to yield a stock solution of 200 μg/mL for the first brand and 100 μg/mL for the three different other brands. These solutions were filtered through Whatmann filter paper and further diluted to obtain six replicates. These were analyzed using the spectrophotometer.

Results and Discussions

The establishment of the parameters for the validation of the method for determining the concentrations of escitalopram oxalates by the spectrophotometric method is based on first obtaining the UV-VIS spectra and then establishing a calibration curve

based on which the initial parameters for the validation of the method should be established. In Fig. 1 are shown the UV-VIS spectra obtained for escitalopram oxalate, which shows a maximum at the wavelength of 238 nm.

In Fig. 2 shows the calibration curve for escitalopram oxalate. The characteristics of the calibration curve are systematized in Table 1. The correlation coefficient of 0.9999 shows that a very good linearity was obtained in the concentration range 2-20 µg/mL.

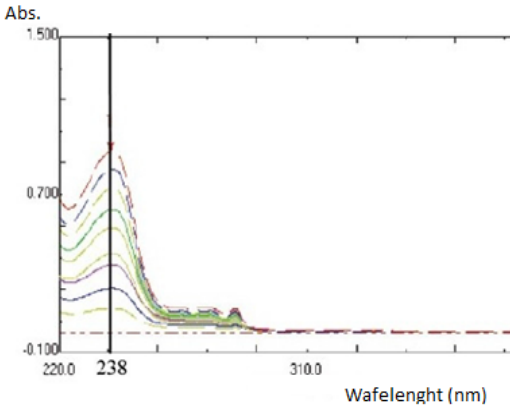

Fig.1 UV-VIS spectra for escitalopram oxalate

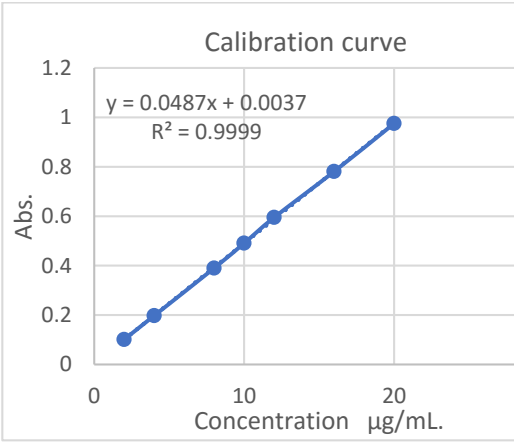

Fig.2 Calibration curve for escitalopram oxalate

Table 1 Characteristics of the calibration curve for escitalopram oxalate

Parameters	UV-VIS Spectrophotometric Methods
The regression equation	Y=0,0487x-0,037
Slope (b)	$48{,}7x10^{-3}$
Intercept (a)	$3.7x10^{-3}$
Linearity (μ_g/mL)	2,003-20,032
Correlation coefficient (r)	0,9999

Table 2 shows the results obtained for the validation parameters of the studied spectrophotometric method. All parameters were analyzed in compliance with the validation standards from the SR-ISO 8466/1990 series and the methodologies in force (Rodica Sîrbu et. al., 2017).

Table 2 Parameters obtained for method validation

Parameters	Result Escitalopram oxalate
Specificity	No interferences other than excipients were found
Linearitaty (correlation coefficient r)	0,9999

Accuracy*(% recovery)	99,72% 99.55% 99.69% and 99,98%
Relative Standard Deviation	0.3105
RSD% Accuracy (Precision)	0.616
Repeatability (n=6)	0,903
Over the course of a day (n=3)	0,176
Over consecutive days (days=3)	0,179
Lower limit of detection (LOD)(μg/mL)	0,191
Minimum limit of quantification (LOQ) (μg/mL)	0,637

*Average of three determinations

To study the validity and reproducibility of the method the recovery of the drug was studied which was determined at 80, 100 and 120% levels. A percentage recovery of 99.72% 99.55% 99.69% and 99.98% was made calculated for all brands. The percentages greater than 99% together with the low standard deviation justify the accuracy of the method. The relative standard deviation of 0.3105 also justifies the good accuracy of the method.

In order for the proposed method to ensure the precision required for an analytical determination, the acceptance criterion is considered met when for each concentration level, 0.616 is obtained, i.e. RSD% < 1%. In the present study, this criterion was fulfilled.

The LOD and LOQ were determined based on the standard calibration curve. To be estimated, the diluent was scanned under the UV region six times and the signal-to-noise ratio was determined. The LOD (0.191 μg/mL) and LOQ (0.637 μg/mL) were regarded as amounts for which the ratio was 3:1 and 10:1, respectively. Confirmation of accuracy is achieved by studying repeatability. This criterion demonstrates the closeness of the measured values to each other, for a number of measurements, obtained under the same conditions. To confirm the accuracy, it is demonstrated that applying the method, repeatedly, for the same samples, generates similar results.

In Table 3, the pharmaceutical formulations (brands) containing Escitalopram are systematized with the most important uses.

Table 3 Systematization of the analyzed drugs according to their uses

Escitalopram Brand	Uses
Escitalopram ATB 20 mg COM	Treatment of episodes of major depression, Treatment of panic disorders accompanied or not by agoraphobia. Treatment of social anxiety disorders (social phobia). Treatment of generalized anxiety disorders. Treatment of obsessive-compulsive disorders.
Escitalopram Aurobindo	Contains the active substance escitalopram. Escitalopram Aurobindo belongs to a group of antidepressant medicines called selective serotonin reuptake inhibitors (SSRIs).

	It acts on the serotonergic system in the brain, by increasing the concentration of serotonin. It is used to treat depression (major depressive episodes) and anxiety disorders (such as panic disorder with or without agoraphobia, social anxiety disorder, generalized anxiety disorder and obsessive-compulsive disorder).
Escitalopram Actavis	It belongs to a group of antidepressant drugs called SSRIs (selective serotonin reuptake inhibitors). It acts on the serotonergic system in the brain by increasing the concentration of serotonin. Disorders of the serotoninergic system are considered an important factor in the onset of depression and similar conditions.
Cipralex contains the active substance escitalopram.	It belongs to a group of antidepressant drugs called selective serotonin reuptake inhibitors (SSRIs). It acts on the serotonergic system in the brain by increasing the concentration of serotonin. It is used in the treatment of depression (major depressive episodes) and anxiety disorders (such as panic disorders with or without agoraphobia, social anxiety disorders, generalized anxiety disorders and obsessive-compulsive disorders).

Table 4. The results obtained and compared with the labels proposed by the brands

Brand	Amount on label (mg)	Amount found*(mg)	% of amount on label	Producer
Escitalopram Atb20	20	19,82±0,022	99,43±0,295	ANTIBIOTICE S.A. - Romania
Escitalopram Aurobindo 10 mg	10	9.95±0,042	99,35±0,275	AUROBINDO PHARMA ROMANIA S.R.L. - Romania
Escitalopram Actavis	10	9.97±0,012	99.69±0,195	ACTAVIS LTD. - Malta
Cipralex	10	9,91±0,032	99,53±0,325	H. LUNDBECK A/S - Danemarca

*Average of six estimates

Table 4 shows the results obtained by applying our validated method. It is found that the results show a good agreement between the active substance levels found in practice and the inscriptions on the product labels. The obtained results are in accordance with the new methodologies used in pharmaceutical control (Rodica Sîrbu et. al., 2017).

Conclusion

The study carried out allows us to highlight the following conclusions:

- Although the causes of depression include a wide range of conditions, from neurodegenerative to thyroid, they all have a common denominator, changes in the brain. These areas include the ventral tegmental area, responsible for the motivation-reward complex, and the insular and anterior cingulate cortex areas associated with psychological pain and suffering.
- Depression can be located within multiple brain regions simultaneously which may be derived from a common molecular abnormality found in neurons in many brain regions. Abnormalities of receptors or impulse transmission could be the answer.

In order to establish a correct diagnosis, the specialist doctor will have to approach the patient in order to obtain data from his history, such as anhedonia, lack of ambition, decreased energy level, social isolation, as well as data from the observation of the present mental state and somatic projections.

As for treatment, it includes both pharmacological and psychotherapy measures. Selective serotonin reuptake inhibitors (SSRIs) are the most prescribed first-line drugs in the treatment of depression, thanks to their safety, effectiveness and increased tolerability.

A simple and accurate spectrophotometric method for the analysis of escitalopram oxalate was developed in the paper. In this method absorbance was measured at 238 nm for escitalopram oxalate. The method can be used for quality control of pharmaceutical forms containing escitalopram. Escitalopram showed linearity at a concentration between 1-20 μg/mL, with a correlation coefficient of 0.9999.

Also, by the same method, the actual concentrations of active substance compared to those listed on the packaging, the standard deviations and the relative standard deviations both during one day and over several consecutive days were revealed.

Future studies should emphasize on studying in more detail various spectrophotometric methods for different drugs.

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Mandatory COVID-19 Vaccination and Free Self-Determination in the Health Field in Italy

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Abstract

The COVID-19 pandemic and the issues related to health emergency management have raised concerns about fundamental rights protection. One of the most complex and contentious issues in doctrine and jurisprudence is the introduction of mandatory COVID-19 vaccination for specified categories of subjects, such as healthcare workers and individuals of a specific age range, as occurred in Italy. The debate in Italy over the mandatory COVID-19 vaccination requirement focuses on whether this obligation interferes with the individual's fundamental right to free self-determination as guaranteed by Article 32 of the Italian Constitution. In jurisprudence and doctrine, conflicting opinions are noted concerning this topic. Part of the doctrine argue that mandatory COVID-19 vaccination compromises some fundamental rights guaranteed by the Italian Constitution, such as the right to free self-determination and respect for human dignity. Other opinions find the basis of the vaccination in the community's best interests, citing Article 32 of the Italian Constitution, which allows for the imposition of compulsory health treatment to safeguard citizens' health. In this regard, the issue of mandatory COVID-19 vaccination requires a reflection on the balance of two fundamental rights: the individual right to free self-determination and the community's interest in terms of protecting public health.

Keywords: Mandatory COVID-19 vaccination, free self-determination, fundamental rights, human dignity, Italian Constitution

Introduction

This contribution analyses the much-discussed issue of the mandatory Covid-19 vaccination requirement that the Italian state adopted during the health emergency caused by the COVID-19 pandemic. The Decree-Law of April 1, 2021, No. 44, converted into Law No. 76 of May 28, 2021, concerning the "*Urgent measures for the containment of the COVID-19 epidemic, in the matter of anti-SARS-CoV-2 vaccinations,*

justice, and public competitions,"[1] provided for the COVID-19 vaccination as an essential requirement for the exercise of the profession for all health professionals who carry out activities in public and private health, social-assistance establishments, as well as in pharmacies, parapharmacies, and professional offices. Subsequently, the mandatory COVID-19 vaccination has been extended to workers in residential and social facilities by Decree-Law No. 122/2021[2] and all Italian and non-Italian citizens residing in Italy of at least 50 years of age by Decree-Law No. 1/2022[3].

This obligation has been the subject of several rulings by some regional courts, which have raised the question of constitutional legitimacy because it interferes with the enjoyment of some fundamental rights guaranteed by the Italian Constitution.

A second relevant profile relating to mandatory COVID-19 vaccination is the provision of informed consent before the vaccination. In this regard, a brief reflection on the nature of informed consent is necessary, considering that the latter assumes relevance only for voluntary medical treatment.

Methodology

This paper carries out a legal and philosophical analysis of the mandatory COVID-19 vaccination requirement introduced by the Italian legislator. For this purpose, the study is composed of three parts: The first section addresses the concept of free self-determination in the health field, its relevance in health protection, and the restrictions placed on this principle by the Italian Constitution. The second section explores the diverse doctrinal and legal perspectives on obligatory COVID-19 vaccination. The last part of the study focuses on discussions regarding the principal concerns and issues raised by various Italian courts about mandatory COVID-19 vaccination.

To better comprehend the topic of compulsory COVID-19 vaccination, the study employs qualitative research methods, taking into account the various perspectives offered by doctrine and jurisprudence.

Free self-determination as a fundamental right

Informed consent is an essential prerequisite for any medical treatment. In line with Article 32 of the Constitution, no medical treatment can be carried out without the person's prior and explicit consent. The necessity of informed consent to medical treatment is foreseen by a variety of sources, including international law sources such

[1] Decree-law 1 April 2021, No. 44, converted into law 28 May 2021, No. 76, "*Urgent measures for the containment of the COVID-19 epidemic, in the matter of anti-SARS-CoV-2 vaccinations, justice, and public competitions*", in GU General Series No. 128 of 31-05-2021.
[2] Article 2, Decree-Law No. 122/2021, "*Urgent measures to deal with the COVID-19 emergency in schools, higher education, and social and healthcare facilities"*, in GU No. 217 of 10-09-2021.
[3] Decree-Law 7 January 2022, No. 1, "*Urgent measures to deal with the COVID-19 emergency, in particular in the workplace, schools, and higher education institutes"*, converted with amendments by Law 4 March 2022, No. 18, in GU No. 56 of 03/08/2022.

as the Convention on Human Rights and Biomedicine, adopted in Oviedo on April 4, 1997, ratified by law No. 145 on March 28, 2001, in Article 5 and Article 3 of the Charter of Fundamental Rights of the European Union, proclaimed in Nice on December 7, 2000.

The patient's free self-determination in the constitutional design includes not only the right to express free consent after being informed but also the right to express dissent and refuse (See Montange, 1973-1974, p. 1664; Furramani & Bushati, 2021, pp. 268 et seq.) or suspend a medical treatment already in progress when possible. The purpose of informed consent is to make the patient aware of his state of health (Bilancetti, 1997, pp. 354 et seq.) while also allowing him to participate in the selection of medical treatment by adequately informing him (See Cass. Pen., Sez. Un., 21 January 2009, No. 2437; Pulitanò, 2007, p. 1209; Rodriguez, 2014, p. 572) on health conditions, diagnosis, prognosis, potential risks of treatment, alternative medical treatments, and the consequences of refusing the medical treatment[1] (See Furramani & Bushati, 2021, p. 266 et seq.; Furramani, 2017, pp. 364-365; Fresa, 2008, p. 67; Ruggiero, 1996, p. 192; Norelli & Mazzeo, 2001, pp. 63 et seq.). It is crucial to recognize that free self-determination is a fundamental right that safeguards a person's health[2], and for this reason, a considerable part of the doctrine considers it an essential instrument for fully implementing the right to health (Casonato, 1995, p. 195; Gennari, 2006, p. 1413; Guerra, 2008, p. 438; Gorgoni, 1999, pp. 488 et seq.; Santossuoso, 1996, p. 16; Furramani, 2017, p. 364).

Limits to the patient's free self-determination: Compulsory health treatment

The implementation of compulsory health treatment is an exception to the general rule that medical treatment must be voluntary. In the constitutional provision, obligatory medical treatment is covered by the absolute legal reserve (Veronesi, 2011, p. 63; Mazzacuva, 1984, pp. 424 et seq.; Gribaudi, 2012, p. 6; Casciaro & Santese, 2012, p. 234; Della Rocca, 2014, p. 394; Vallini, 2008, p. 71; Eusebi, 1995, p. 734; Negroni, 2021, p. 110) to protect public health (Veronesi, 2011, p. 64)[3], while respecting the human person.

On closer inspection, compulsory health treatment must necessarily involve a benefit for the community's health since it compromises the fundamental rights of the individual, such as his free self-determination and his right to health (Casciaro & Santese, 2012, pp. 112–113), and from here we can notice the collective dimension of

[1] Article 1, Law 22 No. 219, December 2017, "*Rules on informed consent and advance directives on treatment",* in GU No. 12 of 16. 01. 2018.
[2] Article 2 of the Italian Constitution: "*The Republic recognizes and guarantees the inviolable rights of man, both as an individual and in the social formations where his personality takes place, and requires the fulfillment of the mandatory duties of political, economic, and social solidarity."*
[3] See Cass., Sez. III, 30 January 2009, No. 2468, in *Giust. civ*., 2009, 4-5, I, p. 885.; Even when public interest is in danger, Law No. 180/1987, in Article 1, Paragraph 5, provides that: "*The compulsory medical examinations and treatments (...) must be accompanied by initiatives aimed at ensuring the consent and participation of those who are obliged."*

the right to health as an interest of the community. According to Italian legislation, to impose compulsory health treatment, it is necessary to present a current, concrete, and direct danger to the community (Veronesi, 2011, p. 69). In addition to protecting collective health, medical treatment must also protect the health of the individual who undergoes it[1] (Veronesi, 2011, p. 69; Mantovani, 1992, p. 61). Only in this way is it permissible to limit a person's free self-determination and ensure a fair balance in the protection of fundamental rights.

In the opinion of Carlassare, "*any limitation to freedom (...) must find an adequate justification in the collective interests*, *taking into account that in principle, the Constitution makes affirmations of freedom and the limitations are not the rule but the exception*" (Carlassare, 1967, pp. 110–111). According to this opinion, the Constitution guarantees individual freedom, and the latter can be limited only in exceptional cases in the presence of a relevant interest of the community (See Negroni, 2021, p. 41).

This orientation finds its basis in the jurisprudence of the Italian Constitutional Court in Decision No. 307 of 1990, in which the Court examines the admissibility requirements of compulsory health treatment. In this case, the Court found that "*compulsory health treatment is applied only when it does not negatively affect the state of health of the person who is obliged, except for those consequences which, due to their temporariness and insignificance, appear normal for any health intervention and therefore tolerable*."[2] The Court also recognized the right of the individual to compensation for damage, even minimal damage, suffered as a consequence of compulsory health treatment (See C. Cost., June 22, 1990, No. 307; Flick, 2013, p. 19; Fineschi, 1990, pp. 924 et seq.).

According to a part of the doctrine that carries out a combined reading of Article 32 of the Constitution, compulsory health treatment necessarily requires the coexistence of individual and collective interests. In this context, it must not have a detrimental impact on personal health and must avoid causing harm or endangering community health[3] (Carlassare, 1967, pp. 109 et seq.; Vincenzi Amato, 1976, p. 172 et seq.; Sandulli, 1978, p. 517; Luciani, 1980, p. 782; Modugno, 1982, pp. 311 et seq.; Veronesi, 2011, pp. 154-155; Negroni, 2021, pp. 39-40).

In this sense, also based on the jurisprudence of the Constitutional Court, compulsory health treatment does not represent any incompatibility profile with Article 32 of the Constitution if the latter is aimed at safeguarding both individual and community

[1] ECHR, *Vavřička and others v. The Czech Republic*, applications nos. 47621/13 and 5 others.

[2] In this sense, Italian Constitutional Court Decisions Nos. 5/2018; 307, 22 June 1990, in *Riv. it. med. leg*., 1990, p. 914; 218/1994, 258/1994, and 118/1996.

[3] Italian Constitutional Court, Decisions Nos. 307/1990; 218/1994; 258/1994; 118/1996; 27/1998; 226/2000; 107/2012; 368/2017; 5/2018.

health[1]. On the other hand, it should be noted that in Decision No. 307 of 1990, the Constitutional Court also stated that: "*The constitutional importance of health as an interest of the community is not alone sufficient to justify the health measure. This observation (...) does not imply that each person's health should be sacrificed for the protection and health of others*"[2].

The mandatory COVID-19 vaccination requirement in the Italian legislation

The imposition of the mandatory COVID-19 vaccination requirement by the Italian legislator, in Article 4 of the law decree of 1 April 2021, No. 44, converted into Law No. 76/2021, has involved great debates in doctrine and jurisprudence, and it must be noted that today there is still no univocal interpretation on the topic. The basic question is whether this vaccination violates some fundamental rights that are constitutionally guaranteed, such as the right to free self-determination in the medical field and human dignity, along with a series of other constitutionally guaranteed rights, such as the right to work and to receive a salary. The issue has been the subject of legal debates and several decisions by regional administrative courts.

Part of the doctrine, contrary to the mandatory COVID-19 vaccination requirement, has advanced the theory that COVID-19 vaccines, such as those from Pfizer BioNTech, AstraZeneca, and Moderna, approved by the European Union's competent authorities, represent a type of experimental medical treatment (Negroni, 2021, p. 128). In support of this thesis, the doctrine underlines that those vaccinations have been authorized by the EMA through conditional marketing authorization, taking into account that it is impossible to report comprehensive and complete data on the drug's effectiveness and long-term side effects. Conditional marketing authorization is usually issued when the pharmaceutical product presents insufficient data and evidence compared to the regular approval, and the producer must submit comprehensive risk and benefit data after the authorization (Cenci, 2021, p. 5). In this respect, part of the doctrine (Negroni, 2021; Cenci, 2021) considers that mandatory vaccination against COVID-19 exposes the person to unknown health risks, given that long-term damage is impossible to predict and the safety and efficacy of vaccines are not certified by complete clinical evidence.

In this regard, the doctrine highlights that the notion of free self-determination in the medical field, as enshrined in Article 32 of the Italian Constitution, contains another fundamental principle according to which no one can be subjected to medical experimentation. This concept emphasizes respect for human dignity and human beings. Consequently, experimental medical treatment, including mandatory vaccination, cannot be imposed even by law as it contrasts with the letter and spirit of Article 32 of the Italian Constitution (Negroni, 2021, pp. 120–122).

[1] Italian Constitutional Court, Decision No. 307/1990.; In the same direction ECHR, *Vavřička, and others v. The Czech Republic*, applications nos. 47621/13 and 5 others.
[2] In the same direction, the Italian Constitutional Court, Decision No. 118/1996.

Another concern about obligatory vaccination is the harm caused by COVID-19 vaccines. Doctrine and jurisprudence perceive these damages as superior to normal tolerance, resulting in a conflict between the individual and collective dimensions (Baccarra & Rinaldi, 2022, p. 64). A part of the doctrine, based on the Constitutional Court's ruling No. 307 of 1990, considers that mandatory vaccination generally affects individuals' health, causing damages in the pursuit of collective interest, but these damages are permissible if they are bearable or of normal tolerance. Accordingly, the doctrine based on statistics, argues that adverse events, including fatal ones caused by COVID-19 vaccines, are significantly superior to those of vaccines practiced for years and cannot fall into the category of events of normal tolerability (Cenci, 2021, p. 19).

The court of Padua intervened on the topic of mandatory vaccination and evaluated the vaccination obligation based on the proportionality of the measure taken in relation to the objective it seeks to achieve. In this sense, the Court of Padua ruled that compulsory vaccination against COVID-19 is "*unsuitable – and thus unreasonable (...) – to achieve the intended purpose*[1]," declaring it in violation of the principles of proportionality, adequacy, and reasonableness guaranteed by Article 3 of the Italian Constitution and Articles 15 and 52, first paragraph, of the European Union Charter of Fundamental Rights (Tundo, 2022).

Contrary to the above, another part of the doctrine believes that vaccines against COVID-19 are not experimental and that even if approved by the competent authorities with conditional authorization, they have exhausted the experimentation phase (Barracca & Rinaldi, 2022, p. 54). In the same direction, we find the decision of the Italian Council of State No. 7045 of 20.20.2021, which highlights that COVID-19 vaccines are effective and not experimental (Baccarra & Rinaldi, 2022, p. 68). With this decision, the Council of State emphasized the significance of two fundamental rights: on the one hand, the right of the individual to free self-determination and, on the other hand, the right to health as a community interest, both guaranteed by Article 32 of the Constitution, accentuating the need for a fair balance between these two fundamental rights[2]. In this regard, the Council of State refers to Decision No. 5 of 2018 of the Italian Constitutional Court concerning the mandatory vaccination requirement for minors introduced in 2017, where the latter declares that the imposition of compulsory health treatment is not incompatible with Article 32 of the Italian Constitution if the medical treatment is directed to recover or preserve the health of the person who is obliged and to protect the community's health[3] (See Pisani, 2022, p. 911); the compulsory health treatment must not adversely affect the state of health of those subjected to it, except for tolerable consequences; and in the case of damage to the subject's health, the law must provide fair compensation in

[1] Court of Padua, Judgment of 28 April 2022. In this direction also T.A.R. Lombardia, Sez. 1, 16 giugno 2022, No. 1397.
[2] Council of State, Third Section, 20 October 2021, No. 7045, para. 35.
[3] Italian Constitutional Court, Decision No. 5/2018.

favor of the latter. However, in all cases, this treatment must respect the human person[1] (Negroni, 2021, p. 38). Therefore, the Council declared that the doubts about the constitutional legitimacy of the mandatory COVID-19 vaccination requirement, introduced by Decree-Law No. 44/2021, were manifestly unfounded.

This decision has been the subject of criticism from a part of the doctrine (Conti, 2022), specifically when it considers manifestly unfounded the question of constitutional legitimacy proposed by the applicants. On this occasion, Article 4 of Decree-Law No. 44 of April 1, 2021, converted with amendments into Law No. 76 of May 28, 2021, was alleged to be in contrast with Article 32 (free self-determination) and Article 3 of the Italian Constitution in terms of reasonableness, proportionality, and equality, together with a series of other articles, including Articles 2, 9, 33, 1, 2, 4, 35, and 36 of the Italian Constitution. As a result, Conti observes that the rejection of the constitutional legitimacy check of the mandatory COVID-19 vaccination requirement reveals the system's poor health in terms of constitutional guarantees (Conti, 2022).

Despite the rejection of the question of constitutional legitimacy by the Council of State, several regional courts have found this question not unfounded[2], leaving the final verdict to the Constitutional Court, which has scheduled a hearing for the following November 29th. The Administrative Justice Council for the Region of Sicily, with Ordinance No. 351 of March 22, 2022, was one of the courts that considered the issue of constitutional legitimacy to be not unfounded. In this ordinance, the Council analyzes the requirements for the legitimacy of obligatory health treatment. Based on Constitutional Court Decision No. 307/1990, the Council reaffirms that compulsory medical treatment must preserve and benefit the health of the obliged subject, as well as protect public health and if adverse events occur, they must enter the limit of normal tolerability.

The Council ordinance raises critical issues concerning mandatory vaccination, including the adverse effects of COVID-19 vaccines. In this regard, the Court refers to the Supervisory Institute's statistics on the side effects of the COVID-19 vaccine, where it is noted that the percentage of adverse events occurring is well above the average of adverse effects occurring with vaccines used for years[3]. The Court's concern is whether adverse effects fall within the normal tolerance, considering the relatively low number of cases with fatal outcomes. In light of the findings of pharmacovigilance controls based on spontaneous reporting of adverse events, the Council concludes that the tolerance limit for adverse events has been surpassed (Gambardella, 2022). Another critical profile that the Council highlights concerns the collection of informed consent, even in the case of mandatory vaccination. The Council

[1] Italian Constitutional Court, Decision No. 307/1990.
[2] See T.A.R. Lombardia, Sez. 1, 16 giugno 2022, No. 1397; Administrative Justice Council for the Region of Sicily, Ordinance No. 351 of March 22, 2022.
[3] Administrative Justice Council for the Region of Sicily, Ordinance No. 351 of 22.03.2022, para. 18.1.

observes that informed consent is relevant in the context of free willful self-determination but not in the context of a legal obligation[1]. In case of compulsory vaccination should be provided an information document[2]. In the opinion of the Council, Article 4, paragraphs 1 and 2 of the Decree-Law No. 44/2021 (converted into Law No. 76/2021) conflicts with some Articles of the Italian Constitution, such as: "*Article 3 (under the parameters of rationality and proportionality); Article 32 (in terms of the impact on free self-determination regarding health treatments that have effects that are neither mild nor transitory); Article 97 (good performance, also about the criticalities of the monitoring system); Article 4 (right to work); Articles 33 and 34 (right to education); Article 21 (right to free expression, which includes the right to dissent)*" [3].

However, the question of the legitimacy of the mandatory COVID-19 vaccine requirement has not yet found a definitive answer. Consider the orientation of the European Court of Human Rights, which has deemed inadmissible the request to suspend the mandatory vaccination requirement under Article 39 of the ECHR, presented by firefighters and first aid workers in France due to the provision of the mandatory COVID-19 vaccination requirement[4]. The Court ruled that the applicant's request was inadmissible because there was no risk of infringement of the rights guaranteed by Articles 2 and 8 of the European Convention on Human Rights[5].

Discussions

To adequately address the topic of compulsory vaccination against COVID-19, we have to consider several issues. In the first place, we have to assess whether this obligation violates human dignity, as noticed by Negroni (See Negroni, 2021). Accordingly, it is necessary to analyze whether vaccination against COVID-19 represents an experimental treatment. In this respect, we have to consider that the vaccines against COVID-19 have obtained conditional marketing authorization from the EMA for the immediate use of drugs due to the grave health situation caused by COVID-19 after having exhausted the experimental phase (Barracca & Rinaldi, 2022, p. 54). Based on these statements, part of the doctrine and jurisprudence exclude that they constitute an experimental treatment (Barracca & Rinaldi, 2022; Council of State, No. 7045/2021). On the other hand, we have to take into account the fact that these vaccines produce adverse effects, and this has been reported by the evidence of the institute of pharmacovigilance based on spontaneous reporting of adverse events[6]

[1] According to Giovanni Maria Flick, informed consent has no relevance in front of a legal obligation (Milella, 2022).
[2] Administrative Justice Council for the Region of Sicily, Ordinance No. 351 of 22.03.2022, para. 18.7.
[3] *Ibid.*
[4] French Law No. 1040/2021, 5 August 2021.
[5] ECtHR, *Abgrall and other 671 v. France*, dec., No. 41950/21, 24 August 2021.
[6] *Rapporto sulla sorveglianza dei vaccini anti COVID-19*, 12, 27.12.2020 – 26.6.2022, retrieved from:https://www.aifa.gov.it/documents/20142/1315190/Rapporto_sorveglianza_vaccini_COVID-19_12.pdf.

and long-term effects are impossible to know, considering the lack of complete and exhaustive evidence.

The second concern is the infringement of the principle of free self-determination in the health field. In this sense, Article 32 of the Italian Constitution guarantees the right to free self-determination in all decisions concerning health and psycho-physical integrity; on the other hand, this article provides a limitation of free self-determination to protect public health in the cases explicitly provided for by law. For this purpose, we should consider the decision of the Italian Constitutional Court No. 5/2018, which recently declared that the vaccinal obligation imposed on minors is not incompatible with Article 32 of the Italian Constitution.

When interpreting this statement, we must consider the requirements that need to be met by mandatory medical treatment. According to the Italian Constitutional Court decision No. 307/1990, compulsory medical treatment must preserve and benefit the health of the obliged subject, as well as protect public health, and if adverse events occur, they must enter the limit of normal tolerability. So the question that doctrine and jurisprudence see as crucial is whether adverse events caused by COVID-19 vaccines, including the rare fatal ones, fall into the category of tolerable events. In this context, the principal question is what should be considered "tolerable" in light of the Italian Constitutional Court's view. On the other hand, this assessment may require complete and exhaustive evidence regarding the long-term adverse effects of these vaccinations, which is currently lacking.

The last concern is whether the legislator's obligation complies with the principles of proportionality, equality, and rationality of the measure adopted concerning the goal to be achieved, as outlined in Article 3 of the Italian Constitution[1]. In this sense, we have to assess whether this is the only measure to protect public health or whether other more appropriate measures with the same purpose are possible. It is crucial to recognize that the issue of mandatory Covid-19 vaccination is a complex and delicate ethical, political, and legal issue with two significant interests at stake that are diametrically opposed: on the one hand, the individual's interest in protecting his health and not being forced to undergo unwanted medical treatment; on the other hand, public health protection.

Conclusions

The aim of the present research was to examine the different concerns that the mandatory Covid-19 vaccination requirement has raised in Italy. The findings of this study indicate that we have to consider the issue of mandatory COVID-19 vaccination in light of two fundamental principles: free self-determination in health choices on one side and public health on the other. In this context, the Italian Constitutional Court has the difficult task of analyzing whether the mandatory COVID-19 vaccination

[1] See T.A.R. Lombardia, Sez. 1, 16 giugno 2022, No. 1397; Court of Padua, Judgment of 28 April 2022.

interferes with the delicate balance between individual and public health, considering the adverse events caused by COVID-19 vaccines.

The most acceptable thesis seems to be the less intrusive policy, which respects individual autonomy in decisions involving health and psycho-physical integrity (Negroni, 2021, pp. 86–87). In this respect, the lawmaker is recommended to consider appropriate measures that respect individual autonomy while also preserving public health.

The issue of the mandatory COVID-19 vaccination requirement in Italy is still controversial, considering that several regional courts have raised doubts about its constitutional legitimacy. Subsequently, the Italian Constitutional Court has set the first hearing for November 29, 2022, in such a way as to express the compliance of this obligation with the fundamental rights guaranteed in the Italian Constitution.

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Evaluation of Gait in Albanian β-thalassemia Patients

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Abstract

One of the most prevalent genetic diseases in the world is beta thalassemia. Hemoglobin synthesis is decreased by the blood condition beta thalassemia. Patients with thalassemia exhibit a range of bone problems, including as spinal deformities, osteopenia and osteoporosis, as well as growth failure. This study's main goal was to evaluate gait and balance in Albanian β-thalassemia patients. In this study, 18 participants aged 17 to 29 years old *(5 males and 13 females)* from three distinct epidemiological cities in Albania took part. Gait analysis was performed using "*T&T medilogic medizintechnik gmbh*" wi-fi insoles. "General Gait Parameters", results of the "Effective Foot Length, left [%]" parameter mean results [67.59%], reveals approx a 2% difference compared with the relative normative mean [69.7%]. Also, "Effective Foot Length, left [%]" mean results [65.49%], reveals approximately a 3.5% difference compared with the relative normative mean [69.7%]. "General Gait Parameters", data comparisons revealed statistically significant differences between "is" and "nom." measurements for the following variables: "Rel Double Step Length", "Double Step Duration", "Effective Foot Length Right" and "Width Of Gait Right" ($p<0.005$). Results for the rest of the variables shwed a not statistically significant difference. The aim of the study was to assess the gait parameter in Albanian individuals with thalassemia. Based on the result of the "General Gait Parameters", data reveal a difference in the % of the average gait line which is related to effective distribution in the insole length showing a specific indication of insecure gait. According to the findings, gait issues are a significant health issue for beta thalassemia patients which are most likely caused by disease-related side-effects such high calcium levels after blood transfusions, inactivity caused by insufficient muscle mass, and all other health-related disease conditions. It is advised that the gait analysis exam to be included in their regular health check-ups. Future studies should be conducted involving larger ß-thalassemia patients take into account the unique state of this category to better evaluate the gait problems related with general static and dynamic posture problems. Clinicians' health specialists and rehabilitation experts when planning B-Thal rehabilitation programs sholud use gait analysis exam as a regular health check-up.

Keywords: Gait analysis, Evaluation, β-thalassemia patients

Introduction

A hereditary condition of hemoglobin synthesis known as beta-thalassemia causes a reduction in the production of the b-globin chain. Intramedullary hemolysis and inefficient erythropoiesis are caused by a relative imbalance of the alpha and beta globin genes. Lifelong transfusion treatment is used to treat this disease, which can cause iron excess and toxicity. It has been demonstrated that multiorgan illness is brought on by iron accumulation in the heart, liver, and endocrine glands. The most severe iron-mediated consequence and the main reason for mortality in those with thalassemia major is cardiomyopathy (Borgna-Pignatti C et al., 2004; Modell B et al., 2000; Zurlo MG et al., 1989). The quality of life (QoL) of thalassemia patients is anticipated to be impacted by some of the major clinical and psychological aspects of the condition, such as: (1) having a chronic condition and the subjective feeling of being different; (2) physical changes, such as bone deformities and short stature, which affect one's self-image; (3) treatment (transfusions and iron chelation therapy); (4) delayed or absent sexual development and issues with fertility; (5) heaving; and (5) he (Telfer P et al., 2005; Mikelli A et al., 2004). Changes in bone architecture, bone quality, and mineral density are among the main impacts of thalassemia on the bones. Other serious morbidities include osteoporosis, fractures, spinal abnormalities, nerve compression, and discomfort. Baldini M. et al., 2017; Steer K. et al., 2017; Baldini M. et al., 2014).

Gait Analysis

The coordination, balance, and synchronization needed for gait are activated by the appropriate operation of the central and peripheral (musculoskeletal) neural systems. Each person's gait characteristics are drastically variable depending on their physical and mental health, resulting in a distinctive pattern (Horst, F et al., 2017). The gait cycle, which is similar to a stride and consists of two subsequent steps, is used to assess gait. A gait cycle has the following phases: (a) the stance phase, which lasts until the same foot leaves the ground, and (b) the swing phase, which starts when the foot leaves the ground. Speed affects a number of gait factors, and speed also depends on body height. On the other hand, when it comes to techniques and modern tools for evaluating gait analysis, sensor insoles are one of the most cutting-edge options available today since each gait event can be defined by a pressure pattern (Daz, S. et al., 2020). Unstable gait is frequently seen in people with neurological and musculoskeletal diseases because it compromises the capacity to control where the body's center of mass (COM) is in relation to the base of support (BOS) (Albertsen, I. M et al., 2017; Kristiansen, M et al., 2019).

Objectives

Main objective of this study was to evaluate gait and balance in Albanian β-thalassemia patients.

Methodology

In this study, 18 participants aged 17 to 29 years old *(5 males and 13 females)* from three distinct epidemiological cities of Albania. Gait analysis was performed using "*T&T medilogic medizin technik gmbh*" wi-fi insoles (Figure 1, 2, 3). For the measuring setup, a 10-m flat indoor walking area was utilized.

Figure: 1, 2, 3. *T&T medilogic medizin technik gmbh*" wi-fi insoles

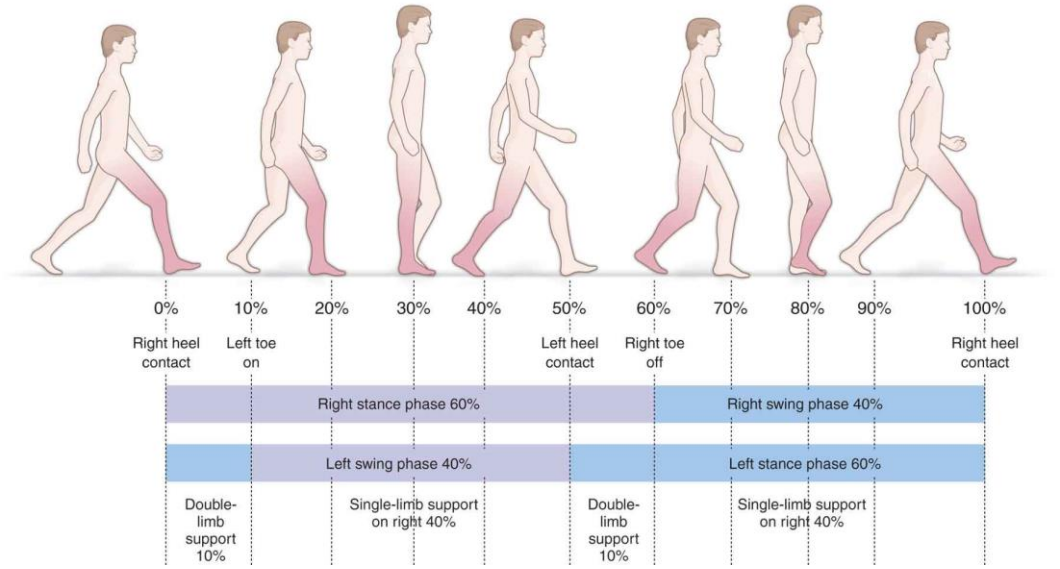

Figure 4. https://musculoskeletalkey.com/gait-and-posture-analysis/

The "Gait Parameter", reading

Gait analysis from T&T Medilogic has identified a few crucial human gait metrics that are helpful for actual evaluation. The distribution of the load under the foot forms a significant portion of the parameters. General factors like speed, step length, and stance phase are included in addition to them. A database of comparison values (Nom.) has been constructed from a set of test subjects with unobtrusive gait patterns for examination and interpretation. The relative location of each parameter in relation to the comparison group is displayed using color. Green denotes a near match to the comparison values, yellow denotes a little difference, and red denotes a significant departure. About 800 measurements from 164 test subjects were used to create the internal database, which removed the speed dependence of several metrics. The quantitative gait analysis's primary metric is speed. A person automatically selects the speed that best suits their locomotor capabilities. Therefore, a significant drop in walking speed is a clear sign of pathology. Many characteristics rely on speed, therefore comparisons utilizing those factors (such before-and-after care) are only valid at walking speeds that are comparable. Determining the relative speed has been beneficial (V_{rel}) taking into account the body height (L_0) of the patient: $V_{rel} = V/L_0$ in [1/s]. From one ground contact of the same foot to the next ground contact of the same foot, a whole gait cycle is formed; this is referred to as a double step. The length of a double step (L) is the distance that is traveled in a single double step. The relative double step length (Lrel), which also depends on body height, is defined as follows: L/L0 = Lrel. This variable is independent of the unit. A short double step length indicates an unstable stride. The reciprocal of the double step frequency, which has the unit min-1, is the double step duration (DSD). The two-footed position,

as defined by %DSD, is when both feet are on the ground. An unstable gait is also indicated by a high value of these parameter. The proportion of DSD during which one leg is on the ground during the stance phase is computed for each leg independently. Patients who only have one side affected (because to an accident or a leg prosthesis, for example) typically have a shorter stance phase length on that side. The effective foot length is calculated as a percentage of the effective insole length using the average gait line. It is a measurement for the entire foot's roll. The variance in the gait line-s medial-lateral direction is known as its width, and is measured as a percentage of the insole's width. Thus, it serves as a gauge for how much the gait line deviates during single steps. Ankle and prosthesis gait injuries can result in an extremely narrow gait line. The same can be said for a gait issue indicated by a very broad gait line.

Measurements

Each subject's age, height, weight, and BMI were noted before to data collection. To establish a secure fixation, the Medilogic insoles were placed directly on the subject's feet while wearing athletic shoes. The same walking pace, cadence, and step length were chosen for each subject to provide a high level of standardization. To begin the test, a "GO" auditory signal was given. All measuring units began as soon as the subject took the first step. Each participant did 4 gait cycles (8 steps). Each participant crossed the walkway two or three times before data collection to become used to it.

Statistical analysis

Statistical analysis was performed via IBM SPSS Statistics 26 using Descriptive Statistics and Student's*t* test. The level of significance was set at 0.05.

Results

Table 1. Anthropometric measurements results

Subject ID	Age (years)	Body Mass (kg)	Height (cm)	BMI
ID L001251 (F)	19	58.3	169	20.4
ID L001250 (F)	19	61.9	160	24.2
ID L001232 (F)	20	53.2	153	22.7
ID L001233 (F)	21	67.6	177	21.6
ID L001237 (F)	20	48.8	164	18.1
ID L001236 (F)	27	56.7	154	23.9
ID L001234 (F)	20	51.6	161	19.9
ID L001235	30	52.3	151	22.9

(F)					
ID (M)	L001238	30	69.2	175	22.6
ID (M)	L001253	17	48.2	149	21.7
ID (F)	L001256	17	55.9	159	22.1
ID (F)	L001257	22	41	145	19.5
ID (M)	L001261	23	53.3	161	20.6
ID (F)	L001259	22	42.7	145	20.3
ID (F)	L001260	22	56.6	164	21
ID (M)	L001255	21	50.7	154	21.4
ID (F)	L001254	26	42.5	154	17.9
ID (M)	L001258	29	67.2	169	22
Mean		22.61	53.76	159.11	

Table 2. General gait Parameters 1

	Speed [km/h]	Rel. Speed [1/s]	Double Step Length [m]		Rel Step Double Length		Double Step Duration [s]		Two Stance DSD]	Leg [%
Subject ID	Is	Is	Is	Nom.	Is	Nom.	Is	Nom.	Is	Nom.
ID L001251 (F)	3.9	0.64	1.35	1.24	0.8	1	0.81	0.71	17.5	21.9
ID L001250 (F)	4.2	0.72	1.27	1.39	0.79	0.76	1.1	1.08	20.5	21
ID L001232 (F)	3.8	0.68	1.17	1.28	0.77	0.73	1.12	1.1	23	21.4
ID L001233 (F)	4.6	0.72	1.57	1.32	0.89	0.76	1.23	1.08	21.5	21
ID L001237 (F)	3.2	0.55	1.2	1.14	0.74	0.65	1.33	1.21	23.5	23.2
ID L001236 (F)	3.6	0.65	1.18	1.24	0.76	0.71	1.17	1.13	24	21.9
ID L001234 (F)	3.6	0.62	1.17	1.22	0.74	0.69	1.18	1.15	18	22.2
ID L001235 (F)	3.6	0.65	1.2	1.24	0.78	0.71	1.2	1.13	24.5	21.8

ID (M)	L001238	4.6	0.73	1.53	1.34	0.88	0.77	1.2	1.07	20	20.9
ID (M)	L001253	3.9	0.71	1.22	1.31	0.8	0.75	1.13	1.09	21	21.1
ID (F)	L001256	4	0.69	1.2	1.29	0.75	0.74	1.08	1.1	23.5	21.3
ID (F)	L001257	4.1	0.78	1.24	1.39	0.84	0.8	1.08	1.04	22	20.4
ID (M)	L001261	3.4	0.58	1.05	1.17	0.64	0.67	1.1	1.18	19	22.7
ID (F)	L001259	3.9	0.75	1.12	1.35	0.77	0.77	1.03	1.06	19	20.7
ID (F)	L001260	4.1	0.7	1.26	1.3	0.76	0.74	1.1	1.1	20.5	21.3
ID (M)	L001255	4.5	0.81	1.48	1.41	0.96	0.81	1.18	1.02	21	20.1
ID (F)	L001254	3.5	0.62	1.09	1.22	0.7	0.69	1.12	1.15	21.5	22.2
ID (M)	L001258	3.8	0.61	1.35	1.2	0.78	0.68	1.29	1.16	19	22.4
Mean		3.905	0.678	1.266	1.281	0.799	0.746	1.144	1.087	21.06	21.53
Standart Dev.		0.40	0.07	0.15	0.08	0.07	0.08	0.11	0.11	2.09	0.82

Table 3. General gait Parameters 1

	Stancephase Duration, Left [% DSD]		Effective Foot Length, Left [%]		Width of Gait Line, Left [%]		Stancephase Duration, right [% DSD]		Effective Foot Length, Right [%]		Width of Gait Line, right [%]	
Subject ID	Is	Nom.	Is	Nom.	Is	Nom.	Is	Nom.	Is	Nom.	Is	Nom.
ID L001251 (F)	58	61.2	80.1	69.7	6.3	3.8	59.5	61.2	73.4	69.7	7	3.8
ID L001250 (F)	60.5	60.7	73.4	69.7	3.1	3.8	60	60.7	71.8	69.7	4.3	3.8
ID L001232 (F)	62	60.9	65.1	69.7	5.9	3.8	61	60.9	57.5	69.7	7.9	3.8
ID L001233 (F)	60.5	60.7	66.8	69.7	5.4	3.8	61	60.7	65.9	69.7	9.9	3.8
ID	61.	61.	59.	69.	7.6	3.8	62	61.	60.	69.	5.3	3.8

L001237 (F) ID	5	9	8	7				9	3	7		
L001236 (F) ID	63	61.2	71	69.7	1.4	3.8	61	61.2	54.8	69.7	2	3.8
L001234 (F) ID	59.5	61.4	56.7	69.7	4.7	3.8	58.5	61.4	53.1	69.7	6.5	3.8
L001235 (F) ID	61.5	61.2	62.6	69.7	5.1	3.8	63	61.2	64.8	69.7	7.1	3.8
L001238 (M) ID	60.5	60.6	70.2	69.7	4.3	3.8	59.5	60.6	76.7	69.7	4.1	3.8
L001253 (M) ID	60	60.8	64.7	69.7	5.1	3.8	61	60.8	69.4	69.7	3.5	3.8
L001256 (F) ID	61.5	60.9	77.6	69.7	2.7	3.8	62	60.9	66.5	69.7	3.5	3.8
L001257 (F) ID	61.5	60.3	65.1	69.7	9.3	3.8	60.5	60.3	62.5	69.7	7.7	3.8
L001261 (M) ID	58	61.7	68	69.7	2.9	3.8	61	61.7	66.7	69.7	5.1	3.8
L001259 (F) ID	58	60.5	63.3	69.7	3.2	3.8	61	60.5	62.6	69.7	3.3	3.8
L001260 (F) ID	61	60.8	71.5	69.7	3.3	3.8	59.5	60.8	70.8	69.7	4.4	3.8
L001255 (M) ID	61	60.1	62.3	69.7	3	3.8	60	60.1	62.7	69.7	5.3	3.8
L001254 (F) ID	59.5	61.4	69.2	69.7	4	3.8	62	61.4	67.7	69.7	6.4	3.8
L001258 (M)	59.5	61.5	69.3	69.7	3.2	3.8	59.5	61.5	71.7	69.7	5	3.8
Mean	60.39	60.98	67.59	69.7	4.47	3.8	60.66	60.98	65.49	69.7	5.4611	3.8
Standart Dev.	1.43	0.48	5.95	0.00	1.94	0.00	1.15	0.48	6.42	0.00	1.98	0.00

T-Tests Results

Ten paired samples t-test were conducted, in order to compare values between "Is" and "Nom" values for each dependent variable (Table 4). These comparisons revealed statistically significant differences between "is" and "nom." measurements for the following variables: "RelDoubleStepLength", "DoubleStepDuration", "EffectiveFootLengthRight" and "WidthOfGaitRight" ($p<0.005$). For the rest of the variables the differences between "is" and "nom" values were not statistically significant based on paired samples t-test results ($p>0.005$). Box-plot for "WidthOfGait" is presented in figure 5.

Table 4. Paired Samples Test results

Paired Samples Test

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	DoubleStepLength_Is DoubleStepLength_Nom.	--.02222	.13095	.03087	-.08734	.04290	-.720	17	.481
Pair 2	**RelDoubleStepLength_Is RelDoubleStepLength_Nom.**	**-.04000**	**.07623**	**.01797**	**.00209**	**.07791**	**2.226**	**17**	**.040**
Pair 3	**DoubleStepDuration_Is DoubleStepDuration_Nom.**	**-.04944**	**.06958**	**.01640**	**.01485**	**.08404**	**3.015**	**17**	**.008**
Pair 4	TwoFootedStance_Is TwoFootedStanced_Nom.	--.4722	2.2510	.5306	-1.5916	.6472	-.890	17	.386
Pair 5	StancephaseDurationLeft_Is StancephaseDurationLeft_Nom.	--.6000	1.5904	.3749	-1.3909	.1909	-1.601	17	.128
Pair 6	EffectiveFootLengthLeft_Is EffectiveFootLengthLeft_Nom.	--2.1056	5.9498	1.4024	-5.0643	.8532	-1.501	17	.152
Pair 7	WidthOfGaitLineLeft_Is WidthOfGaitLineLeft_Nom.	-.6722	1.9423	.4578	-.2937	1.6381	1.468	17	.160
Pair 8	StancephaseDuration_Right_Is StancephaseDurationRight_Nom.	-.3222	1.1533	.2718	-.8957	.2513	-1.185	17	.252
Pair 9	**EffectiveFootLengthRight_Is EffectiveFootLengthRight_Nom.**	**-4.2056**	**6.4249**	**1.5144**	**-7.4006**	**-1.0105**	**-2.777**	**17**	**.013**

Pair 10	**WidthOfGaitRight_Is – WidthOfGateRight_Nom.**	**1.6611**	**1.9814**	**.4670**	**.6758**	**2.6465**	**3.557**	**17**	**.002**

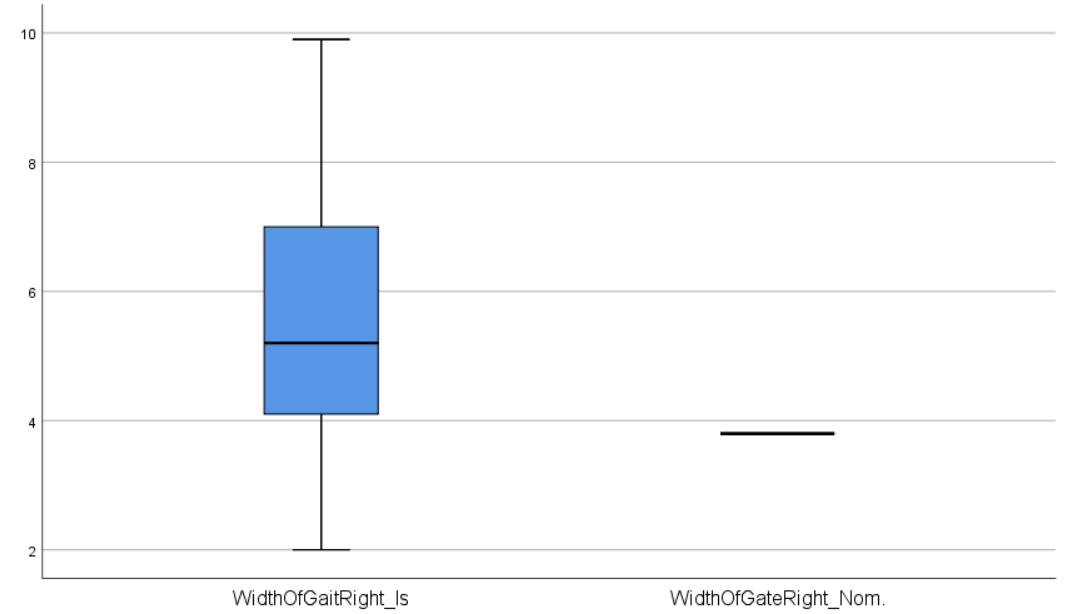

Figure 5. Box-plot for "WidthOfGait" distribution.

Discussion

The aim of the study was to assess the gait parameter in Albanian individuals with thalassemia. Based on the result of the "General Gait Parameters", the "Double Step Length" mean were 1.26[m] and compared with the "Nom." relative mean values that were 1.28[m] we have a diference of 0.2[m] which is an indication of an insecure gait. Nevertheless, mean results of "Double Step Duration [s]" parameter was 1.14[s] compared with the "Nom." relative mean values that were 1.09[s] resulting in a non significant difference 0.05[s]. In the "Two Footed Stance [%DSD]" parameter the mean results were 21.06 [%DSD] compared with "Nom." 21.53 [% DSD], indicating approximately 1[% DSD] which is not a high value to indicate a sign for an insecure gait (Table 2). Regarding the "Stancephase Duration of Left Foot [%DSD]" parameter, mean datas results values were 60.39[%DSD] compared with the "Nom." relative mean values that were 60.98[%DSD], which is not a significant difference to reveal any gait problem. Also "Stancephase Duration of Right Foot [%DSD]" parameter, mean datas results values were 60.66[%DSD] compared with the "Nom." relative mean values that were 60.88[%DSD], also demonstrates a non significant difference for any gait problem. The "Effective Foot Length, left [%]" parameter mean results [67.59%], reveals approximately a 2% difference compared with the relative "Nom." mean [69.7%]. Also, the same parameter for the left foot ("Effective Foot Length, left [%]") mean results [65.49%], reveals approximately a 3.5% difference compared with

the relative "Nom." mean [69.7%]. Both these parameters reveal a difference in the % of the average gait line. Continuing with the "Width of Gait Line, left foot [%]" parameter, results [4.47%] show a a minor difference [0.7%] compared with the relative "Nom." mean [3.8%]. Also, the results of this parameter fort the right foot ("Width of Gait Line, right foot [%]") show a 1.66% difference compared with the relative normative mean [3.8%] (Table 3). These comparisons revealed statistically significant differences between "is" and "nom." measurements for the following variables: "Rel Double Step Length", "Double Step Duration", "Effective Foot Length Right" and "Width Of Gait Right" ($p<0.005$). These parameters reveal a difference in the % of the average gait line which is related to effective distribution in the insole length showing a specific indication of insecure gait most probably related to the mentioned problems associated to this specific category population has. Results for the rest of the variables shwed a not statistically significant difference.

Conclusions

To conclude, our findings showed that, ß-thalassemia patients, based on the results had specific indicatators revealing an insecure gait. Based on the result of the "General Gait Parameters", we can say that gait problems are mostly related to the specific health related problem and side effects like, skeletal deformities and posture related problems, iron overload, high calcium levels related with periodic blood transfusions, inactivity associated with low muscle mass etc.

Reccomendations

Future studies should be conducted involving larger ß-thalassemia patients take into account the unique state of this category to better evaluate the gait problems also associated with general postural problems in static and dynamic phases. Clinicians' health specialists and rehabilitation experts when planning B-Thal rehabilitation programs sholud use gait analysis exam as a regular health check-up.

Study Limitations

Some of this study limitations issues were the small sample size and also the lack of on physical activity intervention program in ordert to compare gait analysis data prior and after the intervention program. Also giving the specifics of the insoles and software usage and the specifities of this population category it was difficult to find normative datas to compare with.

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Plastic Pollution in Albania: Survey on Citizen's Perceptions and Attitudes

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Abstract

Plastics are polymers synthesized from petrochemicals or from biomass raw materials. In recent decades, worldwide plastics production has increased exponentially. An estimated 8.3 billion tons of plastic have been produced worldwide resulting in an estimated 6.3 billion tons of plastic waste disposed in landfill or discarded into the natural environment. Plastic is widely used: is inexpensive, durable, resource efficient and easily attainable. The article makes some preliminary observations based on questionnaire data distributed through probability stratified random sampling method between November 2021 and July 2022. The data provides some useful insights into citizens perceptions and attitudes about plastic pollution. Majority of respondents agree on the fact that plastic pollution is harmful to the environment (94%). Additionally, 77.1% of respondents agree both on the fact that nature conservation activities are necessary and successful if community or citizens are involved. Strategies which can be used to reduce plastic pollution need to provide further incentives for public participation. An example is using citizen science, to address and mitigate plastic pollution both at local and national level.

Keywords: plastic pollution, survey, public participation, citizen science, Albania

Introduction

Plastic pollution is generated by the unsustainable use and disposal of plastic products in modern society, threatening economies, ecosystems, and human health (Prata et al. 2019). The possible risks of microplastics to the health of humans, ecosystems and animals are an increasing concern because they have been detected in human food, the air, and drinking water (Barboza et al. 2018; Gasperi et al., 2018;

Nikiema et al. 2020; Kirstein, Gomiero & Vollersten, 2021; Nikiema & Asiedu 2022). For example, aquatic plastic pollution is entirely due to humans (Pahl, Richter & Wyles, 2020). During the COVID-19 pandemic it is estimated that more than eight million tons of pandemic associated plastic waste have been generated globally, with more than 25,000 tons entering the global oceans (Peng et al.2021).

According to World Wordlife Fund (WWF) Albania is considered one of the most problematic countries with the highest percentage of untreated plastic waste, 73%. According to World Bank (2021) approximattely 65% of marine waste in Albania are synthetic polymers and 58.5% are found along the coast because of the improper waste disposal. Recently, according to a pioneer study in Albania, microplastic pollution in Kune-Vain lagoonary complex was confirmed (Aliko et al. 2022). Given rising concern over plastic pollution, on April 26, 2018, with the decision of the Council of Minister no.232 " For some changes and additions to the Decisions of the Council of Ministers no.177, dated 6.3.2012 "On plastic packaging and their waste" the Albanian government tried to reduce plastic pollution by the introduction of plastic bag charges. The government required that the new plastic material should have no less than 55 percent raw material from recycling or bio-degradable material, which dissolve in the environment and do not remain as a toxic residue. Pricing policy is considered an effective way to reduce plastic bag use (Jakovcevic et al. 2014; Monast and Virdin, 2022). Empowering local communities to actively participate in sustainability efforts is considered very important. Additionally, risk perception of plastic pollution can be improved by the utilization of citizen science which is considered of great value for informing evidence-based policy aimed to address and reduce plastic pollution (Syberg et al. 2018; Nelms et al. 2022).

Materials and methods

Separate visits were conducted from November 2021 to July 2022 as part of the sampling efforts in six major cities in Albania (Tiranë, Durrës, Vlorë, Shkodër, Lushnje and Lezhë). A face-to-face survey was conducted with randomly selected people (N= 947). Within the scope of the survey, 41 questions were directed to participants under three sections: 1- Demographic data, 2- Perceptions and attitudes about plastic pollution, 3- Citizen participation. Statistical Analysis Data was gathered in MS Excel spreadsheet and analyzed using SPSS version 22 software. All assumptions were tested and met using SPSS. A p value of less than 0.05 was considered statistically significant.

Results and discussions

As mentioned, the number of respondents is 947. 60.8% of the respondents are female (F= 576) and 39.2 % male (M= 371). Most of them are young, aged 16 years old to 24 years old (45.3 %) (Table 1). Most of respondents are from Tirana, the capital city of Albania.

Table 1. Demographic data

		Respondent	Percentage
Gender	**Female**	576	60.8%
	Male	371	39.2%
Age group	**16-24**	429	45.3%
	25-34	224	23.7%
	35-44	127	13.4%
	45-54	100	10.6%
	>55	67	7.1%
Education	**1st to 9th grade**	71	7.5%
	High school	280	29.6%
	Bachelor's degree	325	34.3%
	Master's degree	246	26.0%
	PhD	25	2.6%
Working status	**Unemployed**	360	38.0%
	Part-time	148	15.6%
	Full time	409	43.2%
	Retired	30	3.2%

When perception and attitudes about plastic bags were examined (Section 2), it was found that majority of participants (70.2 %) use plastic bags in market and grocery shopping and agree with the fact that plastics is harmful to the environment (Table 2). Risk perception of plastic pollution is high.

Table 2. Relationship between plastic usage behavior and its effect on the environment

		I think plastic is harmful for the environment					
		Agree		***Disagree***		***NR***	
I use plastic bags in market and grocery shopping	***Agree***	664	70.2%	8	0.8%	29	3.1%
	Disagree	126	13.3%	6	0.6%	2	0.2%
	NR	106	11.2%	1	0.1%	4	0.4%

When I buy anything, I take care that the packaging is plastic or not	***Agree***	164	17.3%	1	0.1%	4	0.4%
	Disagree	503	53.2%	7	0.7%	26	2.7%
	NR	229	24.2%	7	0.7%	5	0.5%
I sort my garbage in the house	***Agree***	238	25.2%	5	0.5%	3	0.3%
	Disagree	548	57.9%	9	1.0%	24	2.5%
	NR	110	11.6%	1	0.1%	8	0.8%

This cognitive dissonance is explained by the fact that plastic is easily accessed and despite regulatory law, is still spread in everyday life.

In terms of membership/volunteerism to nature conservation associations, participants were found not especially to be very fond of it. There is both a satisfactory degree on the need of active participation in nature conservation and being an environmentalist (48.3%) (Table 3).

Table 3. Relationship between citizens engagement and pro environmental perceptions.

		Nature conservation activities without citizen's participation are unsuccessful					
		Agree		***Disagree***		***NR***	
In general, I consider myself as an environmentalist	***Agree***	457	48.3%	31	3.3%	66	7.0%
	Disagree	74	7.8%	9	1.0%	24	2.5%
	NR	223	23.6%	19	2.0%	43	4.5%
I actively participate in nature conservation activities	***Agree***	208	22.0%	18	1.9%	25	2.6%
	Disagree	296	31.3%	30	3.2%	51	5.4%
	NR	250	26.4%	11	1.2%	57	6.0%
Nature conservation activities are necessary	***Agree***	729	77.1%	52	5.5%	112	11.8%
	Disagree	4	0.4%	2	0.2%	6	0.6%
	NR	19	2.0%	5	0.5%	15	1.6%

In addition, there is a high degree on the need of nature conservation activities involving citizens (77.1%). Although our respondents are not actively participating in nature conservation activities, they are somehow willing to get involved.

Conclusions

This is the first study to provide useful insights into Albanian citizen's perceptions and attitudes of plastic pollution. Our results suggest that respondents have both a high awareness on plastic pollution and a firm belief on participation. Based on these results, we believe that future plastic pollution mitigation strategies in Albania should take in consideration engagement such as frequent/ constructive dialogue between citizens and local government and the utilization of citizen science to rise participation. Engagement and participation of citizens are seen as key factors in addressing and mitigating the negative effects of plastic pollution both at a local and national level. In addition, we recommend the use of communication strategies involving possible health and touristic advantages for citizens to guide societal response on plastic's negative health and environmental impact.

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Determinants of Patient Satisfaction with Health Care: A Literature Review

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Abstract

This research paper aims to explore the field of patient satisfaction in health care, by performing a literature review on existing healthcare articles that analyse determinants of patient's satisfaction and theories on patient satisfaction assessment. Patient satisfaction is one of the most important factors to determine the success of health care providers. Determining the exact definition, determinants and characteristics of patients that influence satisfaction, as well as different theories on satisfaction, are highly discussed elements in the literature for a long period of time. The research instrument was a literature review by combining different view from many researchers. The literature was searched in databases such as Emerald, Medline/PubMed, Web of Science, ScienceDirect, Scopus. Patient satisfaction appears to play an important role in evaluation of service quality. From the literature review, it was observed that the main determinants of patient satisfaction were the demographic characteristics, expectations and experiences of the patients. Communication is also an extremely important element that affects patient satisfaction. Other research should be conducted to delve even more into this very important area of health care.

Keywords: patient satisfaction, health care, satisfaction theories, literature review

1. Introduction

Satisfying patients' needs is the first step toward having loyal patients. For a long time, the understanding of the relationship that exists between satisfaction and service quality was a key issue in determining development strategies in the health sector. Donabedian (1980), identified the importance of patient satisfaction so well that his work later became the basis for further research on health care. In this sector, the importance of measuring patient satisfaction has been clearly defined (Lin and Kelly, 1995), as it has been measured and studied extensively as an independent factor but also as a component of the quality of health products (Heidergger et al., 2006), and often in special cases also as an important factor in studies on the evaluation of the quality of the health service. Patient satisfaction has begun to be seen as a product of

service quality which also affects clinical outcomes, economic measures and quality of life (Heidegger et al. 2006). The very fact that we have many opposing opinions, satisfied authors or not, shows by itself that the measurement of satisfaction is too complex and regardless of whatever result is concluded, we will still encounter dissatisfaction.

To demonstrate the unresolved conceptual difficulties related to the structure of satisfaction, in the literature it has been defined as: summation between psychological state and specific situations (Oliver, 1989), discrepancies between expectations and actual performance (Yi, 1990), composition of two components together, both emotional and cognitive, as a fulfilling response and a creation of experience (Oliver, 1989), a response to both the process and the end result (Hill, 2003).

Risser (1975) considers patient satisfaction as the degree of convergence between the expectations patients have of ideal care and their perceptions of the care they actually receive. Lochoro (2004) supports this view and also points out that satisfaction corresponds to the gap between expectations and perceived characteristics of a service. Howard and Sheth (1969), explain consumer satisfaction as a cognitive response of consumers. Churchill and Surprenant (1982) define consumer satisfaction based on the cognitive and emotional dimensions of the concept. Later, Oliver (1997) outlined various definitions of customer satisfaction that acknowledged their emotional impact on desired products or services. Mutava et al. (2006) pointed out that the product or service itself is one of the main factors in achieving customer satisfaction, defining it as a system through which the consumer passes for a given value of money.

It is very important to distinguish between interpersonal care and patient satisfaction. Patient satisfaction is usually measured and considered as an indicator of the quality of medical service (Cleary, Mc Neil, 1988). For this, it is necessary to specify the interpersonal aspects of a high quality of service and to ask patients about these experiences. It may also be useful to measure the extent to which care meets patient expectation, always bearing in mind that high satisfaction does not necessarily correlate with the delivery of high quality care (Cleary, et al., 1991). Shi and Singh (2005), from the perspective of patient satisfaction, explain service quality in two ways:

Quality as an indicator for satisfaction, which depends on individual experiences on features such as medical service (comfort, dignity, privacy, security, autonomy in decision-making and attention to personal preferences).

Quality as an indicator of individuals' total satisfaction with life and perceptions of some medical interventions.

On the other hand, Safavi (2006) thinks that patient satisfaction depends on three basic issues of the health care system. They are patients' perceptions of the quality of

care provided, skilled physicians and organizations capable of providing decent service. He reached at the conclusion that satisfaction with hospital service is driven by dignity and respect, speed and effectiveness of service delivery, comfort, information and communication as well as emotional support.

2. Methodology

A narrative review approach was used to achieve the purpose of this study. Out of 84 articles that were read electronically, only 60 of them were taken into consideration for this paper. The studies range in years from 1969 to 2019. No time restrictions were applied. The research instrument was a literature review by combining keywords such as patient satisfaction, health care, satisfaction theories, literature review. The literature was searched in databases such as Emerald, Medline/PubMed, Web of Science, ScienceDirect, Scopus. Inclusion and exclusion criteria were adopted. The articles were selected based on content such as research on patient satisfaction, determinants of patient's satisfaction, health care services, patient's expectations and experiences and theories on patient satisfaction assessment. As sources were reviewed, additional citations were found and explored. The purpose was to see which are the determinants of patient's satisfaction and the theories most used to measure it, more applicable to the health sector.

3. Determinants of patient's satisfaction

Most of the studies reported in the literature showed the relationship between demographic factors such as age, gender, health status and level of education with patient satisfaction, but the results of these studies are different.

Two studies, one followed in Scotland on patients treated on emergency department in hospital between February and March 2002, and the other in 32 tertiary care hospitals in the USA, both showed that male patients older than 50 years , who had stayed a short time in the hospital in a not very serious health condition, as well as those patients with a lower level of education, had reported higher levels of satisfaction compared to other groups of the population. On the other hand, a questionnaire developed in various accredited hospitals in Taiwan showed that characteristics such as age, gender, and level of education had very little influence on patient satisfaction and that patients with less severe conditions had higher levels of satisfaction. Even Nguyen et al. (2002) and Jenkinson et al. (2002) stated from their studies that the two strongest and most consistent determinants of satisfaction were old age and good health status. Meanwhile, two studies conducted in Norway in 63 state hospitals, on overall patient satisfaction and control variables such as age and gender, showed that these variables were not significant determinants of patient satisfaction at all.

In 2004, Otani et al. developed a questionnaire on hospitals in the USA to see the relationship between nursing care, medical care and the surrounding environment, and the overall satisfaction of patients, showing that all these factors were statistically

significant and positively related to patient satisfaction, and nursing care had the greatest impact of these three factors. Research also showed that the courtesy and respect shown by medical staff also affects patient satisfaction, while the way of communication plays a secondary role. On the other hand, a study conducted in Ireland in 13 hospitals showed that effective communication and clear explanation of situations had the greatest impact on improving patient satisfaction (Sweeney et al. 2008). These studies showed the importance of the role of nurses, as the most important determinant in the overall satisfaction of patients.

Other studies showed that the interpersonal communication skills of doctors expressed in variables such as behavior, explaining situations, level of care, emotional support, respect for patients and their preferences, as well as involving patients in decision-making, were more influential factors than competencies, clinical and equipment and technology used, for increasing patient satisfaction (Chang et al. 2006; Andrabi 2012). Research conducted in the USA in various hospitals by Clever, Levinson and Meltzer (2008), also showed that the way of communication played an important role in patient satisfaction. The same results on communication, staff sensitivity and care were achieved by Carlson et al. (2015).

The study conducted in France by Nguyen et al. (2002), and studies conducted in South Korea by Chat et al. (2005), showed that the equipment and technology used were the ones that most affected patient satisfaction, and that the biggest problems came from the lack of these dimensions. Otani et al. (2009) in his study revealed that staff care is the most influential attribute, while Alrasheedi et al. (2019), reported that the greatest dissatisfaction was caused by the long waiting time at the reception and the prolonged time of registration in the hospital. Contrary to them, Jenkinson et al (2002), in his study showed that physical comfort had the highest degree of association with satisfaction, compared to other dimensions such as information, or emotional support.

Patient expectations as a determinant of satisfaction - Different patients have different expectations, based on their knowledge and previous experiences, and consequently these expectations tend to change as their experiences accumulate over time. Patients with low expectations generally have higher satisfaction rates (Jawaid M, et al. 2018). As a quality assurance measurement and evaluation tool, expectations make the concept of satisfaction even more complex. We distinguish three categories of patient expectations identified from the literature: 1) background expectations, which come as a result of knowledge gathered from readings or consultation processes, 2) interactive expectations, which come as a result of the exchange of information between the patient and the provider health care and 3) active expectations, which are created as a result of actions performed by doctors, in providing treatments or counseling in the past (Greenberg RP, et al. 2006).

From a study conducted in Pakistan, emotional support provided by staff, waiting time of no more than 30 minutes, and consultation time of no less than 20 minutes

were the factors most involved in creating patient expectations (Naseer, 2012). These expectations are also influenced by patient characteristics such as age, sex and marital status, as well as psychosocial determinants (Siddiqui et al. 2011; Saleem T, et al. 2009).

Patient experiences as determinants of satisfaction - Patient experiences are a strong predictor of patient satisfaction. Almost all patient questionnaires conducted worldwide attempt to measure patient experiences in the health sector to improve the quality of service in this area. WHO uses the measurement of patient experiences in the health sector as an indicator of the accountability of the health care system.

The performance and consequently the accountability of the system is reflected in a general improvement of the health status of the people who have received the service, ensuring equity and efficiency, while protecting individuals from excessively high costs (WHO, 2009). The level and distribution of accountability is therefore an important determinant of patient satisfaction, related to the performance of the health care system. Responsiveness refers to the ways and environment in which patients were treated when they needed health care. Eight areas of patient experience define health system responsiveness, and all of them are positively related to patient satisfaction.

Other determinants of patient experiences that affect patient satisfaction have also been determined from other studies. Thus, lack of beds, long waiting time at the reception and prolonged administrative procedures, unavailable medical staff, lack of basic medical equipment, cleanliness of rooms have also proved to be as determinants of patient satisfaction (Sajid, Rashid, 2008). Also, focus on the patient, which includes the time dedicated to the patient by the staff, the listening skills of doctors and nurses, the way of communication, has been proven to have a positive relationship with patient satisfaction (Karim, 2003). Lack of autonomy, ineffective communication and non-immediate service are some of the main factors leading to patient dissatisfaction (Oliveira, 2012). We can say that patient trust and doctors' communication behavior and waiting time were more strongly associated and positively affect patient satisfaction with the service received (Chandra, 2018).

Other factors that affect patient satisfaction are the continuity of health care provision and the proximity of health centers to residential areas or work centers (Thornton 2017). Having a hospital center far from the place of residence makes it difficult to attend treatment regularly and leads to unsatisfied patients.

Failure to meet patient expectations also affects satisfaction levels. On one hand, most patients have their own specific expectations about health care services (Kravitz, et al., 1996; Greene, et al., 1980; Sanchez-Menegay and Stalder, 1994; Lazare and Eisenthal, 1997) and on the other hand physicians, not being in aware of these wishes, often fail to provide the right service, causing in most cases dissatisfied patients.

4. Measuring patient satisfaction

Patients, in general, receive various health care services and judge the services provided (Choi et al. 2004). Questionnaires on patient satisfaction have been used to examine quality in health care and have proven the positive or negative relationship of satisfaction with service quality (Brady and Robertson, 2001; Gotlieb, 2000; Rust et al. 1994; Andaleeb, 2007).

Regarding the measurement of patient satisfaction, there are also different opinions in the literature. The best way to measure satisfaction is questionnaires, which have been used very successfully for more than 30 years. Howthorne (2006) after reviewing the literature related to patient satisfaction, concluded that none of the instruments used to measure it could be considered satisfactory.

Major theories on patient satisfaction assessment were published in the 1980s, and later Howthone (2006) synthesized more recent theories that were "restatements" of these assessments.

The Discrepancy and Transgression Theory by Fox and Storms (1981), advocated the idea that as patients' orientations to health care change, so do the conditions of care providers; if the orientations of the patients and the conditions offered are in harmony (match), then the patients are satisfied, if not they are dissatisfied.

The Expectancy-Value Theory of Linder-Pelz (1982), assumed that satisfaction was a combination of beliefs and personal values about care as well as prior expectations about it. They identified the importance of the relationship between expectations and the variance in the evaluation of satisfaction by offering an operational definition of satisfaction as "positive evaluations on various dimensions of health care". In 1983, Pascoe further developed this theory, taking into account the influence of expectations on satisfaction, and then Strasser (1993), based on this theory, developed the model with different psychological factors, which were: formation on cognitive and emotional perceptions, form multidimensional, dynamic process, iterative response behavior and improvement from individual differences.

The theory of Ware et al. (1983) based on determinants and components, which presents patient satisfaction as a function of the patient's subjective responses to health experiences, influenced by personal expectations and preferences.

Multiple Models Theory by Fitzpatrick and Hopkins (1983), according to which expectations are mediated by social factors, reflecting the patient's health goals and the degree to which the illness or health care affects the patient's personal feelings.

Donabedian's (1980) theory of health care quality states that satisfaction was the primary outcome of the interpersonal process of care. He argues that the expression of satisfaction or dissatisfaction is a judgment of the patient on the quality of the service in all its aspects, but especially in relation to the interpersonal components of care.

Brady and Cronin (2001) proposed a hierarchical model to measure satisfaction considering the primary dimensions such as: interactive quality, quality of the physical environment and quality of the results obtained. Some authors showed that studies on patient satisfaction have a real impact on the behavior and habits of health professionals and are likely to stimulate improvement measures in the health service (Greco et al. 2001). A study conducted in France reported that among doctors of a university hospital, 94% of them had a positive opinion on patient satisfaction studies, 60% of them were aware of the results of these studies in their departments, and 40% reported that these studies brought about improvements in service and even modified their own behavior (Boyer et al. 2006).

Aragon et al. (2003) conducted a research in the emergency department of different hospitals and suggested that the main theory for measuring patient satisfaction depended on the measurement of latent variables such as: medical service, waiting time and nursing care. These variables define according to him the characteristics of the quality of health care. They proved that the overall satisfaction of the patient depends on these three variables and that satisfaction is also positively related to two other indicators such as: the desire to recommend the hospital and the degree to which the service justifies the payments made by the patient.

Kano (1979) develops the 'M-H property of quality' by adapting the work of Herzberg et. al.'s (1959) 'Motivation-Hygiene Theory'. He suggest a two-way model on quality based on customers' perception and experience. The Kano model proposes that the relationship between performance of attributes and customers' satisfaction is non-linear. Kano considered five quality elements: Attractive Quality Elements, One-dimensional Quality Elements, Must-Be Quality Elements, Indifferent Quality Elements, Reverse Quality Elements.

CAHPS (Consumer Assessment of Health care Providers and Systems) is another instrument used to measure patient satisfaction and its relationship with service quality. It is a valid international tool for measuring satisfaction, focusing on assessing patients' actual experiences during care, without measuring their expectations.

All of the above methods of measuring patient satisfaction suggested that service quality is one of the main predictors of overall patient satisfaction.

5. Conclusion

In conclusion, from the review of the literature on the factors that influence patient satisfaction in summary, we can say that patients are more satisfied with the health service if these health systems are responsible in terms of dignity, autonomy, prompt service and achievement of patient expectations. Patient expectations, which are influenced by patient characteristics such as age, gender, social class, education, and less so by gender and ethnicity, are very important predictors of patient satisfaction in many important studies. However, patient perceptions and other psychological factors are also potential determinants and should not be neglected.

Patient characteristics such as age, gender, education, ethnicity, marital and socioeconomic status have been widely used to measure patient expectations (Bleich S, et al. 2009). Patient factors that influence their expectations of health care are: older age, male gender, low socioeconomic status, and education, which have been shown to have a positive relationship with patient satisfaction.

Older people have lower expectations and are therefore more satisfied with health care than younger people. They expect less information from doctors and tend to accept treatments more easily than younger people. Gender has been labeled as an inconsistent predictor of patient satisfaction, but the trend is that women are less satisfied with the health service compared to men. High expectations and greater experiences are thought to be the reasons for this result (Sultana A, et al. 2010).

Communication is also an extremely important element that affects patient satisfaction. If the patients did not feel well informed, close to the doctors or unclear about their health status, this could also affect the process of their recovery. Therefore, correct communication and establishment of good cooperative relations, play an important role in increasing patient satisfaction. In particular, patients expect doctors and nurses to communicate clearly, in a friendly manner, and to properly explain test results, diagnoses, medication, health regimens, etc. On the other hand, nurses must also understand patients' problems well and communicate them correctly to doctors. It has already been proven that the better the quality of communication perceived by the patient, the higher their level of satisfaction (Andaleeb, 2010). Patient expectations of health care providers and the health system in general play an essential role in the concept of patient satisfaction. Patients compare their experiences in this sector, with their expectations on this service, allowing the providers of this service to measure their satisfaction (Constantino et al. 2011).

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Uterine Leiomyofibroma in the Gynecological Pathology of the Emergency County Clinical Hospital Saint Andrew the Apostle Constanta

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Abstract

Benign pelvic-abdominal tumor formations are an important part of female pelvic pathology. Uterine fibroids occupy an important place in gynecological benign pathology, among pelvic-abdominal tumors, A retrospective study was performed for a period of 5 years (2015-2019) on a number of 1505 patients admitted to the Clinical Emergency Hospital St. Andrew the Apostle Constanta, on the two departments of Obstetrics-Gynecology OG I and OG II. The patients were followed according to the following criteria: age, origin, symptoms, and the type of surgery performed.

Keywords: Uterine Fibroid, Fractional Biopsy Curettage, Hysterectomy, Hysteroscopy, Laparoscopy, Myomectomy

Introduction

Leiomyomas are benign smooth muscle tumors originating in the myometrium (Hoffman et. al., 2015).

Incidence: 20-25 % (Cramer et. al., 1990). Currently, by using histological and ultrasound criteria, studies demonstrate their presence in approximately 70-80% of women (Day Baird et. al., 2003). Risk factors: older age, nulliparity, early menarche, overweight, polycystic ovary syndrome, and race (African- American women) (Day Baird et. al., 2003), (Wise et. al., 2007), (Ishikawaet. Al., 2009).

Protective factors: combined oral contraceptives, smoking, teenage girls, women who give birth at a young age or with increased parity, Asian or Hispanic women (Parazzini et. al., 1992), (Michnovicz et al., 1986). The European Society of Hysteroscopy divides submucosal leiomyomas into: type 0 - tumor located entirely in the uterine cavity, type I - tumor located less than 50% in the myometrium, type II - more than 50% of the tumor in the myometrium (Wamsteker et. al ., 1993).

In rare cases, the symptomatology may involve **myomatous erythrocytosis syndrome** which is due to the increased secretion of renin by the fibromatous nodule or pseudo-Meigs syndrome, manifested by ascites and even hydrothorax. Both pathologies remit after hysterectomy (Vlasveld et. al., 2008), (Yokoyama et. al., 2003).

The diagnosis of uterine fibroids is clinical and paraclinical through abdominal or transvaginal ultrasound, ultrasonography and hysterosalpingography, hysteroscopy and Nuclear Magnetic Resonance (De Kroon et. al., 2004), (Fleischer et. al., 2003). Currently, in the case of asymptomatic leiomyomas, the main option is their monitoring (Stovall et. al., 1994). Since most leiomyomas develop during menopause, drug treatment is recommended to improve symptoms until the onset of menopause (Hoffman et. al., 2015).

Figure 2. Subtotal histerectomy for a giant myoma developed in the cervix [Personal Collection]

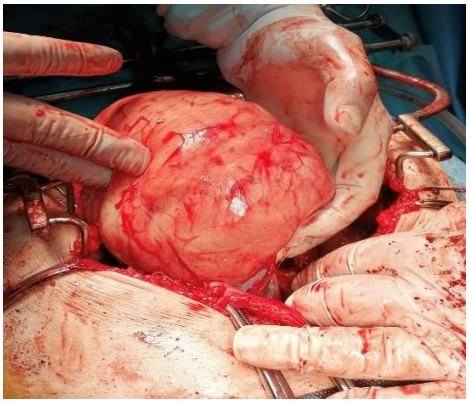


Figure 2. Giant myoma developed in the cervix [Personal Collection]

Drug therapy includes, non- steroidal anti-inflammatory drugs (NSAIDs), androgenic therapy, Gn-Rh agonists, Gn- Rh antagonists, combined oral contraceptives, progestogens, and antiprogesterones (Hoffman et. al., 2015). In the treatment of symptomatic leiomyomas, uterine artery embolization and focused ultrasound therapy under Magnetic Resonance Imaging guidance (MRgFUS) can be performed. The surgical treatment of leiomyomas consists in hysterectomy, which may be performed laparoscopically, abdominally, or vaginally. For patients who wish to preserve fertility a myomectomy can be performed (Whiteman et. al., 2008).

Other possibilities of treatment are laparoscopic or hysteroscopic myomectomy, endometrial ablation, laser vaporization or cryotherapy (Agdi et. al., 2008), (Levy et. al., 2008).

Experimental methods that produce occlusion of the uterine artery or in the application of temporary clips to the level of the uterine arteries have also been used with important success (Hald et. al., 2009), (Holub et. al., 2008), (Vilos et. al., 2010), (Sharp, 2006).

Material and method

The present study was aimed to analyze the incidence of uterine leiomyofibroma in the County Emergency Clinical Hospital "Sfântul Apostol Andrei" Constanţa within the Obstetrics-Gynecology I and II departments.

The study was a retrospective one, over a period of 5 years, between 01.01.2015 and 31.12.2019, and the data were collected from the observation sheets and from the operative protocols of the two departments. Between 01.01.2015 and 31.12.2019, a total number of 10,602 patients were admitted to the Obstetrics-Gynecology I and Obstetrics-Gynecology II departments. The total number of patients included in the study was 1505, all of whom presented to the hospital for symptoms mainly related to the symptoms of uterine leiomyoma.

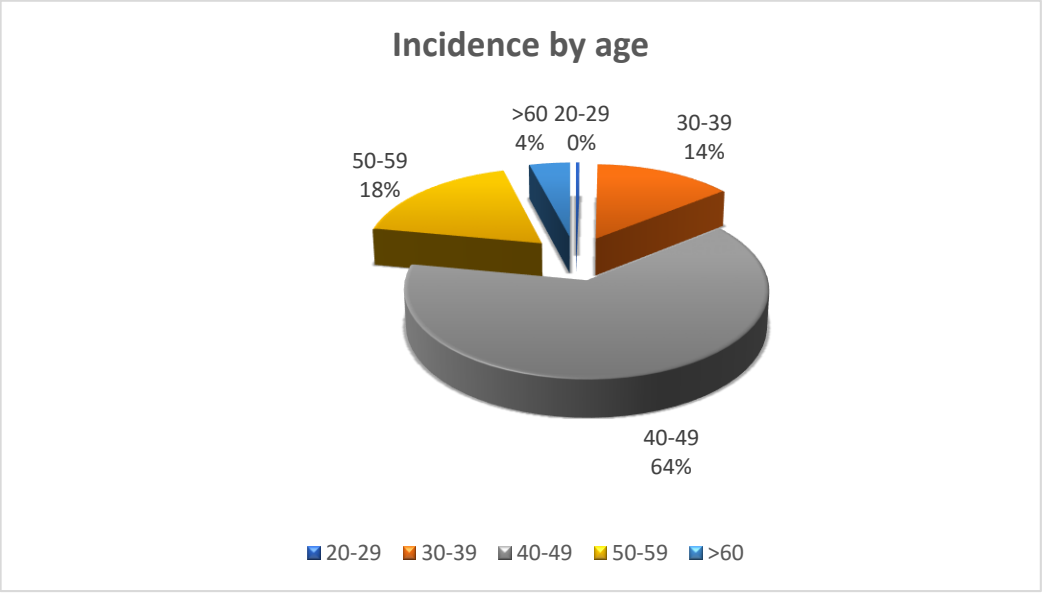
The incidence of uterine leiomyoma in the period presented was **14.19%** of all hospitalizations in the two departments of Obstetrics-Gynecology. Out of 10602 patients, 1505 were diagnosed with uterine leiomyoma.

a) Distribution of the studied group by age groups

The studied over the five years was classified into age groups between 20 and 60 years old.

Table 1. Distribution of the studied group by age

Age	Number of Patients	Incidence
20-29	5	0,33%
30-39	211	14%
40-49	957	63,58%
50-59	270	17,94%
>60	62	4,11%
Total	1505	100%



Graphic 1. Distribution of the studied group by age

It can be seen from the graphic analysis above that the highest incidence of symptoms related to uterine leiomyoma is in **the 40-49 age group**, most of the time, in this age

period overlapping both the associated symptoms leiomyoma and that associated with perimenopause.

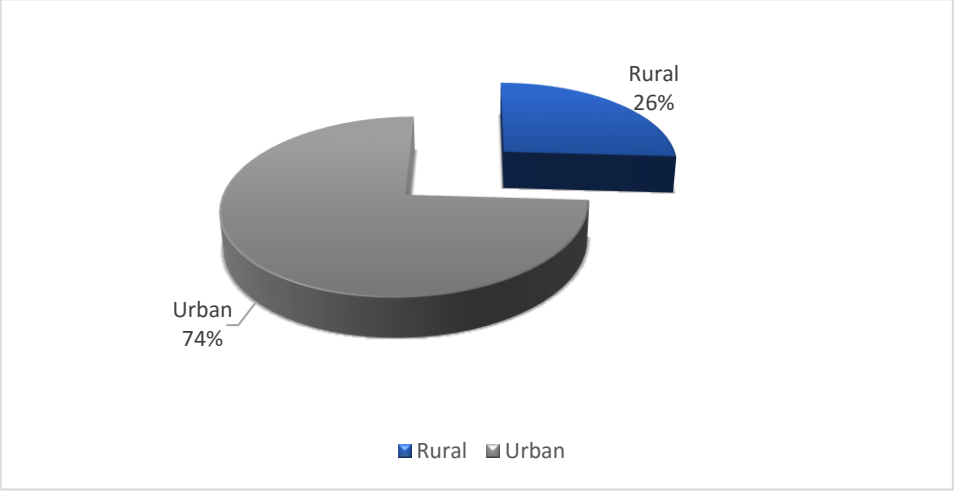
b) Distribution of the studied batch by provenance environment

The studied batch of patients was divided into rural and urban environments.

Table 2. Distribution of the studied group by provenance environment

	Frequency	Procent
Rural	390	26%
Urban	1115	74%
Total	1505	100%

Both from the table 2 above and from the graphic 2 representation, a higher incidence of leiomyofibroma can be observed in the urban population. Urban population incidence is 74%, compared to the rural population, which is only 26% of cases.



Graphic 2. Distribution of the studied group by provenance environment

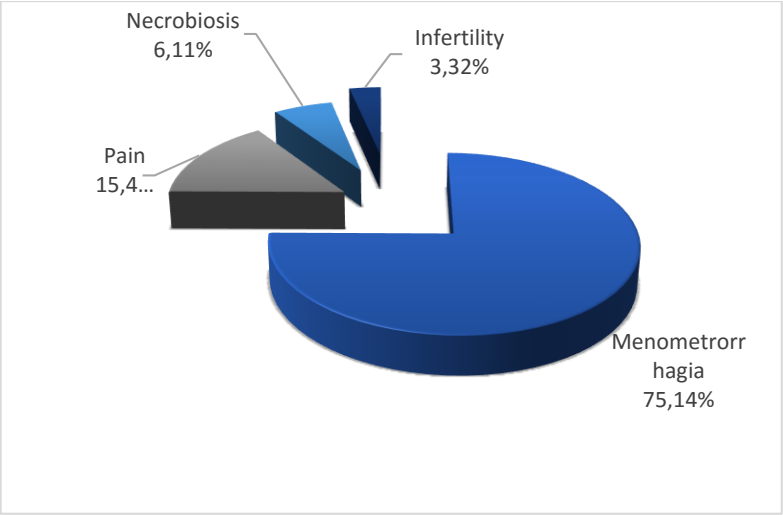
These results can be explained by the low parity in the urban environment compared to the one in the rural environment and also by the low addressability of rural women to the gynecologist compared to urban women.

c) Distribution according to symptoms

The most common reason women presented to the Emergency Department was vaginal bleeding.

Table 3. Distribution of the studied group by symptoms

Main Symptom	Number	Incidence
Menometrorrhagia	1131	75,14%
Compression	232	15,41%
Septic Necrobiotic	92	6,11%
Sterility/ Infertility	50	3,32%
Total	1505	100%



Graphic 3. Distribution of the studied group by symptoms

About 75% of women with leiomyoma experienced abnormal bleeding, followed by those with abdominal symptoms related to compression of surrounding organs, which accounted for 15%. Bleeding can occur both in the context of leiomyoma and because of perimenopause but most of the time the two overlap. Bleeding is often profuse and can lead to severe anemia.

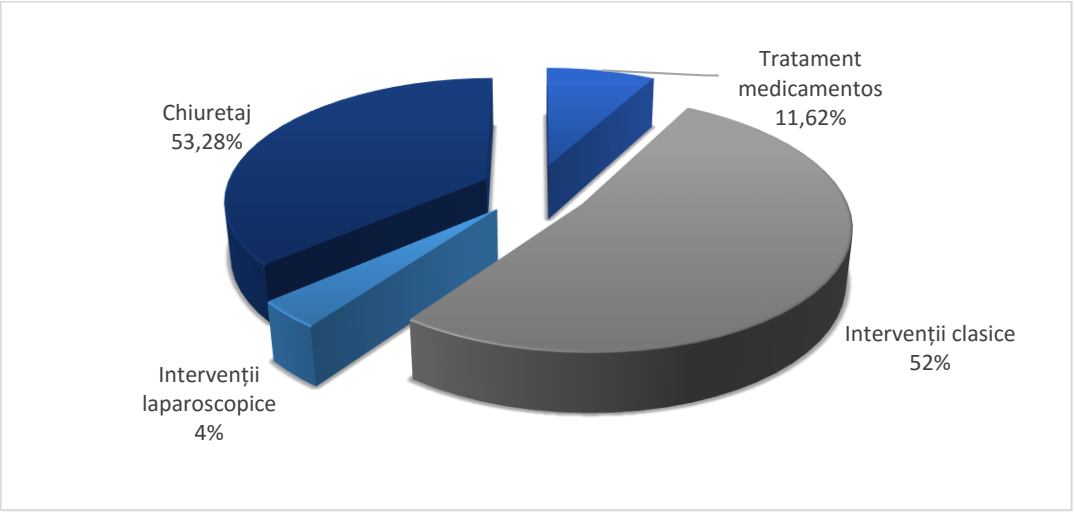
d) Distribution of the studied batch according to the type of surgical intervention performed

After we analyzed the observation sheets and operative protocols, we found that of the 1505 patients hospitalized with the diagnosis of leiomyofibroma, during the years 2015-2019, 528 of them underwent surgical intervention and 802 benefited by a hemostatic uterine curettage and biopsy. Among the 528 patients that had surgical intervention, 361 underwent radical intervention (total hysterectomy) via the abdominal route and 62 via the laparoscopic route. Subtotal hysterectomies by the abdominal way were performed to 41 patients and 2 subtotal hysterectomies using

the laparoscopy. There were also 17 vaginal hysterectomies and 45 uterus-conserving interventions- myomectomies.

Table 4. Distribution of the studied group by intervention type

Medical Treatment	Surgical Intervention 528 (35.08%)							Curettage with biopsy
	Laparoscopy			Abdominal intervention				
	Total HT	Subtotal HT	Miomectomy	Total HT	Subtotal HT	Miomectomy	Vaginal HT	
175 (11,62%)	62 11.74%	2 0.13%	15 2,84%	361 68.37%	41 7,76%	30 5,68%	17 3.21%	802 (53,28%)



Graphic 4. Distribution of the studied group by intervention type

Discussions

Benign uterine pathology occupies a very well-defined place in gynecological practice, given its increasing incidence. The incidence of uterine leiomyoma in the Emergency County Clinical Hospital "Saint Andrew the Apostle" Constanta was 14.19%.

Out of 10602 patients, 1505 were diagnosed with uterine leiomyoma. In terms of age, most of the patients in the studied group belong to the 40-49 years old category, which represents a percentage of 63.58%, followed by the patients located in the 50-59 years old age category with a percentage of 17.94%. Specialized studies have shown a maximum incidence in the 45-50 age group (Marshall et. al., 1997), (Wise et. al., 2016).

The patients from the studied group who came from the urban environment were more numerous, respectively, 1115 patients, which represents 74% of all patients, from this fact a better medical education and a greater addressability to the gynecologist emerges. Data collected from the literature also demonstrated the same increased prevalence of patients from the urban environment, compared to the patients from the rural environment (Wise et. al., 2016), (Parazzini et. al., 1988).

The most common reason for presenting to the emergency room was represented by abnormal vaginal bleeding. That beeinf said, 75.14% of the patients of the studied group presented to the emergency department for appreciable vaginal bleeding, which decreases the quality of life with severe anemia and urgent need for transfusion. Studies have also shown vaginal bleeding as the main reason for presentation to the Emergency Department (Wegienka et. al., 2003), (Schwartz et. al., 2000).

Conclusions

Preferred surgical intervention in the Emergency Clinical Hospital " Saint Andrew the Apostle " Constanta was total abdominal hysterectomy 361, with a percentage of 68.37% of all surgical interventions, followed by laparoscopic hysterectomy 62 cases, with a percentage of 11.74% and 41 cases of subtotal hysterectomy by abdominal way, respectively 7.76%% of all cases.

For patients with special obstetric conditions, especially nulliparous patients, conservative therapy was chosen, 45 cases benefiting of a myomectomy, performed laparoscopically or abdominally, which amounts to 8.52% of all interventions surgeries performed.

However, the Cochrane Meta-analyses demonstrated a better post-operative recovery in the case of patients who opted for laparoscopic intervention, followed by vaginal interventions and, as a last resort, abdominal hysterectomy was reserved for private cases (Nieboer et. al., 2009).

Unfortunately, laparoscopic interventions require qualified personnel and expensive equipment, which are not easily found in all hospital units in the Romanian health system.

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Clinical Course and Treatment of Human Brucellosis in a Sample of Hospitalized Cases in Albania

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Abstract

Brucellosis remains a public health problem in many Mediterranean countries. In this work are presenting data of human brucellosis clinics and treatment in a sample of hospitalized patients. Methods: All patient charts at regional hospital in Gjirokastra, Albania were systematically reviewed, during the period 2016-2021. All hospitalized patients with a laboratory confirmed diagnoses of brucellosis were included in the study. Variables of interest were clinical symptoms, clinical course and treatment provided. Sub-acute brucellosis was defined as clinical persistence of 3-12 months while cases with clinical symptoms persisting for ≥12 months were defined as chronic brucellosis. Results: 79% of the 86 patients were male and residing in rural areas. Fever, profuse sweating and arthralgia were the most common clinical signs. Around 70% of the brucellosis patients showed all these three symptoms. Despite a systematic tendency for more frequent presence of high fever, increased sweating and arthralgia on younger patients we could not find statistically significant differences among demographic categories. 18.6% of cases presented persistence of clinical signs after at least 3 months from the moment of the diagnoses. Almost 7% of the cases were classified as chronic cases. 75.6% of all patients were treated with a combination of doxycycline and ceftriaxone antibiotic regime. Conclusions: The massive use of a cephalosporin in treatment of brucellosis cannot be justified and may reflect a larger problem related to population awareness and health provider attitudes concerning antibiotic use in Albania. The results of this study may assist future interventions to improve brucellosis case management at hospitals or primary health care level as well as national measures at a larger scale for control of the disease.

Keywords: Brucellosis, treatment, antibiotic, Albania

Introduction

Although brucellosis is eradicated in many high-income countries, it remains a common disease in humans and animals in most countries of Eastern Mediterranean.

Humans are infected by direct contact with animal tissues or indirectly by consumption of infected animal foods such as meat or diary. The aerosolized particles containing the brucellae bacteria can also be inhaled by people manipulating the contaminated products (1). Most cases are caused by unpasteurized goat or sheep milk or cheese. Person to person transmission, although rare, is also reported. Brucellosis is considered an occupational hazard to people engaged in certain professions such as veterinarians, slaughterhouse workers, and farmers (2).

The disease may take acute, or chronic forms and usually causes weakness, headache, lethargy, weight loss, fever, and sweating. The fever is typically remittent, giving the disease the name, undulant fever (3,4). Fever with unknown origin, which is the main symptom of brucellosis; can be misdiagnosed with similar pathologies including all fevers of unknown origin that may be caused by infectious diseases such as leishmaniasis, tuberculosis, malaria, as well as other diseases such as malignancies, rheumatic fever etc (5).

The incubation period is usually from 2 to 4 weeks, but may be highly variable. The forms of the clinical course of brucellosis in humans are categorized as acute, sub-acute and chronic (6).

The diagnosis of chronic brucellosis is often based on clinical complaints together with the presence of high immunoglobulin G titers (7).

Treatment in human brucellosis is done by use of a combination of antibiotics. Because the bacteria reside within cells, several weeks of treatment are needed. Treating with a single antibiotic is not recommended as there is a high rate of relapse when a combination regimen is not used (8)

The brucellae are gram-negative aerobic coccobacilli with brucella melitensis the most prevalent species mostly because of difficulties in immunizing goats and sheep (9).

The common reservoirs for Brucellae bacteria that may infect humans are cattle, dogs, sheep, goats, and pigs. Brucellosis remains an important public health problem and being a zoonotic disease, its control in humans implies preventive measures of a veterinary nature.

Although under control during recent years, the brucellosis is far from being eradicated in Albania, with cases reported from more than half of administrative municipalities. Since 30 years ago, the incidence of brucellosis has appeared to be higher in the Southern and Southeastern parts of the country. The risk of infection in the Vlorë, Gjirokastër, and Korçë counties has been significantly higher compared to other parts of Albania (10).

Although well studied in global level, publications about brucellosis clinical characteristics and treatment in Albania are scarce. We have not found any peer reviewed documents during the last decade to describe brucellosis characteristics and treatment in the South of Albania where the risk remains high. The aim of this work is to present data of human brucellosis clinical course in hospitalized patients as well as patterns of treatment of the disease. We compare the data to international clinical profiles and treatment protocols.

Methods

For this study we systematically reviewed all patient charts at Regional Hospital in Gjirokastra, Albania during the period 2016-2021. All hospitalized patients who had a laboratory confirmed diagnoses of brucellosis are included in the study. Laboratory test applied for the diagnoses confirmation has been Wright agglutination essay. This test has been validated and used to assist clinical management of cases (11,12) and epidemiological surveillance.

Although microbiological tests such as isolation of *Brucella* spp. and molecular tests are recommended in brucellosis confirmation, these tests were not available in Gjirokastra hospital.

The clinical chart included data about age, sex and residence of the cases, as well as clinical symptoms, clinical course and treatment provided. All these variables were used for the analyses. Chronic brucellosis was defined as clinical symptoms persisting for ≥12 months (13). A sub-acute category was also added as clinical persistence of 3-12 months (6).

The systematic search was done in statistical unit of the Hospital and an excel matrix was used to retrieve the data from the individual charts

Data were analysed in SPSS software package. When necessary, 95% confidence intervals were applied.

Results

There were 86 patients identified during the systematic search of the period 2016-2021 in the Gjirokastra Regional Hospital. 67 cases were males and 19 cases females, which represents a more than three times risk difference. Almost the same difference was observed between rural and urban residency of patients; 68 cases were from rural areas, in a region where rural and urban populations are similar (Census 2011). The highest number of reported cases during the period of study belonged to the age category of 40-60 years old (32 cases). 23 cases were young adults of age 18-39 years old and 25 cases were over 60 years old. There were only 6 brucellosis cases reported in children and teenagers. No cases under the age of 10 were identified.

Not all of the cases were presented with fever as a symptom of brucellosis. Still, fever along with profuse sweating and arthralgia were the most common clinical signs of brucellosis in Gjirokastra hospital. Around 70% of the brucellosis patients showed all

these three symptoms. Additionally, other relatively common symptoms which have been seen in about 20% of the cases were weakness, back pain and muscle pain referred in clinical charts as myalgia.

Frequency of brucellosis symptoms among hospitalized patients is presented in the Table 1.

Table 1. Symptoms of Brucellosis cases

Symptoms	Cases	%
Fever	63	73%
Sweating	61	71%
Arthralgia	59	68%
Lumbalgia	17	20%
Weakness	16	18%
Myalgia	16	18%
Frissons	9	10%
Headache	9	10%
Nausea	9	10%
Dyspnea	6	7%
Cough	6	7%
Anorexia	4	5%
Stomach ache	3	4%

Note: More than one symptom could be reported in each of the patients.

Distribution of brucellosis clinical signs according to sex, age-groups and residence are presented in Table 2. In young people, there is a systematic tendency of a relatively more frequent presence of high fever, increased sweating and arthralgia. On the other hand, some clinical signs such as lumbalgia, and to a certain degree myalgia (data not shown), were found systematically more often among older patients compared to younger ones. Arthralgia in females seemed to be more common than fever or sweat, compared to males.

Nevertheless, despite all those observed tendencies, our sample failed to prove that the differences were of statistical significance as shown by broad 95% confidence intervals.

Table 2. Most common symptoms distributed according to sex, age and residency

	Fever		Sweating		Arthralgia		Lumbalgia	
	Cases (%)	95% IC	Cases (%)	95% IC	Cases (%)	95% IC	Cases (%)	95% IC
Sex								
Males	52 (78%)	66%-86%	48 (72%)	59%-81%	44 (66%)	53%-76%	15 (22%)	14%-33%
Females	11 (58%)	36%-76%	13 (68%)	40%-84%	15 (79%)	57%-91%	2 (11%)	2%-31%
Age-group								
10-18y	6 (100%)	60%-99%	5 (83%)	43%-96%	5 (83%)	44%-69%	0 (0%)	-
19-39y	19 (83%)	63%-93%	17 (74%)	53%-87%	17 (74%)	53%-87%	3 (13%)	4%-32%
40-59y	24 (67%)	50%-79%	25 (69%)	53%-82%	23 (64%)	47%-77%	8 (22%)	11%-38%
60+y	14 (67%)	45%-83%	14 (67%)	45%-82%	14 (67%)	45%-82%	6 (29%)	13%-49%
Residency								
Urban	8 (53%)	30%-75%	11 (73%)	48%-89%	12 (80%)	54%-93%	4 (27%)	10%-52%
Rural	55 (77%)	67%-85%	50 (70%)	59%-79%	47 (66%)	54%-76%	13 (18%)	11%-28%

In Table 3 are shown the proportions of chronic (over 1 year persistence since diagnoses) and sub-acute cases (3-12 months persistence since diagnoses) of brucellosis. There are in total 18.6% of cases or almost 1 in 5 cases which present persistence of clinical signs and other complications after at least 3 months from the moment of the diagnoses. Almost 7% of the cases are classified as full chronic cases, with clinical signs or complications persisting after 1 year of diagnoses. No significant differences or relative tendencies were found between age, sex and residency categories.

Table 3. Chronic and sub-acute cases of brucellosis

	Chronic		Sub-acute		Total chronic + sub-acute		Total
	Cases (%)	IC 95%	Cases (%)	IC 95%	Cases (%)	IC 95%	Cases (%)
Males	5 (7.5%)	3.2%-16.3%	8 (11.6%)	6,2%-21,8%	13 (19.4%)	12%-30%	67 (100%)
Females	1 (5.3%)	0.9%-24.6%	2 (11.9%)	2.9%-31.4%	3 (15.8%)	6%-37%	19 (100%)
Total	6 (6.9%)	3.2%-14.4%	10 (11.6%)	6.4%-20.1%	16 (18.6%)	11.8%-28.1%	86 (100%)

The overwhelming majority of brucellosis patients or 75.6% of all cases in Gjirokastra regional hospital during the last 6 years under study have been treated with a combination of doxycycline and ceftriaxone antibiotic regime. 16.2% of patients have been treated with the combination of doxycycline in conjunction with another antibiotic (streptomycin 4.6%, or gentamycin 8.1%, or rifampicin 3.5%). In very few patients either monotherapy (4.6%) or tritherapy (3.5%) have been applied. Only 2 cases have not received doxycycline as a part of their standard treatment regime for brucellosis (Table 4).

Table 4. Treatment of brucellosis

	Monotherapy		Bitherapy		Tritherapy		Total	
Treatment regime	cases	%	cases	%	cases	%	cases	%
Doxycycline	2	2.3%						
Ceftriaxone	2	2.3%						
Doxycycline + Streptomycin			4	4.6%				
Doxycycline + Gentamycin			7	8.1%				

Doxycycline + Rifampicin			3	3.5%				
Doxycycline + Ceftriaxone			65	75.6 %				
Doxycycline +Streptomycin+ Ceftriaxone					1	1.2 %		
Doxycycline +Gentamycin+ Ceftriaxone					2	2.3 %		
Total	4	4.7%	79	91.9 %	3	3.5 %	86	100 %

Discussion

In this work we describe some clinical and treatment characteristics in the totality of brucellosis patients hospitalized in the regional hospital of Gjirokastra during the last 6 years.

The hospitalized brucellosis patients under study don't represent all cases of the human infections in the region during the same period as some cases may have experienced few or no symptoms (8) and some may have been treated at home. Nonetheless the overwhelming dominance of male cases in the sample most likely mirror the much higher level of risk of brucellosis infection among males in community. This profile can be explained by gender differences in professional exposure to manipulation with livestock products (14). This is further confirmed by the higher proportion of brucellosis patients of rural residency.

The profile of clinical signs observed is similar with that found in most of literature covering clinical human brucellosis (15,16). Fever, joint pain, increased sweating were the most commonly reported symptoms followed by weakness and muscle pain. In young people, there is a systematic tendency of a relatively more frequent presence of high fever, increased sweating and arthralgia. This profile is found also elsewhere in young patients and children (17,18). Some clinical signs such as lumbalgia, were found systematically more often among older patients compared to younger ones. Arthralgia in females seemed to be more common than fever or sweat, compared to males. Similar profile of symptoms is reported in a systematic review (19). Because of the size of our study sample, it has proven impossible to show statistical significance in differences observed. Other studies in larger samples, or in a more extended time span, would be advisable in the future to confirm some of the trends we report in this work.

Brucellosis can get chronicized in a minority of patients with sick individuals experiencing persistence of symptoms for more than 1 year. Such patients are defined as having chronic brucellosis. There are no objective laboratory methods to confirm the presence of chronic disease and the most common definition is based on the time span of the illness, the return of the symptoms and silent organ complications. In our study we found that in total almost 1 in 5 cases presented with persistence of clinical signs and other complications after at least 3 months from the moment of the diagnosis. These cases could be considered as sub-acute and chronic brucellosis. Almost 7% of the cases were classified as full chronic cases, with clinical signs or complications persisting after 1 year of diagnoses. While the proportion of sub-acute patients is relatively lower than that reported by some other studies in specialized literature (6) the proportion of those with symptoms persisting for more than one year is slightly higher than expected (6,13). We found no significant differences or relative tendencies in chronicization risk between age, sex and residency categories. Often chronic patients suffer delays in both diagnosis and treatment (20). The prognosis is poorer in people who develop organ changes or complications such as heart damage, neurological, or genitourinary problems caused by chronic Brucella infection.

The goal of medical therapy in brucellosis is to control the disease as quickly as possible in order to prevent chronicization and complications. The drug of choice is doxycycline which is used in conjunction with either streptomycin, rifampin, gentamicin, or sulfamethoxazole / trimethoprim (8). The use of doxycycline as the drug of choice for treating brucellosis is confirmed in our study, as doxycycline is not reported only in two patients out of a total of 86 during 6 years. In those two cases the doxycycline might have been avoided because of the young age of patients (8). On the other hand, the combination of doxycycline with ceftriaxone instead of other recommended antibiotics is striking. More than 75% of patients have been treated with this biotherapy. The observed massive use of a cephalosporin in treatment of brucellosis may reflect a larger problem related to population awareness, health provider attitudes and patient's behaviour concerning antibiotic use in Albania (21,22). While the utilisation of ceftriaxone may be effective in treatment of the acute brucellosis, it is recommended only in complicated cases (23) and its massive application can't be clinically justified.

The results of this study may assist any interventions to improve brucellosis case management at hospital or primary health care level as well as national measures at a larger scale for control of the disease.

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Therapeutical Properties of Bioactive Compounds Extracted from *Ganoderma lucidum* Species on Acute and Chronic Diseases

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Abstract

Ganoderma lucidum or Reishi is a medicinal mushroom of significant importance that is being used for the prevention and treatment of various diseases, due to its numerous pharmacological and therapeutic properties. The Ganodermataceae family is diverse, but the focus is on the lucidum species because of its specific biologically active macromolecules such as polysaccharides, triterpenoids, steroids, phenolic compounds, lactones, and fatty acids that were isolated from the mycelia and fruiting bodies. Ganoderma lucidum species have many benefits over the immune system (promoting health) and properties such as: anti-tumoral, anti-inflammatory, anti-allergic, anti-viral, anti-bacterial and antioxidant. Based on the literature, triterpenoids and polysaccharides are the most abundant active compounds that possess anti-diabetic, hepatoprotective, cytotoxic and anti-hypertensive effects. A review of the literature showed that there are very few papers that studied semisolid dosage forms preparations based of Ganoderma lucidum extract to have been used on damaged skin and skin diseases, neither have there been enough studies on its anti-aging properties.

Keywords: Ganoderma lucidum, polysaccharides, anti-inflammatory, anti-tumoral, antioxidant

Introduction

Currently, medicine based on plant extracts or other medicinal herbs is in great demand by the population, since most are looking for a way to live healthy or looking for alternative medicine based on herbal extracts. Still, fungus extracts are in great

demand, especially those from Ganodermataceae family due to its promotions over the years, which shows through statements and studies that is still a wonder-mushroom [1]. It is known that medicinal mushrooms have been used in over the years to treat many disorders, for promoting health and longevity in Asian countries. Among Eastern population, the name Reishi or Mannentake is commonly used by Japanese people and is the symbol of good health. In Chinese and Korea, it is known as LingZhi and represents a combination of spiritual potency and essence of immortality [2]. *Ganoderma lucidum* (*G. lucidum*) also known as Reishi and LingZhi, is the most known medicinal macrofungal species, with a long history in promoting health in different countries, especially in China and Japan. The medicinal activity of *G. lucidum* fungus is localized, for example, inhibiting the activity of the enzyme cyclooxygenase in the anti-inflammatory response [3] and proliferation of the immune system by inhibiting its activity in cases of overstimulation [4].

Various formulations and products based on this fungus species are now available on the market. These are extracted from different parts of the fungus: mycelium, spores, and fruiting body. Most products are in the form of tea, tonics and coffee, powders, and encapsulated food supplements [4].

Botanical description

G. lucidum is a kidney-shaped mushroom [4] with glossy surface and woody texture [5]. *Ganoderma* genus can be found all over the world, mainly in tropical and subtropical climates [6], but different characteristics of the fruiting body, soil specificity and geographical origin are used to identify individual species of this medicinal fungus. The Ganodermataceae family consists of many fungi from the *Ganoderma* genus [6] and the taxonomy classification of this species is related into Table 1.

This species grows on multiple hosts, on living trees or stumps of oak and is growing rarely on coniferous trees [2]. In Europe, specially in Romania, it can be found in the Sub Carpati mountain chain region. The medicinal mushroom, *Ganoderma lucidum*, taken in work, weighs 0.25 grams, is 9-10 cm wide and 9-10 cm long and is quite bulky, has a dark reddish shiny surface and a woody texture, see Fig. 1.

Table 1 Taxonomy classification of species G. lucidum [6, 7]

Kingdom	**Fungi**
Phylum	Basidiomycota
Class	Basidiomycetes
Sub-class	Homobasidiomycetes
Family	Ganodermataceae
Genus	*Ganoderma*
Species	*lucidum*

Fig. 1 Ganoderma lucidum (personal archive)

Polysacharides and Triterpenes of *Ganoderma lucidum* extract

Polysaccharides and triterpenes are the most abundant biocomponents in the composition of *G. lucidum* [1]. The most important functions of polysaccharides are reported by numerous studies, and there are: normalizing blood glucose levels, restoring the enzymatic balance in the intestinal tract due to the probiotic effect [8], reducing damage through antioxidant effect on free radicals formed in oxidation reactions. Anti-diabetic and anti-tumor effects have also been reported for both the polysaccharide and triterpenoid components [9]. In addition to these effects, triterpenoids have an immunomodulatory effect, proven by in vivo and in vitro studies. Beside these two major bioactive compounds, the mushroom contains in significant quantities proteins, lipids, carbohydrates and sodium chloride [10].

Triderpenoids from the *G. lucidum* mushroom

There are many types of triterpenoid compounds that have been isolated from the *G. lucidum* mushroom, the most common are ganodermic, lucidenic, ganoderals, ganoderols and applanoxidic acids. The most characteristic acids in the composition are ganodermic acid F (Fig. 2), lucidenic acid D1 (Fig. 3), ganoderal A (Fig. 4), ganoderol B (Fig. 5) and applanoxidic acid A (Fig. 6) [9].

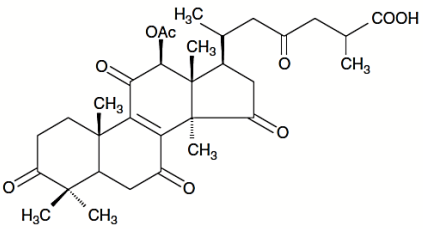

Fig. 2 Ganodermic acid F

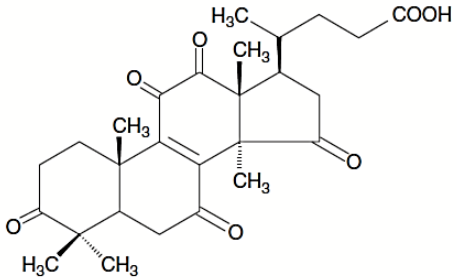

Fig. 3 Lucidenic acid D1

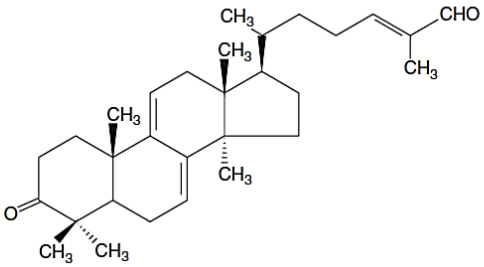

Fig. 4 Ganoderal A

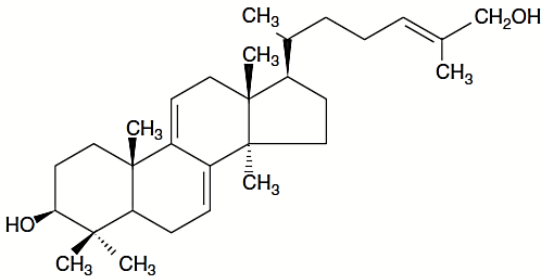

Fig. 5 Ganoderol B

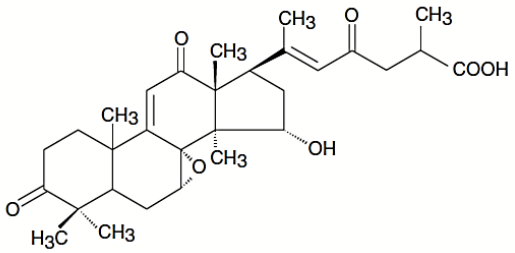

Fig. 6 Applanoxidic acid A

Polysaccharides from the *G. lucidum* species

Polysaccharides are important elements in both the pharmaceutical and food industries, due to their ability to defend the body against pathogens through various mechanisms [11]. These compounds are able to interact with the immune system and to enhance mechanisms of response. Its structure is composed of long-chain sugar molecules linked together by glycosidic bonds. Numerous types of them have been isolated in the *Ganoderma* species. Polysaccharides from this mushroom have heteropolymers with high molecular wight where the major components are glucose, mannose, galactose, and fructose. [12, 13]. Fig. 7 represents the side-chain of β-glucan with protein in polysaccharide-K [14].

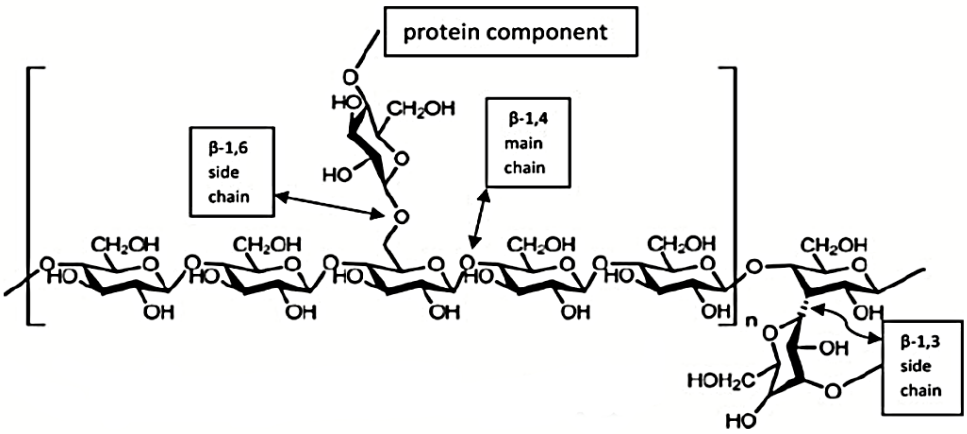

Fig. 7 β-glucan side-chain

Therapeutical applications

Ganoderma lucidum is known to have a very wide range of pharmacological effects. Among these, we list the immuno-modulatory, hypocholesterolaemia, anti-inflammatory and analgesic, antitumor, antibacterial, antiviral, and antifungal, hepatoprotective, antidiabetic, antioxidant effects [5, 12, 15-17]. Today, it is used by a very large number of people especially as an adjuvant medication in the treatment of cancer, hepatitis, diabetes and for anti-aging property [12, 8]. Fig. 8 represents pharmacological actions of both compounds, polysaccharides and triterpenoids.

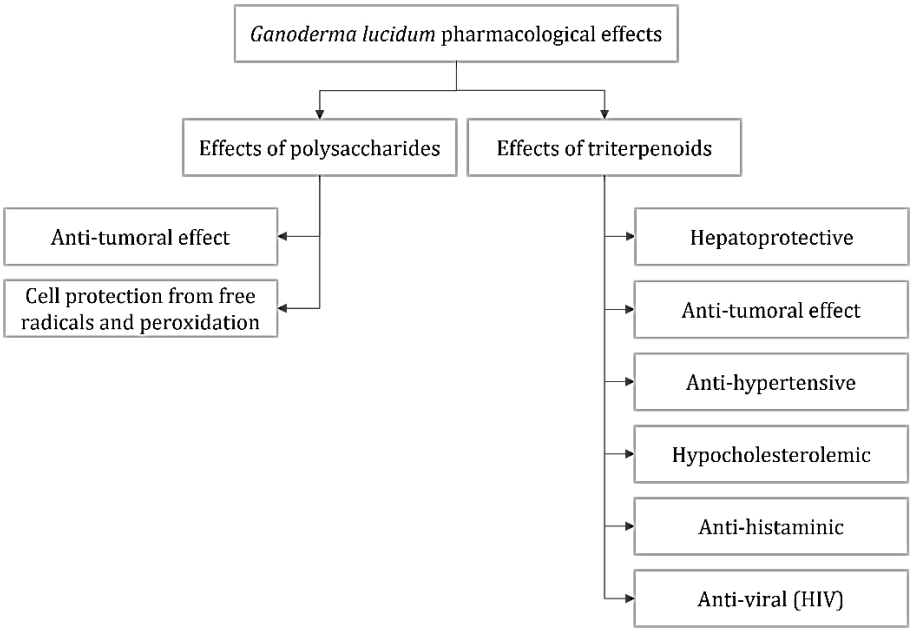

Fig. 8 Ganoderma lucidum pharmacological effects

Anti-inflammatory properties

The anti-inflammatory action is achieved by inhibiting the activity of cyclooxygenase enzymes, which are responsible for the production of prostaglandins. A study related by Joseph et al. [3] showed that the chloroform extract of *G. lucidum* has huge inhibitory potential of the inflammation induced by carrageenan and formalin, in the mice paws. The chloroform extract showed a 73,4% reduction at a concentration of 100mg/kg body weight, and a 63,2% reduction of inflammation at a concentration of 50mg/kg body weight of chloroform extract from the *G. lucidum* for carrageenan-induced inflammation. A reduction in inflammation of 63,4% and 53,4% for the same concentrations was observed in formalin-induced inflammation in the oedematous paw of the mice. For the standard reference, the study used the anti-inflammatory drug diclofenac, which showed an inhibition of oedemas of 53,0% and 40,2% for the same concentrations [3].

Anti-tumoral activity

The anti-tumoral effect of different *G. lucidum* extracts has been intensively studied by several groups of researchers. A study showed that the ethanolic extract of *G. lucidum*, which contains a high number of phenolic compounds, indicates antiproliferative activity in human cervical carcinoma, human alveolar basal adenocarcinoma, and human colon carcinoma [19]. Following the analysis, the author related an important correlation between the antiproliferative activity against human cervical carcinoma and the total phenolic compounds, and antiproliferative activity against human alveolar basal adenocarcinoma and total glucans content. The most abundant phenolic compounds were hesperetin and naringenin, for each analysed sample of the study [19]. In another study, an important part of the antiproliferative activity against cancer cell line is attributed to the high content of total phenolic compounds such as resveratrol and apigenin. Quantitative analysis of the total polyphenolic compounds showed that from 13,991.10 mg of total polyphenolic compounds per 100g of the extract, resveratrol was the most abundant with 5155,70 mg and apigenin with 4039.08 mg [20].

The clinical potential and a wide acceptance of extracts obtained from *G. lucidum* in terms of its antitumoral activity as an alternative therapy have developed interest in the research of its molecular mechanisms. [21]. In table 2 and 3 are related applications, activities, and mechanism of action of different *G. lucidum* extracts and for purified Ganoderic acids on multiple types of cancer cells.

Tabel 2 G. lucidum extracts- applications, activities and mechanism of action

Ganoderma lucidum extracts	Antitumoral applications	Mechanism of action	References
Dried powder of G. lucidum dissolved in boiled water	Invasive breast cancer Prostate cancer	Produces down-regulation of transcription factors AP-1 and NF-κB in cancer cells.	[22]
Hot water extraction	Drug-sensitive (H69) and multi-drug resistant (VPA) human SCLC (small-cell lung cancer) cells.	Both cells show equal cytotoxicity	[23]
Ethanolic extract of G. lucidum	Human Urothelial cells (bladder cancer)	In vitro – ethanolic extract exhibits a potent inhibition and induces	[24]

		growth arrest and cell migration	
Methanolic extract	Inhibit several cancer cell lines including hematological cell lines (lymphomas and multiple myelomas)	Upregulation of p21/p27 Induction of cell line arrest	[25]

Tabel 3 Subtypes of Ganoderic Acids- applications, activities and machanism of action

Subtypes of Ganoderic Acids	Antitumoral applications	Mechanism of action	References
Ganoderic Acid X (GA-X)	Hepatoma cells Colorectal carcinoma Acute promyelocyte leukemia	In Human Hepatoma (HuH-7) cells – GAX caused immediate inhibition of DNA systhesis and activation of ERK and JNK mitogen-activated protein kinases. *In vitro* – inhibate topoisomerases I and IIα	[26]
Ganoderic Acids T (GA-T)	Human metastatic lung carcinoma (95-D)	Apoptosis induction and cell line arrest at G (1) phase Induce cytotoxicity in human carcinoma cell lines in a dose-dependent manner, less toxic to normal human cell lines.	[27]

Anti-allergic property

The first line in the treatment of minor allergies are histamine H1 receptor antagonists. However, they respond poorly to acute hives or pruritic diseases [16]. Thus, studies to develop new adjuvant treatments based on *G. lucidum* extract against symptoms that are resistant to antihistamines are ongoing. Studies have shown that the properties of mushrooms from the Ganodermataceae family are able to restore the balance between TH1 and TH2 immune states [28]. In addition, there have been studies linking the potential of polysaccharides extracted from *G. lucidum* to the process of restoring IL-2 production that has been inhibited by the aging process observed in mice [29]. Powell studied the efficacy of unfractionated *G. lucidum* to determine the role of the medicinal fungus in managing the histamine-mediated immune response. The experiment took two males of different ages into the study. The first man aged 39, who had suffered from hay fever as a child, was initially given a dose of *G. lucidum* tablets of 3g per day (6 tablets x 500mg) with maintenance of this dose until symptoms subsided. The process of maintaining and decreasing the dose to 1. 5g per day (3 tablets x 500mg) showed a significant decrease in symptoms after 10 days of treatment. The second subject, a 5-year-old male who developed hay fever at the age of 4, was given an initial dose of 1g per day (2 tablets x 500mg) maintained until the end of the season. For the youngest subject, a 90% reduction in symptoms was achieved after one week. A review article, reported by Sanodiya et al. 2009 [6], synthesized studies showing inhibition of histamine release by mast cells from rat subjects, finding that ganoderic acids C and D, for the first time, played an important role in the inhibition of histamine release. Also, the compound cyclo-octasulfur found in *G. lucidum* composition inhibited the release of histamine from peritoneal mast cells of rat subjects, justified by membrane proteins interaction for inhibiting the Ca^{2+} uptake causing for the benefit, blockade of histamine release [18].

Anti-bacterial and anti-viral activity

There are many studies based on antiviral, antibacterial and antifungal treatment and they are aimed at discovering new herbal therapies. This approach is being considered due to patient neglect of anti-infectious therapies and the triggering of antibiotic resistance and adverse effects. The aim of these studies is to discover new factors that specifically inhibit antiviral and antibacterial activity without affecting the normal cell [30]. According to the literature, numerous experiments have been conducted to prove the efficacy of *G. lucidum* extract for further use as an antimicrobial or antiviral agent. Kim et al. [31], reported anti-herpetic activity of acidic protein bound polysaccharide, isolated from *G. lucidum*. The results showed potent antiviral activity against HSV-1 and HSV-2 [31]. Many studies have shown that antibacterial compounds in *G. lucidum* have inhibitory potential on gram-positive and gram-negative bacteria [32]. Both polysaccharide bioactive compounds and triterpenoids from *G. lucidum* have been shown to be effective in the antibacterial process [33]. Extracts provided from *G. lucidum* have been shown to exhibit antibiotic

properties by inhibiting the growth of gram-positive and gram-negative bacteria [34]. Many studies reporting that these properties have been conducted mostly on *Escherichia coli* and *Bacilus subtilis* [32]. From the studies, different levels of antibiotic activity against *Staphylococcus aureus*, *Salmonella sp*. and *Pseudomonas aeruginosa* are observed [35]. Another study showed that *G. lucidum* extract inhibits *Helicobacter pylori*, responsible for gastric ulcer formation [32]. Different extracts of *G. lucidum* have also been studied, but the methanolic extract showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [36].

Immuno-modulatory and antioxidant property

According to Joseph et al. 2018 [3] study, chloroform extract of *G. lucidum* shows significant superoxide anion, nitric oxide, and lipid peroxidation inhibition activity. Following these tests, *G. lucidum* presents a source of interest for its natural antioxidant properties for the medical, pharmaceutical, and cosmetic industries.

A lot of research has focused on the immune-modulatory activity of *G. lucidum*, a lot of them have shown that its activity is mainly due to the presence of polysaccharides components, proteins, and triterpenoids [37]. An effect of the activation of immune response stimulating T cells, macrophages and natural killer cells was reported in a study that have been carried out on the subjects, mice [38]. The same result has been shown by Wong at al. 2004, too [39].

A study by Shi et al. 2013 [40], defined the antioxidant and immunoregulatory potential of *Ganoderma* extract, obtaining four types of polysaccharide compounds (GLP-I, GLP-II, GLP-III, GLP-IV). For the structural characterization of the compounds, Fourier transform infrared spectroscopy (FTIR) was used, and to determine the monosaccharide composition. This analysis showed that structurally the four identified compounds are similar, but the monosaccharides composition is significantly different. GLP-III and GLP-IV were found to be composed of six types of monosaccharides while GLP-II was found to be composed of three types. The results showed that all four types of polysaccharides performed antioxidant activity in a dose-dependent concentration, moreover, GLP-III and GLP-IV exhibited a significant scavenging effect on hydroxyl radicals, ABTS radicals and DPPH free radicals. The same study showed that 40 μg/mL of GLP, significantly stimulates macrophage proliferation and higher nitric oxide production [40].

Conclusions

Ganoderma lucidum is a traditional medicinal mushroom, appreciated for its many pharmacological effects and is increasingly consumed by the population to support a healthy life or as an adjuvant treatment in various acute or chronic diseases. Due to the numerous studies that have been carried out on both mice and humans, it can be concluded that the pharmacological effects of the medicinal mushroom are confirmed by the abundant presence of polysaccharides and triterpenoids, and in most studies, there was no evidence of renal or hepatic toxicity or changes in DNA genes [41].

However, there is a lack of studies on topical administration of *G. lucidum* extract and its possible pharmacological effects for epithelial regeneration as well as its anti-aging effect.

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Biomedical Applications Based on Marine Collagen Obtained from the Jellyfish Species Rhizostoma Pulmo Extracted from the Black Sea

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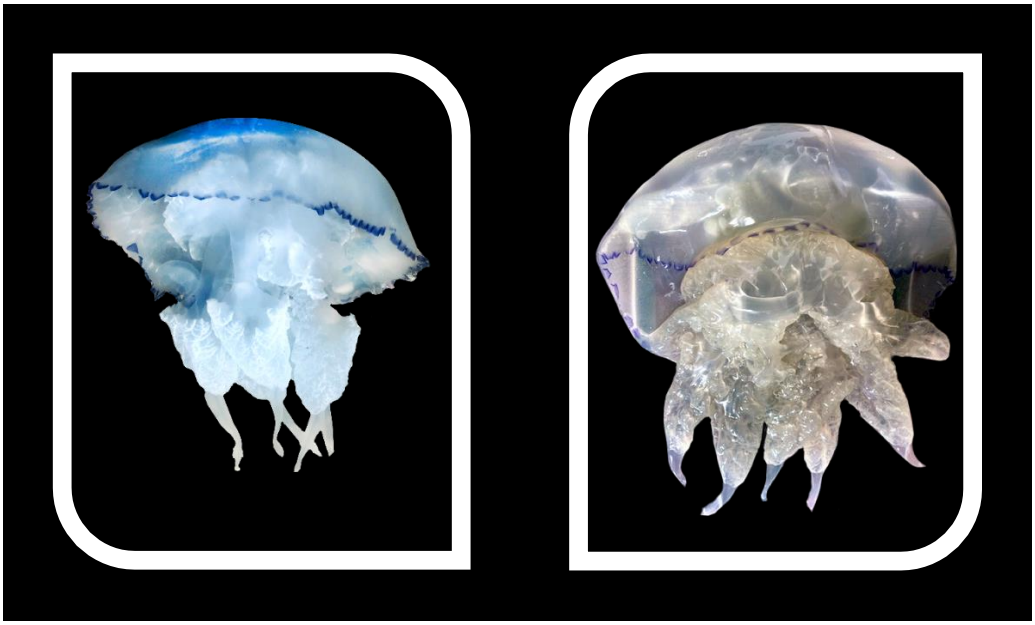
Abstract

Due to its unique properties, collagen is used in various emerging fields such as the pharmaceutical and biomedical device industries, as well as in related fields: nutraceuticals, cosmetics, food, beverages and nutritional supplements. Marine gelatin, one of the biomaterials involved in food and medicinal research, is denatured collagen produced from acid, alkaline, or enzyme hydrolysis. Gelatin is a crucial ingredient for the food, pharmaceutical, medical, biomedical focusing on versatile applications. Jellyfish collagen is a valuable resource for bioplastics and biomaterials used in various health sectors. Recently, marine organisms have been considered viable sources of collagen because they do not harbor transmissible diseases. In particular, fish biomass, as well as the catches of other types of organisms, such as small fish, jellyfish, starfish, sea urchins, sponges, possess a significant content of collagen. The collagen extracted from the species Rhizostoma pulmo from the Black Sea basin is also part of the bioresources that can be used to obtain natural marine collagen from this type of invertebrates. The use of discarded or hither to unused biomass could contribute to the development of a sustainable collagen extraction process with a positive impact on the ecosystem. In the future it is desired to approach a world strategy that minimizes the amount of waste and that supports all three general objectives of sustainability: sustainable economic well-being, social well-being and environmental protection.

Keywords: Rhizostoma pulmo, jellyfish, marine collagen, bioactive compounds, biomaterials, medical apllications

Introduction

Collagen of marine origin can be obtained from various animals, whether they are marine vertebrates - the source of collagen is fish skin, bones, cartilage, including scales, but also from other types of animals such as invertebrates, they are bioavailable compared to collagen bovine or porcine and have a higher absorption capacity (up to 1.5 times more efficient in the body) [1] and faster circulation in the bloodstream due to the low molecular weight and small particle size [2]. Another positive side of collagen of marine origin is the fact that it possesses similarity with conventional bovine and porcine collagen in terms of amino acids in composition and biocompatibility [3].



Rhizostoma pulmo – personal archive photo

Jellyfish collagen can be obtained from different parts of the jellyfish such as the umbrella, oral arms or even from the whole animal. The sources of marine collagen are diverse: fish, jellyfish, sea sponges, sea urchins, starfish, certain species of shells, together forming a biocompatible, natural alternative, without barriers of race, religion, degree of pollution or other inconveniences that standard animal collagen sources can meet them see Fig. 1.

Jellyfish are a rich source of minerals and protein, and collagen is a major protein in these gelatinous sea creatures. Cartilaginous tissue was isolated from jellyfish, later shown to be a type II collagen that can lead to the differentiation of mesenchymal stem cells. From a therapeutic point of view, it is TGF-3 cells in the form of nanoreservoirs that have helped to achieve combined cartilage [4].

Figure 1 Marine collagen sources

The objectives of the study consist in highlighting collagen sources, methods of collagen extraction from these sources and enumerating the most important biomedical applications of collagen from marine resources.

Materials and Methods

Marine collagen extraction methods

In jellyfish, it is common to separate the mouth arms from the umbrella and then divide the umbrella into mesoglea, exumbrella and subumbrella [5].

Reducing the size of these samples is essential to facilitate subsequent chemical (pre)treatment actions, used to remove proteins, pigments or non-collagenous fats. The common method involves the use of basic pretreatment with sodium hydroxide (NaOH), which does not cause structural changes to collagen chains, alcohols (namely butyl alcohol or ethanol) and oxygen peroxide in the process of removing non-collagenous proteins, fats and respectively of pigments [6]. In addition, to remove non-collagenous proteins from cod skin, the use of sodium chloride (NaCl) as an alternative to NaOH has also been proposed [6].

Moreover, to improve the extraction of collagen from bones, cartilage and scales, ethylenediaminetetraacetic acid (EDTA) is recommended for demineralization purposes [8]. For the extraction phase, it is well known that the solubility of collagen in cold water is poor due to the presence of strong crosslinks in its triple helix structure. There are two different conventional methods widely used: acid solubilized collagen extraction and collagen solubilized pepsin extraction see Fig. 2. Using these two methods, the yield, chemical composition and characteristics of the collagen extract differ from each other. The entire extraction phase is carried out at 4 ◦ C. When

collagen extraction is performed using only acid, the product is called acid soluble collagen (ASC). For the extraction of collagen from marine animal tissues, acetic acid is the most used, dilute acid is used (generally at the final concentration of 0.5 M), but also citric acid and lactic acid [7].

About 95% of marine invertebrates such as jellyfish are made up of water, which affects collagen soluble in acetic acid. Therefore, homogenization or lyophilization of jellyfish is necessary to improve the solubility of collagen in dilute acids and consequently increase the extraction yield.

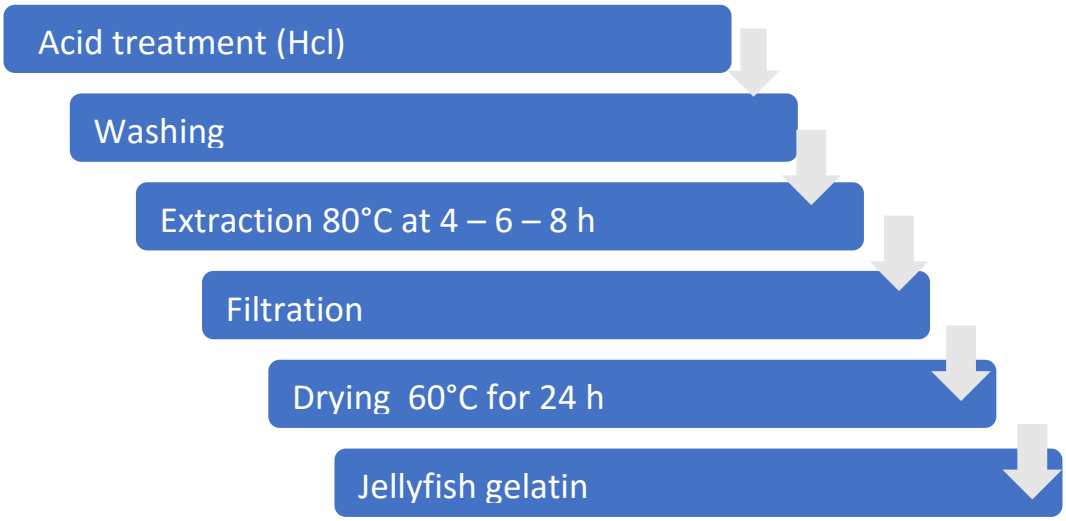


Figure 2 Jellyfish gelatin extraction

Recently, it proposed a new method to extract colagen from aquatic animals, in which acid treatment is combined with a sequence of physical and mechanical treatment, including pH adjustments, homogenization, mixing, and sonication [8]. By increasing physical intervention in jellyfish, the extraction yield increased significantly compared to conventional extraction processes [9].

When the enzyme pepsin is added to the extraction process, the extracted collagen is referred to as pepsin-soluble collagen (PSC). This treatment is very useful because proteases cleave the telopeptide of the cross-linked region without breaking the integrity of the triple helix and thus hydrolyze some non-collagenous proteins and increase the purity of collagen [10]. In most cases, enzymes are used to obtain specific protein products, high yield and reduced waste, as well as a decrease in antigenicity caused by telopeptides [11]. However, when a large amount of pepsin is used for a long time, the yield of PSC may be lower because the collagen is probably cleaved, affecting the integrity of the triple helix. [12].

During the recovery step, collagen is precipitated, generally by adding NaCl to a final concentration of 2.3–2.6 M. The precipitate is collected by centrifugation, dissolved in 0.5 acetic acid, dialyzed, and lyophilized [12].

From jellyfish, collagen is generally extracted by a methodology based on solubilization in 0.5 M acetic acid solution (usually for three days), followed by dialysis against a Na2HPO4 solution. The precipitated collagen is separated by centrifugation, solubilized in acetic acid, and purified by reprecipitation by adding solid NaCl to a concentration of 0.9 M. ASC can also be digested with pepsin to obtain atelo-collagen [12].

Results and Discussions

Pepsin-soluble collagen treated with ultrasound for 15 minutes showed the highest recovery yield (23.8%) as well as the highest amino acid content (18.2%) [13].

Ultrasonic extraction is an efficient and rapid technique to produce collagen from jellyfish in large quantities. Advantages of ultrasound collagen extraction are:

- obtaining food or pharmaceutical products with a high percentage of collagen
- high molecular weight collagen
- good extraction yields
- preserving the composition of amino acids
- fast processing
- easy operation

Ultrasonic extraction can be used in combination with various acid solutions to release acid-soluble collagen (ASC) from jellyfish. Ultrasonic cavitation promotes mass transfer between the jellyfish substrate and the acid solution through cellular structural disruption and acidification of the substrate. Thus, collagen as well as other targeted proteins are transferred into the liquid. In a subsequent step, the remaining jellyfish substrate is treated with enzymes (eg, pepsin) under sonication to isolate pepsin-soluble collagen (PSC) [10].

Sonication is recognized for its ability to increase enzyme activity. This effect is based on ultrasonic dispersion and agglomeration of pepsin aggregates. Homogeneously dispersed enzymes provide an increased surface area for mass transfer, which is correlated with higher enzyme activity. In addition, powerful ultrasonic waves open collagen fibers, so collagen is released see Fig. 3. Research has shown that an ultrasonically assisted enzymatic extraction (with the addition of pepsin) gives higher yields and an extraction process with a shorter execution time [13].

Figure 3 UIP4000hdT (4 kW) ultrasonic extraction system

https://www.hielscher.com/ro/ultrasonic-collagen-extraction-from-jellyfish.htm

In order to use salted jellyfish-derived products as raw material, the presence of 20-25% [13] residual salt from the salting process must first be removed. Traditional washing by desalination with water several times and overnight soaking is required before using it for food [12]. This takes a lot of work and time. Washing machines have been used to reduce the time, but the washing process generates a massive amount of waste water [13]. Research has shown that ultrasound can be used to desalt proteins. The application of ultrasound and microwave pretreatment in the desalination of salted egg white or duck proteins can reduce the salt content from 7.80 to 0.62% [13].

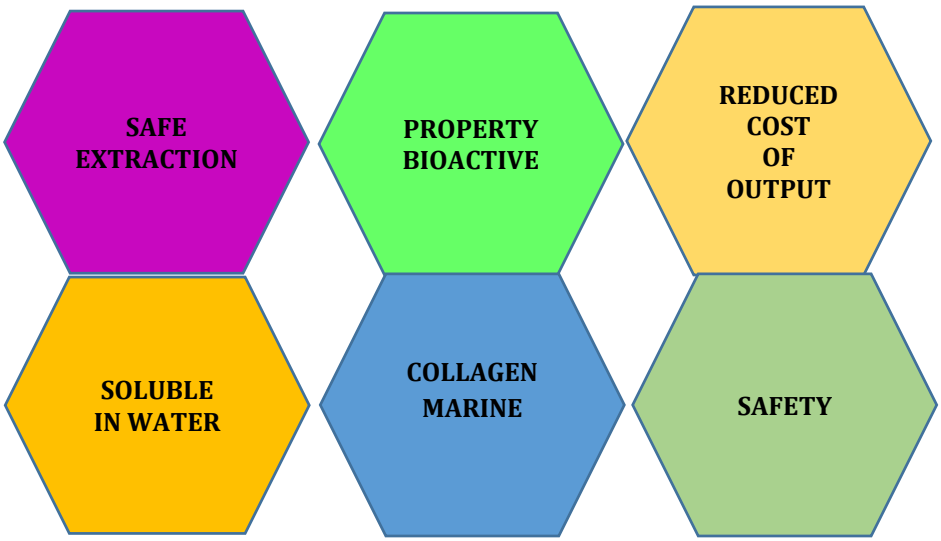
Nowadays, ultrasound, a non-thermal process that generates a sound wave at a frequency of 20-100 kHz, can be applied in food processing, such as in homogenization, emulsification, extraction, crystallization, degassing, marinating or cleaning. Ultrasound has also been investigated for pesticides, mycotoxins, heavy metals and allergen removal [13].

The frequency of ultrasound produces cavitation, crushing, vibration, mixing and heating, thereby reaching the mass transfer to induce the rapid collapse of bubbles and produce shear forces to break covalent bonds in materials [13]. Apply this green technology, ultrasound could be an alternative for removing the remaining salts in salty jellyfish by-products. However, no application of the ultrasonic method for desalination of such a jellyfish by-product sample has been reported.

The structure of type I collagen was observed in the SDS-PAGE pattern of collagens and no structural change occurred during the extraction process. The presence of the helical structure in collagen samples was confirmed by UV and FTIR spectra. The ultrasound cavitation advanced the viscosity of collagens and resulted in excellent solubility in acidic environments and lower salt concentrations.

Ultrasound-treated samples suggested superior water holding capacity along with emulsifying, and foam attributes. The improved gel strength of collagens was parallel with increasing the time of ultrasound up to 15 min. Free radicals scavenging ability and ferric reducing power of collagens were positively stimulated by increasing the time of the ultrasound up to 15 min[13]. Regarding the study it contributes to the development of green technology and the promotion of by-product utilization for collagen recovery as a potential practical protein in biomaterial, wound dressing, drug delivery, food, and cosmetics products[14].

Figure 4 The qualities of marine collagen



Applications of collagen extracted from different species of jellyfish

Jellyfish collagen is an important source of antioxidants. Recently, it was shown that peptide fractions from R. pulmo collagen were able to prevent oxidative stress, collagen peptides exhibit antioxidant and antifatigue activity, they were also identified in R. esculentum [15], as well as collagen hydrolyzate with several activities (including superoxide anion scavenging and melanogenesis inhibition activities) based on the ability of the hydrolyzate to chelate copper by inhibiting intracellular tyrosinase activity [16].

In the case of both jellyfish, it was found that collagen and its hydrolyzate function as protectors against UV radiation, suggesting their possibilities for use in skin care industries [17]. Similarly, collagen peptides from S. meleagris have been shown to be an effective tyrosinase inhibitor by acting on glutathione (GSH) levels [18].

According to recent studies, type II collagen extracted from Rhizostoma pulmo has been used to develop a collagen-based biomaterial. It was implemented using nanoreservoirs containing the growth factor TGF-3 and human stem cells, building a new adaptive device for articular cartilage repair [4].

The immunostimulatory effect of N. nomurai collagen stimulated immunoglobulin and cytokine production, not only in the specific human hybridoma cell line HB4C5, but also in the peripheral blood lymphocyte (PBL) line. In addition to these effects, tumor necrosis factor (TNF) and interferon (IFN) levels were increased in PBL [19].

Thrombosis and hypertension are among the leading causes of cardiovascular disease-related death [20], so research for new treatments remains an active field. In this case, Rhizostoma pulmo collagen was used to fabricate a sensor suitable for the clinical detection of thrombin in blood. Collagen was cross-linked to the designed thrombin amine aptamer using glutaraldehyde.

This hybrid sensor displayed a detection limit of 6.25 nM, largely below the imposed clinical limits, suggesting an interesting future involvement of collagen as a promising candidate for clinical thrombin analysis [20].

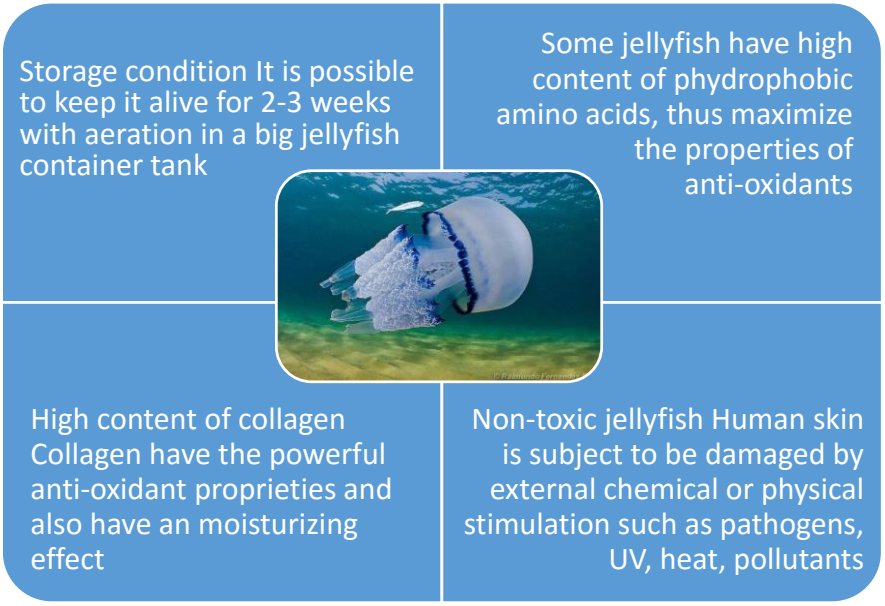


Figure 5 The advantages of collagen extracted from jellyfish

Conclusions

The contemporary world is faced with the urgent need to find alternative, sustainable and ecological resources due to overexploitation of land resources and waste disposal problems [21]. Today, people are living longer than their ancestors, which means they need more support from the medical, pharmaceutical, nutraceutical and biomedical systems to increase people's quality of life and longevity. Collagen from marine sources such as that extracted from jellyfish avoids major problems arising from cultural practices and religious beliefs, which may limit the use of bovine and porcine products by some consumers and in certain parts of the world [22].

Jellyfish collagen is a source of a great number of antioxidants. Recently, it was demonstrated that peptide fractions from *R. pulmo* collagen were able to prevent oxidative stress in HEKa cells treated with H2O2 [23].

Collagen has several applications in different fields, including nutraceuticals, cosmeceuticals, biomedicals, biomaterials and the food industry. Such a large variety of applications means that collagen can be key for the health and well-being of humans. To date, the sources of collagen mainly relied on terrestrial organisms, but they are becoming limited due to the spread of diseases and increasing alternative dietary choices of humans. This review highlights how marine organisms and their wastes can be a sustainable, eco-friendly source of collagen for the applications aforementioned.

Currently, collagen has become a necessary ingredient toward the healthy food development. The production of collagen in the body decreases with age and with an unhealthy diet. As a result, collagen has been added to a variety of foods [24]. Collagens are usually used as food additives to improve the rheological properties and reduce the fat consumption.

Collagens are used also to ensure the presence of adequate amount of animal nutritive fibers [25]. Collagen-based edible films and coatings have already been proposed to protect, maintain and extend the shelf life of different food products. The film or coating acts, in this case, as a barrier layer against the migration of oxygen, moisture and solutes, providing structural integrity and vapor permeability to the food product [26]. Moreover, it prevents fat oxidation, discoloration, microbial growth and preserves the sensory qualities.

In conclusion, research in recent years has focused on marine organisms in order to find new sources, alternatives to those already known and that can be exploited to the maximum with low energy consumption, labor and pollution. Over the past 20 years, more than 28 natural products and 175 chemicals have been found in marine entities, and hundreds of new compounds are still being discovered each year, likely due to advances in collection techniques and molecular biology [26]. To date, there are seven drugs of marine origin approved for clinical use and approximately 26 natural products in phase I to phase III clinical trials [27].

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Brown Seaweeds from Black Sea Coast as an Important Source of Bioactive Compounds of Interest for Human Health

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Abstract

For human nutrition, algae are important organisms that can offer essential compounds and new bioactive substances with pharmaceutical and medicinal value. Macroalgae contain nutritional elements such as lipids, proteins, vitamins and minerals and they are used as food supplements because they are known to be high in mineral content. There are a lot of nutritionally compunds that can be identified in algae such as polysaccharides, polyphenols, diterpenes, sterols, carbohydrates, peptides, polyunsaturated fatty acids, pigments and dietary fibers. Nutraceuticals are confirmed to be used as medicines because they are a good protector against chronic diseases and they have physiological benefits. Algae bioactive compounds could be used as an anticoagulant, antioxidant, anti-inflammatory, antibacterial, antimicrobial, antifungal, anticancer, antiviral, antidiabetic, antiobesity, antihypertensive and hypercholesterolemic nutraceuticals. Although only some of the algae have been studied properly for their chemical composition and properties, they are a considerable biological resource with ability for use as a raw material. In this paper the compounds from red and brown algae from the Romanian seaside are evaluated as sources of biologically active ingredients with benefits in human health.

Keywords: brown algae, antioxidant, Cystoseira barbata, polysaccharides, dietary fibers, fucoidan.

Introduction

One of the most popular organisms that can be found on earth are the algae. They have applications in agricultural, cosmetic and pharmaceutical industries because they have a wide variety of bioactive compounds and they have a rich chemical

composition [1]. Corresponding to many studies, a lot of bioactive compounds extracted from algae such as PUFAs, polysaccharides, peptides, pigments, carbohydrates, polyphenols, vitamins and phytosterols have implicated much interest in recent years for the significant chemical and biological diversity [2]. Anti-inflammatory, antimicrobial, immunostimulatory and immunomodulatory are some of the properties that these compounds had recorded. Both the recent studies about their benefical properties in human health and according to their nutritional composition, has highlighted the growing interest to incorporate algae into human diet [3, 4]. The Black Sea is known to be one of the richest seas with an impressive fauna containing green, red and brown seaweeds, which have not been fully studied for their properties and for their bioactive compounds. For hundreds of years the brown algae have been used by humans. In the recent studies it was highlighted that brown algae are very important sources of bioactive compounds with great nutritional value and they could be considered as functional foods with health benefits [5]. *Cystoseira* genus includes approximately 294 species [6]. *Cystoseira barbata* (Phylum: *Ochrophyta*, Class: *Phaeophyceae*, Order: *Fucales*, Family: *Sargassaceae*) is a genus of brown algae that can be found in the Romanian seacoast of the Black Sea. This brownalgae is defined by apical regions and vastly differentiated basal and catenate pneumatocysts are present too. In time the principal laterals come to be proportionally elongated, but the old plants have an elongated main axis. They have strong flattened lower parts into 'foliar-expansions' or basal leaves. Receptacles are the fertile parts which carry conceptacles and these can be normally situated at the tips of the branches. The apical and basal regions are notably differentiated. This brown seaweed can float in strong currents due to the aerocyst or air vesicles which keep the organism erect [7]. After the investigation of other members of the genus, *Cystoseira* has been discovered to include many biological activities such as: antiprotozoal, antibiotics, anti-inflammatory, antioxidant and cytotoxic [8]. An important source of polysaccharides is represented by the algae, notably the brown ones. The polysaccharides can be found commonly in nature, and these are omnipresent biopolymers. These can be determined in different types. Because of the structural differences, they can have distinct physical and chemical properties. Polysaccharides are nontoxic, biodegradable biopolymers. The sulfated polysaccharides are another essential group. The brown algae are also rich in soluble and insoluble polysaccharides, known as dietary fibres. The soluble polysaccharides that can be found in the brown algae are, fucans, alginates and laminarins. The sulfated polysaccharide that is principally composed of fucose interconnescted by β (1,3) glycoside bonds, alternating β (1,4) and β (1,3) bonds and rarely β (1,2) bonds, is the fucoidan. Its sulfate content is situated between 5% and 38 and it can contain, apart from fucose, other monosaccharides, including xylose, galactose, mannose, glucose, uronic acids and rhamnose [9]. The studies have demonstrated that fucoidan presents biological properties such as anti-inflammatory, antioxidant, liver protection [10] and antitumor [11] and it continues to be the most researched algae molecule. It has an important potential in the production of cosmeticals, functional

foods and pharmaceutical products. A small glucan obtained from the brown algae is the laminaran, which has a molecular weight of 1–10 kDa [12]. The two types that have been defined are the one with the chains terminated by D-glucose residues (G-series) and the other one with the chains that are terminated by D-mannitol residues (M-series). These laminarans have various advantages like biodegradability, low cellular toxicity and high biocompatibility [13].

Algal Material

The algal biomass, see Fig.1 that was collected manually has to be exposed to a pretreatment which is represented by repeated washings with drinking water and in the end with distilled water. On the fresh product only the macroscopic and the microscopic examinations can be performed [14]. For other types of examinations, the marine material has to be dried at temperatures between 25-35 degrees [15]. After that, the dry algal biomass has to be grounded to a powder and to use a sieve of 0.5 mm in order to obtain an uniform powder. May-november is the period that the algal flora was collected from the Black Sea coastline, from water at a distance of 5-25 m from the shore, from the areas of Constinesti, Mangalia, Eforie Sud, Eforie Nord, Navodari, Vama Veche and Constanta Casino.

Fig. 1. Brown algae in algal colony

Macroscopic and microscopic examinations

Macroscopic and microscopic examinations have to be applied on the algae selected for analysis. The first step in the research of the known or untested products was represented by the macroscopic examination [16]. In order to notice its dimension, appereance, taste, colour and smell, this part to be done through the whole plant examination (phylloid, rhizoid, celluloid) with the human eye, as well as with a magnifying glass [17]. The materials that are used in this examination are fragments of thallus from the red seaweed, a Microscope and Micros photomicroscope (10/0.25), forceps, blades, spatulate needles and Petri dishes. The microscopic exam

of the collected algae was realized directly on the fresh thallus fragments which were sliced, because the macrophytic algae species have single-cell or two-layer thallus and brought into a Petri dish with distilled water [18].

Chemical composition determination

Determination of humidity and ash - The humidity was determined in the termoregulator oven by drying the algae at 105 °C. After 12 hours was done, the calculation of the algae calcination at 550 ± 10 °C was performed. The organic substance was calculated by the difference between 100% and the sum of the values (%) of humidity and ash [19].

Determination of the sulfate content – Quantitive chemical analysis was used to determine the sulfate content. According to the national standard STAS 3069-87, the analysis for the determination of sulfates was realized spectrophotometrically [18].

Determination of protein and total nitrogen – Using the UdK DK6 digestor equipment was determined the total nitrogen and protein content of the algae, using the Kjeldahl method. In the presence of sulfuric acid under the catalytic action of selenium and mercury the mineralization was done. Then the alkalization was realized. After that, the ammonia was steam driven and captured in a boric acid solution which was titrated with hydrochloric acid. The result was expressed as a percentage [20].

Determination of lipids – The Soxhlet method was used to determine the lipids from the algae, an extraction method for 5 hours with dichloromethane as solvent. Gravimetrically the lipid content was determined and the results were expressed as a percentage [21].

Determination of carbohydrates – Using 15% acetic acid solution, the carbohydrate extraction was completed. With the Dubois method (1956), at a 190 nm maximum absorption, the carbohydrate content was determined spectrophotometrically. An Aquamate 8000 UV-VIS spectrophotometer was used, which uses spectral bandwidth of 1.8 nm and selectable wavelengths of 190-1100 nm. The results were calculated based on a standard glucose calibration curve [19].

Determination of total dietary fiber – Hipsley used the term "dietary fiber" to involve indigestible constituents of plants that compose the cell wall of plants, known to include hemicellulose, cellulose and lignin. Based on resistance and edibility to digestion in the human small intestine, the definition of dietary fiber, which has been extended by Trowell et al. became principally a physiological definition. In the definition are included indigestible polysaccharides such as modified cellulose, gums, mucilages and pectin and non-digestible oligosaccharides. The method used was the official method AOAC 985.29 "Total dietary fiber in food and the enzymatic-gravimetric method" [20]. The method was extended to allow total measurement of insoluble and soluble fiber in foods (AOAC official method 991.43) and various other modified fiber methods approved by AOAC International [20, 22].

Identification of phenol acids by HPLC

The method that was used to identify the phenol acid was HPLC-DAD. According to the method that was defined by Goupy [23], the HPLC analysis of phenolic compounds was determined in the ethanolic extract of marine brown algae. Using high performance liquid chromatography, the detection was conducted. That was equipped with auto sampler, quaternary pump, auto sampler, multi wavelength detector sett 280nm, 330nm and column compartment set at 35°C. Chem 32 integrator computed and monitored automatically the peak areas and the retention times. The retention time and standard deviations of phenolic standards chosen were: protocatechuic acid (3.130±0.008), pyrogallol acid (0.910±0.025), ferulic acid (8.865 ± 0.06), 4-amino-benzoic acid (3.455±0.005), chlorogenic acid (3.501±0.015), gallic acid (0.990±0.03), vanillic acid (6.919± 0.05), caffeic acid (8.281±0.07), benzoic acid (9.468±0.098), salicylic acid (15.952±0.051) and elagic acid (15.303±0.03), and for which a 0.05 mg/mL concentration was settled.

Extraction of sulfated polysaccharides

Using the method of Ermakova et al. with few modifications, the separation and isolation of water-soluble polysaccharides were realized. Under constant stirring (250 rpm) for 24h at 30 °C, the powdered algal biomass was treated with acetone–methanol (7:3, 500 ml), twice and chloroform (300 ml), twice. Under constant stirring (250 rpm) for 2h at 60 °C, the dried and defatted algal biomass was extracted twice with 0.1 M HCl (500 ml). Using two volumes of absolute ethanol, the supernatant obtained by centrifugation for 10 minutes at 5000 rpm was concentrated and precipitated [24]. The pellet was dissolved in distilled water after centrifugation for 10 minutes at 8000 rpm and 4 °C, dialyzed (cut-off 12–14 kDa) and lyophilized to yield the *C. barbata* sulfated polysaccharides.

Results and discussions

Macroscopic and microscopic examinations

This examination shows that *Cystoseira Barbata* is a large brown monoic alga, 1.5 – 2 m, which grows in the Black Sea on a rocky substratum. It has a thalle with various ramifications, fixed on rocks through a rhizoid as a disclike clip bolt. A lot of cilindric sticks stem from the clip bolt, on which a big number of primary and secundary branches are being developed, flat or cilindric, along which chains of air-bearing vesicles. Shaped as a conic cilinder or as a cilinder, on the top of the branches the receptacles develop. The conceptacles are situated in the receptacles, on the bottom of which various eggs are found, each with a pedicel cell and a single eggsphere. A new plant can form when the eggspheres, eliminated into sea water, merge with the anteroids. The microscopic analysis of the thalli of the brown algae taken in the study showed that *Cystoseira barbata* has a thali consisting of a single layer of cells. In *Cystoseira barbata*, the cells are elongated, arranged in longitudinal rows, they have a membrane on the outside that contains a small amount of cellulose. Inside the cell

there is a nucleus, numerous brown chromatophores with pyrenoids and numerous vacuoles.

Result for the chemical composition determination

The chemical composition of *C. barbata* seaweed is shown in products and affects stability of food materials. The moisture content of C. barbata was 14.45% and the ash content of the brown algae C. barbata was 16.3%. Comparable ash contents were reported by Frikha et al. [25] for C. barbata (14.24%) and by Marinho-Soriano et al. [26] for Sargassum vulgare (14.2%). Generally, the brown seaweed has a higher ash content than red and green algae [27]. C. barbata revealed a total protein amount of 17.9%, which was higher than other seaweeds determination [28]. C. barbata exposed a high level of dietary fibers (TDF= 59.875%), that is why algae are known as a great source of polysaccharides, which could signify a high level of soluble and insoluble dietary fibres. This percentage of dietary fiber from C. barbata was higher than the one recorded for the brown seaweeds Fucus vesiculosus (50.09%), B. bifurcata (37.42%), Undaria pinnatifida (33.58%), and H. elongata (37.14%), L. digi- S. Sellimi et al [32] [33]. The content of lipids present in this brown algae was reltively low, but Himanthalia elongata (L.), L. saccharina, Mastocarpus stellatus and Gigartina pistillata seaweeds showed significanly lower contents of lipids (0.3-0.9%) [28]. In Tabel 1 are presented all the results for this determination. The seaweeds are still an important source of health promoting PUFAs compared with plants and animals, even though they have a total content of lipids generally low.

Table 1. Chemical Composition of algae *Cystoseira barbata* from Romanian Black Sea coast

Parameters	Cystoseira barbata	Literature data values
Ash%	16.3±1.73	12.4-29.9
Moisture%	12.3±0.42	12.6-18.5
Total nitrogen%	2.765±0.34	-
Sulphates%	70.91±1.94	65.3-70.5
Lipid%	1.65±0.54	1.5-3.6
Protein%	17.9±2.11	8-17
Carbohydrate%	59.9±1.06	59.1-61.5
Total dietary fiber%	59.875±1.66	50.3-60.5
Soluble fiber%	30.3±1.33	27.2-30.5
Insoluble fiber%	28.76±1.26	24.2-32.6

HPLC analysis of phenol compounds

The phenol acids in the extracts were identified by HPLC- DAD. In Table 2 are presented the individual phenol concentrations from Cystoseira barbata, determined by HPLC – DAD and they are expressed in mg/100 g f.w. The highest quantity that

Cystoseira barbata from the Romanian Black Sea coast contains is the vanillic acid (99.5 mg/100 g f.w.), followed by benzoic acid (65.7 mg/100 g f.w.) and feluric acid (54.5 mg/100 g f.w.).

Table 2. The individual phenol concentrations determined by HPLC - DAD in brown seaweed from the Romanian Black Sea coast [8]

Type of acid	Mean Value±SD mg/100 g.f.w.	Percentage
Pyrogallol Acid	4.2±0.05	1.10
Protocatechiuc Acid	7.12±0.01	1.85
Gallic Acid	3.5±0.03	0.91
4-Amino-benzoic Acid	5.2±0.09	1.35
p-Hydroxy-benzoic Acid	26.9±0.06	6.97
Chlorogenic Acid	5.3±0.05	1.37
Caffeic Acid	21.2±0.06	5.49
Vanilic Acid	99.5±0.08	25.8
Benzoic Acid	65.7±0.06	17
Feluric Acid	54.5±0.01	14.13
Ellagic Acid	5.6±0.02	1.45
Salicylic Acid	10.5±0.03	2.72
Total	309.22	80.14

The smallest quantities were for 4-aminobenzoic acid (5.2±0.09 mg/100 g f.w.), pyrogallol acid (4.2±0.05 mg/100 g f.w.) and gallic acid (3.5±0.03 mg/100 g f.w.). From the total phenolic content identified, the phenolic acid from hydroethanolic extract of the brown algae, quantified by HPLC-DAD, was 80.14%. Salicylic acid (10.5 mg/100 b f.w.), caffeine (21.2 mg/100g f.w.) and p-hydroxybenzoic acid (26.9 mg/100 g f.w.) were another important compounds that were identified. The remainder phenol acids which were identified and quantified through HPLC-DAD, were found in smaller amounts.

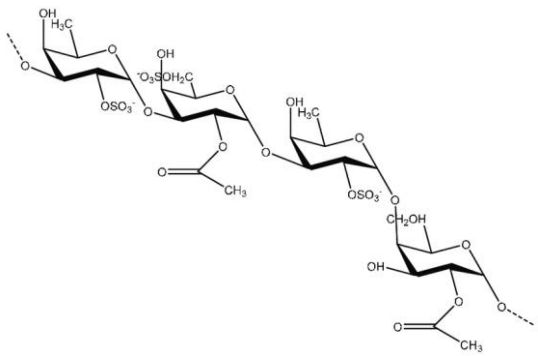


Fig. 2. The chemical structure of fucoidan

Results for the sulfate polysaccharides extraction

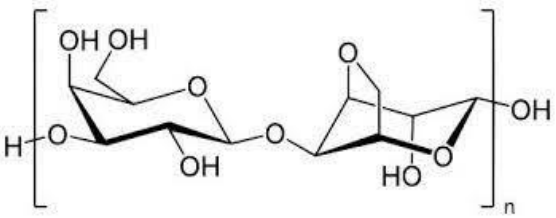
Fucoidans

Fucoidans are sulfated polysaccharides that can be determined in brown algae, see Fig.2. Even if most of them are essentialy made of sulfated α -L-fucose residues, they may also contain xylose, mannose, galactose, uronic acids and acetyl groups, sometimes in substantial amounts. In other algae or terrestrial plants such polysaccharides are absent [30]. Algal fucoidans are a virtually non-toxic bioactive polymers and they can be found in natural sources in high amounts [31]. The most interesting property is their heparin-like anticoagulant and antithrombotic activity, but many other activities, such as anti-inflammatory, antiviral, antitumor, antiadhesive, antiangiogenic, etc., are promising for new drug design [32]. Depending on the species, the harvest period and the part of the thallus, fucoidans usually constitute about 5–10% of dry seaweed biomass [33]. By extraction of algal biomass followed by oxidative decoloration and determination of this sugar with specific color reaction for 6-deoxyhexoses or by using GLC after acid hydrolysis, the fucoidan content may be evaluated by determination of L-fucose. Their biological activity is dependent on anatomical part of the algae, species, extraction procedures, growing conditions and locations and analytical methods and each of these properties is associated with a specific fucoidan [34].

Laminarans

Polysaccharides that are commonly distributed in nature are the one built up predominantly of 3-linked β -D glucopyranose residues [35]. Due to the ordered triple helical molecular conformation, high molecular weight glucans of this type are insoluble in water and therefore serve as structural components, for example, in fungal cell walls. Laminarans are known as similar glucans of lower molecular mass function as storage polysaccharides in brown algae, see Fig.3. Their content can be as high as about 35% of dry biomass in the algae and this percentage is dependent on the environmental conditions and the species. Percival and McDowell monograph reviewed the results of structural investigations of laminarans by classical chemical methods. Containing a backbone of about 20–30 (1 → 3)-linked β -D-glucopyranose residues with some single β -D-glucopyranose stubs attached to the main chain by (1 → 6)-linkages and differing slightly in the degree of branching, polysaccharides from different species were shown to be very similar. The two types that have been defined are the one with the chains terminated by D-glucose residues (G-series) and the other one with the chains that are terminated by D-mannitol residues (M-series). The biological activity can be modified or enhanced by the chemical modification of laminarans. In this way, it has been demonstrated that laminar sulfate causes several plant defense mechanisms and inhibits the proliferation of endothelial cells. Sulfated alkyl laminara-oligosaccharides have potent inhibitory effects on AIDS virus infection [36].

Fig. 3. The chemical structure of laminaran



Biodegradability, low cellular toxicity and high biocompatibility are some of the advantages of the laminarans [37]. The bioactivities that have been identified for the laminarans were anti-inflammatory, anti-apoptotic [38], antitumor, immunoregulatory, antioxidant activities and anticoagulant [39].

Alginic acid and alginates

A polysaccharide composed of two different uronic acids, glucuronic and mannuronic, are one of the principal active compounds of edible brown algae. They act like prebiotics and as potential immunomodulators, supporting the production of short chain fatty acids, because the alginates can not be digested by human enzymes [40]. The alginates have been used for the treatment of gastric reflux, stomach ulcers and heatburn and also as tablet excipients. The absorption, swelling and hemostatic properties of alginates are involved in their mode of action against such health conditions [41].

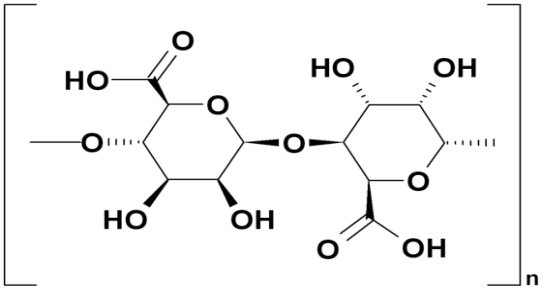


Fig. 4. The chemical structure of the alginic acid

These characteristics also support the use of alginate in wound treatment. They have been linked to many other health effects and their absorption and swelling characteristics. For example, binding of glucose and α-amylase inhibition, which reduce post-prandial glucose levels. Alginates have the property of reducing the level of cholesterol and the level of lipids, because they have the ability to absorb bile acids and lipids. Alginic acids are linear copolymers of two linked uronic acid residues (1 → 4), α-L-guluronic (G) and β-D-mannuronic (M) acids, see Fig.4. They are present as components in the intercellular matrix and cell walls in all known brown algae in the

form of salts mixed with several cations such as Na + , K + , Mg 2+ and Ca 2+ [42]. The polymer is extracted in the form of easily soluble sodium salt by treatment at high temperature with sodium carbonate solution, the algal biomass being treated beforehand with dilute acid to remove the polyvalent cations [43]. Sodium alginate can be precipitated with ethanol, insoluble calcium alginate can be obtained by adding calcium chloride and alginic acids are generally precipitated after acidification of the extract. The alginate content of algae can reach up to 40% of the dry algal biomass and depends on the part of the thallus, the harvest period and the species [44].

Conclusions

Algal biodiversity produces a vast resource of different polysaccharides. Some have been characterized by chemical and physical methods of structural analysis and have found a wide practical application, such as agar-agar and carrageenans from red algae and alginates from brown algae. Several chemical and enzymatic procedures have been developed to improve their practically valuable properties, therefore mariculture seems to be the main source of raw materials for the production of these polysaccharides in the future. This is true even for fucoidans from brown algae, which are the most intensively studied group of biologically active polysaccharides. Their biological activity depends on the composition of monosaccharides, degree of sulfation, conformation and fine structure of polysaccharides. For a high anticoagulant activity of sulfated fucans, the appropriate spatial arrangement of the sulfate groups is necessary, and also the distribution of the molecular mass has a particular importance on the biological properties of fucoidans. The results of this study indicate that Black Sea brown seaweed species *C. barbata* may be a good source of fucoidan. The data also confirmed the long known facts that brown algae cell wall PS are complex, and that their yield and chemical composition are significantly influenced by the algae species and the conditions used to extract them.

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