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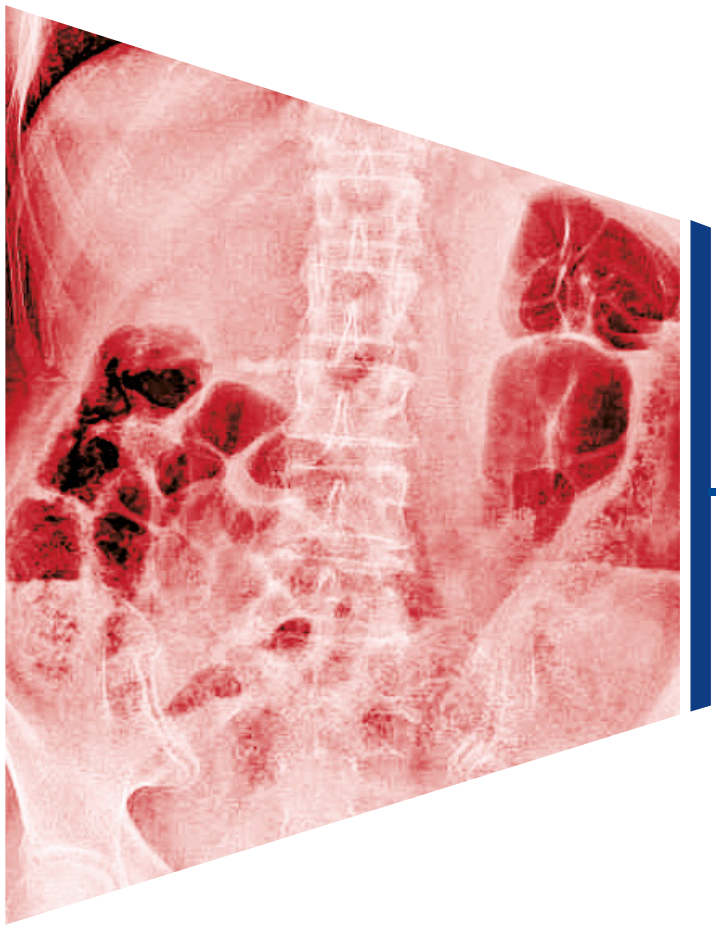

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EUROPEAN JOURNAL OF TRANSLATIONAL
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COMMENTARY

Commentary: The King is Naked – a very subjective look at child and adolescent psychiatry

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Abstract

There is a great crisis in child-adolescent psychiatry, but we will not solve it just only by increasing the number of psychiatrists or psychiatric wards. The young patients described in Agnieszka Wlazło's editorial indeed should not end up in a psychiatry department, but that does not mean they do not need any institutional support at all. In this commentary I would like to add two simple but important remarks: we need a serious discussion of the consequences of childhood trauma and mental problems in adolescence and also a well-functioning prevention system to avoid them.

Keywords: Poland · healthcare · crisis · adverse childhood experiences · prevention

Citation

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Dear Editor,

I read with great interest the Invited Editorial by Agnieszka Wlazło in the latest issue of *European Journal of Translational and Clinical Medicine* [1]. As a psychiatrist who also works with children and adolescents, I share the main observations made by the Author. Indeed there is a great crisis in child-adolescent psychiatry, but as the Author rightly emphasizes, we will not solve it just by simply training more psychiatrists or increasing the number of psychiatric wards. The young patients described in Agnieszka Wlazło's editorial indeed should not end up in a psychiatry department, but that does not mean they do not need any institutional support at all. In this commentary I would like to add two simple but important remarks: we need a serious discussion of the consequences of childhood trauma and mental problems in adolescence and also a well-functioning prevention system to avoid them.

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When the prevalence of suicide attempts and the incidence of mental disorders increases nationalwide [2-3], this is a problem that goes far beyond the psychiatrists' offices. What does this say about the condition of our society and our way of life? Do we really know what we want to teach our children? Do we work in partnership with them to shape their future or do we just want them to be "who they should be"? One half of all serious adult psychiatric disorders start before the age of 14, however the treatment often does not begin until 6-23 years after onset [4]. Thus, child psychiatry is not limited to child issues, because problems that arise at this age often last long into adulthood. There is a term Adverse Childhood Experiences (ACEs) that includes child sexual abuse, physical abuse, verbal threats, living with alcoholic parents etc. A significant group of the patients we work with in child psychiatry clinics and departments have such history. Indeed, a systematic review on ACEs shows their correlation not only to several mental disorders and suicidal behavior but also to physical health consequences and risk factors: obesity, smoking. cardiovascular disease, chronic lung disease, headaches, autoimmune disease and sleep disturbances [5]. It has been suggested that ACEs can also be linked to an increased risk of premature death during adulthood [6].

A large prospective study of 1.420 participants observed from 1993 to 2015 showed that childhood depression has a wide variety of consequences in the subsequent stages of life [7]. The authors found that any episode of childhood/adolescent depression was associated with higher levels of adult anxiety and illicit drug disorders and also with worse health, criminal, and social functioning [7]. A depressed child is then very often an adult with depression. Depression in a parent contributes significantly to a child's emotional problems [8]. In this way, mental disorders seem to be passed down from generation to generation [8]. Therefore, waiting for the children to "grow out of" their problems (and if that does not happen, treating them) is the worst possible option.

Instead of taking this discussion seriously, we would simply like the child and adolescents suicidality crisis to end, psychiatric departments to end beds shortage and everything else to remain the same. It is a bit like the situation where the parents consult a child/adolescent psychiatrist because they wish to "fix the child" and yet refuse to take a closer look at the entire situation and their possible role in the roots of their child's problem/s.

Where is the problem then? The problem is that we don't have much to offer children and their parents to prevent ACEs and mental disorders. Unfortunately, due to frequent lack of other, non-medical forms of sufficient support, parents of a child who is "difficult" or from a "difficult background" receive a request from teachers at kindergarten or school "to go to a psychiatrist." What is missing between everyday life and medicine is a well-functioning social support system. It is easier to refer a child (and its parents) to a psychiatrist or psychologist to start treatment/therapy than to strengthen the systemic (e.g. social or educational) support for families and children in crisis. Many elements of such support are beyond the field of medicine e.g. increasing the number of family orphanages, providing more classes adapted to the needs of children with difficulties, further developing the system of early development support and re-thinking the system of foster care. One of the roles of schools should also be to help young people get to know themselves and their emotions, and teach them to cope with life's difficulties. In addition, it is impossible to talk about child psychiatry without the context of parents and guardians. Last but not least, support should apply not only to the child itself, but also to their caregivers. According to research, Polish parents are burned out more than those from other countries [9]. In a large analysis from 2018-2019, the average level of parental burnout in Poland was higher than in 40 other analyzed countries and very clearly correlated with the intensity of individualism, assessed as loosening of social ties and lack of social support [9].

These examples are as important, if not more important than increasing the number/availability of psychiatric services. In fact, we are already over-medicating even the youngest children, e.g. with antipsychotic drugs, which is also shown by Polish study [10]. As in other fields of medicine, prevention should be the key direction of child/adolescent psychiatry. The World Health Organization also devoted a lot of space to the prevention of mental disorders, with great emphasis on children and adolescents [11]. There is also growing evidence that a large proportion of mental disorders are preventable (e.g. depression, anxiety or substance abuse) but effective strategies are often neglected [12-13].

The reform of child and adolescent psychiatry is currently taking place in Poland and, at least in theory, it takes into account some of the above tenets [14]. The reform put a major emphasis on aid at the community level (e.g. psychologists and community therapists), directing strictly medical issues to higher levels of support when necessary. It also places emphasis on effective communication and cooperation between support levels [14]. The assumptions are important, but in practice, everything can break down at the level of financing from the state budget. It is definitely too early to judge its effectiveness, however it is worth keeping our fingers crossed.

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Conflicts of interest

The author declares no conflict of interest.

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SHORT COMMUNICATION

Use of antihistamines for COVID-19 vaccine recipients with risk of anaphylaxis

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Abstract

Allergic reactions to COVID-19 vaccine ranging from mild to severe have been reported in patients with a history of anaphylaxis. Currently, no guidelines are available regarding prevention of allergic reactions in patients with high-risk of anaphylaxis who plan on receiving the SARS-CoV-2 vaccine. In this case-series study, two patients with a history of anaphylaxis had taken antihistaminic drugs prior to their BNT162b2 vaccinations and experienced no major allergic reactions afterwards. The use of antihistamines prior to COVID-19 vaccination may have affected the outcome of the two subjects with history of anaphylaxis history. However, further studies are needed to evaluate efficacy, generalizability and safety of the approach presented in this case-series.

Keywords: vaccine · anaphylaxis · antihistamine · COVID-19

Citation

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Introduction

Rapid development of vaccines for the coronavirus disease (COVID-19) and a worldwide implementation of vaccination programs have created an opportunity to slow down the pandemic. Currently there are over 100 million confirmed cases of COVID-19 and over 2.5 million deaths globally with several countries entering their third wave of the pandemic [1]. However, several safety issues were noted since the first days of inoculation [2], including reports of anaphylactic shock [2-3]. Observed hypersensitivity reactions resulted in restrictions in the vaccine qualification programs for people predisposed to anaphylaxis. The Medicines and Healthcare products Regulatory Agency (MHRA) and the Food and Drug Administration (FDA) currently disqualify from vaccination people with a history of anaphylactic shock to any of the ingredients contained in COVID-19 vaccines [4-6]. On the contrary, there are no contraindications to inoculation of people with no history of allergic reaction to BNT162b2, mRNA-1273 or ChAdOx1-S vaccine components or severe allergic reaction to the first dose of COVID-19 vaccines [7-8]. Since mRNA vaccines are novel products, the exact mechanism or the root cause of the allergic reaction associated with them is unknown [9]. However, polyethylene glycol (PEG), the inactive ingredient of BNT162b2 and mRNA-1273 vaccines (added for improved mRNA bioavailability) has been associated with anaphylaxis in the past and may be a cause in the recently reported anaphylactic reactions [9-12] Our study examines the implementation of prophylactic doses of antihistaminic drugs for patients with high-risk of anaphylaxis undergoing COVID-19 vaccination. As of our knowledge, there are no current studies conducted on the prevention of anaphylactic responses from COVID-19 vaccines by the use of antihistaminic drugs.

Material and methods

We present two cases of medical professionals with history of severe allergic reactions, including anaphylactic shocks, caused by various factors. Both of them were vaccinated against COVID-19 by BNT162b2 while using an antihistamine drug as preventive measures. Consent to participate in the study was obtained as both subjects are also two co-authors of this paper.

Results

Case 1

A 39-year-old white male, a medical professional, was treated for hypertension with telmisartan (40 mg) and hydrochlorothiazide (12.5 mg). He had a history of multiple severe allergies reactions, including anaphylactic reactions to three different stimuli. History of allergy events included an allergy to penicillin, generalized urticaria of unknown origin and computer tomography (CT) contrast allergy. Allergic reaction to penicillin was observed once during infancy, consisted of diffuse erythematous rash and dyspnea. There is no further data regarding this reaction and penicillin was never administered again.

The patient also reported recurrent generalized urticaria (since the age of 24) with intense itching and blisters up to 3-4 cm in diameter. It occurs once a week to once a month, as a reaction to an unknown trigger (apart from borderline reaction to wheat and rye flour no allergen was identified during routine testing) with tendency to aggravate by distress, physical activity, alcohol use or sleep deprivation. These reactions usually alleviate after the use of antihistaminics (20-40 mg of cetirizine or loratadine). Occasionally severe urticaria was accompanied by mild dyspnea and required administration of intramuscular or intravenous steroids in Emergency Departments. During a single episode of urticaria in 2009, dyspnea and hypotension were observed which resulted in intramuscular administration of adrenaline with a full recovery.

In 2018 this patient developed an anaphylactic shock after intravenous administration of a contrast agent (100 ml of iomeprolum 300 mg/ml) during a CT. Symptoms included generalized erythematosus rash, dyspnea, hypotonia, weakness and confusion. The patient was immediately transported to the Emergency Department where he fully recovered after receiving 300 mg hydrocortisone, 2 mg clemastine and calcium intravenously.

Despite the history of allergic reactions, the patient was qualified for the BNT162b2 vaccination. On the day of the first dose of the vaccine he self-administered 20 mg loratadine (10 mg – 4h before, 10 mg – 2h before) and cetirizine (10 mg – 1h before) as a prophylaxis. Before the injection, his blood pressure was 140/90 mmHg. 15 min after the injection it dropped to 125/80 mmHg and the patient reported slight weakness and a rash limited to three itching lesions. Next, the patient took an additional dose of cetirizine (10 mg) resulting in full recovery. Apart from sedation and sleepiness which lasted for the next 24 hours and local pain in injection site, no additional adverse effects were noted.

Prior to receiving the second dose of the vaccine, the patient took similar prophylactic measures, although with a slightly increased dose (20 mg cetirizine 4 hours before the vaccination and additional 20 mg cetirizine 1 hour before). No local or generalized allergic reactions were observed after the second dose. Reported adverse effects were typical to BNT162b2 (muscle aches, fever for 1-2 days). No additional antiallergic agents were administered post-vaccination. One month follow-up revealed no long-term side-effects.

Case 2

A 54-year-old white male, medical professional with a history of a severe form of atopic dermatitis and a single episode of anaphylactic shock during the process of pollen desensitization. He reported that atopic dermatitis was diagnosed 8 years ago and it is now treated with 100 mg cyclosporine b.i.d. with increase in dosage to 150 mg b.i.d. for exacerbations, with additional use of mometasone and emollients locally. Allergies to several pollen (more severe to alder, birch, weeds, mugwort and cereals; mild to poplar and oak), mold and feline fur were noted since childhood. In 1996, attempts to desensitize to pollen were made but resulted in an anaphylactic reaction and were then discontinued. Symptoms included vertigo, confusion and hypertension. 500 mg hydrocortisone i.v. was administered and the patient recovered fully. Comorbidities included symptomatic epilepsy (probably due to arachnoid cyst) treated with levetiracetam 500 mg b.i.d. (no seizures for the last 7 years), bronchial asthma (only as a reaction to feline fur, not treated), hypertension (probably secondary to cyclosporine) treated with 5 mg amlodipine.

First and second doses of BNT162b2 were both preceded by administration of additional 5 mg desloratadine t.i.d. as prophylaxis. Regular treatment with usual doses of cyclosporine (100 mg b.i.d.) was continued. No allergic reactions were observed after either dose. Additional 5 mg desloratadine was administered a few hours after both inoculations. Adverse effects included only muscle aches, headache and general fatigue for half a day after the first dose of vaccination and muscle pain and mild fatigue after the second dose. Subject's blood pressure before and after vaccinations were within reference ranges. No additional anti-allergic agents were administered post vaccination. One month follow-up revealed no long-term side-effects.

Discussion

The two subjects in our case series both had a history of an anaphylactic reaction. A single center cohort study reported 19 patients who developed anaphylaxis to the COVID-19 vaccine. 31% of the 19 patients also had prior anaphylaxis but did not report using any prophylactic measures before their injections [13]. Anaphylactic response also appears to be more frequent among women. From December 14th to 23rd 2020, the Centers for Disease Control and Prevention (CDC) reported 21 patients (19 of them were female), who developed anaphylaxis from the BNT162b2 vaccine [2]. Similarly, the previously-mentioned single center study reported that 94% of their patients who developed anaphylaxis were females [13]. The subjects in our study were only males and had taken an antihistamine drug prior to their two doses of the vaccination and successfully reported no major allergic reactions after their exposure to both doses of BNT162b2 vaccine. Despite the fact that the patients did not experience anaphylaxis after vaccination, we do not know how the two subjects would have responded if no antihistaminic drug was taken prior to it. However, the mild allergic reaction noted in Case 1 may suggest that the reaction experienced would have been more severe without medications. Currently the CDC does not recommend the use of antihistamines prior to COVID-19 vaccination because the prophylactic use may mask cutaneous symptoms, which could lead to a delay in the diagnosis and management of anaphylaxis. The decision on whether to administer antihistamines prior to vaccination should made individually after assessing the risks and benefits of the patient. Large doses of antihistaminics were fairly safe in our cases, but further studies are needed to evaluate efficacy, generalizability and safety of this approach for people with anaphylaxis history.

Antihistamine agents remain the basis for prevention and treatment of allergies [14], but there are still no studies or guidelines regarding their use by people at risk of a severe allergic reaction from mRNA vaccinations. In regard to other vaccines, one case report found that the prophylactic use of loratadine along with prednisolone may have successfully prevented anaphylaxis to the subject who was injected with a purified chick embryo cell rabies vaccine (PCECV) [15]. However, additional studies on the prophylactic use of antihistamines for high-risk anaphylactic patients during any vaccination in general are limited.

Conclusions

The difficult access to the benefits of COVID-19 vaccination for people with a history of severe hypersensitivity reactions puts them at risk of coronavirus infection with all its possible complications. We believe that the steps towards fighting back against the pandemic by achieving herd immunity should be directed towards reduction of severe allergic reactions and decreasing the social hesitance to vaccinate.

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Conflicts of interest

The authors do not have an association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding).

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SHORT COMMUNICATION

SARS-CoV-2 infection in vaccinated maintenance hemodialysis patients despite anti-spike seroconversion: a report of 3 breakthrough cases

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Abstract

Chronically hemodialyzed (HD) patients are vulnerable population during a SARS-COV-2 pandemic. They are at high risk of developing very severe forms of COVID-19 disease. In this article we describe, for the first time to our knowledge, three HD patients (all males, aged 70, 70 and 74 years) vaccinated intramuscularly with two doses of the mRNA BNT162b2 vaccine (BionTech/Pfizer Comirnaty) in whom subsequent breakthrough SARS-CoV-2 infections developed. All patients achieved post-vaccine seroconversion for anti-spike antibodies with IgG titers of 227 AU/mL (cut-off, 13 UA/ml). SARS-CoV-2 infection was diagnosed 28, 44 and 48 days after the second dose of BNT162b2 and confirmed with the polymerase-chain-reaction (PCR) test. Two patients were asymptomatic of COVID-19 and didn't require hospitalization. The third patient reported only non-significant drop in oxygen saturation and was hospitalized. All patients were characterized by a moderate or even low post-vaccination neutralizing antibody titer but on the contrary a high production of these antibodies after infection. Perhaps this production of antibodies by memory B cells is responsible for the mild course of the disease and the likely

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reduction of mortality. These breakthrough cases in no way undermine the importance of the vaccinations and on the contrary, argue for their urgency.

Keywords: COVID-19 · hemodialysis · breakthrough cases

Citation

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Patients chronically hemodialyzed (HD) in-center are a unique and vulnerable population during a SARS-COV-2 pandemic. Due to the high rate of comorbidity, older age and impaired immunity, they are at high risk of developing very severe forms of COVID-19 disease with fatality rates varying from 16% to 32% [1-2]. Consequently, in most countries HD patients are prioritized to receive vaccines against COVID-19. Vaccinations follow the same schedule as in people without chronic kidney disease. Recently, we showed that the majority of dialyzed patients achieve high seroconversion rates after full vaccination with BNT162b2, with few and mild side effects [3-5]. On the other hand, *Grupper et al.* demonstrated that such vaccine seroconversion is definitely lower than that observed in the general population [6]. Therefore, it is uncertain whether vaccinating with standard schedules will result in sufficient immune response in this population and, by consequence, protection against infection.

In this report, we describe our experience with 3 breakthrough SARS-COV-2 cases in HD patients. Their characteristics are presented in table 1. They had received a two-dose vaccination with the mRNA BNT162b2 vaccine (BionTech/Pfizer Comirnaty) intramuscularly with a 3-week interval between the first and the second doses. All our patients were routinely monitored for anti-spike and anti-nucleocapsid (N)-specific antibody titer before the vaccination, after the first, and the second, dose of BNT162b2. The patients achieved post-vaccine seroconversion for anti-spike antibodies with IgG titers of 227 AU/mL (cut-off, 13 UA/mL); 452 AU/mL and 92.5 AU/mL, assessed 14-21 days after the second dose of BNT162b2 using chemiluminescent immunoassay (The LIAISON® SARS-CoV-2 Trimetric-S IgG, test Diasorin, Italy). SARS-CoV-2 infection was diagnosed 28, 44 and 48 days after the second dose of BNT162b2 and confirmed with the polymerase-chain-reaction (PCR) test. Viral genome sequencing performed in one patient revealed B.1.1.7 – currently the most common variant in Poland.

All three patients were male and suffered from diabetes, two of them had diabetic nephropathy as the underlying renal disease. The Charlson Comorbidity Index was high in all of them, and ranged from 9 to 12. The patients had negative computed tomography. Two patients did not have any symptoms of SARS-CoV-2 infection and did not require hospitalization. The third patient reported fever, fatigue, and a temporary, non-significant drop in oxygen saturation. He was the only one with an elevated inflammation marker (CRP) and was hospitalized. He received antiviral treatment with remdesivir (total dose 600 mg), dexamethasone (6 mg for 10 days), LMWH (enoxaparin 40 mg) and an antibiotic (ceftriaxone). The patient showed a quick improvement in his general condition and on the twentieth day of his hospitalization, he clinically recovered and was discharged home.

In terms of laboratory findings, all patients showed a high titer of anti-spike IgG antibodies, elevated d-dimer level and significantly increased NT pro-BNP, at the time of diagnosis. Fibrinogen level elevation was detected in two out of three patients and only one patient had lymphopenia (lymphocytes < 1.0 x 10^9/L). Oxygen therapy was not required in any of the patients. We observed SARS-CoV-2 clearance (PCR negative) in all patients within 4 weeks of infection. None of the patients had developed any serious complications, and no residual symptoms were observed in any of them.

To our knowledge, this is the first description of three fully vaccinated HD patients in whom subsequent breakthrough SARS-CoV-2 infections developed. It is noteworthy that the infection occurred in patients with confirmed vaccine seroconversion to spike protein. Previous studies demonstrated that sera from individuals vaccinated with BNT162b2, were similarly potent against the B.1.1.7 variant when compared to the reference D614G strain of virus [7]. Although rare, breakthrough infection can occur because vaccines against SARS-CoV-2 do not offer 100% protection according to the pivotal studies. In the recent study the

Table 1. Demographic, clinical, laboratory and radiological features

Variables	Case 1 (MR)	Case 2 (MP)	Case 3 (BF)
Age, yrs	70	70	74
Sex	Male	Male	Male
Body mass index, kg/m^2	31.1	27.7	39.7
Length of HD, months	65	30	23
Cause of kidney failure	ADPKD	DN	DN
Diabetes	Yes	Yes	Yes
Coronary artery disease	No	No	Yes
Charlson Comorbidity Index	9	10	12
Time of diagnosis after 2nd dose of vaccine (days)	44	28	48
Signs and symptoms			
Fever	No	No	Yes
Fatigue	No	No	Yes
Oxygen saturation	98%	93%	96%
Laboratory results			
Anti-S IgG after 1st BNT162b2 AU/ml * [a]	no data	4.18	6.52
Anti-S IgG after 2nd BNT162b2 AU/ml ** [a]	452	227	92.5
Anti-N after 1st and 2nd BNT162b2	negative	negative	negative
SARS-CoV-2 variant	no data	no data	B.1.1.7
Anti-S IgG at diagnosis infection AU/ml *** [a]	795	845	5770
Hemoglobin, g/dl (11.2-15.7)	11.4	8.9	12.7
Lymphocyte count, x109/L (1-3)	1.58	0.84	1.25
CRP, mg/L (0-5)	2.5	3.4	157
Troponin, ng/mL (0-0.014)	0.031	0.078	0.044
NT pro-BNP, pg/mL (0-125)	17556	44984	1623
ALT, U/L (0-33)	8	4	19

Fibrinogen, g/L (1.8-3.5)	5.2	3.49	6.1
D-dimer, ng/mL (0-500)	1571	564	521
Chest CT	**normal**	**normal**	**normal**

DN, diabetic Nephropathy; ADPKD, autosomal dominant polycystic kidney disease; * 21 days after the first dose of vaccine; ** 14-21 days after the second dose of vaccine; * in the first week after diagnosis of breakthrough infection; a - to convert AU/ml into BAU/ml multiple by 2.6**

breakthrough infection rate among 2916 fully vaccinated residents of skilled nursing facilities and staff members did not exceed 1%. Like our report, the majority of the cases were asymptomatic, the rest had a mild or moderate course [8]. Interestingly, all our patients were characterized by a moderate or even low post-vaccination neutralizing antibody titer but on the contrary a high production of these antibodies after becoming infected. Perhaps this production of antibodies by memory B cells is responsible for the mild course of the disease and the likely reduction of mortality. That's why these breakthrough cases in no way undermine the importance of the vaccinations and on the contrary argue for their urgency. However, the described cases in this report raise several new questions: (i) is the breakthrough infection due to the decreased immunity of HD patients or rather immune evasion of new variants, and their ability to create higher viral loads? [9]; what is the optimal vaccination schedule for HD patients? and what is the titer of neutralizing antibodies that protects patients against COVID-19? Clinical studies answering these questions should be a priority.

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Conflicts of interest

None.

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RESEARCH ARTICLE

The importance of obesity and carbohydrate metabolism disorders on the course of gastroesophageal reflux disease – a pilot study

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Abstract

Introduction: Carbohydrate metabolism disorders, obesity and a severe course of gastroesophageal reflux correlate with more frequent development of esophageal complications. The aim of this study was to assess the influence of obesity and carbohydrate disorders on the characteristics of gastroesophageal reflux disease (GERD). **Methods:** The study included 58 patients with excess weight. Anthropometric parameters (including the body mass index, BMI), data regarding GERD (severity of symptoms, gastroscopy and esophageal pH monitoring results) were included in the study. Correlations between obesity and GERD parameters were analyzed. Subjects were divided into a diabetic and a control group and the severity of GERD was compared. **Results:** GERD was diagnosed in 40 patients and occurred more frequently in the obese group (73%) than in the overweight group (57%). Increased GERD severity was associated with increased BMI only for postprandial parameters. GERD was diagnosed in most of the group with carbohydrate disorders (78% vs 63% in the non-diabetic group). No differences in the severity of GERD were observed between groups depending on carbohydrate disorders. **Conclusions:** In our study, GERD was common in obesity and in diabetic disorders. Increased severity of postprandial reflux was associated with an increased BMI. Diabetic disorders were not associated with more severe GERD.

Keywords: gastroesophageal reflux disease · obesity · diabetes mellitus

Citation

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Introduction

Factors linked to modern lifestyle, e.g. physical inactivity, overnutrition and poor sleep quality have led to the widespread incidence of obesity, type 2 diabetes (T2DM) and gastroesophageal reflux disease (GERD) [1-5]. In many countries obesity is an important public health problem and its prevalence results in an increase in the incidence of GERD and type 2 diabetes [6-8]. Gastroesophageal reflux disease is a common health problem which still requires research. Depending on the region, the prevalence of GERD is estimated at 10-20% of the population [2, 5]. Overweight, especially abdominal obesity, correlates with the severity of GERD. The main causes of GERD are impaired function of the lower esophageal sphincter, hiatal hernia, impaired esophageal motility and increased intra-abdominal pressure [9-18]. Gastroesophageal reflux disease leads to the development of Barrett's esophagus (BE) or metaplasia, which is a precancerous condition leading to the development of dysplasia and subsequently of esophageal adenocarcinoma (EAC) [4, 19-20]. Numerous studies have noted that not only the severity of GERD, but the presence of abdominal obesity and T2DM, increase the risk of developing BE and EAC [21-25].

In abdominal obesity, the development of BE and EAC plays a major role in the increased amount of adipokines and inflammatory cytokines produced by visceral adipose tissue, which leads to a chronic inflammatory process, and thus, promotes cancerous transformation [26-28]. Patients with T2DM are often obese, making them more prone to GERD. In T2DM, as in high-grade obesity, the clinical course of GERD is more often atypical. The differences between patients with T2DM result from additional factors contributing to GERD, including esophageal peristalsis disorders, gastroparesis, hyperglycemia, neuropathy and usage of diabetic medications (e.g. GLP-1 receptor agonists and metformin) [29]. The atypical, often mild or asymptomatic course of GERD in T2DM may delay the decision to perform diagnostics, leading to delayed diagnosis of complications, particularly BE and EAC. Therefore, it is important to study the natural course of GERD in obese and T2DM patients in order to reduce the risk of complications through early diagnosis and treatment.

Aim

The aim of our study was to characterize GERD in overweight patients and to assess the correlation between obesity parameters and the severity of GERD. The second aim of this study was to assess the differences in the clinical characteristics of GERD, depending on the diagnosis of carbohydrate disorders, which could explain the increased risk of complications in this group.

Materials and Methods

Study population

Our analysis covers data collected from 58 patients who were diagnosed for sleep breathing disorders at the Department of Internal Diseases of the Institute of Rural Medicine in Lublin. The exclusion criteria were: chronic use of drugs which may interfere with the assessment of GERD parameters (proton pump inhibitors, H2 blockers, alkali, nitrates) or previous significant gastrointestinal surgery (gastrectomy, bowel resection). This patient group also served as study participants in our previous work [30]. We collected anthropometric measurements and the responses from GERD-complaint questionnaires. If GERD was suspected, patients were referred for additional tests (gastroscopy and esophageal pH-measurement). The results from these additional tests were included in the study.

Criteria for the diagnosis of GERD

The diagnosis of GERD was established according to the definitions of the Lyon consensus [4]. Therefore, the diagnosis was based on the combined assessment of clinical symptoms, endoscopic evaluation of the esophageal mucosa, esophageal pH monitoring and response to therapeutic intervention. Clinical diagnosis was made when the patient had persistent symptoms characteristic of GERD which include heartburn and acid regurgitation. Persistence of symptoms was recognized when symptoms of mild intensity occurred at least 2 days a week or when they were more severe and caused deterioration in general well-being and occurred at least 1 day a week. Moreover, the diagnosis of GERD confirmed the presence of inflammatory changes in the esophagus (LA grade C) and the result of pH-measurement with esophageal acid exposure time > 6%, DeMeester Score > 14.72 or > 80 reflux episodes per 24 hours. Additionally, the diagnosis of GERD was confirmed by the reduction of symptoms after starting treatment with a proton pump inhibitor [4].

Anthropometric data

All participants underwent a physical examination. The body mass index (BMI) was calculated as the body weight in kilograms divided by the height in meters squared (kg / m2). The waist circumference (the circumference at midpoint between the lower border of the rib cage and the iliac crest) was measured in the standing position. Overweight was diagnosed if BMI was in the range of 25-29.9 kg / m^2; class I obesity when BMI was 30-34.9 kg / m^2, class II obesity when BMI was 35-39.9 kg / m^2; and class III obesity when BMI was at ≥ 40 kg / m^2 [8].

Survey data regarding the severity of GERD complaints

The severity of GERD clinical symptoms was assesed using a questionnaire about the overall intensity of complaints (within a score range of 0-10) and the intensity and frequency of symptoms considered typical for GERD (within a score range of 0-52). GERD symptoms included the feeling of heartburn and presence of regurgitation typical for GERD situations, acid reflux and dysphagia. In addition, the presence of GERD symptoms was assessed at night, during sleep and after an overnight sleeping period. Patients were instructed to describe the symptoms occurring in the month prior to completing the questionnaire. Survey data from all patients were collected by the primary investigator. The full version of the questionnaire is available in the Supplementary Materials [in Polish]: https://ejtcm.gumed.edu.pl/files/67 .

Gastroscopy and esophageal pH monitoring

All procedures were undertaken by experienced physicians before the introduction of GERD treatment. All gastroscopic examinations were done using the Fujifilm (Japan or Pentax) (Japan). Whereas the esophageal pH monitoring was done using the ComforTEC Plus PHNS single-channel probe and a recorder made by Sandhill Scientific (USA, REF: Z07-2000-A, SN: H109007C). The degree of esophagitis was determined according to the Los Angeles classification, and numerical values with a range of 1 to 4 were given for subsequent grades (grade A-D); 0 was designated as no inflammatory lesions [4, 10].

Diagnosis of pre-diabetes and type 2 diabetes

In order to assess the importance of carbohydrate disorders on the course of GERD, the subjects were divided into a group with carbohydrate disorders (23 patients) and a control group (35 patients). The group with carbohydrate disorders included patients diagnosed with T2DM, impaired glycemic tolerance (IGT) or impaired fasting glycemia (IFG). In all participants with T2DM, diabetes was well-controlled. Diagnoses of carbohydrate disorders were established before inclusion in the study and were based on the Polish Diabetes Association guidelines [31].

Data Analyses and Statistical Methods

All statistical analyses were carried out with the Statistica software package (version 13, TIBCO Software Inc., USA). After confirming that all variables meet the criteria of a normal distribution, Spearman's rank correlation coefficient was used for the analysis. Correlations between variables were calculated using Spearman's rank correlation coefficient, while comparisons between two independent groups were performed using the Mann–Whitney U test. P values < 0.05 were considered statistically significant. Because the distribution of GERD measurements was characterized by a large asymmetry, the median should be taken as the key measurement (possibly including the range of variability in the form of IQR) while assessing its severity depending on the presence of diabetic disorders.

Results

Baseline data

The study population consisted of 58 patients (48 males and 10 females) aged 34-75 years (mean = 54.5 years; Me = 56 years; s = 11.2 years). In the study group, obesity was diagnosed in 44 subjects (75.9%). Of these, class I obesity was seen in 17 subjects, class II obesity was seen in 12 subjects, and class III obesity was seen in 15 subjects. Fourteen (24.1%) patients were overweight. The average patient weight was 104 kg (range 77-161 kg), while average waist circumference was 115.5 cm (range 96-147 cm). The mean BMI was 34.8 kg / m^2 (range 25.1-49.7 kg / m^2). Carbohydrate disorders were reported by 23 subjects (~ 40%), of which 14 subjects (24.1%) had T2DM and 9 subjects (15.5%) had pre-diabetes. Table 1 presents the basic characteristics of the study population.

In our study, GERD was diagnosed in 40 patients (~ 69%). In the obese group, GERD was diagnosed in 32 subjects (73%), while the in overweight group, GERD was diagnosed in 8 subjects (57%). Gastroscopy was performed in 32 patients. In 2 cases, no esophageal inflammatory changes were observed. Most of the gastroscopic examinations revealed esophageal inflammatory changes with grade A, B and C of esophagitis recognized in 22, 7, and 1 subject, respectively. Grade D lesions or peptic stricture were not observed in any of the patients. Moreover, neither BE nor EAC were diagnosed. In 23 patients, pH-metry was performed. Table 1 presents the distribution of the variables.

Apart from obesity, carbohydrate disorders and GERD, the majority participants had comorbidities mainly related to the circulatory system. The most common were: arterial hypertension (47 patients, 81%); dyslipidemia (34 patients, 59%), coronary artery disease (16 patients, 28%); hyperuricemia (10 patients, 17%); chronic obstructive pulmonary disease (5 patients, 9%) and heart failure (4 patients; 7%).

Gastroesophageal reflux in obesity and in diabetic disorders

We investigated the relationship between obesity parameter values (body weight, abdominal circumference, and BMI) and GERD severity parameters. Since all considered

Table 1. Basic characteristics of the study population, severity of GERD symptoms and distribution of esophageal pH monitoring results

Features	Mean	Median	Std. dev.	Min	Max
Age (yr)	54.5	56	11.2	34	75
BMI (kg/m²)	34.8	34.6	6.7	25.1	49.7
Waist circumference (cm)	115.5	115.0	12.8	96.0	147.0
GERD symptoms day	11.9	12	9.2	0	28
GERD symptoms night	2.8	3	2.9	0	12
GERD symptoms overall	14.7	15	11.5	0	40
De Meester index (n = 23)	27.8	15.1	33.2	1.7	136.4
De Meester index – post meal (n = 23)	9.8	7.5	9.0	1.5	37
Reflux episodes – recumbent (n = 22)	34.5	10	57.7	0	250
Reflux episodes – 24/h (n = 22)	136.1	107	127.9	7	570
Mean pH night (n = 23)	6.4	6.5	0.8	4.3	8.1
Mean pH – 24/h (n = 23)	6.2	6.2	0.5	5.0	7.2
Esophageal clearance time (n = 22)	37.0	35.5	21.2	8	91
Esophageal clearance time – recumbent (n = 22)	31.7	22.5	33.1	0	138
Longest reflux episode (n = 23)	13.7	4.9	17.9	0.3	59.3

features were consistent with a normal distribution, Spearman's rank correlation coefficient was used for the analysis. Table 2 presents correlation coefficient values between individual features along with the assessment of their statistical significance. In the studied group, no statistically significant relationships were found between the obesity parameters and the assessed reflux parameters.

A tendency toward greater values with increasing obesity parameters was seen only for the "feeling of heartburn after meals" parameter.

The relationships between GERD parameters from gastroscopy, pH measurement and obesity parameters were calculated. Only the correlation between the number of postprandial reflux episodes and BMI was near statistical significance (test probability values $p = 0.0084$), however its strength was rather small $rS = 0.36$. In addition, there was a trend toward greater values for the postprandial De Meester index and for the duration of gastric acid exposure with increasing BMI (Table 3).

Table 2. Distribution of correlations between obesity parameters and the severity of GERD complaints

GERD symptoms	Obesity parameters (n = 58)		
	Weight	Waist circumference	BMI
GERD symptoms day	0.05 (p = 0.7338)	0.07 (p = 0.6178)	0.03 (p = 0.7973)
GERD symptoms night	0.07 (p = 0.5866)	0.06 (p = 0.6513)	0.04 (p = 0.7892)
GERD symptoms day and night	0.05 (p = 0.7039)	0.07 (p = 0.5779)	0.04 (p = 0.7681)
Overall intensity of GERD-related complaints	-0.01 (p = 0.9461)	0.07 (p = 0.5988)	0.10 (p = 0.4678)
Burning sensation in the chest (Post Meal)	0.16 (p = 0.2302)	0.16 (p = 0.2440)	0.19 (p = 0.1605)

Table 3. Correlations between GERD parameters from gastroscopy, pH measurement and obesity parameters

GERD severity parameters	Obesity parameters (n = 58)		
	Weight	Waist circumference	BMI
Severity of inflammatory changesin the esophagus (gastroscopy assessment)	-0.08 (p = 0.6753)	-0.04 (p = 0.8218)	-0.09 (p = 0.6203)
De Meester Index (Post Meal)	0.02 (p = 0.9340)	0.10 (p = 0.6415)	0.24 (p = 0.2636)
De Meester Index	-0.10 (p = 0.6608)	0,00 (p = 0.9964)	0.11 (p = 0.6198)
Reflux Episodes (Post Meal)	0.16 (p = 0.4610)	0.22 (p = 0.3198)	**0.36 (p = 0.0884)**
Reflux Episodes (24 h)	-0.04 (p = 0.8554)	-0.06 (p = 0.7859)	0.06 (p = 0.8029)
Exposure to gastric acid following a meal	0.03 (p = 0.9055)	0.12 (p = 0.5803)	0.22 (p = 0.3044)
Exposure to gastric acid (24 h)	-0.05 (p = 0.8085)	-0.05 (p = 0.8305)	0.03 (p = 0.8888)
Mean pH (Post Meal)	0.12 (p = 0.5836)	0.00 (p = 0.9839)	-0.11 (p = 0.6025)
Mean pH (24 h)	0.15 (p = 0.4928)	-0.06 (p = 0.7962)	-0.13 (p = 0.5468)
Esophageal Clearance (Post Meal)	-0.13 (p = 0.5452)	0.04 (p = 0.8540)	0.08 (p = 0.7216)
Esophageal Clearance (24 h)	-0.05 (p = 0.8308)	0.07 (p = 0.7636)	0.02 (p = 0.9284)

Next, we analyzed the differences between the parameters of GERD severity depending on the diagnosis of carbohydrate disorders. Among the participants with carbohydrate disorders, GERD was diagnosed in 18 patients (78%), of which 12 (86%) had T2DM, and 6 (67%) had pre-diabetes. Whereas in the group without carbohydrate disorders (n = 35) GERD was diagnosed in 22 participants (63%). No differences in the severity of GERD were observed between the groups with and without carbohydrate disorders. In our study, the GERD parameters in both groups were very similar (Table 4).

Table 4. Comparison of GERD severity parameters depending on the presence of diabetic disorders (p – test probability values were calculated using the Mann-Whitney test)

GERD severity parameters	Diabetic disorders								
	Yes				No				p
	n	Mean	Me	IQR	n	Mean	Me	IQR	
GERD symptoms day and night	23	16.5	18	21.0	35	13.8	14	22.0	0.4206
Severity of inflammatory changes in the esophagus (gastroscopy Assessment)	12	1.1	1	0.0	20	1.3	1	1.0	0.2551
Reflux Episodes (24 h)	10	111.8	66.5	165.0	12	156.4	146.5	151.5	0.4562
Reflux Episodes (Post Meal)	10	45.9	28	49.0	13	69.2	34	119.0	0.7381
Exposure to gastric acid following a meal (%)	10	5.6	2.5	8.5	12	8.1	4.5	9.5	0.8718
Mean pH (24 h)	10	6.1	5.9	0.6	13	6.3	6.4	0.6	0.2316
Mean pH (day)	10	6.1	6.0	0.8	13	6.1	6.3	1.1	0.7844
Mean pH (night)	10	6.1	6.1	0.8	13	6,6	6.7	0.3	0.0666
De Meester Index	10	24.7	10.6	27.9	13	30.2	18.1	29.0	1.0000
De Meester Index (Post Meal)	10	7.8	6.4	9.8	13	11.3	8.4	8.8	0.7844
Esophageal Clearance – 24 h (sec)	10	40.7	38	20.0	12	33.8	33	24.5	0.3136
Overall intensity of GERD-related complaints	23	3.8	2.0	8.0	35	2.5	2.0	5.0	0.3271
GERD symptoms day	23	13.7	15	17.0	35	11.0	10	20.0	0.3041
GERD symptoms night	23	2.8	3	4.0	35	2.8	2	5.0	0.9497
Burning sensation in the chest (Post Meal)	23	2.9	3.0	5.0	35	2.5	3.0	4.0	0.4973

The analysis of the probability of GERD depending on the selected variables (BMI, waist circumference, diagnosis of carbohydrate disorders and diagnosis of diabetes) was performed using the logistic regression model. Based on the analyzes performed, no statistical evidence was found that any of the proposed factors had a significant influence on the diagnosis of GERD (Table 5). Moreover, an attempt was made to search for a model containing statistically significant variables. The best model that included only the diagnosis of diabetes was still not statistically significant: OR (95% CI) = 3,429; p = 0.14.

Discussion

All participants in our study were overweight and in this group the occurrence of GERD was much more frequent than in the general population. Obesity is an important risk factor for GERD and numerous studies demonstrated its more frequent occurrence in people with excess weight [11-17]. In our study, no statistically significant correlation was found between individual obesity measures and GERD parameters. However, there was a trend towards greater severity of postprandial GERD clinical symptoms and worsening of postprandial GERD pH parameters with increasing BMI. Most of the published data demonstrate a more severe course of GERD in people with excess weight.

Obesity, especially the visceral type, causes GERD due to changes in the anatomy and physiology of the gastroesophageal junction (GEJ) [11, 18]. Additionally, it is believed that the pro-inflammatory effects of cytokines synthesized in visceral adipose tissue plays an important role [12, 26]. Akyuz et al. showed a significant correlation between BMI and the severity of GERD in pH measurements. They also found that the severity of esophageal inflammatory changes seen in gastroscopy did not differ significantly in the obese group, however the authors did not assess the severity of GERD in the context of its association with abdominal obesity [11]. In our study, we also did not observe any relationship between obesity and the severity of esophageal inflammatory changes in gastroscopy. The correlation between BMI and GERD severity in pH measurements was also present, but did not reach the level of statistical significance.

We did not observe a correlation between abdominal circumference and the severity of GERD. However, most studies indicate that it does have greater a role than BMI in terms of GERD severity. A study by Wu et al. investigated the correlations of obesity parameters with GERD symptoms and esophageal inflammatory activity via the measurement of glucose metabolism in 18F-Fluorodeoxyglucose positron emission tomography (PET-CT). There was a significant correlation between GERD symptoms and esophageal inflammatory activity in PET-CT with all obesity parameters (BMI, abdominal circumference, and the amount of subcutaneous and visceral adipose tissue) [13]. Nam et al. described the correlations between the amount of visceral fat, concentrations of inflammatory cytokines synthesized within it, and the intensity of esophageal inflammatory changes [12]. Similar results were obtained in large-scale studies in Japan and South Korea. An increased incidence of reflux esophagitis has been observed in obesity and in the metabolic syndrome. Hyperglycemia, high BMI, and in particular, greater abdominal circumference and increased visceral fat, correlated with an increased risk for GERD [14-15].

Gastroesophageal reflux disease is linked to a higher risk of BE [4, 20]. Population-based studies have indicated that the risk of BE and EAC is also significantly increased in obesity, especially in the abdominal type. This risk is increased regardless of the presence of GERD symptoms [21, 23--25]. In a study by Nelsen et al., the risk of developing BE and dysplasia correlated with the amount of visceral adipose tissue and adipose tissue in the GEJ fat area; however, it was independent of the BMI value and the presence of GERD symptoms [24]. Similar conclusions were obtained by El-Seraq et al. [19]. Moreover, in an investigation by Corley et al., abdominal circumference and abdominal obesity (but not BMI) correlated with a greater risk for BE [25].

Table 5. Logistic regression model of the probability of GERD diagnosis depending on selected variables

Independent variables	GERD diagnosis	
	OR (95% CI)	p
BMI	1.060 (0.867-1.295)	0.5704
WC	0.975 (0.880-1.079)	0.6207
Diagnosis of carbohydrate disorders	1.158 (0.244-5.496)	0.8532
Diagnosis of diabetes	3.195 (0.392-26.073)	0.2781

OR – odds ratio; CI – confidence interval; GERD – gastroesophageal reflux disease; BMI – body mass index (kg/m2); waist circumference (cm)

Gastroesophageal reflux disease is common in T2DM and is more likely to be atypical or present with mild symptoms [29]. In addition, these patients are at an increased risk for developing metaplasia [22]. In our study, no differences in the severity of GERD were found between subjects with T2DM or pre-diabetes and the group without these disorders. Notably, the two groups were very similar in terms of GERD characteristics. Lorentzen et al. compared the features of GERD in patients with a high degree of obesity, depending on the diagnosis of T2DM. As in our study, GERD was more common in the obese group than in the general population. However, a large proportion of the respondents had asymptomatic GERD, regardless of whether or not they suffered from T2DM. In this study, clinical symptoms were reported by approximately 29% of the respondents, but esophagitis in gastroscopy was seen in 58% of patients in the T2DM group and in 47% of patients in the non-T2DM group. Among subjects with inflammatory changes in the esophagus, 68-80% did not report symptoms of GERD. In the T2DM group (only T2DM patients underwent pH-metry), 55% of subjects had pathologic acid reflux, whereas 67% of subjects were asymptomatic [16]. In our study, the severity of GERD clinical symptoms was similar in both groups, which may be due to the lower number of severely obese patients when compared to the cited study.

As reported by Ortiz et al., the asymptomatic course of GERD in obese patients may be related to the decreased esophageal sensitivity to acid content observed in this group. The authors indicated that the absence of typical GERD symptoms in these patients may delay the diagnosis of GERD complications, especially BE [32]. Promberger et al. also observed the frequent occurrence of atypical GERD symptoms in T2DM [33]. Furthermore, Lluch et al. found that GERD was common in diabetic patients, but it was more often asymptomatic [34]. The above conclusions are of clinical significance in the context of a report by Leggett et al, which found an increased risk of BE in patients with metabolic syndrome, regardless of GERD symptoms [23]. The role of carbohydrate disorders in the pathogenesis of GERD is unclear. Gokturk et al. observed a more severe course of GERD in subjects with T2DM, but the presence of reflux episodes was associated with obesity rather than hyperglycemia [17]. In the study by Wang et al., the occurrence of GERD symptoms in diabetes was observed more frequently, and their severity clearly increased in the group of patients with diabetic neuropathy [35].

In our study group, we did not observe an atypical course of GERD in T2DM, but in all cases, these were patients with pre-diabetes or well-controlled diabetes without complications. Although the patients with carbohydrate disorders more often suffer from GERD and its complications, the clinical course does not correlate with the risk of complications. Because of this, they may benefit from early gastroscopic evaluation.

The limitations of our study include the small number of participants and the lack of assessment of other obesity parameters, such as the waist-hip ratio. Moreover, not all patients underwent endoscopic examinations or pH-metry. Another limitation was the inclusion of only hospitalized patients with suspected sleep apnea who were mostly obese, which makes it difficult to transfer the obtained conclusions to the general population. Despite these limitations, the collected results allowed us to demonstrate a greater incidence of GERD in obesity and to show that the presence of carbohydrate disorders was not associated with a more severe clinical course of GERD (in the context of clinical symptoms, changes in gastroscopy, and pH measurement).

Conclusions

In our study group, we observed that GERD is more common in obesity and in T2DM; however, the diagnosis of diabetic disorders was not associated with more severe GERD. Our results and a review of the current literature indicate that due to a mild or atypical course, GERD may be underdiagnosed in the group of severely obese and T2DM patients. Finally, although patients with carbohydrate disorders more often suffer from GERD and its complications, the clinical course does not correlate with the risk of complications, and because of this, these patients may benefit from early gastroscopic evaluation.

Acknowledgments

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the bioethical committee at the Institute of Rural Health (Decision No. 6/2014). Informed consent was obtained from all subjects involved in the study.

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None.

Conflicts of interest

There are no conflicts of interest to report for any of the authors.

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RESEARCH ARTICLE

Effect of fenugreek seed supplementation on Hemoglobin and PCV among 20-30 years old females: a pilot study

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Abstract

Background: Females are more likely than males to suffer from anemia. There have been many studies investigating the nutritional qualities of traditional plants. The of this study was to assess the effect of fenugreek seeds on hemoglobin and PCV values in 20-30 years old females. **Material and methods:** Baseline hematological tests were obtained from 10 females aged 20-30 years. Supplementation of the standard amount of fenugreek was given to the population. After 48 days the same hematological parameters were analyzed as a post-test. The results of the pre- and post-test analysis were compared by using a paired-sample t-test. Relevant details were also collected and analyzed. **Results:** The mean value of hemoglobin before supplementation (Hb1) and hemoglobin after supplementation (Hb2) was 12.3200 and 11.3300 respectively and the mean value of packed cell volume before (PCV1) and after supplementation (PCV2) was 36.7970 and 35.4700, respectively. This shows that there is a mild decrease in hemoglobin and PCV values after fenugreek supplementation, but not at a statistically significant level. **Conclusions:** Within the limitations of our pilot study, we conclude that fenugreek has no significant effect on Hemoglobin and PCV values and can't be used as an exact supplement to cure anemia.

Keywords: anemia · fenugreek supplementation · hemoglobin · PCV · hematopoietic stem cells

Citation

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Introduction

Anemia is defined as a decrease in oxygen-carrying capacity and hemoglobin levels from the normal range [1-3]. There are different types of anemia: iron deficiency, hemolytic, aplastic and inflammation-related [4-5]. The most common is iron deficiency anemia and it is becoming increasingly common these days because of lifestyle changes among reproductive females. During this time, physical and psychological activity will be at their peak. The risk of females being neglected by society is higher [6]. The body requires more energy and nutrition during puberty resulting in anemia in about 55% of females around the world [7]. Poverty is also one of the cause of nutritional anemia in females. Furthermore, women have blood loss during their menstrual cycle [8]. Anemia can be diagnosed by looking at the hemoglobin count, red cells count and packed cell volume. The normal value of hemoglobin is 14-16 g/dl, Red cell mass is 4.2-6.7 million cells per microlitre, Hematocrit is 35-48% [9]. Iron deficiency anemia is usually treated with oral iron supplements. Patients are also advised to take regular traditional iron-rich food supplements.

Fenugreek (*Trigonella foenum-graecum*/methi) is a traditional plant with a high nutritional profile that is commonly used to prevent or treat iron-deficiency anemia in the general population. Predominantly most of the spices of the fenugreek family (Fabaceae) serve as a flavoring agent. Although fenugreek belongs to this family, that doesn't mean it can only be used for cooking. Components of fenugreek include fiber, phospholipids, glycolipids, linolenic acid, choline, vitamins A, B1, B2, C, nicotinic acid, niacin, and other basic elements [10]. Additionally, fenugreek seeds are used in the paper, cosmetic, pickle, beverage, perfume and paint industries. Moreover, they are effective hypoglycemic, hypocholesterotic, and antithrombotic agents. The seeds were thought to contain non-nutritional saponins and alkaloids. Fenugreek seeds lower triglyceride levels as well as total cholesterol levels and also lower the density of lipoprotein cholesterol [11]. It was demonstrated in a study that soluble fibers in fenugreek, including glucomannan fiber, have essential biochemical changes like decreasing the intestinal absorption of glucose and sugars (fenugrecin) and possess hypoglycaemic properties [12]. An extract of Fenugreek at a dose of 400 mg daily yielded a positive correlation with hyperlipidemia reduction in the study. Fenugreek extract shows more than 70% inhibition in the growth of tumour cells on repeated control [13]. Recently, insulin resistance and cholesterol metabolism properties of fenugreek were investigated [14] and fenugreek seeds were found to increase erythropoiesis in broiler breeder males [15]. In a previous study published in 2020, fenugreek seeds reversed anemia [16] in dental outpatients. However, there is only a limited amount of evidence about their effect on anemia in adolescent females. The aim of our study was to assess the effect of soaked fenugreek seeds on hemoglobin levels and packed cell volume (PCV) in clinically healthy 20-30 year old females.

Materials and methods

In this prospective study, 10 asymptomatic females 20-30 years of age were selected using the random sampling method using a digital laboratory record system. Females during their menstruation period were excluded from the study. Validation of the study was done by expert pathologists and principal investigators. A written consent form was taken from the study participants before initiating the clinical trial. This form was for explaining the purpose of the study and clarifying their queries about the consumption of fenugreek seeds. Details regarding age, height, weight and general health conditions were recorded in all selected participants.

The fenugreek seeds were cleaned, measured for one teaspoon and soaked in 5 ml of water, and left overnight and supplemented before breakfast. On the first day before fenugreek seed supplementation, intravenous blood samples were collected from participants and analysed for hemoglobin and PCV levels. In the following days, the participants consumed fenugreek seeds orally followed by drinking water every day after breakfast. After a consecutive period of time (48 days) the IV samples were collected. Hemoglobin percentage was analyzed using an automated haematology analyzer. Haematocrit (PCV) is the measurement of the proportion of blood that is made up of cells which were obtained as a calculated value from the automated analyzer. Pair 1 represents the mean value of hemoglobin before supplementation (Hb1) and hemoglobin after supplementation (Hb2). Pair 2 represents the mean value of packed cell volume before (PCV1) and after supplementation (PCV2). The obtained haematological data were assessed using the paired t-test via the SPSS Statistics software (IBM, Armonk, USA).

Results

From the results obtained, pair 1 represents the mean value of hemoglobin before supplementation (Hb1) and hemoglobin after supplementation (Hb2) as 12.3200 and 11.3300 respectively with p value - 0.235 ($p > 0.05$). The pair 2 shows the mean packed cell volume before (PCV1) and after supplementation (PCV2) as 36.7970 and 35.4700 respectively with p value = -0.286 ($p > 0.05$). [Table 1]. After supplementation, hemoglobin and PCV mean values were decreased. However, were no statistically significant differences in the hemoglobin and PCV measurements before and after fenugreek supplementation.

Table 1. The mean, standard deviation and statistical analysis of Hemoglobin and PCV variations before and after supplementation

Study groups	Parameters	Mean	Standard Deviation	Statistical Significance
Pair 1	Hemoglobin g/dL (before supplementation Hb1)	12.3200	1.730	p = 0.236
	Hemoglobin g/dL (after supplementation Hb 2)	11.8300	0.921	
Pair 2	PCV% (before supplementation PCV1)	36.7970	5.158	p = 0.286
	PCV% (after supplementation PCV 2)	35.4700	2.5948	

Discussion

According to the literature, elemental iron contained in fenugreek seeds was effective at boosting functions such as hemoglobin synthesis, oxygen transportation, regulation of body temperature and muscle activity [17]. Vitamin C in fenugreek leaves improves haemoglobin levels in women who suffer from anaemia [18].

In our study, Hemoglobin concentration before fenugreek supplementation seems higher (mean 12.32 gm/dl) than after 2 months of fenugreek supplementation it was insignificantly reduced (mean 11.83gm/dl). This is supported by Verma et al., who showed that fenugreek seeds were effective in decreasing glycated hemoglobin levels (HbA1C) in type 2 diabetic patients [19] (see Table 2). Doshi et al. published that fenugreek seeds increased blood hemoglobin levels and treating anemia in childbearing women [20]. Pregnancy-related hemoglobin increases may be caused by the lack of menstruation during this time. In our current study, the hemoglobin level was lower than expected, which is not consistent with our previous findings. Study by Srinivasan et al. showed that fenugreek reduces menstrual blood loss and thereby it increases the hemoglobin level in females of reproductive age [21].

Table 2. Hemoglobin and PCV values obtained from various previous articles

Authors	Hemoglobin (g dL-1)	PCV (%)	P-value
Roohi et al. [27]	9.45	31.55	p > 0.05
Doshi et al. [20]	12.35	-	p > 0.05
Sakhira et al. [26]	14.75	44.05	p ≤ 0.05
Rao et al. [24]	14.7	36	p > 0.05
Nagammal et al. [23]	11.56	36.76	p ≤ 0.05
Naidi et al. [25]	10.45	-	p > 0.05
Verma et al. [19]	11.23	-	p < 0.05
Our present study	11.83	36.79	p > 0.05

PCV value before supplementation seems high (mean 36.79), when compared with after supplementation values (mean 35.47). Due to a decrease in hemoglobin, the hematocrit level also decreased. In fact, they are directly related as shown in this formula: PCV = 3 x Hb [22]. According to studies conducted by Rao et al. and Nagammal et al., fenugreek increased hemoglobin and PCV values, which is not consistent

with our study [23-24]. A study by Naidi et al. compared the effect of fenugreek with glibenclamide drugs in diabetic patients. A higher RBC count was observed in fenugreek (5.44/mm^3), MCV (88.74 fL) and MCH (27.68 pg) when compared to glibenclamide. Apparently, fenugreek seems to stimulate hematopoietic stem cells, thereby improving hemoglobin levels [25-26]. Roohi et al. observed a reduction in hemoglobin and PCV values in their study on fish diet [37]. A study of fenugreek meal on male rats found significant increases in hemoglobin and PCV levels after supplementation [26].

This is a pilot study and its limitations include a small sample size and short follow-up. This can be overcome by recruiting a higher sample size and a year-long fenugreek supplementation in the future. After analyzing the clinical history of the participants (Table 3), it was discovered that the participants who had a the previous history of anemia and were treated with iron supplements had a mildly higher hemoglobin and PCV value than the other participants. These selection biases can be avoided in future studies.

Table 3. Clinical history and examination findings of the study participants

Clinical History and signs	Present	Absent
Fatigue	20%	80%
Giddiness	10%	90%
Palpitations	60%	40%
Previous history of jaundice	20%	80%
Previous history of treatment for anemia	20%	80%
Pallor (conjunctiva)	50%	50%
Pallor (nails)	30%	70%

Conclusion

With respect to the limitations of this study, we conclude that fenugreek reduced hemoglobin levels and packed cell volume among clinically healthy females of age 20-30 years. However, this reduction is not statistically significant. The data above shows that consumption of a soaked single teaspoon of fenugreek is not an effective supplementation for increasing hemoglobin and PCV and correcting anemia. This might have promising effects when taken with iron supplements. Further research on fenugreek supplementation is awaiting completion by the author.

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Conflicts of interest

None.

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RESEARCH ARTICLE

How to teach pharmacology to medical students during the COVID-19 pandemic?

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Abstract

Background: The COVID-19 pandemic has forced the introduction of many changes into medical student education. The aim of the study was to evaluate medical students' perceptions of a Pharmacology course delivered at a Polish medical university before and during the pandemic. **Materials and methods:** A cross-sectional anonymous survey conducted among medical students. **Results:** 90 out 122 students participated in the study. The vast majority of students found pharmacology to be a difficult subject. The surveyed group of students preferred active methods of learning, including: teacher explanations (86.5%) and discussions (70.8%) during in-person classes, real-time student-teacher meetings via dedicated web-based platforms (73%) during online classes. Students most often described e-learning as interesting (58.9%) and timesaving (52.2%). Less than 30% described it as stressful, difficult, time-consuming and boring. The most commonly reported advantage was the possibility for students to adjust their pharmacology study-time to a more personalised schedule (82.5%). The main disadvantage included the loss of in-person face-to-face contact with the teacher (61.8%). **Conclusions:** Overall, students held positive attitudes towards the new teaching format and adapted well to the new conditions. Modern innovations enabling medical students to continue their studies efficiently and effectively during the pandemic must be developed and introduced into practice.

Keywords: COVID-19 pandemic · medical education · online teaching · pharmacology · undergraduate

Citation

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Introduction

The World Health Organization's (WHO) declaration of the coronavirus disease 2019 (COVID-19) outbreak as a pandemic had serious implications in all areas of human activity – health care systems, travel, employment etc. [1]. The spread of this virus also had significant consequences on higher education as universities were required to shift from in-person teaching to online education [2-6]. This new form of education had to be implemented immediately without any preparation from either the students or staff [7]. In Poland, the closure of all educational institutions, including universities, occurred in mid-March 2020, which was approximately halfway through the second semester of the academic year [8]. The closure of medical schools was considered particularly important, due to concerns around medical students being potential vectors for COVID-19 during their rotations between hospitals and health departments [9]. Thus, all courses taught within the Faculty of Medicine at Polish medical universities were shifted from the in-person modality to online.

Pharmacology is considered a core pre-clinical subject within the curriculum at the Medical University of Gdańsk. It is taught during the 3rd year of the medical studies and consists of 100 didactic hours comprising lectures, seminars and exercises. During this course, students gain basic knowledge about pharmacology, toxicology and prescribing medication. The classes are spread over two semesters and the course ends with a multiple-choice exam. From the beginning of the pandemic, all pharmacology classes, mid-semester exams, oral exams and the final exam were performed online. Since the change to the online modality, both the teaching and studying of pharmacology has been challenging for teachers and students. To achieve the highest standards of education the following methods were applied: individual consultations, classes conducted in real-time via specialised web-based platforms e.g. Zoom, self-directed work, teacher feedback and the delivery of pre-recorded materials. The exams were held using the Moodle and Zoom platforms.

The aim of this study was to evaluate medical students' perceptions of the delivery of the pharmacology course before and during the COVID-19 pandemic (academic year 2019/2020).

Material and methods

This was a cross-sectional survey that was distributed online (via Google Forms). Following the completion of the Pharmacology and Toxicology subject, medical students (from the English Division group) were asked to complete the survey. The link to the survey was made available to students on the online course learning portal, and each student was invited to provide their opinions on both the regular face-to-face and online pharmacology classes. In the introduction of the survey, it was explained that the obtained results will be used for practical and scientific purposes. Participation in the study was voluntary and the survey was anonymous. The respondents did not receive any incentives for participating in the research. The questionnaire was prepared in English and consisted of 12 items which were based on the authors' didactic and research experience. A total of 11 questions were directly related to the examined problem and comprised: 6 multiple choice questions, 2 single answer questions, 2 VAS-scale questions and one open-ended question relating to students overall opinions on the e-learning format. The last question was related to the gender of the participants. The survey was conducted in June-July 2020.

Statistical Analysis

The responses for each question were collected and calculated (frequencies and percentages) using StatisticaTM (TIBCO Software Inc., Palo Alto, CA, USA). For the statistical analysis, the Chi-square test was applied to determine any correlation between the two selected variables, e.g. comparing difficulty of regular classes to that of e-learning (Statistica version 13).

Results

A total of 90 out of a possible 122 medical students participated in the study (response rate 73.8%), of whom 65.6% (n = 59) were female and 34.4% male (n = 31 male).

Perceived difficulty of subject

The first question concerned general information about the course. The intention of this question was to determine the perceived difficulty of pharmacology as a subject for the examined students. Overall, 71 students (78.9%) rated the subject 'difficult', 8 (8.9%) found it very difficult, 10 considered it to be 'easy' and no students rated it 'very easy'. There were no statistically significant differences between the gender of the students and the perceived difficulty of the subject ($p > 0.05$).

Sources of information relied upon by students

The most commonly identified sources of information relied on by students when studying the subject included: teachers, recommended books and classes. In their qualitative answers, 6 students listed the use of the Sketchy Pharmacology program (Fig. 1).

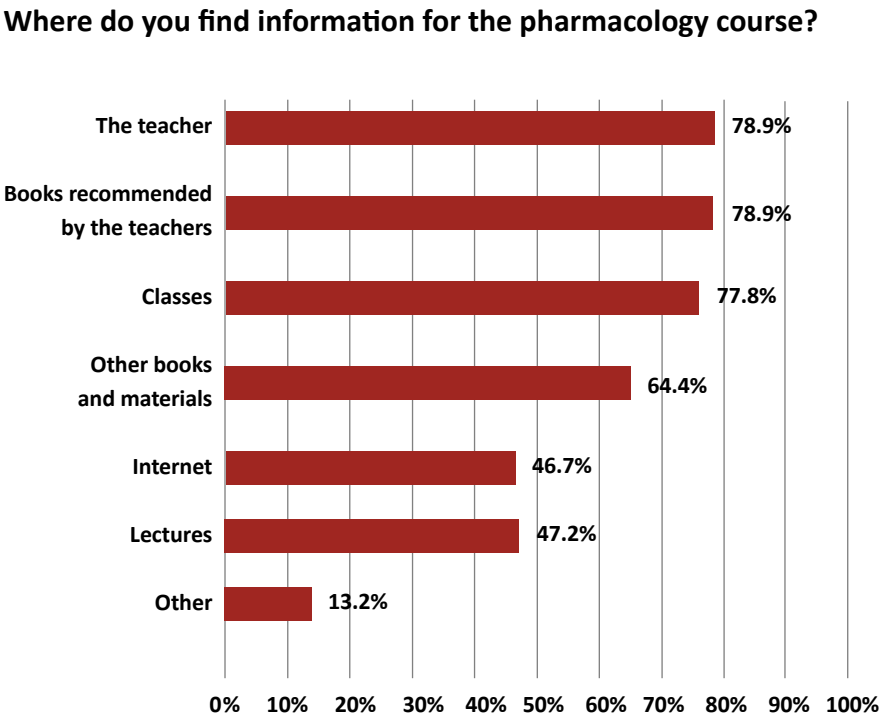

Figure 1. Sources of information used by students (several answers possible, n = 90)

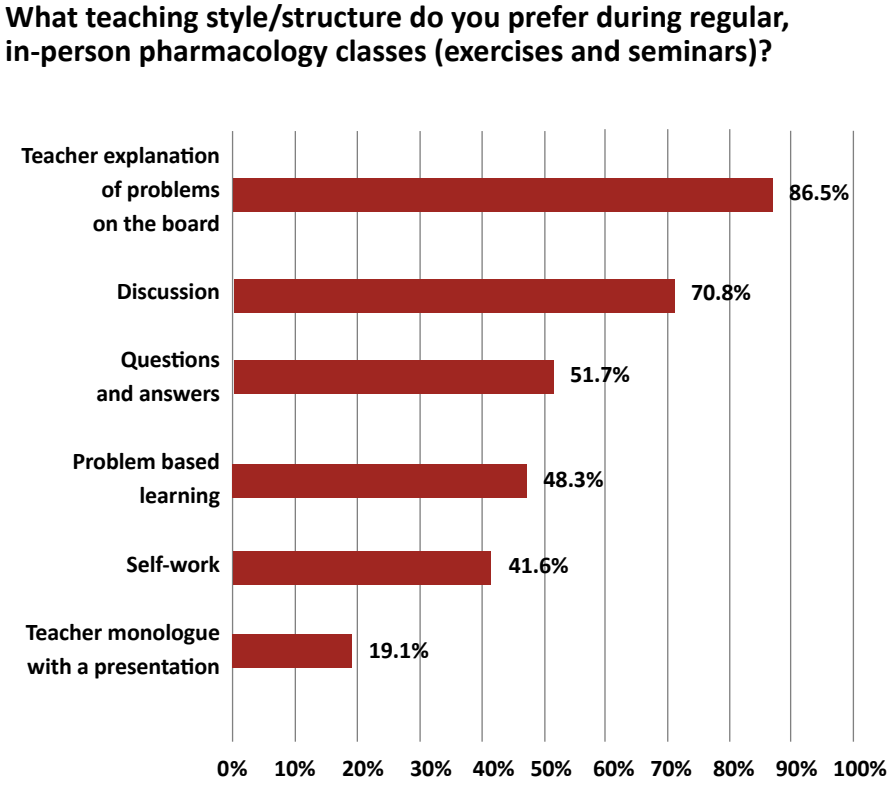

Figure 2. Students' opinions on teaching methods applied during regular classes (n = 89)

Teaching practices utilised in the in-person and online teaching modalities

During the regular (pre-pandemic) classes various teaching methods were applied. Most students preferred active methods such as teacher explanations of problems on the white board (86.5%) and discussion (70.8%). Passive methods involving self-directed work (41.6%) and multimedia presentations (19.1%) were generally not considered to be interesting (Fig. 2).

For online classes (during pandemic), teaching methods were modified and adjusted to the new circumstances. For the majority of students (73%) real-time meetings with the usage of special web-based communication software, e.g. Zoom, was considered the best teaching practice. Similarly, in comparison to the teaching-style/structure preferences of regular in-person classes, self-learning and individual consultations were favoured by less than half of participants (40.4%) (Fig. 3).

Student perceptions of e-learning

Students were asked to choose out of a selection of seven adjectives, which best described their perceptions of e-learning (several answers were possible). The students listed them in the following order: interesting (58.9%), time-saving (52.2%), stressful (27.8%), difficult (26.7%), time-consuming (21.1%), easy (14.4%) and boring (14.4%).

Students were also asked to describe the advantages and disadvantages of e-learning. The most commonly reported advantage related to the flexibility of the studying schedule. Students appreciated being able to adjust their pharmacology study-time to a more personalised timeframe (n = 74; 82.5%). On the

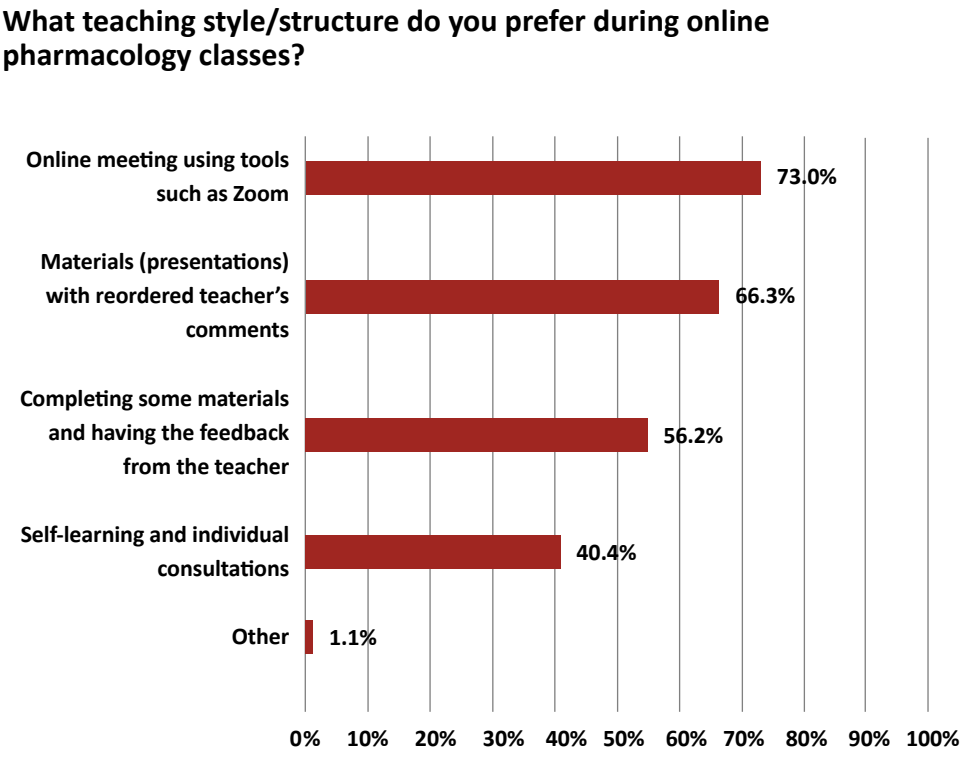

Figure 3. Students' opinions on education methods applied during on-line classes (n = 89)

other hand, the lack of in-person face-to-face contact with the teacher was the most commonly indicated disadvantage (n = 55; 61.2%). Other disadvantages included: increased stress due to having to do more studying alone, disruptions during class-time caused by family members, stress due to technical issues, perceived difficulties with student-teacher communication by e-mail and perceptions that they are learning less than what they would if they were attending normal classes in-person with the teacher. Two students reported no perceived advantages and a further two reported no perceived disadvantages (Table 1).

Student preferences for exams

As far as the mid-semester tests (colloquia) and exams are concerned, 84 out of 90 students (93.3%) preferred having a multiple choice question exam conducted on a virtual platform. Only a few students favoured undertaking an exam in an oral or written form.

Student perceptions of the difficulty of pharmacology learning based on the modality

In the next pair of questions students were asked to assess the perceived level of difficulty in learning pharmacology based on the format in which it is delivered: in-person vs. online. A 10-point VAS scale was used with 0 indicating 'very easy' and 10 indicating 'very difficult'. An overall range of

Table 1. Advantages and disadvantages of e-learning according to medical students

Advantages	Number of students (n = 90)	%	Disadvantages	Number of students (n = 89)	%
Possibility to adjust learning pharmacology to my private time	74	82.5	Impossible to have in-person face-to-face contact with the teacher	55	61.8
Being at home	62	68.9	Stressful exams and colloquia	46	51.7
More comfortable study conditions	58	64.4	Impossible to have real discussions with other students	44	49.4
Other	6	6.6	Other	16	17.6

0-4 denoted 'easy' and a range of 6-10 was 'difficult'; 5 indicated the median, i.e. neither easy nor difficult. Overall, for the face-to-face format of teaching, 24.4% of students considered pharmacology to be easy and 51.1% found it difficult, with the remainder neutral (number 5). However, for the online classes, only 15.6% of students felt that learning pharmacology through a web-based format was easy and 76.4% found it difficult, the remaining 8% felt it was neither difficult nor easy. The mode answer value was calculated as 5 for regular classes (22 students, 24%) and 7 for online classes (20, 22.5%). For regular classes the mean was 5,6 and median 6, and in the case of face-to-face classes the values were 6.5 (mean) and 7 (median). The differences in the perceived levels of difficulty between the two formats was statistically significant ($p = 0.03$). The results are presented in Figure 4.

Feedback from students

In the open-ended question students gave an overall evaluation of the online format of the classes. A total of 13 students reported that they were satisfied with e-learning and thanked the teachers for their good organization, and only two reported that they were dissatisfied, indicating that they preferred regular classes. A further nine students listed some improvements that could be made to the classes including better organization, less material to learn, better access to the information.

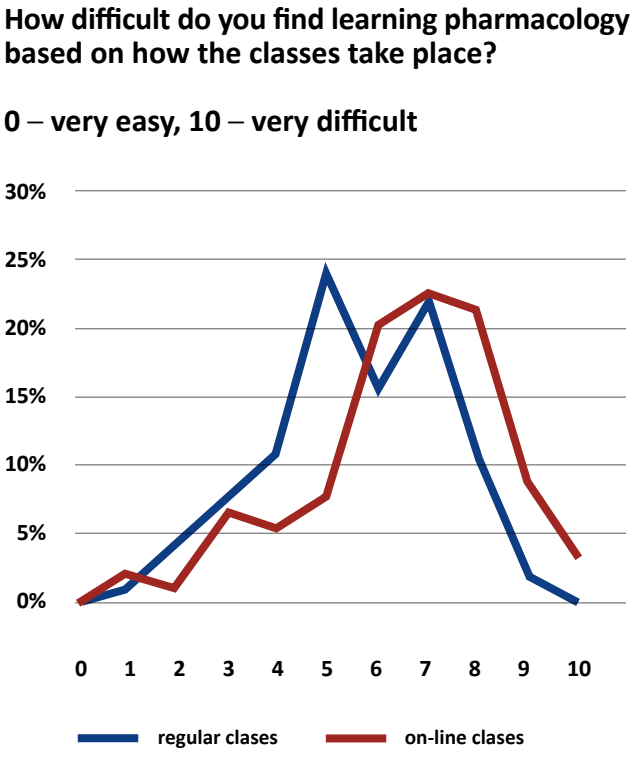

Figure 4. Students' opinions on level of difficulty of pharmacology classes based on how it is delivered: online vs. regular

With reference to future classes, the majority of students (18) agreed that online meetings via a dedicated web-based platform (e.g. Zoom) were the best solution for conducting e-learning. Furthermore, 9 students indicated that pre-recorded lectures or seminars could be helpful in terms of allowing them to re-watch sessions, giving them the opportunity to learn the content at their own pace. A total of 12 students indicated that interactive discussions between teachers and students to solve case-based problems, as well as broader explanations of the material and interactive tasks, were essential for good e-learning. Individual work assignments and working in small groups was perceived as being beneficial for nine students.

Discussion

Our survey describes medical students' perceptions of the differences in studying a pharmacology subject via two different modalities: e-learning during the COVID-19 pandemic vs. regular in-person classes. In general, the vast majority of surveyed students considered the pharmacology subject difficult. This indicates that students may have to put in a lot of effort to learn this subject.

Pharmacology is considered a cornerstone subject in tertiary education health science curricula [10]. Therefore, there is a growing need for the implementation of effective and efficient pharmacology education methods to obtain satisfactory learning outcomes. This challenge during the novel coronavirus pandemic took on a new form; since the start of the outbreak, the priority has been focused on ensuring the safety and well-being of students, patients and staff members. Thus, the universities were forced to introduce online learning, which required a prompt modification of the applied teaching methods and strategies.

When considering the teaching approaches, this study indicated that during face-to-face classes students favoured active forms involving both them and teachers. Similar answers were reported for online teaching – students singled out methods which mimic real-life meetings with teachers. i.e. live classes conducted on a platform that enabled direct communication. Furthermore, prescribed books as well the teachers' own knowledge were considered the most relied upon resources whilst studying.

With respect to the examination methods, alarge number of students indicated that online tests with the usage of multiple-choice questions were the best tools for assessing knowledge. However, some students stressed that these exams formats may generate stress due to the

potential for technical problems. Teachers from the King's College London School of Medical Education proposed an open-book exam (OBE) instead of exam-hall settings, which was adopted by many schools [11]. Such approaches decrease student anxiety and may also have an impact on cheating and plagiarism.

The newly adopted online teaching format has many perceived advantages. Researchers from the UK claimed that the online classes allowed students to return to their homes and families where they felt more comfortable [11]. A study conducted among medical and surgery residents showed that this form of education is less time-consuming and therefore students could spend the time saved on reading academic journals, doing practice questions, completing other hospital work, working on research and volunteering [12]. Similar observations were made by medical students from the Kingdom of Saudi Arabia. Additionally, the authors agreed that online learning was more successful within basic medical sciences or preclinical subjects [13]. Moreover, online learning could decrease the absences of the students at the classes and reduce the pathologies related to their making up [14].

Other advantages listed by students in our survey included a flexible learning schedule as well as being able to study in a more comfortable setting at home. On the other hand, the major disadvantage was a lack of in-person contact with the teacher. Overall, students found online learning interesting and time-saving however, also perceived some aspects of it to be difficult. Similar results were found by researches from another Polish medical university. Medical students most frequently indicated the following advantages: access to online materials, learning on their own peace, ability to stay at home and comfortable surroundings. For these students the technical problems, lack of interactions with patients and decreased interaction with the teacher were the most common disadvantages [15].

A study of 1255 health care students from various countries, showed that positive perceptions and prior experiences of e-learning are closely related to the level of country development. The researchers also claimed that e-learning was more effective in acquiring theoretical knowledge in comparison to clinical and technical skills [16].

The preparation and adaptation of regular coursework to the online format may present a significant challenge for educators and requires comprehensive preparation to ensure that they are simple and uncomplicated for students. This shift to online learning became crucial during the COVID-19 pandemic and imposed on teachers practical and logistic hindrances. Therefore, medical schools and their staff must cooperate together to deliver transparency, communication and online resources for the new type of education [17]. Further research is needed to determine interesting and effective ways of delivery health-based content via online teaching. The implications of the current shift to e-learning for the future of medical education is difficult to predict, however it may change forever the education of subsequent generations of physicians [18].

Limitations

Our study has some limitations. It was conducted only at one medical university, however the study included a variety of participants from many countries, as the study sample comprised the English-language student group, and so none of the students were Polish nationals. Moreover, the study was conducted only among selected medical students from the 3rd year, which limits the generalisability of our findings.

Conclusions

The COVID-19 pandemic had a significant impact on the conditions of academic teaching. Teachers and students were required to shift immediately from in-person to online teaching. In general, pharmacology students at a Polish university were receptive to the new situation and adjusted to the extraordinary conditions. However, it required more effort from both the teaching staff and students themselves to continue with a consistent level of learning. The preferred methods for online learning were those that mimicked regular classes, i.e. student-oriented teaching, real-time classes and discussions. It must be emphasised that the teaching of a complicated subject, such as pharmacology, during this pandemic has been challenging and required the development of new methods of active learning/teaching. The far-reaching consequences of online learning have not yet been discussed and may become the subject of future studies.

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Conflicts of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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REVIEW ARTICLE

Mutual relations between the amygdala and pro-inflammatory cytokines: IL-1β and IL-6

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Abstract

Interleukin 1 (IL-1) and interleukin 6 (IL-6) are typical examples of multifunctional pro-inflammatory cytokines involved in the regulation of the immune response, hematopoiesis, and inflammation. Both peripheral and intraventricular administration of these cytokines causes acute phase symptoms, e.g. fever, activation of the hypothalamic-pituitary-adrenal axis and psychological depression. The amygdala belongs to the strucxtures of the limbic system involved in the regulation of the immune response. Increased activity of immune system may lead to changes in the role of amygdala, medial prefrontal cortex, anterior cingulate cortex or insula. The aim of the study was to present the mutual interactions between the amygdala and pro-inflammatory cytokines such as interleukin 1β (IL-1 beta) and interleukin 6 (IL-6). Most of the data included in this review comes from animal studies.

Keywords: amygdala · inflammation · IL-1β · IL-6

Citation

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Introduction

Interleukin 1 (IL-1) and interleukin 6 (IL-6) are typical examples of multifunctional pro-inflammatory cytokines involved in the regulation of the immune response, hematopoiesis, and inflammation. Both peripheral and intraventricular administration of these cytokines causes acute phase symptoms, fever, activation of the hypothalamic-pituitary-adrenal axis and depression. The amygdala belongs to the structures of the limbic system involved in the regulation of the immune response. Increased activity of immune system may lead to changes in the role of amygdala, medial prefrontal cortex, anterior cingulate cortex or insula [1-4].

Many authors demonstrate that excessive inflammatory responses play an essential role in the pathophysiology of anxiety disorders and depression. Increased serum levels of inflammatory cytokines were observed in patients with depressive disorders. Neuroinflammation is caused by the activation of microglial cells, which stimulate nuclear factor-kappa B (NF-κB) and produces inflammatory cytokines. Systemic inflammation caused by peripheral injection of lipopolysaccharide (LPS) mobilizes the innate immune system, which in turn creates neuroinflammatory responses in the brain.

The aim of this study was to present the mutual interactions between the amygdala and pro-inflammatory cytokines such as interleukin 1-beta (IL-1 beta) and interleukin 6 (IL-6).

Materials and methods

We searched the Medline and Google Scholar databases for articles published in English language, in the years 2015-2020 using the following keywords: amygdala IL 1-beta, IL6, inflammation. The inclusion criteria was that the article focused on the role of amygdala in the inflammatory process, particularly in the context of anxiety disorders.

Results

The search retrieved 294 articles, 45 of which were included in the review. Most of the data included in this review comes from animal studies.

Characteristics of the amygdala

The amygdala (*corpus amygdolideum*) is a group of nuclei deep in the abdominal part of the temporal lobes of the brain. In terms of functional division, the amygdala is included in the limbic system, the part of the central nervous system that is heavily involved in regulating autonomic, secretory and behavioral emotional responses [5].

The amygdala is a heterogeneous structure in terms of morphology, histochemistry and anatomy. It has a rich system of afferent and efferent connections, both subcortical and cortical. It was proposed to divide the amygdala into two parts: phylogenetically older cortico-medial region and phylogenetically younger basolateral-lateral region [6]. According to this division, the cortico-medial part consists of the following nuclei: the middle, medial, cortical and the nucleus of the lateral olfactory tract. On the other hand, the posto-lateral area consists of the basal nucleus, which includes the large and small-cell nucleus and the lateral nucleus. The medial cortex has a stronger connection with the lower levels of the nervous system, which are mainly responsible for triggering autonomic and emotional responses. The basolateral part has stronger links with the higher parts of the nervous system with a rather inhibitory or modulating effect on the motivational behavior of animals [7-8].

Within the amygdala, the nuclei receiving the afferent fibers are located mainly laterally and are usually referred to as the lateral nuclei. Thus, afferent fibers can be divided into cortical and subcortical. The subcorticals are especially important in infancy and childhood, when the amygdala grows faster than the hippocampus. All the associative sensory regions have direct access to the lateral amygdala nucleus. These regions are also connected to the prefrontal cortex by long bundles of associative fibers, giving conscious sensations and objects a cognitive assessment. The activity of the visual association cortex is particularly important in connection with the clinical problem of phobias and anxiety states. The V4 field is connected to the object and face recognition path. The V5 field leads to the motion detection path. Both contact the amygdala via the hippocampus, and therefore current events can evoke fearful memories. The orbital part of the prefrontal cortex of the right hemisphere is usually active along with the right amygdala in stressful situations. The back of the insula has direct access to the amygdala and is likely to be associated with emotional pain assessment. The activity of the Meynert's nucleus increases during the feeling of anxiety, influencing the increase in the autonomic activity that involves the amygdala [7-8].

The afferent fibers from the amygdala run in the form of an marginal line. The second exit projection is the medial discharge pathway, which runs medially and ends in the nucleus accumbens. The amygdala sends projections to the periaqueductal gray (PAG), which is the source of spinal pain suppression [7-8].

An important part of the research on the functional organization of the amygdala concerned the role of the central nucleus and the basolateral part in the mechanisms of classical conditioning of fear reactions. Based on the work on selective damage to the amygdala, it was noted that it is the main recipient of sensory information in the amygdala [9-10] in the area of auditory and contextual stimuli [11-15] The results obtained with the use of selective injury techniques and with the use of electrophysiological methods showed that the basolateral part of the amygdala, espe-

cially the lateral nucleus, is the site of association formation between conditioned and unconditional stimuli during the acquisition of the classic fear response [16-18].

Limbic structures such as the interventricular septum area and the amygdala [1] are particularly associated with emotionally colored behaviors. Damage to the interventricular septum causes the emotional hyperactivity syndrome [19-20]. In humans, stimulation of the amygdala results in fear, depression, feelings of sadness, and disgust, while complete removal of the amygdala reduces fear and aggression. In animals, cortico-medial lesions of the amygdala lead to apathy accompanied by aphagia and adipsia, and basolateral lesions of the lateral amygdala counteract hyperphagia and an increased level of fear [21].

Cytokines as a humoral "feedback" pathway for the immune system to influence the central nervous system

PAMPS (pathogen-associated molecular patterns on infectious C) are a small molecular motifs conserved within a class of microbes that can be found present on the outside (bacterial flagellin) or inside (unmethylated CpG motifs) [22]. They are associated with specific areas recognized on the surface of neutrophils, dendritic cells or macrophages [23]. In this reaction, pro-inflammatory cytokines are released, often referred to as "immuno-transmitters" [24], and they play an important role in initiating the peripheral acute phase of the response [25-26] and activating subsequent immune cells. Toll-like receptors (TLRs) belong to a class of pattern recognition receptors (PRRs), that identify microbes, which infect human organisms by recognizing PAMPs [22]. TLRs are the most studied PPRs. They are significant modulators of the innate immune response, as they explore the intracellular and extracellular space. The localization of TLRs explain their important role in recognizing potential danger [22].

The pro-inflammatory cytokines entering the brain include interleukin 1 alpha and beta (IL-1 α and β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α). Dinarello [26] proved that administration of IL-1 β causes an increase in body temperature, while the blockade of this molecule and its receptors significantly reduces fever induced by administration of LPS. The action of IL-1 β is likely crucial in communication between the immune system and the brain [26]. The transmission of signals from the blood to the brain takes place humoral, which is indicated by the increased level of pro-inflammatory cytokines in the blood, induced by the presence of antigen. However, according to some researchers, the problem may be the relatively large size of cytokines (e.g. IL-1 β - 17.5 kDa), as well as their hydrophilic nature, which makes the passage of these molecules through the blood-brain barrier unlikely by passive diffusion [26].

Possible sites for the transition of cytokines to the parenchyma of the brain are places where the blood-brain barrier is weakened, especially in the periventricular area: the endplate vascular organ, the subfornical organ, the median prominence, the distal field and the vascular plexus. The presence of receptors for pro-inflammatory cytokines, especially IL-1, IL-6 and TNF-α, has been confirmed in many structures of the brain, mainly in the hypothalamus, pituitary gland, amygdala, septum, bed nucleus of the stria terminalis, hippocampus, thalamus, as well as in the ventral and dorsal striatum, pons and cerebellum [27-30] IL-1 β, crossing the blood-brain barrier in the periventricular area or the vascular plexus, stimulates the macrophage-like cells present there to produce IL-1 β, which again can induce the transcription of IL-1 β mRNA in nearby brain cells, e.g. glial and thus spread to further regions of the brain with the possibility of activating other neurons [26, 31]. In addition, it has been shown that IL-1 β produced in the brain can pass into the cerebrospinal fluid or travel through the interstitial space and along fiber bundles [26, 32].

IL-6 plays an important role in physiological homeostasis of neural tissue and in the pathogenesis of inflammatory disorders such as multiple sclerosis, Alzheimer's and Parkinson's disease [33]. It is significant in the regeneration of peripheral nerves and differentiation of oligodendrocytes. IL-6 acts on B-cells, T-cells, hepatocytes, hematopoietic progenitor cells. It can be also secreted by immune system cells - B-cells, T-cells, macrophages, microglia and non-immune cells, e.g. adipocytes, fibroblasts, endothelial cells, neurons [33]. Absence of IL-6 may cause reduced glial activation in traumatic brain injury and changes in sleeping behavior. Overproduction of this cytokine in brain leads to neurodegeneration [33].

In the central nervous system (CNS) pattern-recogntion receptors are expressed by microglia, macrophages and astrocytes [34]. Microglia and macrophages in CNS include multimolecular complexes called inflammasomes. This important protein complex "send a massage" from innate immune system to pathogenic stimuli. An inflammasome causes the activation of caspase 1 and consequently to release of pro-inflammatory cytokines, that lead to inflammatory response [34].

The answer to the LPS in blood-brain barrier (BBB) is available thanks to presence of TLR-4 and other toll-like receptors, which they occur on the membranes of the cells BBB [35]. These particular cells have receptors for chemokines, cytokines and other molecules connected with immune system [35]. The most studied effect of LPS on the BBB after disruption is that of alterations in BBB transport system. Many substances with alterations in their brain/blood ratio is thought to be caused by alterations in the transporters for those substances [35].

Another behavioral role is observed in patients with neoplastic diseases as well as those of viral or bacterial origin.

This set of behavior typical of disease errors is commonly referred to as "sickness behavior" [32]. Subjective feeling of impending illness manifested by malaise, weariness, feeling of cold and numbness, pain in joints and muscles, lack of appetite are very well known to everyone who has experienced a viral or bacterial infection. The psychological and behavioral elements of "behavioral disease" together with the onset of fever and neuroendocrine optimization present an outstanding strategic strategy in the struggle with the disease. Due to their prevalence, these symptoms are often ignored by doctors. These symptoms are often perceived as bothersome, but rather trivial processes with special pathogens affecting the sick person. Peripheral immune activation induces the synthesis of pro-inflammatory cytokines in microglia and macrophages: IL-1 α and IL-1 β, IL-6 and TNF-α. Both peripheral and intraventricular administration of these cytokines causes acute phase symptoms, fever, activation of the hypothalamic-pituitary-adrenal axis and depression.

The "sickness behaviour" syndrome unquestionably proves the relationship between the neuroendocrine and immune systems. This interaction plays a key role in maintaining homeostasis. Understanding the relationship between the neuroendocrine and immune systems is an interstate psychosomatic as well as pathophysiological and pathological basis.

Knowledge of neuroimmunomodulation is extremely important in immunotherapy. Behavioral changes must be made when cytokine therapy is used, e.g. in response to long-term IFN-α, human behavior is "depressed" and hyperalgesic.

Influence of cytokines on amygdala

It is known, that cytokines have impact on affecting fear and anxiety-related structures: amygdala, insula, anterior cingulate cortex [2]. The effects of inflammation and cytokines on previously mentioned regions produce adaptative and beneficial behavioral responses. Researchers suggest that not only does inflammation increase amygdala responses to stress are connected with increased production of inflammatory cytokines [2].

Harrison et al. showed that administration of typhoid vaccination increased IL-6 and also induced behavioral changes and intensify activation of amygdala [36]. High sensitivity in amygdala regarding stress may result in higher inflammatory cytokine production. It is important, when we think about anxiety and post traumatic stress disorder (PTSD) symptoms if amygdala activity is able to create a feed-forward effect of inflammation on neural circuitry [36, 3]. The medial prefrontal cortex is connected to the amygdala. It is said, that these structure is involved in fear extinction and emotional regulation in PTSD. A study using typhoid vaccine as an trigger of inflammation, reported reduced task-related functional connectivity of the subgenual anterior cingulate cortex to the amygdala, nucleus accumbens and superior temporal sulcus, which correlated with the IL-6 concentration in the peripheral blood. Insula is also associated with amygdala and play significant role in emotional distress in anxiety disorders and PTSD [2-3, 36] It is thought that enhanced sensitivity of the insula to the inflammatory cytokines in the periphery, especially in the presence of emotional stimuli may cause altered neural circuitry involving amygdala, medial prefrontal cortex, anterior cingulate cortex to show symptoms of anxiety, fear, emotional disturbance [2-3, 36].

Munshi et al. [37] studied, how repeated social stress impacted the peripheral immune balance. The experiments were performed on Long-Evans and Sprague-Daweley rats. Authors indicated, that chronic stress increased pro-inflammatory cytokines in serum. They indicated, that activated microglia secreted pro-inflammatory cytokines, that generated immune signals within brain [37]. It could then precipitate in the physiological and behavioral responses to neuroimmune activation. Authors highlight, that acute peripheral immune activation by IL-1β lead to increase of the basolateral amygdala neuronal activity. IL-1β can increase electroencephalographic activity in amygdala [37]. This cytokine influence on spontaneous action potential-independent transmission of central amygdala GABAergic neurons via pre- and post-synaptic mechanism. Munshi et al. observed significant increase in the basolateral amygdala neuronal firing after the stress, that correlated positively with severity of the stress. It was reduced by blockade of microglia activation [37].

Yang et al. [38] showed the effect of exogenous insulin-like grow factor 1 (IGF-1) on the cognitive dysfunctions caused by acute ischemic stroke in the rat model. IGF-1 regulates postnatal growth thought mediating effects of growth hormone. It also take part in axon guidance, cell adhesion, differentiation, plasticity. Their study showed [38] that intravenous injection of IGf-1 essentially enhanced the reduced IGF-1 concentrations in the plasma and ischemic brain tissues, hippocampus, amygdala, cortex of ischemic rats in a dose-dependent manner compared with no injection model rats. The results of the Yang's team [38] indicated that intravenous IGF-1 injection decreased cerebral infraction and brain edema in ischemic rats. ELISA assays demonstrated that IL-6, IL-1β and TNF-α in ischemia serum was higher than in sham group, while the administration of IGF-1 decreased the levels of this cytokines in comparison to the model group. These data [38] showed that supplementation of IGF-1 could exert anti-inflammatory effects on ischemic rats. Researchers [38] indicated that, IGF-1 support the neuroprotective effects trough inhibiting the neuroinflammation. They demonstrated [38] that the exogenous administration of IGF-1 improved the neurological dysfunction and cognitive deficits, decreased cerebral infraction and brain edema. It also relieved the systemic and cerebral inflammatory response.

Li et al. [39] showed, that treatment of combined Traumatic Brain Injury (TBI) and hemorrhagic shock and resusci-

tation rat model (HSR) with CORM-3 (a water-soluble carbon monoxide), reduced the impairments of depressive and anxiety-like behaviors post- trauma trough activation of PKG-ERK1/2 signal pathway. The animal model proposed in this study [38] induced depression and anxiety-like behaviors – TBI+HRS rats showed reduced center entries, lower rearing/leaning scores, less time in grooming in open field test compared to sham-treated groups. Researchers indicated [39], that the mean cerebral blood flow arterial spin labeling in the amygdala of the TBI+HRS treated rats was significantly downregulated relative to the Sham group. Their study [39] demonstrated, that trauma significantly attenuated cerebral blood flow and induced vasogenic edema in the amygdala. Animals treated with TBI+HSR showed exposure perturbed the amygdala by induction of significant neuronal apoptosis and pyroptosis in this region of the brain. Authors [39] indicated that neuropsychiatric alterations and neurological dysfunctions post TBI+HRS could be induced by neuronal pyroptosis and apoptosis. The administration of CORM-3 improved the results of TBI+HRS rats than control group in the open field test and elevated plus-maze. Animal from the test group showed improved vasogenic edema and cerebral blood flow in the amygdala. CORM-3 could improved neurological dysfunctions and the mechanism could be involved with improvements of neuronal degeneration in the amygdala. Protein kinase G (PKG) pathway caused the induction of antiapoptic proteins – ERK1/2. It is known that activated PKG promoted an elevation of phosphorylated ERK1/2 in ischemic disorder and neurological degradation. Authors [39] showed that CORM-3 increased levels of phospho-ERK1/2 while KT5823 - pharmacological inhibitor partially reversed the upregulation of phospho-ERK1/2 induced by CORM-3. Scientists demonstrated, that PKG-ERK1/2 signaling pathway could mediate the neuroprotective effects of CORM-3 in rats with TBI+HRS damage [39].

Anesten et al. [40] studied possible interactions between IL-6 and glucagon-like peptide 1 (GLP-1) in central amygdala (CeA). GLP-1 is a post-translational proglucagon product. It is a neuropeptide produced peripherally in ileal L-cells of the intestine and preproglucagon neurons of the nucleus of the solitary tract [40]. Production of GLP-1 is responsible for increase of insulin release and also decrease of glucagon release. Researchers proved that IL-6 was present in approximately 40% of GLP-1R cells in CeA. PPG-fibers appear to synapse with IL-6-immunoreactive cells in this nucleus [40]. In their study about 50% of the neurons in the CeA expressed the ligand binding receptor IL-6Rα. Anesten et al. proved that treatment with IL-6 to CeA overnight fasted mice reduced food intake [40].

Xu et al. [41] indicated that resistant stress mediated time-dependent alterations in the permeability of the BBB, with the modifications in the expressions of the proteins from tight junctions and adherens junctions and also ultrastructural changes in brain microvascular endothelial cells. They indicated showed that resistant stress could induced damage of the BBB in the amygdala [41]. Ultrastructural findings demonstrated detached endothelial cells, defective tight junctions, edematous astrogial endfeet, malformed capillary lumen in the resistant-stressed animals, it proved that resistant stress could induce morphological changes of the BBB in the amygdala [41].

Mehta et al. [42] showed that inflammation negatively correlates with amygdala-ventromedial prefrontal functional connectivity in association with anxiety in patients with depression. The right amygdala is more involved in fear conditioning. They claimed that patients with PTSD during the experiment had higher amygdala reactivity and also decreased right amygdala to left vmPFC functional connectivity in association with symptoms of hyperarousal [42]. They suggest that inflammation might play a role in behavioral symptoms of patients who are more reactive to stress or trauma. At the same time prolonged and exaggerated release of interleukins may feedback on and cause weakness of circuits that drive the symptoms of PTSD, anxiety, depression [42].

Munshi et al. [43] in their studies demonstrated how peripheral inflammatory state affects the activity of the basolateral amygdala (BLA). They showed a link between BLA neuronal firing and triggering of behavioral result of peripheral inflammation. It lead to production and release of IL-1 beta, IL-6, TNF-α [43]. Pro-inflammatory cytokines created features of sickness behavior and depressed mood. Some of these symptoms might be generated by BLA. Sickness behavior may be reason of the emergence of depressive disorders in vulnerable subjects. Hyperactivation of BLA is often observed in patients with depression.

Conclusions

Behavior and immune system, mediated by the endocrine and nervous system create a psychoneuroimmunology. From fever to stress, the impact one system on the other allow to help sense danger and to mount an appropriate adaptive response [44].

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Conflicts of interest

None.

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REVIEW ARTICLE

Cardioneuroablation in neurocardiogenic syncope – hype or hope?

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Abstract

We are presenting the ablation of parasympathetic ganglia in the atria as a new method of treatment of vasovagal and other neurocardiogenic syncope. This method, shifting the balance of the autonomic nervous system in the sympathetic direction, is directed to the immediate cause of syncope which is excessive activation of the vagus nerve. Its effectiveness in the annual observation is within 80-100%. This method offers a great chance to improve the quality of life in patients with reflex syncope what have not been prevented by conventional treatment.

Keywords: atrial fibrillation · vasovagal syncope · sick sinus syndrome · cardioneuroablation.

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Introduction

Syncope is defined as total loss of consciousness characterized by a rapid onset, short duration, and spontaneous complete recovery [1]. Its direct cause is cerebral hypoperfusion [1-2]. There are many reasons of syncope and they are listed in the current ESC guidelines [1]. The most common, however with the lower risk for the patient is the neurocardiogenic syncope. Although they do not significantly increase the risk of sudden death, episodes of syncope can significantly reduce the quality of life and in some patients may be difficult to treat [3-5]. Neurocardiogenic syncope (also called reflex syncope), is caused by a pathological cardiovascular autoregulation. There are two types of syncope: peripheral and central. In the peripheral type, syncope is caused by prolonged standing. In the central type, the cause of syncope is emotional stress [6].

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Pathophysiology of the reflex syncope

The most typical reflex syncope is vasovagal syncope [1] caused by orthostatic stress associated with decreased venous return, resulting in insufficient filling of the ventricles and reflex increase in their contractility. This causes activation of the left ventricular mechanoreceptors that send an impulse via the vagus nerve to the vasomotor center in the medulla oblongata. The efferent pathways consist of the vagus nerve (increased function) to the heart and sympathetic fibers (decrease function) to the heart and blood vessels. The result of this is a sudden and sharp drop in blood pressure and/or heart rate (including asystole), which causes a reduction in cerebral blood flow, leading to the total loss of consciousness [7]. In the central type, the stimulation of centers in the hypothalamus and the cerebral cortex is caused by an emotional stress factor and leads to a vasovagal reaction. [6-8]. Reflex syncope also includes carotid sinus hypersensitivity, excessive orthostatic reaction, and situational fainting, usually occurring in such situations as: coughing, sneezing, stimulation of the back of the throat, micturition, defecation, visceral pain, playing brass instruments, loss of weight or after a heavy meal [1, 4, 8]. Abnormal reactivity of the autonomic nervous system may also predispose to functional disturbances of automatism (some patients with sinus node disease) or atrioventricular conduction [9].

Current treatment methods

Lifestyle modification and non-pharmacological treatment in the prevention of neurocardiogenic syncope are effective in about 80% of patients. The next step is pharmacological treatment, yet there is no clear evidence for its effectiveness [1]. It should also be noted that when choosing a drug, contraindications should be considered, particularly in female patients of childbearing age [10]. Invasive treatment may be considered in patients with refractory syncope or who do not tolerate pharmacological methods. According to the guidelines, in people over 40 years of age with cardiodepressive syncope resistant to the above-mentioned treatment, implantation of a dual-chamber cardiac pacing system may be considered [1].

Genesis of cardioneuroablation

Recently, a new treatment option was introduced: ablation of the atrial parasympathetic ganglia (ganglion plexi – GP) [11-14]. Its genesis was an observation made during ablation of atrial fibrillation [15-16], specifically isolation of the pulmonary veins (PV), that damage of the parasympathetic ganglia (manifested during ablation with bradycardia/sinus asystole or in the course of second- or third-degree atrioventricular block or decrease in arterial pressure; after ablation with an acceleration of sinus rhythm) correlates with higher ablation effectiveness [15]. Hence, the next step was to use targeted ablation of the parasympathetic ganglia to treat reflex syncope [11-14].

Anatomical and histological background

The intrinsic cardiac autonomic nervous system is comprised of an extensive epicardial neural network of nerve axons, interconnecting neurons and clusters of autonomic ganglia, known as GP [17] (Fig. 1). Most of them are located within epicardial fat pads. Ganglion plexi vary in size, contain from just a few neurons to over several hundred neurons [18-19]. The highest density of autonomic innervation

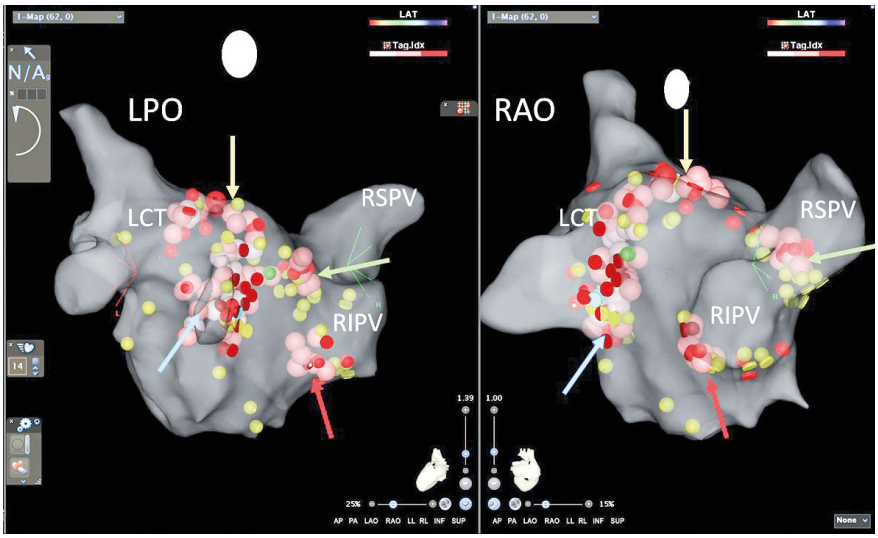

Figure 1. Anatomical CARTO map of the left atrium and pulmonary vein ostia in patient during GP ablation. This patient has anatomical variant with left common trunk. LAO – left posteriori oblique view, LCT – left common trunk, RAO – right anterior oblique view, RIPV – right inferior pulmonary vein, RSPV – right superior pulmonary vein; yellow dots – pacing points without neurocardiogenic reaction, red dots – pacing points with sinus bradycardia induction, green dots – pacing points with vasodepressive reaction, blue dots – pacing points with mixt-type reaction, brown/pink/white – dots – ablation point; red arrow – right inferior GP, green arrow – right superior GP, blue arrow – left inferior GP, yellow arrow – left superior GP

is found at the posterior wall of the left atrium, particularly at the pulmonary vein–atrial junction [18]. The most important anterior right GP is located immediately anterior to the right superior PV and often extends inferiorly, to the region anterior to the right inferior PV. Other important are the superior left GP located at the roof of the left atrium, medial to the left superior PV, the right and left inferior GP located at the inferior aspect of the posterior wall of the left atrium, below the right and left PVs [20].

Cardioneuroablation – challenges for clinicians and electrophysiologists

Cardioneuroablation is a relatively new technique for managing patients with vasovagal syndrome, hence there are no clear indications and contraindications for this procedure [1]. There are many different criteria to qualify patients and many techniques of the procedure, and we need some time to determine in which clinical situations what type of the procedure is extremely effective, beneficial or not at all. So far, several studies were carried out to prove that cardioneuroablation is an effective and safe method of treating vasovagal syndrome [11-14]. The first clinical results are very promising. However, because of the small size of the treatment groups, short observation period and the lack of control groups, the available data are insufficient to confirm the efficacy and safety of ablation of the atrial ganglion plexi [1]. The longest follow-up was published by Sun W. et al. [21] with total cohort of 57 patients over the course of 12-102 months. Syncope and syncope prodromes recurred in 5 and 16 participants, respectively [21]. Because of small study group there were no statistically significant differences between group with ablation based on high-frequency stimulation and based on anatomy. However, all 5 recurrences of syncope were in anatomical group [21]. The time to first recurrent syncopal episode after ablation ranged from 2 to 17 days [21] and such early recurrence suggests that anatomical approach ablation was not enough in some patients. Further studies, particularly randomized multicenter trials, should be performed to assess the long-term effects of the GP ablation strategy in neurocardiogenic syncope [1]. Similar results with 34.1 ± 6.1 months of follow-up were published by Calo L. et al. [22] according to ablation of the right atrial GP only. During this time only 3 of 18 patients (16.6%) experienced syncopal episodes and 5 patients (27.7%) only prodromal episodes (including during the head-up tilt test).

Contrary to pharmacotherapy or pacemaker implantation, ablation aims to get to the source of the problem: disturbances in the intrinsic cardiac autonomic nervous system [17, 23]. The main goal of cardioneuroablation is parasympathetic denervation of the heart (more precisely, shift of the autonomic balance into the sympathetic direction) to treat reflex (neurocardiogenic syncope) or functional (sinus node dysfunction and functional atrioventricular block) bradycardia/asystole [11].

In contrast to the permanent cardiac pacing, the effectiveness of this method is not limited only to cardiodepressive syncope [11, 14, 24], but is also effective in mixed-type of syncope [13, 21, 25]. There are no data on the efficacy of cardioneuroablation in vasovagal syndrome of the vasodepressive type. Theoretically, the chances for success are much lower here, as the effector of this form of syncope is mainly in the vessels. However, it has been shown that the same patient can have various forms of vasovagal syncope (e.g. some episodes vasodepressive, while others cardiodepressive or mixed) [3, 26]. Also, during the isolating pulmonary veins we sometimes observe a blood pressure reaction only, so the pathophysiology of cardioneuroablation in this group is ambiguous.

In most centers, the atropine test is performed prior to cardioneuroablation and only patients with a correct response to the drug are qualified for the procedure [11, 14]. The theoretical basis for this way of proceeding seems to be very strong. However, the question about the dose of atropine remains open (an insufficient dose may lead to a negative test result). Besides, cardiodepressive syncope has an effector in the heart, so in theory it should be sensitive to ablation in all patients. At present it is difficult to predict which treatment approach is appropriate.

Cardioneuroablation – methodology

In order to find GP ganglia and identify the targets of ablation, electroanatomical maps of the right atrium (RA) and left atrium (LA) are created. High-frequency stimulation or anatomical site selection or characteristics of the atrial electrograms can be used to select the site for GP ablation. Various combinations of these methods can be used [14, 21-25]. In our center, we prefer mapping with pacing (100 ms cycle length) (Fig. 2A, 2B). If there is no response to stimulation (we observe this in about half of the patients), we perform ablation using the anatomical method observing the heart rate and pressure response to the applications. If during applications we observe the reaction from GP, we prolong it to obtain a permanent effect [24-25].

Ganglion plexi ablation shifts the balance of the autonomic system towards the sympathetic side. This is related to the improvement in the parameters of automatism and conduction, also observed in our patients [11-14, 24]. In some cases, excessive sinus tachycardia is observed, requiring temporary treatment with a beta-blocker, occasionally in combination with ivabradine (our experience) [24]. Usually, this drug therapy is temporary (few months) because the heart rhythm slows down during follow-up, although it is not as strong as it was before the procedure (probably partial reinnervation) [12, 21, 24]. In study by Sun W. et al [21] the heart rate variability and heart rate demonstrated significant changes at 3 months that persisted at 12 months after the procedure. Compared with the baseline measurement, the time- and frequency-domain heart rate variability was significantly lower (except

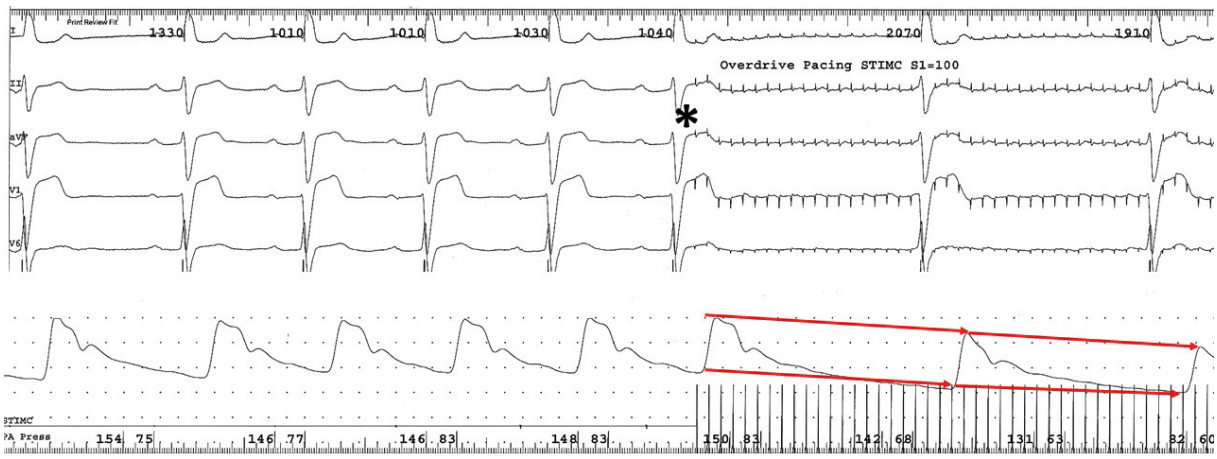

Figure 2A. High frequency pacing (CL 100 ms) of GP with mixt type naurocardiogenic reaction. Beginning of pacing marked with the star. After that there is a significant decreasing of sinus rhythm frequency. Blood pressure line present decreasing of the systolic and diastolic arteriar pressure (red arrow)

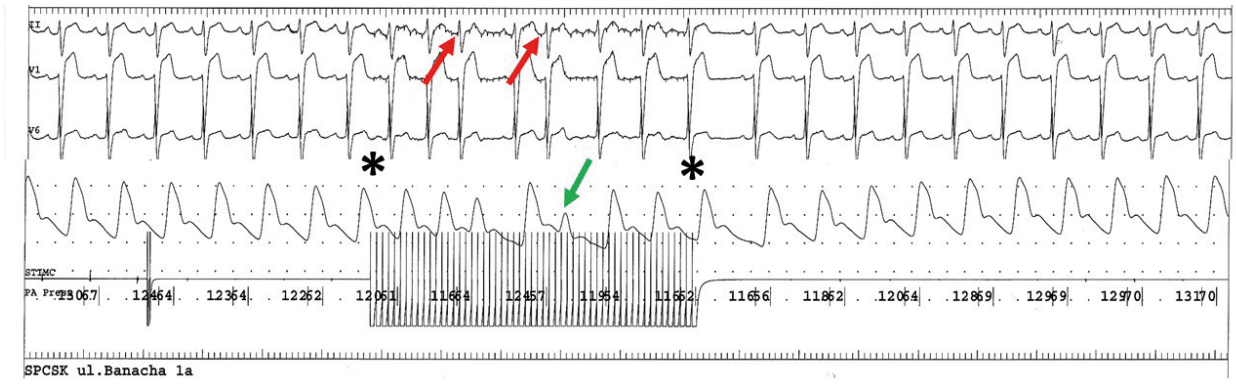

Figure 2B. High frequency pacing (CL 100 ms) of GP region after ablation without important decreasing of sinus rhythm frequency and blood pressure. Stars indicate beginning and termination of pacing. There are two supraventricular extrasystolies induced by pacing (red arrows). After the second one we can observed pulse wave deficyt (green arrow).

at low frequency), whereas the minimum, mean, and maximum heart rates were significantly higher ($P < 0.01$). After an average period of 28.7 ± 9.8 months after ablation, only the minimum heart rate remained higher than before the ablation ($p = 0.022$). This also confirms partial reinnervation hypothesis [12].

Cardioneuroablation – outcomes

At present, the effectiveness of GP ablation in preventing recurrence of vasovagal syncope is within the range of 80-100% [11-14, 21, 23]. In order to assess the direct effectiveness of the procedure, it is worth considering several endpoints, defined as an increase the frequency of the sinus rhythm or a decrease in the degree of atrioventricular (AV) block [12, 14] or lack of response to stimulation in places where it induced a neurocardiogenic reaction [12, 21, 24-25]. It may also be useful to record the lower variability of automatism and conduction parameters observed in patients with vasovagal syndrome [27]. We can also receive valuable information from HRV analysis based on 24-hours ECG Holter monitoring [28]. Performing a control head-up tilt tests raises doubts. Due to the fact that this tests provokes reflex reaction in a very aggressive, exceeding the real-life conditions manner, it is not recommended to assess the effectiveness of any form of treatment of vasovagal syncope [1].

In most studies, however, the control head-up tilt test is performed because for research purposes. Changes in test results that were interpreted as a benefit of cardioneuroablation include a change in the detected hemodynamic response to a vasodepressive form or a completely negative test result, as well as an increase in the time of symptoms or syncope onset [17]. The behavior of hemodynamic parameters during upright standing may also be helpful in the assessment [29-23].

Summary

At present, cardioneuroablation is still an experimental method, although it has a strong pathophysiological basis and much indicates its high effectiveness. For this reason, it should be reserved for patients with multiple syncope, especially traumogenic, which cannot be prevented by lifestyle modification. The effectiveness of this method has been documented in patients with cardiodepressive and mixed syncope.

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Conflicts of interest

None.

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REVIEW ARTICLE

Urolithins and their possible implications for diabetic kidney

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Abstract

The increasing prevalence of diabetic kidney disease (DKD), a common complication of type 1 and type 2 diabetes, is becoming a leading risk factor of developing end stage renal disease (ESRD). The multiple mechanisms involved in renal tissue damage are a challenge for effective targeted therapy. Urolithins are metabolites generated by gut microbiota upon dietary intake of plant-derived ellagitannins. Multidirectional effects of these compounds include their anti-inflammatory, antioxidant, anti-proliferatory, anti-migratory and antiglycative properties that are mediated by modulation of signaling pathways and gene expression. Biochemical properties of urolithins indicate their capacity to regulate numerous mechanisms responsible for developing the hyperglycemia-induced tissue injury. The potentially beneficial effects of urolithins on podocytes, the most vulnerable renal cells should be particularly considered. The purpose of this review is to provide the evidence from the *in vivo* and *in vitro* studies showing that urolithin-based therapy could be a useful tool for protecting the kidneys from damage in diabetes.

Keywords: urolithins · diabetes · kidney · podocytes

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Introduction

Urolithins (hydroxylated dibenzo [*b,d*]-pyran-6-one derivatives) are a family of bioactive compounds which were first isolated from beaver scent glands in 1949 [1]. Their presence in human and animal intestines is a result of bacterial metabolism in gastrointestinal tract of dietary ellagitannins (ETs) and their constituent, ellagic acid (EA) [2]. ETs and EA are naturally occurring polyphenols found in numerous fruits and vegetables, e.g. pomegranates, raspberries and nuts [3]. Their beneficial health properties including anti-inflammatory, antioxidant and antiproliferative effects

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have been proven in both animal and human models. Various studies demonstrated the protective effects of ETs and EA against chronic diseases of the cardiovascular system, neurodegenerative diseases, diabetes and cancer. However, ETs and EA have poor bioavailability and their biological activity is associated with urolithins that are more easily absorbed in gut [4-8]. The synthesis of urolithins depends on the composition in the gut of specific bacteria that varies between the individuals. The identification of the microorganisms responsible for the complete transformation of EA into the final urolithins is still under research. According to the recent findings, different numbers of *Synergistetes phylum* and members of *Coriobacteriaceae* (genus *Gordonibacter urolithinfaciens* and *Gordonibacter pamelaeae*) and *Lachnospiraceae* families can be used to discriminate between individuals producing certain urolithin forms [9-10]. Thus, after absorption and passage through the liver, different urolithins can be found in human body fluids and tissues at nanomolar to micromolar concentrations. Absorbed urolithins undergo phase I and phase II metabolism, resulting in glucuronide, sulfate and methylated derivatives, while small amounts can be found in the form of free aglycones [2, 11-12].

Both the conjugated and unconjugated forms of urolithins can be detected in human plasma and urine even 48 hours after consumption of ET-rich food [6, 13-14]. It is clear therefore that bioactive urolithins directly contact renal tissue during the passage through the nephron. It seems plausible that the cells constituting the glomerular filter, as well as epithelial cells lining the urinary tract may be affected by these compounds. Antioxidant, anti-inflammatory and antimicrobial properties of these compounds could be beneficial in treating several renal diseases, including diabetic kidney disease (DKD) [15-16]. Nevertheless, so far there are not many data on the effects of urolithins on the renal tissue. The purpose of this review is to provide the evidence from the *in vivo* and *in vitro* studies showing that urolithin-based therapy could be a useful tool for protecting the kidneys from damage in diabetes.

Material and methods

We searched the Medline, Scopus and Science Direct databases for articles published from 2011 to April 2021 using the following keywords: kidney disease, urolithins, ellagic acid, elagitannins, podocytes, polyphenols, diabetes, nephropathy, transforming growth factor and relevant abbreviations (e.g. TGFβ, CKD, DN). Older articles describe pathomechanisms and serve as context for the presented new information. The inclusion criteria were: full-text article, on-topic. Case reports and letters to the Editor were excluded from the review.

Results

Bioavailability and metabolism

Similarly to other phytochemicals, ETs are poorly absorbed in the gut. However, following consumption the ETs undergo spontaneous hydrolysis into EA in the upper gastrointestinal tract. Microbes residing in the intestine further transform EA yielding a series of bioactive compounds including urolithins that are characterized by a dibenzopyranone structure and a decreasing number of phenolic hydroxyl groups (Figure 1) [4, 17-18]. Due to their high lipophilicity, urolithins are much more readily absorbed than the original polyphenols.

After absorption in the gut, urolithins rapidly undergo metabolism by phase II enzymes in enterocytes [8, 19]. Once in the bloodstream, conjugated and unconjugated metabolites reach liver via portal circulation and are further subjected to phase II metabolism in hepatocytes. Due to enterohepatic recirculation, part of urolithin conjugates can be secreted with bile back to the small intestine [5, 20]. Analyses of human and animal blood and urine samples indicate that urolithins A and B and their glucuronide and sulfate conjugates are predominant urolithin isoforms. In particular, Uro A is considered to be a major metabolite in humans. However, depending on the study design, the measured final plasma concentrations vary substantially reaching 0.1–35 μM [4,8,15]. Human and animal studies have shown that urolithin metabolites accumulate in the gall bladder and urinary bladder [21], prostate gland [22-23], colon and intestinal tissues [22], whereas no accumulation in other tissues (e.g. muscles, adipose tissue, heart, liver, kidney) was observed [21].

Individual variability in the composition of gut microbiome results in significant differences in ET metabolism and urolithin type production, which is defined as different metabotypes [8]. Specific gut microbiota profile or illnesses generating dysbacteriosis may contribute to different bacteria composition and levels, consequently leading to various potential health effects [24-26]. Therefore, it is essential to consider the influence of factors such as age, gender, race, health condition and geographic origins on the polyphenol profile after consumption of ETs-rich products. Based on the amount and type of urolithins excreted in the urine from healthy volunteers, three different urolithin metabotypes have been described: metabotype A (only Uro A metabolites excreted), metabotype B (Uro B and/ or Uro B metabolites excreted in addition to Uro A and isourolithin A) and urolithin metabotype 0 (no urolithins/ urolithin metabolites excreted) [13]. Significant interindividual variability was also reported in the first pharmacokinetic study, that showed for the first time that EA bioavailability was not increased after intake of a high free EA dose. It was concluded that factors such as pH and food protein content have a strong impact on EA bioavailability [19].

Biological activity

The direct biological effects of urolithins have been examined in different cell models, such as various cancer cell lines, fibroblasts, immune, endothelial and epithelial cells [4, 27-28].

The studies on the chemopreventive potential of EA and its metabolites Uro A and Uro B revealed that their anti--tumor properties, including the influence on cancer cell apoptosis and proliferation involve alterations in the expression of genes involved in signaling (MAPK) pathways, oncogenes (K-Ras, c-Myc), suppressors (DASP6, Fos), p53 protein, growth factor receptors (FGFR2, EGFR) and multiple genes involved in cell cycle [11, 29-33]. EA, as well as urolithins (and principally Uro A) exhibit proapoptotic activity via caspase-dependent pathways, in which activation of caspases 3, 8 and 9 has been reported [34-35]. Inhibition of cancer growth is mediated by suppression by Uro A and Uro B of Wnt/ β-catenin signaling [36-37].

Moreover, anti-tumor and anti-metastatic effects of urolithins include inhibition of migration of cancer cells by diverse mechanisms such as suppressing the K-ras/HMGA2 expression or by decreasing actin polymerization [38-39].

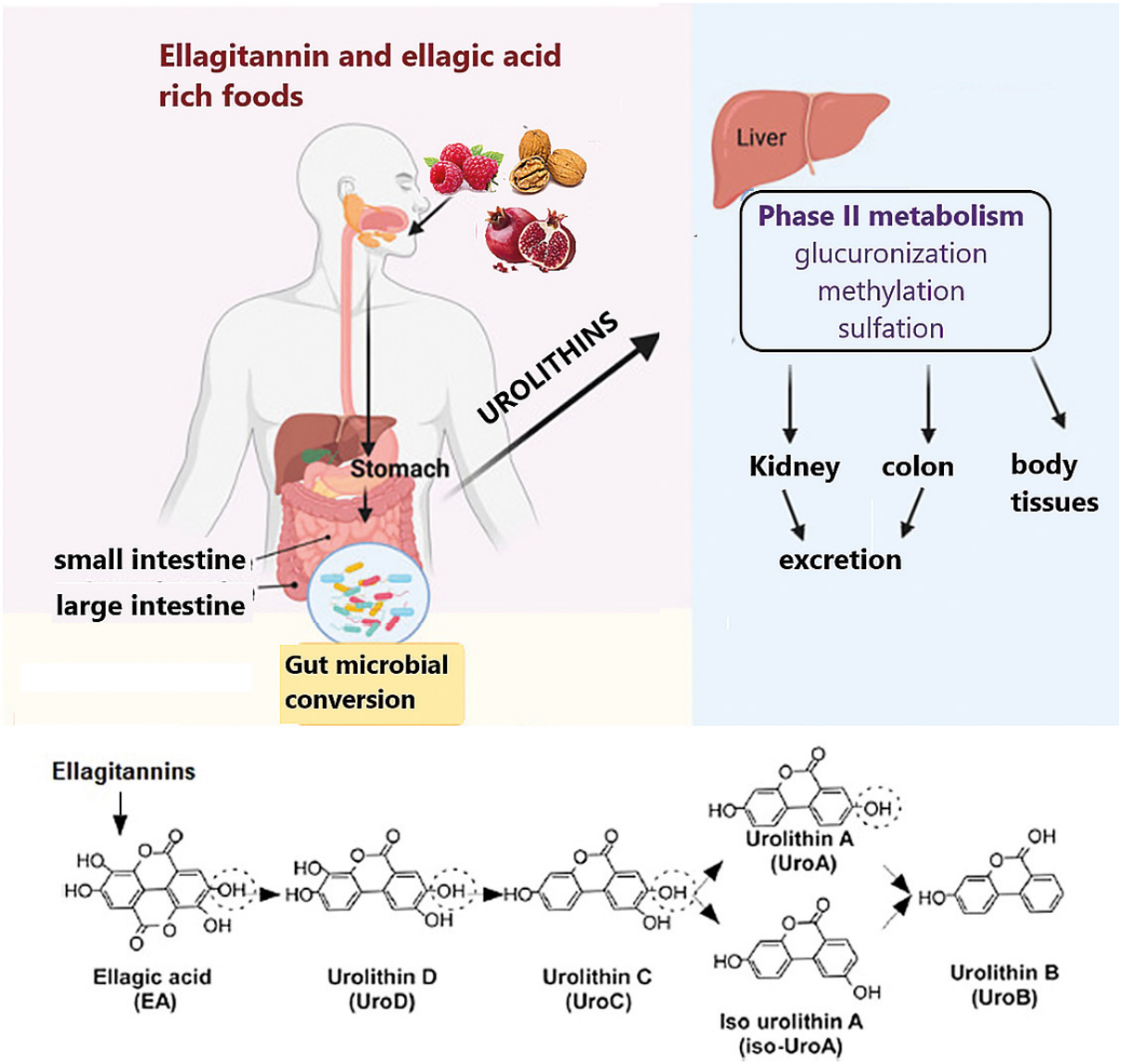

Figure 1. Dietary elagitannins and ellagic acid are converted by the gut microbiota to urolithins that are readily absorbed to the bloodstream. Most of circulating urolithins undergo II phase metabolism in liver and the conjugates as well as free aglycones reach various peripheral tissues. Urolithins from filtered plasma pass the nephron to be excreted in urine. Some urolithin metabolites are also excreted in faeces [18, 75]

Suppression by urolithins of cell motility accounts also for the mechanisms of their anti-inflammatory activity. By moderate down-regulation of chemokine C-C motif ligand 2 (CCL2), plasminogen activator inhibitor-1 (PAI-1) and decreased expression of IL-8, Uro-A and Uro-A glucuronide inhibited induced by TNFα monocyte adhesion and human fibroblast and aortic endothelial cell migration [40]. The anti-inflammatory and antioxidant activities of Uro A and Uro B involve inhibition of the nuclear factor kappa-B (NF-κB) pathway and modulation of phosphorylation of diverse kinases such as AMPK and MAPK pathway members [41]. In the IL–β1 stimulated rat chondrocytes and in LPS-stimulated macrophages, Uro A pre-treatment inhibited NF-κBp65 translocation into the nucleus [42-43], while in the HepG2 hepatic carcinomas cell line, Uro A decreased p65 expression and increased the activity of intracellular antioxidant enzymes SOD and GSH-Px [41]. Suppression of NO production, decrease of proinflammatory molecules such as TNF-α, IL-6 and IL-β at the mRNA and protein levels was observed in the presence of Uro B in the LPS-activated mouse microglial cells and in J774 murine macrophages stimulated with LPS [28,43]. Another study demonstrated that Uro A, by regulating miR-10a-5p, reduced the proliferation of murine CD4+ T cells, that are a trigger to immune response [17]. Furthermore, Uro A exhibited anti-inflammatory effects by directly binding the aryl hydrocarbon receptor (AHR). Acting as a selective AHR antagonist, Uro A inhibited its transcriptional activity which resulted in attenuating cytokine-induced inflammatory signaling in Caco-2 cells [3].

Table 1. Summarizes diverse biological effects exerted by urolithins

Biological activity	Cell line	Signaling pathway	References	Urolithin	Urolithin Effect
Anti-tumor (anti--proliferative, anti--metastatic, pro-apoptotic)	Caco-2 cells Caco-2 cells Caco-2 cells SW 480 Caco-2 cells HCT-116, Caco-2 cells HCC cells HepG2 Ishikawa cells (ECACC)	MAPK kinase Oncogenes (K-Ras, c-Myc) Suppressors (DASP6, Fos) p53 protein Growth factor receptors (FGFR2, EGFR) Cell cycle genes (CCNB1, CCNB1IP1) Wnt/ β-catenin K-ras/HMGA2 Actin polymerization	[30] [30] [30] [11, 33] [30] [30] [36-37] [35] [38]	UroA, UroB UroA, UroB UroA, UroB UroA UroA, UroB UroA, UroB UroA, UroB UroA UroA	↑/↓ ↑/↓ ↑/↓ ↑ ↑/↓ ↑/↓ ↓ ↓ ↓
Proapoptotic	T24 cells, HepG2, Caco-2 cells, SW 480, HT-29	Caspases: 3, 8, 9	[34-35]	UroA, UroB, 8-OMe UA	↑
Inhibition of the cell motility	Human aortic endothelial cells, monocytes, human fibroblasts	C-C motif ligand 2 (CCL2) plasminogen activator inhibitor-1 (PAI-1) IL-8	[40]	UroA UroA UroA	↓ ↓ ↓
Anti--proliferative, anti-renal fibrosis	HK–2 cells	TGF- β1/ Smad NF-κB	[66]	UroB	↓
Anti--inflammatory and anti-oxidant	Chondrocytes, HepG2 T24 cells HepG2 Murine CD4+ T cells Caco-2 cells Murine kidney HK–2 cells	NF-κBp65 SOD and GSH-Px AMPK and MAPK kinases MicroRNA-10a-5p Aryl Hydrocarbon Receptor (AHR) TNF-α, IL-18, IL-23, MIP2 TGF- β1, IL-6, NF-κBp65	[41-43] [41] [41] [17] [3] [63] [66]	UroA	↓
Suppression of NO release	Murine microglial cells, macrophages	TNF-α, IL-6 and IL-β	[28, 43]	UroB, UroA	↓
Autophagy	Murine hippocampal tissue J774.1 murine macrophages N2a cells, primary murine cortical neurons Murine kidney	MicroRNA-34a , SIRT1 Akt/mTOR signaling Endoplasmic reticulum stress TFEB → CLEAR motif-containing genes	[44] [43-44] [45] [46]	UroA UroA UroA UroA	↑ ↓ ↓ ↑

It has been demonstrated that Uro-A exhibits protective properties against brain aging [44] and against ischemic neuronal and ischemia reperfusion renal injury (IRI) [45-46]. These anti-inflammatory and cytoprotective effects include induction by Uro A of autophagic flux, which was confirmed by observed expression of the autophagic markers LC3-II and p62 [43-45]. Some recent studies show that Uro A activates autophagy by upregulating Sirtuin 1 (SIRT1) signaling [44] and by impairing Akt/mTOR signaling [43-44]. Within the kidney, Uro A attenuated IRI by inducing autophagy through activation of transcription factor EB (TFEB) followed by regulation of target genes of Coordinated Lysosomal Expression and Regulation (CLEAR) network [46].

Safety of urolithin administration

Considering that Uro A is the most representative urolithin form and potential therapeutic agent, safety profile of this compound was evaluated in several studies. A comprehensive study by Heilman et al. [47] indicated that in both 28-day and 90-day observations in rats, orally administered synthetic Uro A did not modify any clinical and blood parameters, did not disrupt homeostasis and did not indicate any specific toxic mechanisms. The 4-week clinical trial in which up to 2000 mg oral Uro A doses were administered to elderly volunteers confirmed that the treatment had no adverse health effects. The observed 31 unfavorable effects were determined to be unrelated to the compound tested [48]. On the basis of the above findings, the US Food and Drug Administration already issued a favorable review for using Uro A as a food ingredient [49].

Urolithins in diabetes

Pathophysiology of diabetes is strictly linked to metabolic changes and chronic inflammation. Overproduction of multiple pro-inflammatory cytokines, growth factors and reactive oxygen species (ROS) account for the diabetes- related damage of tissues and organs [50-51]. Thus, antioxidant and anti-inflammatory properties of urolithins may exert a protective role in diabetic state [52]. It was reported recently that while the *in vivo* occurring extensive conjugation severly hampers the activity of urolithins, systemic inflammation triggers tissue deconjugation of Uro A glucuronide, yielding free aglycone with remarkably higher biological activity [53]. Indeed, the *in vivo*, as well as the *in vitro* studies confirmed the beneficial effects of unconjugated Uro A and Uro B administration to diabetic rats. Urolithin injections prevented the early cardiac inflammatory response as well as the occurrence of cardiac dysfunction in the streptozocin--induced (STZ) type 1 diabetes rats [54]. In cardiomyocytes cultured in the presence of 25mM glucose, Uro B significantly reduced the glucose-induced high levels of monocyte chemoattractant protein-1 (MCP-1), the pro-inflammatory cytokine fractalkine and vascular endothelial growth factor (VEGF). In fibroblasts exposed to high glucose, expression of fractalkine was reduced by Uro A, Uro B, Uro C and Uro D [55]. In diabetes, hyperglycemia is also a causative factor for neurodegeneration and development of Alzheimer's disease. Uro A injections in a STZ- induced diabetic mouse prevented mitochondrial ROS accumulation, amyloidogenesis and neuronal cell death, which indicates that Uro A-based therapy may be useful in prevention and treatment of diabetes-associated neuronal impairment [56]. Viability of neuronal cells exposed to oxidative stress was significantly increased in the presence of Uro A and Uro B [57]. In addition, via facilitating L-type Ca2+ channel opening, Uro A and Uro C have been shown to enhance insulin secretion in cultured INS-1 beta-cells and isolated rat islets of Langerhans [58].

One of the hallmarks of diabetes mellitus (DM) is spontaneous non-enzymatic glycation of proteins, lipids and nucleic acids. Dependent on the level and duration of hyperglycemia this process leads to formation of advanced glycation end products (AGEs) that permanently modify protein structures and functions, contributing to oxidative stress and development of chronic diabetic complications [57, 59-60]. Anti glycative properties of Uro A and Uro B were shown in several *in vitro* experiments in which glycation of the bovine serum albumin (BSA) was strongly hampered by these compounds. The effects were concentration-dependent and the mechanisms included urolithins scavenging for reactive carbonyl species [57, 61].

Urolithins in diabetic and non-diabetic kidney disease

Although urolithins do not accumulate in the kidney, the circulating urolithin-rich plasma has continuous contact with renal structures [2, 6, 14], possibly affecting the renal tissue. Indeed, in the experimental rat model based on cisplatin-induced nephrotoxity, Guada et al. revealed that Uro A effectively attenuated kidney damage by inhibiting the inflammatory cascade and apoptosis pathway. Furthermore, anti-inflammatory cytokine IL-10 was markedly increased in the kidneys of Uro A-treated animals [62]. Similarly, in the cisplatin-induced acute kidney injury (AKI) mouse model, orally given nanoparticle-encapsulated Uro A not only reduced mortality but also protected the kidneys from oxidative stress and cytotoxic injury including necrosis, tubular atrophy and glomerular hypertrophy [16]. Also, in another experiment, Uro A pretreatment of mice receiving cisplatin for 3 days not only improved renal parameters but also attenuated oxidative and nitrative stress and downregulated the expression of pro-inflammatory cytokines and chemokines TNFα, IL-23, IL-18 and MIP2 [63] Beneficial properties of Uro A were also documented in the kidney ischemia

reperfusion injury (IRI) in mice. Attenuation of renal injury was associated with Uro A – dependent reduction of pro-inflammatory cytokines TNFα, IL1β, MIP1α and promotion of autophagy [46]. Subcutaneously administered Uro A also increased activity of antioxidant enzymes and attenuated expression of pro-inflammatory cytokines in the kidneys of aging mice [64].

Modulation of the renal TGFβ system

The transforming growth factor beta (TGFβ) family of multipotential cytokines controls numerous physiological and pathological events such as embryogenesis, carcinogenesis and the immune response [15, 65]. Regulation of cell proliferation, differentiation, migration and apoptosis involves ability of TGFβ to affect the transcription and translation processes. The hyperglycemia and inflammation associated with diabetes strongly activate the TGFβ system, resulting in undesirable changes within tissues. In diabetic kidney disease (DKD), overactive TGFβ plays a prominent role in promoting renal cell hypertrophy, fibrosis and stimulating extracellular matrix (ECM) accumulation. So far, urolithins have been shown to counteract the TGFβ-dependent effects in unilateral ureteral obstruction (UUO) rats and in cultured renal epithelial cells. In the UUO model, Uro B treatment abolished renal damage and fibrosis, maintained tubular and glomerular structure and reduced inflammatory cell infiltration. Moreover, expression levels of TGFβ1, NF-κB p65, angiotensin II, collagen IV and several pro-inflammatory factors was significantly reduced. In cultured proximal tubular HK-2 cells, Uro B inhibited stimulated by TGFβ cell proliferation and restored cell morphology. It was demonstrated that in the in vivo, as well as in the in vitro experiments, protective effects of Uro B were related to the down-regulation of TGF-β1/Smad, most likely via inhibition of the NF-κB signaling [66]. Overproduction of the plasminogen activator inhibitor (PAI-1) results in accumulation of ECM in acute and chronic kidney diseases, including diabetic nephropathy. In stimulated by TGFβ renal epithelial NRK-52e cell line significant increase of PAI-1 release was inhibited by Uro A in a dose-dependent manner [67]. The positive feedback loop between TGFβ and PAI-1 has also been documented in diabetic kidney [68]. Hence, targeting the TGFβ- and PAI-1-related pathways by urolithins might be an effective aproach in treating renal fibrosis and inflammation, particularly in the DKD.

Potential effects of urolithins on podocytes

Podocytes are terminally-differentiated, highly specialized cells of epithelial origin covering the outer aspect of glomerular capillaries. Due to their inability to replenish in mature kidney, podocyte loss is believed to initiate irreversible impairment of the glomerular filter [69-70]. Clinical and experimental data suggest a key role of podocytes in the development of diabetic nephropathy (DN) [71]. Podocyte depletion is considered to be the first indicator of glomerular destruction in diabetic patients, even before the appearance of proteinuria [72]. For preventing detachment, podocytes rearrange their structure and migrate to seek attachment in other sites of glomerular basement membrane However, increased migration may disrupt the slit diaphragms between neighboring cells and the tightness of glomerular filter resulting in proteinuria [73]. Recently performed in our laboratory experiments showed that Uro A effectively inhibited induced by high (30 mM) glucose motility of mouse podocytes (unpublished data), which could be beneficial in the diabetic kidney.

Diabetic milieu induces multiple mechanisms in podocytes that directly affect functions and viability of these cells. In addition to AGEs and direct cytotoxic effects of hyperglycemia, primarily via increased production of ROS, deleterious for podocytes is up-regulation of their local renin-angiotensin and TGFβ systems and increased synthesis of VEGF. Moreover, angiotensin II, TGFβ and VEGF reciprocally modulate their production, this way perpetuating podocyte and glomerular impairment [74]. So far, our knowledge on urolithin-mediated effects on the podocytes is very limited. However, it seems likely that similarly to other cells, urolithins in podocytes may regulate the activities of TGFβ, VEGF, antioxidant systems and multiple signaling pathways and kinases that are sensitive to urolithins in other cell types. The urolithins' ability to attenuate the harmful hyperglycemia-induced effects could protect these vulnerable cells from injury.

Conclusions

A growing body of evidence suggests that urolithins are potent multifunctional compounds capable of regulating a variety of cellular processes. Since they do not act through specific receptors, urolithins may affect various cell and tissue types. Not all body tissues accumulate urolithins but all urolithins present in plasma pass the glomerular filter, thus directly contacting kidney cells. However, so far relatively little is known about the effects of urolithins on kidney function and data concerning action of urolithins on glomerular cells is particularly sparse. Yet, the available reports definitely show that urolithin administration prevents inflammation and diabetes-induced changes in renal tissue. Figure 2 summarizes the current state of knowledge on urolithin-dependent protective effects in the kidney. The research on urolithins is growing and it can be expected that in the near future the beneficial effects of urolithins in kidney disease will be supported by ample evidence.

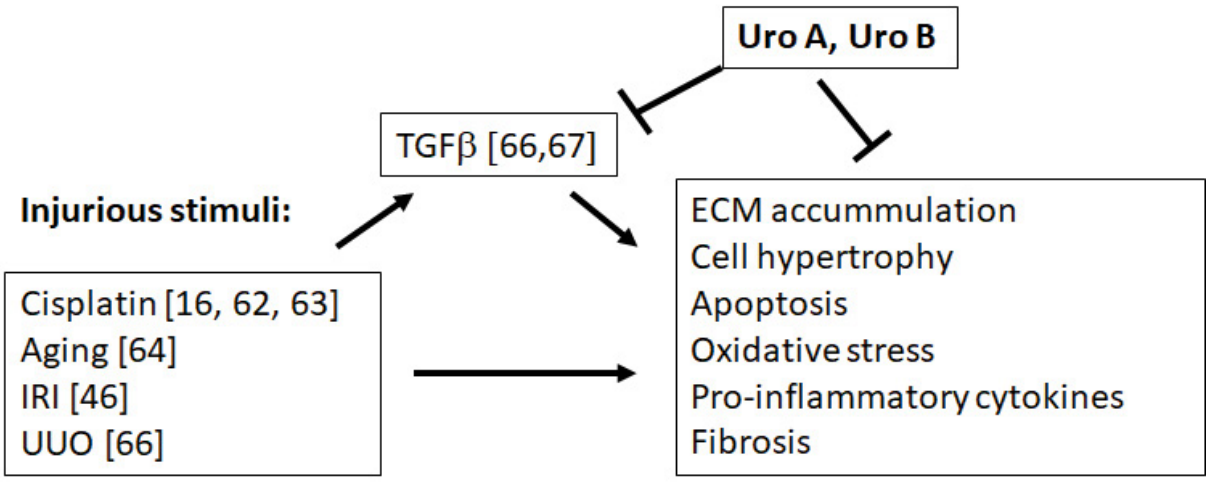

Figure 2. Protective effects of urolithins in the kidney. IRI -ischemia-reperfusion injury. UUO – unilateral ureteral obstruction [66]

Funding

None.

Conflicts of interest

None.

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REVIEW ARTICLE

Duodenal diverticulum perforation mimicking acute cholecystitis – case reports and a literature review

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Abstract

Background: Duodenal diverticula affect a large part of the population. It is a congenital abnormality that develops over time. The incidence of duodenal diverticulum is estimated at 22% of the population in autopsies. Only 5% of patients present symptoms and of those only 1-2% require surgery. **Material and methods:** We describe two patients who underwent surgery due to duodenal diverticulum perforation mimicking acute cholecystitis. **Results:** Perforation of the duodenal diverticulum, combined the difficulty of treatment and potential for complications, is a disease with a high mortality rate. It is a subtle and difficult diagnosis due to the unspecific symptoms and a lack of generalized peritonitis. The rarity and the wide spectrum of the disease, in combination with additional factors to be considered, mean there is no standard treatment. Depending on the patient's general condition, disease advancement, age and pathological findings observable only during surgery, we can choose between conservative treatment and a wide spectrum of surgical methods. **Conclusions:** Duodenal diverticular disease rarely gives any symptoms. However, even after the onset of symptoms, only 1-2% of patients require surgery. Our work is unique because we present two cases, each treated via different approach, conservative and surgical.

Keywords: acute cholecystitis · diverticulectomy · perforation · duodenal diverticulum

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Introduction

Duodenal diverticula are a congenital abnormality that develop over time and affect a large part of the population [1]. The incidence of duodenal diverticula is estimated at 22% of the population in autopsy[2,3], with only 5% of those presenting symptoms [3] and only 1-2% of patients requiring surgery [4]. Duodenal diverticula can demonstrate a wide spectrum of symptoms due to compression of the surrounding organs, cholestasis, haemorrhage, inflammation

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or perforation [3, 5-12]. Perforation is an extremely rare complication and until 2013 only 162 cases were described in the literature [3, 13]. Symptoms of perforated duodenal diverticulitis are non-specific with very subtle signs in the radiological images [14]. Uncharacteristic symptoms delay the diagnosis and appropriate treatment. Therefore, perforation of the duodenal diverticula, combined with the difficulty of treatment and potential for complications, is a disease with a high mortality rate. Due to above, the most serious complications of duodenal diverticular disease are associated with a mortality rate of up to 30% [15]. In this work we present 2 cases of perforation of the duodenal diverticulum mimicking acute cholecystitis.

Materials and methods

Two patients are described, who underwent surgery due to duodenal diverticulum perforation mimicking acute cholecystitis.

Case presentations

Patient 1

A 70-year-old woman arrived at the Emergency Department complaining of an abdominal colic pain lasting for two hours. Upon admission, the patient was in a good general condition, conscious, with effective breathing and circulation and respiratory efficiency. Her abdomen was tense, painful, with vivid peristalsis but without peritoneal symptoms. The patient did not take any medications on a daily basis and did not undergo appendectomy in the past. Her abdominal X-ray did not show any signs of perforation. See Fig 1. The gallbladder projection showed several calcified deposits of up to 8mm. The gallbladder lumen contained numerous concrements of up to 11 mm, with a normal gallbladder wall. An ultrasound examination showed no other pathological changes. The laboratory tests showed no deviations from the norm. The clinical picture suggested symptomatic biliary colic without signs of inflammation, probably due to wedging of the concrements in the neck of the follicle. Due to the persistence of the symptoms despite adequate analgesic and antispasmodics therapy, the patient was qualified for surgical treatment of acute cholecystitis and was admitted to the Department of General, Endocrine and Transplant Surgery. Less than 8 hours elapsed between the patient being admitted and the start of the surgery.

Surgery

Laparoscopic cholecystectomy was performed. A large amount of purulent fluid in the upper abdominal cavity and an unchanged inflammation of the gallbladder was observed. Therefore, it was decided to convert from the laparoscopic to the open method. An unchanged gallbladder filled with stones was found. An inflammatory infiltration with a large amount of fibrin and a significant swelling of the duodenum, hepatic flexure of the transverse colon and mesentery of the large intestine were revealed. The retroperitoneal space was opened, Koher's manoeuvre was performed, and the transverse hepatic ligament was cut. The pancreas and the posterior wall of the stomach were unchanged, with fibrous infiltration posterior to the head of the pancreas. After its removal, a fluid-filled reservoir (diameter ~3 cm) was visible, with a crackling sound during palpation. After opening it, a large amount of purulent content with an admixture of bile flowed out. The duodenal diverticulum in its further part was visible at the bottom of the reservoir. The perforated diverticulum was dissected. Good quality edges of perforated diverticulum, without inflammatory infiltration was observed. It was decided to perform primary suturing. Two layers of non-absorbable sutures were applied on the base of the diverticulum. A fragment of the tissue was collected for culturing and histopathological examination. A feeding probe was inserted posterior to the ligament of Treitz and a duodenostomy with a 14" Foley catheter was established. After isolating and cutting both the cystic artery and cystic duct, the gallbladder was removed subcapsularly. The peritoneal cavity

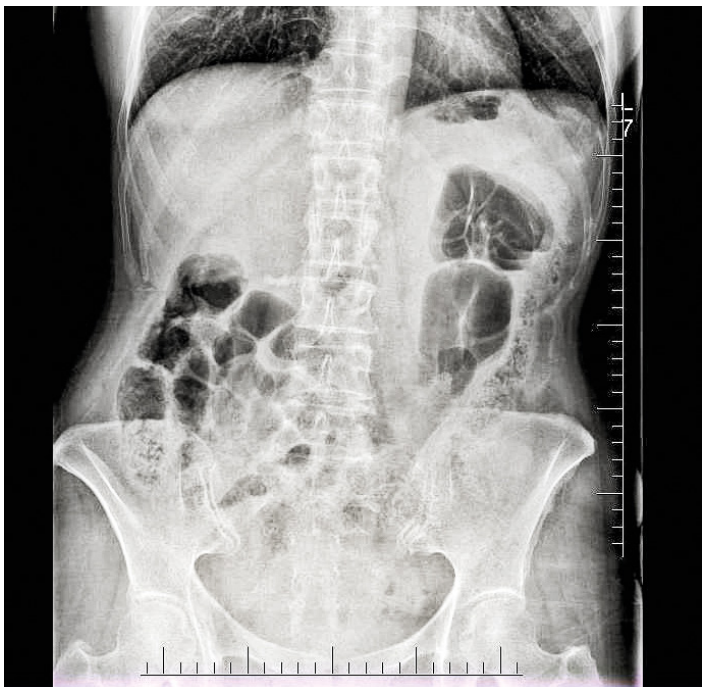

Figure 1. Patient 1 – abdominal radiograph, no signs of perforation

was rinsed extensively. Redon drainage was placed in the right upper abdominal quadrant.

Hospitalization

A gastrointestinal (GI) leakage test (administering contrast medium via the duodenostomy catheter) was performed 5 days after the procedure and no signs of gastrointestinal leakage were found and an undisturbed intestinal passage was shown. During the examination, a duodenal diverticulum, 23x20 mm in size, was found in the proximal duodenum. See Fig.2. During the hospitalization the inflammatory parameters decreased and the postoperative period was uneventful. The patient was discharged home after 9 days in good condition. Three days after the discharge the patient returned to the hospital due to fever and weakness. A fistulography by duodenostomy was performed. No signs of gastrointestinal tract perforation were shown. The drain was removed. Symptom relief was observed during the hospitalization. The patient was discharged home after 3 days in good condition.

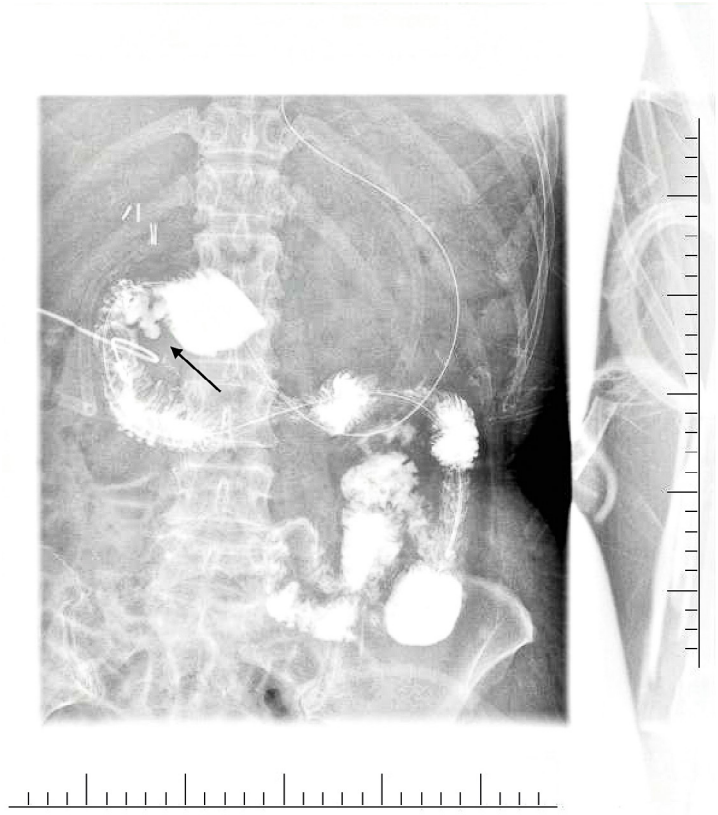

Figure 2. Patient 1 – **abdominal radiograph with contrast, duodenal diverticulum marked with the arrow**

Patient 2

A 77-year-old woman was admitted to the Department of General, Endocrine and Transplant Surgery for surgical treatment of acute cholecystitis. The patient presented with significant epigastric pain, positive peritoneal symptoms and vomiting. Abdominal ultrasound revealed fluid in the subhepatic region and the features of acute cholecystitis. The patient suffered from diabetes mellitus, hypertension and chronic renal failure.

Upon admission, the patient was in good general condition, conscious, with effective breathing and circulatory. The abdomen was tense, painful, with guarding over the entire surface. Peristalsis was reduced.

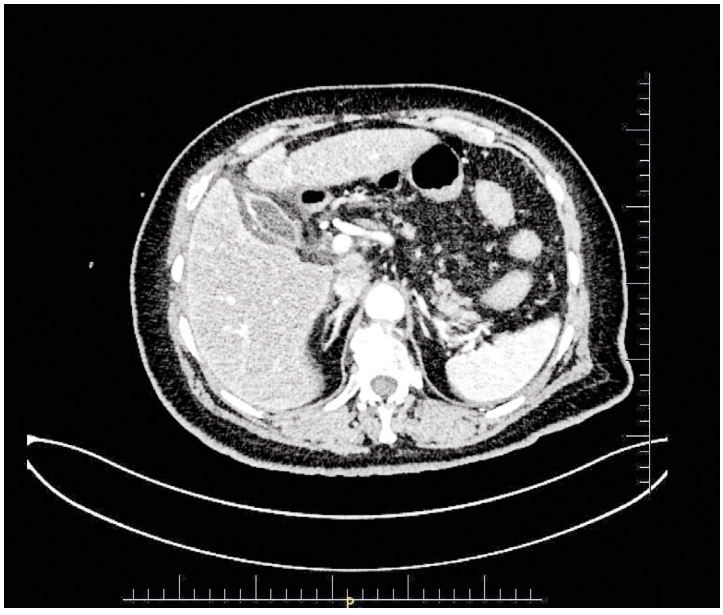

Figure 3. Patient 2 – **computed tomography image**

There were no radiological signs of GI obstruction or perforation in the X-ray examination. The CT scan revealed a non-dilated gallbladder, with wall thickening of up to 8 mm. There was a small amount of perivesicular fluid and obliteration of the fatty tissues around the liver cavity. The liver was not enlarged, without signs of cholestasis, without focal lesions and with features of steatosis. The pancreas was normal in size, lobular, without secreting lesions. A small amount of intrahepatic fluid (layer thickness of up to 1 cm) was observed, and a small amount of fluid was observed in the abdomen. The conclusion was that the CT image might correspond to cholecystitis. A small amount of fluid was observed in the abdomen and the small pelvis. See Fig. 3.

Surgery

Less than 8 hours elapsed between the patient's admission to the hospital and the start of the planned laparoscopic surgery. Large amount of purulent fluid with bile was found in the epigastric region. A thin-walled gallbladder with bile leakage from single point wall perforation was seen. Therefore, it was decided to convert to the open surgery method. No further changes were noted in the gallbladder, no stones were found. The appearance of fluid in the abdomen and the sequence of clinical symptoms indicated the possibility of GI perforation. Koher's manoeuvre was performed, and a GI leak test was performed by injecting air into the nasogastric tube, with no GI leakage being found. The abdominal cavity was revised. Except the perforation of the gallbladder, no pathologies were seen. The gallbladder was removed, and underwent histopathological examination. The abdominal cavity was rinsed extensively, and peritoneal drainage was attached.

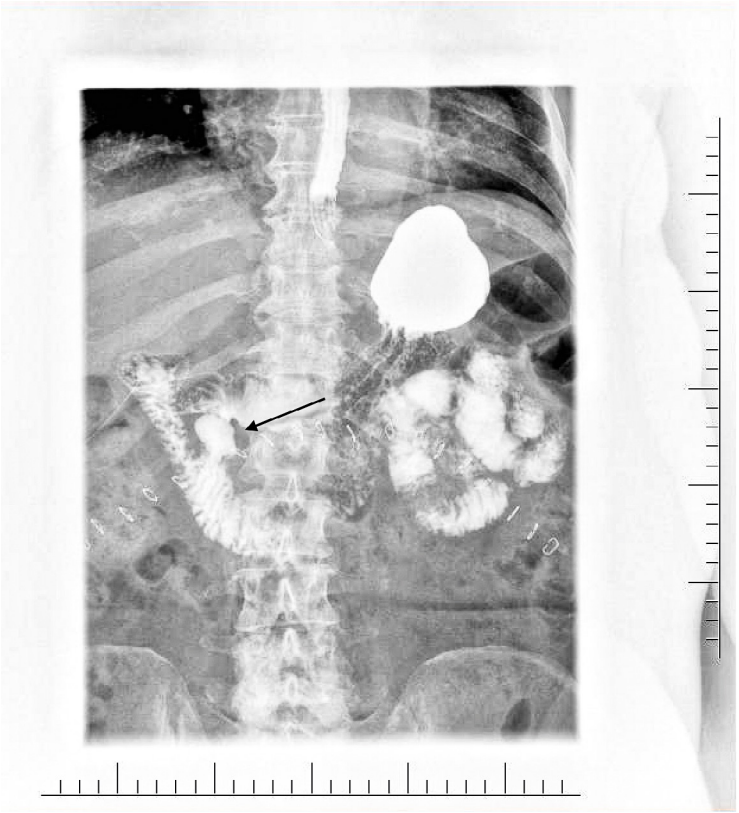

Figure 4. Patient 2 – abdominal radiograph with contrast, duodenal diverticulum marked with the arrow

Hospitalization

The histopathological examine showed Balser's fat necrosis with an abscess that could be caused by pancreatic fluid. Due to not finding the site of the perforation during surgery, an upper GI X-ray with contrast was performed on the 5th post-operative day. A duodenal diverticulum, 27x17mm in size, with a 12mm wide base was seen on the medial part of the duodenum. See Fig. 4. No GI leakage was observed. Decreases in the inflammatory parameters were observed during hospitalization. The patient was discharged home in good condition after 8 days of hospitalization.

In this case, the entire clinical picture supported the following sequence of events. First, perforation of the duodenal diverticulum caused the release of digestive fluids and pancreatic fluid into the peritoneal cavity. The pancreatic fluid later caused Balser's necrosis within the omentum and necrotic gallbladder damage. Necrosis perforation also occurred on the gallbladder wall. Non-specific symptoms led to a misdiagnosis of acute cholecystitis. After opening the peritoneal cavity, perforation of non-inflamed gallbladder wall and symptoms of GI perforation were seen. At the same time, GI perforation was not confirmed during the radiological diagnosis nor during the laparotomy, which prompted the implementation of conservative treatment. The X-ray with contrast showed a duodenal diverticulum. It can therefore be assumed that the perforation of the duodenal diverticulum had been taped already during the surgery. Only the entire clinical picture, with observations from surgery combined with the CT scan and subsequent X-ray GI leakage test, allowed definitive diagnosis.

Surgical treatment did not concern the treatment of the perforation, but only its complications: damage of gallbladder wall and bile leakage. This seems to suggest that the problem of perforated diverticulum was successfully resolved by conservative treatment.

Discussion

Perforation of the duodenal diverticulum, combined with the difficulty of treatment and potential for complications, is a disease with a high mortality rate. It is a subtle and difficult diagnosis due to the unspecific symptoms and the lack of generalized peritonitis [16]. The diagnosis can be so difficult that, as in the case of our Patient 2, perforation of the duodenal diverticulum may not be diagnosed either in radiological imaging before surgery or even during laparotomy. Only the entire clinical picture, postoperative imaging examination and the presence of Balser's fat necrosis in the histopathological examination of the gallbladder allowed the diagnosis of perforation of the duodenal diverticulum. In this case the only radiological examination that could facilitate the diagnosis before surgery was X-ray examination with a sip of

contrast. This is a procedure that could be taken into account in the event of an atypical clinical picture. However, due to the rarity of the disease, it should not be a routine procedure.

These unique cases allowed us to collect material for this insidious and dangerous disease mimicking one of the most common diseases in the population – acute cholecystitis. Due to the rarity of the disease and the additional factors to be considered when treating this disease, there is no standard treatment protocol. Depending on the patient's general condition, disease advancement, age of the patient and changes often only seen during surgery, we can choose between conservative treatment or a wide spectrum of surgical methods.

Non-operative (conservative) management can be considered for carefully-selected, stable patients, after quick and accurate diagnosis of low disease severity [17]. In a 1992 review of the world literature on this subject, only 2 of the 101 published cases were managed by nonoperative methods [14, 18]. Conservative treatment consists of fasting, nasogastric tube, a wide spectrum antibiotic therapy, and parenteral nutrition combined with vigilant observation. The occurrence of complications, e.g. retroperitoneal abscess, requires intra-abdominal drainage [19-21].

Surgical intervention should be considered in unstable patients, in septic shock with haemorrhage. It is also optimal if the disease can be diagnosed only during laparotomy. The choice of the optimal surgical method depends on the local advancement of the disease. During the procedure, the surgeon must decide based on his/her experience whether local repair is possible by removing the diverticulum and suturing the duodenum or whether it is necessary to temporarily (or permanently) bypass the duodenum or even to remove it. A very wide spectrum of surgical procedures has been described in the literature that have been performed due to duodenal diverticulum perforation. These surgical treatments include bilio-enteric bypasses, Roux-Y choledochojejunostomy, duodenojejunostomy and even pancreatoduodenectomy [22-23]. Sometimes surgical treatment requires duodenal diversion: distal gastrectomy, truncal vagotomy or gastrojejunostomy [24]. The standard operative treatment option is diverticulectomy [16, 25]. If locally advanced inflammation permits, the treatment of choice is diverticulectomy with repairs to the defect involving two layers of sutures [19, 26]. Interestingly, although diverticulectomy is considered the procedure of choice, it carries a high risk of duodenal fistula formation, which is associated with a mortality rate of up to 30% [17].

In the literature, diverticulectomy is burdened with a high percentage of complications. In our case, there were no complications related to this procedure. It should be assumed that success in this case was a careful assessment of the healing of the edges of the duodenal wall, otherwise the seemingly simplest procedure for the surgeon (in that case diverticulotomy) should be abandoned. However, it should be remembered that the emergency laparotomy was performed due to suspected cholecystitis. Not every surgeon will take this step and remove the duodenum if necessary due to local advancement of the disease. Therefore, it cannot be ruled out that a large percentage of complications after diverticulectomy may be due to an over-optimistic assessment of the sutured tissue or lack of experience.

Patient 1 was in good general condition, with no comorbidities and no signs of malnutrition. Several hours had passed since the onset of the symptoms. This, combined with low local advancement of the inflammation, allowed the implementation of the standard treatment: diverticulectomy. In this case, it was correctly suspected during surgery that the cause of the symptoms was not gallbladder-related. After a thorough abdominal examination, the actual cause of the symptoms was revealed. Following careful assessment by the surgeon, it was decided to remove the diverticulum with initial duodenal wall suturing.

In Patient 2, even during the laparotomy and despite the leak test, the perforation site was not visible. This means that during the surgery the perforation site was already covered with adjacent tissues. As a result, only the gallbladder was removed. A subsequent histopathological examination showed that the perforation of the gallbladder wall was caused by the release of digestive fluids and pancreatic juice into the peritoneal cavity. In both cases, the further course of hospitalization was uncomplicated, with the patients discharged home in good general condition.

Conclusion

Our work is unique because we present two cases of duodenal diverticulum perforation treated via two different approaches: conservative and surgical. In nearly 90% of cases reported in the literature, the definitive diagnosis was not made until the operation. It can be seen, therefore, that the perforation of the duodenal diverticulum is such an insidious disease that even during laparotomy it can sometimes be impossible to make the final diagnosis. Doctors ordering and assessing the abdominal CT rarely suspect perforation of the duodenal diverticulum. This means it can be easy to overlook subtle symptoms that may be visible during the radiological examination. At the same time, we hope that after reading the description of the above cases, surgeons and radiologists will not be surprised by discovering perforation of the duodenal diverticula.

Funding

None.

Conflicts of interest

None.

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RESEARCH ARTICLE

Efficacy of biocide-based sanitizer in daily use during the COVID-19 pandemic

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Abstract

Background: The objective of this study was to evaluate the effectiveness of a biocide-based disinfectant against pathogenic flora on the skin of the hands. **Methods:** This is a prospective study of 30 participants from the general population. The questionnaire, interview data and results of two swab analysis were collected. All the data were statistically analyzed. **Results:** The results demonstrated that after using a biocide-based disinfectant, the number of bacteria colonies on the participants' skin decreased or completely disappeared, and a significant correlation was found between the number of colonies before and after disinfection. In case of coagulase-negative staphylococcus the number of colony – forming units (CFU) significantly decreased [p < 0,001]. Also, Bacillus ssp. and Acinetobacter spp. were also found in 17.6% (n = 3) of the participants after the disinfection, however the number of the colonies was significantly smaller [p = 0,001; p = 0,008]. **Conclusions:** Our study demonstrates that a biocide-based disinfectant has high effectiveness against Gram-positive, Gram-negative, as well as fungal pathogens.

Keywords: disinfectants · biocide · hand sanitizer

Citation

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Introduction

Biocides are chemical substances intended to kill or limit the growth of bacteria, fungi and viruses. Biocides are used worldwide as antimicrobials which can further be divided into oxidizing and non-oxidizing subtypes. The main difference between these two groups is a mechanism of action and activity against microorganisms. Oxidizing biocides tend to attack microorganisms by oxidizing cell structures causing an electron transfer reaction which then doesn't

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allow nutrients to cross the cell wall. The other type of the biocide action is a non-oxidizing pathway in which the microorganism's respiratory reproductive processes are interrupted, in addition to damaging the cell wall [1-2].

Several factors can affect the activity of a biocide, e.g. pH, concentration, duration of exposure and temperature. Biocides can be divided in four groups. The first biocide group includes biocide-based disinfectants which are safe for humans or animals and are commonly used in public and private spaces or to disinfect food and water. The second group are preservatives which prevent bacteria biofilm formation and feeding on the organic plasticisers. Finally, there are pesticides and other selective biocides [3].

Disinfectant effectiveness is as important as skin health using disinfectants on daily basis. According to the National Health Service of the United Kingdom, one of the main causes for irritant contact dermatitis are disinfectants and antiseptics [4]. However, according to Slotosch et al., alcohol-based disinfectants are not the primary cause of irritant contact dermatitis. Instead, alcohol based disinfectants sting already damaged skin and develop first signs of the skin disease. In the ongoing COVID-19 pandemic, skin disinfection has become an element of our daily routine, which leads to development of different types of skin damage. Thus, disinfectants need to be equally effective and safe for the skin [5].

Material and methods

This is a prospective cross-sectional study. We collected data from 30 participants (n = 30). The eligibility criteria were age (> 18 years old), no history of active or acute contact dermatitis, hand eczema or other acute hand skin condition. The trial was performed in two centers: Jurmala Hospital (Jurmala, Latvia) and a private clinic in Riga, Latvia. We tested a biocide-based hand sanitizer gel, which contained Alkyl (C12-C16) Dimethylbenzylammoniumchloride (0,2%) and unknown concentrations of Aloe-Vera and D-panthenol.

To test action and effectiveness of the sanitizer we performed a microbiological analysis in accordance with the EN 1500 standard (a European Standard test method used to evaluate the efficacy of hand sanitizers and hand rubs). Microbiological swabs were taken from 5 cm x 5 cm large palmar and dorsal hand surfaces from one hand using the "Cliniswab TS" and the same technique was used to all participants. After the first swab, 5 ml of the tested hand sanitizer was placed on the participants' skin using a pipette. Next, the participants disinfected their hands for 30 seconds and after finishing disinfection, the investigator waited for 30 seconds to take second swab (the exposition time to the disinfectant was 30 seconds). The sanitizer was dosed using a pipette and the same amount of the disinfector (5 ml) was placed to the participant's skin. The participants disinfected their hands in accordance with the World Health Organization (WHO) recommendations: hands palm to palm, then left palm over right dorsum with interlaced fingers, continued with palm to palm with fingers interlaced, then backs of fingers to opposite palms with fingers interlocked, continued with rotational rubbing of left thumb clasped in right palm, then rotation rubbing, backwards and forwards with clasped fingers of right hand in left palm [6]. The hand disinfectant covered the entire hand surface.

Before taking the second swab, the participants' hand surface was completely dry. After the exposition time, investigators repeated the microbiological swab from palmar and dorsal hand surfaces. All samoles were stored in room temperature (+23°C) and transported to the same clinically-accredited microbiological laboratory for culture and analysis.

For statistical analysis, the SPSS v.23 was used (IBM, Armonk, USA). Parametric statistical tests (Student's t-test, ANOVA) were used to evaluate the microbiological culture results. Written informed consent was obtained from all the study participants before enrolment into the study. The Ethics Committee of the Faculty of Medicine at the University of Latvia approved the study protocol.

Results

Table 1 illustrates demographic data of the trial population and hand disinfection habits. The study included data of 30 participants, of whom 66.7% (n = 20) were women. Majority of the participants were 40-49 years of age (n = 11) and reported that they disinfected their hands up to 10 times per day (36.6%, n = 8). An important data was collected about a type of an occupation. The field of work correlated with the frequency of hand disinfection on a daily basis. In majority of cases (66.7%, n = 20) the frequency of daily hand disinfection was influenced by work. Nearly the same number of participants worked in offices as in health care (respectively 26.7%, n = 8 vs 23.0%, n = 7) and 23.0% (n = 7) worked in the beauty industry. Participants expressed their opinion about the importance of basic chemicals contained in hand sanitizers and their influence on skin after frequent use. 98.7% (n = 29) of the participants stated that since the start of winter and the pandemic, they are using hand sanitizers everyday and 90.0% (n = 27) used alcohol-based sanitizer. Only one participant reported using a biocide-based hand sanitizer (Table 1).

Microbiological cultures of the first swabs (before hand disinfection) were positive for coagulase-negative staphylococcus (CoNS, 46.0%, n = 23), Bacillus spp. (14.0%, n = 7), Acinetobacter spp. (14.0%, n = 7) and Enterobacter spp. in (16.0%, n = 8). Among the less commonly noted species were Staphylococcus aureus (4.0%) and Klebsiella spp., Pseudomonas spp. and Candida albicans (2.0% each). Whereas the

cultures from the second swabs (after hand disinfection), significantly less microorganism colonies were detected. In 10 cases (58.8%) CoNS was still noted, however with significantly fewer colony-forming units (CFUs) [p < 0,001]. Also Bacillus spp. and Acinetobacter spp. were found in 17.6% (n = 3) of all cases [p = 0,001; p = 0,008], and similar to CoNS, the number of CFUs decreased (Table 2, Figure 1).

Table 1. Socio-demographic characteristic of trial population

Variable	Levels	Number/Precent
Gender	Missing Male Female	0 10 (33.3%) 20 (66.7%)
Age	Missing Mean (±SD) Median Min-Max	0 42.63 40.0 22.0-77.0
Profession	Missing Health care professionals Beauty industry Public catering Business Office worker Construction Housewife Senior	0 7 (23.3%) 7 (23.3%) 1 (3.3%) 1 (3.3%) 8 (26.7%) 1 (3.3%) 3 (10.0%) 2 (6.7%)
Hand	Missing > 10 times per day Up to 10 times per day Once in a day 1-2 times per week	0 8 (26.7%) 16 (53.3%) 2 (6.7%) 4 (13.3%)
Active compound of sanitizers	Missing Alcohol Chlorin Chlorhexidine Biocid	0 27 (90.0%) 1 (3.3%) 1 (3.3%) 1 (3.3%)

Discussion

In Majority of cases colonies of pathogenic microorganisms were either completely removed or there was a significantly decreased amount of CFUs. According to the literature the total bacteria count on hand surfaces of normal human skin ranges from 3.9×10^4 to 4.6×10^6 CFU/cm2 [7-10]. Hand disinfection is an important step in the prevention of infections and complicated diseases. One of the most common bacteria appearing in similar trials was CoNS (76.7% of cases). This gram-positive microorganism is a part of normal human skin flora but in some cases it can cause potentially life-threatening complications and infections, e.g. endocarditis, peritonitis, as well as complicated skin infections [11-13]. The commonly detected bacteria in our study were Bacillus spp. which is an aerobic or facultative anaerobic, endospore-forming, usually Gram-positive bacteria which can cause a wide range of infections including abscesses, bacteremia/septicemia, wound and burn infections, ear infections, endocarditis, meningitis, ophthalmitis, osteomyelitis, peritonitis and even cutaneous anthrax and a major risk factor is contact with animals [14-15].

We noted similar results about Acinetobacter spp. and Pseudomonas spp. Acinetobacter spp which are saprophytic organisms that can live in soil, water and food. Acinetobacter spp. is a Gram-negative, strictly aerobic bacteria. Frequently, these bacteria colonize the oral cavity, respiratory tract and gastrointestinal tract. Acinetobacter spp. very often cause nosocomial infections, mainly infections of blood, urinary tract, skin infections including wound infections, pneumonias and meningitis. Similar to Acinetobacter spp., Pseudomonas spp. also belong to Gram-negative bacteria. Under huge risk for Pseudomonas spp. infection development are immunosuppressive patients and cancer patients [16-17]. According our trial data, number of CFU decreased after skin disinfection with the biocide based skin sanitizer.

After disinfection the following colonies completely disappeared: Klebsiella spp., Enterobacter spp. and Candida albicans. Klebsiella spp. is Gram-negative rod shaped anaerobic bacteria. Klebsiella spp. cause urinary tract infections, as well as wound and skin infections and respiratory tract infections. Similar to Klebsiella spp., Enterobacter spp. belong to Gram-negative bacteria, theses bacteria can be isolated from water, soil, commensals. This bacteria is now emerging a pathogenic, under the risk are immunocompromised patients [18]. An opportunistic fungal pathogen was detected in our study: Candida albicans. This fungus has ability to colonize in almost all human body tissue and organ, which can cause serious and complicated infections [18]. In all the cases, when these three pathogens were detected before disinfection, after using the biocide-based disinfection all of the three were entirely killed and absent on hand skin surface.

During the COVID-19 pandemic hand disinfection became a part of many people's daily routine. The main reason for choosing alcohol-based skin disinfectant is the cliché belief that ethanol is effective against most of microorganisms and viruses, despite the fact that ethanol has a negative

influence on skin condition. Majority of our trial participants noted that after the use of the ethanol disinfectant, they feel dryness, itchiness and irritation on the surfaces of their hands. Additionally, there is a little research about alcohol's influence on skin, however some sources report that allergic contact dermatitis to alcohol-based antiseptics is uncommon. The main ingredients in alcohol-based hand rubs are n-propanol and/or isopropanol. A systemic review showed that in majority of studies in the period from 2000 to 2019 n-propanol and isopropanol in different concentration were related to irritation. N-propanol caused significant skin barrier damage, however, studies showed that n-propanol alone has low irritation potential [19]. Most common local skin allergic reaction to alcohol-based formulations is a sign of allergy against an impurity or an aldehyde metabolite. An immediate allergic is mostly caused by ethanol or isopropanol, but Irritant contact dermatitis mostly is caused by chemical or physical agents and it increases with the duration, intensity and concentration of the substance [6, 20-22].

Table 2. Effectiveness of biocide based hand sanitizer is proven by colonies changes before and after disinfection

Pathogen	Before disinfection	After disinfection	*P*-value
Staphylococcus aureus	2 (6.7%) 28 (93.3%)	0 30 (100.0%)	0.157
Coagulase – negative staphylococcus	23 (76.6%) 7 (23.3%)	10 (33.3%) 20 (66.7%)	< 0.001
Bacillus spp.	7 (23.3%) 23 (76.6%)	3 (10.0%) 27 (90.0%)	0.017 p < 0.05
Acinetobacter spp.	7 (23.3%) 23 (76.6%)	3 (10.0%) 27 (90.0%)	0.034 (p < 0.05)
Enterobacter spp.	8 (73.3%) 22 (73.3%)	0 30 (100.0%)	0.005 (p < 0.05
Klebsiella spp.	1 (3.3 %) 29 (97.6%)	0 30 (100.0%)	0.157
Pseudomonas spp.	1 (3.3%) 29 (97.6%)	1 (3.3%) 29 (97.6%)	0.157
Candida albicans	1 (3.3%) 29 (97.6%)	0 29 (97.6%)	0.157

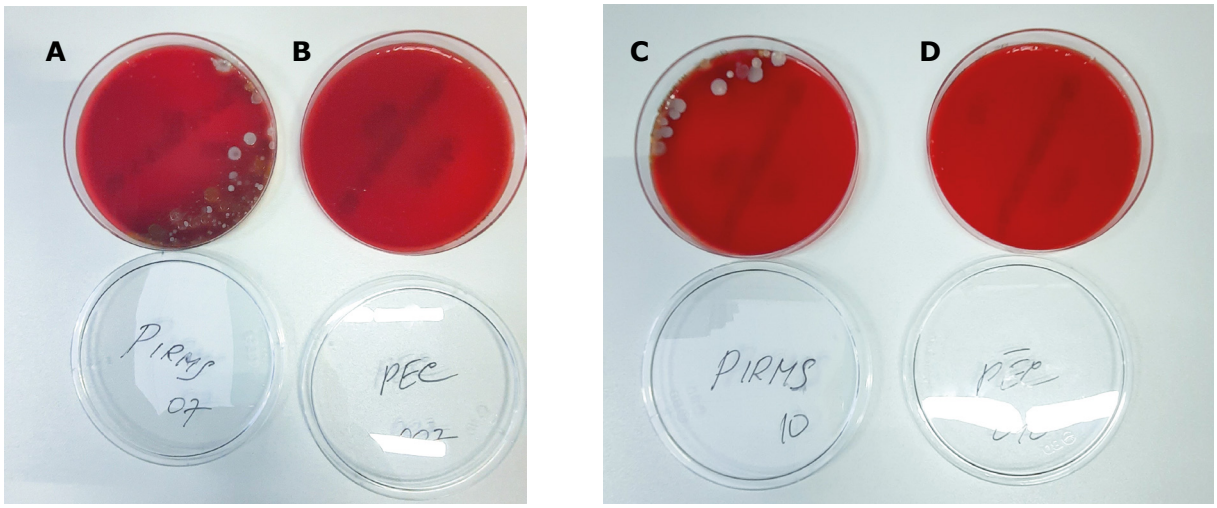

Figure 1. (a) Colonies detected before hand disinfection in swab from a patient Nr. 7; (b) Colonies detected after hand disinfection in swab from a patient Nr. 7. (c) Colonies detected before hand disinfection in swab from a patient Nr. 10; d) Colonies detected after hand disinfection in swab from a patient Nr. 10

Use of the biocide-based gel disinfectant with Aloe Vera and D-panthenol, does not irritate or damage skin. Our trial proves that a biocide based disinfectant has high effectiveness against Gram positive, Gram negative, as well as fungal pathogens, and even more protects skin from developing allergic reactions and worsening of chronic skin diseases.

On the other hand, our study has some limitations due to the small amount of participants included. However, the study was performed in two centers in different cities, with randomly selected participants. Nevertheless, conventional microbiology methods have some limitations in detecting microorganisms. Microscopy with staining and detection of cultures allows identifying only a limited number of colonies and could not recognize the full spectrum of microbiota of human skin. For further investigation it will valuable to use polymerase chain reaction (PCR) and conduct a trial in a multiple medical centers and hospital departments. Unfortunately, in this study we did not test the biocide-based sanitizers' efficacy against viruses, such as SARS-CoV-2 (causative pathogen of the COVID-19 disease). However, the United States Environmental Protection Agency (EPA) maintains a database of sanitizers which are effective against viruses, particularly SARS-CoV-2 [23]. This database contains information only about substances with proven efficacy and the criteria are as follows: substance is effective against SARS-CoV-2, demonstrates efficacy against any pathogen which is harder to kill than SARS-CoV-2 or shows efficacy to kill coronaviruses similar to SARS-CoV-2. Based on this database, the active substances of sanitizers with proven efficacy against SARS-CoV-2 or SARS-CoV-2-like viruses are: hydrogen peroxide, quaternary ammonium, tetraacetylethylenediamine, dodecylbenzenesulfonic acid and others.

Conclusions

Our study demonstrates that a biocide-based disinfectant has high effectiveness against Gram-positive, Gram-negative, as well as fungal pathogens. Further research should be done with the use of PCR and detection of viral particles to determine the significance of biocide use for all pathogens that can be transmitted through the skin directly or indirectly.

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None.

Conflict of interest

The authors do not have any conflicts of interests to report.

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REVIEW ARTICLE

Bromatological, analytical and chemometric assessment of animal and plant foods based on mineral composition

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Abstract

There are several examples of numerous applications of analytical and multivariate techniques useful in investigations of varied assortment of food products. The successful use of chemometrics in study of food such as meat and its products, fish, seafood, milk and dairy products, honey, cereal products, oils, oilseeds and nuts, vegetables, fruits, mushrooms, tea, coffee, confectionary products, mineral waters and alcoholic beverages deserves attention. RDA indicated exceeded its normative values for Se, Cu, Mn, Fe and Cr in some groups of animal food and Cr, Mn, P and Fe in some assortment of plant food. Based on PTWI values for Pb, Cd and Hg, there is no threat to human health resulting from the consumption of the investigated food products. It is concluded that the proper use of analytical and chemometric tools is useful for assessing nutritive and health quality of animal and plant foods. They play an important role in quality control, and their classification in view of geographical origin, confection and degree of environmental pollution. Both, instrumental and multivariate techniques would be useable in differentiating unprocessed and technologically processed food as well as detecting fraud to preserve the brand name of the original product. The aim of this study is to give an overview of the crucial issues associated with the implementation of chemometrics in food research and development.

Keywords: assessment of food quality · analytics · chemometrics · bromatology

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Introduction

In order to perform adequate analytical and chemometric assessment of original data it is important to use skillfully modern both analytical and computational tools following strictly the rules and research criteria. In recent times dynamic progress of analytical and chemometric techniques has been observed owing to new and advanced informative technologies. They make it possible to reliably obtain useful information from an experimental data set. Therefore, multivariate techniques appeared to be the key statistical and mathematical approach to explore extensive data base being highly helpful in simple and quick explorations as well as identification of similarity between samples (objects) and measured parameters (variables). The aim of this study is to give an overview of the crucial issues associated with the implementation of chemometrics in food research and development.

Material and methods

The Scopus, ScienceDirect, Medline and Web of Science databases were searched for literature published from 1989 to 2021 using the following keywords: Chemometric evaluation; Analytical evaluation; Food authenticity; Food adulteration; Meat; Fish flesh; Seafood; Milk and dairy products; Honey; Grain products, Olive oils and oilseeds; Vegetables and legume seeds; Mushrooms; Fruit and its products; Tea and its infusion; Coffee and its infusion; Cocoa and its products; Sweets; Mineral and drinking water; Alcoholic beverages. The main inclusion criterion was whether the article contained evaluation of data obtained by using combination of more advanced analytical and chemometric (mainly multivariate) methods/techniques. The exclusion criteria were: small number of the samples studied, lack of sufficiently advanced analytical and chemometric methods or techniques.

Results

The search retreived 1478 articles of which 341 were included in the review.

Analytical and chemometric methods

The following analytical methods have been routinely applied: Atomic Absorption Spectrometry with four techniques, i.e. flame technique (FAAS), electrothermal (ET-AAS), cold vapor (CV-AAS), hydrogen generation (HG-AAS) as well as Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). Reliability and correctness of concentration data were checked under the quality assurance test by the use of appropriate certificate reference materials (CRMs) with declared, known concentration of analytes [1-2]. The validated analytical data were then processed chemometrically by means of univariate, bivariate and other multivariate techniques, e.g. bar charts, histograms, one-way ANOVA, correlation and regression analysis and other, advanced and frequently applied techniques such as Principal Component Analysis (PCA) or Factor Analysis (FA) and Cluster Analysis (CA) or Hierarchical Cluster Analysis (HCA) [3-12]. Among the different criteria for determining the number of components/factors, the Kaiser criterion was selected and therefore factors greater than 1 were exclusively considered and interpreted. The aim of multivariate data analysis is to divide data matrix into its components to reduce relatively numerous variables to a smaller number of orthogonal factors. Such approach guarantees achieving a high degree of generalization of registered tendencies or statistical relationships, and what more, at a high level of statistical significance [2-3, 13].

Meat

Bromatological, analytical and chemometric assessment of foods of animal origin based often on stable chemical elements has attracted a special attention of environmentalists as well as scientists specializing in food. Such investigations resulted in recognition of environmental parameters differentiating geographical distribution and diverse assortment of defatted mutton samples [14], pork belly [15], poultry breast meet and dried beef samples [16-17], labeling lamb meat [18], bovine muscles [19-22], liver, kidney and muscle of sheep [23], beef steak [24-25], pork [26], pork, beef and chicken [27-28] and others.

Fish and other seafood

Interesting information is also available on the application of chemometrics in assessment of world-wide populations of fish; such investigations were performed by many researchers, e.g. Julshamn and Grahl-Nielsen [29], Szefer et al. [30], Molkentin et al. [31], Ye et al. [32], Yamashita et al. [33], Li et al. [34], Ahmed et al. [35], Rahman et al. [36] and Marpaung et al. [37]. Chemometric processing of mineral composition related to vendace caviar has been performed by Rodushkin et al. [38]. Edible mussels were also analysed and assessed chemometrically, e.g. by Struck et al. [39], Favretto et al. [40], Bechmann et al. [41], Julshamn and Grahl-Nielsen [29], Szefer and Wołowicz [42], Szefer et al. [4-5, 42-45], Mesa et al. [46], Bartolomé et al. [47], Przytarska et al. [48], Chen et al. [49] and Bennion et al. [50, 51]. Among marine organisms also edible crustaceans were investigated by Li et al. [52-55]

and Nędzarek et al. [56-58]. Kwoczek et al. [59] analysed different assortment of seafood available in Poland exported from different geographical regions.

Milk and dairy products

Milk and its products from different geographical regions were analysed for mineral component composition to assess their authenticity in the view of chemometric evaluation, among others milk [18, 60-67], commercial skim milk powders sweet whey and different milk-based infant formulae [68-79], cheeses [80-85], butter, margarine, and peanut butter [37] and eggs [86-89].

Honey

Honey samples were also chemometrically classified relative to their type and origin based on the content of chemical elements [7, 90-114].

Cereal products

Application of the chemometric techniques in evaluation of cereal products in view of their mineral composition was performed by many researchers, e.g. wheat samples [115--117], rice [118-125], wheat, barley and faba bean [126], buckwheat [127-128] and sorghum [129-130]. Different kinds of grain products (bread, cereals, rice, flour, pasta) purchased from the local market in Poland originated from 14 different countries were analysed and evaluated chemometrically by Grembecka [8] and Grembecka et al. [131].

Oils, oilseeds and nuts

Mineral components of edible oils [132-137] as well as oilseeds and nuts [138-145] were analysed and evaluated chemometrically. Six different kinds of oilseeds purchased from the local market in Poland originated from 6 different countries were investigated and assessed by Grembecka [8].

Vegetables and fruits

Different types of vegetables originated from various geographical regions were studied in accordance of chemometric evaluation of chemical elements concentration, namely potato [146-152], tomato [153-155], cabbage [156--157], broccoli [158], caper [159, 160], carrot [161, 162], onion [163-169], garlic [170-172], beetroot [173], pea, bean, faba bean [164, 174-175], lentil [176], parsley, carrot, onion, carrot, cabbage, lettuce, cucumber, green bean [177], parsley [178], paprika [179], chili pepper [180], Sechium edule fruits [181], Caigua [182], Taro [183] and sea cucumber [184]. Twenty five different kinds of commercially available fresh and processed vegetables and 5 kinds of leguminous vegetables purchased from the local market in Poland originated from 4 different countries and other EU countries were investigated and assessed by Grembecka [8] and Grembecka et al. [185].

The following assortment of fruits was assayed and estimated: pear [186], apple [187], lemon pulps samples [188], orange [189], pomelo [190], kaki fruit [191] and fruit juice, i.e. lemon juice [192, 193], grape juice [194, 195] and orange juice [196]. Twenty four different kinds of commercially available fresh fruits purchased from the local market in Poland originated from different countries were analysed and assessed by Grembecka and Szefer [9].

Tea and coffee

Different kinds of tea originated from various countries were analysed and chemometrically classified in view of chemical elements composition by Marcos et al. [197], Wong et al. [198], Fernández-Cáceres et al. [199], Herrador and González [200], Moreda-Piñeiro et al. [201, 202], Fernández et al. [203], Chen et al. [204], McKenzie et al. [205], Mbaye et al. [206], Marcelo et al. [207], Paz-Rodríguez et al. [208], Brzezicha-Cirocka et al. [11, 209-211], Ma et al. [212], Milani et al. [213], Ye et al. [214], Lou et al. [215], Zhao et al. [216--219], Malinowska et al. [220], Zhang et al. [221], Idrees et al. [222], Liu et al. [223-226] and Motta et al. [227].

There are also numerous available literature data on application of the chemometric techniques in analytical evaluation of coffee in view of their mineral composition. Differentiation and classification of coffee samples have been achieved by Krivan et al. [228], Martin et al. [229-231], dos Santos et al. [232], Anderson and Smith [233], Fernandes et al. [234], Filho et al. [235], Grembecka et al. [236], Akamine et al. [237], Muñiz-Valencia et al. [238], Valentin et al. [239], Barbosa et al. [240], Liu et al. [241], Oliveira et al. [242], Szymczycha-Madeja et al. [243], Habte et al. [244], Mehari et al. [245], Pohl et al. [246], Zhang et al. [247], Al-Jaf and Saydam [248], Cloete et al. [249], Worku et al. [250], Endaye et al. [251], Voica et al. [252] and Bitter et al. [253].

Confectionary products

Different beet and cane sugar products (cane sugar plants, maple syrup, crude and syrup juices, molasses, the end products of consumer sugar) have been investigated and assessed chemometrically in aspect of the mineral composition by several authors, e.g. Awadallah et al. [254], Nunes et al. [255], Rodushkin et al. [256], Grembecka and Szefer [257], Barbosa et al. [258], Andrade et al. [259] and Guedes and Pereira [260]. Different ingredients such as sugarcane, soy, citrus, coffee, maize, eucalyptus, mango, bean, banana, lettuce, brachiaria, pearl millet, grape,

rubber tree and tomato were analysed by de Carvalho et al. [261]. Chemometric estimation of both the confectionary and geographical provenance of cocoa and chocolate was performed by some authors, e.g. Pedro et al. [262], Grembecka and Szefer [257], Bertoldi et al. [263], Junior et al. [264], Kruszewski and Obiedziński [265] and Vanderschueren et al. [266].

Mushrooms

Chemometric techniques have been used to explore the elemental data for different species of mushrooms coming from various geographical areas, e.g. by Malinowska et al. [10], Cocchi et al. [267], Chudzyński et al. [268, 269], Falandysz et al. [270-273], Pająk et al. [274], Drewnowska and Falandysz [275], Kojta et al. [276-277], Mleczek et al. [278], Niedzielski et al. [279], Brzezicha-Cirocka et al. [280], Wang et al. [281], Zsigmond et al. [282], Buruleanu et al. [283] and Nowakowski et al. [284].

Mineral water

Application of the chemometric techniques in analytical evaluation of mineral water in view of their elemental composition has been performed by Misund et al. [285], Versari et al. [286], Kraic et al. [287], Bityukova and Petersell [288], Birke et al. [289-291], Cicchella et al. [292], Demetriades et al. [293], Fugedi et al. [294], Dinelli et al. [295-297], Grošelj et al. [298], Kermanshahi et al. [299], Peh et al. [300], Avino et al. [301], Bertoldi et al. [302], Cidu et al. [303], Souza et al. [304], Banks et al. [305], Flem et al. [306], Pantić et al. [307], Khan et al. [308] and Bodor et al. [309].

Alcoholic beverages

Different investigators have processed chemometrically the concentration of chemical elements in different kinds of red and white wines, including must, e.g. Latorre et al. [310], Barbaste et al. [311], Pérez Trujillo et al. [312], Marengo et al. [313], Coetzee et al., [314], Gonzálvez et al. [315], Rodríguez et al. [316], Aceto et al. [317], Durante et al. [318], Catarino et al. [319], Pořízka et al. [320], Cruz et al. [321], Dembroszky et al. [322], Shimizu et al. [323] and Grembecka et al. [324]. Chemometric evaluation of mineral composition of beers appeared to be useful in the classification of different features of this alcoholic beverage as has been proved by Bellido-Milla et al. [325], Alcázar et al. [326], Wyrzykowska et al. [327], Mahmood et al. [328], Carter et al. [329], Voica et al. [330], Rodrigo et al. [331], Styburski et al. [332] and Redan et al. [333]. Ciders were also studied and chemometricaly considered relative to their mineral composition [334-335] as well as to different kinds of Scotch whisky [336], sherry brandy, whisky [337-338] and traditional Galician orujo alcoholic distillates with and without a certified brand of origin (CBO) [339].

Differentiation of geographical origin

For instance, a clear discrimination between soft tissue of Mytilide originated from different coastal regions of subarctic, temperate, subtropical and tropical marine ecosystems was achieved owing to use of FA technique [45]. Object samples are separately distributed along F1 axis (relative to F1 score values) corresponding to edible mollusks inhabited coastal regions of marine ecosystems including also intertidal zones of Atlantic, Pacific and Indian Ocean (Table 1).

Table 1. Application of the chemometric techniques in analytical evaluation of animal food in view of its mineral composition

Kind of animal food, no of samples, country of origin	Chemical elements as descriptors	Analytical technique	Chemometric /statistical technique	Reference
81 samples of perch (muscle and liver) from Pomeranian Bay and Szczecin Lagoon (n = 162) Southern Baltic	Hg, Cd, Pb, Cu, Zn	FAAS, ET-AAS, Hg analyser	Correlation and regression analysis, One and two-way ANOVA, FA	[30]
Cockle *Cerastoderma glaucum* from the Bay of Gdańsk (Baltic Sea), Marennes-Oleron and Arcachon Bay (French Atlantic coast), Embiez Islands (Mediterranean Sea) (n = 50 pooled samples) Poland, France	Cd, Cu, Fe, Mn, Ni, Zn	AAS	PCA	[42]

Kind of animal food, no of samples, country of origin	Chemical elements as descriptors	Analytical technique	Chemometric /statistical technique	Refe-rence
800 specimens of *Mytella strigata* (soft tissues and byssus), and 200 specimens of *Chione subrugosa* and the associated sediments from 4 sampling sites in a tropical mangrove lagoon (n = 1000 specimens) Mexico	Cd, Pb, Zn, Cu, Ag, Cr, Co, Ni, Mn, Fe	FAAS	Correlation and regression analysis	[44]
Samples of cockle from 6 sites along the Mediterranean Lagoon Etang de Thau (n = 12 pooled samples) France	Ag, Cd, Co, Cr, Cu, Ni, Pb, Zn, Mn, Hg	FAAS, CV-AAS	Correlation and regression analysis	[5]
About 4200 specimens of Blue mussel from 23 sites from in 3 sectors of the southern Baltic Sea; i.e. pooled samples of soft tissues (ca. 350), shells (ca. 200) and byssus (26) (n ≅ 580) Poland	Ag, Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, Zn, Hg	FAAS, CV-AAS	Correlation and regression analysis, FA	[4]
3600 specimens of *M. galloprovincialis* from 7 sampling sites in Masan Bay and Ulsan Bay, i.e. pooled samples of soft tissue (119), byssus (30); moreover water (24) and suspended matter (26) (n ≅ 2 00) South Korea	Cd, Co, Cu, Cr, Fe, Hg, Mn, Ni, Pb, Sn, Ti, Zn	ICP-MS, AFS, HRICP-MS, AAS	Correlation and regression analysis, two-way ANOVA, DFA	[6]
About 5000 specimens of Mytilids (101 soft tissue samples and 48 byssus samples) from different geographical coastal regions all over the world, i.e. North Sea, Mediterranean Sea, Atlantic coast, Southern Baltic, Northern Baltic, White Sea, Arabian Sea, East Sea, Indian Ocean coast, Pacific coast (n = 150) France, Holland, Poland, Sweden, Russia, Spain, Yemen, South Korea, Japan, Mexico, Brazil	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn	FAAS	FA	[45]
Pooled samples of edible parts of shellfish products (shrimps, surimi products, octopus, mussels, squids, octopuses, crabs, lobsters) from 8 countries (n = 88) Great Britain, Norway, Spain, India, Philippines, Thailand, Canada, New Zealand	Cu, Zn, Fe, Mn, Co, Ni, Cr, Mg, Na, K, Ca, Cd, Pb, Se, Hg	FAAS, GF-AAS, HG-AAS, CV-AAS	FA, correlation analysis	[59]

Kind of animal food, no of samples, country of origin	Chemical elements as descriptors	Analytical technique	Chemometric /statistical technique	Reference
Approximately 1000 specimens of blue mussel *M. edulis* from east coast of Kyushu Island (n = 46 pooled samples) Japan	Cd, Pb, Zn, Cu, Ag, Cr, Co, Ni, Mn, Fe	FAAS	Correlation and regression analysis	[43]
260 specimens of *Perna perna* from 3 locations of Gulf of Aden (n = 8 pooled samples) Yemen	Cd, Pb, Zn, Cu, Mn, Fe	FAAS	Correlation and regression analysis	[340]
Samples of 4 bivalve species (800 specimens) and associated sediments from 7 locations of Gulf of Aden (n = 94) Yemen	Cd, Pb, Zn, Cu, Ni, Co, Cr, Mn, Fe	FAAS	ANOVA, FA, correlation and regression analysis	[341]
Several brands of commercially available honeys and bee products of different botanical origins from 3 different countries and other EU countries (n = 66) Poland, Italy, Hungary, EU	Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Zn	FAAS, spectro-photometric method	CA. FA, ANOVA	[7]

Interesting results were obtained in investigations of cockle (C. edule) from the Bay of Gdansk (Southern Baltic), Marennes-Oleron Bay, Arcachon Bay (French Atlantic coast) and Embez Islands (Mediterranean Sea). The PCA data displayed that Mn and Fe are responsible for discrimination between individuals originated from Marennes-Oleron Bay and Arcachon Bay whilst Zn, Cd and partly Ni have a main contribution in separation of the Bay of Gdansk from the others [42].

It was also stated that FA technique is helpful in discrimination of the Korean Peninsula mussel *M. galloprovincialis* with respective to its geographical origin, i.e. from the Masan Bay and the Ulsan Bay, i.e. more and less polluted regions with heavy metals, respectively [6].

Another exemplar concerns application of CA technique in processing of concentration data obtained for *Boletus edulis* mushroom and the adjacent soil as substratum from 12 different forest regions of Poland (Table 2). There is a significant grouping of samples collected in the Tricity Landscape Park, adjacent to the Tricity agglomeration and Pb is a main descriptor responsible for separation of this region from other 11 Polish forest sampling sites as protected areas, deprived of industrial and urban influences [10]. Based on obtained concentration data corresponding to 22 different species of mushrooms collected from different forest regions of Poland it is found that *C. cibarius*, *B. edulis* and *L. scabrum* were diversified relative to their geographical provenance [280].

Interesting results were reported for tea samples imported from Asiatic countries which allow differentiation of object scores corresponding to Japan, India and China as well as it was possible to identify particular varieties of the green tea studied (Sencha, Kokeicha, Bancha, Darjeeling, Gunpowder, Chun Me and Yunnan) [11]. Two multivariate techniques, i.e. FA and CA were applied to differentiate black tea samples and their infusions in view of their geographical origin. These chemometric tools proved to be able to discriminate samples according to their provenance as well as plantation within the common regions [12].

CA technique allowed differentiation of teas relative to the country of origin, i.e., China, India, Ceylon and Kenya as well as it was useful in distinguishing of teas originated from various plantations within a single country. Thus, chemometrics proved to be effective tool to discriminate these samples in view of their provenance as well as plantation within the common region. Moreover, FA technique appeared to be useful in differentiating of various wine varieties in aspect of their geographical origin [324].

Table 2. Application of the chemometric techniques in analytical evaluation of plant food in view of its mineral composition

Kind of animal food, no of samples, country of origin	Chemical elements as descriptors	Analytical technique	Chemometric /statistical technique	Refe-rence
Different kinds of grain products (bread, cereals, rice, flour, pasta) purchased from a local market in Poland, originated from 14 different countries (n = 146) Poland, Germany, Italy, Spain, India, Pakistan, Singapore, Thailand, China, Vietnam, Japan, Borneo, Guyana, USA	Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Zn	FAAS, spectro-photometric method	CA. FA, ANOVA	[8, 131]
25 different kinds of commercially available vegetables (fresh and processed) and 5 kinds of leguminous vegetables purchased from the local market in Poland originated from several EU countries and Canada (n = 129) Poland, Spain, France, Italy, EU	Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Zn	FAAS, spectro-photometric method	CA, FA, ANOVA	[8, 185]
Samples of instant and ground coffee (dry coffee and infusions) (n = 120) South America, Africa, Asia	Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, Na, P, Pb, Zn	FAAS	Correlation coefficients, ANOVA, CA, FA	[236]
25 different kinds of commercially available fresh fruits purchased from the local market in Poland originated from different countries (n = 98) Poland, Europe, Asia, Africa, America	Mg, Ca, K, Na, P, Zn, Cu, Fe, Cr, Co, Ni, Mn	FAAS, spectro-photometric method in the form of phospho-molybdate	ANOVA, FA, CA	[9]
Several types of green tea including their infusions from 3 countries (n = 41) China, India, Japan	Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb Zn	FAAS, spectro-photometric method	Kruskal-Wallis test, FA, CA	[11]
Black tea samples including their infusions from different areas of 4 countries (n = 118) China, India, Ceylon, Kenya	Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb Zn	FAAS, UV-Vis spectrometry	Spearman rank correlation analysis, Kruskal-Wallis test, FA, CA	[12]
Samples of dark (Pu-erh) and fruit tea leaves including their infusions from one country of declared origin (n = 32) China	Mg, Ca, K, Na, Mn, Cu, Fe, Zn, Cr, Ni, Co, Cd, Pb	FAAS, spectro-photometric method in the form of phospho-molybdate	Spearman rank correlation analysis, Kruskal-Wallis test, FA, CA	[210]

Kind of animal food, no of samples, country of origin	Chemical elements as descriptors	Analytical technique	Chemometric /statistical technique	Reference
Samples of teas (black, dark and green) from 3 different countries of declared origin (n = 30) China, India, Sri Lanka	Oxalate, Mg, Ca	Mangano-metric method, FAAS	Spearman rank correlation analysis, Kruskal-Wallis test, CA	[209]
Infusions of commercially available teas (black, green, oolong, Pu-erh and white), herbal infusions, instant tea and ready-to-drink tea beverages from 13 different countries in Eurasia, Asia, Southeast Asia and Oceania, Africa, South America (n = 64) China, India, Sri Lanka, Nepal, Vietnam, Taiwan, Japan, Java, Kenya, Georgia, Brazil, Argentina, South Africa	F-	Ion-selective electrode	CA	[220]
Samples of cocoa and chocolates (dark and milk) (n = 33) Poland, USA	Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Zn	FAAS	ANOVA, CA, FA	[257]
Samples of sugar (from beet and cane), molasses, maple syrup (n = 15) Poland, India, Republic of Mauritius, Hawaii, Argentina, Canada	Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Zn	FAAS	ANOVA, CA, FA	[8, 257]
166 samples of fruiting bodies (caps and stalks) of *Xerocomus badius* and 31 soil samples from 12 different 12 regions (n = 197) Poland	Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Na, K, Ca, Mg	FAAS	Correlation analysis, HCA, FA	[10]
Samples of 22 different species of mushrooms collected from different forest regions (n = 1500) Poland, Sweden	Ca, Na, K, Mg, Zn, Fe, Mn, Cu, Cr, Cd, Ag, Ni, Pb, Al, Ba, Sr	FAAS, ICP-AES	Non-parametric R-Spearman test, ANOVA Kruskal-Wallis test, post-hoc Dunns test, FA	[280]
6 different kinds of oilseeds purchased from the local market in Poland originated from 6 different countries (n = 14) Poland, Czech Republic, Hungary, Belgium, India, China	Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Zn	FAAS, spectro-photometric method	CA, FA, ANOVA	[8]
Different types of red wines dry, semi-dry, sweet and semi-sweet wine) obtained at retail (n = 32) Austria, France, Chile, Hungary, Italy, USA, Portugal, Spain, Bulgaria, Germany, Poland, Moldavia	Mg, Ca, Na, K, Zn, Cu, Fe, Mn, Co, Cr, Ni, Cd, Pb, P	FAAS, spectro-photometric method with phospho-molybdate	ANOVA, Kruskal-Wallis test, FA	[324]

Differentiation of varietal origin

As shown in Table 1, the FA technique appeared to be helpful research tool in analysis of diverse assortment of seafood (oysters, mussels, prawns, surimi products, octopus, squids, octopuses, crabs, lobsters) originated from various over-worlds waters bordering 8 countries, i.e. Norway, England, Spain, India, Thailand, Canada, Philippines and New Zealand. Obtained data documented significant discrimination between factorial distribution of scores with respect to a degree of technological processing (described by F1 values) and taxonomic features of seafood [59].

Based on the FA technique, it is found that samples of artificial honey are separated from samples of natural honey. Moreover, natural honeys indicated a clear differentiation relative to their botanical origin. CA technique resulted in the dendrogram consisted of two main clusters, i.e. representing dark and light color honeys. The dark color honeys cluster contains generally samples corresponding to honeydew, buckwheat and heather honeys, while the second cluster is consisted of acacia, lime, rape and multifloral honeys. The FA technique appeared to be effective chemometric tool to separate the data concerning artificial honey samples (described by the highest levels of Ca and Na) whilst natural honeys and those with natural additives were characterized by K, P, Cu, Mn and Mg. Moreover, F2 achieved the lowest values for natural and syrup-feed honeys identified by high levels of Fe and Zn [7].

The analytical data obtained for different kinds of grain products (bread, cereals, rice, flour, pasta) purchased from the local market in Poland were also processed chemometrically (Table 2). FA appeared to be helpful technique in differentiating these products according to their type, especially in case of flour and rice [8, 131].

It was possible to discriminate numerous types of vegetables and fruits relative to their botanical type. Vegetables, legumes and oilseeds were characterized by the most effective discrimination by means of FA technique [8, 185]. There is differentiation of fruits in view of their belonging to botanical type (accessory, berry, pip and stone fruits) and family, i.e. Grossulariaceae (GR), Actinidiaceae (AC), Musaceae (MU), Bromeliaceae (BR), Cucurbitaceae (CU), Caricaceae (CA), Anacardiaceae (AN), Rosaceae (RO), Rutaceae (RU), Lauraceae (LA), Vitaceae (VI) and Ericaceae (ER) [9].

Chemometric analysis (ANOVA Kruskal Wallis test, Dunn's test, R-Spearman correlation, FA) of 6 species of mushrooms from 2 forest regions of Poland indicated that Ca, Na, K, Mg, Zn, Fe, Mn, Cu and Cd are effective descriptors of interspecies differentiation [280].

Authenticity of confectionery products was assessed based on CA data which distinguished samples of varied types in view of their botanical origin. According to [257], hierarchical dendrogram distinguishes two main clusters corresponding to the analyzed chocolate samples. The first cluster of scores represents dark chocolates, while the second one grouping milk chocolates. Dark chocolates are adjacent to subclusters representing cocoa products with content > 70% (C2–C4, C13) and others. However, dark chocolates, with declared cocoa content at least 45% (C7, C11), are ascribed to the grouping of milk chocolates (C15-C21), which means that these products contain less of cocoa than was stated on the label by the producer. Therefore, CA technique appeared to be effective tool in fraud detection [257].

As for coffee analysis (Table 2), chemometric assessment was performed in aim of categorization of samples in view of varieties characteristics. Based on FA data, classification of object samples and variables (loadings) relative to numerous coffee samples was achieved [236]. Analysis of their different technological forms (ground, instant coffee and coffee infusions) resulted in clear discrimination of the particular varieties of this assortment. Interesting results were obtained based on FA data which makes possible to distinguish arabica from robusta coffees. Higher values of F1 corresponded to 100% coffee of one bean type. Expensive brand coffee samples are generally situated near arabica coffee scores whilst less expensive brands are corresponded to robusta ones. It is pointed out that Mn is the best descriptor for identification of arabica samples whilst P identifies robusta ones [236].

Chemometric assessment was also helpful in classification of different types of teas based on their mineral composition (Table 2). For instance, CA technique was successfully used to identify several varieties of tea, e.g., Earl Grey, Assam, Ceylon and English Breakfast. Moreover, based on content of oxalates, Ca and Mg, it was possible to differentiate three types of tea according to their degree of technological processing (fermentation), i.e. black, green and Pu-erh tea [209].

Promising results were obtained for different assortment of wines (Table 2). A statistically significant correlation was found between the type of wine and the content of alcohol, K, P, Co and Pb concentrations. Moreover, FA technique allowed differentiation of individual types of wine based on its elemental composition. Macroelements such as Ca, K and P were responsible for distinguishing the group of dry wines and semi-dry wines corresponded to Pb, Cr, Co and Mg, while sweet wines contained the highest levels of Zn and Ni [324].

Differentiation of degree of pollution and other parameters

Bearing in mind the need to guarantee the quality of food, several multivariate techniques have been applied in identification of the sources of chemical pollutants in food. For instance, PCA data concerning heavy metals in molluscs of the Bay of Gdansk, French Atlantic coast and the Mediterranean Sea (Table 1) allowed for identification of the population of zoobenthos exposed to Zn, Cd and partly Ni in the

Gulf of Gdansk. It means that anthropogenic sources could be responsible for higher levels of these three elements in contrast to specimens inhabited the French aquatic regions. Moreover, besides the inter-regional differentiation also seasonal factors have an important influence on the heavy metals content in the cockles [42]. Seasonal variations were also observed in case of mussel *M. galloprovincialis* from the southern part of the Korean Peninsula [6]. ANOVA data indicated that seasonal variations in the both regions, i.e. the Ulsan Bay and the Masan Bay are statistically significant for mussel content of Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Ti, Pb, Sn and Zn. Moreover, based on FA data, the Masan Bay and partly of the Ulsan Bay samples were identified by the lowest values of F1, whilst most mussel samples originated from the Ulsan Bay situated near heavily industrialized area were described by the highest values of F1 [6].

For instance, concentration data for perch from southern Baltic was processed by chemometric techniques (Table 1). It is concluded that Hg in muscle and Cd, Pb and Cu in liver are descriptors for factorial differentiation of age groups of the fish investigated. The positive relationship between muscle Hg and age (weight-length) seems to be associated with the specific bioaffinity of CH_3Hg with a high biological half-life. Moreover, FA technique supported seasonal differences in muscle and especially hepatic samples; specifically, summer muscles were clearly separated from winter ones [30].

RDA and PTWI

The concentration data obtained for food have been frequently applied for an assessment of the hypothetical percentage realization of the recommended dietary intakes (RDA) for the essential elements in question and of provisional tolerable weekly intakes (PTWI) of toxic elements from the consumption of 100 g food product. For instance, Cd and Pb levels in muscle of perch are significantly lower than the PTWI and do not constitute any threat to man [30].

RDA and PTWI values of 15 elements were assessed for edible parts of 8 types of shellfish products. As for the former one, higher percentages of Ca (38. 8), Mg (17.2-22.5), Zn (88.2-121), Cu (88.2) and Se (155) were generally achieved for crabs. High values were also observed for lobsters, i.e. Ca (18.2), Mg (8.1-11.9), Zn (38.8-53.4), Cu (204) and Se (120). It should be emphasized that high RDA was also obtained for Mn (174-222) and Fe (52.6-118) in great scallop and mussels in shell, respectively. In view of the PTWI assessed for seafood products characterized by the highest levels of Hg, Cd and Pb, no health hazard is posed by exposure to these toxic heavy metals through seafood consumption [59].

Assessed RDA values for bee honey and syrup-feed honeys ranged from 0.35% (Na) to 5.84-23.4% (Cr) and from 0.20% (Na) to 3.85-15.4% (Cr), respectively. Consumption of bee products supplies human organism with the lowest and the highest percentages of RDA, i.e. from 1.21% (Na) to 68.5--103% (Mn) and 119-478% (Cr) [7].

It is reported that consumption of 100 g different kinds of grain products provides daily human organism with bioelements within a range of 0.33% (Na) to 321 (Cr) and 353--530% (Mn). It means that the highest average percentages of RDA were observed for Cr and Mn in bran and germs [8]. Assessment of PTWI for Pb and Cd in different cereal products (bread, cereals, rice, flour, pasta) allowed to conclude that consumption of 100 g of these products did not exceed allowed daily intake of both toxic heavy metals.

RDA values of 11 essential elements ranged from 1.65 to 2.07% (Cu) for fresh vegetables, 1.26-1.58% (Cu) to 40.8% (Na) for processed vegetables and 18.3% (Na) to 211-269% (Fe), 149-224% (Mn) and 72.7-291% (Cr) for dried vegetables. The latter three elements exceeded recommended dietary intakes resulting from daily consumption of 100 g different vegetables. As for RDA for legumes, minimum values achieved for Na (0.25%), whilst maximum values for P (94.7%). In case of oilseeds they oscillated between 2.29% (Na) and from 168% (P) to 173% (Mn) [8]. Referring to PTWI it is found that daily consumption of 100 g fresh, processed or dried vegetables poses no health hazard relative to Pb and Cd of food origin.

It is shown that RDA reached the highest values for K, Mg and Cu in 22 species of mushrooms. Based on PTWI it was concluded that the consumption of mushrooms collected from different forest regions of Poland poses no risk to human health [280].

Confectionary products (beet and cane sugar, molasses, maple syrup, cocoa, dark and milk chocolates) were also categorized according to hypothetical percentage realization of the recommended dietary intakes (RDA) for the essential elements in question. RDA values obtained for 11 elements in beet and cane sugar and its products such as molasses and maple syrup varied from 0.37% (Zn) to 8.19-32.8% (Cr) and from 2.28% (Ca) to 78.6-118% (Mn), respectively. Among all the analyzed confectionary products, cocoa was characterized by the highest RDA values, i.e. 41.6-51.9% (Na), 61.1% (K), 97.7-147% (Mn), 129% (P), 149-186% (Cu), 262-333% (Fe) and 215-862% (Cr). Dark and milk chocolates contained accordingly less essential elements than cacao. Relatively high RDA values were obtained for dark chocolate, i.e. 15.8-22.1% (Zn), 16.5% (P), 20.6% (K), 34.4-43.0% (Mg), 44.5-66.8% (Mn), 60.2-241% (Cr), 63.2-80.4% (Fe) and 67.1-83.9% (Cu). RDA values for milk chocolate were appropriately lower as compared with those obtained for dark chocolate [257]. Based on PTWI values for Pb and Cd, there is no threat to human health resulting from the consumption of honey [7] and confectionary products [257].

Concerning RDA estimated for essential elements, it is concluded that consumption of instant coffee supplies human organism with the highest average percentages of realization

of this index for adult [236]. Based on assessed PTWI for Cd and Pb corresponding to their content in 2 cups of coffee, it is shown that daily consumption of coffee did not exceed the tolerance limit (0.21% for Pb and 0.22% for Cd) [236].

Bearing in mind that the RDA of Mn approximately amounted to 15 % and 28.3% for black tea and green tea, respectively, it seems that black and green teas could be a good source of Mn. However, its bioavailability to the human body needs to be considered [11-12]. It is reported that one cup of black tea or green tea provided very low levels of Pb and Cd suggesting that consumption of both tea varieties does not exceed the PTWI recommendation for these toxic heavy metals.

Conclusions

Instrumental methods, e.g. spectroscopy combined with multivariate analysis techniques, appear to be helpful in quantitative food authentication, identification of adulterants/mislabeling and determination of food safety. The proper use of analytical and chemometric tools for assessing nutritive and health quality of animal and plant foods plays an important role in quality control, their classification in view of geographical origin, confection and degree of environmental pollution. Both these techniques would be useful in differentiating unprocessed and technologically processed food as well as detecting fraud to preserve the brand name of the original product. Application of chemometric tools leads to a deeper understanding of the distribution of mineral components in foods, what is especially important feature in the bromatological and ecotoxicological aspect.

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Conflicts of interest

None.

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