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SEEMEDJ SOUTHEASTERN EUROPEAN MEDICAL JOURNAL

Editorial SEEMEDJ 2022;6(1): 1-139

Dear colleagues,

Hereby, the new, 11th issue of SEEMEDJ is presented to you. This issue brings 14 articles with the main topic of arterial hypertension. Arterial hypertension is the leading cardiovascular disease, but also a risk factor for other cardiovascular and cerebrovascular disease and it is major public health problem due to its high prevalence. Thus, this issue of SEEMEDJ is co-edited with member of Croatian Academy of Science, prof. Bojan Jelaković. Topics covered in the present issue are hypertension management with SGLT2 (e.g. invited review of Bilić-Ćurčić et al), articles that assessed comorbidities and outcomes of arterial hypertension, particularly in relation to COVID-19, still an actual topic (articles by Gvozdanović et al and Bukal et al). Several reviews address other topic related to arterial hypertension, such as effects of anxiety, depression and antidepressant use on blood pressure by Ivanušić-Pejić et al, and the relationship of dehydroepiandrosterone sulfate and arterial hypertension (Jug et al) and new markers for hypertension and cardiovascular diseases, non-coding parts miRNAa (Kolobarić N and Drenjančević I). Adeleke presented a study on relationship between alcohol use and patterns of blood-pressure change due to examination stress among university academic stuff. In relation to the world and national campaigns for dietary salt intake reduction it is an intriguiging question if consumption of iodine is too high (elaborated by Vasiljev et al). Lack of physical activity is also an important contributing factor to bad hypertension outcomes. On the other hand, physical activity is necessary to preserve functional ability of elderly, which is studied by Bilajac et al. Kurup et al investigated transfusion transmissible infections among blood donors in the National Blood Transfusion Service in Guyana. Mišković et al reviewed relationship of iron deficiency and recurrent aphtous stomatitis, while Liović-Milec et al presented their work on transient corneal edema after phacoemulsification, demonstrating that older patients, higher grade of NO and amount of energy consumed during procedure are predictive factors for the severity of the corneal edema. During intramuscular vaccine injections, aspiration has always been performed, to ensure that the needle does not puncture one of the blood vessels. Whether the lack of this simple procedure is related to side effects of vaccination

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(against COVID-19, for example) is reviewed by Kajan et al. Finally, Slivšek and co-authors introduce to us to the concept of deep ecology.

We are happy to inform our potential authors that SEEMEDJ has been included to another citation base: OAJI- Open Academic Journal Index and also pending to Cabell's database, thus widening our accessibility to readers.

The art work at the cover page is a painting – a collage of Osijek artist Branimir Kusik.

I hope that readers will find relevant published articles for their work. On the behalf of the editorial board and my own, I warmly greet our readers and invite them to join us in the endeavor of publishing their own scientific work in SEEMEDJ.

Ines Drenjančević, MD, PhD
Editor-in-Chief
Southeastern European Medical Journal (SEEMEDJ)

Contents

Invited review

Changing the Landscape of Hypertension Management With SGLT2i

Ines Bilić Ćurčić [1,2], Vjera Ninčević [3], Silvija Canecki Varžić [2,4], Ivana Prpić Križevac [2,4], Jasminka Milas Ahić [2,5], Ivica Mihaljević [6,7]

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Abstract

Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are a newer class of drugs that have primarily been used in the treatment of type 2 diabetes. However, as new findings from clinical trials have become available, their indication has been expanded to include treatment of heart failure and chronic kidney disease without the presence of diabetes. The pathophysiological mechanisms of extraglycemic effects of SGLT2i are still being unraveled, but one of the most prominent consequences is a decrease in blood pressure, which has implications for hemodynamics and arterial stiffness. Recent findings indicate that this class of drugs has a beneficial effect on lowering nocturnal blood pressure (BP), with special importance in type 2 diabetes (DMT2), since unregulated nocturnal hypertension is associated with an increased incidence of cardiovascular (CV) events. In this mini-review, we have summarized current knowledge about the effects of SGLT2i on blood pressure, including office, home, and ambulatory BP, and potential implications for treatment of hypertension in diabetic and non-diabetic individuals, with positive effects on cardiorenal outcomes.

(Bilić Ćurčić I, Ninčević V, Canecki Varžić S, Prpić Križevac I, Milas Ahić J, Mihaljević I. Changing the Landscape of Hypertension Management With SGLT2i. SEEMEDJ 2022; 6(1); 1-11)

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KEYWORDS: SGLT2i, blood pressure, type 2 diabetes, cardiovascular disease

Introduction

SGLT2i are a class of newer therapeutic agents in diabetes treatment with proven cardiorenal benefits, which reduce the risk of cardiac failure and death from cardiovascular disease (CVD) and slow the progression of diabetic kidney disease [1–5]. This class of agents is therefore recommended for diabetes treatment in high-risk patients with heart failure, CVD or kidney disease, based on recent guidelines [6]. In addition, their role in the treatment of heart failure and kidney disease independent of the presence of diabetes has also been recognized recently [7, 8].

Possible mechanisms responsible for cardiorenal benefits are numerous, including positive effects on hemodynamic parameters, reduction of arterial stiffness, improvement of chronic inflammation, metabolic fuel switching, along with traditional mechanisms, such as lowering of blood glucose and reduction of blood pressure and body weight [9–12].

Diabetic patients have a specific 24-hour BP profile with a non-dipper pattern of nighttime BP likely caused by increased circulating volume [13–16]. The presence of nocturnal hypertension is more common in patients with diabetes compared to those without it [17–19] and is associated with higher mortality rates [20]. Likewise, the frequency of masked hypertension is relatively high in diabetic patients, ranging from 27% to 47% [21–24], and it is one of the independent predictors of CVD [24]. Recognizing and treating masked hypertension in patients with DMT2 is imperative, and out-of-office BP measurement using home BP monitoring (HBPM) or ambulatory BP monitoring (ABPM) is therefore recommended in the diagnosis of hypertension (HTN) [25–27].

Effects of SGLT2i on BP measurements

SGLT2i agents, such as empagliflozin [28–34], canagliflozin [35–39], ertugliflozin [40–42] and dapagliflozin [43–47], have been shown to lower office blood pressure in patients with DMT2 and HTN in a number of studies. According to several published meta-analyses, marked reductions in systolic BP (SBP) ranged from -2.45 mmHg to -4.45 mmHg compared to active comparators, with diastolic BP (DBP) reductions from -1.46 mmHg to -2.01 mmHg [48, 49]. Changes in BP with empagliflozin and existing antihypertensive therapy were examined in the SACRA (Sodium-Glucose Cotransporter-2 [SGLT2] Inhibitor and Angiotensin Receptor Blocker [ARB] Combination Therapy in Patients With Diabetes and Uncontrolled Nocturnal Hypertension) study. The study included Japanese patients with type 2 diabetes and poorly controlled nocturnal hypertension who were receiving standard antihypertensive therapy and were randomized to the empagliflozin or the placebo group. Clinic BP, 24-hour ABPM and morning home BP was monitored, while the primary endpoint was change from baseline in nighttime BP (ABPM). At 12 weeks, empagliflozin significantly reduced daytime, 24-hour, morning home, and clinic SBP (-9.5, -7.7, -7.5, and -8.6 mmHg, respectively). Body weight and glycosylated hemoglobin reductions between groups were significant, albeit minor (-1.3 kg and -0.33 percent, respectively). In addition, marked reductions in N-terminal pro-B-type natriuretic peptide levels were observed in the empagliflozin versus the placebo group (12.1%; P = 0.013); reductions were likewise observed in atrial natriuretic peptide levels (9.7%; P = 0.019) [50]. However, there was no statistically significant reduction in overnight BP compared to the placebo group, with a drop in nighttime SBP of 6.3 mmHg from the baseline. Given that a 5-mmHg decrease in mean overnight SBP has been linked to a 20% reduction in CVD risk [51], the nighttime BP reduction observed with empagliflozin could have clinical importance regardless of the lack of statistical significance.

As previously mentioned, 24-hour ambulatory BP is a better predictor of CV risk than office BP [20, 24]. A recently published meta-analysis including randomized, double-blind, placebo-controlled trials reporting 24-hour ABPM data demonstrated the lowering effect of SGLT2i on ambulatory systolic and diastolic BP by 3.76 mmHg (95 percent CI, 4.23 to 2.34; I2 = 0.99) and 1.83 mmHg (95 percent CI, 2.35 to 1.31; I2 = 0.76),

respectively, over a 24-hour period. There were also significant reductions in systolic and diastolic BP during the day and at night, independent of body weight change [52].

Another meta-analysis examining the effects of SGLT2i inhibition on ambulatory BP aimed to assess the relationship between dose and ambulatory BP response to SGLT2 inhibition and to compare it to low-dose hydrochlorothiazide. According to this meta-analysis, in 24-hour ABPM, SGLT2 inhibitors caused an average reduction of 3.62/1.70 mmHg in systolic/diastolic BP, which is equivalent to the BP-lowering efficacy of low-dose hydrochlorothiazide, regardless of the SGLT2 inhibitor dose. However, SGLT2 inhibition reduced blood pressure more effectively during the day than at night [53]. In a post-hoc analysis of the EMPA-REG BP trial, empagliflozin was found to lower nocturnal BP more than daytime BP in patients with DMT2 and a non-dipper HTN profile [54].

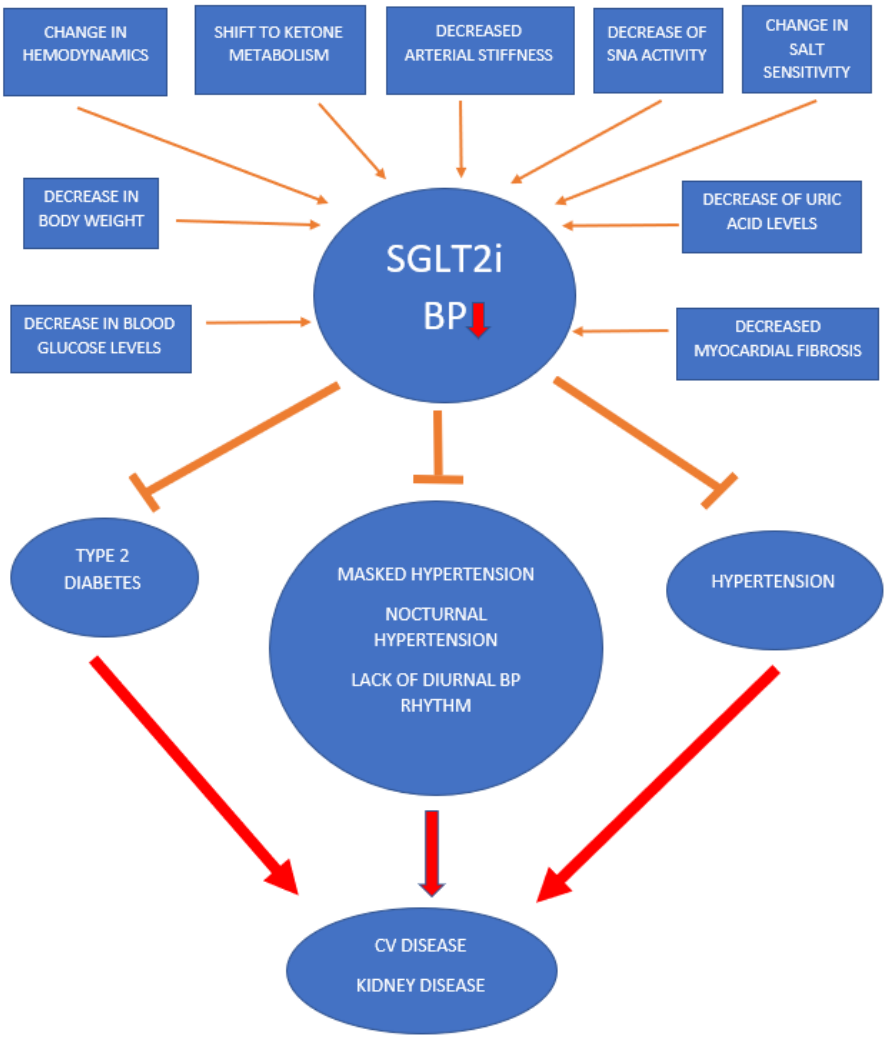

Figure 1. Pathophysiological mechanisms involved in blood pressure-lowering effects of SGLT2i and their role in preventing CV and kidney disease

The presently published data regarding the effects of SGLT2i on home BP are limited to the Japanese population. In addition to the results of the previously mentioned SACRA study, which showed a lowering effect of empagliflozin on morning home BP [50], the results of the SHIFT-J study demonstrated a beneficial effect of canagliflozin on nocturnal, morning and evening home SBP (-5.23, -6.82, -8.74, respectively) compared to the control group of patients with uncontrolled DMT2 and nocturnal BP [55]. In addition, beneficial effects of dapagliflozin on morning, evening and nocturnal home SBP (-8.32; -9.57 and -2.38 ± 7.82 mmHg, respectively)

were reported in Japanese patients with DMT2 [56].

Based on the available evidence, we can conclude that the drop in 24-hour ambulatory BP seen with SGLT2 inhibitors is a class effect and is comparable to low-dose hydrochlorothiazide. Likewise, it seems that SGLT2i have a significant BP-lowering effect at night, suggesting that these drugs have the potential to repair altered circadian BP rhythms in hypertensive patients with DMT2, thus improving cardiovascular and kidney outcomes (Figure 1).

Proposed mechanisms of SGLT2 inhibition on blood pressure

The effect of SGLT2i on lowering blood pressure could be explained by several mechanisms involving changes in hemodynamics due to a decrease in effective circulating volume caused by diuresis and natriuresis, reduction in body weight and uric acid levels, antihyperglycemic effects, shifting metabolic fuel from glucose to ketones, inhibition of sympathetic nervous system activity, change in arterial stiffness and switching of salt-sensitive to non-salt sensitive BP phenotype due to osmotic diuresis [11, 57, 58] (Figure 1).

Microvascular and macrovascular dysfunction leading to increased arterial stiffness is common in diabetic patients [59, 60], meaning that an increase in blood pressure and blood pressure variability cannot be attenuated in these patients' major arteries and are instead conveyed to atherosclerotic plaque sites in the periphery. As a result, the combination of HTN and DMT2 creates hemodynamic stress that predisposes patients to the development of CVD [61–63]. According to preclinical evidence, improvements in arterial stiffness and endothelial function may contribute to the CV advantages of SGLT2i therapy [64]. This is supported by evidence obtained in clinical trials, suggesting that currently available SGLT2i medications reduce arterial stiffness in individuals with DMT2 [65–68]. Furthermore, dapagliflozin also reduced cardiac fibrosis in infarcted rat hearts by controlling macrophage polarization via STAT3 signaling, as well as by altering epicardial fat tissue distribution and cytokine and adipokine secretion [69–71].

Clinical evidence of beneficial effects of SGLT2i on cardiorenal outcomes

Large cardiovascular outcome trials (CVOT) have shown the beneficial effects of SGLT2i on improving CV outcomes, largely due to reduction in hospitalizations caused by heart failure and, in some studies, decreasing mortality. In the EMPA-REG OUTCOME trial, empagliflozin significantly reduced the occurrence of primary endpoint events (3-point major cardiovascular event (MACE) consisting of non-fatal myocardial infarction, non-fatal stroke and CV death) compared to placebo, with significantly lower rates of CV death, hospitalization for heart failure (HF) and death from any cause [1]. Similar results were obtained in the CANVAS trial [2], which examined the cardiovascular safety of canagliflozin, demonstrating a reduction in 3-point MACE, but no effect on CV death, as opposed to the EMPA-REG OUTCOME trial. Dapagliflozin, on the other hand, reduced the risk of HF, but had no effect on atherosclerotic CVD, as shown in the DECLARE-TIMI 58 trial [3].

According to available evidence, the protective effect of SGLT2i on 3-point MACE is limited to patients with previously existing CVD, while class benefits exist for HF regardless of CVD presence [72]. Furthermore, in patients with DMT2 and chronic kidney disease (CKD) (eGFR 60 mL/min/1.73 m2), a favorable effect on HF and 3-point MACE has been confirmed [73]. Recently, results from two large-scale studies were published, demonstrating improved outcomes in patients with HF with reduced ejection fraction (HFrEF) treated with dapagliflozin and empagliflozin (DAPA-HF and EMPEROR-Reduced, respectively) independent of the presence of diabetes [7, 8].

Based on the evidence obtained in the above trials, the guidelines of the European Society of Cardiology (ESC) propose SGLT2i and/or

glucagon-like peptide-1 receptor agonists (GLP-1 RA) as the treatment of choice for patients with diabetes and proven CVD or risk thereof, which is contrary to the common practice of using metformin as the first choice for DMT2 treatment [6].

Nearly all patients included in the CREDENCE study, which examined the effect of canagliflozin on renal outcomes, were treated with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs), with eGFR from 30 to 90 ml per minute [74]. The experiment was halted early due to favorable interim analysis results, showing that the relative risk of composite renal outcomes was reduced by 34%, while the relative risk of end-stage renal disease was reduced by 32%. These findings reveal that renoprotection was accomplished across the whole range of eGFR levels, re-establishing the nephroprotective effect regardless of baseline renal function.

The probability of dialysis, transplantation, or death due to kidney disease (hard renal outcomes) was considerably reduced in individuals treated with SGLT2i, as was recently validated in a meta-analysis of all studies using SGLT2i in diabetic patients [72]. Because of their reduced antihyperglycemic efficacy, SGLT2i were previously not recommended for diabetic patients with eGFR of less than 45 ml/min per 1.73 m2 [75, 76]. Such restrictions became debatable after the evidence of renoprotective effects emerged in the mentioned trials [77]. This was confirmed in the latest clinical trials involving diabetic and non-diabetic patients with heart failure and preserved ejection fraction [7, 8], demonstrating improvement of hard renal outcomes in the lower specter of eGFR, 30 and 20 mL/min/1.73 m2 in the EMPEROR-Reduced and DAPA-HF trials, respectively. The final proof that the renoprotective effect is independent of the antihyperglycemic effect was provided by the DAPA-CKD study, which indicated that dapagliflozin reduced renal events in patients with CKD with or without DM treated with maximum tolerated doses of ACE inhibitor/ARB [78]. Beneficial effects were observed on non-CV and all-cause mortality.

Conclusions

The effects of SGLT2i on blood pressure also appear to be largely responsible for the beneficial effects of this class of drugs on cardiorenal outcomes. They play a particularly important role in the control of nocturnal blood pressure, which is associated with increased CV risk, especially in patients with DMT2. Thus, the use of SGLT2i in patients with DMT2 and hypertension is certainly justified regardless of glycemic control in order to reduce the risk of CV and renal complications and improve the patient's quality of life. The extent to which the effects of SGLT2 inhibition on blood pressure are responsible for reduction of risk of heart failure, cardiovascular and renal disease in non-diabetic individuals with arterial hypertension remains a question to be answered.

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Disclosure

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Competing interests. None to declare.

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Review article

Overview of Iodine Intake

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Abstract

Iodine is an essential element for human health. Food is the primary source of iodine, but the iodine content of local foods depends on the iodine content of the soil. Therefore, a low iodine concentration in soil and water results in plants and animals with low iodine content. Numerous effects of iodine deficiency on growth and development are known as iodine deficiency disorders. Iodine deficiency has been identified as the most common cause of brain damage in the world and is linked to its effects on infant and child growth and development. Supplementation of table salt with iodine was introduced in the 20th century. Croatia was one of the first countries to introduce the supplementation of table salt with potassium iodide at a concentration of 10 mg/kg in 1953 and 25 mg/kg in 1993. In 2003, the Croatian population reached iodine sufficiency, but given the excessive salt intake (11.6 g/day) and additional sources of iodine in the diet, the question arises, are we consuming too much iodine? This article gives a short overview of iodine intake.

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Discovery of iodine and history of iodine supplementation

Purple vapour was first discovered in 1811 by the chemist Courtois during the production of saltpeter. The production of saltpeter required soda, which was obtained from the ashes of seaweed. The chemical reaction required to make saltpeter resulted in the formation of insoluble material at the bottom of metal vats. The material was cleaned out using acid and heat, which led to purple vapour crystallising on the bottom of the vats. However, iodine was only discovered two years later by Sir Humphry Davy and Gay-Lussac (1). The use of iodine in medicine started in 1819 with Coindet, a physician from Geneva who administered a tincture of iodine to goitre patients, which resulted in the swelling going down within a week. The link between iodine and the environment was not confirmed, although the French chemist Chatin proved that the iodine content in water and food was insufficient in the areas commonly affected by goitre. The first paper on the link between iodine deficiency and goitre was published in 1851 (2). Chatin's discoveries on the link between environment and iodine deficiency were neglected until the end of the 19th century. In 1896, the presence of iodine in the thyroid gland was discovered by Baumann and Roos (3). Switzerland was the first country to introduce iodine as prophylaxis against goitre and cretinism. Since 1922, Switzerland has had a continuous program of supplementation of salt with iodine, with the level of iodine eventually being raised from 15 mg/kg in 1980 to 20 mg/kg in 1998 (3, 4).

Role of iodine, iodine deficiency disorders and optimal iodine intake

The thyroid gland of a healthy adult stores 70-80% of the total iodine content in the body, which ranges from 15 to 20 mg. It also uses about 80 µg of iodine per day for the synthesis of thyroid hormones. Iodine is essential for the synthesis and production of thyroid hormones (triiodothyronine – T3 and thyroxine – T4) and for normal thyroid function. The thyroid hormones control the metabolic processes in our body. Their production is controlled and influenced by the pituitary gland and its hormone thyrotropin (thyroid-stimulating hormone – TSH). TSH increases the uptake of iodine from the blood into the thyroid gland and stimulates the production of thyroid hormones, which is regulated through feedback. When the level of thyroid hormones in the blood is low or decreases, increased TSH is released from the pituitary gland. TSH stimulates the function of the thyroid gland, causes cell growth and proliferation of thyroid tissue, and, in case of chronic iodine deficiency, can lead to an enlargement of the thyroid gland known as a goitre. Goitre is one of the most common diet-related diseases (5). In addition, when iodine intake is very low, thyroid hormone production decreases despite elevated TSH levels, leading to hypothyroidism (6, 7). Numerous effects of iodine deficiency on growth and development are known as iodine deficiency disorders. Iodine deficiency has been identified as the most common cause of brain damage in the world and it is related to its impact on the growth and development of infants and children (8,9).

Table 1. Recommended daily intake of iodine (12)

	Children 0-5 years	Children 6-12 years	Adults	Pregnant and lactating women
Intake (µg)	90	120	150	250

The spectrum of iodine deficiency disorders includes mental retardation, hypothyroidism, goitre and varying degrees of other growth and developmental disorders (10). Chronic iodine deficiency is also associated with an increased risk of developing follicular thyroid cancer (11).

According to Zimmerman and Andersson, the recommended daily dietary intake for iodine is as shown in Table 1.

Iodised salt regulations

One of the greatest public health challenges worldwide is the lack of essential vitamins and minerals in the daily diet. This issue is widespread among women and young children in low- and middle-income countries (13). This can lead to serious health issues and economic consequences, which only adds to the global burden of disease (14,15). This also applies to iodine and the issue of high prevalence of disorders caused by insufficient iodine intake (16). To overcome this issue, many national nutrition strategies started promoting programs to eliminate iodine deficiency disorders in the 1990s, after the World Summit for Children and the Joint United Nations Children's Fund (UNICEF)/WHO Committee on Health recognised the benefits of iodisation of table salt (17-19). Iodisation of salt has been recognised as the best preventive measure to eliminate iodine deficiency disorders at the population level (20). Not only is the iodisation of salt technically feasible, but salt is also consumed in standard quantities by all segments of the population worldwide (21).

Between 1942 and 2021, 123 countries worldwide established a legal framework for mandatory iodisation of table salt and 21 countries introduced legislation for voluntary iodisation of table salt (22). According to estimates made by UNICEF, an average of 88.7% of households worldwide consumed table salt with some form of iodine in 2021. Most households using iodised table salt are in the East Asia and Pacific region (92%) and in South Asia (89.9%) (23). On the other hand, there are also countries with excessive iodine intake, such as South Korea (449 µg/L), Djibouti (335 µg/L), Cameroon (>300 µg/L), Honduras (356 µg/L) and Colombia (407 µg/L). The reasons for the increased iodine concentration are related to diet, groundwater and drinking water, which are naturally rich in iodine, as well as to high amounts of iodine added to salt considering the per capita intake of salt (22).

Although the legal regulations on compulsory or voluntary iodine intake via table salt have greatly reduced the issue of iodine deficiency disorders, there are still regions where almost one billion people do not have access to iodised table salt(22). One way to ensure the necessary supply of iodised table salt for such persons is to promote the Universal Salt Iodization (USI) Initiative, which is one of the most economical, convenient and effective strategies to increase the intake of iodised table salt (24). The initiative could be improved through cooperation between relevant stakeholders at local, regional and national levels, in the food industry and in the scientific community. Nowadays, EU member states introduce various strategies and legal frameworks to combat iodine deficiency, but there are still countries such as Norway (75 µg/L), Finland (96 µg/L) and Germany (89 µg/L) that are deficient in iodine from table salt (22). To combat iodine deficiency, it is necessary to identify potential barriers and adopt a uniform approach to the development of a single regulatory framework at the EU level based on the guidelines of WHO, without exception. Furthermore, such a uniform approach should allow national governments to implement measures in line with their culture and to remove barriers to marketing for the purpose of raising awareness of iodine deficiency disorders among the general public (25).

1. *Dietary sources of iodine and fortification of salt with iodine*

Unlike most essential nutrients, the status of iodine in our body is not related to socioeconomic factors, but rather to the climate in which we live(16). The iodine content of local foods depends on the iodine content in the soil. Therefore, a low concentration of iodine in the soil and water will result in plants and animals with low iodine content. Most of the iodine on our planet is found in the oceans. Also, the iodine

content in the soil varies depending on the region. If the soil is older and more exposed to external factors, it is more likely that iodine will be washed out by soil erosion. People who depend on local foods produced in iodine-deficient areas must also rely on foods fortified with iodine(26, 27). Humans receive iodine through food, food supplements and water, mainly in the form of iodide (27, 28). Iodine concentrations vary both between food groups and within the groups themselves. Foods containing iodine include seafood, eggs, milk and dairy as well as iodised salt. The iodine content of milk and eggs depends on how animals are fed (iodine-enriched feed) and on the hygiene on the farm (28). The natural iodine content in most foods and beverages is low, and the most commonly consumed foods provide 3 to 80 µg per meal (5). In Croatia, all table salt used for food is iodised by adding 25 mg of iodine per kilogram of salt in order to prevent diseases related to insufficient iodine intake and Croatia is recognised as an iodine sufficient country (29, 30). The urinary iodine test is a well-known, inexpensive and easily accessible method of determining iodine status (31). A nationwide project named Epidemiology of Hypertension and Salt Intake (EH-UH 2) is currently active in Croatia. One of its main objectives is to determine iodine intake from a 24-hour urine sample and to assess whether there is a risk of exposure to low iodine concentrations if the recommended daily salt intake limit of 5 grams is observed (Strategic Plan for Reduction of Salt Intake) (32, 33).

Iodine toxicity

Excessive iodine intake usually occurs when people take iodine supplements to improve thyroid function. Several types of seafood, including shrimp, cod, tuna and seaweed, are rich in iodine. In cultures where a lot of seaweed is eaten, people sometimes consume thousands of milligrams of iodine per day. It is estimated that people in Japan consume between 1,000 and 3,000 mg of iodine daily, mainly from seaweed. This leads to iodine-induced hyperthyroidism and goitre in Japan. However, the same research also points out that the higher iodine intake may play a role in the low cancer rates and high life expectancy in Japan (8). Exceeding the maximum permissible amount of iodine can lead to poisoning and iodine toxicity may eventually lead to iodine goitre, hypothyroidism, or myxoedema (35). Also, excessive iodine intake can have a negative impact on patients with breast cancer due to the stimulation of the transcriptional activity of oestrogen receptor α (ER-α), resulting in an elevated risk of developing thyroid cancer (36). Chronic toxicity develops only if iodine intake exceeds 2 mg/day. In some sensitive individuals, ingestion of iodine-containing substances may lead to thyroid dysfunction due to high iodine exposure. Under certain circumstances, excessive iodine intake can have harmful effects on the thyroid gland after just one exposure to an iodine-containing substance (35). Patients with iodine deficiency and patients with a pre-existing thyroid condition may be sensitive to iodine levels considered safe for the general population. Neonates, older people and pregnant women may also be more susceptible to iodine excess (37). It is very difficult to get iodine poisoning from food alone, so iodine poisoning is usually a result of taking too many iodine supplements (38). Acute iodine poisoning is rare and the symptoms of iodine poisoning range from relatively mild to severe, depending on the amount of iodine. Mild symptoms of iodine poisoning include diarrhoea, burning in the mouth, nausea and vomiting. Very large amounts of iodine can cause a metallic taste in the mouth, increased salivation, irritation of the digestive system and acne-like skin changes. Severe symptoms of iodine poisoning include swelling of the airways, blue skin discoloration (cyanosis) and low heart rate. Iodine poisoning can also lead to kidney failure (39).

Certain medications can also increase the amount of iodine in the body. Amiodarone, a drug used to regulate heart rate and rhythm, contains 75 mg of iodine in each 200 mg tablet, which is one hundred times more than the standard recommended daily intake (40). Various drugs, such as propranolol (in high

doses), the anti-thyroid drug propylthiouracil, dexamethasone, the cholecystographic agents (ipodate and iopanoic acid) and the previously mentioned amiodarone can inhibit the conversion of T4 into T3 (41). Potassium iodide supplements and the contrast medium used for CT scans also contain iodine (9). Elemental iodine is an oxidising irritant and can cause lesions in case of direct contact with the skin, while exposure to iodine vapours causes irritation of the lungs, eyes and skin.

Diagnosis and assessment of iodine toxicity is an important part of the health care team's approach to providing treatment, enhancing care coordination and establishing communication necessary to improve patient outcomes. Iodine toxicity is a rare condition that requires a comprehensive initial diagnosis and a heightened level of suspicion. Patients may present with vague signs and symptoms. Although medical history may reveal toxicity, the cause is difficult to determine without further investigation. The consequences of iodine toxicity depend on the cause and severity. However, to improve outcomes, it is recommended that an interprofessional group of experts be consulted to monitor the patient's vital signs and educate the patient and their family (42).

Discussion

Iodine is an essential mineral for healthy functioning of the human body, but only in moderate amounts. Iodine boosts thyroid function and consequently increases metabolism, supports a healthy pregnancy and prevents cretinism, and promotes heart health by stimulating the production of hormones that regulate heart rate and blood pressure. Over one billion people around the world still do not have access to iodised salt and therefore suffer from iodine deficiency (10, 11). After the Second World War, the prevalence of goitre and cretinism in the endemic areas of the Republic of Croatia was high (43, 44). The highest prevalence was found in the village of Rude and in the Samobor and Žumberak mountains, where almost 85% of school children suffered from goitre and 2.3% of children suffered from cretinism. The first intervention involving iodised salt was made in 1953 and set at 10 mg per kilogram of salt. This intervention led to a threefold reduction in goitre in children and complete elimination of cretinism (44, 45). Fifteen kilometres from the Adriatic coast, in the region of Grobnik, Croatia, goitre was endemic before iodised salt prophylaxis. In 1963, the prevalence of goitre was 63% in school children and 34% in adults. A second survey was conducted in 1981 and the prevalence of goitre was 18% in school children and 11% in adults. In 2001, another survey on the prevalence of goitre was conducted in the Grobnik region. The results showed that the prevalence of goitre was 6.6% among school children and 6.4% among adults. A significant decrease in prevalence was achieved among school children, but not among adults due to the hereditary thyroid disease in the indigenous population (11.7%) (46). The high prevalence of goitre led to an increase in the potassium iodide content in salt from 10 mg/kg to 25 mg/kg (47). In the period from 2002 to 2009, urine samples of school children were analysed to determine the iodine concentration using a median urinary iodine concentration [UIC] of 68 μg/L. The results showed that the iodine concentration in 2009 was significantly higher than in 2002, indicating that there were hidden sources of iodine in the diet besides salt (29).

The first legal document regulating the general iodisation of salt in the Republic of Croatia was introduced in 1953, prescribing 10 mg of potassium iodide (KI) per kilogram of salt (48). In the 1990s, at the initiative of the then chairman of the National Committee for Eradication of Goitre and Control of Iodine Prophylaxis, Professor Zvonko Kusić, two new legal documents were introduced: Instructions on Iodisation of Table Salt (Official Gazette 84/96) and the Salt Iodisation Regulation (Official Gazette 15/97), which led to a further increase in the prescribed amount of iodine in table salt (48). The initiative for universal iodisation of salt in Croatia allows for iodisation of table salt to be applied at three levels: in households, food industry and animal feed production. The Croatian model of table salt iodisation complies

with all guidelines of the WHO, International Council for the Control of Iodine Deficiency Disorders (ICCIDD) and UNICEF and it has been internationally recognised as one of the models for addressing this emerging public health issue (48, 49).

Conclusion

This article provides an overview of iodine discovery and its physiological role in our bodies, at the same time explaining the optimal iodine intake in relation to different dietary sources and environments. Iodine toxicity and iodine deficiency disorders are also addressed, which raises the question of whether we really know how much iodine we consume per day. Another question is, if we ingest too much salt, are we placing ourselves at the risk of excessive iodine intake? The answer to those questions could be found in different initiatives and projects, such as the EH-UH 2, for which a comprehensive and detailed strategic approach should be defined to assess the amount of iodine intake across the population.

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Original article

Association Between Common Comorbidities and Outcomes in COVID-19 Patients Hospitalised in General Hospital Našice – a Cross-Sectional Study

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Abstract

Aim: The aim was to define the impact of comorbidities, specifically hypertension as one of the most common chronic diseases, on the outcome and length of stay for COVID-19 patients.

Methods: The cross-sectional study, carried out from October to December 2021, included 129 hospitalised COVID-19 patients who presented to the Emergency Department and were hospitalised and treated in the COVID ward in the General Hospital Našice. All patients tested positive for COVID-19 with a polymerase chain reaction (PCR) test. Clinical parameters were also recorded and they included demographic factors, comorbidities, type of antihypertensive therapy, new-onset hypertension, length of stay and the overall outcome.

Results: The most common comorbidity was hypertension (86, 66.7%). Hypertension was associated with women ($P = 0.03$) and age over 65 years ($P < 0.001$). Length of stay was longer for patients with hypertension ($P = 0.04$) and/or diabetes mellitus ($P = 0.04$). Higher mortality was associated with age over 65 years ($P < 0.001$) and a higher number of comorbidities ($P = 0.01$). New-onset hypertension was recorded in three patients. There was no significant difference in the outcome in relation to antihypertensive therapy.

Conclusion: Hypertension is the most common comorbidity in hospitalised COVID-19 patients. Although treated hypertension did not have a negative impact on the outcome, other potential risk factors, including a higher number of comorbidities and older age, are associated with mortality in COVID-19 patients.

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KEYWORDS: COVID-19, comorbidity, hypertension, mortality

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes pneumonia and flu-like symptoms. The first case was reported in Wuhan, China in late 2019, after which the virus has spread around the world. Shortly afterwards, the coronavirus pandemic has been declared.

Since the first confirmed case of COVID-19, there have been approximately 418,650,474 confirmed cases worldwide, including 5,856,224 deaths, as reported by the WHO (1). The first case of COVID-19 in Croatia was reported in February 2020 in Zagreb. At the time of writing, there have been approximately 1,041,212 confirmed cases, 14,815 of which were fatal (2).

Several variants of SARS-CoV-2 with the far more dominant Alpha strain were recorded in early May 2021. In June, the Delta strain began to spread more rapidly, and by early September, it was responsible for more than 99% of all COVID-19 cases. In December 2021, the Omicron variant began to break through within the Delta strain and a few weeks later, it became the dominant strain (3).

Although the prognosis for patients with COVID-19 is generally good, it has been observed that some patients with comorbidities have a poor outcome (4,5). Previous studies on COVID-19 have reported that over 60% of COVID-19 patients have associated comorbidities (4,6). The most common comorbidities are hypertension (7–9), diabetes mellitus (DM) (8,10), cardiovascular diseases (10), chronic obstructive pulmonary disease (7), chronic kidney disease (11) and malignancy (12), which are associated with severe disease and mortality. However, there is conflicting data in this regard. A poor outcome in COVID-19 patients is also associated with a higher number of comorbidities (8). It has been observed that three or more comorbidities are the cut-off point for severe COVID-19 cases, while four or more comorbidities indicate a high rate of fatal outcomes (4). Older age is an independent risk factor for disease progression and higher mortality rates (13). It has been observed that many patients with rapid disease progression are 45-65 years old, while severe disease and most deaths were reported in patients aged 70 and above (14). In general, both age and the number of comorbidities could have a predictive role in disease severity and in the final outcome of COVID-19.

The length of stay for COVID-19 patients depends on several factors. Analyses have shown that age, gender, hypertension, DM, cardiovascular disease and renal failure are significantly associated with the length of stay (15). It has been observed that every additional comorbidity was significantly associated with an increase in the expected length of stay by 2%. Similarly, every one-year increase in age is also significantly associated with an increase in the expected length of stay by 2% (16).

Hypertension is one of the most common chronic diseases associated with hospitalised patients, but its impact on COVID-19 patients has not been well-defined (17). Previous studies indicate that hypertension is associated with a higher risk of all-cause mortality regardless of age, gender and other comorbidities (9). It is also assumed that antihypertensive drugs play a major role in the mechanism of interaction between the coronavirus and target cells in the body through the angiotensin-converting enzyme 2 (ACE2) (18,19). This hypothesis has come to light when the following two facts were related: the finding that the renin-angiotensin-aldosterone system (RAAS) inhibitors increase the expression of ACE2 and the observation that hypertension and diabetes were the most common comorbidity among patients with severe COVID-19, for which RAAS inhibitors are widely used (20). Although the treatment of hypertension with these drugs is thought to increase the risk of developing severe and fatal COVID-19, studies have found no such association so far (20–22).

The COVID-19 pandemic is a global health issue that has spread rapidly around the world with varying degrees of severity. This study aimed to define the significance of comorbidities in

hospitalised COVID-19 patients, specifically hypertension as one of the common chronic diseases worldwide. Understanding these factors is key to improve the accuracy of predicting disease outcomes as well as the length of stay for the purpose of optimising resources and providing better care for patients.

Patients and Methods

This cross-sectional study, carried out from 1 October to 31 December 2021, included 129 hospitalised COVID-19 patients who presented to the Emergency Department in the General Hospital Našice, which has been a designated hospital for treating COVID-19 patients since November 2020. Inclusion criteria were as follows: patients admitted from the Emergency Department, hospitalised and treated in the COVID ward, who tested positive for SARS-CoV-2 with a nasal swab polymerase chain reaction (PCR) test (23) during the admission. Exclusion criteria included the following: eight patients transferred from the COVID ward to the University Hospital Centre Osijek during hospitalisation.

The data were reviewed based on the electronic medical records kept by the Emergency Department. Clinical parameters were also recorded, including demographic factors (age and gender), comorbidities (hypertension, DM, atrial fibrillation and respiratory comorbidities – asthma or chronic obstructive pulmonary disease), type of antihypertensive therapy (angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), calcium channel blockers and diuretics), length of stay and outcome (discharge or fatal outcome). With regard to the number of comorbidities, the patients were divided into five groups (0, 1, 2, 3 and 4 comorbidities). Depending on the combination of antihypertensive therapy, the patients were divided into four groups (1, 2, 3 and 4 types of antihypertensives). New-onset hypertension was defined as hypertension in patients who did not have hypertension as a comorbidity or who did not have any antihypertensive treatment during admission, but received hypertensive therapy at discharge. Regarding age, two groups of patients were defined (<65 and ≥65 years old). In terms of the length of stay, the patients were divided into two groups based on the number of days spent in the COVID ward (≤10 or >10 days). The study was approved by the Ethics Committee of the General Hospital Našice (Official Gazette 100/18, 147/2020).

Statistical Analysis

Categorical and numerical data were used for the statistical analysis. Categorical data were presented as absolute and relative frequencies. Numerical data were presented in the form of an arithmetic mean and standard deviation in case of normal distribution, and as a median and interquartile range for data not following the normal distribution. Differences in categorical variables were tested by the χ2-test. The Student's t-test for independent values was used in case of normal distribution, while the Mann-Whitney U test and the Kruskal–Wallis test were used in case of deviations from the normal distribution to test the numerical data. All P-values were two-tailed. The significance level was set to Alpha = 0.05. The statistical analysis was performed using the MedCalc Statistical Software, version 20.027 (MedCalc Software Ltd, Ostend, Belgium, https://www.medcalc.org/, 2022).

Results

The study included 129 patients, of whom 61 (47.3%) were male and 68 (52.7%) were female. The median age was 70 years (interquartile range from 60 to 83 years), while 46 (35.7%) patients were under 65 years of age. The most common comorbidity was hypertension (86, 66.7%), followed by diabetes mellitus (36, 27.9%) and atrial fibrillation (27, 20.9%), whereas only 14 patients (10.9%) had no comorbidities. The diagnosis of hypertension was significantly associated with gender – 75% of women had hypertension, in contrast to 57.4% of men (P = 0.03, χ2 test), and with age – 80.7% of the patients aged 65 and above had hypertension (P < 0.001, χ2 test) (Table 1).

Table 1. Demographic findings in relation to presence of hypertension

		Hypertension N (%)		P*	Total N (%)
		Yes	No		
Gender	Male	35 (57.4)	26 (42.6)	**0.03**	61 (47.3)
	Female	51 (75)	17 (25)		68 (52.7)
Age	< 65	19 (41.3)	27 (58.7)	**<0.001**	46 (35.7)
	≥ 65	67 (80.7)	16 (19.3)		83 (64.3)
Total N (%)		86 (66.7)	43 (33.3)		129 (100)

* χ 2 test

The patients stayed in the hospital for a median of 9 days (6 – 12 days). The majority of patients were discharged from the hospital (94, 72.9%), whereas 35 patients (27.1%) had a fatal outcome. A total of 83 patients (64.3%) stayed in the hospital less than 10 days, while 46 (35.7%) stayed longer. The length of stay was significantly associated with the presence of certain comorbidities. In particular, patients with hypertension (P = 0.04, χ 2 test) and diabetes mellitus (P = 0.04, χ 2 test) had a significantly longer stay. Healthy patients had a shorter length of stay, but there was no statistical significance (Table 2).

Table 2. Length of stay in relation to different comorbidities

		Length of stay (days) N (%)		P*	Total N (%)
		≤10	>10		
Comorbidity					
None	Yes	11 (78.6)	3 (21.4)	0.24	14 (10.9)
	No	72 (62.6)	43 (37.4)		115 (89.1)
Hypertension	Yes	50 (58.1)	36 (41.9)	**0.04**	86 (66.7)
	No	33 (76.7)	10 (23.3)		43 (33.3)
Diabetes mellitus	Yes	18 (50)	18 (50)	**0.04**	36 (27.9)
	No	65 (69.9)	28 (30.1)		93 (72.1)
Respiratory†	Yes	6 (60)	4 (40)	0.77	10 (7.8)
	No	77 (64.7)	42 (35.3)		119 (92.2)
Atrial fibrillation	Yes	17 (63)	10 (37)	0.87	27 (20.9)
	No	66 (64.7)	36 (35.3)		102 (79.1)
Total N (%)		83 (64.3)	46 (35.7)		129 (100)

* χ 2 test; † Asthma and chronic obstructive pulmonary disease

The majority of patients used a combination of two antihypertensive drugs (34, 28.3%), the most common of which were ACE inhibitors (55, 45.8%) and diuretics (43, 35.8%). There was no significant difference in the outcome with regard to the type of antihypertensive therapy and the number of antihypertensives used (Table 3).

New-onset hypertension was detected in three (2.3%) patients. All three patients were under 65 years of age and were discharged. The patients with new-onset hypertension had a shorter mean length of stay.

Table 3. Antihypertensive therapy in relation to outcome

		Outcome N (%)		*P**	Total N (%)
		Discharge	Fatal		
Antihypertensive therapy					
None	Yes	33 (78.6)	9 (21.4)	0.42	42 (35)
	No	56 (71.8)	22 (28.2)		78 (65)
ACE inhibitor	Yes	41 (74.5)	14 (25.5)	0.93	55 (45.8)
	No	48 (73.8)	17 (26.2)		65 (54.2)
ARB	Yes	5 (62.5)	3 (37.5)	0.43	8 (6.7)
	No	84 (75)	28 (25)		112 (93.3)
Beta blocker	Yes	24 (70.6)	10 (29.4)	0.58	34 (28.3)
	No	65 (75.6)	21 (24.4)		86 (71.7)
Calcium channel blocker	Yes	21 (70)	9 (30)	0.55	30 (25)
	No	68 (75.6)	22 (24.4)		90 (75)
Diuretic	Yes	29 (67.4)	14 (32.6)	0.21	43 (35.8)
	No	60 (77.9)	17 (22.1)		77 (64.2)
Combination of antihypertensives					
0		33 (78.6)	9 (21.4)		42 (35)
1		11 (68.7)	5 (31.2)		16 (13.3)
2		26 (76.5)	8 (23.5)	0.16†	34 (28.3)
3		19 (73.1)	7 (26.9)		26 (21.7)
4		0	2 (100)		2 (1.7)
Total N (%)		94 (72.9)	35 (27.1)		129 (100)

ACE = angiotensin-converting enzyme; ARB = Angiotensin receptor blocker; * χ 2 test; † Kruskal–Wallis test

Patients aged 65 and above had significantly higher mortality rates (P <0.001, χ 2 test), while gender-related differences in the outcome were not significant. There was no significant association between the investigated comorbidities and outcomes. Out of the 14 healthy patients, 1 (7.1%) had a fatal outcome. Regarding the hypertensive patient population, 61 patients (70.9%) were discharged and 25 (29.1%) had a fatal outcome. There was a significant association between the number of comorbidities and fatal outcome (P = 0.01, Kruskal–Wallis test), with patients with one and three comorbidities having the worst outcome (Table 4). In addition, the patients with a fatal outcome had a significantly shorter length of stay (median of 7 days), in contrast to discharged patients, whose length of stay was 10 days (P< 0.001, Mann–Whitney U test).

Table 4. Demographic findings, presence and number of comorbidities in relation to outcome

		Outcome N (%)		*P**	Total N (%)
		Discharge	Fatal		
Gender	Male	44 (72.1)	17 (27.9)	0.86	61 (47.3)
	Female	50 (73.5)	18 (26.5)		68 (52.7)
Age	< 65	42 (91.3)	4 (8.7)	**<0.001**	46 (35.7)
	≥ 65	52 (62.7)	31 (37.3)		83 (64.3)
Comorbidity					
None	Yes	13 (92.9)	1 (7.1)	0.08	14 (10.9)
	No	81 (70.4)	34 (29.6)		115 (89.1)
Hypertension	Yes	61 (70.9)	25 (29.1)	0.49	86 (66.7)
	No	33 (76.7)	10 (23.3)		43 (33.3)
Diabetes mellitus	Yes	25 (69.4)	11 (30.6)	0.59	36 (27.9)
	No	69 (74.2)	24 (25.8)		93 (72.1)
Respiratory‡	Yes	8 (80)	2 (20)	0.6	10 (7.8)
	No	86 (72.3)	33 (27.7)		119 (92.2)
Atrial fibrillation	Yes	18 (66.7)	9 (33.3)	0.42	27 (20.9)
	No	76 (74.5)	26 (25.5)		102 (79.1)
Number of comorbidities					
0		13 (92.9)	1 (7.1)		14 (10.9)
1		27 (62.8)	16 (37.2)		43 (33.3)
2		45 (80.4)	11 (19.6)	**0.01**†	56 (43.4)
3		6 (46.2)	7 (53.8)		13 (10.1)
4		3 (100)	0		3 (2.3)
Total N (%)		94 (72.9)	35 (27.1)		129 (100)

* χ 2 test; † Kruskal–Wallis test; Difference between 0 and 2 to 1 and 3 comorbidities (Post hoc Conover); ‡ Asthma and chronic obstructive pulmonary disease

Discussion

This research studied the demographic characteristics and comorbidities in COVID-19 patients hospitalised in the General Hospital Našice from 1 October to 31 December 2021, with a focus on the hypertensive population, in relation to the length of stay and outcome. Of 129 hospitalised COVID-19 patients included in the study, hypertension was identified as the most common chronic disease, followed by diabetes mellitus and atrial fibrillation, which is consistent with previous studies (6,7,10,11,17,24), including studies in Croatia (25–27).

In Croatia, the prevalence of hypertension is high, namely 37% (M – 35.2%, F – 39.7%) according to the 2005 EH-UH study (28). Accordingly, in this study, hypertension was significantly more common in females, which is consistent with research conducted in China (17) and the United States (16). Hospitalized COVID-19 patients with hypertension were also significantly older than those without hypertension, which is consistent with research conducted in Iran (6). The patients stayed in the hospital for a median of 9 days (6 – 12 days). The study found that comorbidities played a significant role in the length of stay. It was observed that hypertension and diabetes mellitus were more associated with a longer

stay than other detected comorbidities, corresponding with research conducted in the United States (16) and Ghana (15).

In this study, the majority of hospitalised COVID-19 patients with hypertension were treated with ACE inhibitors (55, 45.8%), but there was no significant association between the type of antihypertensive therapy and the outcome, which is consistent with previous studies (20–22). Since ACE2 plays a negative role in the RAAS system, a decrease in ACE2 and an increase in ANG2 levels can lead to higher blood pressure values (29). In this study, new-onset hypertension was detected in three (2.3%) patients. All three patients were under 65 years of age and were discharged. In the study conducted in Turkey (30), of 153 subjects, new-onset hypertension was observed in 18 (12%) patients, which is comparable to our study.

There are other potential risk factors, including age and the number of comorbidities, that could lead to more severe disease and increased mortality rates in hospitalised COVID-19 patients. The association between a fatal outcome and specific comorbidities was not determined in this study. However, a significant association was detected between a higher number of comorbidities and older age, which is consistent with previous research (7,24,31,32), including studies in Croatia (25,27).

This study identified hypertension as the most common comorbidity in hospitalized COVID-19 patients. Even though treated hypertension did not affect the outcome negatively, other potential factors, including the number of comorbidities and age, were shown to be associated with mortality among COVID-19 patients. Further studies of the mechanism in hypertensive COVID-19 population are needed.

Study limitations

This study has a few limitations. First, only 129 subjects with confirmed COVID-19 were included in the study and more extensive research would help gain a better understanding of the role of hypertension in COVID-19 patients. Second, the study was conducted in a short period of time without a control group. Finally, the data on outcomes in a short-term and long-term follow-up period after COVID-19 are limited.

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Review article

Hypertension in Association With Anxiety and Depression – A Narrative Review

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Abstract

Hypertension is recognized as a multifactorial disorder. Anxiety disorders, depressive disorder, psychosocial stress and certain individual psychological characteristics can influence the development and course of hypertension. Likewise, certain antidepressants can impact blood pressure. Association of anxiety disorders and depression with hypertension is bidirectional, so hypertensive patients are at risk of anxiety or depression. Monitoring the blood pressure of patients with anxiety disorders and depression, screening for anxiety and depression in patients with arterial hypertension and understanding pathophysiological mechanisms is important for future prevention and treatment strategies. This narrative review will briefly summarize current knowledge about the association of anxiety and depression with the risk of development of hypertension. Likewise, certain psychological factors and pathophysiological mechanisms in stress that are of importance for the association of hypertension with anxiety and depression are pointed out in this review, and effects of commonly used antidepressants are also considered.

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KEYWORDS: blood pressure, hypertension, anxiety, depression, antidepressants, adverse effects

Introduction

Hypertension is a common chronic disorder in the general population and is itself an important risk factor for cardiovascular disease (1). According to WHO reports, in 2015, about ¼ of the world's adult population suffered from hypertension and about 40% of cardiovascular deaths were connected to hypertension (2). Blacher et al. showed that each increase of blood pressure by 10 mmHg raises the risk of severe cardiovascular disease complications and death by almost 20% (2).

Multifactorial aetiology of hypertension includes genetic predisposition as an unmodifiable factor and several potential modifiable factors. The risk of development of hypertension increases with age, especially in women, who are under lower risk in the reproductive period than men. The most important risk factors are smoking, sedentary lifestyle, excessive salt intake and high-calorie food intake, adiposity, as well as stress, anxiety and depression (3). Although the association of hypertension with stress, anxiety and depression has been studied for decades, study data are controversial and the underlying pathophysiological mechanisms have not been completely understood. However, it is known that hypertension is understood as a psychosomatic disorder, where psychological factors can play an important role in its development and can have an impact on its course and treatment. There is an impact of psychological factors, psychosocial stressors and mental disorders on the cardiovascular system (4). Type A personality, emotional distress, anxiety and depression have the greatest impact on blood pressure (4).

Recommendations for management of hypertension published in 2018 by the European Society of Cardiology/European Society of Hypertension (ESC/ESH) include psychosocial factors as risk factors for hypertension, while the British National Institute for Health and Clinical Excellence (NICE) recommended that management of hypertension in adults in primary healthcare settings include interventions for reducing stress and achieving relaxation (5).

The aim of this review is to summarize current knowledge about the association of anxiety and depression with the risk of development of hypertension. Likewise, certain psychological factors and pathophysiological mechanisms in stress that are of importance for the association of hypertension with anxiety and depression are pointed out in this review. Effects of commonly used antidepressants are also considered.

Methods

In order to summarize current knowledge about the association of anxiety and depression with the development of hypertension, we searched for relevant literature using PubMed, ScienceDirect, SpringerLink, PsycNet and Elsevier until February 2022. Keywords included were blood pressure, hypertension, anxiety, depression and stress.

Blood pressure control

The physiological process of blood pressure control is complex and involves regulation of volume and natriuresis, as proposed by Guyton, with rapid control (within seconds or minutes) of vessel resistance by the central nervous system (CNS) and the sympathetic nervous system (SNS), with circadian control (within hours) mostly dependent on activity of the renin-angiotensin-aldosterone-system (RAAS) and with long-term control (within days) mostly reflecting salt intake and activity of RAAS (6).

Antihypertensive drugs act through sodium/volume regulation, renin-angiotensin system and the sympathetic nervous system. Antihypertensive drugs that act through sodium/volume regulation (diuretics and calcium-channels blockers) or through the renin-angiotensin system (angiotensin converting-enzyme inhibitors and angiotensin receptor blockers) manage to control

hypertension in at least 75% or more cases (7). 76% of adult patients diagnosed with hypertension in the USA have suboptimally regulated blood pressure (BP) despite appropriate medication and changes in lifestyle (8). In a minority of hypertensive patients, drugs that act through SNS manage to control hypertension better than the drugs mentioned above, so the sympathetic nervous system seems to have a key role (known as neurogenic hypertension in earlier literature) (7). Severe hypertension of non-secondary origin, treatment-resistant hypertension (failure of drug combinations that affect sodium/volume and RAAS) and paroxysmal hypertension (unprovoked severe BP elevation in patients without pheochromocytoma) are considered to be associated with psychological factors (7). Certain studies revealed high levels of anxiety among patients with treatment-resistant hypertension (8). Paroxysmal episodes of high BP have been observed in patients with unusually severe trauma who deny any emotions and in patients that minimize emotional distress (7). Paroxysmal hypertension is often managed acutely with anxiolytics and/or alfa/beta-blockers, and these drugs are also effective in long-term treatment by reducing the magnitude of BP elevation attacks (7). Likewise, antidepressants are shown to be successful in preventing paroxysmal BP elevation (7). Patients who complain of frequent variations in BP, with particularly high levels accompanied by rapid heartbeat, may also benefit from SNS-targeting medications (7).

In the 1930s, Franz Alexander considered the importance of psychological variables in hypertension and he pointed out the relationship of hypertension with repressed hostility (9). Expressing anger was shown to be inversely related to blood pressure (9). Likewise, reporting less emotional distress and less anxiety negatively affects the blood pressure (9). Individuals who are defensive also tend to have higher blood pressure (7).

Psychosocial stress is thought to be involved in the development of hypertension (10, 11). The risk of developing hypertension is associated with chronic stress, where the intensity of stress is more important than the specific type of stress (10). Higher levels of perceived chronic stress, rather than low intensity and short-term stress, are more likely to be accompanied by psychological and behavioural changes that increase the risk of hypertension (10). Stronger effects of chronic stress on blood pressure and incident hypertension are seen in women than in men (10). These effects are possibly linked to women being more exposed to psychosocial stressors and having more intense and prolonged emotional and physiological reactions (10).

In the Jackson Heart Study, a community-based cohort of black people, moderate and high perceived stress over time were associated with a significantly higher risk (15% and 22%, respectively) of incident hypertension over a median of seven years, compared to sustained low perceived stress (10). Consistent results were demonstrated in the Coronary Artery Risk Development in Young Adults study (CARDIA). The CARDIA study among black and white participants showed that increasing or sustained levels of stress are associated with incident hypertension among young adults (10). Therefore, evaluating prolonged and repeated stress over time can be an important part of primary prevention of hypertension and subsequent risk of cardiovascular disease (10).

Psychological stressors, according to Lazarus, are defined as perceived threats to a person's well-being that tax or exceed own coping capacity (12). Individual differences in coping resources are based on appraisal processes in forebrain neural circuits, where information is assessed in terms of personal relevance and potential threat, after which peripheral physiological reactions are initiated (12). The main response to stressors are immediate changes in the sympathetic and parasympathetic nervous system and hypothalamic-pituitary-adrenal axis, which then influence cardiac output and peripheral vascular resistance to redirect blood flow in peripheral tissue according to behavioural needs (12). Cortical and subcortical circuits, according to appraised stressors, generate anticipatory visceromotor commands to change

cardiovascular reactions in order to prepare individuals for a behavioural response, and those reactions can be exaggerated in the form of excessive increase of heart rate or blood pressure (12). Such exaggerated cardiovascular reactions, when repeated, can have cumulative consequences for cardiovascular disease and cardiovascular incidents among vulnerable individuals (12). Brain-imaging studies show that individuals with excessive increase of heart rate and blood pressure in stressful situations exhibit higher activity in the anterior cingulate cortex, medial prefrontal cortex, insula, hippocampus, basal ganglia, periaqueductal grey matter, pons and amygdala (12).

Short-term psychological stressors evoke cardiovascular reactions which are adaptive, providing hemodynamic and metabolic support for surviving behaviour (known as the fight or flight reaction) (12). However, cardiovascular reactions that are evoked by stressors for a long period of time or in a repeated manner initiate or exacerbate pathophysiological changes in the heart and vasculature (12). Blood pressure is controlled by baroreceptors mainly distributed in the bulbus of the carotid artery and the aortic arch (12). Increase of blood pressure causes stretching of the baroreceptors, followed by transmission of information through vagal and glossopharyngeal pathways toward nucleus tractus solitarius in the brainstem, which makes neural connections to pre-autonomic nuclei in the brainstem and to the forebrain, such as the ventromedial prefrontal cortex, anterior cingulate cortex, insula and amygdala, which are key regions in threat appraisal (12). These pathways enable modifications in the operating characteristics of the baroreflex through various behaviours, psychological stress, physical activity and particular stages of lifespan and can have a role in disease risk (12). Given these findings, it is necessary to study the potential genetic and developmental causes of individual differences in central control of cardiovascular reactions and to prevent psychological reactions and behaviours that increase the risk of cardiovascular disease in response to stress.

Changes of hypothalamic pituitary adrenal (HPA) axis functioning also underlie stress-induced cardiovascular reactions. Chronic psychosocial stress causes continuous secretion of HPA axis hormones, such as cortisol, which lead to resistance of glucocorticoid receptors and consequently reduce sensitivity of the immune system to anti-inflammatory action, thus promoting mild chronic pro-inflammatory state (11).

Hypertension then develops in the manner that high levels of cortisol inhibit the expression of nitric oxide synthase and thus decrease the availability of endothelial nitric oxide and increase regional vascular resistance (11). Chronic low-intensity inflammation also leads to the development of obesity, primarily with intra-abdominal accumulation of visceral fat, increased salt retention and insulin resistance (11). Increased levels of cortisol and catecholamines in blood, urine and saliva are considered markers of chronic psychosocial stress, but are influenced by physiological fluctuations in circadian rhythms and transient stressors, while hair cortisol concentration is probably a more reliable biological marker reflecting chronic psychosocial stress since cortisol accumulates in the hair for several months (11). In the study by Bautista, individuals with high hair cortisol concentrations were twice as likely to be hypertensive compared to those with low levels of cortisol in their hair (11). There exists also an association of alexithymia, which is characterized by an inability to recognize and express emotions, with hypertension (7).

Hostility, as a personality trait, is a long-term risk factor for hypertension in the general population (CARDIA study), which also negatively affects the prognosis of coronary heart disease (13). Hostile individuals have higher blood pressure and higher rates of cardiovascular morbidity and mortality (13). Hostile individuals are cynical and suspicious toward others, their emotions and behaviour cause interpersonal conflicts and lead to a possible lack of social support with perceived fewer coping resources, which can result in difficulties for managing chronic conditions like hypertension and encourage unhealthy habits such as tobacco or alcohol consumption (13). Type A personality, characterized by anger, hostility and impatience,

increases the risk of coronary heart disease and is a predictor of sudden cardiac death (4).

Association of anxiety and hypertension

Anxiety disorders are the most common mental disorders today, with a lifetime risk of occurrence of about 13.5% (2). Likewise, according to data of the European Study of the Epidemiology of Mental Disorders, the most frequent disorder is the panic disorder – 12.8% will be diagnosed with it (2). Anxiety disorders seem to have a central role among all mental disorders associated with hypertension (1). Systematic review and meta-analysis of cross-sectional and prospective studies by Pan et al. supported the association between anxiety and increased risk of hypertension (3). Pan et al. conducted a meta-analysis and found significant correlation between anxiety and hypertension in cross-sectional studies and longitudinal association in prospective studies, supporting earlier findings of anxiety as an independent risk factor for incident hypertension (14). In the Mechanisms and Outcomes of Myocardial Silent Ischemia (MOMSI) Study, incident hypertension in the evaluated period of one year was highly associated with baseline anxiety in middle-aged women, even after adjusting for factors of age, sex, BMI, smoking and psychopharmacotherapy (15). Data from World Mental Health Surveys show that of all anxiety disorders, diagnoses of panic disorder, social phobia and specific phobia have been associated with developing hypertension in the period of 11.7 to 34.2 years (15). In the study by Wu et al., one year prevalence in 2005 and average annual incidence of hypertension in the period 2006–2010 in patients with anxiety disorders were higher than in the general population (37.9% vs. 12.4% and 3.63% vs. 1.95%), seen in all age and sex groups (16). In the National Health and Nutrition Examination and Epidemiologic Follow-up Study, in a cohort of initially normotensive men and women that were followed up for 7 to 16 years, anxiety and depression, especially high levels at baseline, were found to be predictive of later experience of hypertension (17). Among outpatients with anxiety in the National Ambulatory Medical Care Survey and the National Hospital Ambulatory Medical Care Survey, up to 32.5% had unrecognized elevated blood pressure (18).

According to O'Donovan et al., individuals that suffer from anxiety disorders show greater threat-related vigilance and sustained threat perception, followed by prolonged activation of the HPA axis and the autonomic nervous system (15). At first, anxiety increases blood pressure transiently; in the long term, it may cause persisting vascular resistance, higher levels of angiotensin II, sustained sympathetic nervous activity and HPA axis activity as the major stress response system (14). Sustained activation of SNS reduces renal blood flow, increases sodium and water retention and through abnormal hemodynamic and dysregulating lipid metabolism causes damage and dysfunction of the endothelium, with consequent increase of the risk of atherosclerosis (14). Prolonged activation of the neurobiological stress system in different anxiety disorders can potentiate chronic inflammation along with functional and structural damage and, finally, cardiovascular, autoimmune and neurodegenerative diseases can develop (19).

Anxiety disorders are recognized as associated with higher blood pressure variability (BPV) (15). Likewise, the relationship between anxiety and BPV is bidirectional (20). In the study by Zhou et al., predictive values of long-term BPV for incident anxiety were studied among patients in family medicine clinics (20). Incident anxiety was significantly predicted by higher BP and higher BPV in female and older patients (20). Higher BPV is connected to reduced baroreflex sensitivity and it shows sympathetic predominance over decreased parasympathetic activity, which is also found in patients with anxiety disorders (20). Higher BPV is a predictor of development, progression and severity of organ damage associated with hypertension (15). Small cross-sectional and other studies have shown greater BPV and lower heart rate variability (HRV) among adults with higher levels of anxiety (15).

Sinhba et al. reported that anxiety levels are negatively correlated with heart rate and HRV in the stress response, and Yu et al. found that anxiety may diminish the cardiovascular response to stress by desensitizing beta-adrenergic receptors (1). An ambulatory blood pressure monitoring study reported that anxiety disorders were associated with nocturnal and early morning hypertension in hypertensive outpatients (21). Numerous studies showed a higher prevalence of panic disorder in hypertensive patients than in the control groups, e.g. Davies et al. found that 13% of hypertensive patients in primary care, in contrast with 8% of normotensive patients, suffer from panic disorder, and another study reported that 12% of patients with resistant hypertension have the diagnosis of panic disorder, along with 14% of those without treatment resistance (2). Increased lifetime risk of development of hypertension in patients with the diagnosis of panic disorder was reported in prospective studies by Grimsrud et al., Chou et al. and Stein et al. (2). Patients with panic disorder show autonomic dysfunction with increased sympathetic and decreased parasympathetic activity, so that increased heart rate and blood pressure and decreased HRV are observed (2).

However, some researchers have not found a link between anxiety and hypertension; this was reported in the Nord-Trøndelag Health Study (HUNT) in Norway (8). Patients who participated in the HUNT study from 1984 to 1986 were re-examined 11 years later and it was reported that high levels of anxiety and depression at baseline predicted low systolic blood pressure at follow-up (22). Researchers also found that increase in symptoms of anxiety and depression from baseline predicted a decrease in blood pressure in men and women of all adult age groups (22). It is important to note that those effects were not explained by the use of antidepressant or antihypertensive medication (22). Likewise, patients in the HUNT study were re-examined 22 years later and it was found that high levels of combined anxiety and depression at baseline were associated with lower mean systolic and diastolic blood pressure (22). It was reported that the risk of developing hypertension at year 22 was 20% lower and that both anxiety and depression separately contributed to the lowering of blood pressure (22). It is noteworthy that there was no evidence of mediating effects of heart rate changes and that the association of lower blood pressure with anxiety and depression persisted after excluding individuals who used antidepressants or antihypertensive medications (22).

It is known that individuals with anxiety disorders, compared to those without such diagnosis, are more likely to smoke, excessively consume alcohol and be obese, but there are studies that show the association of anxiety disorders and prevalent hypertension that is independent of unhealthy behaviour patterns as risk factors for hypertension (15). Likewise, patients with anxiety disorders may poorly adhere to medication treatment for hypertension, which makes blood pressure control difficult (11). However, numerous epidemiological studies concerning potential association of anxiety and hypertension have given inconsistent results. In addition to researchers that found that patients with anxiety are at higher risk of developing hypertension, others did not support the role of anxiety in development of hypertension and, finally, a few reported on lower blood pressure in patients with anxiety (14). The Australian Longitudinal Study of Women's Health (ALSWH) reported incident hypertension in 29.8% of women in 15 years of follow-up, but the association was not significant after adjusting for covariates (15). Similar findings of generalized anxiety disorder not being significantly correlated with incident hypertension in the elderly after adjusting for covariates were shown in the ESTHER study (15).

Association of depression and hypertension

Depressive disorders are the most widespread mental disorders (23). Depression is common among patients with cardiovascular diseases, with prevalence estimated between 15% and 50% (4). It is an important risk factor for coronary heart disease and is strongly associated with

angina pectoris, myocardial infarction and cardiac death (4). Depression leads to greater disability and lower quality of life in cardiovascular patients (23). Major depressive disorder is also associated with development of hypertension (1). There are reports of 1.4 times greater chance of having hypertension when depressed (8). The average annual incidence of hypertension in patients diagnosed with major depressive disorder was higher than in the general population (3.96% vs. 2.90%) when studied by Liang-Wu et al. in the period 2006–2008, and the 1-year prevalence was also shown to be higher than in the general population (21.21% vs. 13.28%); the results were comparable regardless of sex and age (16). One study conducted on women showed significant increases of systolic blood pressure in relation to increasing depressive symptoms, while increasing intensity of anger and anxiety and decreasing levels of social support showed a greater likelihood of developing hypertension in middle-aged women (24). A meta-analysis by Meng et al. showed an increased risk of hypertension incidence in depression, and it was significantly correlated with the baseline intensity of depressive symptoms and length of follow-up (9.6 years) (25). Depression is recognized as a significant and independent risk factor for hypertension, especially in younger people, and inducing lower heart rate variability is accompanied by greater mortality of cardiovascular disease (3). In the field of physiological research, a significant correlation has been found between depression, arterial stiffness and hypertension, with the intensity of depressive symptoms independently associated with arterial stiffness (8).

There is also growing interest about the role of intracellular calcium homeostasis dysregulation and calcium/cAMP signalling pathway in the pathogenesis of depression and hypertension (26). Calcium channel blockers are known as antihypertensives, but are also reported to have beneficial effects on certain symptoms of depression, such as cognitive dysfunction, and the possible link lies both in regulation of neurotransmitters such as serotonin and sympathetic outflow (26).

Gonzalez-Sanchez et al. reported about increased sympathetic vasomotor tone in patients with depression, association of depressive symptoms with an increase of SNS activity and/or decreased PNS, cardiac hyperactivity during SNS stimulation, morning SBP surge in positive correlation and nocturnal SBP dipping in negative correlation with depressive symptoms (1). The exact mechanism of hypertension development in depression, whether joint with anxiety or separate, still remains incompletely understood (1). The incidence of depression in hypertension patients is 27%, higher than in the general population (13). Depression increases the risk of hypertension complications (13). In hypertension patients that also suffer from depression, hypertensive crises are more common than in those without depression (23).

Villarreal-Zegarra showed that the association between hypertension and depression changes as time passes after diagnosing hypertension (27). Less than one year after hypertension diagnosis, patients were twice as likely to experience depression compared to individuals without hypertension, and as time passes, the risk of depression in patients with hypertension decreases, but it remains increased even in the period of five years or longer after hypertension is diagnosed (27). Possible explanations for this effect can be biological, such as chronic vascular and brain damage under high blood pressure during long periods, and/or psychological, mainly as emotional reactions to awareness of diagnosis and adaption difficulties to personal abilities and need for lifestyle changes. It is probable that patients with arterial hypertension adapt better after a longer period with the diagnosis and thus the risk of depression decreases (27).

In the study concerning the impact of anxiety and depression on quality of life among patients with arterial hypertension by Polishchuk et al., the majority of hypertensive patients and patients with nonpsychotic mental disorders had high levels of trait and state anxiety, and there was a direct correlation between trait anxiety and severity of depression. Levels of anxiety and depression were higher and quality of life was

lower in female patients with arterial hypertension, with significantly lower quality of life in patients with mixed anxiety and depressive disorder, as the most common diagnosis in the observed population (28).

Effects of antidepressants on blood pressure

In patients suffering from depressive disorders and anxiety disorders, blood pressure changes have to be considered in the context of taking antidepressants. The longitudinal National Study of Adolescent to Adult Health (ADD Health) that followed teenagers to adulthood concluded that taking antidepressants is an independent factor for development of hypertension in men, but not in women, with diastolic BP increased by an average of 1.6 mmHg (8). It has to be questioned whether elevations in blood pressure in patients taking antidepressants are a result of the medication or of the disorder (depression and anxiety disorder) itself.

Possible effects of antidepressants on blood pressure may be associated with their mechanism of action through serotoninergic, adrenergic, dopaminergic, histaminergic and cholinergic systems (29). Selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) may lead to changes of blood pressure through basic mechanisms of inhibiting the serotonin transporter (SERT) and the norepinephrine transporter (NET). SSRIs are the most frequently used antidepressants in treatment of depression, but also of anxiety disorders. SSRIs selectively inhibit SERT, but also act through presynaptic serotonin (5-HT) receptor desensitization, mostly 5-HT1A, thus enhancing serotoninergic neurotransmission (29). SSRIs show limited effects on the autonomic nervous system, which is why they are considered to be a safe class of antidepressants, especially for patients with cardiovascular disorders and older patients; additionally, their long-term use is accompanied by an increase in HR variability, which results in reduced cardiac morbidity and mortality (29). However, Humbert et al. analysed the WHO pharmacovigilance database and showed that the use of SSRIs significantly increases the risk of hypertension (30). Humbert et al. suggested that hypertension can be a possible adverse effect explained by SERT inhibition and its vasoconstrictor effects and/or inhibited nitric oxidase vasodilatation effect, while the pharmacoepidemiological-pharmacodynamic study found a positive correlation of the NET/SERT pKi ratio with the occurrence of hypertension in patients treated with SSRIs, as well as SNRIs (31). One cohort study in primary care in London was conducted to assess the risk of incident hypertension over 10 years and it showed the correlation of antidepressant use with developing hypertension (and other cardiovascular risks such as DM and hyperlipidaemia), possibly regarding the use of SSRIs in anxiety treatment (14).

Citalopram and its enantiomer escitalopram, the most selective SSRIs, are considered among the safest SSRIs due to not changing either systolic or diastolic BP nor HR and QTc interval in studies conducted on the older population with or without coronary artery disease (29). Sertraline is one of the commonly used SSRIs and it has the ability to increase dopaminergic neurotransmission. Sertraline is also considered one of the safest SSRIs, even in patients with unstable angina pectoris or after myocardial infarction and among older hypertensive patients (29). Fluvoxamine is an SSRI with agonistic effect on the sigma-1 receptor. The sigma-1 receptor is believed to mediate vasodilatation and decrease systolic BP in the presence of nitric oxide; however, fluvoxamine is not associated with changes to blood pressure or other significant cardiac effects, although it can cause mild bradycardia and rarely QTc prolongation (29). Fluoxetine is an SSRI with antagonist effects on 5-HT2C receptors that could enhance noradrenergic and dopaminergic neurotransmission. With short-term use of fluoxetine (12 weeks), depressive patients, either hypertensive or normotensive, show a modest decrease of systolic and diastolic BP, although there are some reports about a larger BP decrease in hypertensive than normotensive

patients (29). Paroxetine is an SSRI that additionally inhibits norepinephrine reuptake and has anticholinergic properties. In patients with ischaemic heart disease and depression, paroxetine shows a significant increase of supine systolic BP after a period of six weeks of administration (29).

Depressive patients on SSRI therapy compared to those on placebo in a meta-analysis by Zhong et al. did not show significant changes in blood pressure, while patients on SNRIs compared to patients on SSRIs experienced a modest increase in systolic and diastolic BP in short-term and long-term treatment (less or more than eight weeks) (32). On the contrary, psychiatric evaluation and ambulatory blood pressure measurements taken in the Mayo Clinic in Florida in the period 2012–2016 showed that patients taking SSRIs or SNRIs had higher nocturnal systolic and diastolic BP compared to individuals without a diagnosis of mental disorder and without psychopharmacotherapy (33). A single-centre retrospective study among patients with mental disorders who were taking SSRIs or SNRIs, a group of patients with mental disorders who were not taking SSRIs or SNRIs and a group of individuals without a mental disorder diagnosis who were not taking psychopharmacotherapy used information obtained from 24-hour ambulatory blood pressure monitoring and showed that SSRIs and SNRIs were significantly associated with higher nocturnal systolic and diastolic BP (34). That finding is important because nocturnal BP is reported to be a value predictor of cardiovascular mortality regardless of sex and age (34). Among SNRIs, venlafaxine shows a significantly higher risk of hypertension in a dose of 150 mg per day, but the risk is reduced by its extended release (ER) form (31). It is recommended that venlafaxine users be screened and monitored for hypertension, while duloxetine users are advised to monitor their BP if they have a prior diagnosis of hypertension or CVD (35). In a meta-analysis of 17 randomized clinical trials, Park et al. showed that duloxetine does not increase HR and BP in short-term therapy (20). Other concerns regarding duloxetine and venlafaxine include their effects on HR and QTc interval because of their described effects on sodium or potassium channels (35), but SNRIs are generally considered safe.

Tricyclic antidepressants (TCAs), however, are connected with serious arrhythmias; they are considered more toxic for the cardiovascular system and should not be administered to cardiac patients (30). TCAs inhibit 5-HT reuptake (mainly amitriptyline, imipramine, clomipramine) and NE reuptake (mainly nortriptyline and desipramine), but are also antagonists on cholinergic M1 receptors, histaminergic H1 and adrenergic $\alpha 1$ receptors, and orthostatic hypotension is one of the common side effects. Randomized trials have shown that SSRIs and SNRIs have better safety profiles than TCAs, although some meta-analyses reported that citalopram prolonged the QTc interval (36). The use of TCAs was accompanied by increased systolic and diastolic BP and patients suffered from hypertension in the study by Licht et al., which is why it was concluded that there is an increased risk of hypertension (30). Patients with major depressive disorder had a significantly lower systolic BP and anxious patients had a significantly higher diastolic BP compared to healthy controls, while for patients taking TCAs, systolic and diastolic BP were significantly higher (37).

Mirtazapine, a noradrenergic and specific serotoninergic antidepressant (NaSSA), seems to have a significantly lower risk of adverse cardiovascular effects (36). Mirtazapine has antagonistic properties on adrenergic alpha2-autoreceptors and alpha2-heteroreceptors as well as 5-HT2 and 5-HT3 receptors, thus enhancing the release of norepinephrine and 5-HT1A-mediated serotonergic transmission. In a meta-analysis of 25 randomized and controlled trials by Wanabe at al., significantly lower risk of hypertension was shown for mirtazapine than for TCAs (30).

Trazodone is an antidepressant with partial agonistic activity at 5-HT1A receptor and antagonism at 5-HT2A, 5-HT2C, $\alpha 1$ and H1 receptors. Trazodone is described as safe for the cardiovascular system, although mild

orthostatic hypotension can occur, so the general recommendation is to take blood pressure before administration and to avoid getting up abruptly from a lying or seated position (38).

Vortioxetine is an antidepressant with multimodal activity; it has antagonistic properties on 5-HT3, 5-HT7 and 5-HT1D receptors, a partial agonistic effect on 5-HT1B and an agonistic effect on 5-HT1A receptors, as well as an inhibitory effect on SERT. According to Baldwin et al., vortioxetine has a favourable cardiovascular safety profile when compared to the placebo in acute randomized controlled trials (six to eight weeks in duration) and in long-term open-label treatment (52 weeks of duration) (39).

Agomelatine is an antidepressant with agonistic properties at melatonin (MT1 and MT2) receptors and antagonistic properties at 5-HT2B/5-HT2C receptors. In addition to the effect on the circadian rhythm, agomelatine can influence the autonomic output to the cardiovascular system; however, agomelatine seems to have a safe cardiovascular profile (29). Clinical studies suggest agomelatine as a safe option among antidepressants in patients with cardiovascular disease; additionally, its cardioprotective and anti-inflammatory effects are described (40).

Moclobemide is a reversible inhibitor of monoaminoxidase (MAOI) and through that mechanism, breakdown of monoamines is reduced. Earlier MAOIs were irreversible and had a greater risk of increasing blood pressure, even causing a hypertensive crisis, when taken together with tyramine-rich food and medications like phenylephrine and pseudoephedrine. The general recommendation is to avoid such food and medications when taking MAOIs (30). Further studies for the assessment of effects of commonly used antidepressant medications are needed – particularly an analysis of potential association of changes in blood pressure to the prescribed doses of antidepressants.

Conclusion

Aetiology of hypertension has been widely studied in the last few decades. It is known that hypertension is the result of complex interaction of genetic and environmental risk factors, especially lifestyle and psychosocial factors. Mental disorders, emotional distress and certain individual psychological characteristics have been associated with increased incidence of hypertension. Anxiety disorders and depression have been established as risk factors for hypertension. As hypertension is itself a major preventable factor for cardiovascular disease, cerebrovascular diseases and chronic kidney damage and is associated with earlier mortality, better understanding of the pathophysiological mechanisms underlying the association of anxiety and depression with hypertension could improve prevention and treatment of hypertension. Implementing systematic screening for hypertension among patients suffering from anxiety disorders or depression can be questioned. Because of the bidirectional relationship and possible negative effects of anxiety disorders and depression on hypertension, treatment and quality of life, patients should be screened for mental disorders and, if needed, referred to clinical psychological interventions and administered psychiatric medication, i.e. antidepressant therapy. Knowledge about the effects of antidepressants on blood pressure is of importance and enables the optimal selection of medications for patients with anxiety disorders or depression, especially those for whom other risks of development of hypertension are present or those that are already hypertensive.

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Original article

Investigating the Relationship between Alcohol Use and Patterns of Blood Pressure Change Due to Examination Stress among Adekunle Ajasin University Academic Staff

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Abstract

Aim: This study examined the relationship between alcohol use and patterns of blood pressure changes due to examination stress among academic staff at the Adekunle Ajasin University (AAUA).

Methods: It involved using concurrent mixed methods with quantitative and qualitative approaches. Both the questionnaire and the blood pressure and pulse rate reading were used as instruments in data collection. The examined population includes all academic staff of AAUA. Multistage sampling techniques were used to select participants for the study. In stage one, a simple random sampling technique was used to select five faculties of the university. In stage two, systematic sampling techniques were used to select participants for the study; academic staff in every 5th academic staff office at the selected faculties were selected as a sample frame. Two instruments were used in gathering information for this study. The instruments were a self-constructed questionnaire and an electronic sphygmomanometer. Data were analyzed using mean and standard deviation at alpha level of 0.05.

Results: Findings revealed that there is a significant difference in the pattern of blood pressure before – $F(3, 46) = 4.260$, $P < 0.05$; during – $F(3, 46) = 3.570$, $P < 0.05$; and after the examination period – $F(3, 46) = 3.131$, $P < 0.05$, based on the respondents' level of alcohol intake.

Conclusions: It is recommended that academic staff should be educated on the detrimental health consequences of consuming alcohol to avoid high blood pressure before, during and after the examination period.

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Introduction

Blood pressure can be defined as the physical pressure of blood flowing through the arteries, i.e. the pressure that blood exerts on the inner walls of the blood vessels; it varies in different phases of the cardiac cycle and under different conditions of exertion. High blood pressure accounts for high mortality in the form of coronary heart disease and usually has no warning signs. Any form of stress is known to cause definable mental and physiological reactions in the body in the form of alteration of different biological functions, especially the heart rate and blood pressure (1). Hypertension is one of the most common health problems with negative consequences, which have devastating effects on human health. In 2016, in the Global Burden of Disease study by Risk Factors Collaborators, high systolic blood pressure was reported as the leading cause of global disease burden in both men and women (2). Today, stress has become prevalent in everyday human life, especially among different employees at various job levels. On the one hand, stress is a motivational force and on the other hand, it is a cause of depression. In fact, lack of stress represents the end of life, as there is no enthusiasm for accomplishment of goals. When an employee is at the workplace, there are different stressors present that can have a direct impact on the employee's performance (3). Following this view, work stress emerges when people perceive that they have difficulties in coping with the demands related to work and that their sense of well-being is threatened. Many stressors associated with academic staff have been identified. Examples include shortage of staff, work overload, too much administrative work, lack of support from superiors and peers, irregular payment of salaries, marking of scripts, lecturing, supervising duty during the examination period, carrying out various research works, etc., as some of the stressors that the academic staff deal with in the university system. Stress process is complex and dynamic, fluctuating over the time, places, and persons (4). Stress produces a series of distinct biological processes, such as hypothalamic-pituitary-adrenal (HPA) axis activation and associated autonomic nervous system responses (5). Many other researchers have found that work stress arises when an individual experiences a demand that exceeds his/her real or perceived abilities to successfully cope with the requirements of the job, resulting in a disorder to his/her emotional and physiological balance (3).

Examinations are frequently used to evaluate the academic ability of students in a university's education system. Apart from the students, the staff are responsible for smooth running of the examination process, for instance through setting of examination questions, invigilating and marking of scripts to results processing, which represents the academic staff's multiple roles and responsibilities within the universities. All these responsibilities and many more cause stress, which is a major concern in many, if not all educational institutions around the world. At the university level, administrative duties have increased alongside the escalating demands associated with teaching and research responsibilities. Administratively, the lecturers work as counselors, examination officers, staff advisers, department heads, members of various committees at the faculty and department level and they hold many other responsible positions in addition to their teaching duties. Thus, the lecturers work under increasing pressure to meet targets set by the university. Due to all this workload, some lecturers use alcohol to unwind and also see it as a means of socialization and relaxation. However, this may have a great effect on their blood pressure pattern. For example, it was revealed that examination stress is significantly associated with increased pulse rate, systolic and diastolic arterial pressure (6). This was further affirmed by the finding that high-dose alcohol has a biphasic effect on BP; it decreases BP up to 12 hours after consumption and increases BP > 13 hours after consumption. High-dose alcohol increases HR at all times up to 24 hours (7). The rate of alcohol consumption is very high among adults, and two out of every five people are heavy episodic drinkers (8). High blood pressure is particularly prevalent among

blacks and serves as a major risk factor for coronary heart disease, stroke, and heart failure (9). As a result, prevention of high blood pressure early in life is essential for reducing the burden of hypertension for blacks later in life; it likewise serves as a form of primary prevention for heart disease and stroke (8).

Alcohol consumption is positively associated with BP (10). Consequently, alcohol consumption and high blood pressure are among the top five risk factors responsible for the growing global burden of non-communicable diseases (NCD), and are key parts of the World Health Organization (WHO) goals to reduce NCD mortality by 25% by 2025. From a public health perspective, both alcohol consumption and high blood pressure are among the most important risk factors for the global burden of NCDs (11).

A reduction in alcohol consumption will aid a reduction in blood pressure, which has the potential for substantial synergistic health gains in terms of morbidity, mortality, and healthcare costs; yet only about half of the hypertension guidelines worldwide recommend a reduction in alcohol consumption to reduce high blood pressure (12). This would be an important contribution to reaching the goals of the WHO Global Action Plan for prevention of NCDs (13), which stipulates a 10% relative reduction of harmful alcohol use and a 25% reduction in raised blood pressure by 2025 to reduce NCD mortality by 25%.

Lecturers at the Adekunle Ajasin University are no exception. Due to these reasons, the aim of present study was to establish the relationship between alcohol use and patterns of blood pressure changes due to examination stress among academic staff at the Adekunle Ajasin University. The main objective of this study is to investigate the relationship between alcohol use and patterns of blood pressure changes due to examination stress among academic staff at the Adekunle Ajasin University. We hypothesized that alcohol use will have no significant effect on patterns of blood pressure changes due to examination stress among academic staff at the Adekunle Ajasin University.

Material and Methods

Descriptive research design of the survey type was used in this study. This method involves observing and describing a subject's behavior without influencing it in any way. It involves gathering data that describe events and then organizing, tabulating, depicting, and describing the data collection (14). Furthermore, it serves to organize findings in order to fit them with explanations, and then to test or validate those explanations (15).

The population for this study consisted of all academic staff of the Adekunle Ajasin University, Akungba Akoko. Multistage sampling techniques were used to select participants for the study. In stage one, a simple random sampling technique was used to select five faculties of the university. In stage two, systematic sampling techniques were used to select participants for the study; academic staff in every 5th academic staff office at the selected faculties were selected as a sample frame. A total of fifty (50) participants were enrolled as the sample size for this study.

Two instruments were used in gathering information for this study. The instruments are a self- constructed questionnaire and an electronic sphygmomanometer used to measure the participants' blood pressure. The questionnaire examined the demographic data of the respondents (age, gender and length of service) and how often the academic staff at the Adekunle Ajasin University, Akungba Akoko (AAUA), consume alcohol, which is defined in categories as follows – do not consume, occasionally, moderately and a lot. Likewise, blood pressure pattern values were measured using a sphygmomanometer and recorded in millimeters of mercury (mmHg) before, during and after the examination period. The pattern of blood pressure distribution is determined in accordance with the categories stated below: Normal – below 120 mmHg; Elevated – 120–129/80 mmHg; High Blood Pressure Stage 1 (HBP 1) – 130/80–139/89 mmHg; High Blood Pressure Stage 2 (HBP 2) – 140/90 mmHg and higher.

Items in the self-constructed questionnaire were carefully reviewed and submitted to experts in the related field for their review of the instrument; comments and corrections made by experts were carefully incorporated by the researcher. Likewise, the sphygmomanometer was calibrated to ensure its adequacy for use.

The survey was conducted within a semester that lasted for 4 months. The first application of the instrument occurred one month before the examination period, since not many academic activities were happening at the time. The examination period was a 14-day written exam period, while two weeks after the examination period, another set of data was retrieved and recorded. The survey was also based on continuous alcohol consumption, indicating that before, during or after the examination period, alcohol drinking or lack thereof is a peculiar lifestyle. The instruments were applied by the researcher and two trained research assistants with a 100% guarantee of keeping information confidential. The instrument was applied at each selected faculty at intervals before, during and after the examination period. All questionnaire forms, which also contain information about blood pressure patterns of individual participants, were distributed, retrieved and screened after the last administration. After allowing the respondent to rest for about five minutes, blood pressure was rechecked; the average scores were recorded thereafter. This was repeated when the respondent completed the filling of the questionnaires.

Ethical Consideration

Informed consent of each subject, ethical and official approval from the local research ethics committee of the Department of Human Kinetics and Health Education, Faculty of Education, Adekunle Ajasin University Akungba Akoko was obtained for the study and the investigation was performed in accordance with the principles outlined in the study.

Statistical Analysis

To determine the reliability of the instrument, a test-retest technique for a two-week interval was employed. This was applied on 5 randomly selected respondents from the target population. These respondents were excluded from the main study. A reliability of 0.89 was obtained using the Pearson product-moment correlation coefficient, which was considered adequate for the study. Alpha was set at 0.05. The IBM SPSS version 25 was used for statistical analysis in this study. Frequency counts and percentages were used to describe the participants' information, level of alcohol intake and blood pressure changes due to examination stress. On the other hand, ANOVA was used to test the hypothesis at 0.05 alpha level. Systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) records among AAUA academic staff based on level of alcohol intake were measured using the t-test. The Kolmogorov-Smirnov test was used to test for normality of distribution.

Results

Table 1 shows that 34 (68%) respondents do not consume alcohol, 12 (24%) consume alcohol at times, 2 (4%) consume alcohol moderately, while 2 (4%) drink a lot.

Table 1: Frequency distribution of respondents by level of alcohol intake

	Frequency	Percent
Do not consume	34	68.0
Occasionally	12	24.0
Moderately	2	4.0
A lot	2	4.0
Total	50	100.0

Results in Table 2 reveal that the blood pressure measurement of 24 (48%) respondents before examination was normal, for 22 (44%) it was elevated, 4 (8%) recorded high blood pressure stage 1, while none was at high blood pressure stage 2. Furthermore, the blood pressure measurement of 15 (30%) respondents during examination was normal, for 15 (30%) it was elevated, 17 (34%) recorded high blood pressure stage 1, while none was at high blood pressure

stage 2. Likewise, the blood pressure measurement of 22 (44%) respondents after examination was normal, for 22 (44%) it was elevated, 6 (12%) were at high blood pressure stage 1, while none was at high blood pressure stage 2.

Table 2: Frequency distribution of respondents by blood pressure measurement

Pattern of blood pressure	Before examination		During examination		After examination	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
normal (below 120 mmHg)	24	48.0	15	30.0	22	44.0
elevated (120–129/80 mmHg)	22	44.0	15	30.0	22	44.0
HBP 1 (130/80–139/89 mmHg)	4	8.0	17	34.0	6	12.0
HBP 2 (140/90 mmHg and higher)	0	0	0	0	0	0
Total	50	100.0	50	100.0	50	100.0

Table 3: Systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) records of AAUA academic staff

Parameters		Do not consume alcohol (n = 34) Mean ± SD	Occasionally (n = 12) Mean ± SD	Moderately/a lot (n = 4) Mean ± SD	Total (n = 50) Mean ± SD
SBP	Before examination	112.26 ± 21.84	122.75 ± 5.64	125 ± 2.83	115.80 ± 18.88
	During examination	119.85 ± 9.76	126.25 ± 8.09	200 ± 4.25	122.40 ± 9.86
	After examination	117.18 ± 8.83	122 ± 6.22	129 ± 4.24	119.28 ± 8.67
DBP	Before examination	70 ± 14.61	72.75 ± 5.91	78 ± 11.31	71.30 ± 12.93
	During examination	74.03 ± 8.88	75.42 ± 6.56	115.5 ± 12.34	74.54 ± 8.40
	After examination	72.62 ± 8.82	73.50 ± 8.15	77.25 ± 5.31	73.20 ± 8.44

Systolic blood pressure of all individuals when there were no examinations was 115.80 ± 18.884 mmHg; during examinations, it rose to 122.40 ± 9.86 mmHg; while after the examinations, it decreased to 119.28 ± 8.67 mmHg. Diastolic blood pressure recorded when there were no examinations was 71.30 ± 12.93 mmHg; during examinations, it was 74.54 ± 8.40 mmHg; while after the examinations, it decreased to 73.20 ± 8.44 mmHg.

Table 4: ANOVA showing the differences between patterns of blood pressure changes due to examination stress based on alcohol intake

	Alcohol intake	N	Mean	Std. deviation		Sum of squares	df	Mean square	F	Sig.
Average BP measurement before examination	Do not consume alcohol	34	1.41	.609	Between groups	4.348	3	1.449	4.260	.010*
	Occasionally	12	1.92	.515	Within groups	15.652	46	.340		
	Moderately	2	2.50	.707	Total	20.000	49			
	A lot	2	2.00	.000						
	Total	50	1.60	.639						
Average BP measurement during examination	Do not consume alcohol	34	1.91	.900	Between groups	8.068	3	2.689	3.570	.021*
	Occasionally	12	2.58	.793						
	Moderately	2	3.50	.707	Within groups	34.652	46	.753		
	A lot	2	2.50	.707	Total	42.720	49			
	Total	50	2.16	.934						
Average BP measurement after examination	Do not consume alcohol	34	1.50	.663						
	Occasionally	12	2.00	.603						
	Moderately	2	2.50	.707	Between groups	3.880	3	1.293	3.131	.035*
	A lot	2	2.00	.000	Within groups	19.000	46	.413		
	Total	50	1.68	.683	Total	22.880	49			

P < .05 * – significant, ** – not significant

Table 4 shows that there is a significant difference in the patterns of blood pressure before (F (3, 46) = 4.260, P < 0.05), during (F (3, 46) = 3.570, P < 0.05) and after the examination period (F (3, 46) = 3.131, P < 0.05), based on the respondents' level of alcohol intake.

Discussion

At many Nigerian universities, administrative duties have increased alongside the escalating demands associated with teaching and research responsibilities. Lecturers work as counselors,

examination officers, staff advisers, department heads, members of various committees at the faculty and department level and they hold many other responsible positions in addition to their teaching duties. Thus, lecturers work under increasing pressure to meet targets set by the university. Due to all this workload, some lecturers use alcohol to unwind and also see it as a means of socialization and relaxation. However, this may have a great effect on their blood pressure pattern.

This study revealed that the systolic blood pressure (SBP) of individuals who drink moderately/a lot (125 ± 2.83 mmHg) is higher than of those who drink occasionally (122.75 ± 5.64 mmHg) and those who do not drink alcohol at all (112.26 ± 21.84 mmHg) before examinations commence; SBP of individuals who drink moderately/a lot (200 ± 4.25 mmHg) is higher than of those who drink occasionally (126.25 ± 8.09 mmHg) and those who do not drink alcohol at all (119.85 ± 9.76 mmHg) during examinations; while SBP of individuals who drink moderately/a lot (129 ± 4.24 mmHg) is higher than of those who drink occasionally (122 ± 6.22 mmHg) and those who do not drink alcohol at all (117.18 ± 8.83 mmHg) after the examinations. The findings of this study indicate that systolic blood pressure of all individuals was low when there were no examinations; it increased during examinations and decreased after the examinations. Systolic blood pressure was significantly higher in the period during examinations than when there were no examinations (before and after). The study was in line with the findings of (6) that revealed in their study that examination stress is significantly associated with increased pulse rate, systolic and diastolic arterial pressure.

Diastolic blood pressure (DBP) of individuals who drink moderately/a lot (78 ± 11.31 mmHg) is higher than of those who drink occasionally (72.75 ± 5.91 mmHg) and those who do not drink alcohol at all (70 ± 14.61 mmHg) before examinations commence; DBP of individuals who drink moderately/a lot (115.5 ± 12.34 mmHg) is higher than of those who drink occasionally (75.42 ± 6.56 mmHg) and those who do not drink alcohol at all (74.03 ± 8.88 mmHg) during examinations; DBP of individuals who drink moderately/a lot (77.25 ± 5.31 mmHg) is higher than of those who drink occasionally (73.50 ± 8.15 mmHg) and those who do not drink alcohol at all (72.62 ± 8.82 mmHg) after the examinations. In other words, diastolic blood pressure record when there were no examinations was normal, it increased drastically during examinations and became normal again after the examinations. Diastolic blood pressure, which is defined as the minimum pressure during ventricular diastole, with a normal range of 60–90 mmHg and an average of 90 mmHg in adults, was within the normal value during the examination period and the period when there were no examinations (before and after). This could be explained by stimulation of the adrenergic nervous system, which leads to release of catecholamines, in particular noradrenaline at the postsynaptic neuron and adrenaline or epinephrine from the adrenal medulla, which results in activation of α 1, β 1 and β 2 receptors, consequently elevating systolic blood pressure (16). Likewise, the number of hours stayed for examination and invigilation, where many staff members may supervise two to three examinations in a day, long distance covered before reaching the examination center, sitting arrangements of students, counting and marking of exams could be underlying factors as to why systolic blood pressure is elevated. On the other hand, the decrease of systolic blood pressure after examinations have passed can be explained by stating that the decrease results from decrease in peripheral arteriolar resistance and/or cardiac output by a variety of mechanisms at different sites, such as dilatation of resistance vessels, heart pumping against lower resistance, dilatation of capacitance vessels, reduction of venous return to reduce cardiac output, reduction of sympathetic drive to the heart which leads to lower cardiac output, especially in response to examination stress (16).

The findings from the analysis of hypothesis one showed that there is a significant relationship between alcohol and patterns of blood pressure changes due to examination stress among academic staff at the Adekunle Ajasin University. The results also showed that academic staff who consume alcohol moderately had the highest

blood pressure before examinations (mean = 2.50), followed by those who consume it a lot (mean = 2.00) and those who drink alcohol often (mean = 1.92), while people who do not consume alcohol at all (mean = 1.41) had the lowest blood pressure before examinations. During examinations, staff who consume alcohol moderately had the highest blood pressure (mean = 3.50), followed by those who consume it a lot (mean = 2.50) and those who drink alcohol often (mean = 2.58), while people who do not consume alcohol at all (mean = 1.91) had the lowest blood pressure during the examination period. After the examinations, staff who consume alcohol moderately had the highest blood pressure (mean = 2.50), followed by those who consume it a lot (mean = 2.00) and those who drink alcohol often (mean = 2.00), while people who do not consume alcohol at all (mean = 1.50) had the lowest blood pressure after the examinations. Thus, academic staff of the Adekunle Ajasin University, Akungba Akoko, who consume alcohol a lot and moderately are at greater risk of having high blood pressure than academic staff who consume a little or who do not consume alcohol at all, regardless of whether this occurs before, during or after the examination period, which is in agreement with (7).

Conclusion

There is a significant relationship between alcohol and patterns of blood pressure changes due to examination stress among academic staff at the Adekunle Ajasin University; those who consume alcohol a lot and moderately are at higher risk of having elevated blood pressure than academic staff who consume a little or do not consume alcohol at all, regardless of whether this occurs before, during or after the period of examination. Based on the findings of this study, the following recommendations are made: a) Health educators should design effective awareness programs on blood pressure management to educate staff on blood pressure and work stress; b) Stress management techniques and a healthy lifestyle through education about health should be made available to the academic staff; and c) Academic staff should be educated on detrimental health consequences of consuming alcohol to avoid high blood pressure before, during and after the examination period.

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Review article

MicroRNAs and Hypertension

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Abstract

MicroRNAs (miRNAs) are non-coding, highly conserved RNAs found in all biological fluids, that are emerging as master regulators of gene expression, consequently impacting a variety of biological processes in both healthy and diseased environments. There are still certain limitations regarding analysis of circulating miRNAs, specifically concerning standardisation and accuracy of obtained data. However, there is an indisputable therapeutic and diagnostic potential, confirmed by recent research. Hypertension, as one of the leading causes of death in modern world, has been in the focus of scientific society for several decades now. So, it is of outmost importance to investigate and pinpoint appropriate miRNAs for early indication and diagnosis of hypertension in general population. More in vivo and clinical research is necessary in animal and human models in order to exploit the full potential of this novel technology.

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Introduction

MicroRNAs (miRNAs) represent a class of short length (20-24 nucleotides), highly conserved, non-coding RNAs that have a role in regulation of gene expression through inhibition of transcription, translation or degradation of target genes, depending on their origin (1,2). MiRNAs can be categorized as tissue-derived miRNAs (t-miRNAs) and freely circulating miRNAs (c-miRNAs), with t-miRNAs being mainly associated with hypertension and cardiac function (more evidence needed), while the latter are described as a potential specific biomarkers for early disease detection, with their abnormal expression being largely associated with diseases in humans (1,3).

C-miRNAs or extracellular miRNAs can be found in all biological fluids, mainly carried in vesicles exosomes, such as serum, plasma, saliva, urine, while serving a hormone-like purpose in processes of signalisation, mediation and regulation of a variety of biological and cellular activities, physiological responses and pathological conditions (3–5). Even though the sampling is minimally invasive and there is a potentially high reproducibility of results, analysis of miRNAs still represents a challenge due to low concentrations of target compounds, lack of standardized methodology and eventual accuracy obtained regarding their in vivo role and therapeutic properties (1,6).

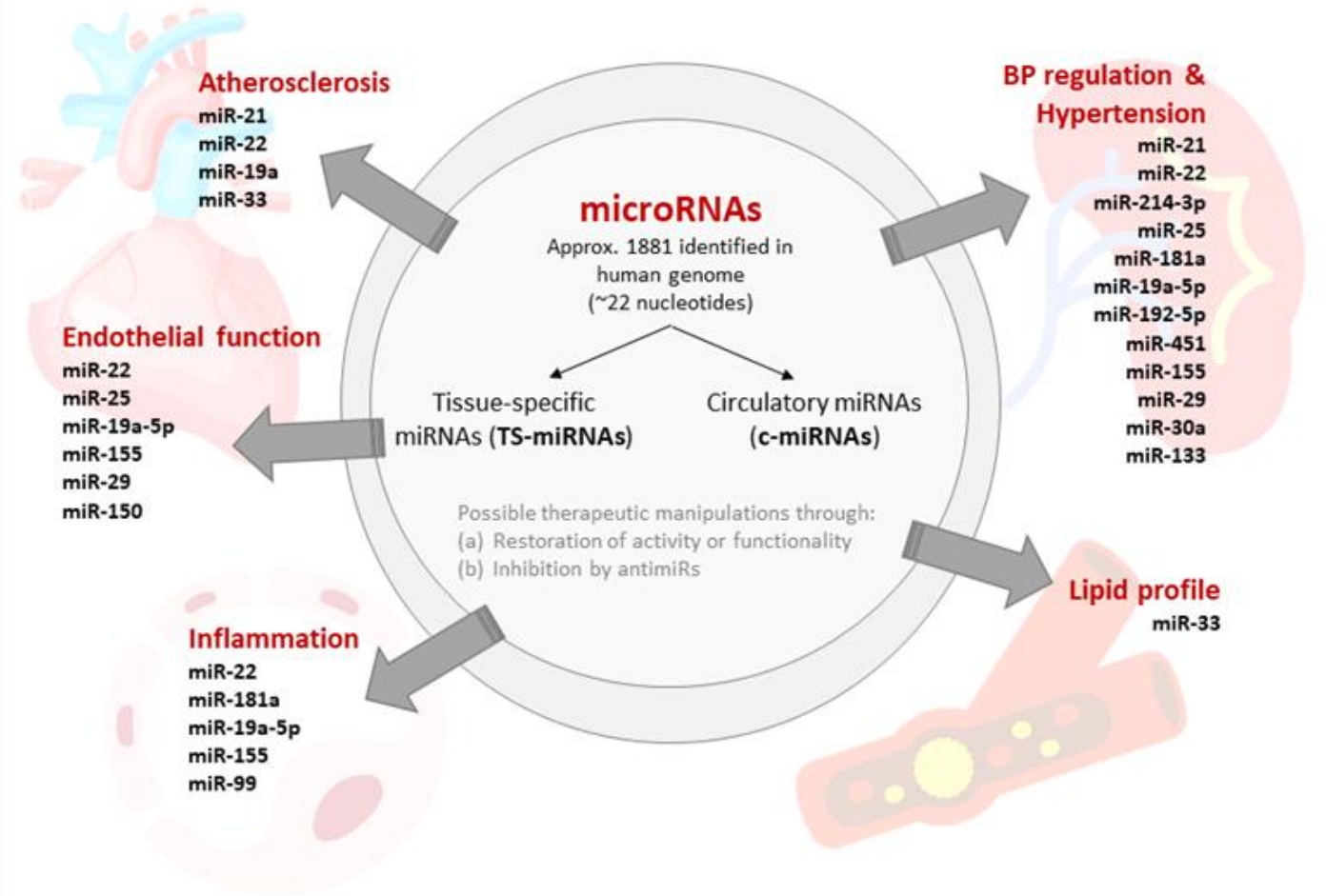

Figure 1. Schematic presentation of therapeutic and diagnostic potential of microRNAs in atherosclerosis, blood pressure regulation and hypertension, endothelial function, inflammation and lipid profile.
miR – microRNA; BP – blood pressure.

These highly stable regulators are primarily found in human serum and saliva (a total of 1881 miRNAs identified in human genome) and they can reduce gene expression post transcription by binding to the 3' untranslated region (UTR) of a messenger RNA (mRNA) leading to repression of translation and/or degradation of target (2,7). Lately, efforts are being made regarding investigation of therapeutic use of miRNAs in a wide variety of diseases, including cardiovascular disease, immune disorders, rheumatoid arthritis and cancer (8). The fact that mature miRNAs are highly conserved and short, makes them perfect for therapeutic manipulations towards modulation of cellular pathways and networks (9). There are two possible proposals for such actions: (1) therapeutic restoration of miRNA activity/functionality; and (2) inhibition of miRNA function by so-called antimiRs – antisense oligonucleotides (4,9,10). In order to draw firm conclusions, it is necessary to take into account that there are great variations in miRNA expression levels present, depending on their origin (tissue or cell type) and pathological state, which requires further extensive in vivo experiments in appropriate animal models. Schematic representation of miRNA breakdown regarding therapeutic/diagnostic potential is presented in Figure 1.

Emerging role of non-coding RNA in endothelial function and blood pressure regulation

Hypertension is, alongside diabetes and cancer, the most common chronic disease and leading cause of death in modern society (11). Yet, in a large number of affected individuals, the main cause of elevated blood pressure (BP) cannot be determined with certainty. Some of the risk factors associated with hypertension include genetic predisposition, advanced age, low physical activity, obesity and overall poor dietary habits characterized by high sodium chloride (NaCl) and low potassium intake (12,13). High-salt (HS) diet normally has a suppressive effect on renin-angiotensin system (RAS) – a physiological system that regulates BP – and leads to reduced renin, angiotensin II (Ang II) and aldosterone plasma levels (14,15), inciting endothelial dysfunction (16–18). However, in hypertensive and diabetic patients, RAS activation is not suppressed following salt loading, thus salt loading tends to aggravate the cardiovascular risks in those particular individuals (14,19,20).

So far, animal studies have shown that Ang II, HS diet and exercise change miRNA levels in hypertension (6). Several studies listed miR-22 (21), miR-181a (22) and miR-25 (23) as potential therapeutics in stress response, hypertension and vascular remodelling. Furthermore, it is necessary to address environmental factors in future research and find out what influence they could potentially have on miRNAs expression, especially in case of hypertension and related complications (6). Table 1 represents a cross-section of research studies regarding non-coding RNA role in endothelial function and BP regulation.

Non-coding RNAs in hypertension treatment

Currently, there is a lack of evidence supporting a significant role of non-coding RNAs in BP regulation and hypertension but it has been frequently mentioned that miRNA activity is deregulated in the state of illnesses.

MiR-21 is among the first identified non-coding RNAs (24) and has been suggested by several studies as a biomarker for early atherosclerosis and hypertension (25–27). In a study by Kara et al. (2021), significantly increased levels of miR-21 and aldosterone were found in patients with resistant hypertension compared to newly diagnosed hypertensive patients and healthy controls (28). Further, higher levels of miR-21 were reported in hypertensive, stroke and atherosclerotic patients compared with healthy controls, while it also negatively correlated with plasmatic levels of eNOS in hypertensive patients (29–31). Renal miR-214-3p has also been mentioned as a contributor to hypertension by directly targeting eNOS in rats and potentially humans (32,33).

Table 1. Cross-section of research studies regarding non-coding RNAs role in endothelial function and BP regulation

miRNAs	Study model	Targets	Effects	Reference
miR-21	Human model – hypertensive, ischemic stroke and atherosclerotic patients Rat model – SHR rats Mice model - C57BL/6J, TAC mice	mt-Cytb eNOS	↑ Mir-21 and aldosterone levels in RHT group Positive correlation with aldosterone, age, office SBP, 24-h ABPM all-day SBP; ↑ CRP level, plasma miR-21 expression level and CIMT in HT group ↓ NOx and eNOS levels in HT group ↑ miR-21 in stroke and atherosclerotic patients	(28) (29) (25) (27) (31)
miR-214-3p	Human model - HT and hypertensive nephrosclerosis patients Rat model – Dahl SS rat, SS.13^{BN26}(L26) rat	eNOS	Upregulated in kidneys of HT patients and HS–fed SS rats ↓ hypertension and albuminuria in SS model following inhibition of miR-214-2p	(33)
miR-22	Rat model – SHR rats Mice model – cardiac-specific knockout	Chga TGFβR I	↓ BP following inhibition ↓ cardiac hypertrophy and ↑ fibrogenesis of cardiac fibroblasts in knockout mice ↑ cardiac contractility and function following inhibition	(34) (36) (37)
miR-181a	Mice model – BPH/2J mice, miR-181a knockout	RAS	↑ BP and salt-sensitivity in knockout mice ↓ BP and renal renin mRNA following treatment with miR-181a mimic	(40) (41)

miR-19a	Human model – AMI and CAD patients, Rat model – Dahl SS rat Mice model - ApoE$^{-/-}$ mice, c57BL/6 mice	ADRB1 HBP-1	↑ proliferation and arteriogenesis ↓ apoptosis in endothelial cells ↓ atherosclerotic plaques and lipids load in mice fed with high-fat diet following administration of antagonist	(1) (83) (47) (84)
miR-192-5p	Human model - hypertensive and hypertensive nephrosclerosis patients Rat model – Dahl SS rat, SS.13^{BN26}(L26) rat Mice model – mir-192 knockout	Atp1b1	↓ miR-192-5p in hypertension animal model and following HS diet ↑ MAP following HS diet in antimiR-treated L26 model ↑ MAP, SBP, DBP following HS diet in knockout mice	(32)
miR-25	Human model – diabetic patients Rat model – SD rat Mice model – WT mice, C57BL/6 mice, *db/db* mice	CDC42	↑ RAS, hypertension, renal dysfunction following inhibition in normal mice ↓ glomerular fibrosis and BP following inhibition in *db/db* mice	(49)
miR-451	Human model – PAH and HCM patients Rat model – Wistar rat Mice model - miR-451 knockout	TSC1	Negatively correlated with mPAP, BNP and ADMA ↓ development of PAH in hypoxia-exposed rats following inhibition ↑ development of HCM following down-regulation	(51) (52)
miR-29a	Human model – hypertensive patients Rat model – SHR rat	PTEN/AKT /mTOR signalling pathway	Negatively correlated with the glomerular filtration rate, but positively with CRP, TGF-β1, and UACR ↓ hypertrophy and associated indices following inhibition	(57) (59)

	Mice model – TAC mice	(85)

SHR rats – spontaneously hypertensive rats; TAC mice – transverse aortic constriction mice; mt-Cytb - mitochondrially encoded cytochrome B; eNOS - endothelial nitric oxide synthase; HS – high-salt; RHT – resistant hypertension; SBP – systolic blood pressure; ABPM - ambulatory blood pressure monitoring; CRP – C-reactive protein ; CIMT - carotid intima-media thickness test; HT – hypertension; NO_x – nitric oxide; SS rat – salt-sensitive rat; SS.13^{BN26}(L26) rat – salt-insensitive rat; BP – blood pressure; Chga – chromogranin A; TGFβR I - transforming growth factor βR I; BPH/2J mice – hypertensive mice; RAS - renin–angiotensin system; AMI - acute myocardial infarction; CAD - coronary artery disease; ApoE$^{-/-}$ mice – model of atherosclerosis; ADRB1 - adrenoceptor beta 1; HBP-1 - HMG-box transcription factor 1; Atp1b1 - ATPase Na+/K+ transporting subunit beta 1; MAP – mean arterial pressure; DBP – diastolic blood pressure; SD rat – Sprague Dawley rat; WT mice – wild type mice; C57BL/6 mice – basic background mouse strain; *db/db* mice – type II diabetes model; CDC42 - cell division cycle 42; PAH – pulmonary arterial hypertension; HCM – hypertrophic cardiomyopathy; TSC1 - TSC complex subunit 1; mPAP - mean pulmonary artery pressure; BNP - brain natriuretic peptide; ADMA - asymmetric dimethylarginine.

The above mentioned miR-22 targets chromogranin A (Chga) mRNA – a protein expressed peripherally and in the central nervous system (CNS). Chga influences BP, vasodilatation, insulin sensitivity, and inflammation (34). Animal studies report overexpression of Chga and polymorphism of untranslated binding region (UTR) for miRNAs in Spontaneously Hypertensive Rat (SHR) animal model (13), which consequently increases the binding of miR-22 (34,35). Inhibition of miR-22 caused a decrease in BP of SHRs, which potentially makes it a therapeutic agent in terms of navigating hypertension treatment (7,34). Furthermore, results show that miR-22 is an essential regulator of cardiac function and remodelling, since its' genetic ablation suppresses induced cardiac hypertrophy and enhances fibrogenesis of cardiac fibroblasts in murine model (21,36,37). Wahlquist et al. (2014) suggested miR-25 inhibition as a treatment strategy for heart failure, since it improves cardiac contractility and function. It is a repressor of cardiac function and has been upregulated in heart failure events in both murine and human model (7,23).

MiR-181a is another miRNA that has a potential influence on BP. It is the most abundant miRNA in lymphoid tissue and it regulates T cell function (38). It showcases somewhat anti-inflammatory effect and contributes to adaptive immunity (39). In knock-out mice, deletion of miR-181a led to salt sensitivity and increased BP while it was downregulated in hypertensive murine and human model (40,41). In mice model of hypertension (Schlager BHP/2J mouse), treatment with miR-181a mimics resulted in decreased BP and renal renin mRNA (6). Recently, we published several papers concerning effects of high-salt intake on inflammation and endothelial function and found altered leukocyte activation status followed by advancement of vascular low-grade inflammation in both animal and human model (16,42), and also impaired microvascular reactivity in healthy individuals (43).

Langlo et al. (2021) exposed Dahl salt-sensitive (Dahl/SS) rats to low-salt (LS) and HS diet, with first resulting in mild to moderate hypertension over time, while the latter resulted in severe hypertension following severely increased systolic blood pressure (SBP) (1). Out of 145 studied c-miRNAs assessed in that study, 68 of them were associated significantly with hypertensive complications and can potentially serve as biomarkers for diagnostic purposes. Among others, miR-19a-5p was suggested as a biomarker in cases of hypertensive encephalopathy and endothelial dysfunction (ED), since it was recognized as the main regulator of platelet activation, coagulation and inflammation (44). Enhanced expression of c-miR-19a was reported in cases of pulmonary arterial hypertension (45), acute myocardial infarction (46), coronary artery disease and atherosclerotic patients (47).

Baker et al. (2019) reported antihypertensive effects of renal miR-192-5p in animal hypertension models with an emphasis on the role of Atp1b1 target genes (32). They noted decreased levels of miR-192-5p in L26 (SS.13BN26; mild hypertension) and Dahl/SS rats following HS loading, with more pronounced effect of HS diet in the latter model. Furthermore, when treated with antimiR-192-5p, L26 rats had increased mean arterial BP (MAP) following HS diet. Similar effect was detected in miR-192 knockout mice where MAP, systolic (SBP) and diastolic BP (DBP) increased significantly compared to wild type (WT) mice as a response to a HS diet. These results suggest that deletion or a decrease in miR-192-5p levels leads to exaggerated renal damage and hypertension, suggesting protective role of miR-192-5p against hypertension development.

In another study, high salt loading caused renal and cardiac dysfunction in uninephrectomized Sprague Dawley rats (SD) to a larger extent compared to normal rats fed a HS diet. This was accompanied by an increase in levels of miR-25, miR-451, miR-155 and a decrease in levels of miR-99 of the heart, with opposite effect on same circulatory miRNAs (48). According to Liu et al. (2017), miR-25 levels are lower in blood and tissue samples from diabetic patients/animals and cell cultures exposed to glucose when compared to controls (49). They investigated the effect of knock-down/inhibition of miR-25 on

BP, among other parameters, since hypertension is associated closely to diabetic nephropathy. In WT mice, such venture resulted in RAS activation and hypertension contributing to renal dysfunction. MiRNA-25 has also been described as an oncogenic miRNA and an important regulator in acute myocardial infarction, left ventricular hypertrophy and heart failure (50). Expression levels of miR-451 have been cited in literature as a diagnostic reference in pathogenesis of pulmonary hypertension (51–53), while circulating miR-155 has been positively correlated with BP (both SBP and DBP) and inflammatory markers, with significantly higher expression levels in hypertensive patients compared to healthy controls (54). As suggested by Sun et al. (2012), miR-155 acts as a key regulator of cardiovascular functions, since, when overexpressed, it targets endothelial nitric oxide synthase (eNOS) expression, decreases it and impairs endothelium-dependent vasorelaxation (55).

Another potential therapeutic target and diagnosis biomarker (e.g. early stages of hypertensive nephropathy) is miR-29, depicted as necessary for both normal endothelial function and its restoration in animals and humans (56–58). For example, hypertensive patients with left ventricular hypertrophy had significantly higher levels of miR-29a compared to patients with hypertension, while anti-miR inhibition in transverse aortic constriction (TAC) mice model resulted in suppressed hypertrophy and associated indices (59). Alongside miR-29, miR-30a and miR-133 were also assessed for diagnostic accuracy in case of white-coat hypertension, where their expression levels were associated with BP-related parameters and BP monitoring (60).

Potential of non-coding RNAs in endothelial dysfunction treatment

In the last decade, there has been a lot of research dealing with therapeutic inhibition of miR-33 in animal models. There are two isoforms of miR-33 present in humans, miR-33a and miR-33b, embedded within SREBF1 and SREBF2 genes (SREBP family of transcription factors), while there is only one isoform present in rodents and non-human primates – miR-33a (9,61,62). These serve a purpose in progression of cardiometabolic diseases such as atherosclerosis and obesity and are crucial factors in lipid metabolism regulation, since they are responsible for maintenance of cholesterol, fatty acid and triglyceride homeostasis (62,63).

Previous studies reported improved lipid profile (increased circulatory HDL-cholesterol levels), mitigated inflammation and decreased formation of atherosclerotic lesions following inhibition or genetic ablation of miR-33 in mice (63–65). Similar effects were also detected in case of inhibition by antimiR when administered subcutaneously in Ldlr-/- mice (deficient for LDL receptor) which resulted in reduced plaque size and atherosclerosis regression as well as improved HDL-cholesterol functionality (66,67). On another note, in non-human primates, both normal and metabolic disease model, treatment with miR-33 targeting antimiRs resulted in increased HDL-cholesterol levels, while in normal males' pharmacological inhibition also resulted in decreased VLDL triglycerides (68,69). These results suggest potential therapeutic utility of miR-33s in treatment of atherosclerosis, dyslipidaemia and related metabolic disorders.

Nuclear factor kappa B (NF-κB), a fairly general transcription factor, regulates a variety of biological processes, particularly in stress response and progression of inflammation, both playing large roles in vascular damage and onset of cardiovascular diseases (70,71). Downregulation or decreased expression of miR-150 occurs in acute coronary syndrome and correlates with its onset (72). It targets pentraxin-3 (PTX3) and negatively regulates it through inhibition of NF-κB signalling pathway, furthermore attenuating vascular remodelling and restoring endothelial cell function. Mir-150 has also been associated with therapeutic potential for thrombosis treatment, since its upregulation has an effect on endothelial progenitor cell differentiation and increases angiogenic potential (73). MiR-155, a master

regulator of inflammation, plays an important role in regulation of endothelial inflammation through targeting NF-κB pathway and suppression of inflammatory factors, since its inhibition results in significant inflammatory response (74,75). Another non-coding RNA potentially attenuating endothelial inflammation through effects on NF-κB pathway is miR-99 (76).

MicroRNA identification and screening

Choosing the right miRNA for diagnostic/therapeutic purposes and identification of key targets responsible for specific phenotype is somewhat of a challenge of its own (77). Understanding of phenotypes evoked by certain miRNAs requires usage of computational tools (databases of validated miRNAs, prediction algorithms) as well as an experimental approach (gene expression analysis and proteomics), in order to perform functional cell-based screenings in both health and disease conditions (77,78). Several reviews and research articles listed functional genomics for appropriate validation of critical proteins in biological networks for the purpose of creating prognostic risk models using miRNA data (77–79).

Eulalio et al. (2015) (77) provided a review of functional cell-based screening technologies for miRNA function, aimed at biological processes and illness-related events, including proliferation, signaling, cell maintenance and differentiation. For characterization of mechanisms of action, it is of crucial importance to identify potential key targets for different miRNAs. Such information can be detained from computational, prediction algorithms based on complementarity between miRNAs and target sequences (80–82).

Several prediction tools for miRNA targets have been developed in the last two decades (miRanda, miRanda-mirSVR, TargeScan, DIANA-microT-CDS, MirTarget2, rna22-GUI, TargetMiner, SVMicrO, PITA, RNAhybrid), although it should be clarified that despite the predictive power each approach has, there are also limitations and weaknesses calling for future efforts and research towards the upgrade of available tools (79,81).

Conclusions

Circulating miRNAs could have a potential for early diagnosis of end-organ injury in hypertension and hypertensive emergency. Pathway prediction tools elucidate possible mechanisms in hypertensive emergency that may be the subject for further investigations. Further mechanistic studies are needed (e.g. with miRNA-214-3p and miRNA-29, which have recently been shown to be involved in the development of hypertension). However, currently there are several limitations in using miRNAs in clinical diagnosis and therapy, such as large number of miRNAs as potential biomarkers that require high-throughput technology (e.g. functional screenings) for investigation, followed by mechanistic studies in animals and humans (the latter much more difficult).

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Review article

Dehydroepiandrosterone Sulfate and Arterial Hypertension

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Abstract

Dehydroepiandrosterone sulfate (DHEAS) is a steroid molecule whose function and mechanism of action in the human body are still inadequately researched. A potential protective function for the cardiovascular system can be explained by activation of nitric oxide production, impact on endothelial and mitochondrial function, and inhibition of proinflammatory cytokine production (IL-6 and TNF- α). Some research shows the beneficial effects of DHEA/DHEAS on many bodily functions, especially in the cardiovascular and the neurological systems. However, we need to be careful with interpretation of the results because of different criteria used for defining arterial hypertension, the race that was observed, and reproductive status of women, as these factors can change the conclusion. Due to a lack of evidence, DHEAS supplementation is still not recommended. We need multicentric prospective and randomized studies on DHEAS to examine its potential impact on blood pressure regulation and cardiovascular risk.

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Introduction

Dehydroepiandrosterone sulfate (DHEAS) is a steroid hormone created by adding a sulfate group to dehydroepiandrosterone (DHEA) in the zona reticularis of the suprarenal gland (90%) and the gonads (10%). DHEA is also synthesized in the central nervous system, where its concentration is 6–8 times higher than in the serum, which is why it is also called a neurosteroid hormone (1).

Depending on age, DHEAS can serve as a precursor in the production of other hormones (2). Accordingly, in the reproductive age, 40–75% of testosterone is synthesized from DHEAS. On the other hand, more than 90% of circulating estrone is created from DHEAS (3). DHEA

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concentration in the serum is 10 times higher than cortisol and it shows weak androgenic and estrogenic activity. On the other hand, DHEAS is a hormonally inert molecule with the greatest activity in the central nervous system; it is 100 times more concentrated in the serum than cortisol. This concentration makes it the steroid with the highest ratio in the serum (4). In addition, its levels significantly depend on gender and age, with the lowest levels found in childhood and the period before puberty, while in puberty males have DHEAS levels twice as high as females, with the maximum reached in their early 20s. In women, these levels are not dependent on the menstrual cycle. Total DHEAS concentration decreases by 2% annually and after the age of 70, it reaches pre-puberty levels. The half-life of DHEAS in the serum is around 10 hours. In comparison, DHEA has a 20 times shorter half-life than DHEAS (30 minutes) (5).

In recent research, DHEAS has been shown to have a positive impact on learning and memorizing due to allosteric modification of N-methyl-D-aspartate (NMDA) receptors. Likewise, DHEAS can exaggerate neuron excitation through negative allosteric modulation of gamma-aminobutyric acid (GABAA) receptors (6). By activating sigma-1 receptors and producing nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), DHEAS serves a neuroprotective function (7–9).

In addition to effects on the central nervous system, DHEAS has other pleiotropic effects, including modulation of mitochondrial and endothelial function, as well as bone metabolism (10). One minute of stimulation of bovine aortic endothelial cells (100 nm for 5 min) with DHEA (abolished responses with nitro-L-arginine methyl ester (L-NAME) or phosphatidylinositol (PI) 3 – kinase inhibitor) increased nitric oxide production, stimulated endothelial cell growth, decreased the growth of smooth muscles in blood vessels and the levels of plasminogen activator inhibitor type 1 (PAI-1), and inhibited leukocyte adhesion (11–13). Likewise, DHEA can inhibit the production of proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) (11, 14). The release of NO is independent of intracellular calcium mobilization but depends on G protein tyrosine and mitogen-activated protein (MAP) kinases (15). This mechanism of action could be the key to the cardioprotective effect proposed for DHEA.

DHEA and DHEAS levels are often elevated in polycystic ovary syndrome (PCOS), congenital adrenal hyperplasia (CAH), Cushing's syndrome, and in the presence of hormonally active tumors (16).

History of DHEAS research and cardiovascular risk

In 1975, by examining DHEA and DHEAS concentrations by compartmentalization in urine and blood, Feher et al (17) suggested that a significant uptake of DHEA by adipose tissue occurs, causing lower sulfation into DHEAS and its faster metabolism in obese female patients. They also connected low DHEAS concentration with increased insulin resistance in three obese individuals. Later, in 1987, Rotter et al (18) examined the distribution of DHEAS levels in 178 individuals from 26 families and correlated low DHEAS levels with low testosterone levels. Barret-Connor et al (19) examined the connection between low DHEAS levels and 12-year mortality in 242 men aged 50 to 79. DHEAS levels below 3.8 μ mol/L showed a relative risk of 1.5 for death from any cause (without statistical significance), 3.3 ($P < 0.05$) for death from cardiovascular (CV) disease, and 3.2 ($P < 0.05$) for death from ischemic heart disease in males. There was still a lot to learn about DHEAS in the coming years, and Barret-Connor et al (20) expanded their cohort to 1,029 men and 942 women in California, USA, in 1995, linking low DHEAS levels with coronary ischemic disease in postmenopausal women, but without increased CV mortality.

The next significant research was conducted in 1997, when Barna et al (21) found a significant correlation between serum DHEAS concentration and blood pressure (including the dipping profile), from which they concluded that

lower DHEAS levels are associated with higher blood pressure. This research was conducted on 387 white subjects (86 normotensive and 301 hypertensive), suggesting a positive effect of DHEAS supplementation on CV risk.

Two years later, in 1999, Schunkert et al (22) made the opposite conclusion in their study conducted on 646 subjects, associating higher DHEAS levels with higher blood pressure and higher levels of aldosterone. The debate was resolved in 2004 with the discovery by Liu et al (15), who found that DHEAS increases the activity of nitric oxide synthase (eNOS) mediated by tyrosine and MAP kinases in both bovine aortic endothelial cells and human umbilical vein endothelial cells. Further research on DHEAS was rarely conducted until 2010, when the WISE study (Women's Ischemia Syndrome Evaluation), conducted on 270 postmenopausal women, showed that lower DHEAS levels are linked to higher CV mortality in postmenopausal women who underwent coronarography due to myocardial ischemia (23). Furthermore, in 2019, De Paiva Lemos et al (24) found a correlation between DHEAS and lower heart rate variability in old age, but this causality can hardly be confirmed since heart rate variability decreases physiologically with aging, much like DHEAS serum concentration. The other problem with this study was that the sample consisted of only 45 men, divided into three age groups. However, today we assume that lower heart rate variability is linked to a 32–45% higher risk of CV events (25).

Possible reasons why the aforementioned researchers came to different conclusions about DHEAS and its impact on blood pressure may lie in the choice of methods and patients. To date, only three studies have examined the correlation between DHEAS and blood pressure levels. Differences in the methodology used in these studies are shown in Table 1.

Table 1. Methodology of the most prominent studies about DHEAS and arterial hypertension

Authors and year	Correlation between DHEAS and AH	Correlation with nocturnal indices	AH criteria	Race (country)
Barna et al, 1997 (21)	Negative	Positive	> 130/85 mmHg	White (Hungary)
Schunkert et al, 1999 (22)	Positive	/	> 160/95 mmHg	White (Germany)
Jimenez et al, 2019 (26)	Nonexistent	/	> 130/80 mmHg	Latino (Puerto Rico)

AH = arterial hypertension

DHEAS supplementation

Due to the previously mentioned benefits for the CV system and the brain, scientists have been discussing DHEAS supplementation in postmenopausal women for many years. Yet, meta-analyses conducted by Boxer et al. (27) in 2010 and by Wang et al. (28) in 2020 did not show any clear benefits of DHEAS supplementation in postmenopausal women. Longer prospective cohort studies are needed to accurately demonstrate the benefits of this kind of supplementation in women.

Medication and DHEAS

Higher DHEAS serum levels were observed in hypertensive patients treated with beta-blockers and calcium channel blockers (16). On the other hand, lower DHEAS levels were observed in patients treated with ACE inhibitors (29).

Lifestyle changes and DHEAS

Despite the clear evidence of effects of a healthy diet and weight loss on blood pressure, the impact of lifestyle changes on DHEAS levels remains unknown. The exception is smoking, since nicotine can stimulate the production of corticotropin-releasing hormone (CRH) and vasopressin, which stimulate DHEAS synthesis (30). Likewise, smoking can decrease DHEAS clearance, as well as its binding to serum proteins, which also increases its serum concentration (31). Diet rich in pectin can likewise increase DHEAS levels in the serum, but excessive protein intake may reduce DHEA levels (32, 33). An increase of DHEA levels immediately after exercise was found in both genders, but DHEAS levels increased only in women (34). A very small number of studies have examined the impact of exercise on these hormones, with many methodological differences, which complicates their comparison.cardiovascular and renal disease in non-diabetic individuals with arterial hypertension remains a question to be answered.

Conclusion

Although there is clear evidence that DHEAS can stimulate NO production in blood vessels, the conclusions of relatively small and methodologically different studies about its impact on blood pressure are still contradictory. However, some studies have shown increased CV mortality in subjects with low DHEAS levels, but its supplementation is still not recommended. There is a need for larger prospective multicentric studies to be conducted in order to clarify the connection between DHEAS and arterial hypertension.

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Review article

Arterial Hypertension and Risk of Mortality in Patients with COVID-19 Infection

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Abstract

COVID-19 is currently a major global health concern. Among many unanswered questions related to COVID-19, some of the most debated ones are those concerning arterial hypertension. Arterial hypertension is a major risk factor for mortality worldwide and its importance has been emphasised even further in light of COVID-19. The most common antihypertensive drugs are ACE inhibitors and angiotensin II type-I receptor blockers. SARS-CoV-2 utilises the angiotensin-converting enzyme-2 (ACE2) for cell entry and therefore has a direct effect on the renin–angiotensin system (RAS). In terms of arterial hypertension and COVID-19, there are three main issues which have been the focus of extensive debates. First, is arterial hypertension a predisposing factor for COVID-19 infection? Second, does arterial hypertension affect the severity of COVID-19 infection and increase the risk of all-cause and cardiovascular mortality? And finally, how important is the interaction of COVID-19 infection and the renin–angiotensin system for clinical outcomes? Is RAS blockade beneficial or harmful? The aim of this brief review was to provide substantiated answers to these questions.

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Introduction

Infection with SARS-CoV-2 is associated with high mortality rates and it is currently the most important global health concern. The risk of severe outcomes of COVID-19 infection increases with patients' age and prevalence of comorbidities (1). Arterial hypertension is one of the most prevalent chronic conditions (2) and it is often treated with renin–angiotensin system (RAS) blockage. Angiotensin-converting-enzyme 2 (ACE2) is the pivotal receptor for SARS-CoV-2 to enter host cells (3) and as such, it provides a link between COVID-19 and RAS. The association between arterial hypertension, its treatment, the risk of SARS-CoV-2 infection and clinical outcomes of COVID-19 infection has been a concern and a matter of debate for both patients and physicians. The aim of this paper was to review our knowledge about this issue by focusing on the most important publications issued so far and by answering the following key questions. First, does arterial hypertension predispose individuals to COVID-19 infection? Second, does arterial hypertension increase the risk of all-cause and cardiovascular mortality? Third, how important is the interaction of COVID-19 infection with the renin–angiotensin system? And finally, is RAS blockade beneficial or harmful in respect to COVID-19 outcomes?

However, before discussing arterial hypertension and COVID-19 infection, it is necessary to point out some important facts about arterial hypertension, RAS and respiratory infections independent of COVID-19 infection. It is well known that people over the age of 65 have a higher risk of a lower respiratory tract infection (LRTI) as well as a higher prevalence of arterial hypertension (4,2). In addition, a beneficial effect of RAS blockade associated with LRTI was detected in terms of lower incidence and lower mortality rates (5). Based on the above, it can be concluded that arterial hypertension is not an independent risk factor for LRTI and that the use of RAS blockers decreases the incidence and improves the outcomes of LRTI (6).

Is arterial hypertension a predisposing factor for COVID-19 infection?

According to the first data collected in Lombardy in early 2020, almost 73% of patients with COVID-19 had arterial hypertension (7). At that point, arterial hypertension was often indicated as a risk factor for COVID-19, together with cardiovascular and cerebrovascular diseases, chronic kidney disease, malignancies and obesity. However, it was initially overlooked that the average age in the Lombardy group was 81. Having that in mind, it is not surprising that the prevalence of arterial hypertension was so high. Other data from the first reports from Lombardy were related to the intake of antihypertensive drugs. Almost 47% of COVID-19 patients were taking RAS blockers. This information, along with some pathophysiological considerations, aroused suspicion that drugs which block RAS could be related to the higher incidence of SARS-CoV-2 infection, more severe forms of the disease and increased mortality. To support those data, Mancia et al. analysed the data concerning 6,272 people with COVID-19, together with a control group of 30,759 subjects paired based on age and gender (8). The first important fact they discovered was that the COVID-19 patients more often had cardiovascular diseases in their medical history, especially coronary heart disease and heart failure. Moreover, they also suffered from respiratory diseases, chronic kidney disease and, to some extent, carcinomas.

The second important piece of data concerned the association between RAS blockers and COVID-19 infection – no significant correlation of RAS blockers with COVID-19 infection was detected in the study, neither with regard to the severity of the clinical features of COVID-19 infection nor with regard to age or gender. In relation to COVID-19 in this group of subjects, RAS blockers had the same status as all other

classes of antihypertensive drugs. The final conclusion was that the observed relationship between arterial hypertension and COVID-19 infection was blurred by the influence of age and comorbidity. A number of other studies have confirmed that the prevalence of arterial hypertension in COVID-19 patients does not differ significantly from the prevalence in the general population (9).

Does arterial hypertension affect the severity of COVID-19 infection and increase mortality?

A number of researchers have noticed that patients with more severe forms of COVID-19 infection suffer from arterial hypertension significantly more frequently (Table 1). By analysing a group of patients from Wuhan, China, Shi et al. found that COVID-19 mortality was significantly higher in patients with myocardial damage and that arterial hypertension was also much more common among them (10). In a retrospective study by Wu et al., patients with hypertension were more likely to have increased oxygen demand, myocardial injury and a greater risk of developing severe COVID-19, suggesting that hypertension might play an important role in COVID-19 (11). These findings are consistent with the idea that hypertension cases involve an increased risk of comorbidity, infection and multiple organ function damage (12,13). In a multicentre retrospective cohort study of 1,833 COVID-19 patients, Mubarik et al. found that the prevalence of hypertension was 40.5% and that patients with hypertension were more likely to have severe COVID-19 illness than patients without hypertension (14). Evidence from a meta-analysis which included 24 observational studies with 99,918 COVID-19 patients suggested that hypertension was independently associated with a significantly increased risk of critical COVID-19 and in-hospital mortality of COVID-19 (15). Guan et al. detected a higher risk of severe COVID-19 in older individuals and those with underlying health conditions such as arterial hypertension (16). Gao et al. observed that mortality was much higher in patients with uncontrolled hypertension compared to those who achieved target blood pressure values (17). They also noticed that mortality was significantly lower in patients treated with RAS blockers.

Interesting results were obtained from the HOPE COVID-19 registry (Italy, Spain, Germany), where out of 5,937 patients with COVID-19 infection, 48.8% had arterial hypertension and 70.3% were treated with RAS blockers (18). As in the Lombardy group, the patients with COVID-19 infection more frequently had comorbidities associated with an increased risk of death. Over 40 days of follow-up, a significantly higher mortality rate was noticed among patients with arterial hypertension (29.6% vs 11.3%; $p<0.001$). Based on this finding, the authors expected that the increased mortality in COVID-19 infection could be related to arterial hypertension. However, when data was analysed in more detail using the multivariate Cox regression analysis, the authors found that the age over 65, respiratory infections and sepsis were independent predictors of mortality. A very important finding derived from their analysis was the fact that the use of RAS blockers had a protective effect on mortality, consistent with the findings by Gao et al. (17). Sheppari et al. have recently published an interesting finding concerning a group of patients with stage 1 uncontrolled arterial hypertension that there were no differences in mortality compared to the patients with controlled hypertension (19). They did not observe any associations between the control of arterial hypertension and COVID-19 infection or the need for hospitalisation. They concluded that the association between arterial hypertension control and mortality could be explained by advanced atherosclerosis and pre-existing target organ damage. The authors pointed out a probable key problem associated with arterial hypertension and COVID-19 infection, and that is the issue of irregular medical check-ups. Additionally, it should not be forgotten that the immune system in patients with arterial hypertension is already promoted and as such, it might represent the mechanisms through which arterial hypertension could be associated with severe forms of COVID-19 infection. This is an area which requires further research.

Is the interaction of COVID-19 infection and RAS important for the clinical course of the disease?

Table 1. Publications related to the association between severe COVID-19 and arterial hypertension

Authors	Findings
Shi et al. [10]	Cardiac injury is a common condition among hospitalised patients with COVID-19 in Wuhan, China and it is associated with a higher risk of in-hospital mortality. A total of 82 patients (19.7%) had cardiac injury. Compared with patients without cardiac injury, these patients were older and had more comorbidities (e.g. hypertension in 49 of 82 [59.8%] vs 78 of 334 [23.4%]; P < .001).
Wu C et al. [11]	Arterial hypertension, especially poorly controlled hypertension, may play an important role in the severity of COVID-19.
Guo T et al. [12]	This retrospective single-centre case series analysed patients with COVID-19 in Wuhan, China, from 23 January 2020 to 23 February 2020. Myocardial injury is significantly associated with a fatal outcome of COVID-19, while the prognosis for patients with underlying CVD, but without myocardial injury is relatively favourable. 66 patients (35.3%) had underlying CVD, including hypertension, coronary heart disease and cardiomyopathy.
Dessie GZ et Zewotir T. [13]	Chronic comorbidities, complications and demographic variables, including acute kidney injury, COPD, diabetes, hypertension, CVD, cancer, increased D-dimer, male gender, older age, current smoker and obesity are clinical risk factors for a fatal outcome associated with coronavirus.
Mubarik S et al. [14]	Prevalence of hypertension in 1,833 studied COVID-19 patients was 40.5%. Hypertension was associated with the severity and mortality of COVID-19 infection.
Du Y et al. [15]	Evidence from this meta-analysis suggested that hypertension was independently associated with a significantly increased risk of critical COVID-19 and in-hospital mortality of COVID-19.
Guan WJ et al. [16]	Advanced age was associated with a higher prevalence of other comorbidities such as diabetes, renal impairment, arterial hypertension and obesity, which altogether increased the proportion of hypertensive patients.

Angiotensin-converting enzyme 2 (ACE2) is a protein which has a crucial role in the entry of the COVID-19 virus into the cells. It is the key matter of debate on the benefits or dangers of RAS blockade in COVID-19 infection. Verdechia et al. labelled COVID-19 an ACE2-centric infectious disease (20). Recently, the results of a study that used human cardiomyocytes as a model for researching the entry of SARS-CoV-2 into the cells have been published. After the viral infection, there is an increase in the synthesis of ACE2, type 1 receptors for angiotensin II, but no change in the synthesis of the angiotensin-converting enzyme (ACE) (21). The same group of researchers have observed that the synthesis of ACE2 in ACE2-knockout (ACE2-KO) cardiomyocytes was almost immeasurable, and, after the infection, these cells also had a negligible quantity of SARS-CoV-2. Furthermore,

the authors treated human cardiomyocytes and human endothelial cells either only with the virus or with the virus combined with lisinopril or losartan. The amount of the virus was not increased in endothelial cells or in cardiomyocytes after the use of ACE-inhibitors or sartans and the expression of proteins (ACE2) did not significantly change after the use of these medications. This research provides an additional confirmation that ACE-inhibitors or sartans do not promote SARS-CoV-2 infection.

The hypothesis that advocated a negative role of RAS blockade in SARS-CoV-2 infection was based on the fact that RAS blockers, by inducing an enhanced ACE2 expression, facilitate the entry of the virus into the cell (22). Supporters of the opposing hypothesis, however, believe that the enhanced ACE2 expression, along with RAS blockade, facilitates increased synthesis of angiotensin 1-7, promoting its beneficial anti-inflammatory and antifibrotic effects and preventing lung injury (Figure 1) (23).

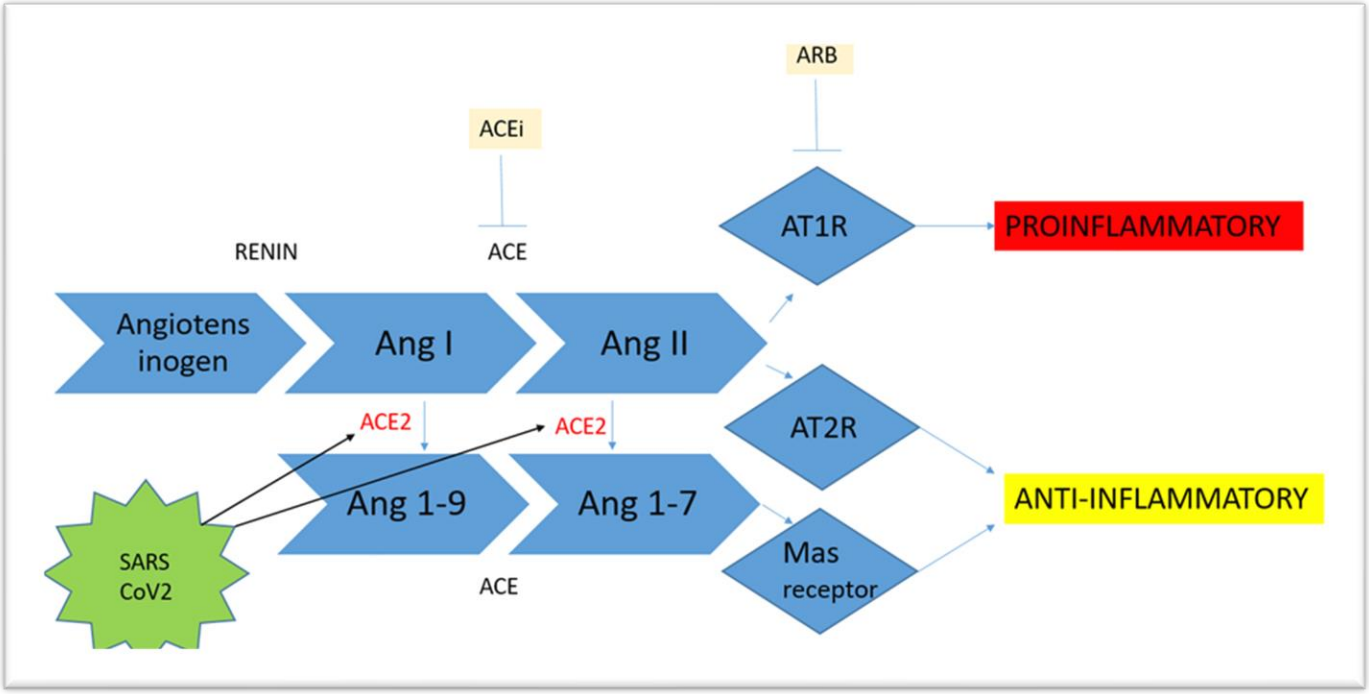

Figure 1. Association between SARS-CoV-2 and renin angiotensin system

ACE – angiotensin-converting enzyme, ACEi – angiotensin-converting enzyme inhibitor, Ang – angiotensin, ARB – angiotensin type II receptor blocker, AT1R – angiotensin II type 1 receptor, AT2R – angiotensin II type 2 receptor

It has already been mentioned that RAS blockers have a positive effect on the clinical course of pulmonary diseases without COVID-19 infection (5,24). The significance of the beneficial impact of ACE2 and angiotensin 1-7 on COVID-19 infection is evident from the fact that, among other drugs which are synthesised for the treatment of COVID-19 infection, scientists have been intensively working on recombinant ACE2 and angiotensin 1-7 (20).

The results of basic research are bringing us back to the epidemiological clinical research by Mancia et al., which proved in a group of patients with confirmed COVID-19 infection that neither ACE-inhibitors nor sartans were associated with a higher probability of COVID-19 infection (8). This was also confirmed in a meta-analysis by Kurdi et al., which involved 72,372 patients from 27 studies (25). Analysing a group of 1,002 patients with severe COVID-19 infection, Reynolds et al. concluded that RAS blockade is not associated with severe forms of COVID-19 infection (26). Those data were confirmed in the previously mentioned meta-analysis by Kurdi et al., where no association was observed between RAS blockade and severe forms of pneumonia (25). The HOPE COVID-19 registry, Zhang et al. and research by Gao et al. indicated a lower mortality rate in patients treated with RAS

blockers in comparison to those who did not receive these medications (18,27,17). Finally, the meta-analysis by Kurdi et al. also showed that the use of RAS blockers was associated with a lower mortality rate and a lower risk of admission to intensive care units (25).

Another meta-analysis was published in 2021 and it included 101,949 patients from 52 studies, 26% of whom were treated with RAS blockade (28). In this meta-analysis, the authors did not find an association between the use of RAS blockers and higher mortality. On the contrary, they found that RAS blockade has a positive effect, i.e. there were less deaths and severe complications after adjusting for all risk factors.

In an extensive observational research, Semenzato et al. noticed that patients treated with ACE-inhibitors or sartans for a very long time had a lower risk of COVID-19 infection than those treated with calcium channel blockers (29). This finding corresponds to the previously published results, according to which the use of ACE-inhibitors or sartans increases survival, i.e. reduces mortality rates in hypertensive patients with COVID-19 infection (30). The authors have not found any differences between ACE-inhibitors and sartans. However, a group of authors has recently raised an intriguing question whether sartans are the drug of choice for the treatment of arterial hypertension, and moreover, during COVID-19 infection (31).

There is obviously no hard evidence for this approach and further research is needed to either confirm or reject this premise. Nevertheless, it is a good example of how the paradigm has switched from the fear of possible danger of applying RAS blockers in patients with COVID-19 infection to completely the opposite – exploring the details of their benefits. However, as mentioned, apart from sartans that obviously precede ACE inhibitors, recombinant ACE2 and angiotensin 1-7 are potential treatment options that are currently being explored (20,32).

The real consequences of COVID-19 infection for patients with arterial hypertension

Inadequate access to medical care, either due to the fear of infection or the medical personnel's preoccupation with an enormous number of COVID-19 cases, will lead to numerous negative impacts. The real consequence of COVID-19 infection is a decline in the control of treated hypertensive patients, decline in adherence to medications, irregular blood pressure measurements and an increase in morbidity and mortality from cardiovascular, cerebrovascular and renal causes. Instead of an isolated pandemic, a syndemic, clustering COVID-19 and the excess burden of cardiovascular, cerebrovascular and kidney disease/deaths will become our harsh reality.

Conclusion

In our opinion, the answer to the first question – does arterial hypertension increase the risk of COVID-19 infection? – is most probably not, although there has been insufficient evidence so far. The answer to the second question – does arterial hypertension increase the risk of severe infections? – might be that it does, but only if arterial hypertension is not controlled well and treated properly. If it is controlled poorly, then there is a higher possibility of a fatal or more severe cytokine storm due to the chronic latent stimulation of the immune system among patients with arterial hypertension. The answer to the third question – is the risk of severe COVID-19 infection associated with the use of drugs that block RAS? – is that there is no evidence to suggest so.

Finally, are RAS blockers beneficial or harmful? According to the recent studies, which indicate that patients treated with RAS blockers might have a better prognosis, RAS blockers might be beneficial. Therefore, patients with stable arterial hypertension must continue taking RAS blockers. However, in severe COVID-19 infections accompanied by hypotension, patients must stop using RAS blockers as well as any other antihypertensive drugs.

Numerous questions related to these issues are still left unanswered. As a stimulus for further considerations, we decided to select these two questions: should we introduce RAS blockers for

lung protection in case of COVID-19 and are there really any differences between ACE-inhibitors and sartans?

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Original research article

Physical Activity as Prediction of Functional Ability Among Elderly

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Abstract

Aim: Falling can have serious consequences for older persons, but fear of falling is also a current problem. The aim of the study is to assess the incidence of falls and fear of falling among older adults, as well as to study the connection of falls and fear of falling with functional ability, body mass index (BMI) and age.
Methods: The participants of this study are older persons (> 60 years) who live in the city of Rijeka, are mobile and have no symptoms of dementia. The relationship between falls, fear of falling, body mass index (BMI) and functional ability was measured using various indicators. Assessment of physical functioning and pain relief was made using the COOP/WONCA questionnaire. Falls and fear of falling were assessed using questions with different response options.
Results: The results of the study showed that participants who reported experiencing fear of falling (FoF) had a higher BMI (p = 0.018) and did not feel physically healthy (70%). Participants who lived with a partner reported experiencing FoF at a lower rate (36.4%). The most frequently reported functional problems were visual problems (46.2%) and walking difficulties (40.0%).
Conclusions: A large percentage of older persons struggle with the effects of aging, which include various health problems that can increase the risk of falling and FoF. Prescription of physical activity and engaging in it could improve functional ability and have an important effect on healthy aging. This could be the starting point from which key stakeholders can plan different types of public health interventions, prescribing physical activity to maintain functional ability that enables well-being in old age.

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Introduction

The aging process brings with it many physiological changes that can subsequently lead to the development of risk factors and chronic diseases. According to the World Health Organization, most changes begin after the age of 60 and usually include functional impairment (such as hearing loss, visual impairment, reduced mobility) and the onset of non-communicable diseases (heart disease, stroke, chronic lung disease, malignancies and dementia) (1). All these conditions lead to functional impairment and consequently cause dependence.

Falls are a major cause of morbidity and mortality in older persons. It is estimated that 30–40% of persons over the age of 65 fall at least once a year (2). The number of fall-related deaths in persons over 75 years of age in the US increased from 8613 to 25189 from 2000 to 2016, which means that the number of older persons who died due to falling nearly tripled in 2016. Several studies in Europe report a similar increase in the number of deaths among older persons due to falling (3, 4). Despite this, most falls in the general population do not endanger the individual's life, but 37.3 million falls per year require some form of medical intervention (5) and 70% of these result in some degree of disability (6). Falling can lead to moderate to severe musculoskeletal injuries, loss of independence and even death. However, one of the most important psychological consequences of falls is the appearance of fear of falling (FoF) (2).

FoF leads to physical, functional, psychological and social changes in older persons, which are associated with a decrease in physical activity and health. Falls could also be a consequence of FoF. According to a 2013 study, persons who have fallen in the past year are usually over 85 years old, have cataracts, significant circulatory, respiratory, musculoskeletal and nervous system disorders, take four or more different medications, use walking aids and are overweight (6). Obesity is associated with a 25% higher risk of falling than in persons of normal weight. This information is explained by the fact that being overweight affects the postural stability of older persons, which can lead to more frequent falls (7). Despite all these facts, there is a simple way to prevent most of these conditions. Physical activity has significant health benefits for hearts, bodies and minds. Regular physical activity provides significant benefits for health and can improve muscular and cardiorespiratory fitness, improve bone and functional health, reduce the risk of hypertension, coronary heart disease, stroke, diabetes, various types of cancer (including breast cancer and colon cancer) and depression, reduce the risk of falling as well as hip or vertebral fractures and help maintain a healthy body weight (1, 8).

The aim of this article was to show the functional ability of elderly persons who engage in physical activity, as well as to study and establish a relationship between risk factors that influence the possibility of falling and the prevalence of fear of falling in the elderly as a function of functional ability.

Materials and Methods

The survey is retrograde and cross-sectional and takes into account the first measurements of functional status of the elderly from 2016. The sample includes respondents/elderly persons who participated in group exercise sessions which were organized as part of the Urban Health Centres Europe project and were maintained as continuation of the project. The group exercise sessions were organized twice a week and lasted for one hour. The exercises were led by physiotherapy students who were trained to lead exercise sessions for the elderly. Older persons also participated in educational programs for active and healthy aging.

Participants

The participants in this study are older persons (over 60 years old) who live in the city of Rijeka, are mobile, live independently and have no dementia symptoms. Recruitment was organized during project activities, and visiting nurses recruited participants based on the

inclusion criteria. All participants were informed about the study and signed an informed consent form in order to participate in the study. Confidentiality was maintained in the storage, retrieval and analysis of the data. The study was approved by the Ethics Committee for Biomedical Research of the Faculty of Medicine, University of Rijeka.

Methods

The Dartmouth Functional Health Assessment Charts (COOP/WONCA questionnaire) were used to assess physical functioning and pain. The COOP questionnaire is used for a quick subjective assessment of the subjects' functional health. The COOP charts reflect the patient's assessment of their functional capacity (9). The instrument consists of six charts. For this study, we used physical functioning and pain charts. Respondents rated the intensity of physical activity they had been capable of in the last two weeks on a scale of 1 to 5, where 1 is very heavy activity and 5 is very light activity. In addition, participants rated the intensity of pain in the last four weeks on a scale of 1 to 5, where 1 means that there was no pain and 5 means that the pain was severe. Participants' height and weight were measured at baseline and BMI was calculated as weight (in kilograms) divided by the square of height (in meters) (kg/m2). In order to detect falls in the past 12 months, respondents were asked "Have you fallen in the last 12 months?", where the answer that could be given was yes or no. If the answer was yes, another question was asked, "How many falls did you have in the last 12 months?". FoF was measured by asking the question "In the last months, did you worry about falling down?" The responses were yes or no, and we classified it as a binary variable.

Measurements of restrictions on daily activities and data on the use of medical devices such as walking sticks, glasses etc. were collected.

Sociodemographic data

Information on age group, gender, education, marital status and living arrangements were collected. Results were analyzed in terms of experience of falls, demographic characteristics (age, gender and marital status) and personal perceptions of health and functional ability.

Statistical analysis

Statistical analysis included a descriptive analysis of participants' baseline characteristics. Pearson's chi-squared test and Fisher's exact test were used to compare the characteristics of those with and those without fear of falling, as reported. These tests were used to determine the level of statistical significance at the 0.05 (5%) level. For $p \leq 0.05$, the difference in arithmetic mean is considered significant. Data were processed using Statistica (version 13.5.0.17, 1984–2018 TIBCO Software Inc) and Microsoft Office Excel 2016.

Results

Sixty-five older persons participated in the study, of whom 93.9% (N = 61) were women and 6.1% (N = 4) were men. The mean age of the participants was 69.8 (± 7.3) years, with the youngest participant being 60 years old and the oldest 92 years old. Subjects were divided into three age groups: younger than 65, 66 to 70 and over 70 years old. The average BMI of the subjects was 27.5 (± 3.5) kg/m2; for men, it was 28.8 (± 4.7) kg/m2 and for women, it was 26.6 (± 3.3) kg/m2. Half of the subjects were classified as pre-obese. Most of the respondents live with a partner (Table 1).

Of the health problems observed in the lives of the respondents, visual impairment (46.2%) and walking difficulties (40.0%) were the most frequently reported. In addition, almost 40.0% of respondents reported problems in everyday life due to physical fatigue. Problems with balance (15.4%) and hearing (12.3%) were mentioned less frequently (Table 1).

Table 1. Characteristics of the study population groups

	Overall	Male	Female
N	65 (100.0)	4 (6.1)	61 (93.9)
Average age	69.83 (± 7.3)	81 (± 7.3)	69.17 (± 6.7)
Age group			
Younger than 65	20 (30.8)	-	20 (32.8)
65 to 70	18 (27.7)	-	18 (29.5)
Older than 70	27 (41.5)	4 (100.0)	23 (37.7)
BMI (kg/m²)*	27.5 (± 3.5)	28.8 (± 4.7)	26.6 (± 3.3)
Normal weight	22 (34.4)	1 (25.0)	21 (35.0)
Pre-obesity	32 (50.0)	2 (50.0)	30 (50.0)
Obesity class I	10 (15.6)	1 (25.0)	9 (15.0)
Marriage status*			
Unmarried	-	-	-
Married	34 (53.1)	3 (75.0)	31 (51.7)
Extramarital union	1 (1.6)	-	1 (1.7)
Divorced	9 (14.1)	-	9 (15.0)
Widow/widower	20 (31.2)	1 (25.0)	19 (31.6)
Household composition**			
Living alone	22 (34.9)	1 (25.0)	21 (35.6)
Living with a partner, no children	26 (41.3)	2 (50.0)	24 (40.6)
Living with a partner and children	6 (9.5)	1 (25.0)	5 (8.5)
Living without partners and children	6 (9.5)	-	6 (10.2)
Living in a household that I share with others	3 (4.8)	-	3 (5.1)
Problems in everyday life resulting from			
Walking difficulties	26 (40.0)	3 (75.0)	23 (37.7)
Balance problems	10 (15.4)	2 (50.0)	8 (13.1)
Impaired hearing	8 (12.3)	1 (25.0)	7 (11.5)
Impaired vision	30 (46.2)	1 (25.0)	29 (47.5)
Hand weakness	23 (35.4)	3 (75.0)	20 (32.8)
Physical fatigue	25 (38.5)	3 (75.0)	22 (36.1)

* one subject did not respond ** two subjects did not respond

FoF is present in half of the respondents and increases with age. For example, more than 60.0% of respondents over 70 years of age reported FoF, while FoF was present in 25.0% of respondents under 65 years of age. Less than a quarter of the respondents reported experiencing a fall in the past year. There was no difference in the presence of FoF between the respondents who had experienced a fall in the past 12 months and those who had not ($p < 0.05$).

The results of the study showed that the respondents who reported experiencing FoF had a statistically significantly higher BMI than those who did not report experiencing this fear ($p = 0.018$). Despite the higher BMI among subjects who reported experiencing a fall in the past 12 months compared to those who did not, this difference was not statistically significant. Subjects who were married reported experiencing FoF at a significantly lower rate (36.4%) than those who were unmarried (63.3%).

They also reported falling at a lower rate (20.6%) compared to the unmarried respondents (26.7%). However, no statistically significant difference was found between the subjects in that regard (Table 2).

Table 2. Comparison of functional ability, falls and fear of falling in the study group

	Fear of falling*			Fall in the last 12 months		
	Present N (%)	**Not present N (%)**	**p**	**Present N (%)**	**Not present N (%)**	**p**
Participants	32 (50.0)	32 (50.0)		15 (23.1)	50 (76.9)	
Male	3 (75.0)	1 (25.0)		1 (25.0)	3 (75.0)	
Female	29 (48.3)	31 (51.7)		14 (23.0)	47 (77.0)	
Age group						
Younger than 65	5 (25.0)	15 (75.0)		5 (25.0)	15 (75.0)	
65 to 70	10 (58.8)	7 (41.2)		2 (11.1)	16 (88.9)	
Older than 70	17 (63.0)	10 (37.0)		8 (29.6)	19 (70.4)	
BMI (kg/m²)	27.7 (± 3.6)	25.6 (± 3.1)	< 0.05	27.2 (± 3.7)	26.6 (± 3.4)	
Marriage status*						
Married	12 (36.4)	21 (63.6)	< 0.05	7 (20.6)	27 (79.4)	
Other	19 (63.3)	11 (36.7)		8 (26.7)	22 (73.3)	
Perception of physical health N (%)						
Feel physically healthy	25 (46.3)	29 (53.7)		14 (25.5)	41 (74.5)	
Do not feel physically healthy	7 (70.0)	3 (30.0)		1 (10.0)	9 (90.0)	
Pain in everyday life N (%)						
Do not report pain or report mild pain	17 (39.5)	26 (60.5)	< 0.05	7 (15.9)	37 (84.1)	< 0.05
Moderate to severe pain	14 (70.0)	6 (30.0)		8 (40.0)	12 (60.0)	
Problems in everyday life resulting from: N (%)						
Walking difficulties						
Present	17 (65.4)	9 (34.6)	< 0.05	5 (19.2)	21 (80.8)	
Not present	15 (39.5)	23 (60.5)		10 (25.6)	29 (74.4)	
Balance problems						
Present	9 (90.0)	1 (10.0)	< 0.05	3 (30.0)	7 (70.0)	
Not present	23 (42.6)	31 (57.4)		12 (21.8)	43 (78.2)	
Impaired hearing						
Present	5 (62.5)	3 (37.5)		3 (37.5)	5 (52.5)	
Not present	27 (49.1)	29 (52.7)		12 (21.1)	45 (78.9)	
Impaired vision						
Present	20 (66.7)	10 (33.3)	< 0.05	8 (26.7)	22 (73.3)	
Not present	12 (35.5)	22 (64.7)		7 (20.0)	28 (80.0)	

* one subject did not respond

Subjects who felt physically unwell reported experiencing FoF at a higher percentage (70.0%) compared to subjects who felt physically healthy (46.3%). FoF occurs significantly more often in subjects reporting moderate to severe pain in everyday life (70.0%) compared to subjects reporting no pain or mild pain. In addition, subjects reporting moderate to severe pain reported a significantly higher percentage of falls (40.0%) compared to subjects reporting

no pain of this intensity in everyday life (15.9%). Subjects reported a significantly higher percentage of falls when they had problems with walking (65.4%), balance (90.0%) and vision (66.7%) in everyday life compared to subjects who reported no such problems (Table 2).

Discussion

According to the results of our study, the presence of FoF is associated with several factors. Elderly persons with FoF have higher BMI, are not married, live alone, have moderate to severe pain and functional problems in everyday life, have a negative perception of their physical health and are of advanced age. The risk factors for FoF occurrence are interrelated and interdependent. Studies have shown that the annual incidence of falls in older persons is between 30 and 40% (2), but it was less than 25% in our study population. We can assume that the reason for this could be the participation in group exercise sessions twice a week.

The association between female gender and more frequent falls is frequently mentioned in the literature (10, 11). In our study, the small number of male subjects must be taken into account, along with their higher average age compared to the female subjects. A higher incidence of falls is also associated with older age (12). A psychological consequence of the fall could be the appearance of FoF, and on the other hand, FoF could be the reason for the fall. Falls occurred significantly more often in those subjects who suffer from moderate to severe pain in everyday life. Older persons who report pain in everyday life are more likely to report experiencing a fall in the past 12 months than those who report no pain. The results of our study show that respondents with moderate to severe pain reported a higher percentage of falls in the past 12 months compared to those who did not report such pain. The study associated an increase in the incidence of falls with pain affecting activities of everyday life (13). A 2018 study states that pain is one of the most important factors in the occurrence of moderate to severe mobility impairment in older persons (14).

Elderly women who live alone are more likely to suffer from FoF, which is associated with impaired quality of life and reduced functionality in performing activities of everyday life. We can say that less than 40% of older persons limit their activities due to FoF. FoF can occur before a person experiences a fall (12). Non-married respondents have a significantly higher incidence of FoF than married respondents; they also report a higher percentage of falls. This is consistent with the findings of a 2010 study, which found that persons who live alone are at higher risk of falling than persons who live with family (15).

Walking is one of the basic activities of everyday life. The presence of gait instability can be a significant risk factor for the occurrence of falls, especially in older persons (16, 17). The results of our study show that persons who reported problems with gait difficulty were less likely to have fallen in the past 12 months than persons who reported no such difficulties. We can assume that they are more careful in their everyday movements, but the results show that FoF occurs in more than 65% of respondents who reported walking difficulty. The findings can be explained by a 2017 study which found that postural instability and gait problems associated with FoF are linked to a higher incidence of falls in older persons. Research has shown that the occurrence of FoF only predicts future falls in persons with postural instability and gait problems (18).

In 2014, Hoang et al. associated FoF with previously experienced falls, poor balance and negative perceptions of physical health (19). In our study, results also showed that FoF was more common in subjects who had previously experienced a fall. The subjective feeling of balance problems in everyday life is more pronounced in persons who have a fear of falling. According to our research, 90% of persons who reported balance problems have FoF. The results suggest that persons who have a negative perception of their physical health are more likely to report experiencing FoF than persons who have a positive perception of their health, which is related to the study by Hoang et

al. (19). FoF was present in 70% of subjects with a negative perception of health.

FoF and its frequency are associated with visual and hearing impairments (20, 21), especially when a person has both, together with balance problems (22). In addition, persons who perceive their hearing impairment as a significant disability are at higher risk of experiencing two or more falls within five years than persons who do not have hearing problems (21). Participants who reported having hearing problems reported a higher percentage of falls in the past 12 months. Choi and Ko published a paper in 2015, in which they found that persons with vision problems fall more often than persons who do not have such problems (12). The reason for this could be a reduced perception of environmental signs. In addition, subjects who perceived problems related to visual impairment reported a higher percentage of FoF when performing functional activities of everyday life. Studies show that visual impairment is an independent risk factor for the occurrence of falls, their recurrence and the occurrence of fractures (20, 21, 23, 24).

Obesity is associated with an increased risk of falls, as well as a higher risk of disability in activities of everyday life following falls in older adults (25). In addition to the incidence of falls and disability, obesity is associated with a greater likelihood of pain and reduced mobility after falls when compared to peers with normal body weight (26). A 2011 study found no significant difference between fall experiences in individuals with normal weight and overweight individuals (27), in contrast to a 2017 study, which found that undernourished and obese individuals had a higher percentage of falls compared to individuals with normal weight and overweight individuals (28). Older persons who have postural instability and are obese are at higher risk of falling because obesity affects balance and performance of dynamic tasks (29). Some studies state that older women who are obese have less postural stability than those with normal body weight, which increases the fear of falling (30). The results of our study showed that subjects who reported experiencing a fall in the past 12 months had, on average, a higher BMI than those who did not report experiencing a fall. Furthermore, FoF increased with increasing BMI, meaning that subjects who reported experiencing a fall had a higher BMI on average. Research suggests that obese persons are at increased risk of falling, but that such falling does not necessarily lead to serious injury. In such cases, obesity becomes a protective factor against musculoskeletal injuries (6, 25, 27, 30). A disadvantage of this study is the small number of subjects and the disproportionate number of male and female subjects, as well as the fact that the severity of falls, the number of falls and the severity of FoF were not considered.

FoF is higher in subjects reporting problems in everyday life related to walking difficulty, balance problems, vision problems, BMI and moderate to severe pain, which is consistent with the published literature. The falls themselves are more likely to occur in persons who report moderate to severe pain. Physical activity can improve balance and thus prevent the possibility of falls and increase the self-confidence of the elderly and reduce the incidence of fear. The findings and data from other studies suggest that a large percentage of older persons struggle with the effects of aging, which includes various comorbidities and health problems that can increase the risk of falling and FoF. The results of the research showed that both previous falls and fear of falling are important predictors of functional ability. They equally impact the development of everyday activity limitations. Physical activity can help the elderly stay physically independent.

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Original article

Trends in Transfusion-Transmissible Infections Among Blood Donors at the National Blood Transfusion Service, Guyana

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Abstract

Aim: The most adverse effect of blood transfusion is the acquisition of transfusion-transmissible infections (TTIs), which poses a serious threat in developing countries. This study aims to identify the trends of transfusion-transmissible infections among blood donors.

Materials and Methods: This study was a laboratory-based retrospective study conducted using blood donors' records from January 2015 to December 2018, collected at the National Blood Transfusion Service, Guyana (NBTS). Analysis of data was performed using the Statistical Package for the Social Sciences (SPSS) version 22.0 software and the results were presented in tables and graphs. Chi-square and logistic regression were used to identify trends and influencing factors.

Results: A total of 39,308 blood donors were included in this study, of whom 2,418 (6.2%) donors tested positive to at least one pathogen. Among those donors, 4.4% were coinfected with at least one of the sixteen dual infection combinations. The overall seroprevalence of HIV, HTLV, syphilis, HBV, HCV, Chagas, microfilaria, and malaria was 0.8%, 0.8%, 0.6%, 1.5%, 1.3%, 1.2%, 0.0%, and 0.0%, respectively. Trends of transfusion-transmissible infections showed an overall increase from the lowest prevalence, 5.1%, in 2015 to 7% in 2016, followed by decreases in 2017 (6.8%) and 2018 (5.8%).

Conclusions: Even though 98.6% of the donor population are volunteers, this study has shown that a significant percentage of blood donors harbour transfusion-transmissible infections. Stringent screening and preventive measures are very important to ensure the safety of the transfusion recipient.

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Introduction

Globally, transfusion of human blood is an essential medical procedure. Although it can save lives, blood and/or its products are not 100 percent safe for transfusion. In some cases, there is about one percent (1%) chance of adverse immunological, physiological, and infectious complications in the recipients. Of all adverse effects of transfusion, transfusion-transmissible infections (TTI) are the most significant and represent a risk for blood safety in developing countries like Guyana. TTIs include human immunodeficiency virus (HIV), human T-cell lymphotropic virus (HTLV), hepatitis B virus (HBV), hepatitis C virus (HCV), malaria parasite (MP), microfilaria parasite (MFP), Trypanosoma cruzi (Chagas), and syphilis. As a result, the World Health Organization (WHO) recommends that all donated blood be tested for TTIs caused by these pathogens (1). In addition to serological screening, all campaigners for blood donation should be submitted to clinical screening, which consists of an interview with a trained professional, in order to establish the clinical history and life of the donor concerning exposure to risk factors for TTIs.

In Guyana and other countries around the world, voluntary donors and replacement donors represent the main source of blood for transfusion. For better patient safety, the World Health Organization (WHO) recommends that before blood is released for clinical use, it should be screened for evidence of any possible infection. Countries with stringent routine serological screening have achieved a very impressive reduction of TTI cases. However, the risks persist due to the limited virus detection techniques (2–4). The magnitude of the TTI problem varies from country to country; even within a country, the magnitude could vary between different regions depending on the load of transfusion-transmissible infections in that particular population. Blood transfusion, in general, is a very expensive process, both as the entire procedure and in case any infection. Any infected blood that might reach a patient could lead to morbidity and mortality risks. This could pose an economic burden on the recipients themselves, as well as their families (5). TTIs have a higher chance of reaching a wider population since some infections have a long asymptomatic period or some persons could act as the carrier. The costs that would be added to such transmission include an aggressive treatment plan and short or long-term dependency, further burdening the country's economy (6).

Viruses like HIV, HBV, and HCV can cause long-term carrier states, prolong viremia and infectivity, lead to chronic disorders with high rates of morbidity and mortality because of chronicity, liver cirrhosis, hepatoma, and other opportunistic infections (7–9). These viruses have a direct transmission route with blood products during transplantation, hemodialysis, intravenous drug use, tattooing as well as sexual intercourse (10). The chance of transmission of those viruses through transfusion of infected blood is higher than with other routes of transmission, mainly due to transmission of a high viral load per transfusion. Regardless of whether the viral load is low within the blood, the possibility of infection remains high (9). However, currently, transfusion has a relatively low contribution to the transmission of virus infections since the obligation of screening blood donations for viral infections before transfusion is of the highest priority (2).

Similarly, parasites are rare, but commonly recognized infectious microbes worldwide, and several protozoans are known to be transmitted through blood transfusion. Parasites such as Plasmodium spp. (P. falciparum, P. vivax, P. malariae, and P. ovale) and Trypanosoma cruzi are causative agents of malaria and Chagas disease, respectively (11). The prevalence of these infectious agents among blood donors varies from nation to nation, depending on the specific population from which the blood units are collected. In sub-Saharan Africa, 5–10% of HIV infections are caused by transfusion of infected blood and 12.5% of patients who receive such blood transfusions are at risk of developing post-transfusion hepatitis (12). The choice of blood donors with low TTI risk was followed by

effective laboratory screening of blood transfusion units (8, 13). Those activities were extremely effective; however, the transmission of diseases nevertheless rose due to the incapacity of laboratory testing to detect the donors with an infection during the window period, a lack of budget for all general laboratories for TTI testing and trained manpower, the presence of immunologically variant viruses, the presence of non-seroconverting silent carriers, laboratory testing errors and poor quality control of laboratory tests (14, 15).

Variations in donor screening strategies and the predominance of risk factors in society might explain the changes in prevalence rates of TTIs over time. It is, therefore, necessary to assess the prevalence of these TTIs among blood donors at regular intervals to estimate the current most prevalent risk factors and to evaluate the effectiveness of blood safety strategies employed in the blood banks (16). Evaluation of trends in the prevalence of TTIs among blood donors is not only essential for estimating the effectiveness of blood safety strategies (10, 13); it also provides information to policymakers for the purpose of improving strategies for minimizing the potential risk of acquiring such infections through blood transfusion (17).

As a result, to our knowledge, there are no comprehensive data to determine the trends of transfusion-transmissible infections among blood donors at the NBTS, Guyana. Therefore, the primary objective of this study was to determine the prevalence and trends of major TTIs among blood donors at the NBTS, Guyana.

Subjects and Methods

The study was carried out at Guyana's main blood bank, the National Blood Transfusion Service (NBTS) in Georgetown, Guyana, by extracting data from the NBTS database. NBTS is the only blood bank in Georgetown, the capital city of Guyana. All blood donors are screened for infectious diseases at the NBTS. The facility provides TTI-tested blood and blood products for many referral hospitals in the region. The center has several departments, sections, and subsections. It comprises the Donor Clinic, Laboratory (sub-sections: TTI, Immunohematology and Component Preparation), Quality Management and Data Management sections. This research used the retrospective descriptive study method, in which the donor data were collected from the period between January 2015 and December 2018.

Screening Methods

The donors' blood was screened for TTIs after donation. Blood samples were tested using two kits, based on the recommendation by WHO, using two different testing strategies involving enzyme-linked immunosorbent assay (ELISA) and/or simple or rapid assays for surveillance. Cortez Diagnostic Inc. RPR test was used for screening for syphilis. The positive RPR test was confirmed by a quantitative RPR test. The presence of malaria and microfilaria parasites was established using the peripheral blood smear test. The hepatitis B surface antigen (HBsAg) was detected using the Murex HBsAg Confirmatory Version 3.0 ELISA infectious disease; the kit had a sensitivity of 100% and specificity of 99.97%. Antibodies to HCV were detected using the Murex anti-HCV version 4.0 ELISA infectious disease, which had a sensitivity of 100% and specificity of 99.88%. Antibodies to HIV types 1 and 2 were screened using the Murex HIV Ag/Ab combination ELISA infectious disease. The kit had a sensitivity of 100% and a specificity of 99.78%. Antibodies to HTLV types 1 and 2 were screened using the Murex HTLV I/II ELISA infectious disease. The kit had a sensitivity of 100% and a specificity of 99.88%. Antibodies for Chagas were screened using the GrupoBios S.A. test ELISA Chagas III. The kit had a sensitivity of 100% and a specificity of 100%.

Ethical approval

The study was approved by the Institutional Review Board (IRB) of the Ministry of Public Health, Guyana. Permission was also obtained from the Director of the National Blood Transfusion Service.

Statistical analysis

All data were retrieved from the anonymized blood donor register, donor cards, and logbooks. Delphyn blood bank software was used by the principal researchers with the help of the blood bank staff. Donors' records containing specific sociodemographic information (age, sex, ethnicity, donor type, seropositivity for TTIs, blood type, and blood donor code), the number of donations and the serostatus of TTIs were transferred to an Excel Spreadsheet (Microsoft Inc.) and analyzed using SPSS (Statistical Package for the Social Sciences) software version 22.0.

Table 1: Demographic characteristics of blood donors (2015–2018), NBTS Guyana

CHARACTERISTIC		n (%)
SEX	Male	23766 (60.6)
	Female	15458 (39.4)
AGE (YEARS)	15–20	3652 (9.3)
	21–30	12776 (32.5)
	31–40	10191 (25.9)
	41–50	7728 (19.7)
	> 51	4958 (12.6)
DONOR TYPE	Voluntary – 1st time	12211 (31.1)
	Voluntary – Regular	26537 (67.5)
	Family Replacement –1st time	363 (0.9)
	Family Replacement – Regular	163 (0.4)
ETHNICITY	Afro-Guyanese	11346 (28.9)
	Indo-Guyanese	18407 (46.8)
	Mixed	8482 (21.6)
	Amerindian	615 (1.6)
	Caucasian	187 (0.5)
	Chinese	28 (0.1)
	Others	232 (0.6)
BLOOD GROUP (ABO + RHESUS)	A+	7943 (20.2)
	B+	9041 (23.0)
	O+	18133 (46.2)
	AB+	2106 (5.4)
	A-	387 (1.0)
	B-	424 (1.1)
	O-	1086 (2.8)
	AB-	119 (0.3)
LOCATION	Inhouse	16909 (43.0)
	G/Town Mobile Drive	14739 (37.5)
	Region 2	640 (1.6)
	Region 3	948 (2.4)
	Region 6	5047 (12.8)
	Region 9	16 (0.0)
	Region 10	1005 (2.6)

The seroprevalence of HIV, HTLV, HCV, HBsAg, Chagas, syphilis, malaria, and microfilaria was expressed in percentages for the entire study group and based on different sociodemographic characteristics (age, sex, ethnicity, region, donor blood type, and donor category) and frequency of donation. Descriptive statistics were performed, and the results were presented as percentages in tables and graphs. The chi-squared test for trend was applied to examine the variation in trends. Logistic regression was used to explore the association between dependent and independent variables. The associations are presented as odds ratios (OR), together with 95% confidence intervals (CI). P-values of less than 0.05 were considered statistically significant.

Results

Sociodemographic characteristics of blood donors

A total of 39,308 blood donors were screened at the National Blood Transfusion Service (NBTS) from January 2015 to December 2018, with 98.6% of those donors being volunteers (Table 1 and Table 2). Of the total, 43% of blood donors donated blood at the central blood bank location. Overall, a larger percentage (60.6%) of blood donors were male, while most (32.5%) of the study subjects were in the 21–30 age group. Of all donors, 46.2% of them had the O-positive blood type. Most of the donor population were Indo-Guyanese (46.8%) followed by Afro-Guyanese (28.9%) (Table 1).

Table 2. Total blood donors, volunteer vs replacement, in the years 2015–2018 at the National Blood Transfusion Service (NBTS), Guyana.

Type of donor	2015	2016	2017	2018	p-value
VF	3270 (31.6)	6401 (32.6)	2903 (29.7)	2839 (30.3)	0.00
VR	6910 (66.8)	12934 (65.9)	6751 (69.2)	6407 (68.3)	0.00
RF	101 (1.0)	224 (1.1)	69 (0.7)	81 (0.9)	0.003
RR	56 (0.5)	58 (0.3)	29 (0.3)	49 (0.5)	0.001
Total	10337	19617	9752	9376	

Voluntary first time – VF; Voluntary repeat – VR; Replacement first time – RF; Replacement repeat – RR

Seroprevalence of transfusion-transmissible infections (TTIs)

A total of 2,418 (6.2%) blood donors tested positive for at least one TTI agent. The positivity rates of HIV, HTLV, syphilis, HBV, HCV, Chagas, microfilaria, and malaria were 0.8%, 0.8%, 0.6%, 1.5%, 1.3%, 1.2%, 0.0%, and 0.0%, respectively. Overall, HBV was the most prevalent TTI (Figure 1). Among the 2,418 blood donors, 107 (4.4%) donors were coinfected. Sixteen dual infection combinations were observed, with Chagas and HCV being the most common (0.7%), followed by HBV–Chagas (0.5%), HTLV–HBV (0.5%), and HIV–HTLV (0.4%) (Figure 2).

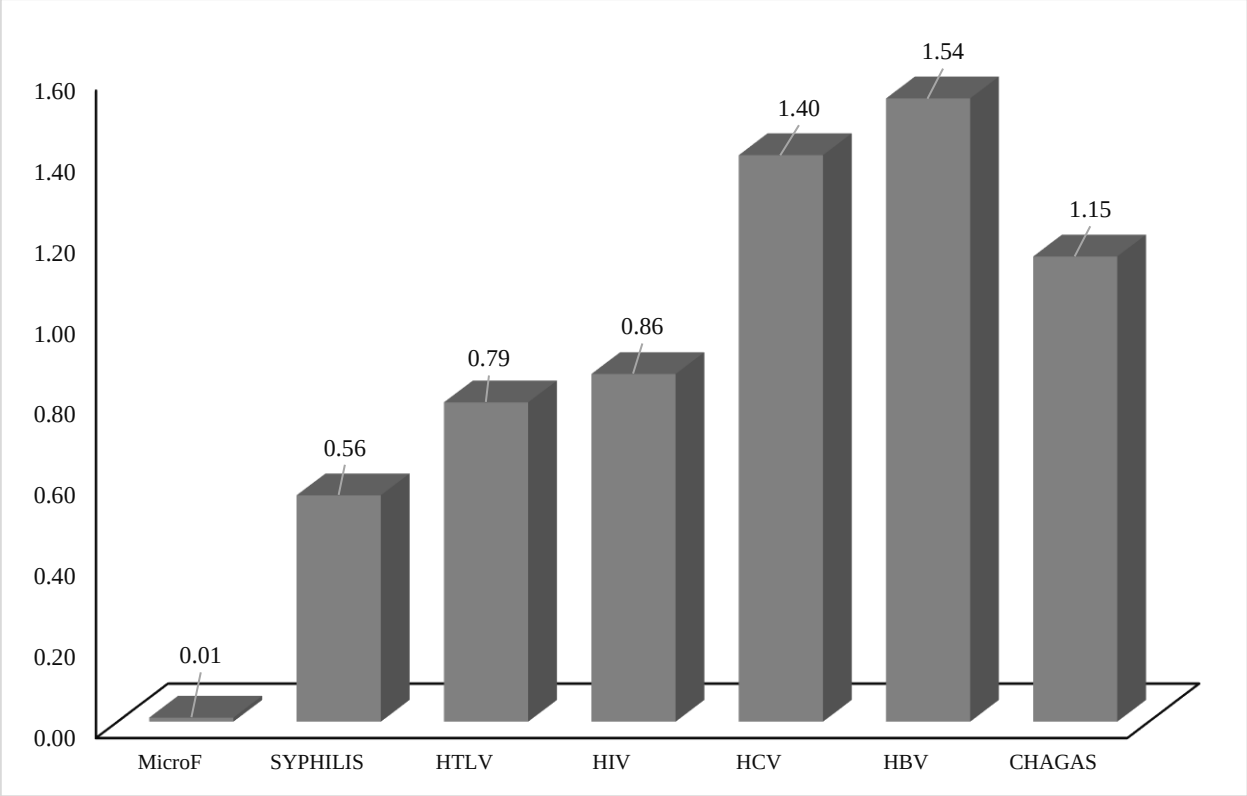

Figure 1. Prevalence of transfusion-transmissible infections in 2015–2018 at the National Blood Transfusion Service (NBTS), Guyana.
(MicroF: microfilaria; HTLV: human T-lymphotropic virus type 1; HIV: human immunodeficiency virus; HCV: hepatitis C virus; HBV: hepatitis B virus)

Trends and associated sociodemographic factors of blood donors regarding TTIs (2015–2018)

The overall trend of TTIs showed an increase from the lowest prevalence, 5.1%, in 2015 to 7% in 2016, followed by decreases in 2017 (6.8%) and 2018 (5.8%). HBV was the most prevalent TTI (1.5%), with a significant ($p \leq 0.05$) progressive increase from 2015 (1.3%) to 2017 (1.8%), followed by a subsequent decrease in 2018 (1.3%) (Table 3).

Overall, in regard to donor types, no TTI was significantly prevalent in both first-time and regular family replacement blood donors ($p \geq 0.05$). HTLV, syphilis, HBV, and microfilaria were significantly ($p \leq 0.05$) prevalent in both first-time and regular voluntary donors, while HIV was significantly ($p \leq 0.05$) prevalent in just first-time voluntary donors. Chagas was not significantly present in any of the blood donor types. HTLV (43.9%) and HBV (45.8%) were the significantly prevalent TTIs in Afro-Guyanese donors ($p \leq 0.05$), while Chagas (37.6%, $p \geq 0.05$) was statistically prevalent in Indo-Guyanese donors. TTI positive blood donors with O positive (O+) blood type had a statistically significant prevalence of syphilis (40.9%, $p \leq 0.05$). All TTIs except HIV, syphilis, and microfilaria showed significant prevalence in blood donors who donated mostly at the blood bank or via mobile blood drives (Table 3).

Table 3. Reactive Serological Marker, Type of Donor, Sex, Age Group at NBTS (2015–2018)

Characteristic n (%) Reactive Serological Marker	Total	2015	2016	2017	2018	*p* Value
HIV	307 (0.8)	75 (0.7)	116 (1.2)	66 (0.7)	50 (0.5)	0.00
HTLV	312 (0.8)	68 (0.7)	76 (0.8)	86 (0.9)	82 (0.9)	0.2
Syphilis	227 (0.6)	30 (0.3)	51 (0.5)	66 (0.7)	80 (0.9)	0.00
HBV	600 (1.5)	139 (1.3)	158 (1.6)	179 (1.8)	124 (1.3)	0.01
HCV	510 (1.3)	122 (1.2)	180 (1.8)	106 (1.1)	102 (1.1)	0.00
Chagas	458 (1.2)	89 (0.9)	108 (1.1)	159 (1.6)	102 (1.1)	0.00
Microfilaria	4 (0.0)	1 (0.0)	3 (0.0)	0	0	0.1
Malaria	0	0	0	0	0	
Sex						
Male	23766 (60.6)	6213 (60.1)	5749 (58.8)	5901 (60.6)	5903 (63.0)	
Female	15458 (39.4)	4121 (39.9)	4027 (41.2)	3842 (39.4)	3468 (37.0)	0.00
Age group						
15–20	3652 (9.3)	1098 (10.6)	982 (10.0)	839 (8.6)	733 (7.8)	
21–30	12776 (32.5)	3263 (31.5)	3407 (34.7)	3080 (31.6)	3026 (32.3)	
31–40	10191 (25.9)	2632 (25.4)	2384 (24.3)	2614 (26.8)	2561 (27.3)	
41–50	7728 (19.7)	2075 (20.0)	1879 (19.1)	1936 (19.8)	1838 (19.6)	
> 51	4958 (12.6)	1283 (12.4)	1164 (11.9)	1292 (13.2)	1219 (13.0)	0.00
Type of donor						
Voluntary – 1st time	12211 (31.1)	3270 (31.6)	3199 (32.6)	2903 (29.7)	2839 (30.3)	0.00
Voluntary – Regular	26537 (67.5)	6910 (66.8)	6468 (65.9)	6752 (69.2)	6407 (68.3)	0.00
Family Replacement – 1st time	363 (0.9)	101 (1.0)	112 (1.1)	69 (0.7)	81 (0.9)	0.01
Family Replacement – Regular	163 (0.4)	56 (0.5)	29 (0.3)	29 (0.3)	49 (0.5)	0.004

HTLV was significantly ($p \leq 0.05$) more prevalent among female donors (58%) compared to HBV, which was significantly ($p \leq 0.05$) more prevalent in males (65.7%). A significant prevalence of TTIs – HBV (35.7%), HCV (31.2%), and Chagas (37.8%) – was seen especially in the 21–30 age group, while HTLV (26.9%) was significantly observed in the > 51 age group ($p \leq 0.05$) compared to other age groups (Table 4). Overall, in regard to donor types, no TTI was significantly prevalent in both first-time and regular family replacement blood donors ($p \geq 0.05$). HTLV, syphilis, HBV, and microfilaria were significantly ($p \leq 0.05$) prevalent in both first-time and regular voluntary donors, while HIV was significantly ($p \leq 0.05$) prevalent in just first-time voluntary donors.

Chagas was not significantly present in any of the blood donor types. HTLV (43.9%) and HBV (45.8%) were the significantly prevalent TTIs in Afro-Guyanese donors ($p \leq 0.05$), while Chagas (37.6%, $p \geq 0.05$) was statistically prevalent in Indo-Guyanese donors. TTI positive blood donors with O positive (O+) blood type had a statistically significant prevalence of syphilis

(40.9%, p ≤ 0.05). All TTIs except HIV, syphilis, and microfilaria showed significant prevalence in blood donors who donated mostly at the blood bank or via mobile blood drives (Table 4).

Table 4. Blood donor characteristics and chi-square for transfusion-transmissible infections (TTIs), 2015–2018, NBTS, Guyana.

Characteristic	HIV (307)	HTLV (312)	Syphilis (227)	HBV (600)	HCV (510)	Chagas (458)	Microfilaria (4)
Sex							
Male	185 (60.3)	131 (42.0)	145 (64.2)	394 (65.7)	317 (62.2)	274 (60.0)	1 (25.0)
Female	122 (39.7)	181 (58.0)	81 (35.8)	206 (34.3)	193 (37.8)	183 (40.0)	3 (75.0)
χ^2	0.1	45.6	1.2	6.6	0.5	0.08	2.1
***p* Value**	0.9	0.00	0.3	0.01	0.5	0.80	0.1
Age (years)							
15–20	28 (9.1)	17 (5.4)	17 (7.5)	58 (9.7)	36 (7.1)	66 (14.4)	0
21–30	104 (33.9)	78 (25.0)	80 (35.4)	214 (35.7)	159 (31.2)	173 (37.8)	3 (75.0)
31–40	63 (20.5)	73 (23.4)	54 (23.9)	185 (30.8)	103 (20.2)	96 (21.0)	0
41–50	76 (24.8)	60 (19.2)	41 (18.1)	95 (15.8)	122 (23.9)	87 (19.0)	1 (25.0)
> 51	36 (11.7)	84 (26.9)	34 (15.0)	48 (8.0)	90 (17.6)	36 (7.9)	0
χ^2	7.9	62.3	3	22.4	24.7	29.8	4.2
***p* Value**	0.1	0.00	0.6	0.00	0.00	0.00	0.4
Donor Type							
Voluntary – 1st time	113 (36.8)	121 (38.8)	92 (40.5)	244 (40.7)	172 (33.7)	159 (34.7)	4 (100.0)
χ^2	4.6	8.7	9.5	26.2	1.7	2.9	8.9
***p* Value**	0.03	0.003	0.002	0.00	0.2	0.09	0.00
Voluntary – Regular	192 (62.5)	185 (59.3)	132 (58.1)	346 (57.7)	335 (65.7)	294 (64.2)	0
χ^2	3.5	9.7	9.1	26.9	0.8	2.3	8
***p* Value**	0.06	0.002	0.003	0.00	0.4	0.1	0.00
Family Replacement – 1st time	0	4 (1.3)	3 (1.3)	8 (1.3)	2 (0.4)	3 (0.7)	0
χ^2	4.1	0.4	0.4	1.1	1.6	0.4	0.04
***p* Value**	0.04	0.5	0.5	0.3	0.2	0.5	0.8
Family Replacement – Regular	0	2 (0.6)	0	1 (0.2)	2 (0.4)	1 (0.2)	0
χ^2	1.2	0.4	0.9	0.9	0.006	0.4	0.03
***p* Value**	0.3	0.5	0.3	0.3	0.9	0.5	0.8
Ethnicity							
Afro-Guyanese	89 (29.0)	137 (43.9)	81 (35.7)	275 (45.8)	142 (27.8)	153 (33.4)	2 (50.0)
Indo-Guyanese	142 (46.3)	77 (24.7)	105 (46.3)	196 (32.7)	253 (49.6)	172 (37.6)	1 (25.0)
Mixed	74 (24.1)	84 (26.9)	36 (15.9)	122 (20.3)	102 (20.0)	118 (25.8)	1 (25.0)
Amerindians	1 (0.3)	12 (3.8)	3 (1.3)	6 (1.0)	10 (2.0)	10 (2.2)	0
Caucasians	0	1 (0.3)	0	0	2 (0.4)	3 (0.7)	0

Others	1 (0.3)	1 (0.3)	2 (0.9)	0	1 (0.2)	2 (0.4)	0
χ^2	6.0	72.9	8.8	93.7	3.9	17.5	1.2
***p* Value**	0.40	0.00	0.2	0.00	0.7	0.01	0.9
Blood group							
A+	59 (19.2)	79 (25.4)	55 (24.4)	117 (19.6)	114 (22.4)	88 (19.2)	1 (25.0)
A-	2 (0.7)	1 (0.3)	3 (1.3)	3 (0.5)	5 (1.0)	4 (0.9)	0
B+	72 (23.5)	72 (23.2)	51 (22.7)	135 (22.6)	100 (19.6)	112 (24.5)	1 (25.0)
B-	7 (2.3)	1 (0.3)	0	4 (0.7)	6 (1.2)	3 (0.7)	0
O+	136 (44.3)	131 (42.1)	92 (40.9)	297 (49.7)	243 (47.6)	218 (47.6)	2 (50.0)
O-	15 (4.9)	9 (2.9)	7 (3.1)	15 (2.5)	8 (1.6)	12 (2.6)	0
AB+	15 (4.9)	15 (4.8)	12 (5.3)	26 (4.4)	32 (6.3)	19 (4.1)	0
AB-	1 (0.3)	3 (1.0)	5 (2.2)	0	2 (0.4)	2 (0.4)	0
χ^2	10.1	13.0	33.7	7.3	7.6	3.3	0.5
***p* Value**	0.2	0.07	0.00	0.40	0.40	0.90	1
Location							
Inhouse	118 (38.4)	96 (30.8)	100 (44.1)	198 (33.0)	207 (40.6)	142 (31.0)	1 (25.0)
G/Town	136 (44.3)	145 (46.5)	82 (36.1)	282 (47.0)	185 (36.3)	241 (52.6)	3 (75.0)
Mobile Drive							
Region 2	4 (1.3)	11 (3.5)	4 (1.8)	13 (2.2)	18 (3.5)	7 (1.5)	
Region 3	10 (3.3)	13 (4.2)	13 (5.7)	20 (3.3)	8 (1.6)	9 (2.0)	
Region 6	36 (11.7)	35 (11.2)	26 (11.5)	73 (12.2)	83 (16.3)	48 (10.5)	
Region 9	0	0	0	0	0	0	
Region 10	3 (1.0)	12 (3.8)	2 (0.9)	14 (2.3)	9 (1.8)	11 (2.4)	
χ^2	9.9	31.5	13.6	32.7	20.2	46.5	2.6
***p* Value**	0.2	0.00	0.06	0.00	0.005	0.00	0.9

Logistic regression analysis shows that male donors have a significant (OR = 0.57, CI: 0.45–0.71) chance of presenting with HTLV and HBV, with odds of 0.51 and 1.51, respectively, when compared to female donors. Additionally, blood donors in the 41–50 age group compared to those in the > 51 age group, with blood types O+, A-, and B-, compared to AB-, had significant ($p \leq 0.05$) risk of presenting with HTLV. Blood donors who donated in Region 2 were at a significantly higher risk of HTLV and HCV infection compared to those in Region 10. Furthermore, logistic regression analysis showed that the odds of blood donors presenting with syphilis significantly ($p \leq 0.05$) increased with age, while all age groups except 31–40 ($p \geq 0.05$) had a significant chance of presenting with Chagas.

Discussion

Over the observed four years, this study showed that 2,418 donors tested positive for at least one of the eight TTI markers that were screened. The study had similar findings as observed in the studies conducted in eastern Ethiopia by Teklemeriam et al (18) and by Birhaneselassi et al (19). Our findings included a higher percentage compared to similar studies conducted in the neighboring Brazil (20), in South India (21), in Andaman and Nicobar Islands (22), in South Gujarat, India (23), in Kano, Nigeria (8) and in South-South Nigeria (24).

The differences in prevalence among these studies may be due to the existence of different magnitudes of risk factors for contracting transfusion-transmissible infections,

discrepancies in the overall sample size, donor enrolment, the observed period, as well as factors associated with testing procedures used for screening, storage, and validation of test kits.

Of the eight TTIs that were studied, the most common was HBV, followed by HCV, Chagas, HIV, HTLV, syphilis, and malaria. The rate of TTI variation depends on improvement in analytical technology, which could improve the current screening reagents, not only to make them more specific, but also more reliable (25). Likewise, our study revealed that HBV was significantly more prevalent among males when compared to female donors; this might be due to the fact that sex is a genetic factor of disease consequence. Concerning HBV infection, when this virus infects an adult, most subjects will produce protective antibodies and fully fight off the infection. But in a few subjects (5%–10%), the virus will establish a chronic infection. Moreover, because of the slow plasma disappearance rate for HBsAg in males compared to females, males are more likely to develop chronic HBV infection. In addition to the above-mentioned reasons, behavioral risk factors such as having multiple sex partners could be the cause of the increase in prevalence of HBV among male donors (26).

HIV's seroprevalence in this study is lower compared to a previous study performed at the same blood bank during 2010–2011 (1.42%) (27) and the 2016 national estimated HIV prevalence (1.6%) (28). Many factors could be the cause of the differences in seroprevalence recorded in some developing nations, such as cultural and religious differences, differences in education level, and different socioeconomic structure (8, 24). Having multiple or concurrent sex partners, dependence on unskilled persons for childbirth, instruments used for female genital mutilation, cultural tattooing or markings on the body are some of the sociocultural and religious practices involved in the risk of transmission (29). A lot of effort needs to be put in to reduce such practices and to prevent the spread of infections. Introducing aggressive campaigns and counseling before and after blood transfusions might in some way help reduce transmission (2).

This study shows an increasing trend in blood donations testing positive for TTIs in the last four years. One of the reasons associated with the increasing trend of TTIs in donated blood during 2016 could be the increased number of first-time blood donors in that particular year, since studies have identified that first-time blood donors always tend to pose a greater risk of infectious donation than repeat donors. One assumption is that repeat donors are well-informed of the risky behaviors that might cause blood infections and as such reduce the probability of blood infections in the window period. Furthermore, if a donor tests negative prior to a donation, it is likely that most repeat donors will not suddenly engage in high-risk behaviors. Therefore, it is very important to ensure the safety of blood supply through careful recruitment of new donors and maintaining the donation pool (2).

This study had a few limitations. Many epidemiological data were not collected since this was a retrospective study. There could be much more information if it is collected with the aim of better establishing the risks of TTI infection. This study did not do any confirmatory tests nor were any molecular data evaluations done. Repeated positive tests were considered positive for the purposes of this study. As such, false positive cases cannot be completely excluded. Despite the limitations, this study highlighted the trends and associated sociodemographic factors of major TTIs.

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Review article

The Correlation between Iron Deficiency and Recurrent Aphthous Stomatitis: A Literature Review

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Abstract

Aphthous lesions of the oral mucosa are a very common symptom and can be seen in both family medicine practice, dental medicine practice, and dermatology or otorhinolaryngology clinics. Some patients develop a chronic recurrent condition, which is clinically known as recurrent aphthous stomatitis (RAS). These ulcers are round, clearly defined, and can be visible on the movable part of the oral mucosa, with variations in size. A prodromal symptom like the burning or stinging sensation can precede the appearance of lesions. The main reason why patients seek medical help is oropharyngeal pain with lack of appetite.

The exact etiopathogenesis of RAS remains unknown. Immune disorders, nutritional deficiencies, allergies, mechanical injuries, and even psychological disorders are being studied as potential causes of this condition. Some authors claim that iron deficiency may be a possible causative factor of RAS due to its role in DNA synthesis, mitochondrial function, and enzymatic activity. In iron deficiency, epithelial cells turn over more rapidly and produce an immature or atrophic mucosa. Such mucosa is vulnerable and can be a fertile soil for chronic inflammation and development of aphthae.

Finally, our goals were to describe the clinical aspects and etiology of RAS, as well as to determine whether RAS may be related to iron deficiency, in order to identify potential patients with iron deficiency in everyday work.

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Introduction

Recurrent aphthous stomatitis (RAS) is a frequent oral disorder characterized by multiple painful ulcers in the oral cavity and oropharynx. The prevalence of this condition in the general population is high and varies from 5% to 66%, with a mean of 20% (1). It can be seen in both family medicine practice, dental medicine practice, and dermatology or otorhinolaryngology clinics. We therefore wanted to describe the clinical aspects and etiology of RAS, as well as determine whether RAS may be related to iron deficiency, in order to identify potential patients with iron deficiency in everyday work.

Methods of literature search

We performed a literature search using PubMed and Google Scholar and we used the search filter with the following specific terms: recurrent aphthous stomatitis, aphthae, iron deficiency, and anemia, to find works published in the last twenty years, from 2000 until now. In searching these databases, we focused on meta-analyses, systematic reviews, randomized controlled trials, and landmark studies that have previously focused on similar topics (Table 1).

Table 1. Results of international research on hematinic deficiency in RAS

Author and year of study	Country	No. of subjects			Serum iron level (µg/dl)		Ferritin (ng/ml)		Hemoglobin (g/dl)		Anemia (N)
				ALL	M	W	M	W	M	W	
Slebioda et al. 2018	Poland	RAS	71	141	104.96	78.56	x		x		7
		C	70		115.85	102.43	x		x		1
Sun et al. 2014	Taiwan	RAS	273	546	96.8	83.9	x		14.1	12.8	57
		C	273		104.1	97.9	x		15.1	13.6	0
Piskin et al. 2001	Turkey	RAS	35	61	91.39	71.06	63.78	53.47	x		x
		C	26		84.23	75.31	59.74	67.73	x		x
Al-Amad et al. 2019	United Arab Emirates	RAS	52	104	81.0		x		14.0		13
		C	52		89.3		x		14.5		9
Koybasi et al. 2006	Turkey	RAS	34	64	67.82		x		13.53		x
		C	32		71.16		x		13.42		x
Babaee et al. 2015	Iran	RAS	28	56	x		115.64		12.87		x
		C	28		x		55.42		12.98		x
					LOW, number		LOW, number				
Lopez-Jornet et al. 2013	Spain	RAS	92	186	low (< 60 µg/dl) = 7		low (< 12 ng/ml) = 6		x		x
		C	94		low (< 60 µg/dl) = 2		low (< 12 ng/ml) = 5		x		x
Compilato et al. 2010	Italy	RAS	32	61	low (< 40 µg/dl) = 11		low (< 10 ng/ml) = 13		x		11
		C	29		low (< 40 µg/dl) = 2		low (< 10 ng/ml) = 0		x		2

C = control group, RAS = recurrent aphthous stomatitis, M = men, W = women, x = no data.

Classification of recurrent aphthous stomatitis

The onset of RAS is usually during childhood, with symptoms decreasing with age. The ulcers are round or oval and very painful. When looking closely at each aphtha, an erythematous lesion with a raised edge and inner necrosis covered with a yellow pseudomembrane can be seen. A

burning or stinging sensation can precede the appearance of erythematous macules and consequently very painful ulcers are formed (2).

Clinically, RAS can be classified into three types: minor, major, and herpetiform. Small aphthous ulcers (minor RAS), which are smaller than 10 mm in diameter, appear in more than 80% of patients and heal without a scar in 7 to 14 days. The most commonly affected sites are buccal and labial mucosa. Large aphthous ulcers (major RAS), which are bigger than 10 mm, heal in 2 to 6 weeks and often leave scars (3). The soft palate, tonsillar fossa, and labial mucosa are frequently affected. Herpetiform RAS is rare and represents a condition in which a great number of small oral lesions is seen. The typical onset of herpetiform RAS is in adulthood and it resembles herpes simplex virus type 1 infection, which is usually seen on the gingiva and hard palate (1).

Etiology of recurrent aphthous stomatitis

Recurrent aphthous stomatitis is one of the recurrent ulcerative disorders of the oral cavity. The exact causative mechanism remains unclear, but experts agree on its multifactorial origin (4). Possible etiologic factors of RAS include dysregulation of the immune system, hematologic diseases, hypovitaminosis and mineral deficiencies, allergies, mechanical injuries, mental disorders, etc (5) (Figure 1).

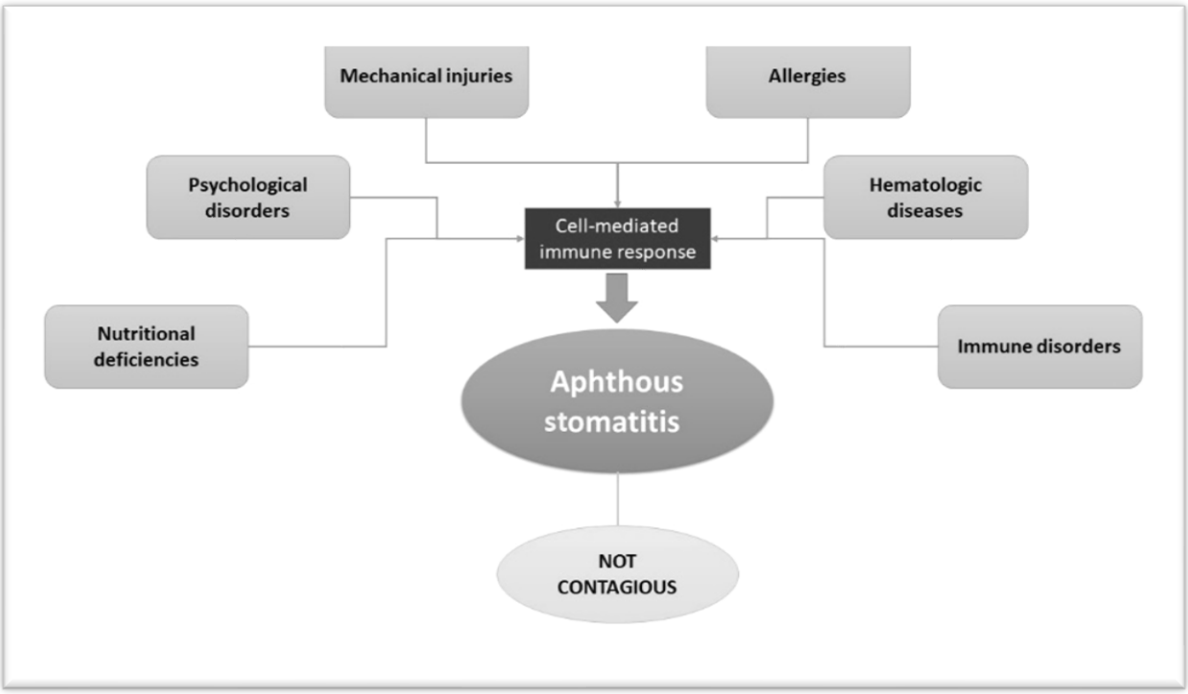

Figure 1. Etiology of recurrent aphthous stomatitis

Some studies suggest that local trauma may be the main cause of aphthae development, but only in susceptible individuals with a hereditary predisposition to the disease (6). Genetic predisposition plays a significant role in the development of RAS. The eruption of aphthae is more common in patients with positive family history. Likewise, it is followed by a severe clinical presentation (4). Guimaraes et al. examined whether DNA polymorphism related to the cytokine interleukin-1 beta (IL-1 β) may be associated with RAS. Their work showed a positive correlation and demonstrated how a genetic risk factor can determine susceptibility to the disease. Aberrant cytokine cascade induces an amplified cell-mediated immune response and activation of T-lymphocytes and phagocytes. These immune cells are directed

toward focal areas of the oral mucosa, resulting in ulceration (7).

Iron in recurrent aphthous stomatitis

In some patients, the possible causative factors are vitamin and mineral insufficiency, such as lack of serum iron, folate, zinc, or vitamin B12, and their frequency is estimated at 5%–15% (8). Iron is a very important element in the human body. Hemoglobin, myoglobin, and a variety of enzymes contain the majority of the iron. The main storage of iron within the human body is the liver, where it is stored in the form of ferritin and hemosiderin. Transport of iron through the plasma is performed by the transport protein transferrin, which binds to its receptor when it reaches the tissue (9). When looking intracellularly, iron is essential for DNA replication, enzyme and mitochondrial action, and neurotransmitter function (10). When iron intake is too low to fulfill normal bodily demands or to satisfy a pathological deficit, stored iron is consumed, leading to a lack of available iron for hematopoiesis and erythropoiesis. Production of hemoglobin without adequate concentrations of iron culminates in hypochromic microcytic anemia (9). Moreover, a lack of iron will disrupt lymphocyte development and impair cytokine production, leading to decreased cellular immunity (11).

Cytochrome oxidase, the iron-dependent enzyme, is required for the development of epithelial cells (12). In iron deficiency, epithelial cells turn over more rapidly and produce an immature or atrophic mucosa (13). Such impaired mucosa is more sensitive and susceptible to a pathological process like ulcer development. In addition, lower oxygen levels in anemic patients mean lower oxygen levels in different tissue, as well as the mucosal epithelium of the oropharynx. Inadequate oxygen saturation contributes to the disintegration of cells, which provokes atrophic mucosa. Atrophic oral epithelium may be the cause of increased susceptibility to RAS development in anemic patients (14).

The influence of age-related changes on the immune system and development of recurrent aphthous stomatitis

Aging is related to a higher level of systemic inflammation and persistent activation of non-specific immune cells due to lifelong exposure to external antigens and raised stimulation with own antigens (15). Disbalance in the inflammatory response leads to a variety of common chronic diseases due to dysregulation of immune cells and lack of their self-limiting feature, although in the elderly, the driving mechanism is more complicated, considering the vast number of comorbidities (16, 17). Unfortunately, current understanding of the relationship between the physiological processes in aging and the development of age-related illnesses remains inconclusive, with a lack of standardized methodology to clinically evaluate the inflammation. Assessment of iron status is challenging when concomitant inflammation is present. Identifying how to optimally adjust conditions linked with chronic illnesses and how to select realistic target outcomes at various stages of disease progression is becoming increasingly difficult (18). Furthermore, difficulties in stress-induced physiological mechanisms result in a detrimental allostatic load, a cumulative burden of chronic stress and life events (20), eventually precipitating a negative stress response along with disease progression, especially in chronic medical conditions (19). Due to its association with endothelial dysfunction and chronic inflammation, it can be assumed that the frequency of RAS would be higher in the elderly, but this would be incorrect. The occurrence of RAS decreases with age, while its severity increases (21). However, there is no clear understanding of the pathophysiological mechanism. The first clinical presentation of aphthous lesions in the elderly can easily be a local sign of systemic disease, which is why the term “aphthous-like ulcers” is used (22).

Discussion

The effect of iron deficiency in the biology of RAS has been studied by various authors with inconclusive results (Table 1).

Koybasi et al. (23) examined the possible etiologic factors of RAS in a controlled prospective study by analyzing 34 patients with recurrent aphthous stomatitis and 32 healthy subjects. They identified some important predisposing factors, such as positive family history and vitamin B12 deficiency. Interestingly, their study showed that nonsmoking status is connected with a higher chance of aphthae development, which means that smoking can have a protective effect. This can be explained by increased keratinization of the oral cavity due to cigarette use. Finally, their results did not show a statistically significant correlation between iron, ferritin, hemoglobin and RAS. Piskin et al. (24) studied 35 patients with RAS and 26 control subjects. They found that patients with oral ulcers have iron, ferritin, and folic acid insufficiency more frequently, but that this is statistically irrelevant ($p > 0.05$). Vitamin B12 level was the only measured variable that showed significance ($p = 0.005$).

Al-Amad and Hasan (25) conducted a case-control study on 52 patients with stomatitis and 52 age-matched healthy subjects. Their results showed a high percentage of deficiencies in the overall study population. Low hemoglobin was seen in 22%, vitamin B12 deficiency in 15%, iron deficiency in 11%, and vitamin D deficiency surprisingly in 53% of the subjects, with no difference between the diseased and healthy cases. They pointed out that vitamin D deficiency makes ulcers more severe. Lopez-Jornet et al. (26) evaluated laboratory tests of 92 patients with RAS and 94 healthy controls. They measured the concentration of iron, ferritin, folic acid, and vitamin B12. Their results showed that the patients with RAS have hematinic deficiencies more often compared with healthy controls. However, this result was also statistically insignificant, with overall frequency of hematinic deficiencies in the RAS group amounting to 14.14% and in the control group to 6.39% ($p = 0.086$).

Compilato et al. (27) studied 32 adults with RAS and 29 healthy controls, measuring full blood count, hemoglobin, serum folate, vitamin B12, iron, and ferritin levels. Their study showed statistical significance between the observed groups, with overall deficiencies amounting to 56.2% in patients with RAS versus 7% in healthy age-matched individuals ($p < 0.0001$). When looking at anemia alone, 34.4% of diseased subjects had anemia, compared with 6.9% of healthy individuals. They implied that ulceration could anticipate the onset of anemia, which is why routine laboratory blood tests should be performed in patients with RAS to promptly diagnose hematinic deficiency and prevent more severe clinical manifestations caused by iron, folic acid, or vitamin B12 deficiency. Their study established a firm connection between positive family history and the onset and severity of symptoms. No significant differences were found in the body mass index in all observed groups. The second part of their research involved administering adequate replacement therapy for a month to patients with ulcers who had some hematinic deficiencies, after which they were re-evaluated in a 3-month follow-up period. Their results strictly classified two groups of people, one with total remission of ulceration in the oral cavity and another without remission. The variable by which they were divided was family history of RAS. People with negative family history completely recovered after replacement therapy, but in patients with positive family history, ulceration was present despite therapy. This fact emphasizes the importance of detailed case history examination and leads us to adequate therapeutic options for different patients with the same disease.

Sun et al. (14) piloted a 6-year study with 273 patients with RAS and the same number of controls. This was the longest study with the highest number of participants regarding this issue. They showed statistically significant differences between the observed groups, with lower mean hemoglobin in patients with RAS ($p < 0.001$) and lower iron levels for women in the diseased group ($p < 0.001$). Male subjects also had lower iron levels, but this was not statistically significant. In addition, there was no

distinction in the levels of vitamin B12 and serum folate between groups. However, when adjusted in hemoglobin, iron, vitamin B12, or folic acid deficiency groups by the World Health Organization criteria, patients with recurrent stomatitis had a significantly higher frequency of observed hematinic deficiencies (20.9%, 20.1%, 4.8%, 2.6%, respectively) than healthy control subjects ($p < 0.05$). Nevertheless, approximately 60% of people who suffer from recurrent aphthous stomatitis had blood test results within normal limits, which can be explained by genetic susceptibility and other possible causes, such as immunological, hormonal, and emotional factors.

Slebioda et al. (11) analyzed blood samples of 71 subjects with RAS and 70 individuals without the disease. They measured hemoglobin, hematocrit, mean corpuscular volume, red blood cell count, iron, and vitamin B12 levels. When comparing those two groups, hematinic deficiencies were more common in patients with aphthae. The mean serum iron levels were lower in the RAS group than in the control group (88.6 lg/dL vs. 105.88 lg/dL). However, there was no observed correlation between hematinic deficiencies and clinical presentation and the severity of aphthae in the tested sample. Therefore, these results may easily describe a confounding factor, and not the actual cause of the disease.

Babaee et al. (28) conducted a research about oxidative stress in 28 patients with RAS and the same number of healthy individuals. They compared markers of oxidative stress in the saliva and routine hematological tests in both groups. Salivary malondialdehyde is the main product of lipid peroxidation and it indicates oxidative stress. The oral cavity is the first entry point for outside pathogens, which is why saliva contains variant antioxidants to help fight infection. Their overall activity coincides with the total antioxidant capacity (TAC). When comparing the group with RAS and the healthy group, salivary malondialdehyde level was considerably higher ($p < 0.001$) and TAC level was significantly lower ($p < 0.042$). The imbalance between oxidants and antioxidants may cause many diseases of the oral mucosa. Several studies also acknowledge that tissue damage in patients with recurrent stomatitis is caused by disruption of the oxidant/antioxidant balance (29). According to hematological parameters, only the ferritin levels were significantly higher in RAS patients ($p < 0.008$). Ferritin is an indicator of the body's iron reserves and can expedite oxidative stress. Production of ferritin can initiate lipid peroxidation and the formation of reactive oxygen species (30). Therefore, high serum ferritin in RAS can be used as an inflammatory measure, comparable to c-reactive protein or erythrocyte sedimentation rate. Another hypothesis is that ferritin may be a protective attribute by making chelation with free iron during prolonged oxidative stress (28).

Conclusion

Numerous controlled studies analyzing the hematological status and iron levels of patients with recurrent aphthous stomatitis have been conducted. However, the methods, number of participants, including criteria, referent numbers, gender distribution, and other factors are not standardized. Therefore, the results remain inconclusive, with some authors reporting a strong connection between iron levels and the development of RAS, while others disagree. Nevertheless, routine hematological screening in patients with RAS may help reveal hidden nutritional deficiencies and prevent related systemic manifestations. Additional studies are required to improve comprehensive understanding and clarify the impact of serum iron levels on recurrent aphthous stomatitis.

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Original article

Postoperative Corneal Edema After Phacoemulsification

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Abstract

Aim: To determine the effect of nuclear opalescence (NO) on intraoperative parameters during phacoemulsification using the WhiteStar Signature® PRO and to show the impact of preoperative and intraoperative parameters on postoperative corneal edema.

Methods: This prospective study included 267 patients selected to undergo phacoemulsification using the WhiteStar Signature® PRO system at the Department of Ophthalmology of the General Hospital "Dr Josip Benčević", Slavonski Brod, Croatia. NO was graded using the Lens Opacities Classification System III. Preoperative parameters were age, sex, NO and preoperative central corneal thickness. Intraoperative parameters of phacoemulsification included in the study were ultrasound time (UST), phaco time using Ellips FX technology (EFX) and average phaco power (AVG). Patients were followed up on postoperative days 1 and 7 and after two months. The state of the cornea was noted in each follow-up.

Results: There was a statistically significant increase of intraoperative parameters with NO. Postoperative corneal edema depended on all measured intraoperative parameters (UST, EFX and AVG, all $p < .001$), patient's age ($p < .05$) and NO ($p < .001$) on postoperative day 1, while on postoperative day 7, it depended on UST ($p = .011$) EFX ($p = .012$) and NO ($p < .05$).

Conclusion: Older patients, higher grade of NO and amount of energy consumed during phacoemulsification using the WhiteStar Signature® PRO are predictive factors for severity of transient corneal edema. We found this information important for better preoperative planning of phacoemulsification, as well as for better postoperative results.

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Introduction

Continuous advances in phacoemulsification have made safer and more efficient cataract surgery possible. There is a need for objective and transparent research in order to observe the impact of new technology on surgery outcomes, patient safety and satisfaction. The most frequent complication after phacoemulsification is corneal edema (1). The main factor that determines the function of the cornea as clear and transparent tissue is a metabolically active monolayer, corneal endothelium (2). Corneal endothelial cell loss after phacoemulsification leads to corneal edema (3), which may be due to perioperative and intraoperative risk factors. Intraoperative risk factors which can lead to acute corneal edema after phacoemulsification are endothelial injury by consumed ultrasound energy, Descemet's membrane detachment, toxic anterior segment syndrome (TASS) and IOL instability with endothelial touch (1).

Around 2010, Abbott Medical Optics (AMO) introduced the new Ellips FX system, which combined both longitudinal and elliptical motion into a single handpiece to improve phaco efficiency. The WhiteStar Signature® PRO was introduced by AMO in 2015 and has demonstrated improved nucleus followability, cutting efficiency and proactive intraocular pressure management with automatic occlusion sensing (4, 5).

In a previously published article by Sekelj et al., corneal edema recovery was compared between patients with type 2 diabetes mellitus and patients without type 2 diabetes (6). After that, we wanted to analyze the use of the new phaco technology in our hospital on postoperative corneal edema recovery. The aims of this study were to determine the effect of nuclear opalescence (NO) on intraoperative parameters during phacoemulsification using the WhiteStar Signature® PRO with the Ellips FX handpiece and to determine the impact of preoperative and intraoperative parameters on postoperative corneal edema recovery.

Patients and Methods

This prospective study included 267 patients selected to undergo phacoemulsification using the WhiteStar Signature® PRO system. The phacoemulsification surgery was performed on all patients by a single experienced surgeon during the study period of 7 months at the Department of Ophthalmology in our hospital. The study was performed in accordance with the principles of the Helsinki Declaration. Informed consent was obtained from all study participants. Out of 267 patients who were included in the study, 266 patients came to checkup on the first postoperative day, 265 patients came to checkup on the seventh postoperative day and 205 patients came to checkup after 2 months. Other study participants were under care in other hospitals.

We performed preoperative examination and used exclusion criteria (previous ocular infection or ocular surgery and Fuchs' endothelial corneal dystrophy) on all patients using the previously described methods.6 Three independent observers graded cataracts using a slit lamp and the findings were compared based on the Lens Opacities Classification System III (LOCS III).7 Eyes with NO grades between 1 and 6 were included in the study and were subdivided into mild (NO1, NO2), moderate (NO3, NO4) and hard (NO5, NO6) cataracts.

Phacoemulsification was performed with a 2.75 mm clear corneal incision. Sodium hyaluronate (Healon GV) was injected in the anterior chamber. One side port was created using a 30 degree knife, followed by continuous curvilinear capsulorhexis and hydrodissection. Phacoemulsification was performed using the WhiteStar Signature® PRO system, using the phaco chop technique with the Ellips FX handpiece (AMO, Inc.).

Coaxial irrigation/aspiration of the remaining cortical lens material was performed. Intraocular lens was implanted in the capsular bag after injecting hydroxypropyl methylcellulose 2.0% (Celoftal). The "rock 'n' roll" (RNR) technique was used for removing ophthalmic viscosurgical devices at the end of the surgery. The clear

corneal incision wound was hydrated and prophylactic intracameral injection of cefuroxime or vancomycin was given. Postoperative treatment included the use of dexamethasone/neomycin/polymyxin B (Maxitrol) drops and bromfenac drops in all type 2 diabetic patients based on our previously described protocol (6).

Ultrasound time (UST), phaco time using Ellips FX technology (EFX) and average phaco power (AVG) were intraoperative parameters for phacoemulsification. The EFX is a parameter of effective phaco time (EPT), with the specific coefficient for transversal movement expressed in seconds. Patients were followed up on postoperative days 1 and 7 and after 2 months. Two independent observers graded postoperative corneal edema recovery using three grading scales: clear cornea, focal corneal edema and diffusive corneal edema, as previously described (6).

Statistical analysis was performed using SPSS software (version 24, SPSS INC, IBM Corporation, Chicago, USA). The Shapiro–Wilk test was used to check if a continuous variable follows a normal distribution. Significance was tested using the Wilcoxon signed-rank test, the Kruskal–Wallis H test, the Friedman test and the chi-squared test. A P value of less than 0.05 was considered statistically significant.

Results

The study was conducted in order to determine the effect of NO on intraoperative parameters (UST, EFX, AVG) during phacoemulsification using the WhiteStar Signature® PRO with the Ellips FX handpiece. The other reason was to establish the impact of preoperative (age, sex, NO, preCCT) and intraoperative parameters on postoperative corneal edema recovery.

There were 267 patients included in the study; 164 were females (61%) and 103 were males (39%). The average age of the study population was 73.0 ± 8.76 years. Most patients had moderate cataract (67%). Mean preCCT was 556 (range 445–697). Intraoperative parameters are shown in Table 1. UST, EFX and AVG were statistically significantly different based on estimated nuclear hardness (Kruskal–Wallis H test, all $p < .001$) (Table 1); all intraoperative parameters increased significantly with NO. UST, EFX and AVG were statistically significantly different between mild and moderate cataract (Wilcoxon signed-rank test, all $p < .001$, post hoc test), moderate and hard cataract (Wilcoxon signed-rank test, all $p < .001$, post hoc test), as well as mild and hard cataract (Wilcoxon signed-rank test, all $p < .001$, post hoc test). Significantly higher mean UST, EFX and AVG were observed in hard cataracts, indicating a greater amount of energy during phacoemulsification.

Table 1. Ultrasound time (UST), elliptical motion energy (EFX) and average phaco power (AVG) values for various grades of cataract (n = 267)

	Mild cataract (average ± SD)	Moderate cataract (average ± SD)	Hard cataract (average ± SD)	Kruskal–Wallis H test
UST (seconds)	5.6 ± 8.25	37 ± 20.25	87.2 ± 33.3	$p < .001$
EFX	1.3 ± 2.25	12.7 ± 9.33	38.7 ± 17.45	$p < .001$
AVG (%)	1.8 ± 2.25	5.8 ± 2.32	8.2 ± 1.54	$p < .001$

Table 2. Intraoperative parameters (UST, EFX, AVG) in connection with postoperative corneal edema on postoperative day 1 (n = 266)

	+ (*n* = 173) average ± SD	++ (*n* = 68) average ± SD	+++ (*n* = 25) average ± SD	Kruskal–Wallis H test
UST (sec)	30.8 ± 28.22	49.2 ± 30.85	68.4 ± 32.88	$p < .001$
EFX	11.0 ± 12.59	19.7 ± 17.14	25.4 ± 15.71	$p < .001$
AVG (%)	4.8 ± 2.94 (5)	6.4 ± 2.74	7.0 ± 1.92	$p < .001$

+ Clear cornea, ++ Focal corneal edema, +++ Diffusive corneal edema

Another area of interest was postoperative corneal edema, as well as determining the impact of preoperative and intraoperative parameters on postoperative corneal edema recovery. In our study, postoperative corneal edema depended on all measured intraoperative parameters on postoperative day 1 (all Kruskal–Wallis H test, p < .001) (Table 2), while on postoperative day 7, it depended on UST and EFX (Wilcoxon signed-rank test, p = .011, p = .012), with no correlation with AVG (Wilcoxon signed-rank test, p = .179) (Table 3).

Table 3. Intraoperative parameters (UST, EFX, AVG) in connection with postoperative corneal edema on postoperative day 7 (n = 265)

	+ (*n* = 255) average ± SD	++ (*n* = 10) average ± SD	Wilcoxon signed-rank test
UST (seconds)	37.4 ± 30.42	72.2 ± 43.61	$p = .011$
EFX	13.9 ± 14.54	27.9 ± 19.72	$p = .012$
AVG (%)	5.4 ± 2.94	6.6 ± 2.41	$p = .179$

+ Clear cornea, ++ Focal corneal edema

In the study, we observed a statistically significant reduction in postoperative corneal edema (Friedman test = 126.4, p < .001). There were statistically significant differences between postoperative days 1 and 7 (Wilcoxon signed-rank test = -8.514, p < .001), as well as between postoperative day 7 and two months after the operation (Wilcoxon signed-rank test = -2.828, p < .01). In a previously published article, we compared corneal edema recovery and visual acuity between patients with type 2 diabetes mellitus and patients without type 2 diabetes (6).

Furthermore, in this study, we analyzed the impact of preoperative parameters on postoperative corneal edema. Corneal edema depended on the patient's age and NO on postoperative day 1 (Table 4), while on postoperative day 7, it depended only on NO (Table 5). In this study period, there were no cases of bullous keratopathy.

Table 4. Preoperative parameters (sex, patient's age, NO, preCCT) in connection with postoperative corneal edema on postoperative day 1 (n = 266)

	+ (*n* = 173)		++ (*n* = 68)		+++ (*n* = 25)		
	%	average ± SD	%	average ± SD	%	average ± SD	
Sex							
female	64.0		28.7		7.3		p = .162 *
male	66.7		20.6		12.7		
Patient's age		72.2 ± 8.13		73.6 ± 9.89		76.8 ± 8.75	p < .05 **
NO							
mild	91.5		8.5		0		
moderate	63.7		27.9		8.4		p < .001 *
hard	40.0		35.0		25.0		
preCCT		555.9 ± 37.37		558.1 ± 41.09		552.9 ± 30.52	p = .791**

+ Clear cornea, ++ Focal corneal edema, +++ Diffusive corneal edema, χ 2*, Kruskal–Wallis H test**

Table 5. Preoperative parameters (sex, patient's age, NO, preCCT) in connection with postoperative corneal edema on postoperative day 7 (n = 265)

	+ (*n* = 255)		++ (*n* = 10)		
	%	average ± SD	%	average ± SD	
Sex					
female	96.9		3.1		p = .462 *
male	95.1		4.9		
Patient's age		73.1 ± 8.7		70.7 ± 10.39	p = .58 **
NO					
mild	100		0		
moderate	96.6		3.4		p < .05 *
hard	89.7		10.3		
preCCT		555.7 ± 38.03		568.9 ± 28.24	p = .2**

+ Clear cornea, ++ Focal corneal edema, +++ Diffusive corneal edema, χ 2*, ** Wilcoxon signed-rank test

Discussion

Transient postoperative corneal edema leads to dissatisfied patients, which is why there is a need to evaluate preoperative and intraoperative risk factors for endothelial pump failure in the use of modern phaco technology. In the present study, 67% of the patients had moderate cataracts, 17.6% of the patients had mild cataracts and 15% of the patients had hard cataracts. Significantly higher mean UST, EFX and AVG (mean 87.2, 38.7 and 8.2, respectively) were observed in hard cataracts than in mild cataracts (mean 5.6, 1.3 and 1.8, respectively) or moderate cataracts (mean 37, 12.7 and 5.8, respectively). This is consistent with what has been found in previous studies, showing that the average phacoemulsification time increases due to increasing grades of lens hardness (1, 2, 8-15). In conclusion, we can assume that cataract density is a predictive factor for the amount of energy used during phacoemulsification.

Cataract surgery is one of the most common surgical procedures in the world, with a positive effect on the patients' quality of life. Since postoperative corneal edema leads to dissatisfied patients, another area of interest was postoperative corneal edema and the time needed for corneas to become clear after phacoemulsification using the WhiteStar Signature® PRO with the Ellips FX handpiece. Postoperative corneal edema was present in 93 eyes (35%) on postoperative day 1, and most of the patients had focal corneal edema (25.6%), which decreased to 3.8% on postoperative day 7. A similar pattern of results was observed by Kausar et al., who showed that postoperative corneal edema was present in 44% of the patients and that most of the patients had focal corneal edema (24.7%) (8).

Based on the results of our study, predictive factors for assessment of postoperative corneal edema are NO and the patient's age. This is consistent with what has been found in a previous study (8). This observation might be explained by the theory that significant factors for corneal clarity are the number and condition of endothelial cells. Other than quantity, the quality of endothelial cells is also an important factor for corneal clarity (2). Gradual corneal endothelial cell loss occurs with increasing age and studies estimate the cell loss rate at 0.3% to 0.5% per year (3, 16). With regard to the corneal endothelial barrier and pump function, our study suggests that there is a higher risk of transient postoperative corneal edema in older patients. The limitation of the present study is subjective evaluation of postoperative corneal edema using slit lamp biomicroscopy due to a lack of resources.

One of the intraoperative risk factors which can lead to transient corneal edema after phacoemulsification is endothelial injury caused by consumed ultrasound energy (1). In order to reduce the amount of energy used during phacoemulsification, we used the phaco chop technique, since it has been shown in previous studies that less ultrasound energy is needed when using phaco chop in comparison with other techniques (8, 17,18). In our study, we used the Ellips FX handpiece with a combined longitudinal and transversal movement of the tip, which demonstrates safe performance in comparison with the standard longitudinal phaco handpiece, especially when dealing with hard cataracts (5). Likewise, in our study, only 3.8% of the patients had focal corneal edema on postoperative day 7, and all the patients had clear cornea two months after the operation, which confirmed the surgical efficiency of the Ellips FX handpiece. The results of this study showed that corneal edema depend on measured intraoperative parameters (UST, EFX and AVG), showing that consumed energy is a predictive factor for the severity of corneal edema, as shown in the study by Kausar et al (8). On the other hand, the study by Tsaosus and al. showed that ultrasound energy is not the determining factor for corneal edema (2).

This study aimed to analyze the use of advanced phaco technology with the WhiteStar Signature® PRO system with the Ellips FX handpiece, based on cataract density and its effect on postoperative corneal edema. To conclude, using advanced technology makes every surgeon's life easier. The WhiteStar Signature® PRO with the Ellips FX handpiece showed effective surgical performance with good corneal edema recovery in our study population. Despite continuous advances in cataract surgery, there is still a higher risk of transient postoperative corneal edema in harder cataracts, which require a greater amount of energy use during phacoemulsification, especially in the older population. Further research is needed to better understand the risks associated with postoperative corneal edema.

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Review article

Aspiration During Vaccination: Evidence for SARS-CoV-2 Vaccination

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Abstract

Aspiration has always been performed during intramuscular vaccine injections to ensure that the needle does not puncture one of the blood vessels. However, at the beginning of the twenty-first century, this procedure became debatable.
Using an advanced search builder and logical operators, we searched the PubMed database for all articles about aspiration guidelines. The deltoid blood vessels are large and diverse, with potentially dangerous changes occurring in certain groups such as athletes or people with connective tissue diseases. The pharmacokinetics and reported side effects of improperly applied vaccines differ. Some reported vaccine-related injuries, such as subacromial bursitis, can be avoided by using the aspiration technique. We discussed experiments that provide evidence that intravenous administration of mRNA vaccines can cause myopericarditis. Aspiration during vaccination is not technically demanding and does not require much time. Previous arguments against aspiration were based on efforts to make the procedure of vaccinating children less painful. In response to public concern about vaccine-induced thrombotic thrombocytopenia as a possible side effect, Denmark issued a guideline on mandatory aspiration during vaccination in March 2021.
Guidelines vary by country, and there is a need for an updated and globally applicable instruction manual. Countries should carefully document vaccine side effects so that they could be compared between countries that aspirate and those who do not. More focused research experiments are needed to determine the relationship between aspiration and side effects. We propose a randomized study to compare the effectiveness of aspiration.

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Introduction

The vaccination campaign against COVID-19 focused public attention on the safety of vaccines and vaccine side effects. Not only is the vaccine being called into question due to side effects, but so is the method of administering the vaccine. The aim of this article is to encourage reflection on vaccination techniques in the hope that a discussion on this issue will increase confidence in COVID-19 vaccination and increase vaccination uptake.

Twenty years ago, aspiration was a common procedure during vaccination

Aspiration used to be a common procedure during intramuscular vaccine injections to ensure that the needle did not puncture any blood vessels. However, the guideline recommending this procedure was not evidence-based, and the procedure was disputed at the beginning of the twenty-first century (1). Because of its unproven value, the WHO removed aspiration from intramuscular vaccine administration procedures in 2004 (2). According to Plotkin and Orenstein, aspiration was unnecessary because there were no large blood vessels at the site intended for intramuscular injections. In a more recent edition of their book, they cited the 2007 Ipp study, which found the procedure to be painful, especially for infants (3,4). Based on the WHO guidelines and the 5th edition of Plotkin's book (5,6), the official 2013 UK immunization guidelines, the Green Book, stated that aspiration was unnecessary. The Centers for Disease Control and Prevention guidelines confirmed this, citing an article that makes no mention of aspiration or the distance of large blood vessels from the injection site (7,8). Similarly, the Canadian Government's Canadian Immunization Guide does not recommend aspiration (9).

Search strategy related to aspiration procedure

Using the AND and OR logical operators, we searched the PubMed database using the advanced search builder. The following search strategy was used: ((("intramuscular administration" [All Fields]) OR ("intramuscular injection" [All Fields])) AND (("aspiration" [All Fields]) OR ("technique" [All Fields]))) AND (("vaccine" [All Fields]) OR ("medication" [All Fields])).

Table 1. Search results with mentioning of aspiration

S.no	Author	Publication year	Type of article	Sample size
1.	Nicoll and Hesby(12)	2002	Review	-
2.	Wynaden et al.(50)	2006	Review and questionnaire	-
3.	Hunter(51)	2008	Review	-
4.	Taddio et al.(52)	2009	Review	-
5.	McDonnell et al.(53)	2010	Research	-
6.	Ogston-Tuck(54)	2014	Review	-
7.	Taddio et al.(55)	2015	Review	-
8.	Silva et al.(56)	2021	Research	148*

*Randomized clinical trial that compared adverse events with or without aspiration

There were 77 results in total, with 8 articles mentioning aspiration and 17 articles discussing medication and vaccine administration but not mentioning aspiration (see Table 1). We also searched the reference and citation lists of the studies that were included. Aspiration as part of intramuscular injection was first mentioned in John H. Stokes' 1945 book Modern Clinical Syphilology: Diagnosis, Treatment, Case Studies (10). Although the book was not available to us, we found a figure from it in a 1961 article by Zelman, which simply stated the importance of aspiration (11). The most detailed instructions for needle aspiration were provided by Nicoll and Hesby in 2002: after inserting the needle into the muscle, the first step is to pull back the plunger for 5-10 seconds; if blood is aspirated into the syringe, the administration should be stopped and the needle withdrawn from the muscle; the syringe with blood should be discarded; new syringe should be prepared and administered at a new administration site (12).

The blood vessels in the muscle are large and diverse

The deltoid muscle is a common vaccination site. It is well supplied with blood from several sources: the deltoid and acromial branches of the thoracoacromial artery, anterior and posterior branches of the humeral circumflex artery and deltoid branches of the deep brachial artery. These arteries are accompanied by large veins that drain into the axillary and cephalic veins (13,14). The intersection of the anteroposterior axillary line and the perpendicular line from the mid-acromion is the safest area for intramuscular injections. The lower half of the muscle is avoided due to the significant risk of injury to the arteries and the axillary nerve (15), whereas the upper third is avoided due to the risk of hitting the subacromial bursa. Complications involving the subacromial bursa have been reported after unintentional injection of COVID-19 vaccines into the bursa (16). The deltoid's excellent blood perfusion alters the pharmacokinetics of the injected medication and causes absorption at this site to be faster than at other intramuscular injection sites (17). Furthermore, athletes have a thinner arterial wall and a larger vessel diameter, which might make them more susceptible to aneurysms and ruptures (18,19). The importance of blood supply to muscles is also demonstrated by the avoidance of intramuscular administration in people with hemophilia due to frequent formation of intramuscular hematomas (20).

Renewed interest in vaccine administration during COVID-19 pandemic

The ongoing worldwide vaccination campaign against COVID-19 has reignited the interest in vaccine administration (21). Newspaper articles, letters to the editor, and scientific discussions on YouTube channels have all addressed the topic of aspiration (22–26). We searched the PubMed database for articles published between 2019 and the end of 2021 that contained one of the following terms: vaccine administration method, vaccine administration methods, vaccine administration practices, vaccine administration recommendations, vaccine administration technique, vaccine administration techniques. There were fifteen studies found, four of which discussed vaccine administration and one of which discussed COVID-19 vaccine administration (27–30).

Pharmacokinetics of mRNA vaccine during intravenous and intramuscular administration

During intramuscular administration, the peak concentration of any drug is lower and delayed, and it is higher and instantaneous during intravenous administration (31). To induce immunity, mRNA vaccines must be translated into spike protein (32). There have been no studies that compare the pharmacokinetics of mRNA vaccines administered intramuscularly versus intravenously, but an in vivo study examined the pharmacokinetic translation of mRNA luciferase delivered by lipid

nanoparticles (33). Intravenous administration led to a shorter translation half-life and higher total protein formation (33). Because solid lipid nanoparticles, carriers for the mRNA of the spike protein, have different affinities for various tissues due to their charge (34), they should be carefully monitored in trials. For BNT162b2 vaccine, the distribution of lipid nanoparticles has been reported only for the liver, spleen, adrenal glands and ovaries, while the rest of the data is not publicly available (35). All of these differences raise the question of whether they are significant enough to affect immunogenicity and thus create more potent side effects.

Intravenous injection of mRNA vaccine may induce myopericarditis in a mouse model

An increased incidence of myocarditis and pericarditis was observed following mRNA-based COVID-19 vaccination (36). The link between myopericarditis and vaccination has yet to be established. Li et al. compared intramuscular and intravenous vaccine administration in a mouse model with saline as a control. Despite the fact that the intramuscular group exhibited significant weight loss and higher serum cytokine concentrations after the first dose, only the intravenous group developed a histopathological picture of myopericarditis. Histological changes in the intravenous group lasted for two weeks and worsened significantly after the second dose, regardless of whether the injection was intramuscular or intravenous. According to the authors, these in vivo findings show that intravenous administration of mRNA vaccines can cause myopericarditis (37). There is also evidence that Spike protein can cause pericarditis on its own via CD147 receptor signaling (38).

Intravenous injection of adenovirus causes thrombocytopenia in a mouse model

Adenovirus vectors are another type of COVID-19 vaccine vectors. Adenovirus-induced thrombocytopenia was first observed some twenty years ago, with the introduction of gene therapy (39,40). Vaccine-induced thrombotic thrombocytopenia has also been observed as a side effect of adenovirus-based COVID-19 vaccines (41). These vectors were first effective in in vivo gene delivery transduction, and their advantage is that most cells have adenovirus receptor on their membrane, making adenovirus vectors easily infectable (42). Gene therapy requires high adenovirus concentration via intravenous injection because of the quantitative transduction of the entire organ (40). This high viral particle concentration in the blood may cause adenovirus-induced thrombocytopenia via the following mechanism: The virus attaches to and activates platelets via the Coxsackie adenovirus receptor. After activation, platelets expose P-selectins on their membranes and aggregate with leukocytes. Platelet and leukocyte aggregates then activate other leukocytes and endothelial cells. This type of abnormal platelets is phagocytosed by macrophages. Macrophages and activated endothelial cells release large amounts of high-molecular-weight forms of von Willebrand factor and microparticles. As a result, Von Willebrand factor and microparticles cause a strong pro-coagulant stimulus (39). There is also a more recently proposed mechanism of action that is similar to heparin-induced thrombocytopenia and is caused by the presence of anti-platelet factor 4 (43). The mechanism of vaccine-induced immune thrombotic thrombocytopenia is initiated by the complex of adenovirus vector and platelet factor 4. This complex induces lymphocytes B cells, which produce anti-platelet factor 4. This type of antibody stimulates platelets, monocytes and neutrophils. Activated platelets and monocytes release pro-coagulant microparticles, while activated neutrophils exert a pro-inflammatory effect (44). This complex interaction between adenovirus vector ChAdOx1 and platelet factor 4 was confirmed by Baker et al in 2021 (45).

At the end of March 2021, Denmark issued a guideline for mandatory

aspiration during intramuscular vaccine administration

Following the increased public concern about vaccine-induced thrombotic thrombocytopenia, Denmark issued a guideline on mandatory aspiration during vaccination. The English version of the guideline can only be found in the Janssen vaccination procedure instructions on the Statens Serum Institute website (46). A population-based cohort study from Denmark that followed participants from 1 October 2020 to 5 October 2021 found 69 cases of myocarditis or myopericarditis within 28 days of vaccination. Data from this study does not correspond to the data from United States and Israel indicating an increased rate of myocarditis and myopericarditis in men aged 12-39 (47). On 17 February 2022, the Robert Koch Institute published an update for COVID-19 vaccination, stating that vaccines should only be administered intramuscularly and that aspiration during vaccination makes sense for increased safety to avoid intravascular injection (48).

Conclusion

Aspiration during vaccination is neither technically demanding nor time-consuming. Arguments against it were based on efforts to alleviate a painful procedure when vaccinating children, thereby reducing stress and achieving better child cooperation. Although one study examined the safety of the injection site on the deltoid muscle, its sample size was insufficient and unrepresentative (49). Larger sample size studies are required to support or refute the safety of aspiration omission during vaccination. There are two study types that could be used. The first type should be designed to determine the prevalence of positive blood aspirations during intramuscular injections. The second type should compare the number of side effects associated with intramuscular injections with and without aspiration. The previous study, which found that intravenous injection of mRNA vaccine caused myocarditis in mice, failed to establish a cause-and-effect relationship, but did indicate the potential presence of a preventable factor. Furthermore, the results of that experiment cannot be translated to humans. All countries should document vaccine side effects much more thoroughly, allowing comparisons to be made between countries where aspiration is performed and those where it is not. Although findings obtained in this manner would not be sufficient to demonstrate a causal relationship between side effects and aspiration, they would provide sufficient evidence to begin a more direct research experiment.

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Review article

Deep Ecology: Contemporary Bioethical Trends

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Abstract

Deep ecology emphasizes the importance of the ecological problems as a practical issue, and its importance is in changing the human understanding of everything, including even man's understanding of who he is.
The aim of this paper was to present deep ecology, what it represents and how it has become a significant ecological movement of the 20th century and to indicate the connection between bioethics as new environmental ethics and deep ecology, as well as other environmental movements which, in the contextualization of bioethics, emphasize changing the outlook on life, giving a better knowledge of it, and allowing questioning of social actions and looking at events from different aspects. The idea is to emphasize that man is not only an active, but also a responsible being which is capable of making a paradigm shift in responsibility, and therefore, taking responsibility for all life on Earth.
Content analysis and comparative method were introduced and applied for the requirements of making this review.
Based on the obtained results, the review points to the need to create new ethics which could introduce a general value system for all living and non-living things - a paradigm shift involving man as part of nature and not opposed to it, and to successfully address these complex issues. It will take a profound shift in human consciousness to fully comprehend that it is not only plants and animals that need a safe habitat - because they can live without humans, but humans cannot live without them.

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Introduction

From the beginning of man's life on Earth, every invention and discovery he had made to ease life was about subduing nature for his benefit (1). The reason why the problem began to appear, back in ancient times, is the importance of the presentation of the course of human thought and how changing this thought has led to the consciousness that in its expression subjugated the entire world around itself (2). Deep ecology emphasizes the importance of ecological problems as a practical issue, and its importance is in changing the human understanding of everything, including man's awareness of himself (3). The result produced would be that deep ecology, pointing to the value of all living things, also wants to point to the responsibility that people have in their environment. The new ethics must also have the dimension of sustainability, which can be accomplished in the frame of bioethics, as an interdisciplinary area of science. It is necessary to change awareness so that people can re-establish a relationship with nature without perceiving nature as a resource from which man will have a (short-term) benefit (4). In that sense, international nature and environment protection laws are deficient in practice, and citizens also need to contribute to ecological awareness.

By unifying human approaches in the relationship to nature, this review aims to show that this relationship has become threatened. The aim was to determine whether deep ecology finds its justification in the change of awareness regarding human relationship to people and nature and to show how and to what extent environmental and nature protection which exceeds ecology in its complexity is carried out.

Deep Ecology

Scientists have the most significant responsibility when it comes to preservation and strengthening of the ethical principles in their research and institutions, to act beneficially upon this crossroad of fate from where one can either crash into eternal doom or finally get into the haven of peace (5). Increased interest in the problem of the environment (i.e., the ecological problem) began to appear during the 1970s, and considering the need for new ethics, some scientists and ecologists came up with the idea of said ethic. That considered, Rand Aldo Leopold, a forester, philosopher, writer, teacher, and one of the greatest American biologists called such ethics the ethics of the Earth, which would, by expanding the boundaries of the community, contain everything - from earth to animals (1). He explained the base of his ethics, which was to protect wholeness and stability, and only then can the righteousness of the matter itself be discussed. Arne Næss expanded the thought behind such ecological movement with the diversity between surface and deep elements, where the surface elements mark our avoidance to contaminate the environment exclusively for our own benefit. In contrast, the deep elements represent the protection of the whole biosphere, regardless of the benefits a human being could have (6). This division in the surface and deep elements, that is, shallow and deep ecology, points to the meaningful division within contemporary ecological thought (7). According to that, shallow ecology represents the anthropocentric thought in which a human being is above nature, and nature has only instrumentalist value, while deep ecology goes for the highest ecological norm: preservation of the vital needs of everything living (8).

The maker of the term deep ecology, Arne Dekke Eide Næss, who was born in 1912 and died in 2009 in Oslo (1). He was one of the most famous Norwegian philosophers, who taught at the University of Oslo between 1937 and 1970, where he also graduated and completed a master's degree. He taught semantics and gathered a group of young philosophers and sociologists who were applying empirical methods to affirm the meaning of philosophical terms. He also taught the philosophy of science and the philosophy of Spinoza and Gandhi (9), who also had a significant impact on him. As a hiker and a tour guide of the first expedition to the Tirich Mir mountaintop in the Islamic Republic of Pakistan, his motivation for nature and environmental protection was no wonder.

Although it is not about the motivation founded on the reformist current of the ecological movement, which only wants to prevent contamination, Naess should be given a closer look as a supporter of the revolutionary current, who supports the original current, but who also builds his philosophy seeking for new metaphysics, cognitive theory, and ethics which would solve the relationship between a human being and nature. He called this (eco)philosophy, which is contained in the term deep ecology and synonymous with the terms fundamental ecology, a new philosophy of nature, ecosophy, or ecophilosophy T. In that regard, ecosophy T is built starting with oneself, the change within oneself – to act upon welfare as a whole (1). The core of Næss's philosophy is about connecting everything into a whole, that is, the idea that nothing works independent of the whole, meaning that the relationships between people, plants, and animals depend on one another. According to that, two fundamental principles of that philosophy stand out, as well as those of the ecological movement: self-fulfilment and biospheric equality (5). Contrary to health and welfare of the population, more precisely the population which lives and acts in the developed industrial countries as a central theme of the contemporary society fighting against the contamination of the environment, Næss turns to the inner knowledge of norms, values and ethics, meaning that ecological science will bleed into interdisciplinary practical life wisdom (3). Naess called that transition deep ecology (9). Furthermore, Næss and the American philosopher George Sessions (who also referred to the new ecological ethic which Næss discovered in 1972 and referred to as deep ecology) shaped and exposed the principles which would work for the deep ecology platform, in eight chapters in an article from 1984. Some of those principles are:

1. The welfare and the success of human and non-human life on Earth have their own values (synonyms: intrinsic value, inherent value). Those values do not dependent on the usefulness of the non-human world for humans.

2. The richness and diversity of life forms contributes to the realization of these values. People have no right to jeopardize that richness and diversity unless the goal is to satisfy life needs (6).

The authors state that, although those principles relate to life when we talk about the term biosphere, they are also meant to include the unliving, like rivers, environment, and finally the ecosystem. Naess replaces the term biosphere with the term ecosphere, and that way he does not limit himself to the form of life in the immediate or global surroundings (9). In addition, he replaces the term environment with the term co-world to mark the place of a human in the most truthful way possible.

Deep ecology increases the meaning of the principle of letting the being be (10) while trying to bring ecological consciousness to a higher level and achieve a healthier ecological life. Among other things, deep ecology is founded on Darwinist thought, which tries to move the human away from the centre of life and into a natural circuit of existing (9). Because of that, the Darwinist element presented in the deep ecology builds a complex and contradictory relationship. Deep ecology postulates that exiting from evolutional and acceptable circumstances, which Darwinism sets as an imperative in the way of life, damages the human civilization and nature (1). It exposes the human being and breaks the illusion that humans are wise enough to rationally manage their physical and social environment, not taking into account the evolutionary processes (9). Another relevant characteristic of deep ecology is its attitude towards wilderness, the only real-world left, around which, because of its ecocentric orientation, exists a cult of wilderness (11). According to that, it advocates ecoregionalism and condemns urbanization and hypermobility. It is clear that deep ecology nearly revises that pantheistic belief and divinifies nature, but what needs to be underlined is that it does not replace religion, cults, or a mystical worldview, even though it has mystical aspects. The possibilities and the controversy of deep ecology are manifested even in its basic statement about the concept of intrinsic values, which states that every part of nature is valuable in itself, and not because of

higher goals (human, for instance). In that regard, humans are a part of nature and not its highest achievement (9). However, nature is formed hierarchically, with humans on top, which subjects this concept to criticism and doubt (11). By replacing the term biospheric egalitarianism – in principle – with the term biospheric equality, Næss equalizes all the organisms in the biospheric community, and their equality is a consequence of a relational interconnection, which gives them an intrinsic value. The fact that humans are at the top of the pyramid does not mean that they are not responsible for it. Understanding that a human being must satisfy its needs to survive, Næss does not deny those needs, but only for existential purposes, and when human secondary needs and vital needs of another species come into conflict, a human being should sometimes abandon egoism before the needs of other living beings (12).

The authors of the book Deep Ecology, Bill Devall and George Sessions, think that all organisms and entities in the ecosphere, as parts of an interconnected whole, are equal by intrinsic value. A question arises how all these living, but diverse beings are equal by their intrinsic value. Furthermore, one criticism may be that even if there is an intrinsic value relating to the whole, the book does not say anything about the values of individuals. No individual is a necessity for the survival of the ecosystem as a whole (6). It is concluded that the ethics of the deep ecology does not answer the questions concerning the value of life of individual living beings. The reason may be that the wrong questions are being asked: ecological ethics might be more acceptable when applied to the level of species and ecosystem. In trying to establish that value based on the ecological ethics, a certain holistic feeling arises, a feeling that a species or the ecosystem is not just a total of individuals, but an entity in itself (3).

Authors like Lawrence E. Johnson, Frey Mathews, and James Ephraim Lovelock include species and ecosystems as holistic entities or selves with their own form of realization (6). If the species and the ecosystem can be considered a type of an individual with its own interest, the ethics of deep ecology must face the problems of determining the moral value of the species or the ecosystem again, regardless of the value which it has because of its importance for sustaining life (9). The fact that the biosphere can react to events in ways that look like a self-sustainable system does not show that the biosphere wants to contain itself consciously (1). This fact underlines that the ethics of deep ecology must reject its moral base because the argument stemming from the intrinsic value of plants, species, and the ecosystem is problematic (6). This, of course, does not mean that the argument for protecting intact nature is weak, but the argument based on the difference between the feeling and non-feeling creatures is firmer than the division between the living and non-living (5). The arguments should show that the value of preservation of the last significant areas of untouched nature significantly overcomes economic values (6).

A human must acknowledge that value as an ethical category for that to happen, and therefore, confirm its responsibility (13). If a human's realization of interests for his benefit is acknowledged as an intrinsic value, then it must also be acknowledged for other living beings who are ensuring their well-being (11). Also, the concept of the "right of nature" is doubtful because it enters into a new manipulation. The right to preserve natural resources is contradictory to the concept of preservation of intrinsic values (13). The task of intrinsic values is building the marvel towards the wholeness of existence which is independent of humans (11). It stems from the fact that due to the prevalence of big cities and mechanicalized environment, such marvel cannot be seen or felt towards the non-human, which is what the deep ecology wants to revive. One of the objections to deep ecology is humanist voluntarism, which postulates that humans can change things by their own will. Nevertheless, ecological destruction occurred because of actions of generations, and that is also why one generation cannot change it.

The stumbling stone of deep ecology is that if it cannot change people's awareness, it cannot lead to radical change (10). Modern ecology states that nature existed before the first

humans and that it will continue to exist, which is different from the understanding of tribal societies, and this is something that can be the encouragement for treating nature with more respect. Tribal life, which deep ecologists advocate, is unacceptable for most people. In that regard, bioregionalism is unenforceable in the global world (11). Talking about a relationship of a human being towards nature that is filled with awe, a German physician, theologian and philosopher Ludwig Philipp Albert Schweitzer is the most noted expert in defending ethics by expanding on sensitive beings (9). Using the phrase "awe before life", he builds the ethics of awe, which is based on having equal awe before every life, as well as one's own life (11). He shaped the first and extensive attitude of philosophical biocentrism (7), but his ethics finds itself before the question: What is it like in the cases in which human life can be preserved only then when another human life has to be destroyed instead (14)?

Deep ecology sets a unique view of the relationship towards evolutionism. Generally, the attitude of the deep ecologists is that modern life in industrial societies is not evolutionarily adjusted (11). Tomislav Markus understands that people did not kill nature, but they abandoned the environment of evolutionary adaptation. As the author points out, deep ecology is closer to science and philosophy, and it is not a moral lesson for wealthy individuals (10). Markus points out that knowledge in biology and ecology is essential for understanding the relationship between humans and nature. So is the awareness of the pressure modern industrial societies put on the environment, which means that evolutionary adjustment to the environment is impossible. Therefore, the author sets an imperative in creating a new view of nature, human nature, and human inadaptability to evolution (11).

Since the base of the humanist disciplines lies in dualism, a human as a being is separated from nature with its history about the self-creative process, which is founded on biophobia and ecophobia. The solution is found in the human need to escape into the circumstances of an organic existence (9), representing the escape from environmental destruction. According to that, deep ecology is the escape from consumerism, hyperurbanism, hyper-population, and all other significantly destructive orders of the modern industrial society (15). The solution might be seen in accepting naturalness as a characteristic of human nature, which could decrease environmental destruction. To stop environmental destruction, in favour of life preservation, deep ecology emphasizes the change of the paradigm (1). That would mean that the paradigm, which positions the human being in a superior position looking at nature exclusively as a resource, should change by accepting the evolutionary insights about people's lives. It is trying to rise above consumerism as one of the characteristics of technical civilization. Markus thinks that there are too many people living on this Earth who are not one with nature and who, by that, challenge it by destruction (11). The solution is in the tribal communities, and the precondition is decreasing the population. It is the tribal communities who have the lowest rate of intervention in the environment, as opposed to industrial societies which replace life through the technical and, by doing so, they put pressure on the environment. According to Næss, the quality of life of an individual and of an entire population cannot be considered if the size of that population is excessive. He agrees with decreasing the population in a non-violent way through voluntary birth control (12). Also, he thinks that there should be a 100 million people less on Earth. Numerous deep ecologists believe that diseases, wars, and lack of food will more likely lead to decreasing the population than the rational, controlled way (10). For instance, when Næss wrote about the solutions for depopulation, there were six billion people in the world, while today that number has exceeded seven billion and is still growing. As partially shown before, the two attitudes were determined according to ecoethics: shallow and deep ecology, which try to solve the problems regarding human violations against nature (16). Various ecological ethics or ecoethics appeared because of the care for nature and the paradigm change, as is the case with deep ecology. Deep ecology, by pointing to the value of all living and

non-living beings, also wanted to indicate the responsibility of all towards the environment (17). That term, as well as others, lay the foundation of bioethical principles, and the relationship between bioethics and deep ecology (5).

Bioethics and Deep Ecology

Bioethics is a term that came into use in the 1970s, relating to ethical questions in the areas of biology, medicine and psychology in order to provide answers to the challenges of new knowledge. Although the term bioethics, i.e., "bioethik", was first used by Paul Max Fritz Jahr in an article from 1927, the credits for conceptualizing and preparing the term go to Van Rensselaer Potter II, who built the foundation for the development of bioethics in his work in the 1970s (15). Since the meaning of life is broader than the human or medicinal aspect, bioethics questions the responsibility of human action towards humans themselves, but also towards all life on Earth, or better said towards the biosphere (18). Namely, Potter thought that ethical values cannot be separated from biological facts, and he considered bioethics to be a bridge between science and humanity (19) which includes all living beings or, in other words, a biosphere essential for guaranteeing a future (20). Numerous discoveries have brought new knowledge, which he believed could not in itself be completely bad or good, but that it represented power, and, therefore, once available, it would mostly be used for power (21). It is therefore essential to know how to use new knowledge, and that is possible only by possessing the wisdom on how to use new knowledge (22). On that end, he believed that bioethics as a science of survival would provide the wisdom on how to ensure sustainability (21). However, despite that, bioethics is often synonymous with clinical, medical or, the commonly called, biomedical ethics, which is wrong and inconsistent with Potter's original idea of a global bioethics which deals with man's relationship with himself, but also with the ecosystem (23). Bioethics cannot be only clinical ethics because the concept simultaneously contains elements of environmental ethics — it is concerned with the survival of man, but not any survival - the survival which considers the survival of the ecosystem that has its value, entirely independent of man (24).

Finally, according to Potter, bioethics implies the inevitable interconnectedness of man and the rest of the living world (25), or in other words, an interconnected biosphere (20). Deep ecology as a part of environmental ethics understands people as an indispensable part of nature or a link in the chain of life, it points to the interconnectedness and interdependence of all parts of the ecosphere, emphasizes the primordial value of all species regardless of human needs, and it focuses on wisdom and balance (26). Deep ecology can be seen as a form of a radical environmental critique of the technological civilization which reacts to technolatry, anthropocentrism, instrumentalism and resourcism, consumerism, and linear progressivism which overtook society with the emergence of new knowledge (27). Naess considered deep ecology to be an ecosophy developed under the influence of Leopold, focused on wisdom, that is, the wisdom of the Earth, which focuses on ecologically wise and healthy living (28). It is shown that ecological ethics, ecoethics, or environmental ethics gather different theories, some of which are mentioned here. For example, ecocentrism, biocentrism, pathocentrism, or their mixed forms such as ecocentrism and ecofeminism, as well as the ethics of deep ecology from which each of them stems, try to set a frame in order to discuss the moral relationship between humans and inhuman entities, by expanding the human moral obligation to animals, plants or certain areas of nature or life in general (29). Despite the critics and the deficiencies to which deep ecology subjected, the framework for building a new theory is the concept of responsibility, more precisely the responsibility of acting, as in lighting the effects of knowledge (30). Also, new ethics must have a dimension of sustainability, which bioethics as an interdisciplinary field of science can realize within the scope of its content, and its strength can be seen in generating a new sensibility and creating a new awareness which goes past particular

dimensions and tries to preserve life to stabilize all the segments of society (29).

In the works of Leopold and Potter, it is evident that bioethics and environmental ethics share a common source. The connection between bioethics and deep ecology as part of the environmental ethic is in their vision of an interconnected biosphere (20). People are a part of the natural world, and not just bystanders, and based on that, the responsibility towards the world around and towards each individual is evident (31). Bioethics and environmental ethics also share wisdom as a common root (21), mostly because of new knowledge. It is precisely because of that high complementarity between bioethics and environmental ethics that, in 1988, Potter proposed the introduction of the new term global bioethics (32). Potter coined the term global bioethics in an attempt to protect the new science of survival from a growing transition into a predominantly clinical ethics, but also to further expand it with even more elements of environmental ethics, especially under the influence of Leopold's legacy (33). However, despite all that, bioethics and deep ecology have over time developed into two separate fields (20), which has led to the creation of a gap between bioethics and environmental ethics (34). Namely, bioethics has mostly developed into clinical ethics, where the focus is on the individual health of a human patient, while environmental ethics has developed more with the focus on biosphere health and not on individual health, that is, on the health and sustainability of the overall ecosystem (35).

Public Health Ethics as a Bridge Back to Potter's Bioethics

Public health ethics is a relatively new field, coming into its own somewhere at the beginning of the 21st century, and it is still in its developmental stage but in recent years it has become one of the fastest growing subdisciplines of ethics (34). It is deeply rooted in bioethics, clinical and research ethics, and also in environmental ethics (36). Public health ethics is primarily focused on policies, programs and laws for the protection and promotion of public health, and the focus is not on the individuals but on the community (i.e., the population) when it comes to achieving the common good (34). Since health is a state of complete physical, mental and social well-being and not merely the absence of diseases or infirmity (20), the complexity of public health, and thus of public health ethics, is evident. The fact that human health depends on the environment has been known since the beginning of time, and today it is increasingly clear that it also depends on animal health, because the convergence of humans, animals, and their products is more pronounced than ever before (37). The current coronavirus pandemic shows the importance of interconnectivity of the domains of people, animals, and the environment as a group of interconnected circles when it comes to public health, but also when it comes to the future of all living things (38). Severe acute respiratory syndrome coronavirus 2 is most likely the product of ecological conditions created by humans, while the related pandemic is a product of the number, density, and connectivity of the human species and its interaction of the environment (39). It is obvious that the health of humans is connected to the health of animals and the environment, and, therefore, we can say that the health of each of those three domains is the product of interactions of triangles of their health which, in fact, forms public health (40). That kind of public health – the One Health approach (41) – is in line with Potter's vision of an interconnected biosphere; hence it can be considered as a planetary vision of One Health (42) or Global One Health, and, consequently, we can talk about the global public health ethics (Figure 1) (43).

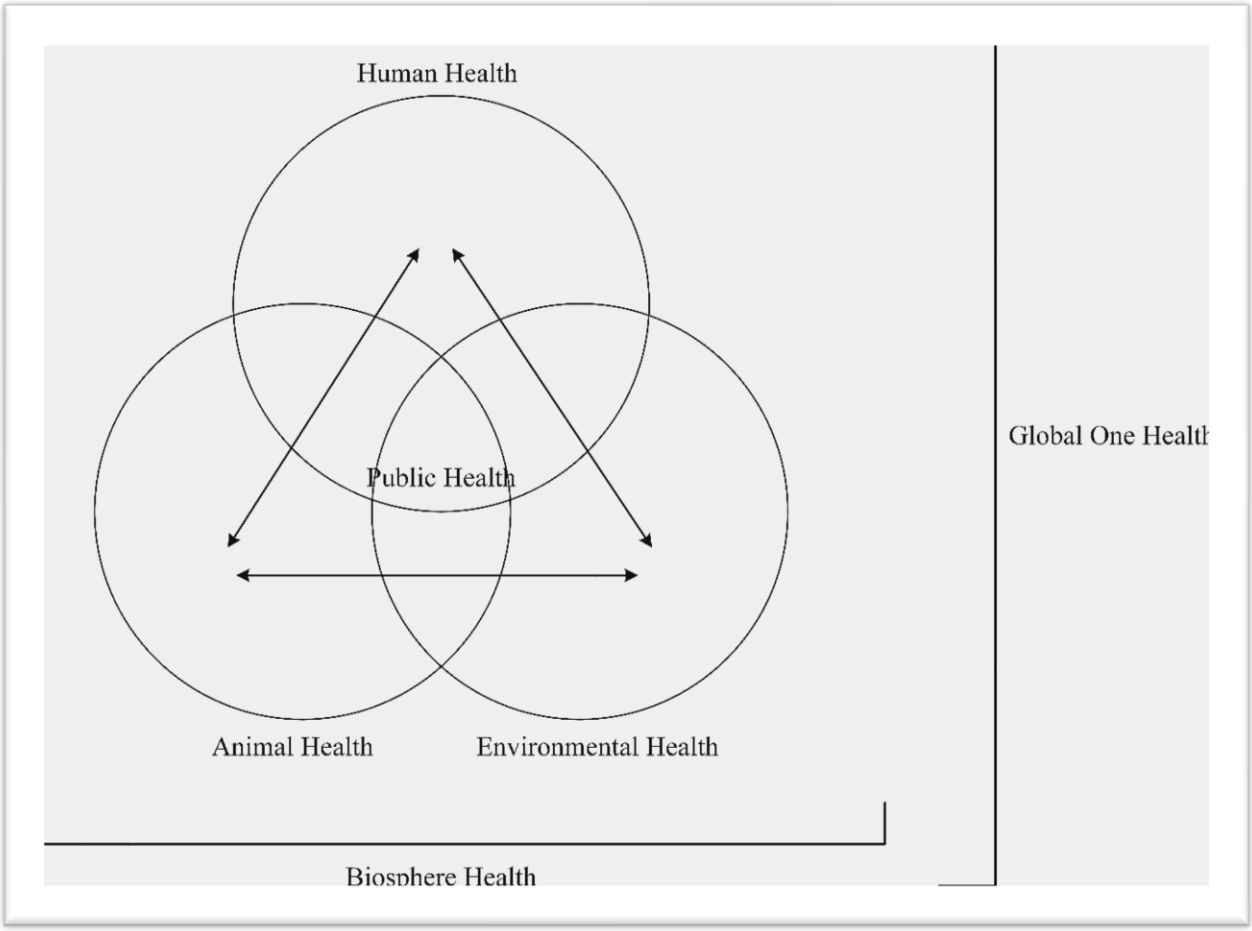

Figure 1 The expanded model of the Global One Health concept

Potter sought to include health, survival, and the environment in the new ethics, which will combine knowledge and deliberation in the human constant quest for wisdom, that is, the knowledge of how to use new knowledge for the survival and progress of humankind (44). Those qualities are contained and encouraged by public health ethics, which on one hand overlaps with bioethics, and on the other hand with deep ecology as part of the environmental ethics, while in its origins contains features of global ethics (20). Public health ethics shows that human health is strongly and inseparably linked to the health of the planet (the biosphere) and that the health of the community is essential for the health of individuals, which in turn has a strong impact on the health of the population (45). That is not surprising since public health deals with the health of the individual, but also with the health of the environment, in order to achieve the best possible health of the population (20). The case of the coronavirus pandemic underlines the need for a fundamental shift in the human conception of health, sustainability, and humanity, which is only possible by returning to Potter's bioethics, which evaluates and considers all living beings, or in other words, the biosphere (46). Based on everything mentioned above, public health ethics can be used to bridge the gap between bioethics and deep ecology as part of the environmental ethics to restore the values of Potter's bioethics for a brighter future of all living things (34).

Conclusion

The history of ecology starts with the Neolithic Revolution, although it seems that it was only after the revolution that we heard about ecological problems. It has been confirmed that, at the same time when the human

anthropogenic activities started to change his organic and wild environment, to which he is genetically adjusted, began the alienation of the wilderness that he has gotten used to (13). Of course, it was not just humans who conditioned the (negative) changes in nature; there were also volcanic eruptions, asteroid collisions, earthquakes, and floods – in other words, a multitude of natural disasters to which most of the living world is not adjusted and most of which happened long before human existence.

With the development of civilization, the shaping of cultures, and usage of technology, human beings genuinely become active factors in affecting nature. From Greek philosophy to Cartesianism, nature was thought to be the starting point for questioning everything (47). Experiencing nature as a devalued magnitude and the subject of knowledge conditions the forming of new things, more specifically new age humans. The new age products are modern science and technology, in which science is the beholder and technology is the executioner (48). The role of technology is to satisfy the needs of life as quickly and pleasingly as possible, and through that, the consumer society is created, which also affects the expansion of the ecological crisis. It is no wonder that the relationship of a human being and nature is altered because of the eternal nature of modern science and technology (49). Numerous archaeological studies have shown that the ecological problems started with the Neolithic domestication, which has increased in intensity in the last few centuries and led to an ecological crisis (50). Although the ecological crisis does not affect everyone equally, it is a problem that significantly influences life and demands an urgent solution, regardless of those who think that the ecological crisis is either a reflection of capitalism or industrialization, contrary to those who believe that technology could solve the problems of humanity (51).

Ecology contains many areas affected by biosphere processes, which should be contained to access the solution to its problems. This should be done with the help of sustainable development, which presents the principles of sustainability of the system, a way of development that does not degrade or violate nature (50). However, to achieve progress, it is people's attitude towards nature that must change, not their attitude towards themselves, which is how Næss formulated it in his philosophy, known under many other terms, but mentioned here most often under the term "deep ecology" (52).

The concept that emphasises the value of every life — in (new) bioethics, ethics of life, which due to its interdisciplinary area of impact can be applied in reality, is enriched through that responsibility (53). In recent years, it has come to light that public health ethics can be used to bridge the gap between bioethics and deep ecology as part of the environmental ethics, thus enabling the return to Potter's bioethics which has built-in values of deep ecology (54).

Although much has been done in recent years, deep ecology is to a great extent still in its very beginnings.

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