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Dear Readers,

This is my great privilege to deliver to you the very first issue of *Arterial Hypertension* that I have been entrusted the responsibility for.

Facing the expiry of term of the former Editor-in-Chief Professor Krystyna Widecka, Board of Polish Society of Hypertension accepted my vision and plans focused on our Journal development. Within the next 4 years I will strive to vastly improve visibility of our Journal, and to make way for further indexations in large databases.

I cordially invite the authors from Poland and neighboring countries to disseminate your work via our platform. This will not only help to strengthen our position but also facilitate tightening our bonds on the scientific grounds.

As I reviewed all articles published in Arterial Hypertension for the last three years I believe that some valuable works have still a very strong citation potential. I therefore encourage the authors to bring them back to light and cite them upon closest convenient occasion. Of more, back in 2019 two essential documents were publicized in *Arterial Hypertension* i.e.: Polish Society of Hypertension official guidelines on the management of hypertension, and the position paper coordinated by three influential medical societies. The latter comprehensively clarifies aspects of hypertension management in pregnancy. I kindly invite you to recollect these extremely important statements, what has been made easy for you, as both are open access documents. Therefore, including these references in your manuscripts would be of huge benefit for our Journal.

Taking this rare opportunity of a first letter from Editor-in-Chief I would like to share just one closing remark with you. I want to warmly invite you to start or continue the cooperation with us. It is very likely that with your even minimal input we can get wider visibility in the world's scientific community. Let's make this happen, together.

Jacek Wolf, M.D., Ph.D.
Editor-in-Chief 'Arterial Hypertension'
March 31, 2021

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Contents

The evolutionary development of the renin angiotensin aldosterone system and its importance for the survival of the human species

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Abstract

Kidneys produce a number of substances that affect intrarenal blood circulation; however, the key system that regulates blood flow in both general and local circulation (including the renal circulation) is the renin-angiotensin-aldosterone system (RAAS). Individual elements of the RAA system are synthesized in separate tissues of the body under the influence of specific local factors. The system functions as a whole due to mutual relations based on feedback and it consists of three basic elements: renin, angiotensin and aldosterone.

The history of research on the RAA system dates back to the late 19th century. One of the important stages of exploring the mechanisms related to RAA system functioning was the publication (in 1898) of the results of research on the hypertensive effect on blood pressure of rabbit kidney extracts (containing renin). The observations from 1934 were of similar significance: the correlation between dog kidney ischaemia and the occurrence of hypertension was found. In the following years, the enzymatic properties and structure of renin and angiotensin peptides, resulting from the action of renin and the enzyme converting angiotensin I (Ang I) to its active form — angiotensin II (Ang II), were clarified. The latter belongs to the most important regulators of aldosterone secretion. In 1939, it was proved that under the influence of renin blood pressure-rising peptides are formed. Consequently, it was documented that angiotensin was the cause of hypertension in animals with ischaemic kidney, and in 1954 the sequence of angiotensin I and II was described. In 1960–1961 systemic RAA occurrences were identified.

However, to provide the insight of evolutionary significance of the RAA system for humans, the phylogenetic development of this enzyme-endocrine system in vertebrates should be investigated. The largest database of information regarding this system in the aforementioned group of animals is the research of Hirofumi Sokabe and Hiroko Nishimura, which, among others, is the basis for this manuscript.

Key words: renin; angiotensin; angiotensin; aldosterone; vertebrates

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Introduction

Kidneys produce a number of substances that affect intrarenal blood circulation; however, the key system that regulates blood flow in both general and local circulation (including the renal circulation) is the renin-angiotensin-aldosterone system (RAAS). Individual elements of the RAA system are synthesized in separate tissues of the body under the influence of specific local factors. The system functions as a whole due to mutual relations based on feedback and it consists of three basic elements: renin, angiotensin and aldosterone [1–3].

The history of research on the RAA system dates back to the late 19th century. One of the important stages of exploring the mechanisms related to RAA system functioning was the publication (in 1898) of the results of research on the hypertensive effect on blood pressure of rabbit kidney extracts (containing renin) [4] obtained by prof. Robert Tigerstedt and his assistant Per Bergman. Goldblatt observations from 1934 were of similar significance. He found a correlation between dog kidney ischaemia and the occurrence of hypertension [2]. In the following years, the enzymatic properties and structure of renin and angiotensin peptides, resulting from the action of renin and the enzyme converting angiotensin I (Ang I) to its active form — angiotensin II (Ang II), were clarified. The latter belongs to the most important regulators of aldosterone secretion (discovered by Simpson, Tait and Wetstein in 1953). In 1939, Braun-Menandez and Page proved that under the influence of renin blood pressure-rising peptides are formed. Consequently, it was documented that angiotensin was the cause of hypertension in animals with ischaemic kidney, and in 1954 Skeggs described the sequence of angiotensin I and II. In 1960–1961, Davis, Genest, Laragh and others identified systemic RAA occurrences.

However, to provide the insight of evolutionary significance of the RAA system for humans, the phylogenetic development of this enzyme-endocrine system in vertebrates should be investigated. The largest database of information regarding this system in the aforementioned group of animals is the research of Hirofumi Sokabe and Hiroko Nishimura, which, among others, is the basis for this manuscript.

Evolution of RAA system in vertebrates

Structure of the juxtaglomerular apparatus in vertebrates

In the kidneys of mammals, the juxtaglomerular apparatus consists of:

- renin-secreting granular epithelial cells in the central part of the afferent glomerular arterioles;
- *macula densa* composed of specialized cells originating from epithelial cells of the closer part of the distal tubule;
- extraglomerular mesangial cells;
- sympathetic nerves, densely innervating the afferent glomerular arterioles [6].

The structure of granular cells evolves at various stages of phylogenesis. In primitive vertebrates, i.e. jawless fishes, elasmobranches, holocephali, primary bony fishes, the structure and development of granular cells is different than in higher vertebrates. Granular cells in fish are localized externally to the afferent and efferent arterioles, whereby renin is secreted interstitially and enters the lymphatic circulation. In amphibians, reptiles and birds, granular cells occur in the area of the juxtaglomerular apparatus. In addition, the avian kidney has dense macular cells that are characteristic of macula dense in mammals. In mammalian kidneys, renin-secreting cells are present in the efferent arterioles; however, their number is smaller than in the afferent arterioles. As for the vascular component of the juxtaglomerular apparatus, it evolved earlier than the *macula densa* [6].

There is a well-described sympathetic innervation of glomerular cells and afferent arterioles in the mammalian group, unlike primitive vertebrates. In the arterioles of the batrachoidiformes, despite the abundant presence of renin-secreting cells, sympathetic nerve fibres have not yet been determined. In amphibians and birds, similarly as in mammals, the presence of nerve fibres has been confirmed histochemically and ultrastructurally.

Biochemical and molecular structure of RAA system in vertebrates

Angiotensinogen and renin

The occurrence of various forms of Ang I and Ang II in vertebrates (including the primary forms of Ang I and Ang II in elasmobranches) suggests the existence of many forms of angiotensinogen. Angiotensinogen concentrations, determined by maximum Ang I plasma production, are higher in higher vertebrates. In contrast, the ability to produce renin was found in all representative vertebrate species. There is a view that the RAA system, or its major components, developed in the early stages of vertebrate evolution. Unfortunately, the molecular identification of renin is significantly limited in primary vertebrates [5].

Angiotensin

Primary Ang I (decapeptide) and Ang II (octapeptide) are found in all vertebrates. The variability of the primary structure of Ang II in the course of phylogenesis relates to amino acids in position I (Asn, Asp, Tyr or additional chain), 3 (Val, Ile, Pro), 4 (His) and 5 (Ile, Val). Asn1AngII is the original form of Ang II. Changes at the first and ninth amino acid positions of Ang I result in a decrease in contractile activity, as does the removal or change of the first amino acid of Ang II [5].

Angiotensin-converting enzymes and angiotensinases

There are two biologically active ACEs in vertebrates, i.e. ACE 1 and ACE 2. Angiotensin converting enzymes are found in plasma, lungs, kidneys, gills, brain and other tissues of various vertebrate species, including lampetra fluviatilis, separate the last two amino acids of angiotensin (*in vitro*). Angiotensinase activity was also found in plasma, kidneys and other tissues in non-mammalian vertebrates [5].

Angiotensin receptors

Angiotensin AT1 and AT2 receptors were detected in only few non-mammalian vertebrate species. The first primary vertebrate belonging to agnatha superclass, in which angiotensin, ACE, and angiotensin receptors were found, is lampetra fluviatilis [5].

Tissue RA

The tissue renin angiotensin system exerts autocrine or paracrine effects in vertebrates, and the whole system or its components are found in the adrenal glands (adrenal tissue) and heart [5].

Function and regulation of RAA system in vertebrates

Mammals have four main mechanisms to control renin secretion:

- intrarenal baroreceptors that detect changes in blood pressure in the renal artery or nearby glomerular cells;
- a *macula densa* in the proximal part of the distal tubule, detecting the speed of NaCl ionic transport and transmitting information via specific mediators to the juxtaglomerular cells;
- sympathetic conduction *via* β-adrenoreceptors;
- many other, various humoral factors, including prostanoids, angiotensin, atrial natriuretic peptide (ANP), nitric oxygen (NO) and other mechanisms (no evidence of their occurrence in teleostei) [5].

Renal artery baroreceptors due to reduced blood pressure, also in fish, reptiles and birds, secondarily lead to a significant increase in plasma renin activity.

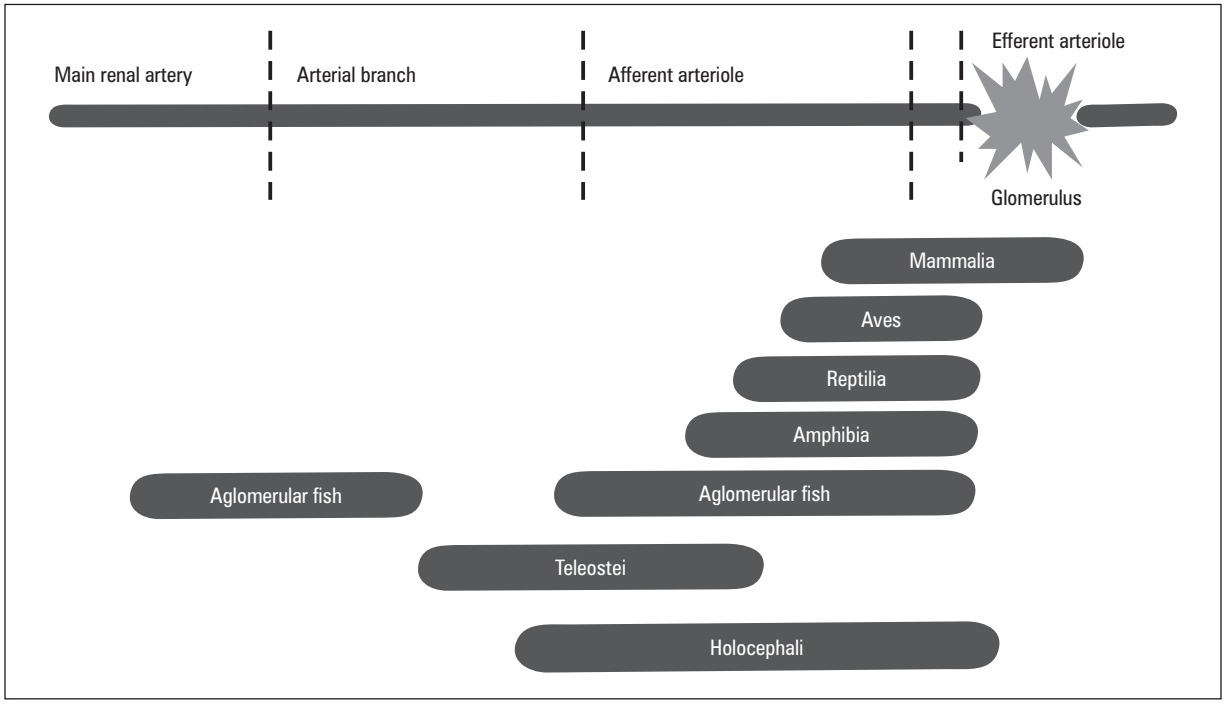

Figure 1. Distribution of granular cells along the arteries and arterioles of the kidneys in vertebrates. During the progress of phylogenesis in vertebrates, granulosa cells gradually

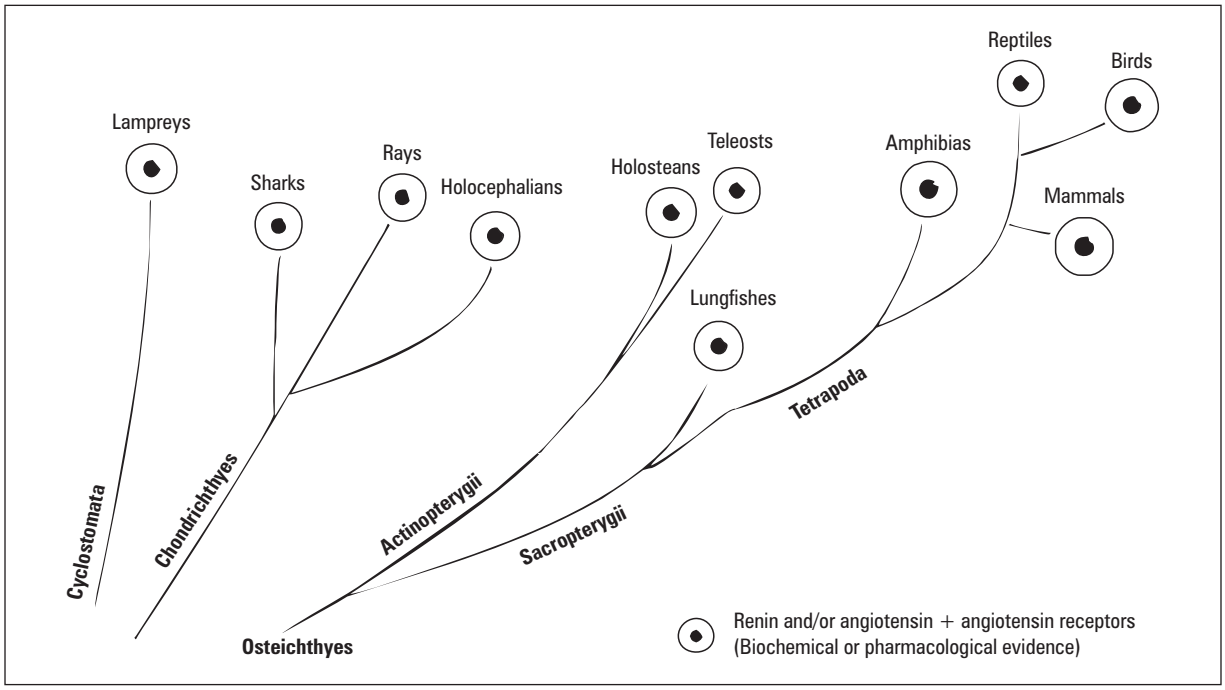

Figure 2. Renin/angiotensin and angiotensin receptors presence in vertebrates

This means that the lack or incomplete juxtaposition apparatus in lower vertebrates has been replaced by a renal mechanism of activating baroreceptors to control renin secretion. The exception are birds in which much less activity of the renin–angiotensin system in sudden onset of hypotension or hypovolemia is observed. This is due to the unique mechanism of rapid blood volume replenishment by absorbing fluid from interstitial tissue into the vessels.

Referring to *macula densa* mechanism, an increase in NaCl concentration in its cells causes an increase in the afferent arteriole resistance in a few seconds, followed by a reduction in glomerular filtration and a decrease in capillary glomerular pressure. This negative feedback that stabilizes the glomerular filtration is caused by a decrease in renin secretion by the juxtaglomerular cells. Although the renin–angiotensin system is only a negative feedback modulator, a decrease in renin secretion may lead to a decrease in salt concentration and reabsorption of water in the proximal tubule. The role of central baroreceptors and sympathetic nervous system in lower vertebrates is still being studied [5].

Biological effects of angiotensin in vertebrates

Ang II increases blood pressure in individual vertebrate species by increasing peripheral vascular resistance, indirectly, by releasing catecholamines and by central activity. In the most primitive vertebrates, the vasopressor effect of Ang II (Asn1, Val5) occurs indirectly by releasing catecholamines by stimulating sympathetic nerve endings or ganglia. Among teleostei, amphibians, reptiles and birds Ang II (Asp1) or (Asn1) increases blood pressure depending on the amount of angiotensin-dependent vasoreceptors. The effect of Ang II on catecholamine secretion by the sympathetic nervous system, adrenal medullary cells or chromophilic cells was developed in early phylogenesis.

Lower vertebrate kidneys are not or are only slightly self-regulated — changes in blood pressure in the aorta easily affect glomerular filtration and renal blood flow, which seems to be a mechanism controlling fluid and electrolyte excretion. Even if the birds evolved from ancient reptiles and descended from a line leading to mammals, the vascular effect of avian Ang II is significantly different from that of reptiles and mammals. The stimulated by Ang II vasopressor effects in adult birds is attributed solely to the stimulation of secretion of catecholamines from the adrenal medulla and adrenergic nerve endings.

Ang II in mammals stimulates aldosterone production in the early and late stages of steroidogenesis, by stimulating the conversion of cholesterol to pregnenolone and by stimulating the synthesis of active aldosterone. Similarly, to the concentration of potassium ions, it is an important regulator of aldosterone secretion. In anamnia belonging to vertebrates, such as elasmobranches, bony fishes, anurana

and caudate amphibians, the adrenal cortex and medulla are located in and on the abdominal surface of the kidney (this is more of the adrenal gland than the adrenal cortex). In contrast, reptiles and birds have discreetly separated the adrenal glands into the adrenal cortex and medulla area, but it is not as visible as in mammals.

In elasmobranches, the main corticosteroids in the intrarenal tissue and circulation are 17-hydroxylase 1α-hydroxycorticosterone, while in teleostei cortisol is the quantitatively dominant steroid hormone synthesized in the intrarenal tissue and has mineralocorticosteroid activity. Alderone and corticosterone occur in amphibians, and corticosterone is the main adrenal steroid in reptiles. Among birds, Ang II stimulates the synthesis of aldosterone in the adrenal glands [5].

Conclusion

It is believed that each component of the juxtaglomerular apparatus developed at a different stage of phylogenesis. Granular cells producing renin or renin-like enzymes developed earlier, while macula dense and extraglomerular mesangial cells appeared in higher vertebrates. It is unclear when abundant adrenergic innervation appeared in the juxtaglomerular cells (at the level of teleostei there is no evidence of communication between renin-secreting cells and nerves or adrenergic receptors). The distribution and location of the juxtaglomerular cells seem to have been shifted during phylogenesis.

In primitive vertebrates, renin-secreting cells are scattered along the small arteries and arterioles in the kidney. Along with the phylogenetic advancement of vertebrates, renin-secreting cells moved to the area of the juxtaglomerular apparatus. The presence of prorenin and active renin as well as its regulatory mechanisms are probably the remains of non-mammalian vertebrates.

It is a fact that angiotensin is present in all vertebrate species. Primary Ang II is a stable molecule that occurs during phylogeny, with variation in amino acids at positions 1, 3, 4 and 5. Lampetra fluviatilis, similarly to teleostei, has Ang II of a unique structure. AT receptors are found in most vertebrates (the problem with the determination of receptors in lower vertebrates is related to the different structure of the junction site with Ang). The degree of similarity increases along with the development of vertebrates and suggests that all AT1 receptor homologues can derive from the same protoplast. Unfortunately, there is no confirmed data on the simultaneous evolution of the angiotensin receptor with the development of the RA system (under study).

The relationship between the RA system and steroid hormones of the adrenal glands occurs in every class of vertebrates, except for jawless fishes. The adrenal tissue of reptiles and birds is defective, only in mammals it is complete and has a layer producing glyco- and corticosteroids. Ang II stimulates the release of the production of 1α-hydroxycorticosterone (elasmobranches), cortisol (teleostei) and corticosterone/aldosterone (amphibians, reptiles, birds). Adrenocorticotropic hormone (ACTH) is less often the main regulator of adrenal (intrarenal) hormones than Ang II. The role of mineralocorticoids, including aldosterone, in sodium renal tubule transport is unclear in non-mammalian vertebrates.

Summarizing the evolutionary aspects of RAA system development in individual animal classes, Ang II causes an increase in blood pressure in representative vertebrate species directly through vascular smooth muscle spasm or indirectly by the secretion of catecholamines from the adrenergic nerve endings and the adrenal medulla/chromatophilic cells. Haemorrhage or pharmacologically induced hypotension causes an increase in renin secretion or the production of angiotensin.

Maintaining normal blood pressure can be one of the fundamental roles of the RAA system in human. In mammals, including humans, an immediate response to dramatic drops in blood pressure or volume occurs in a neurological mechanism through baroreceptors that stimulate the RAA system to restore blood pressure. Long-term recovery or maintenance of blood pressure and volume is constantly controlled by the kidneys. In addition, it is even more complex due to the fact that apart from the systemically acting RAA, there are also local counterparts, acting in such tissues as the brain, heart, blood vessel walls or kidneys, causing an increase in blood pressure.

Unfortunately, during long-term stimulation of RAA, Ang II systemically increases the synthesis and secretion of aldosterone as well as growth factors and pro-inflammatory cytokines, causing cardiovascular remodelling along with hypertension leading to secondary damage to such organs as heart or kidneys. Locally acting Ang II (in tissues) has a mitogenic effect on cardiac myocytes and vascular smooth muscle cells; it also increases collagen synthesis in the myocardium and vessels, contributing to the development of left ventricular hypertrophy, thickening of the arterial walls and end organ damage.

At the end of the discussion, it should be stated that the RAA system, which in the course of evolutionary development protected against a decrease in blood pressure, is currently the reason for the excessive persistence of increased blood pressure values and secondary organ damage. For this reason, the inclusion of drugs that inhibit RAA components is currently the primary treatment for cardiovascular and renal diseases.

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Blood parameters, symptoms at presentation and adverse in-hospital outcomes of COVID-19 pneumonia in patients with hypertension

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Abstract

Background: We aimed to explore the association of clinical symptoms of COVID-19 pneumonia, blood parameters on admission, and anti-hypertensive drugs with in-hospital outcomes, including length of hospital and intensive care unit (ICU) stay, receiving mechanical ventilation, degree of lung injury, and in-hospital death among patients with hypertension.

Material and methods: This retrospective study conducted in patients with newly diagnosed COVID-19 pneumonia from August 20, 2020 to September 25, 2020.

Results: A total of 182 patients with COVID-19 pneumonia were included in the present study. The patients were categorized into those with hypertension (n = 82) or without hypertension (n = 100). Patients on angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs) showed no significant increase in the risk for all in-hospital outcomes. Old age [0.6 (0.5–2) $p < 0.00$], fever [0.3 (0.2–1.8), $p < 0.00$] and low lymphocytes percentage [0.3 (0.2–1.2), $p < 0.00$] were associated with increased risk for extensive lung injury. Old age [0.4 (0.1 = 0.7) $p < 0.01$], high neutrophil count [0.3 (0.2–2), $p = 0.02$] and low lymphocyte percentage [0.3 (0.1–0.7), $p = 0.01$] were associated with prolonged hospital stay while low lymphocytes percentage [0.7 (0.6–0.9), $p < 0.00$], old age [1.2 (1–1.4), $p = 0.01$] and fatigue [2 (1–4), $p = 0.04$] showed significant association with prolonged length of ICU stay. Low lymphocytes percentage [0.7 (0.6–1), $p < 0.00$], old age [1.1 (1–1.2), $p = 0.01$] and fatigue [2 (1.7–4), $p = 0.02$] were associated with increased risk for receiving mechanical ventilation. Risk for in-hospital death was associated with increased neutrophil percentage [1.2 (1–1.5), $p = 0.01$] and old age [1.1 (1–1.2), $p = 0.03$].

Conclusions: ARBs and ACEIs showed no significant association with adverse in-hospital outcomes. Old age, low lymphocytes percentage and high neutrophils percentage on admission were independent predictors for increased risk of in-hospital mortality and morbidity among COVID-19 pneumonia patients with hypertension.

Key words: COVID-19; blood parameters; hypertension; in-hospital outcome

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), was identified as the cause of an outbreak of acute respiratory illness in Wuhan City, China. The World Health Organization (WHO) declared that the SARS CoV-2 causes the CoV disease 2019 abbreviated as COVID-19 [1, 2]. The severity of the COVID-19 infection can vary from patient to patient. Some cases of COVID-19 infection were associated with pneumonia and shortness of breath. On the other hand, some patients developed respiratory failure, septic shock, or multiple organ failure leading to death [2–4].

Previous clinical studies and reports on SARS and Middle East respiratory syndrome found that hypertension is associated with increased rate of morbidity and mortality in infected patients [5, 6].

Emerging data about COVID-19 pandemic reports that the morbidity and mortality rates of COVID-19 infection in patients with cardiovascular diseases, including hypertension, are much higher than those of patients without comorbidities [7, 8]. The available evidence has suggested that COVID-19 severity and prognosis are significantly associated with cardiovascular disorders, but the specific mechanisms and link between the two are unclear [8]. Some initial reports have suggested that hypertension or its treatment may be involved in the pathogenesis and progression of COVID-19 due to interaction between SARS-COV-2 and angiotensin-converting enzyme 2 (ACE2), a potential receptor for SARS-COV-2 to enter human cells [2, 9]. However, the potential effects of hypertension and its treatment on COVID-19 pneumonia severity and prognosis remain an area for active research. Also, there is limited evidence that determines the predictors of poor outcomes among COVID-19 pneumonia patients with hypertension during in-hospital course.

The main aim of the present study was to explore the association of clinical symptoms of COVID-19 pneumonia, blood parameters on admission, and anti-hypertensive drugs with in-hospital adverse outcomes, including length of hospital and intensive care unit (ICU) stay, receiving mechanical ventilation, degree of lung injury, and in-hospital death among patients with hypertension.

Material and methods

In this retrospective study, we recruited patients with newly diagnosed COVID-19 infection who presented with features suggestive of pneumonia to the Al-Sader teaching hospital in Al-Najaf governorate from August 20, 2020 to September 25, 2020. All patients were presented with features consistent with COVID-19 pneumonia based on clinical symptoms (fever, dry or productive cough, fatigue, or shortness of breath) and radiological findings. Diagnosis of COVID-19 infection was based on positive nasopharyngeal swab by real time polymerase chain reaction (PCR). At hospital admission, the baseline clinical characteristics and complete blood count were recorded using medical records and collected by attending physicians. The baseline clinical characteristics including age, sex, hypertension, diabetes mellitus, smoking, body mass index (BMI), previous coronary artery disease, anti-hypertensive drugs, complete blood count, and in-hospital clinical outcome. Complete blood parameters included white blood cell count (WBC), lymphocyte count and percentage, neutrophil count and percentage, red blood cell count (RBC), hemoglobin (Hb), red blood cell mean volume (MCV), red blood cell width distribution (RDW), platelet count, platelet distribution width (PDW), and platelet mean volume (PMV). Patients who were receiving antihypertensive therapy with the diagnosis of hypertension at the time of hospital admission were labeled as patients with hypertension. The severity of lung injury by COVID-19 pneumonia was assessed by CT scan examination score at the time of hospital admission. Patients were followed up in hospital until discharged or died. The main in-hospital outcome for the study was defined as receiving mechanical ventilation, length of hospital and ICU stay, degree of lung injury according to CT score and in-hospital death. Approval of this study was provided by our medicine College Board.

Statistical analysis

Statistical analysis was performed using SPSS ver. 23.0 (SPSS Inc., Chicago, IL, USA). P-value of < 0.05 was chosen for statistical significance. Baseline clinical data of the patients and blood parameters were expressed as mean ± standard deviation for continuous variables and compared by Student t-test or as numbers with percentages for categorical data and compared by chi-square test. Univariate analysis was used to calculate the odds ratio and confidence intervals [OR (CI)] and assess the association of anti-hypertensive drugs with in-hospital outcomes. Baseline clinical characteristics, including age, sex, diabetes mellitus, smoking, body mass index (BMI), previous coronary artery disease, clinical symptoms on admission and complete blood parameters underwent univariable logistic regression to the in-hospital outcomes. Those with a p value of < 0.05 were found

eligible for inclusion in the final multivariable logistic regression analysis to assess their independent association with in-hospital outcome.

Results

A total of 182 patients with COVID-19 pneumonia were included in the present study. The patients were categorized into those with hypertension [age (years) 58 ± 9, 33 (40%) were males] or without hypertension [age (years) 42 ± 15, 49 (49%) were males). In patients with hypertension, fever was the most common clinical symptom followed by dry cough, shortness of breath, fatigue, productive cough, taste and smell loss. Thirty nine (48%) patients with hypertension were on angiotensin receptor blockers (ARBs) while 18 (22%) patients were on angiotensin-converting enzyme inhibitors (ACEIs). Patients' characteristics are shown in Table 1.

Patients with hypertension were older (58 year versus 42 year, $p < 0.00$) and had higher BMI values (30 versus 28, $p = 0.01$) than patients without hypertension. The prevalence of diabetes mellitus ($p < 0.00$), smoking ($p = 0.02$), coronary artery disease ($p < 0.00$) was higher among patients with hypertension compared to patients without hypertension. Shortness of breath ($p < 0.00$) and loss of smell and taste ($p < 0.00$) were more frequently observed among patients with hypertension while no significant change in the frequency of fever, dry and productive cough, and fatigue between the groups with and without hypertension. Regarding blood parameters distribution, higher values of white blood cells (1000 *vs.* 8000, $p = 0.03$), neutrophil count (8000 *vs.* 5000, $p < 0.00$), neutrophil percentage (77 *vs.* 62, $p < 0.00$), and PDW (13 *vs.* 12, $p = 0.01$) and lower values of lymphocyte count (1.4 *vs.* 2.3, $p < 0.00$) and lymphocytes percentage (16 *vs.* 29, $p < 0.00$) were observed among patients with hypertension compared to patients without hypertension. With the exception of in-hospital death, the remaining in-hospital outcomes, including length of hospital and ICU stay, receiving mechanical ventilation, and lung injury were more common in patients with hypertension than patients without hypertension ($p < 0.00$) (Tab. 1).

Predictors of in-hospital outcome among patients with hypertension

Patients on ARBs and ACEIs showed no significant increase in the risk for all in-hospital outcomes in univariate analysis (Tab. 2) Baseline clinical symptoms, comorbidities and blood parameters which were significant in univariate analysis were selected for final multivariate analysis.

Old age [0.6 (0.5–2), $p < 0.00$], fever [0.3 (0.2–1.8), $p < 0.00$] and low lymphocyte percentage [0.3 (0.2–1.2), $p < 0.00$] were associated with increased risk for extensive lung injury as assessed by CT scan examination. Old age [0.4 (0.1–0.7), $p < 0.01$], increased neutrophil count [0.3 (0.2–2), $p = 0.02$] and low lymphocyte percentage [0.3 (0.1–0.7), $p = 0.01$] were associated with prolonged hospital stay while low lymphocyte percentage [0.7 (0.6–0.9), $p < 0.00$], old age [1.2 (1–1.4), $p = 0.01$] and fatigue [2 (1–4), $p = 0.04$] showed significant association with prolonged length of ICU stay. Low lymphocyte percentage [0.7 (0.6–1), $p < 0.00$], old age [1.1 (1–1.2), $p = 0.01$] and fatigue [2 (1.7–4), $p = 0.02$] were associated with increased risk for receiving mechanical ventilation. Risk for in-hospital death was associated with increased neutrophil percentage [1.2 (1–1.5), $p = 0.01$] and old age [1.1 (1–1.2), $p = 0.03$].

Discussion

The major findings of our study were: (1) comparative analysis of COVID-19 pneumonia patients with and without hypertension showed that patients with hypertension had higher prevalence of age > 45 year, obesity, diabetes mellitus, history of coronary artery disease, and smoking than patients without hypertension; (2) in-hospital outcomes, including length of hospital and ICU stay, receiving mechanical ventilation, and lung injury were more common in patients with hypertension than patients without hypertension($p < 0.00$); (3) ARBs and ACEIs were not associated with increased risk of adverse in-hospital outcome; (4) old age, low lymphocyte percentage, and high neutrophil count and percentage were associated with increased risk for adverse in-hospital outcome among patients with hypertension.

During the previous outbreak of Middle East Respiratory Syndrome (MERS), Metabolic disorders and clinical predictors of morbidity and mortality outcomes among MERS-CoV infected patients were hypertension, old age, diabetes, obesity, and coronary artery disease. These comorbidities can be involved or linked etiologically to the pathogenesis of MERS-CoV through modulating the innate immune response of the host [1, 10, 11].

According to data from china, COVID-19 patients with metabolic disorders showed adverse clinical outcome [2, 12]. It is speculated that systemic inflammation may be the major factor for development and progression of COVID-19. Several studies

Table 1. Patients characteristics

Variables	Hypertension (n = 82)	Without hypertension (n = 100)	p value
Age (years)	58 ± 9	42 ± 15	< 0.00
Male sex, n (%)	33 (40%)	49 (49%)	0.23
Diabetes mellitus, n (%)	41 (50%)	19 (19%)	< 0.00
Smoking	20 (24%)	12 (12%)	0.02
Coronary artery disease, n (%)	19 (23%)	4 (4%)	< 0.00
BMI	30 ± 5	28 ± 4	0.01
Fever, n (%)	70 (85%)	82 (82%)	0.54
Dry cough, n (%)	63 (77%)	75 (75%)	0.77
Productive cough, n (%)	23 (28%)	24 (24%)	0.53
Smell loss, n (%)	14 (17%)	43 (43%)	< 0.00
Taste loss, n (%)	18 (22%)	45 (45%)	< 0.00
Fatigue, n (%)	37 (45%)	45 (45%)	0.98
Shortness of breath, n (%)	56 (68%)	47 (47%)	< 0.00
Anti-hypertension drugs			
ARBs, n (%)	39 (48%)	–	–
ACEIs, n (%)	18 (22%)	–	–
Ca channel blockers, n (%)	18 (22%)	–	–
Beta-blockers, n (%)	8 (10%)	–	–
> one drug, n (%)	15 (18%)	–	–
Blood parameters			
WBCs [$\times 10^9$/L]	10 ± 5	8 ± 3	0.03
Lymphocyte (%)	16 ± 5	29 ± 13	< 0.00
Neutrophil (%)	77 ± 10	62 ± 17	< 0.00
Lymphocyte count [$\times 10^9$/L]	1.4 ± 0.5	2.3 ± 1	< 0.00
Neutrophil count [$\times 10^9$/L]	8 ± 3	5 ± 2	< 0.00
Neutrophil/lymphocyte	7 ± 0.9	5 ± 1	0.22
Platelet/lymphocyte	196 ± 15	200±33	0.91
RBC [$10^6/\mu$L]	4.5 ± 0.6	4.6±0.6	0.25
Hb [g/dL]	12 ± 2	13 ± 2	0.31
MCV [fl]	85 ± 7	87 ± 60	0.11
RDW (%)	45 ± 4	44 ± 4	0.78
Platelet count [$\times 10^9$/L]	251 ± 50	264 ± 25	0.57
PDW (%)	13 ± 3	12 ± 2	0.01
PMV (fl)	9 ± 1	9.1	0.13
In-hospital outcomes			
In-hospital death, n (%)	13 (19%)	9 (9%)	0.15
Hospital stay [day]	9 ± 2	3 ± 1	< 0.00
ICU stay [day]	6 ± 1	3 ± 1	<0.00
Lung injury	42 ± 22	20 ± 12	< 0.00
Mechanical ventilation use, n (%)	26 (31%)	12 (12%)	< 0.00

ARB — angiotensin receptor blocker; ACEI — angiotensin-converting enzyme inhibitor; BMI — body mass index; ICU — intensive care unit; MCV — mean cell volume; PDW — platelet distribution width; PMV — platelet mean volume; RDW — red blood cell distribution width; WBCs — white blood cells

have been reported that patients with underlying chronic metabolic disorders, such as diabetes, obesity and hypertension, can modulate the function of the innate and humoral immune systems leading to

Table 2. Univariate analysis of angiotensin receptor blocker (ARBs) and angiotensin-converting enzyme inhibitor (ACEIs) with in-hospital outcome

	Death		Mechanical ventilation use		Length of ICU stay		Length of hospital stay		Lung injury	
ARBs	0.6 (0.1–4)	0.67	1 (0.3–4)	0.78	0.2 (–12–2)	0.18	0.3 (–17–2)	0.13	0.1 (–22–8)	0.34
ACEIs	0.4 (0.0–5)	0.55	1 (0.2–6)	0.70	0.1 (–12–4)	0.33	0.2 (–18–3)	0.17	0.0 (–16–20)	0.81

ICU — intensive care unit

Table 3. Multivariate regression* **

Lung injury		
Predictor	**OR (CI)**	**p value**
Old age	0.6 (0.5–2)	< 0.00
Fever on admission	0.3 (0.2–1.8)	< 0.00
Low lymphocyte %	0.3 (0.2–1.2)	< 0.00
Length of hospital stay		
	OR (CI)	**p value**
Old age	0.4 (0.1–0.7)	< 0.00
Low lymphocyte %	0.3 (0.1–0.7)	0.01
High neutrophil count	0.3 (0.2–2)	0.02
Length of ICU stay		
	OR (CI)	**p value**
Low lymphocytes %	0.7 (0.6–0.9)	< 0.00
Old age	1.2 (1–1.4)	0.01
Fatigue on admission	2 (1–4)	0.04
Mechanical ventilation use		
	OR (CI)	**p value**
Low lymphocyte %	0.7 (0.6–1)	< 0.00
Old age	1.1 (1–1.2)	0.01
Fatigue on admission	2 (1.7–4)	0.02
In-hospital death		
	OR (CI)	**p value**
High neutrophil %	1.2 (1–1.5)	0.01
Old age	1.1 (1–1.2)	0.03

*significant variables, including baseline blood parameters, comorbidities and clinical symptoms with p value < 0.05 in the univariate logistic regression model were entered as predictors in the final multivariate regression model; **only variables significant with p value < 0.05 are displayed in the table; OR (CI) — odd ratio (confidence interval)

cytokines imbalance and chronic low-grade systemic inflammatory phenotype. As such, this chronic low-grade systemic inflammation in COVID-19 patients who have underlying chronic diseases could promote systemic inflammatory response when SARS-CoV-2 infected [8, 9, 13].

Another major mechanism by which COVID-19 can lead to adverse outcome is via virus binding to ACE2 receptors, which is required for virus entry into target cells in the cardiovascular system and lungs [2, 14]. Patients with hypertension, diabetes and coronary artery disease are the most consumers of ACEIs and ARBs. There is evidence from experimental studies that inhibition of ACE2 production by negative feedback from the use of ACEIs and ARBs for treatment of hypertension and other cardiovascular diseases increases the expression of the ACE2 receptors particularly in the cardiac tissues, thus increasing the availability of target molecules for SARS-CoV-2 [2, 15]. However, the upregulation in ACE2 following ARB or ACEI use is observed after high dose administration of these drugs in animals and not in doses commonly used in humans. Also, this upregulation has been documented mainly in cardiac tissues and not in the lungs [16].

In our study, ARBs and ACEIs showed no significant association with adverse in-hospital outcome, although about 70% of patients with hypertension enrolled in the study were on ACEIs or ARBs prior to hospital admission. In the literature, the role of ARBs and ACEIs in the pathogenesis and severity of COVID-19 infection is obscure and controversial [8]. In the early phase of COVID-19 infection, there has been concern regarding the potential harmful role of ACEIs and ARBs based on previous animal studies showing that these drugs could alter tissue expression of ACE2 leading to enhance susceptibility to viral host cell entry and propagation [17]. On the other hand, no effect of ACEIs or ARBs on ACE2 activity was found in recent reports [14, 18]. Consistent with our results, Guo et al. reported that ARB and ACEI use was not associated with increased patients' mortality rate, although more patients were using ACEI and ARB medications prior to COVID-19 infection [19]. Also, use of ACEI or ARB among hospitalized COVID-19 patients with hypertension was associated with lower risk of all-cause mortality compared with ACEI/ARB non-users [8, 20].

Regarding blood parameters' role in COVID-19 patients with hypertension, low lymphocyte count or percentage and high neutrophil count or percentage may be considered as a cardinal finding in COVID-19 infection with prognostic implication in determining disease severity and progression [21].

High neutrophil count reflects the intensity of inflammatory response during COVID-19 pneumonia, while low lymphocyte count reflects the damage of immune system and may perpetuate a harmful inflammatory status [22]. Chronic cardiovascular disease, such as hypertension, may influence neutrophils count and function [23]. The predictive role of lymphocyte and neutrophil counts in assessing the severity of COVID-19 pneumonia is consistent with previous reports that patients infected with SARS-CoV-2 had a high neutrophil count and a low lymphocyte count during the severe phase [24, 25]. In line with a previous report, Zhu Z et al. found that high level of peripheral blood cytokine IL-6, significant increase in neutrophil count, significant decrease in lymphocyte percentage, and hypertension were independent risk factors for assessing the severity of COVID-19 [22].

The present study has several limitations. The sample size was relatively small recruited from single hospital. The design of the study was retrospective, thus residual confounding might exist.

Conclusion

COVID-19 pneumonia patients with hypertension were likely to have adverse in-hospital outcomes compared to COVID-19 pneumonia patients without hypertension. ARBs and ACEIs showed no significant association with adverse in-hospital outcomes. Old age, low lymphocyte percentage and high neutrophil percentage on admission were independent predictors for increased risk of in-hospital mortality and morbidity among COVID-19 pneumonia patients with hypertension.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Myeloperoxidase (MPO) and high sensitivity C-reactive protein (hsCRP) as inflammatory biomarkers of endothelial and leukocyte activation in overweight hypertensive patients

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Abstract

Background: Low-grade inflammation mediates the relation between overweight and the development of cardiovascular diseases. The study aimed to examine if myeloperoxidase (MPO) and hsCRP (high-sensitivity C-reactive protein) in overweight hypertensive patients can be used as biomarkers of endothelial and leukocyte activation.
Material and methods: Seventy-five subjects were included in the study; 38 had essential arterial hypertension (AH) and 37 were normotensive controls (NC), subsequently divided into overweight (OW; BMI ≥ 25 kg/m^2) and normal weight subgroups (NW; BMI < 25 kg/m^2). Body mass index (BMI), inflammatory markers concentrations, association of MPO and hsCRP with AH and/or overweight were assessed.
Results: AH patients had higher MPO (median 132.5 pmol/L, IQR: 53.8–691.9) ($p < 0.001$), while hsCRP did not significantly differ compared to normotensive controls (NC). NW-AH patients had higher MPO ($p = 0.02$) than normotensive NW patients. MPO was similar between normotensive patients OW and NW, while hsCRP concentration was significantly higher in the OW (median 1.85 mg/L, IQR: 0.47–7.19) ($p = 0.01$) compared to NW. OW-AH patients had significantly higher MPO (median 137.4 pmol/L, IQR: 53.80–703.4) ($p = 0.002$) compared to normotensive NW and OW ($p < 0.001$) patients, likely reflecting neutrophilic activation in hypertension. Additionaly, OW-AH patients had significantly higher hsCRP (median 1.71 mg/L, IQR: 0.22–14) ($p = 0.005$) than normotensive NW patients. hsCRP significantly positively correlated with BMI in both AH ($\rho = 0.41$, p = 0,009) and NC groups ($\rho = 0.38$, $p = 0.01$), while MPO did not correlate, supporting inflammation in OW, particularly in OW with AH.

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Conclusions: All together, the results suggest that inflammation may mediate mutual association of AH and OW, suggesting MPO as inflammatory biomarker for AH and hsCRP for overweight.

Key words: arterial hypertension; overweight; myeloperoxidase; high sensitivity C-reactive protein; inflammation; biomarker

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Introduction

Arterial hypertension (AH) is a major independent risk factor for development of cardiovascular (CV) diseases, such as heart failure and myocardial infarction, as well as the primary cause of stroke which makes it a significant public health problem [1]. Another CV risk factor and a global health epidemic is overweight (OW), a chronic disease with distinct metabolic and endocrine disorders, which result in a number of severe chronic complications [2]. The common denominator of the both, AH [3, 4] and overweight, is inflammation, which causes endothelial dysfunction [5, 6] and has a key role in the development and progression of atherosclerosis [7].

C-reactive protein (CRP) is an inflammatory biomarker synthesized in hepatocytes in response to primary stimulation by interleukin 6 (IL-6) [8]. A recent study demonstrated that high sensitive CRP (hsCRP) (which is a term for a more sensitive assay of CRP) is involved in mechanisms that lead to development of AH [9]. hsCRP can be considered as biomarker of the process of endothelial dysfunction and, at supraphysiological concentrations, as a predictor of vascular disease [10].

Several studies have shown that CRP impaired endothelial vasoreactivity *in vivo* [11, 12] by inhibiting endothelial nitric oxide synthase (eNOS) activity *in vitro* and *in vivo*, reducing NO availability, and by predisposing blood vessels to chronic vasospasm, leading to an increase in total vascular resistance and AH [13]. Interestingly, there are evidences that hsCRP may be also secreted by other cells than hepatocytes, such as smooth muscle cells and adipocytes [14]. In addition, prolonged periods of low-grade systemic inflammation may explain the higher risk for high blood pressure observed among overweight people [15].

Myeloperoxidase (MPO) is a lysosomal enzyme present in neutrophils and monocytes, released during their activation to the surface of vascular endothelial cells and around them, following leukocyte degranulation [16]. MPO has affinity to both, the endothelial and the leukocyte's surface and may contribute to leukocytes attraction and recruitment to endothelial cells by its electrostatic effect of positively surface charge, which is a catalysis-independent function of the enzyme [17].

MPO catalyzes the production of hypochloric acid and a range of other highly reactive species. These MPO-derived reactive substances may damage the arterial wall, thereby reducing its elasticity [18]. Another potentially important consequence of MPO activity is consumption of NO and induction of endothelial dysfunction [19].

MPO is rapidly taken up by endothelial cells by a transcytotic process and accumulates within the subendothelial space, positioning it anatomically to interfere with the effects of NO in the vessel wall [20]. Together, these mechanisms may lead to endothelial dysfunction and subsequently increased blood pressure. And while some reports of studies in human subjects have shown a significant association between blood pressure and hs-CRP elevation in people with hypertension [21, 22] other studies showed no significant relation between hypertension and hs-CRP [23, 24].

The aim of the study was to examine if myeloperoxidase (MPO) and hsCRP in overweight hypertensive patients can be used as biomarkers of endothelial and leukocyte activation and their interaction.

Material and methods

The study included 75 examinees, 43 men and 32 women, ranging in age from 20 to 70 years (median 42). Thirty-eight patients were recently diagnosed having essential AH that lasted less than a year (AH stage 1 according to the European Hypertension Guidelines 2013 – systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg), while 37 were normotensive controls. The study was designed as a cross-sectional investigation. The examinees were recruited from the ambulatory office for nephrology and hypertension, in the Department of Nephrology, Osijek University Hospital, in Osijek, Croatia (Fig. 1).

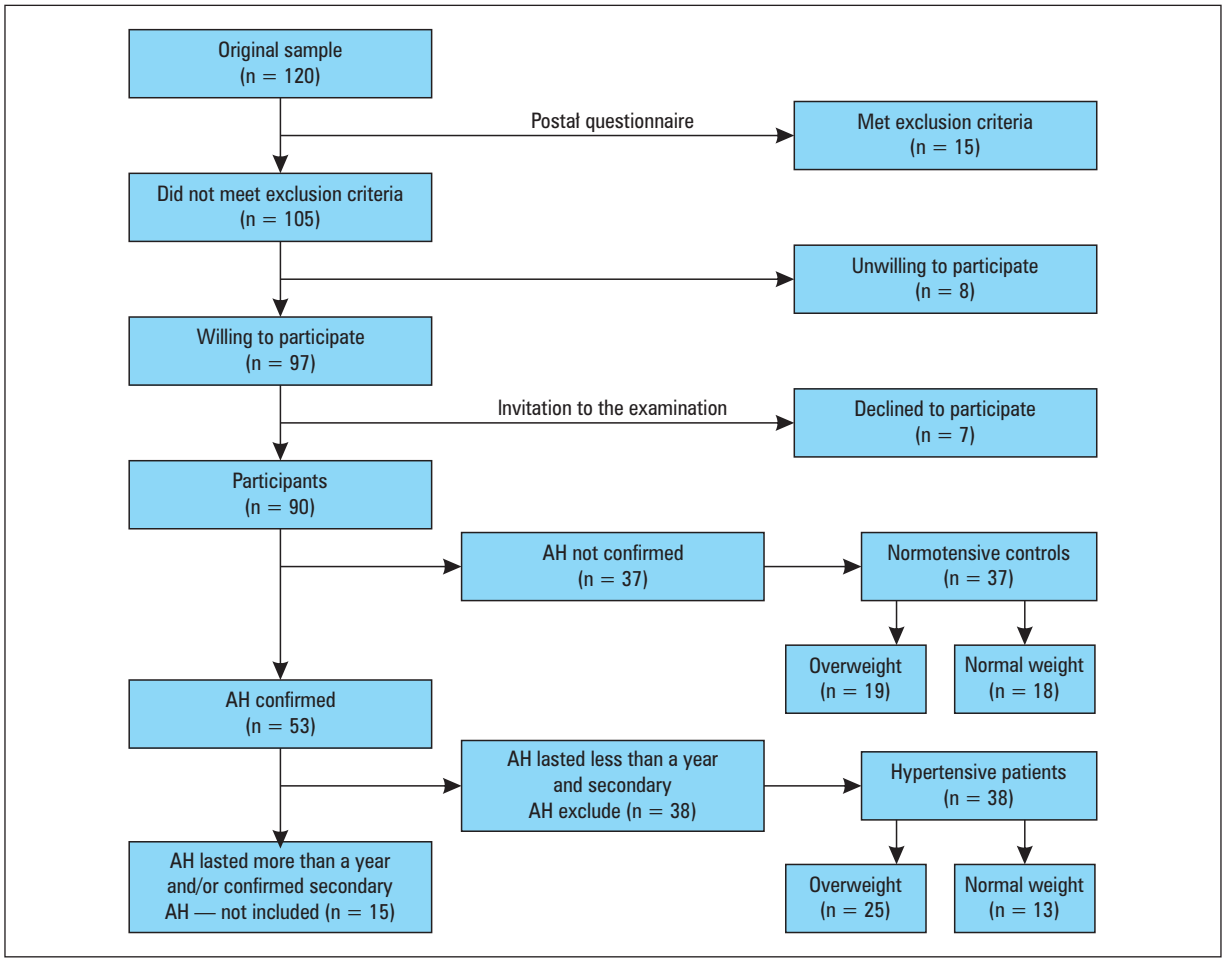

Figure 1. Formation of the sample in the study

Exclusion criteria were diabetes, liver disease, renal disease and/or serum creatinine above 97 μmol/L for men and 80 μmol/L for women, myocardial infarction, stroke and use of nonsteroidal anti-inflammatory drugs or glucocorticoids.

The groups were further subdivided according to the body mass index (BMI) into an overweight (OW) group, with BMI ≥ 25 kg/m^2, and a normal weight (NW) group, with BMI < 25 kg/m^2. The studied groups' characteristics are shown in the Table 1.

The previous workup for diagnosis of essential AH was as following: all patients were examined for medical history, then underwent physical examination that included arterial blood pressure measurement using calibrated mercury sphygmomanometer with the suitable dimension cuff; blood pressure (BP) readings were taken while patients were in the sitting position and rested for 5 minutes; their BP was taken three times consecutively and the mean value was calculated for systolic and diastolic BP, respectively. The same procedure was performed in the controls group to exclude AH. During the study, the hypertensive patients did not receive anti-hypertensive therapy for 2 weeks before the blood sampling. In patients with AH additional laboratory tests were done to exclude secondary AH: plasma renin activity, aldosterone, metanephrine and normetanephrine in the 24-hour urine, ACTH, circadian rhythm cortisol, duplex sonography of the kidneys, endogenous creatinine clearance and 24-hour proteinuria to assess renal function. Patients' height and weight measurements were taken in the morning and the following laboratory tests were performed: total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, plasma glucose, urea, creatinine, urate and urinalysis. All examinees were informed about the research methods, ethical principles and the purpose of the research prior to its beginning and they all gave their written informed consent.

MPO and hsCRP concentration measurements

Concentrations of MPO and hsCRP were determined by aArchitect 1000i (ABBOT, United

Table 1. Demographic and laboratory characteristics of arterial hypertension (AH) patients and normotensive controls (NC) (n = 75)

	Group		Test value	p
	AH (n = 38)	NC (n = 37)		
Age [yrs]	42 ± 14 (20–70)	40 ± 9 (24–55)	t = 0.704	0.48
Sex (men/women) (n)	21/17	22/15	χ^2 = 0.13	0.71
Cholesterol [mmol/L]	5.44 ± 1.05	5.55 ± 1.81	t = –0.33	0.74
HDL [mmol/L]	1.31 ± 0.34	1.4 ± 0.34	t = –0.86	0.39
LDL [mmol/L]	3.38 ± 1.03	3.35 ± 1.44	t = 0.10	0.92
Triglycerides [mmol/L]	1.38 (0.37–5.65)	1.62 (0.34–9.94)	z = –0.81	0.42[a]
BMI [kg/m^2]	27.97 (19.33–45.61)	25.2 (19.49–34.29)	z = –3.38	**0.001**[a]
hsCRP [mg/L]	1.62 (0.22–14)	1.05 (0.13–7.19)	z = –1.63	0.09[a]
MPO [pmol/L]	132.5 (53.8–691.9)	73 (28.6–859.3)	z = –4.60	**< 0.001**[a]

HDL — high density lipoproteins; LDL — low density lipoproteins; BMI — body mass index; hsCRP — high-sensitivity C-reactive protein; MPO — myeloperoxidase; [a]Mann-Whitney test

States) analyzer in EDTA plasma, applying chemiluminescence microparticle immunoassay (CMIA). Serum hsCRP was determined using immunoturbidimetry test for quantitative determination on Olympus AU-680 analyzer (Beckman Coulter, Switzerland).

Statistical analyses

The data were statistically analyzed by SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as proportions and percentages. Normality of distribution of numerical data was analyzed by Kolmogorov-Smirnov test. Differences in categorical variables were examined by chi-square test. Differences in numerical variables were tested by Student's t-test for normally distributed variables and by Mann-Whitney test for nonparametric statistics. Binary logistic regression (Hosmer-Leveshow goodness of fit test) was used for multivariate analysis. Spearman's ρ was determined for correlations between numeric variables. Values of $p < 0.05$ were considered statistically significant.

Results

Myeloperoxidase and hsCRP concentrations

Table 1 shows the general demographic characteristics of the groups. The patients with AH were not different from normotensive individuals in age, gender, plasma concentration of cholesterol and triglycerides. AH patients had higher BMI (p = 0.001) and higher MPO concentrations (median 132.5 pmol/L, 53.8–691.9) compared to the normotensive group (NC) (median 73 pmol/L, 28.60–859.3) (p < 0.001). No difference in hsCRP concentrations were found between the two groups (p = 0.09).

Overweight hypertensive (OW-AH) and overweight normotensive (OW-NC) examinees together had higher hsCRP (median 1.8 mg/L, 0.22–14) (p = 0.01) compared to normal weight hypertensive (NW-AH) and normal weight normotensive (NW-NC) examinees taken together (Tab. 2). MPO did not differ between all OW and all NW examinees.

MPO concentration and hsCRP concentration were not different in hypertensive OW patients compared to NW-AH patients (MPO p = 0.87 and

Table 2. Differences in high-sensitivity C-reactive protein (hsCRP) and myeloperoxidase (MPO) within the entire and within the hypertensive group according to body mass index (BMI)

	BMI [kg/m^2]		z	p
Entire group (n = 75)	**≥ 25 (n = 48)**	**< 25 (n = 27)**		
hsCRP [mg/L]	1.80 (0.22–14.00)	1.04 (0.13–5.50)	–2.42	**0.02**[a]
MPO [pmol/L]	100.55 (28.6–703.40)	98.90 (30.8–859.3)	–0.57	0.57
Hypertensive group (n = 38)	**≥ 25 (n = 25)**	**< 25 (n = 13)**		
hsCRP [mg/L]	1.71 (0.22–14.00)	1.55 (0.29–5.50)	–0.49	0.62
MPO [pmol/L]	137.4 (53.80–703.4)	132.50 (37.75–691.90)	0.16	0.87

[a]Mann-Whitney test

Table 3. Differences in myeloperoxidase (MPO) and high sensitivity C-reactive protein (hsCRP) between arterial hypertension (AH) patients and normotensive controls (NC) divided according to body mass index (BMI)

BMI [kg/m^2]	MPO [pmol/L]		z	p
	AH (n = 38)	NC (n = 37)		
< 25	132.5 (37.75–691.9)*†	78.5 (30.8–59.3)*‡	*–2.21,	0.03[a]
≥ 25	137.4 (53.8–703.4)‡§	73 (28.6–131.5)†§	§ –4.04,	< 0.001[a]
	hsCRP [mg/L]			
	AH (n = 38)	NC (n = 37)		
< 25	1.55 (0.29–5.5)*†	0.89 (0.13–4.64)*‡	*–1.42	0.15
≥ 25	1.71 (0.22–14)‡ §	1.85 (0.47–7.19)†§	§ –0.34	0.73

[a]Mann Whitney test; MPO: †z = –0.64, p = 0.008; ‡z = –3.15, p = 0.002; hsCRP: †z = –0.566, p = 0.572, ‡z = 2.778, p = 0.005

hsCRP p = 0.61, respectively) (Tab. 2). OW normotensive subjects did not significantly differ from NW normotensive subjects in MPO concentrations (median 73 pmol/L, 28.60–131.50 in OW compared to median 78.05 pmol/L, 30.80–859.30 in NW) (p = 0.56), while significantly higher concentrations of hsCRP were found in overweight normotensive subjects (OW-NC) (median 1.85 mg/L, 0.47–7.19 compared to median 0.89 mg/L, 0.13–4.64 in NW) (p = 0.01).

OW-AH patients had significantly higher MPO (median 137.4 pmol/L, 53.8–703.4) (p = 0.002) and hsCRP (median 1.71 mg/L, 0.22–14) (p = 0.005) compared to NW-NC patients. OW-AH patients had significantly higher MPO compared to normotensive OW subjects (p < 0.001), while hsCRP did not differ between those two OW subgroups (p = 0.72) (Tab. 3).

Multivariate analysis

Multivariate analysis (binary logistic regression) with overweight, hsCRP and MPO as covariates showed that overweight was independently significantly predictive for AH, unlike hsCRP, while higher MPO increased risk for AH with statistically marginal significance (Tab. 4).

Correlations between MPO and hsCRP

Within the entire examined group (n = 75) Spearman Rank Correlation Analysis of body mass index (BMI, kg/m^2) with serum concentrations of hsCRP and with MPO showed that hsCRP and MPO positively correlated with BMI (for hsCRP correlation coefficient (ρ) = 0.44, p < 0.001; for MPO ρ = 0.24, p = 0.038).

hsCRP significantly positively correlated with BMI in hypertensive (n = 38, ρ = 0.42, p = 0.01) and in normotensive (n = 37, ρ = 0.39, p =0.01) groups respectively, while MPO did not correlate with BMI in any of these two groups (in the hypertensive group ρ = 0.18, p = 0.26, whereas in the normotensive group ρ = –0.12, p = 0.48).

Discussion

The main findings of our study include: a) significant elevation of MPO concentration in hypertensive patients, irrespective of their BMI; b) significantly increased hsCRP in overweight subjects, irrespective of AH; and c) hsCRP significantly positively correlates with BMI in all participants, irrespective of AH. Nevertheless, MPO and hsCRP were significantly increased at the same time only in patients who were both hypertensive and overweight compared to the normotensive overweight and normal weight subjects. Our results are in concordance with the data obtained in study by Heine et al. [25] in which the concentrations of MPO in the circulation positively associated with systolic and diastolic blood pressure, and in concordance with the data obtained in study by Yudkin et al. [26] which showed relationships between levels of CRP and measures of overweight in healthy subjects without hypertension.

Table 4. Binary logistic regression (Hosmer-Lemeshow goodness of fit test) for arterial hypertension (n = 38) as dependent variable (n = 75)

Covariate	p	Exp (B)	95%CI
Obesity	0.044	3.00	1.03–8.75
hsCRP [mg/L]	0.247	1.19	0.88–1.61
MPO [pmol/L]	0.069	1.00	1–1.01

hsCRP — high-sensitivity C-reactive protein; MPO — myeloperoxidase; CI — confidence interval

Our results suggest that hypertensive patients might have higher neutrophil activation than normotensive subjects, reflected by MPO increase, and also suggest potential role of inflammation in the development of AH, by MPO affecting the local vascular level of oxidative stress, thus possibly altering the synthesis and degradation of vasodilating and vasoconstricting factors, as discussed in [27].

Previous studies confirmed that inflammatory process is an integral part of AH by showing elevated concentration of CRP in hypertensive patients [28]. Moreover, it has been suggested that elevated CRP in healthy humans lead to an increased risk of developing AH [29]. CRP in arterial wall promotes expression of adhesion molecules E-selectin, cell adhesion molecules wall 1 (VCAM-1) and intercellular adhesion molecule1 (ICAM-1) on endothelial cells. Dysfunction of endothelial cells, as induced by CRP, renders reduced NO synthesis, resulting in enhanced reactivity of blood vessel wall [30].

Overweight *per se* is a condition of chronic inflammation which could contribute to a further endothelial damage, worsening the vascular disease and promoting development or maintenance of AH [31]. Thus, one of the goals of the present study was to evaluate the potential mutual interaction of overweight and hypertension.

Plasma concentration of hsCRP has been shown to be strongly associated with OW and OW-related diseases, including diabetes mellitus, and hyperlipidemia [31]. In the present study, overweight hypertensive and normotensive patients together had significantly increased hsCRP, which is in accordance with the literature [31, 32]. Moreover, there was a significant positive correlation between the concentrations of hsCRP and BMI within the entire examined group. All together, these findings support hypothesis that hsCRP is a good marker of overweight-associated inflammation, independently of the blood pressure.

Although there was a positive correlation between hsCRP and BMI in the group of hypertensive patients, there was no difference in hsCRP levels between OW and NW-AH patients. In support of this, the results of The Relationship between Blood Pressure and C-Reactive Protein in the Multi-Ethnic Study of Atherosclerosis (MESA) study [33] demonstrated that some hypertensive groups showed a greater association, while some groups showed no difference in CRP in respect with the hypertension status. MESA showed that Chinese participants had the lowest CRP concentration but the largest difference in CRP by hypertension status, followed by Caucasians and African Americans, whereas Hispanics had no significant difference in CRP by hypertension status. It might be that some other factors, besides being OW, affect the value of CRP in AH. Nevertheless, the level of systemic inflammatory markers such as CRP, white blood cell count and albumin are at least partially determined by genetic factors [34].

Adipose tissue has been characterized as a dynamic endocrine organ that produces proinflammatory cytokines. Overweight is a well-known predictor of AH and may confound the association between inflammation and hypertension [35]. However, the finding that hsCRP statistically significantly positively correlated with BMI in normotensive patients might support a previously published research which found that the protein may be released by adipocytes. Our data also showed a positive correlation between hsCRP and BMI in normotensive group. Therefore, it can be concluded that overweight, as a chronic persistent process, affects CRP levels, thus increasing the risk of developing CV disease in normotensive participants, i.e. independently of AH. In support of this thesis is large prospective epidemiological study that have shown that plasma level of hsCRP was a strong independent risk for a future myocardial infarction, stroke, peripheral arterial disease and vascular death among individuals without known previous CV disease [36].

While concentration of MPO activity was found significantly elevated in hypertensive patients in comparison with the normotensive, regardless of BMI, high-sensitivity C-reactive protein between those groups were different only in respect to BMI. That means that only OW-AH patients had significantly higher hsCRP compared to the NW-normotensive subjects, while hsCRP did not differ between OW-AH patients and OW-normotensive subject, suggesting that the association between hsCRP and OB could be stronger in hypertensive individuals, which makes it more difficult to distinguish independent effects of inflammation or overweight on AH.

Our results showed a positive correlation between BMI and hsCRP in hypertensive and normotensive patients, but with no impact on MPO, suggesting that the increase in body weight induces endothelial inflammation processes, which may contribute to the development of hypertension.

Taken together, the results of our study lead to the conclusion that the effect of hsCRP and MPO in the development of AH might be strengthened by the increase in body weight, due to the observed fact that only patients who are hypertensive and overweight at the same time had significantly increased both hsCRP and MPO compared to all normotensive

subjects, regardless of their BMI. Additionally, the findings could reflect that overweight might participate in the development of AH due to the proinflammatory processes.

Conclusion

In the present study, MPO was significantly higher in patients with AH, irrespectively of BMI, which indicates neutrophilic activation concomitant to hypertension. OW is associated with proinflammatory effects, as evident by increase in hsCRP in OW participants (both hypertensive and normotensive), but without effect on leukocyte activations in any of the studied groups. Only in patients with both hypertension and overweight, there were significantly higher concentrations of MPO and hsCRP compared to the healthy normal weight subjects who had either AH or OW solely. Taken together, our data provide evidence that inflammation may be an important link between overweight and AH and that MPO and hsCRP may serve as good markers for inflammatory processes common to these two diseases. Altogether, results suggest that inflammation may mediate mutual association of AH and OW, suggesting MPO as an inflammatory marker for AH and hsCRP for overweight.

Ethical Standards

The study protocol and procedures conformed to the standards set by the latest revision of the *Declaration of Helsinki*. The study was approved by the institutional Ethical Committee and by Ethical Committee of the Faculty of Medicine Osijek, University Josip Juraj Strossmayer Osijek, Croatia.

Informed consent

Informed consent was obtained from all patients for being included in the study.

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Neck circumference, visceral adiposity, and hypertension: does upper body adiposity outperforms visceral adiposity in terms of hypertension predictions?

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Abstract

Background: This study set up to determine which of the neck circumference (NC), as a predictor of upper body sub-cutaneous fat, or visceral adipose tissue, as an indicator of intra-abdominal fat mass, can be the better predictor of hypertension.

Material and methods: 130 overweight/obese women took part in this cross-sectional study conducted in November 2017. Blood pressure, anthropometric measurements, and body composition were determined. Pearson's correlation coefficients, multivariate logistic regression, and the area under the curve of the receiver operator characteristic curves analyses were performed.

Results: Mean age, weight, and neck circumference were 39.93 ± 8.71 years, 74.26 ± 9.86 Kg, and 35.06 ± 1.74 cm, respectively. There was a significant correlation between neck circumference and visceral adipose tissue with systolic blood pressure ($r = 0.32$, $p = 0.001$) ($r = 0.57$, $p < 0.001$) and diastolic blood pressure ($r = 0.23$, $p = 0.008$) ($r = 0.45$, $p < 0.001$), in the respective order. According to the results of the ROC curve analysis, visceral adipose tissue and neck circumference predicted hypertension with an accuracy of 81 and 65 percent, respectively. In addition, the probability of having increased blood pressure increased with higher visceral adipose tissue (OR = 1.22, $p < 0.001$).

Conclusions: According to our findings, abdominal obesity and high NC in implication with overweight or obesity can more exactly evaluate hypertension risk.

Key words: obesity; hypertension; neck circumference; visceral adiposity; body fat distribution

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Introduction

Hypertension is one of the risk factors to predict cardiovascular diseases (CVD) including ischemic or hypertensive heart disease, as well as chronic kidney diseases [1]. Globally, 1.4 billion people had hypertension in 2010 [2], and is anticipated to affect 30% of the worldwide population by the year 2025. Hence, hypertension remains as one of the serious issues of medical and public health and its burden is

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remarkably greater than before throughout the world both in developed and developing countries [2].

The causes of elevated blood pressure (BP) are multifarious and related to numerous environmental and genetic factors [3]. Some studies have reported that obesity and adiposity are the common risk factors for hypertension [4–6]. Several epidemiological studies also demonstrated significant associations between anthropometric measurements and hypertension [7, 8]. It has been shown that waist circumference (WC) significantly associated with abdominal adiposity and it is also highly correlated with CVD risk factors [9]. This anthropometric tool is generally an acceptable measure, but it has some drawbacks, for instance, the anatomic marker of waist size could not be observed easily and could vary greatly particularly in obese persons, which is influenced by nutrition, breathing, and diseases. Furthermore, the clients may not be comfortable being measured by exposing the midsection and their privacy should be respected [10].

Recently, neck circumference (NC) has received abundant attention from the researchers. It is suggested that NC would be a better measure than waist measurements since it is simple, inexpensive, not time-consuming, and not invasive. NC is also a more reliable and advantageous anthropometric parameter applied as an alternative to determine the upper body subcutaneous adipose tissue distribution [11, 12]. Numerous studies in large populations, demonstrated that NC is capable of predicting cardiovascular risk factors, fatty liver disease, type 2 diabetes, insulin resistance, and metabolic syndrome in adults [11–14].

Another measurement is visceral adipose tissue (VAT) which significantly involved in central obesity and is located in the abdomen and intra-abdominal contents [15]. VAT is known as an exceptional pathogenic fat depot and giving metabolic risk beyond normal anthropometric measures like body mass index (BMI) and WC [16]. Besides, recent studies have shown that VAT is linked to a greater atherosclerotic danger profile [17], adverse cardiovascular events [7], development of insulin resistance [16, 18], and incidence of diabetes among obese adults [19].

Studies show that NC and VAT are associated with CVD by contributing to the development of hypertension. Some former investigations have explored the relationship of NC [11, 14, and 20] and high VAT [21–23] with hypertension, and often the results were inconsistent. Also, none of these researches have compared NC with VAT, with respect to their link with BP. Early identification of pre-hypertension and hypertension will help in decreasing the incidence of hypertension in the adult population in the near future, thereby significantly reducing the hypertension-related health burden. Therefore, this study was set up; firstly, to evaluate the association of NC, as an indicator of upper body subcutaneous adipose tissue, and VAT, the key indicator of intra-abdominal fat mass, with hypertension; and secondly, to specify the most favorable cut-off points of NC and VAT to show one is better in terms of hypertension prediction.

Material and methods

Study participants

This cross-sectional study was conducted on 130 overweight/obese (BMI > 25) women aged 19–64 years that were living in Sardrood-Tabriz, Iran during November 2017. The research aims were described to participants individually before entering the study and informed written agreement was acquired from all participants. Ethical committee of Tabriz University of Medical Sciences, Tabriz, Iran, certified the study protocol (reference number: IR.TBZMED.REC.1396.291).

Anthropometric measurements

Weight and height of the participants were measured by a balance beam scale (SECA) and a portable stadiometer, with the accuracy of ± 0.1 cm and ± 0.1 kg, in the respective order; they clothed scantily wearing no shoes. NC was measured in the standing position, head at the level of the thyroid cartilage. WC was measured in the middle of the inferior rib margin and the iliac crest. Hip circumference (HC) was measured at the maximum circumference around the buttocks. A flexible measuring tape with the accuracy of ± 0.5 cm was used to measure NC, WC, and HC. In addition, waist to hip ratio (WHR) and waist-to-height ratio (WHtR) were determined.

Body composition assessments

Body composition parameters including fat mass (FM), skeletal muscle mass (SMM), and VAT were measured by the application of A hand to-hand impedance analyzer (OMRONBF511, made in Germany) [24]. They were told to empty their bladder prior to measurements. They were also requested to first wipe the sole of the feet using a damp tissue and then stand over the electrodes of the instrument. Details such as weight, height, age and gender were given as input into the machine and outputs were registered. This device was held while both arms were strained straight in front of the body.

BPs measurements and definition

BP was measured by following standard guidelines. The individual was made comfortable and sit at least for 5 minutes on chair. A mercury sphygmomanometer was used for measuring systolic blood pressure (SBP) and diastolic blood pressure (DBP) on the right arm with and stethoscope and the average of the two measurements was taken. Participants were advised not to drink alcohol, tea or coffee, smoke and to take exercise for at least 30 minutes before measuring BP. The "2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults" were used to set elevated BP (SBP between 120- and 129-mm Hg and DBP lower than 80 mm Hg) and stages 1 and 2 of hypertension (SBP of 130 mm Hg or greater or DBP of 80 mm Hg or greater) [25].

Statistical analysis

Since all the variables have normally distribution, descriptive statistics, mean and standard deviation (SD) were calculated for the quantitative variables (age, weight, BMI, NC, Fat mass, VAT, SBP and DBP). Pearson's correlation coefficients were used to study the relations between NC with SBP and DBP, as well as the link between NC, anthropometric measurements (BMI, WHtR, WHR and WC) and body composition (FM and VAT). The receiver operating characteristics (ROC) analysis was applied to define the performance and cutoffs of variables. The total biased power of a diagnostic test was shown by the ROC curves. A test appearance its curve nearer to the upside left corner. The area under the curve (AUC) is a scale of the predictive ability of test. An ideal test has an AUC of 1.0, and an AUC of 0.5 indicates the test achieves no preferable than accident. Variables sensitivity and specificity were determined for every feasible cutoff point to distinguish the most satisfactory cutoff value. The best sensitivity and specificity were the contents providing topmost amounts from the ROC curves [26]. Also, multivariate logistic regression analyses were calculated. These analyses were adapted for confusing variables such as weight and age. Odds ratios (OR) with 95% confidence intervals (CI) were determined. Statistical analyses were done by SPSS version 23. P values less than 0.05 were considered as statistically significant.

Results

In our studied women, the mean of age, weight and NC were 39.93 ± 8.71, 74.26 ± 9.86 and 35.06 ± 1.74, respectively. Sixty-three-point one percent (n = 82) of the participants had elevated BP or hypertension. Table 1 presents other descriptive and anthropometric measures of the study population.

Table 1. Clinical characteristics of the study participants (n = 130)

Variables	Mean (SD)
Age [years]	39.93 (8.71)
Weight [kg]	74.26 (9.86)
Height [cm]	155.46(5.12)
BMI [kg/m^2]	30.65 (3.94)
NC [cm]	35.06 (1.74)
Fat mass (%)	43.81 (4.94)
VAT	8.47 (2.008)
SBP [mm Hg]	114.00 (14.93)
DBP [mm Hg]	75.38 (9.61)

BMI — body mass index; NC — neck circumference; VAT — visceral adipose tissue; SBP — systolic blood pressure; DBP — diastolic blood pressure

Pearson's correlation coefficients between BP and independent variables are shown in Table 2. According to the results, age ($r = 0.59$, $p < 0.001$) ($p = 0.53$, $p < 0.001$), BMI ($r = 0.37$, $p < 0.001$) ($r = 0.25$, $p = 0.004$), WC ($r = 0.34$, $p < 0.001$) ($r = 0.20$, $p = 0.01$), NC ($r = 0.32$, $p < 0.001$) ($r = 0.23$, $p = 0.008$), FM ($r = 0.26$, $p = 0.003$) ($r = 0.20$, $p = 0.01$), and VAT ($r = 0.57$, $p < 0.001$) ($r = 0.45$, $p < 0.001$) were considerably associated with SBP and DBP, in the respective order.

Table 3 presents that using the ROC Curve Analysis, VAT ≥ 10 with 81% accuracy were calculated to be the best cutoff levels to detect patients with hypertension for overweight and obese women (95% CI: 0.74–0.88). In addition, NC cutoff values for hypertension were determined to be ≥ 38 cm with 65% accuracy (95% CI, 0.56–0.76). FM cutoff values for overweight and obesity were determined to be ≥ 44 % with 65% accuracy (95% CI: 0.56–0.75). The ROC Curve of NC, BMI, FM and VAT presented in Figure 1.

As shown in Table 4, the odds of having elevated BP increased significantly with higher VAT ($OR = 1.22$, $p < 0.001$), following the adjustment for weight and age. In the other word, the participants with higher VAT significantly had a greater risk of having elevated BP.

Discussion

It is very important that we recognize the elevated BP, before progress to hypertension. To our best knowledge, this study is the first to investigate the

Table 2. Pearson's correlation coefficients between variables and blood pressure; Diastole blood pressure

Variables	SBP		DBP	
	r	p	r	p
Age [years]	0.59	**< 0.001****	0.53	**< 0.001****
Weight [kg]	0.22	**0.01***	0.13	0.11
Height [cm]	–0.21	**0.01***	–0.19	**0.02***
BMI [kg/m^2]	0.37	**< 0.001****	0.25	**0.004***
WC [cm]	0.34	**< 0.001****	0.20	**0.01***
HC [cm]	0.21	**0.01***	0.09	0.30
WHR	0.27	**0.002***	0.20	**0.01***
WHtR	0.40	**< 0.001****	0.26	**0.002***
NC [cm]	0.32	**< 0.001****	0.23	**0.008***
FM (%)	0.26	**0.003***	0.20	**0.01***
SMM (%)	–0.21	**0.01***	-0.17	**0.04***
VAT	0.57	**< 0.001****	0.45	**< 0.001****

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed); BMI — body mass index; NC — neck circumference; WC — waist circumference; HC — hip circumference; WHR — waist to hip ratio; WHtR — waist-to-height ratio; FM — fat mass; SMM — skeletal muscle mass; VAT — visceral adipose tissue; SBP — systolic blood pressure; DBP — diastolic blood pressure

Table 3. Area under the curve (AUC), sensitivity, specificity and cutoff points for determining the hypertension, in overweight and obese women, with using ROC analysis

Variables	AUC	(95% CI)		Cutoff	Sensitivity (%)	Specificity (%)
		Lower	Upper			
Age [years]	0.82	0.75	0.89	≥ 47	52.08	90.24
Weight [kg]	0.65	0.56	0.76	≥ 74.4	66.67	68.39
BMI [kg/m^2]	0.71	0.62	0.81	≥ 34.6	39.58	90.24
WC [cm]	0.68	0.59	0.78	≥ 105	60.42	75.61
HC [cm]	0.63	0.53	0.73	≥ 116	29.17	91.46
WHR	0.63	0.53	0.73	≥ 0.99	27.08	84.15
WHtR	0.68	0.59	0.78	≥ 0.71	35.42	89.02
NC [cm]	0.65	0.56	0.76	≥ 38	18.75	97.56
FM (%)	0.65	0.56	0.75	≥ 44	66.67	65.65
VAT	0.81	0.74	0.88	≥ 10	54.17	89.02
RMR (%)	0.63	0.53	0.73	≥ 1589	14.58	96.34

BMI — body mass index; NC — neck circumference; WC — waist circumference; HC — hip circumference; WHR — waist-to-hip ratio; WHtR — waist-to-height ratio; FM — fat mass; VAT — visceral adipose tissue; AUC — area under the curve

links between NC and VAT with hypertension, in Iranian overweight/obese women. Our findings revealed that there was a relationship between NC with SBP and DBP. This result was in accord with previously published data in Asian and European populations on different age groups and sample sizes [27, 28]. In the study on Chinese population, Zhou et al. found that NC had significant association with BP and hypertension [11]. In another study, Assyov et al. reported that there was a positive association between NC and hypertension in univariate analysis. Though, when adjusted for age and WC, the association lost its statistical significance in females [29]. The results of a cross-sectional study in the US also demonstrated that in each category of BMI, participants with high NC had a Greater risk for high BP [30]. The specific mechanisms justifying the relations of NC with hypertension are not completely confirmed. It has recommended that upper-body subcutaneous fat potency influences the arterial BP and the progress of hypertension by discharging considerable amounts of systemic free fatty acid, which could to bring vascular damage, aggravate endothelial cell dysfunction and insulin resistance, and increase oxidative stress and very-low-density lipoprotein cholesterol production [31–34].

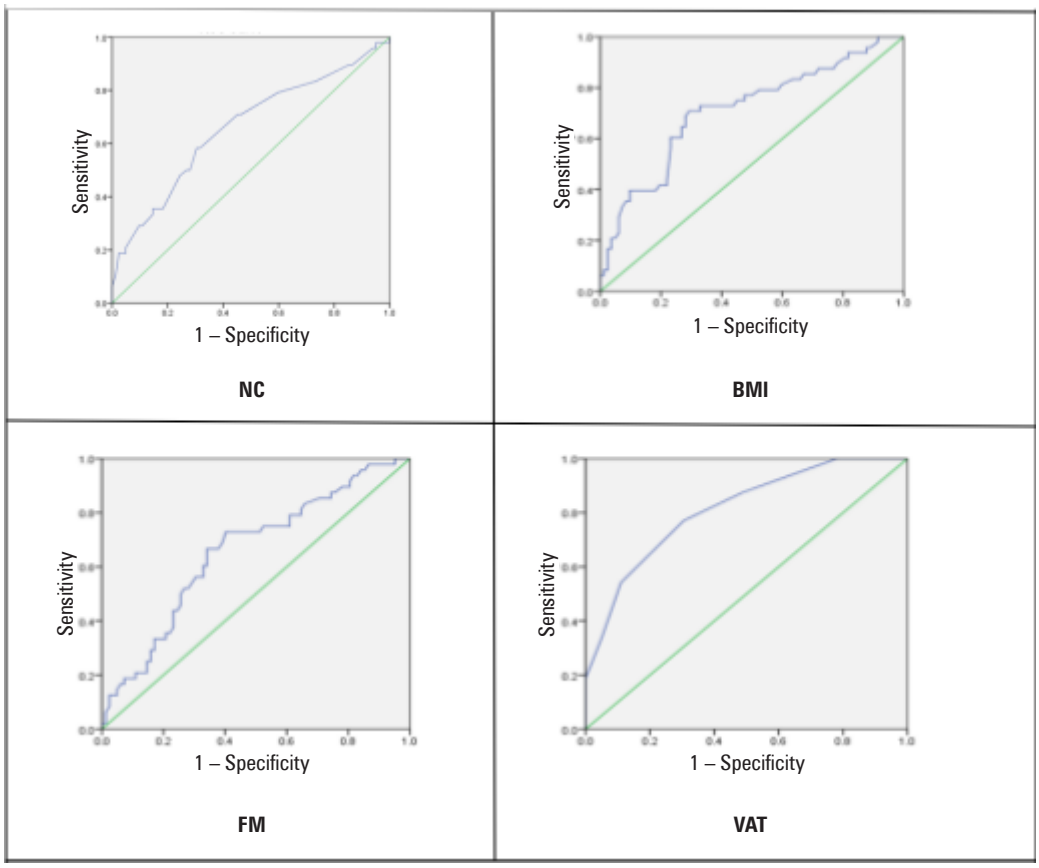

Figure 1. The ROC curve of neck circumference (NC), body mass index (BMI), fat mass and visceral adipose tissue (VAT)

Table 4. Multivariate logistic regression analysis of associations between the selected risk factors and hypertension

Variables	β	OR	CI	p value
Height [cm]	–0.23	0.78	0.24–2.56	0.69
BMI [kg/m^2]	0.12	1.13	0.30–4.26	0.85
WC [cm]	0.37	1.46	0.11–17.92	0.76
HC [cm]	0.28	1.33	0.44–4.01	0.60
WHR	17.83	1.1	0–2.44	0.78
WHtR	–64.11	0.00	0–8.78	0.70
NC [cm]	–0.245	0.78	0.54–1.11	0.17
FM (%)	0.20	1.23	0.92–1.64	0.15
SMM (%)	0.45	1.57	0.84–2.93	0.15
VAT	1.47	1.22	0.11–1.44	**< 0.001***
SMM (%)	0.45	1.57	0.84–2.93	0.15
RMR	–0.01	0.98	0.94–1.03	0.56

Adjusted for age and weight; *Correlation is significant at the 0.05 level (2-tailed); BMI — body mass index; NC — neck circumference; WC — waist circumference; HC — hip circumference; WHR — waist-to-hip ratio; WHtR — waist-to-height ratio; FM — fat mass; SMM — skeletal muscle mass; VAT — visceral adipose tissue; SBP — systolic blood pressure; DBP — diastolic blood pressure

Findings about the association of independent variables and hypertension using ROC Curve Analyses demonstrated that VAT is a strong predictor of hypertension and it has finest cut-off points with best-balanced specificity and sensitivity for the hypertension. We evaluated the prognostic power of anthropometric measurements for hypertension and found that the AUC for VAT was more than AUC for total body fat mass and other anthropometric indices. Similar to current study, Cassano reported

an epidemiological association between adiposity and hypertension [35]. Also, the results of George researches support our research hypotheses: there was a link between measures of central adiposity with larger risks (50–65%) of hypertension than total adiposity (44–45%) [36].

Results of our study about odds ratios for factors associated with hypertension highlights that increased VAT was robustly associated with elevated BP. Similar to our study, in a cohort of Japanese Americans, the intra-abdominal fat area was reported as a major risk factor for hypertension, even after adjustment for WC, abdominal subcutaneous fat area and total subcutaneous fat area [21]. In addition, Dallas Heart Study revealed that both baseline and gain of visceral fat were linked to a greater relative risk for hypertension. In this manner, gain of visceral and abdominal subcutaneous fat was also considerably linked to higher SBP, even after 7 years follow up [15, 22]. Likewise, in a middle-aged Chinese population, there was a significant link between excess VAT and higher risk of hypertension and prehypertension [23]. Some studies proposed that local influences from fat surrounding the kidneys might influence the progress of hypertension [15].

Although this study is limited by the cross-sectional nature and small sample size, it does provide direction and insight for future researchers to build upon. Additional large-scale, prospective studies could assistance to improve explain and confirm associations between anthropometric measurements and hypertension.

Conclusion

The current study reported the value of the interactions of various anthropometric indices of obesity for assessing the risk of hypertension. As BMI is a weight-for-height measure, it is not capable of showing the difference between FM and FFM. Moreover, WC measurements are not capable of differentiating VAT and subcutaneous adipose tissue. In fact, abdominal obesity and high NC in implication with overweight or obesity can more exactly evaluate hypertension risk. Our findings also propose that advanced imaging tools can prepare a more detailed phenotypic characterization of obesity than usual anthropometric indices, consenting greater distinction of hypertension and cardiovascular complications. Also, we propose that treatments pointed at redistribution of fat mass; away from the VAT toward the lower body subcutaneous depot. This manner may be more helpful than only aiming decrease body mass, for stopping CVD in obesity.

Conflict of interests

There are no conflicts of interest in terms of the publication of this paper.

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Authorship

M.E.V. and M.A. designed research; M.E.V. conducted research; L.F.G. analyzed data; and M.E.V., R.M.G. and M.A. wrote the paper. The final manuscript was read and approved by all authors.

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Blood pressure lowering effects of alpha-lipoic acid supplementation: a meta-analysis of randomized controlled trials

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Abstract

Background: The aim of the present meta-analysis was to detect the effect of α-lipoic acid (ALA) supplementation on systolic and diastolic blood pressure (BP).

Material and methods: The related records were selected from several electronic databases from the earliest date 1980 until October 2019. The heterogeneities were assessed by I2 test (I2 < 50%) and χ^2 test on Cochrane's Q statistic. Standardized mean difference (SMD) and their 95% confidence intervals (CIs) were considered for net change in systolic blood pressure (SBP) and diastolic blood pressure (DBP). Subgroup analyses were also conducted by baseline BP, health status, doses of supplementation, study duration and supplement utilization.

Results: As a result, a total of 10 studies with 612 subjects were included in the final analysis. Alpha-lipoic acid supplementation significantly reduced SBP (SMD = –0.50, 95% CI: –0.84, –0.16, p = 0.004) and DBP (SMD = –0.40, 95% CI: –0.71, –0.09, p = 0.01), compared to the controls, with the reduction of 6.1 mm Hg and 3.6 mm Hg of the mean SBP and DBP, respectively. Heterogeneities were explored in both SBP and DBP. Moreover, a statistically significant reduction in BP was detected in elevated BP and hypertensive patients as compared with the normotensive subjects.

Conclusion: ALA supplementation could be considered as a BP-lowering agent, especially in subjects with higher blood pressure.

Key words: α-lipoic acid; blood pressure; systematic review; meta-analysis; RCT

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Introduction

Alpha lipoic acid (ALA), as a naturally occurring dithiol compound and a organosulfur compound is essential as a cofactor for mitochondrial α-ketoacid dehydrogenases [1].

It is synthesized by the liver [2] and is present in animal and vegetable sources [3]. The antioxidant effect of ALA has been demonstrated by the ability of ALA to clear reactive oxygen species (ROS) and activating the endogenous antioxidant system [4, 5]. Additionally, it has been shown that ALA could improve endothelium function and play a role in nitric oxide synthesis [6, 7]. According to the latter role, different studies were conducted in vivo, which emphasizes the effect of ALA supplementation on blood

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pressure (BP) [4, 8, 9]. It is well established that hypertension condition (BP) can results in several cardiovascular disorders while decreasing blood pressure reduces cardiovascular risk, myocardial infarction, heart failure, and stroke [10, 11]. The administration of different doses of ALA has been examined in several studies. Ergür et al. conducted a study in which ALA was administered orally in rats at a dosage of 100 mg/kg, and reported a significant reduction of secondary hypertension [8]. However, the findings of human trials are controversial [12–14]. In a recent study by Mohammadi et al., the daily supplementation of ALA with a dose of 600 mg on subjects with cardiovascular risk factors and chronic spinal cord injury revealed the systolic and diastolic blood pressure lowering effects [12]. However, other trials did not observe any significant blood pressure-lowering effect of ALA supplementation, after four months of oral supplementation with 800 mg ALA [15] and after twenty months of oral supplementation with 1800 mg ALA [16]. Although there is a systematic review showed that supplementation with ALA did not appear to be efficient on BP [4], we conducted the present meta-analysis to better clarify the potential effect of ALA supplementation on BP.

Material and methods

We carried out the present meta-analysis and designed the strategies according to the PRISMA guidelines [17].

Literature search strategy

Several electronic databases including Pubmed™, Scopus™, EMBASE™, and Google Scholar™ were searched from inception until October 2019. In addition to electronic searching, we tried to handsearch the reference list and citations of papers to detect more potential eligible studies. Search terms used were as follow: (ALA OR "α-lipoic acid" OR "lipoic acid" OR "alpha lipoic acid") AND ("Hemodynamic parameters" OR "Blood pressure" OR "Systolic blood pressure" OR "Diastolic blood pressure" OR "BP" OR "SBP" OR "DBP").

Selection criteria

To be included for meta-analysis, the studies had to meet the following inclusion criteria: 1) being a trial in human species with either cross-over or parallel design, 2) providing the possible effects of ALA supplementation on either systolic or diastolic blood pressure, 3) reporting sufficient data on SBP/DBP including baseline/end of supplementation or mean changes and related standard deviations in both intervention and placebo groups, 4) the study had to use a control or placebo group for the treatment group and, 6) subjects had to take ALA supplements for at least 2 weeks. Studies were excluded according to the following criteria: 1) lack of a control group, 2) lack of adequate data regarding SBP or DBP in each group, or lack of required data for computing the indices

Data abstraction

The data from all included articles were extracted independently by two authors. Any possible disagreement was solved by a third author through consensus and discussion. The following data was obtained from the each of eligible studies: author identification, publication year, study design and location, duration of supplementation and follow-up, the dose of ALA supplementation, the sample size in both intervention and control groups, demographic indices (age, gender), clinical condition, baseline SBP, and DBP values, and observed significant outcomes.

Validity assessment

We estimated the quality of studies according to the Jadad scale with the following criteria: (1) randomization (one score for mentioning random allocation and one more score for explaining the method of randomization appropriately), (2) blinding (One score for stating that the trial was blinded and one more score for describing the method of blinding properly, and (3) reporting of dropouts, in addition to reasons for withdrawals (one score for reporting of dropouts and the withdrawal reasons). The total score varies between 0 to 5, in which the trials with the score of ≥ 3 are considered as high-quality trials.

Data synthesis

We analyzed the data using two software including RM Software (Review Manager 5.3) and Biostat Comprehensive Meta-Analysis. We defined the treatment effects by standardized mean difference (SMD) and 95% confidence intervals (CIs) of outcomes. Moreover, we assessed the possible heterogeneity by χ^2 test on Cochrane's Q statistic and I2 test, by which $p < 0.05$ or $I2 > 50\%$ was considered as heterogeneous. We used the random-effects model to calculate the pooled effect size. Moreover, we conducted subgroup and sensitivity analyses in accordance with the Cochrane guidelines for exploring any possible sources of heterogeneity between included studies [18]. We performed the sensitivity analysis by removing a single included trial and re-calculating the effect

size to detect any potential effect on the final overall effect size [19].

To find any potential publication biases, Begg's rank correlation test, funnel plots, and Egger's regression test were used. A p value < 0.05 was considered as statistically significant.

Results

Selection of trials

Figure 1 shows the process of trial selection. In general, a number of 218 articles were primarily detected, in which 194 articles were excluded due to duplication (n = 101) or were irrelevant to the current meta-analysis including non-original research (letters, case reports, and series, reviews, experimental or animal studies) (n = 93). More studies were excluded because of the following reasons: inappropriate reporting data on SBP or DBP, supplementation of ALA in less than two weeks, improper study design such as non-randomized trial, and lack of control group. Finally, ten studies were included in the meta-analysis [12–16, 20–24].

Characteristics of included trials/quality assessment

The descriptions of all included studies of the current meta-analysis are shown in Table 1. The year of publication of included studies varied between 1997 and 2019, of which five studies were conducted in Iran [12–14, 21, 24], two in the USA [20, 22], and the remaining included studies were carried out in the Republic of Korea [16], Italy [23] and Germany [15].

Totally, ten clinical trials with 612 subjects (Intervention, n = 311, and control, n = 301), were included in the present synthesis. The number of participants in included trials ranged from 7 to 82 subjects. Supplementation duration was between 8 weeks to 20 weeks, and the dose of supplementation varied from 300 to 1800 mg/d. Of the ten included trials, five recruited Type 2 Diabetic patients [13–16, 20], one trial was performed in chronic spinal cord injury patients [12], one trial included subjects with stroke [24], two studies elected participants with obesity [16, 23], the patients with metabolic syndrome and coronary artery disease were used in one study [22] and one more trial was conducted in patients with rheumatoid arthritis [21]. The mean age of subjects varied between 11.5 to 62.3 years. Among included trials, in accordance with the updated guidelines of the American College of Cardiology/American Heart Association (ACC/AHA); two have been categorized as elevated blood pressure subjects [20, 21], one study as normotensive subjects [23], and seven remaining studies as hypertensive patients which ranged from 117 to 144 and 69.4 to 87.85 for systolic and diastolic blood pressure, respectively [12–16, 22, 24]. The quality of studies was assessed using the Jadad score scale. According to Jadad scale method, all included studies were categorized as high-quality studies (Tab. 2). Six out of 10 included trials described the blinding method

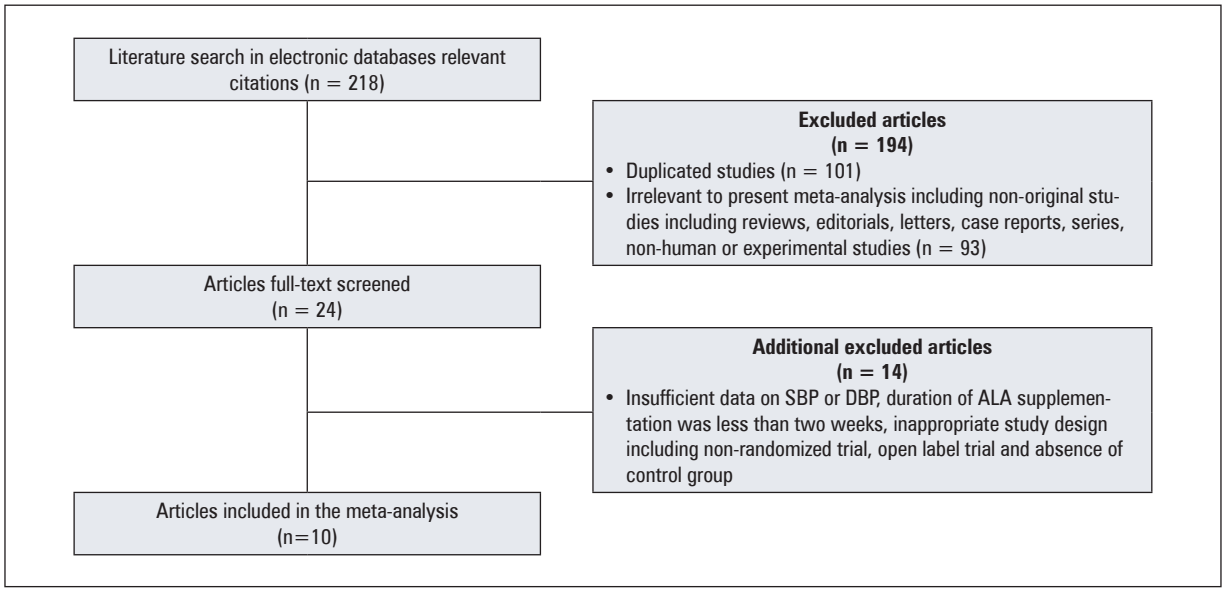

Figure 1. The flow diagram of literature search and study selection of the meta-analysis. SBP — systolic blood pressure; DBP — diastolic blood pressure; ALA — α-lipoic acid

Table 1. Characteristics of included research

Author	Year	Design of studies	Country	No. of subjects in case group	No. of controls	Gender	Age of intervention (mean) ± SD	Follow-up duration (weeks)	Clinical condition	Dosage [mg/d]	Significant outcome	Baseline BP (SBP/ /DBP)
Koh	2011	Randomized, double-blind, placebo-controlled	Republic of Korea	82	73	M F	41.4	20	Obese, HT, DM, or hypercholesterolemia	1800	Treatment with ALA was not associated with significant reductions in BP	132.97 82.4
Lukaszuk	2009	Double-blind study pre-/post-test control group	USA	13	7	M F	56	13	T2DM	600	There were minor, not significant, downward trends in the ALA group (as opposed to upward trends in the placebo group) in DBP	127.6 78.3
Mazloom	2009	Randomized, double-blind, placebo-controlled trial	Iran	29	28	M F	49	8	T2DM	300	A significant decrease in both SBP and DBP at the end of study in the ALAd	133.4 80.1
McMackin	2007	Double-blind, placebo-controlled, crossover	USA	15	21	M F	62	8	Coronary artery disease, metabolic syndrome	400 mg of ALA and 1000 mg of acetyl-L-carnitine	Active treatment tended to decrease SBP for the whole group	131 71
Mohammadi	2015	Randomized, double-blind, placebo-controlled clinical	Iran	28	30	M	39	12	Chronic spinal cord injury	600	There was significant reduction in BP	126.43 87.85
Mohammadi	2018	Randomized, double-blind, placebo-controlled clinical	Iran	33	34	M F	62.33	12	Stroke	600	After the intervention period, SBP, DBP reduced in ALA group compared with placebo group, significantly	133.18 84.24
Noori	2013	Randomized, double-blind, placebo controlled trial	Iran	17	17	M F	60.0	12	Diabetic nephropathy	800 mg ALA and 80 mg pyridoxine	SBP decreased significantly in the supplement group compared to the placebo group	142 77
Pourghasem	2015	Randomized, double-blinded, placebo controlled clinical trial	Iran	33	32	F	36.09	8	Rheumatoid arthritis	1200	SBP and DBP in ALA group was significantly decreased in comparison with placebo group ($p < 0.05$). But, CRP and IL-6 serum levels did not indicate any significant within- and between group changes	121.59 77.04
Tromba	2019	Double-blind, placebo-controlled randomized trial	Italy	32	32	M F	11.5	12	Overweight/obese children	800	There were no significant differences within each group for BP	117 69.4
Ziegler	1997	Randomized, double-blind placebo controlled multicenter trial	Germany	29	27	M F	57.9	4 months (16 wks)	NIDDM patients with cardiac autonomic neuropathy	800	Mean BP did not differ between the groups at baseline and during the study	144 81.4

HT — hypertension; DM — diabetes mellitus; T2DM — type 2 diabetes mellitus; NIDDM — non-insulin-dependent diabetes; ALA — alpha-lipoic acid; BP — blood pressure; DBP — diastolic blood pressure; SBP — systolic blood pressure; CRP — C-reactive protein; IL-6 — interleukin 6

Table 2. Quality of the 10 studies as assessed by the Jadad score

Study [year]	Blinding	Randomization	Withdrawals and dropouts descriptions	Score
Koh [2011]	2	2	1	5
Lukaszuk [2009]	2	1	1	4
Mazloom [2009]	1	2	1	4
McMackin [2007]	2	2	1	5
Mohammadi [2015]	1	2	1	4
Mohammadi [2018]	2	2	1	5
Noori [2013]	1	2	0	3
Pourghasem [2015]	2	2	1	5
Tromba [2019]	2	2	1	5
Ziegler [1997]	1	2	1	4

appropriately [16, 20–24] and most of the included studies (9 of 10) provided a sufficient and acceptable description of the method of randomization [12–16, 21–24]. All studies except one study [13] stated the dropouts descriptions and the associated reasons.

Blood pressure-lowering effects of ALA supplementation

The synthesis was carried out based on the data of 612 participants from 10 clinical trials reporting blood pressure values (intervention, n = 311, and placebo, n = 301). As it has been shown in Figure 2, ALA supplementation statistically significantly reduced both SBP (SMD = –0.50, 95% CI: –0.84, –0.16, p = 0.004) with the reduction of 6.1 mm Hg in the SBP mean and DBP (SMD = –0.40, 95% CI: –0.70, –0.09, p = 0.01) with the reduction of and 3.6 mm Hg in the DBP mean. Additionally, a significant heterogeneity was observed between the included studies regarding both SBP and DBP (SBP: p < 0.001, I2 = 74% and DBP: p < 0.001, I2 = 69%). According to the Cochrane guidelines, we conducted a stratified analysis to detect possible sources of heterogeneity.

Stratified analysis

Stratified analyses according to baseline blood pressure, follow-up duration, the dosage of supplementation, clinical conditions, and supplement utilization were conducted to explore the effect of ALA supplementation on blood pressure (Tab. 3). In accordance with the updated guidelines of the American College of Cardiology/American Heart Association (ACC/AHA), three subsets were investigated as normotensive, elevated BP, and hypertensive patients. Considering the baseline blood pressure, subgroups of elevated BP and hypertensive patients showed statistically significant reduction in blood pressure as compared with normotensive patients [elevated BP: SMD of DBP, –0.6 (95% CI: –1.04, –0.16, p = 0.008), hypertensive subjects: SMD of SBP, –0.64 (95% CI: –1.03, –0.25, p = 0.001), SMD of DBP, –0.44 (95% CI: –0.83, –0.05, p = 0.03)].

The supplementation duration was separated into ≤ 12 weeks and > 12 weeks. With respect to clinical condition of subjects, two specific groups were divided to non-diabetic/diabetic subjects. As far as the dose of supplementation was considered, higher-dose (> 600 mg/day) and lower-dose (≤ 600 mg/day) were separated as two distinct subsets. As presented in Table 3, There was a considerable reduction in subgroups of studies categorized in respect to duration of supplementation of ≤ 12 weeks [SMD of SBP, –0.67 (95% CI: –1.10, –0.23, p = 0.003), SMD of DBP, –0.52 (95% CI: –0.92, –0.13, p = 0.01)], ALA dose of ≤ 600 mg/day weeks [SMD of SBP, –0.80 (95% CI: –1.22, –0.37, p < 0.001), SMD of DBP, –0.71 (95% CI: –1.14, –0.29, p < 0.001)], and non-diabetic subjects [SMD of SBP, –0.62 (95% CI: –1.22, –0.02, p = 0.04), SMD of DBP, –0.63 (95% CI: –1.15, –0.11, p = 0.02)]. Subgroup analysis according to different utilization (single/multi-component) of supplements revealed both systolic and diastolic-lowering effect of single component supplementation [SMD of SBP, –0.48 (95% CI: –0.87, –0.08, p = 0.02), SMD of DBP, –0.46 (95% CI: –0.82, –0.10, p = 0.01)].

Sensitivity analysis

We performed a sensitivity analysis to investigate the effect of each trial on the estimated overall pooled effect size. Removal of each study and re-calculating the effect size did not show any significant alteration in the overall effects of ALA supplementation on SBP

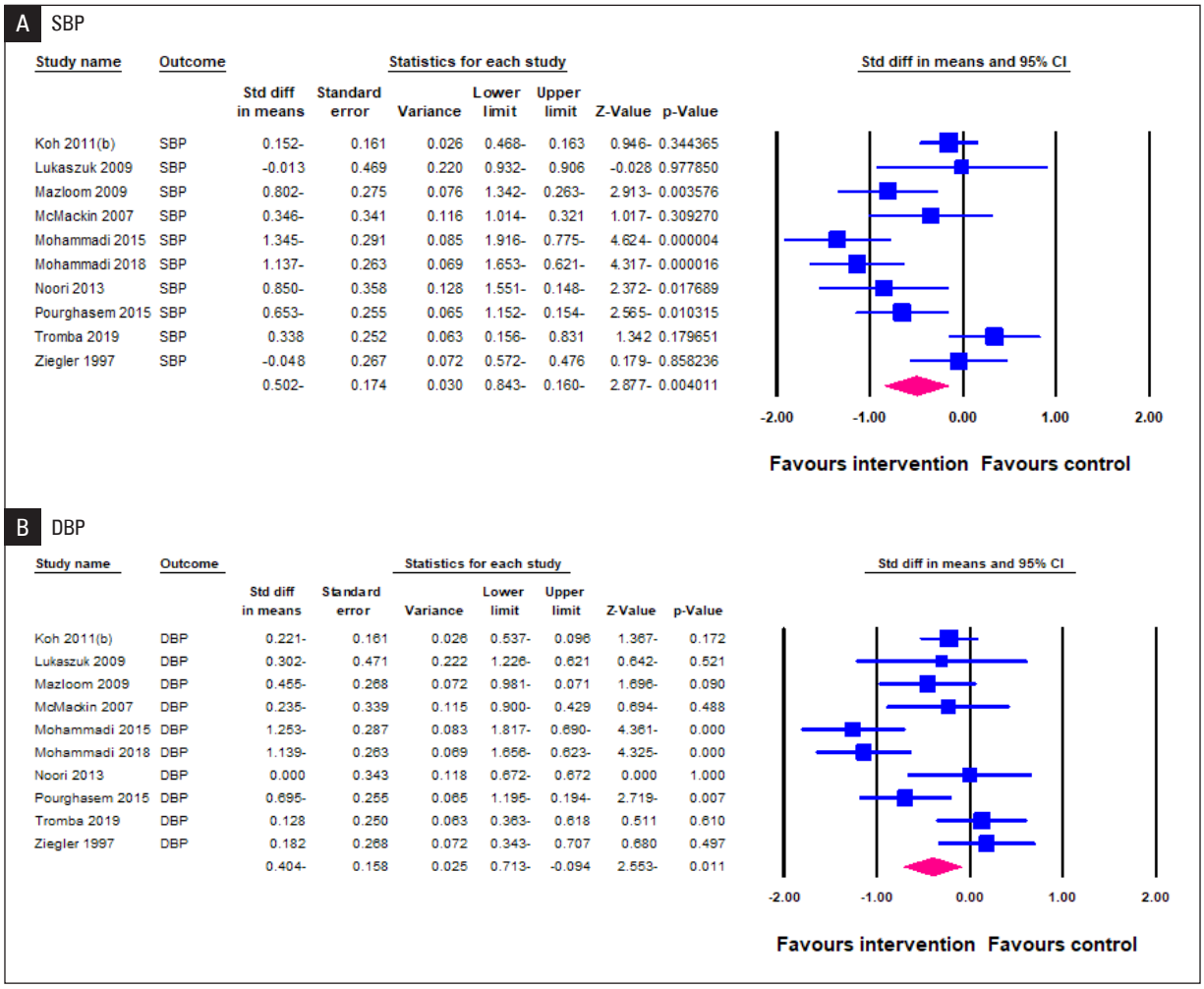

Figure 2. Forest plot of comparison of blood pressure between ALA supplementation and control groups. **A.** Systolic blood pressure (SPB); **B.** Diastolic blood pressure (DBP). Random effects model was used to pool the standard mean differences of indicators. CI — confidence interval; I — squared inconsistency

with the range of –0.40 (95% CI = –0.72, –0.08) to –0.60 (95% CI = –0.92, –0.27) and DBP with the range of –0.31 (95% CI = –0.59, 0.02) to –0.47, 95% CI = –0.79, –0.15) (Fig. 3).

Publication bias

We investigated the publication bias of the present meta-analysis by the funnel plot analysis. As it has been shown in Figure 4**,** the symmetrical pattern of funnel plots regarding to both SBP and DBP hypnotized that there are no specific potential biases in the included trials. Moreover, Egger's linear regression for both SBP (intercept: –1.98; standard error: 2.41; 95% CI: –7.54, 3.58; t = 0.82, df = 8; two-tailed p = 0.43) and DBP (intercept: –0.71; standard error: 2.31; 95% CI: –6.05, 4.62; t = 0.30, df = 8; two-tailed p = 0.76) confirm the finding. Additionally, Begg's rank correlation test did not explore potential publication bias (SBP: Kendall's Tau with continuity correction: –0.22; z = 0.89; two-tailed p = 0.37); DBP: Kendall's Tau with continuity correction: –0.08; z = 0.35; two-tailed p = 0.72).

Discussion

This is the comprehensive systematic review and meta-analysis which summarizes the data from 10 trials involving a total of 612 subjects.

The results of the present meta-analysis reveal that ALA supplementation causes a significant reduction in DBP in elevated BP subjects and a reduction in SBP and marginally DBP in hypertensive subjects. The definition and explanation of hypertension have been changed in the past years. One of the most recent definitions belongs to the American College of Cardiology and American Heart Association (ACC/AHA). This is an updated version of the guidelines

Table 3. Subgroup analysis

			WMD (95% CI)	Test for overall effect	Test for heterogeneity	I^2 (%)
Baseline BP	Normal	SBP	0.33 [–0.16, 0.83]	p = 0.19	Not applicable	Not applicable
		DBP	0.13 [–0.36, 0.62]	p = 0.61	Not applicable	Not applicable
	Elevated	SBP	–0.45 [–1.02, 0.12]	p = 0.12	p = 0.24	29
		DBP	–0.60 [–1.04, –0.16]	p = 0.008	p = 0.46	0
	Hypertension	SBP	–0.64 [–1.03, –0.25]	p = 0.001	p < 0.001	74
		DBP	–0.44 [–0.83, –0.05]	p = 0.03	p < 0.001	74
Follow-up duration [weeks]	≤ 12	SBP	–0.67 [–1.10, –0.23]	p = 0.003	p < 0.001	76
		DBP	–0.52 [–0.92, –0.13]	p = 0.010	p = 0.001	72
	> 12	SBP	–0.11 [–0.37, 0.14]	p = 0.39	p = 0.92	0
		DBP	–0.13 [–0.39, 0.13]	p = 0.34	p = 0.42	0
Dosage [mg/d]	≤ 600	SBP	–0.80 [–1.22, –0.37]	p < 0.001	p = 0.06	57
		DBP	–0.71 [–1.14, –0.29]	p < 0.001	p = 0.05	58
	> 600	SBP	–0.23 [–0.59, 0.13]	p = 0.21	p = 0.03	64
		DBP	–0.14 [–0.43, 0.16]	p = 0.36	p = 0.11	47
Clinical condition	Diabetic patients	SBP	–0.35 [–0.69, –0.01]	p = 0.04	p = 0.11	46
		DBP	–0.17 [–0.39, 0.05]	p = 0.13	p = 0.53	0
	Non-diabetic subjects	SBP	–0.62 [–1.22, –0.02]	p = 0.04	p < 0.0001	84
		DBP	–0.63 [–1.15, –0.11]	p = 0.02	p = 0.001	78
Supplement	Single supplement	SBP	–0.48 [–0.87, –0.08]	p = 0.02	p < 0.001	79
		DBP	–0.46 [–0.82, –0.10]	p = 0.01	p < 0.001	75
	Multi supplement	SBP	–0.57 [–1.06, –0.09]	p = 0.02	p = 0.32	0
		DBP	–0.12 [–0.59, 0.36]	p = 0.63	p = 0.63	0

BP — blood pressure; SBP — systolic blood pressure; DBP — diastolic blood pressure; SMD — standard mean difference; CI — confidence interval; I^2 — percentage score for heterogeneity

related to the prevention, evaluation, detection, and management of hypertension in adults. This is distinguished by removing the category of prehypertension and separating it into two distinct levels: elevated blood pressure/stage 1 hypertension [25].

The association of hypertension and elevated blood pressure with morbidity and mortality of cardiovascular disorders has been well established [11, 26]. Abundant Epidemiologic data support that the risk of cardiovascular disorders disease increases with elevating blood pressure values. It has been reported that the blood pressure starting at ≥ 115/75 mm Hg results in such a manner [11, 27, 28]. There are evidence that both oxidative stress and a diminished capacity for scavenging free radicals play major roles in the development of hypertension and cardiovascular disorders. Moreover, SBP and DBP have been reported to positively relate to oxidative stress markers and negatively relate to plasma antioxidant capacity and free radicals could participate in the development of hypertension complications [29, 30].

Disruption of endothelial function leads to a diminished production or availability of NO and subsequent impaired NO bioactivity. This results in an imbalance between the endothelium- vasoconstrictors, and vasodilators derived from endothelium [30]. Multiple cardiovascular risk factors are related to the possible changes in endothelial function including sedentary and inappropriate lifestyle, hypercholesterolemia, aging, arterial hypertension, and a family history of atherosclerotic disorders [31]. Thus, most updated guidelines suggest improvements in lifestyle including limiting daily dietary sodium, exercise, and reasonable weight-loss in high-risk patients.

According to the previous evidence, ALA is considered as an efficient antioxidant with both lipid and aqueous solubility [4]. Furthermore, ALA supplementation may exert anti-inflammatory and hypoglycemic characteristics of the subjects with various conditions [32]. Beyond the main function of ALA as an antioxidant [33, 34], ALA can also increase NO synthesis which results in an improvement in endothelial function [35]. Moreover, many enzymatic and metabolic reactions are dependent on ALA as a potential co-enzyme, and enhancing agent in the

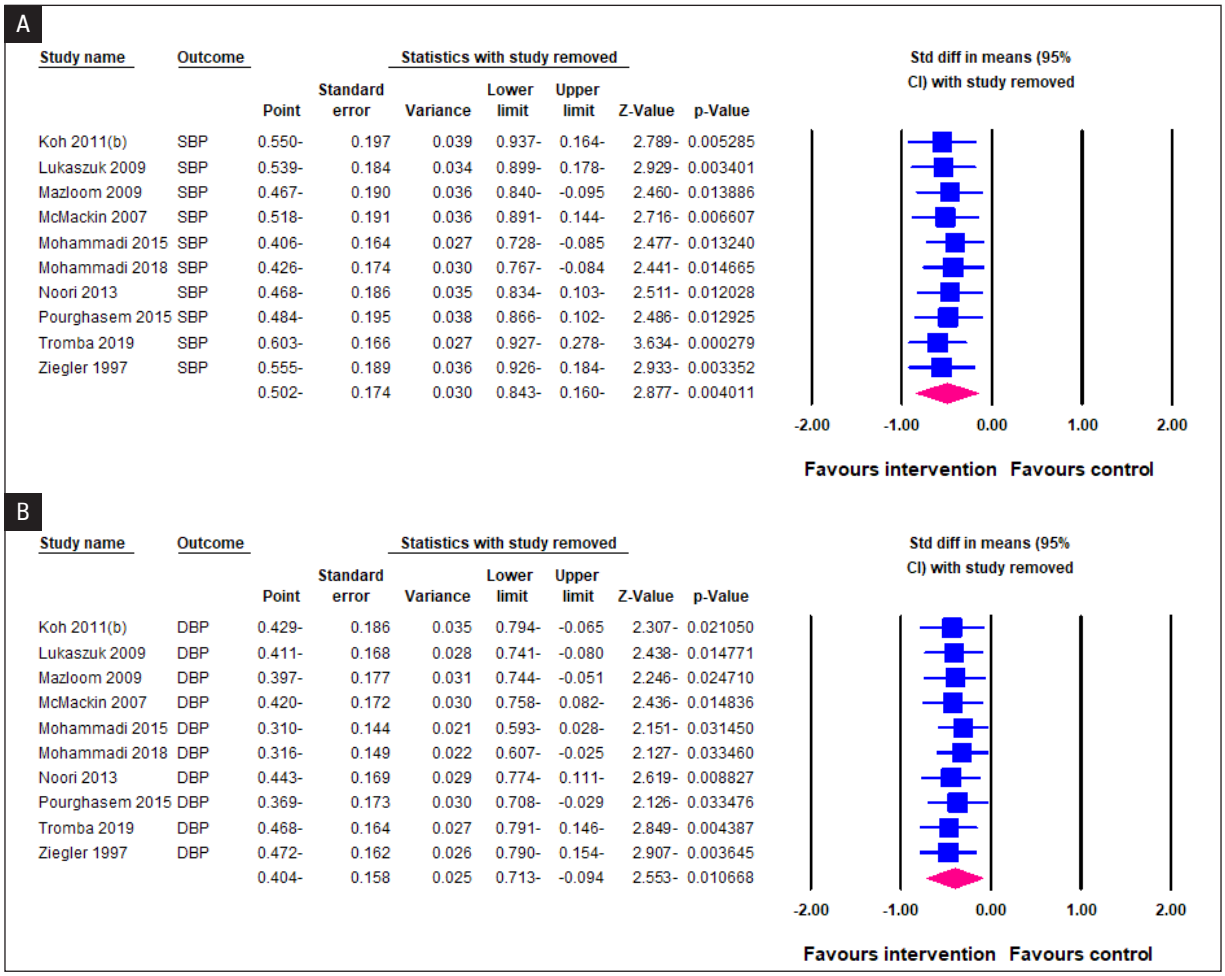

Figure 3. Sensitivity analysis for the effect of α-lipoic acid (ALA) supplementation on systolic blood pressure (SBP) (**A**) and diastolic blood pressure (DBP) (**B**)

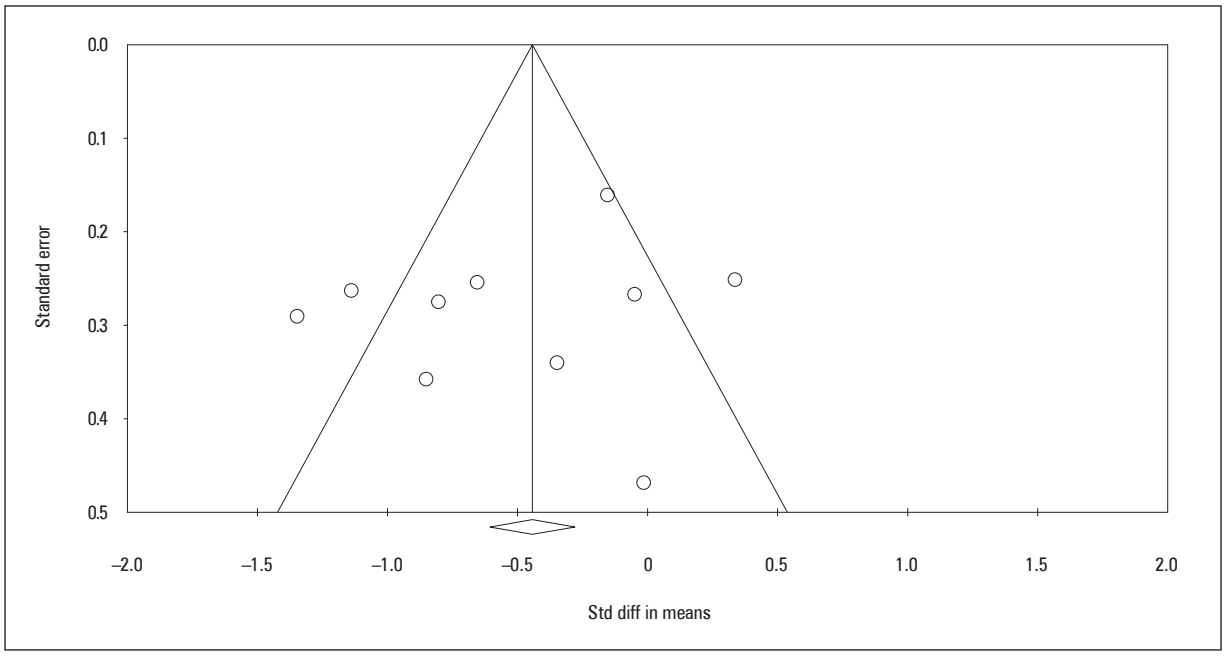

Figure 4. Funnel plot detailing publication bias of included studies. SMD — standard mean difference; SE — standard error

regeneration of endogenous antioxidants including vitamin C, vitamin E, and glutathione [36]. As stated before, ALA supplementation may play a role in anti-inflammatory mechanisms. Interestingly, these mechanisms are considered as the potential predictors of cardiovascular disorders and type II diabetes development [37–39]. In general, our meta-analysis reveals the reductions in DBP and SBP after ALA supplementation in elevated BP and hypertensive subjects, respectively; however, the blood lowering effect of ALA supplementation was not observed in normotensive subjects. Therefore, this could be assumed that ALA supplementation may have the beneficial effects on lowering blood pressure when the hypertension is manifested as both subclinical and clinical condition. ALA may exert its antihypertensive and hypoglycemic effects and the subsequent anti-cardiovascular effects by an attenuation of the oxidative stress which is reflected by the decrement in the basal O2– synthesis in vessels and by the retention of the gluthatione-peroxidase activity of the plasma [9].

Despite some strengths of the present meta-analysis, multiple limitations should be noted. First, most of the included trials enrolled limited participants, which result in misleading in final estimates of treatment effects, as trials with small sample sizes might be methodologically less considerable and are prone to report the values somewhat larger than their actual effect sizes. Moreover, the heterogeneity between included studies was considerable even after performing subgroup analyses which may potentially reduce the influence of the final results.

Nonetheless, the present analysis had some strength: the first point is that we systematically and comprehensively searched through several databases. Defined inclusion criteria/clear approach in gathering data and in-depth quality assessment of studies are considered as other points. Finally, lacking the potential biases should be considered as another strength point.

Conclusion

The current meta-analysis observed a beneficial effect of ALA supplementation in lowering BP in subjects with elevated blood pressure. The beneficial effect of this compound in alleviating blood pressure is maybe by its anti-oxidative and vascular endothelial properties. However, future precision randomized trials should establish whether different doses of ALA or longer-term supplementation of ALA could provide a hypotensive role and reduce cardiovascular risk.

Conflict of interest

No conflict of interest was declared regarding the present manuscript.

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Therapeutic strategy in patients with long-lasting essential hypertension with comorbid type 2 diabetes mellitus and obesity

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Abstract

Background: Presently the level of blood pressure (BP) control is extremely unsatisfactory in hypertensive patients throughout the world. The aim of our study was to find the optimal drug therapy for patients with hard-to-treat essential hypertension (EH) associated with type 2 diabetes mellitus (T2DM) and obesity, namely the comparison of strategies of fixed and non-fixed combination.

Material and methods: Eighty-seven patients with EH, T2DM and obesity were enrolled into the study. Two groups were formed: the 1st group — 41 patients received antihypertensive therapy in the form of unfixed combination of drugs ("multi-pill") perindopril, indapamide and amlodipine; the 2nd — 46 patients, who received the same drugs, but in a fixed-dose combination ("single pill").

Results: A favorable treatment result was found for fixed-dose combination of antihypertensive drugs, with significant reduction in the frequency of visits to the doctor: relative risk (RR) — 1.27 (95% CI: 1.01–1.61), p = 0.045, and odds ratio (OR) — 3.10 (95% CI: 1.05–9.13), p = 0.04. That indicates that patients on fixed-dose combination were less likely to visit a doctor with complaints. Patients on single-pill therapy were less likely to get to progression (worsening) group in contrast to multi-pill non-fixed combination: RR — 1.37 (95% CI: 1.02–1.84), p = 0.03; OR — 2.91 (95% CI: 1.12–7.59), p = 0.03.

Conclusion: The single-pill triple combination has significant advantage compared to multi-pill regimen in hard-to-treat hypertensive patients with comorbid T2DM and obesity. Fixed-dose triple combination leads to significantly faster achievement of blood pressure control.

Key words: single-pill; essential hypertension; comorbidity; treatment

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Introduction

A high level of blood pressure is among the main reasons of early disabling cardiovascular complications (CVC) such as myocardial infarction and stroke. Poor control of blood pressure (BP) remains among the major problems in contemporary cardiology. At the moment BP control in hypertensive patients is extremely unsatisfactory [1]. Unsatisfactory national cardiovascular regulations, low patients' adherence to prescribed treatment schemes, and the so-called specialists' inertia are factors responsible for the mentioned problem. Also it is worth to point out the next important factor promoting inappropriate BP control is the confined use of antihypertensive drug combinations. Despite the fact that there is enough evidence that combination treatment leads to more effective BP control in comparison to monotherapy [2].

Nowadays, there are drugs that can effectively affect blood pressure and therefore reduce the risk of stroke, kidney and cardiovascular disease in the arsenal of the clinician, but uncontrolled blood pressure and low adherence to antihypertensive drugs remains one of the main clinical problems [3].

The treatment of essential hypertension (EH) predominantly includes well-known medications from five main classes of drugs: angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), diuretics, calcium channel blockers (CCB), and β-blockers. There is bounded variation by race, age, gender in choosing of antihypertensive agents. Only 58% of treated patients had SBP < 140, 24% had unsatisfactory controlled BP with SBP ≥ 150, evidencing the effectiveness of treatment needs to be improved [4].

Long-term prospective studies have shown that patients with hypertension did not receive effective monotherapy and needed an average of two or three drugs for adequate control [5].

Despite mentioned data, BP control is poor in majority of countries. Contemporary investigations revealed, regardless the location (country) and irrespective to the high- or low-income economies, just about 40% of patients with hypertension are treated; of these approximately about 35% have well controlled blood pressure with BP values less 140/90 mm Hg [6].

All mentioned above justifies the search for adequate methods of treatment of patients with EH, especially in the presence of polymorbidity: type 2 diabetes mellitus (T2DM) with obesity. Special difficulties are present when solving the problem of antihypertensive therapy in high-risk patients with additional comorbidities — T2DM with obesity. Thus, if in the general population of patients with EH effective control is achieved in approximately 15–20% of such patients, then when combined with T2DM and obesity, this percentage becomes even more unfavorable, even dramatic values, which in turn leads to an increase in adverse events, CVC and reduced life expectancy and earlier disability [6, 7].

The question arises, what strategy of drug treatment in such patients is the most optimal? The problem is exacerbated by the fact that the clinician often decides to prescribe antihypertensive therapy to a patient who has additional pathology (T2DM, obesity) and who has suffered from EH for many years and the antihypertensive treatment used so far has been ineffective. Thus, it is not a question of the so-called first line therapy, but of solving the question of finding an effective strategy in such (polymorbid) patients with EH who have experienced treatment failure and have poorly controlled blood pressure despite prescribed therapy.

The aim of the study was to find the optimal drug therapy for patients with long-lasting essential hypertension associated with type 2 diabetes mellitus and obesity, namely the comparison of strategies of fixed combination of antihypertensive drugs with the strategy of non-fixed combination.

Material and methods

The 87 patients with EH, type 2 diabetes and obesity were enrolled into the study. Examined subjects were patients admitted to Cardiology Departments of O.I. Meshchaninov City Clinical Hospital of Ambulance and Emergency Care and Kharkiv Regional Hospital who had elevated arterial blood pressure and were over 37 years of age. Two groups of medicament strategy were formed: the 1st group — 41 patients with EH, T2DM and obesity, who received antihypertensive therapy in the form of unfixed combination of drugs or "multi-pill" (NFC) — perindopril at a daily dose of 4 mg, indapamide (1.25 mg per day) and amlodipine (5 mg); the 2nd group — 46 patients with EH, T2DM and obesity, who received the same combination of drugs, but in the single pill — a fixed combination. The drug was used in a dose of 1 tablet per day, regardless of food intake, preferably in the morning.

In the study, the combination of EH with T2DM and obesity required the application of the antidiabetic and hypolipidemic therapy in addition to the antihypertensive treatment. Metformin or, if necessary, a combination of metformin with gliclazide, and

atorvastatin, or rosuvastatin was used. The patients with the stable coronary artery disease were prescribed acetylsalicylic acid. Thus, the treatment strategy was to act on the normalization of blood pressure and blood glucose, lowering total cholesterol and triglycerides, and included diet aimed at weight loss.

Two treatment regimens were evaluated by comparing their positive and negative effects. After that frequencies of good and negative results in the groups were calculated. The scheme with an unfixed combination of antihypertensive drugs was considered as a control to the scheme with a fixed combination. When calculating the relative risk (RR), visiting a physician due to any kind of complaints associated with hypertension-mediated organ damage (headache, chest pain, shortness of breath, fatigue, the appearance of flicker before the eyes, difficulty falling asleep, mood swings, etc.) was referred as negative results.

The both groups were matched by all investigated parameters — age, gender, additional risk factors, physical state etc.

All patients signed informed consent and Institutional Ethical Committee of the participating center approved the protocol of the study (number 2018/05/02), that was performed according to the requirements and norms of the ICH GCP (2002), and following the Declaration of Helsinki, as well as standard provisions on ethics of the Ministry of Health of Ukraine No. 66 13.02.2006.

The examination of hypertensive patients with T2DM and obesity was carried out 3 and 6 months after treatment. The target level of SBP was considered as 130/80 mm Hg.

Statistical analysis. The data is presented as mean ± standard deviation (M ± SD) or percentage (%). To determine the association between treatment strategy and outcome (result of the therapy) the relative risk and the odds ratio (OR) with 95% confidence interval (CI) were calculated. OR is most often used in case-control studies, but it can also be used in a one-step intergroup study and in a cohort study (with some assumptions) [8]. The target levels' frequencies and the medicament treatment regimen were compared between two groups by the χ^2 test. Statistical significance was considered as p value < 0.05. Med Cal (v. 19.3) program was used to calculate and analyze obtained data.

Results

The baseline characteristics of enrolled patients are presented in Table 1.

The baseline characteristics of enrolled patients show that patients from both treatment regimens

Table 1. Baseline characteristics of patients in the study groups (M ± SD)

Parameters	All patients	Fixed combination (single-pill) (n = 46)	Non-fixed combination (multi-pill) (n = 41)
Age [yrs]	59.67 ± 9.32*	58.87 ± 8.19*	59.53 ± 9.51*
Gender	Males: 39 (45.4%)* Females: 48 (54.6%)	Males: 21 (45.6%)* Females: 25 (54.4%)	Males: 18 (43.9%)* Females: 23 (56.1%)
Systolic blood pressure [mm Hg]	161.32 ± 7.65*	161.56 ± 6.98*	160.89 ± 7.49*
Diastolic blood pressure [mm Hg]	96.78 ± 4.92*	96.89 ± 4.87*	95.91 ± 5.01*
HbA_{1c} (%)	7.1 ±1.41*	7.0 ±1.42*	7.1 ± 1.33*
EH duration [yrs]	11.12 ± 4.34*	10.91 ± 4.02*	11.65 ± 3.97*
Obesity duration [yrs]	13.32 ± 4.43*	14.03 ± 3.53*	13.11 ± 4.39*
T2DM duration [yrs]	7.83 ± 3.32*	8.05 ± 2.81*	7.66 ± 2.90*
BMI [kg/m^2]	35.43 ± 3.46*	36.11 ± 3.52*	35.03 ± 3.43*
Heart failure [NYHA FC]	0: 5 (5.8 %)* I: 19 (21.8%)* II: 63 (72.4%)*	0: 3 (6.5 %)* I: 10 (21.7%)* II: 33 (71.8%)*	0: 2 (4.9%)* I: 9 (21.9%)* II: 30 (73.2%)*
Smokers (%)	28 (32.2 %)*	15 (32.6 %)*	13 (31.7%)*
Dyslipidemia (%)	60 (69.0%)*	31 (67.4 %)*	29 (70.7%)*
LV hypertrophy (%)	87 (100.0%)*	46 (100.0%)*	41 (100.0%)*
Carotid intima-media thickness > 0.9 mm (%)	55 (63.2%)*	29 (63.0%)*	26 (63.5%)*
Microalbuminuria (20 to 200 mg/L) (%)	71 (81.6 %)*	38 (82.6%)*	80.5%)*

*the p level significance p > 0.1 (differences between groups); EH — essential hypertension; T2DM — diabetes mellitus type 2; BMI — body mass index; LV — left ventricle; NYHA FC — New York Heart Association Functional Classification

Table 2. Influence of single-pill (fixed combination) and multi-pill (unfixed combination) strategies on the frequency of visits to the doctor, 6-minute walking test, and clinical state in patients with essential hypertension, type 2 diabetes mellitus (T2DM) and obesity

	RR (95% CI)	OR (95% CI)
Visiting a physician	1.27 (1.01–1.61)	3.10 (1.05–9.13)
p value	p = 0.045	p = 0.04
6-minute walking test	0.99 (0.83–1.18)	0.96 (0.29–3.11)
p value	p = 0.94	p = 0.94
Clinical worsening (progressing)	1.37 (1.02–1.84)	2.91 (1.12–7.59)
p value	p = 0.03	p = 0.03

RR — relative risk; CI — confidence interval; OR — odds rate

were consequently matched by all investigated parameters.

A favorable treatment result was found in the polymorbid group receiving fixed antihypertension combination that was proved by significant reduction in the frequency of visits to the doctor, with calculated RR — 1.27 (95% CI: 1.01–1.61), p = 0.045 and OR — 3.10 (95% CI: 1.05–9.13), p = 0.04 (Tab. 2).

Thus, this calculation indicates in favor of a scheme with a fixed combination compared to a non-fixed one. Thus, values of relative risk of 1.27 (95% CI: 1.01–1.61) indicate that patients who received a fixed combination of drugs were less likely to visit a doctor with complaints on hypertension-mediated target organ damage (HMTOD).

The influence of the two treatment options on the dynamics of the clinical condition assessed by the 6-min walking test was also analyzed. Improving the performance of the 6-minute walking test were recorded in 96% of patients taking a fixed triple combination, and in the group taking three separate drugs; the increase in test scores was observed in 85% of patients.

Comparison of 6-min walking test results did not showed significant advantage of the triple regimen in one tablet over taking three separate antihypertensive drugs. It was found, that calculations of RR — 0.99 (95% CI: 0.83–1.18), p = 0.94 and OR — 0.96 (95% CI: 0.29–3.11), p = 0.94 indicate that the probability of clinical improvement did not reach statistical significance between groups (Tab. 2).

Progression (worsening) of patients' condition with EH, T2DM mellitus and obesity was assessed by the dynamics of structural and functional parameters of the target organs (heart, kidneys and blood vessels). It was revealed (Tab. 2) that patients taking fixed combination were less likely to get to progression group (group with worsening of condition) than those on non-fixed combination: RR — 1.37 (95% CI: 1.02–1.84), p = 0.03; OR — 2.91 (95% CI: 1.12–7.59), p = 0.03.

This result of calculations of HR and OR indicates that the use of triple-drug single-pill combination (SPC) is much more effective in preventing deterioration of the target organs in hypertensive patients with polymorbidity. We can assume that this is due to more careful control and faster achievement of target blood pressure levels.

After 3-month treatment (Fig. 1) with a three-component antihypertensive regimen the target levels of systolic blood pressure were achieved in 33 hypertensive patients on unfixed combination, and number of patients on fixed combination who reached target levels was 44 (p < 0.001). An important result is the data of blood pressure control after 6 months, which demonstrate the effectiveness of both selected strategies in achieving the target levels of blood pressure — 97.83% and 92.68% of patients receiving fixed and non-fixed combinations, respectively (Fig. 1). Thus, there is a significant advantage of antihypertensive therapy with the use of a fixed combination, which led to a much faster achievement (p < 0.05) of blood pressure — 130/80 mm Hg.

Thus, taking a fixed combination (an ACE inhibitor — perindopril, a diuretic — indapamide and a calcium channel blocker — amlodipine) along with the antihyperglycemic therapy (metformin or a combination of metformin and gliclazide) and recommendations for lifestyle modification was significantly better compared with non-fixed combinations of antihypertensive drugs (Figure 1): after 3 months it lead to significantly higher percentage of patients achieving target blood pressure — 95.65% (44 patients) against 80.49% (33 patients) (χ^2 = 4.84; p = 0.03) and after 6 months 97.83% (45 patients) against 92.68% (38 patients) (χ^2 = 1.30; p = 0.26). The fixed combination definitely reduces the risk of progression (clinical worsening) of the hypertensive patient with T2DM mellitus and obesity

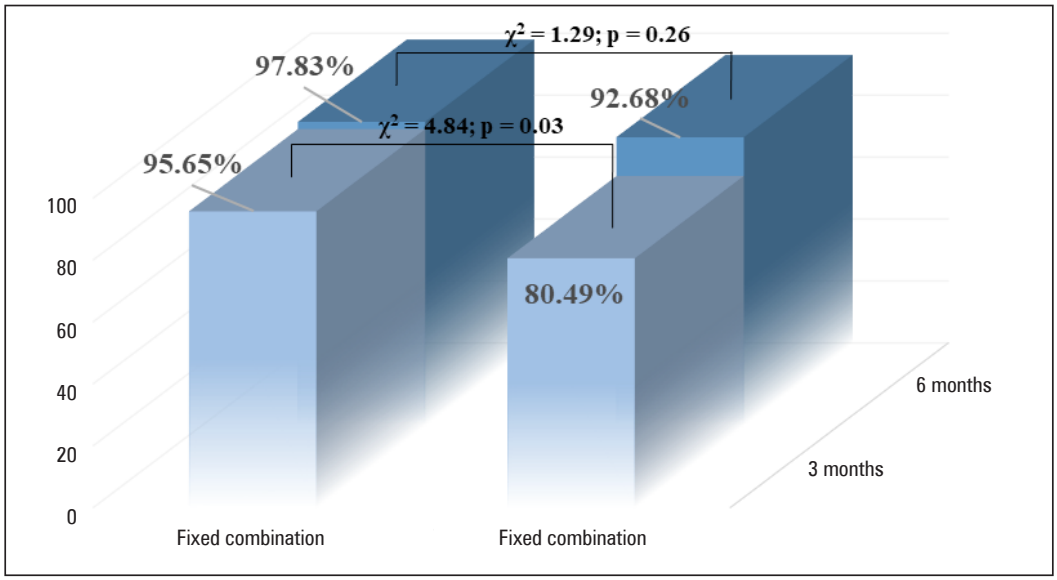

Figure 1. Results of studied treatment strategies: after 3 months and after 6 months. Results are presented as proportions of patients (%) achieved target blood pressure (BP)

during 6 months of treatment: 80.43% vs. 58.54% (χ^2 = 4.90; p = 0.03).

Discussion

The mentioned triple combination was chosen for the study because of the best evidence based data among the drugs used in hypertension. Perindopril is one of the best representatives of the ACE inhibitor class. It showed high hypotensive efficacy, reduction of overall mortality in patients with EH, as well as a probable reduction in the overall risk of cardiovascular complications in patients with stable coronary heart disease and preserved left ventricular function (ASCOT-BPLA, ADVANCE, HYVET, EUROPA, PERSPECTIVE, PERTINENT). Indapamide is also the optimal drug in its class. It is a metabolically neutral diuretic that has a significant antihypertensive effect and is proven to reduce the incidence of cardiovascular complications in patients with hypertension (HYVET, ADVANCE, PROGRESS, PATS). Amlodipine, in turn, is one of the most powerful CCB. It showed a marked reduction in cardiovascular complications (both cardiac and cerebral) in patients with EH, especially in combination with ACE inhibitors (ASCOT and ACCOMPLISH). In addition, both perindopril and amlodipine have pronounced renoprotective properties [6, 9].

It is desirable to underline that the main feature of our study was that medicament treatment in hypertensive patients with high CV risk and with previous history of ineffective BP lowering treatment was investigated. The majority of publications are devoted to the problem of the first-line therapy [10–12]; in contrast, we tried to find out better strategies in those patients who were unsuccessful with recommended first-line treatments. Also we tried to evaluate (to measure) exact preferences of single-pill triple regimen over multi-pill format.

Nowadays the issue of antihypertensive therapy in diabetics remains the cornerstone, and a lot of research has been devoted to this aspect, and often their results are contradictory. In general, it can be concluded from meta-analyses that antihypertensive treatment significantly reduces cardiovascular outcomes in diabetic and non-diabetic patients. Diabetic patients have uncertain benefits in intensive SBP lowering to less 120 mm Hg, while such intensive BP control is associated with evident benefits in those without diabetes mellitus [13]. At the same time, reducing ESRD risk has been evidenced only in diabetics (when SBP < 130 mm Hg). Meta-analyses showed that all BP-lowering drugs are effective in diabetic patients, with renin–angiotensin system blockers preferably included in the treatment schemes of patients with diabetes mellitus [9, 14, 15].

On the contrary, Bangalore's study state that in diabetics RAS blockers have no superior effects compared to other antihypertensive medicaments, such as diuretics, calcium channel blockers, or β-blockers, in diminishing the risk of undesirable cardiovascular and renal events. These resulted in recommendations of the guidelines of the European and American

societies on management of hypertension to use any antihypertensive drugs in diabetic people but with normal renal function [16].

Grossman et al. assessed evidence supporting different BP targets in diabetics and reviewed the various guidelines on this topic. Moreover, they discussed the different variants available for the BP lowering in diabetics. The authors are more inclined to choose RAAS blockers as the first-line agents among anti-hypertensive drugs. In this study, as in our investigation, calcium channel blocker and/or thiazide were preferable as second line medications in case it was necessary to use more than one drug for the BP control in patients with diabetes. In Grossman et al.'s study it was stated that new anti-diabetic agents could be more effective in antihypertensive treatment and might change the physician's choice in the treatment of T2DM. On the contrast, we used traditional well-recommended drug as metformin in our study. In addition, it is essential to manage all other risk factors in hypertensive patients with T2DM when decreasing BP. Grossman et al. concluded that individual flexible treatment model, reflecting the general modern trends in medicine, proved the need for individually tailored medicine, adapted specifically for the particular anthropological and morphological characteristics of every person [17]. Such result is also supported by our team, but at the same time, we understand that it is necessary to find optimal treatment recommendation for specific cohort of hypertensive patients — with prolonged history of EH with comorbid T2DM and obesity.

The systemic review by Brunström Mattias et al. indicates that the available data point out the need for a decrease in SBP to more than 140 mm Hg in people with T2DM. At the same time, these authors did not find differences in the effectiveness between the main antihypertensive drugs: angiotensin-converting enzyme inhibitors, ARBs, β-blockers, calcium channel blockers and diuretics in the prevention of general or cardiovascular mortality, as well as coronary artery disease and ESRD. Furthermore, minor additional effects have been found for stroke and heart failure. It is important to note that in this study there were limitations on the inclusion criteria and patients with type 1 diabetes and very elderly patients with T2DM were excluded from the study, which differs from our study [18].

The initial treatment should be started with two drugs or a single-pill combination of drugs proven to reduce cardiovascular events in patients with diabetes. Recommendation of class A insist on poly-drug strategy to control blood pressure targets (but not usage of ACEI with ARB combination), herewith there is no indication on previous treatment history. The initial treatment for diabetic patients depends on the severity of hypertension, but in our study it is defined by previous treatment effectiveness. In diabetic patients with BP over 160/100 mm Hg, initial pharmacological treatment is recommended with two antihypertensive medications, whereas the presence of such additional risk factor as obesity is not discussed deeply [19].

Also, we want to point out the need for wider implementation of single-pill combination in the routine practice, according to hypertension treatment algorithms included in the 2018 ESC guidelines [6]. Combination treatment leads to a faster achievement of BP control in comparison to monotherapy. That is essential in many cases and can be crucial. Herewith, the implementation of the fixed combination approach into everyday clinical practice should be supported by initiatives aimed at resolving physicians' and patients' barriers such as low experience of use, miscellaneous perception of SPC, lack of confidence in the independence of recommendations, patient-physician communication. The concept of first-line antihypertensive therapy needs to be promoted with help of national CME programs and lists of available SPCs, print media and digital materials [20].

Fixed-dose combinations as initial therapy are often needed to achieve fast blood pressure control [21]. On the other hand, European guidelines do not recommend initial triple therapy under any circumstance, but here we firmly underline that only in initial (starting) phase, but not in general. Moreover, we also emphasize in our work more firm and definite usage of fixed triple combination in high-risk hypertensive patients with diabetes and obesity who had prolonged ineffective treatment history.

There are limited investigations of antihypertensive treatment in polymordid patients who have a lot of additional risk factors, which forced us to do presented study.

The distinguishing feature of our work was that the proven benefits of prescribing a fixed triple combination were calculated. It was also emphasized that the effect of a triple fixed combination was noted only in this high-risk group (that is, with multimorbidity) with a documented history of long-term hypertension and ineffective blood pressure control. In other cases — for example, in patients naïve to antihypertensive therapy, it is most likely to start with a fixed two-component combination.

It is fundamentally important to note that we included patients with both type 2 diabetes and obesity.

It was an obligatory inclusion criterion to have previous experience of ineffective drug therapy, which, from our point of view, represents a huge proportion of hypertensive patients. In addition, these patients are most often people with modest financial resources.

And, most importantly, specific benefits from a fixed combination were calculated — significantly fewer visits to the doctor, decreased numbers of complaints and concerns raised, improved prognosis — reduction of target organ damage and improved quality of blood pressure control.

The presented study had some limitations — a small sample and comparison of frequencies in this case can be regarded as the limit of the study. It is also worth noting the relatively short-term of the study. It can be assumed that a longer observation would make it possible to find additional advantages of a fixed triple combination or, on the contrary, to reduce them.

Further research is needed in this cohort of patients, who are at increased risk — with a large sample size and prolonged exposure.

Conclusion

The non-first line antihypertensive regimen with single-pill triple combination has significant advantage compared to multi-pill (non-fixed) regimen in hard-to-treat hypertensive patients with comorbid 2 type diabetes mellitus and obesity. Such one-pill combination leads to significantly faster achievement of blood pressure control and decreases the chances of worsening of patients' state or/and diminishes chances to disease development.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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Primary aldosteronism and coronary-pulmonary artery fistula: coincidence or causal link? A case report and literature review

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Abstract

Background: Primary aldosteronism (PA) is the most frequent form of potentially reversible hypertension and coronary-pulmonary fistulas are increasingly recognized during routine coronary angiography or multidetector computed tomography for analysis of chest pain in hypertensive patients. Aldosterone hypersecretion has been associated with endothelial proliferation and pathological remodeling of the heart and arteries, though coronary artery fistulas have never been reported in patients with PA.

Case presentation: The authors report the first case of PA with dilated cardiomyopathy unusually associated with electrocardiographic changes after normalization of hypokalemia and with the finding of a coronary-pulmonary fistula during coronary angiography. The clinical presentation and our diagnosis and treatment decision-making in the COVID-19 era are discussed below.

Conclusions: Our case suggests a potential link between hypertensive patients with coronary artery fistulas and PA.

Key words: aldosterone; cardiomyopathy; coronary-pulmonary fistula; hyperaldosteronism; hypertension; ECG

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Introduction

Although primary aldosteronism (PA) was initially considered a rarity, now it is one of the most common causes of secondary hypertension in which aldosterone production is inappropriately high and partially autonomous from the renin-angiotensin system. Broadly, PA can be dichotomized to unilateral aldosterone production from the left or right adrenal gland (aldosterone-producing adenoma; APA, or Conn's syndrome) or bilateral hyperaldosteronism (BHA). APA can be cured surgically and it is therefore important to distinguish it from BHA, which is managed medically with mineralocorticoid receptor antagonists. Appropriate diagnostic management of PA consists of three consecutive steps: first, screening test with the aldosterone to renin ratio, then case confirmation through the aldosterone suppression tests and last, discrimination of unilateral from bilateral forms of PA by adrenal venous sampling.

Our case highlights an atypical clinical presentation of PA and our choice of a diagnostic therapeutic decision-making plan amid the delicate and complex first spread of COVID-19 in Italy.

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Case presentation

A 53-year-old Caucasian obese man with a history of modest fatigue and subjective dyspnea was admitted to our hospital after undergoing an outpatient transthoracic echocardiography (TTE) that showed cardiac dysfunction in March 2020, amid the pandemic of COVID-19. The patient had been in good health until 2 months before the current admission, when he saw his primary care physician for the first time presenting an asthenia lasting some weeks. Blood pressure (BP) taken in office was repeatedly noted to be 210/110 mm Hg regardless ongoing therapy with three blood-lowering drugs

Owing to his recent history of fatigue and frequently reoccurring atrial and ventricular extrasystoles (the patient was scheduled for 24-hour ECG monitoring, TTE and exercise stress test (EST).

As the TTE documented moderately dilated and hypertrophic left ventricle with a decreased contractility of the inferior and septal walls as well as modestly reduced ejection fraction (Tab. 1) the patient after the negative result for SARS-CoV-2 RNA testing was admitted in our department for detailed evaluation.

On admission, the patient was obese (BMI = 37 kg/m^2) and had a two-month history of poorly controlled hypertension, treated with amlodipine 10 mg, ramipril 5 mg and bisoprolol 1.25 mg a day. His father had ischemic cardiomyopathy but there was no family history of sudden cardiac death. He did not drink alcohol, smoke tobacco or use illegal drugs and reported drinking about 2.5 liters of water a day and snoring during the night without experiencing daytime sleepiness. On physical examination, heart rate was 104 beats per minute and the BP was 140/110 mm Hg, respiratory rate of 16 breaths per minute and the oxygen saturation 97% while the patient was breathing ambient air. Laboratory tests (Fig. 1) revealed mild to moderate hypokalemia and the arterial blood gas showed hypoxemia and primary metabolic alkalosis with a secondary respiratory alkalosis. HbA_{1c} levels were diagnostic for new onset type 2 diabetes mellitus. A chest X-ray and ECG performed at the time of admission were unremarkable as well (Fig. 2A).

Investigations

During the wide spread of coronavirus, it was necessary to invest few resources in a targeted manner and to contain the risk of hospital infections with rapid and effective hospitalizations. In such circumstances, we studied the renin-angiotensin-aldosterone system after only one week of therapy with a calcium channel blocker and a serum potassium > 4 mmol/L with oral potassium supplements.

The aldosterone/renin ratio screening test was compatible with a suspected PA, later proved by the lack of aldosterone suppression after intravenous saline load (2 L of 0.9% saline infused over 4 h) (Fig. 1). The subtype diagnosis of PA was made by means of abdomen CT scan, which showed an adenoma of 7 mm in diameter in the lateral limb of the right adrenal gland and a slight thickening of the contralateral gland (Fig. 3A).

It was surprising to notice that, 18 hours after hospitalization and oral potassium supplementation, in the absence of symptoms or secondary causes, significant changes were observed in the ventricular repolarization on ECG (Fig. 2B) with indications by the cardiologist to perform a coronary angiography and start dual antiplatelet therapy with aspirin and clopidogrel. Selective coronary angiography showed a right dominant coronary circulation and a slightly reduced left ventricular function due to diffuse hypokinesia, without evidence of significant stenoses. Furthermore, the presence of a fistula from the left anterior descending coronary draining into the pulmonary branch was detected (Fig. 3B). After a week from admission, we observed a progressive and complete normalization of ventricular repolarization on the ECG and a twenty-four hour Holter monitoring showed a sinus rhythm with no significant arrhythmias.

Table 1. Change in echocardiogram parameters before and after 9 weeks of medical treatment†

	Before treatment March 9, 2020	After treatment May 15, 2020	Reference range, Adults
LVEDVI [mL/m^2]	95	67	< 75
LVEF (%)	43	51	≥ 52
LAVI [mL/m^2]	39	25	≤ 34
PWT [mm]	12	10	< 11
IVS [mm]	13	12	< 11

†Spironolactone 100 mg, amlodipine 5 mg and bisoprolol 2.5 mg daily. LVEDVI indicates LV end-diastolic volume index; LVEF — LV ejection fraction; LAVI — LA volume index; PWT — posterior wall thickness; IVS — interventricular septal thickness

	8 Wk before Current Admission		6 Wk before Current Admission	On Admission	14 hours after Admission	Hospital Day 6	
Date (year 2020)	January, 10		January, 27	March, 9	March, 10		
	Medical practitioner	**ER 1st access**	**ER 2nd access**	**ER 3rd access**	**Hospitalization**		
Symptoms and remarks	Fatigue, dyspnea		Palpitations, fever	Cardiac dysfunction			
Relevant testing	None		Laboratory, abdominal US	Echocardiography, laboratory, ECG		Abdominal CT scan, coronary angiography	
Clinical and Laboratory data							***Reference range†***
Clinic SBP/DBP (mmHg)	210/110	140/70	140/77	140/90	140/110	135/86	
Antihypertensive drugs (n)	0	0	1	3	3	1	
White-cell count (x10^9/liter)			**12.6**	9.9	7.9		*3.5 - 11.0*
Hemoglobin (g/liter)			133	149	144		*125 - 169*
C-reactive protein (mg/dL)			**26.0**		**1.9**		*0.0 - 0.5*
Creatinine (g/dL)			1.17	0.83	0.77		*< 1.30*
Sodium (mmol/liter)			136	142	145		*135 - 145*
Potassium (mmol/liter)			**2.8**	**3.0**	4.0	4.1	*3.3 - 5.0*
Magnesium (mg/dL)					2.1		*1.7 - 2.6*
Glucose (mg/dL)			**121**	97	**120**		*70 - 99*
Glycated hemoglobin (mmol/mol)					**48**		*20 - 41*
Total cholesterol (mg/dL)					178		*< 190*
LDL-cholesterol (mg/dL)					119		*<130*
Triglycerides (mg/dL)					99		*< 150*
TSH (mUI/liter)			2.2		1.1		*0.4 - 3.7*
NT pro-BNP (ng/liter)			**3681**	**4049**		**399**	*< 300*
Troponin I (ng/liter)			47	16	17		*0 - 60*
Aldosterone (ng/liter)					**294**		*30 - 150*
Renin (µU/mL)					**1.0**	**0.7**	*3.0 - 29.0*
ACTH (ng/liter)					57		*< 95*
Cortisol (µU/mL)					235		*50 - 250*
ABG: pH				**7.5**			*7.35 - 7.45*
PaO2 (mmHg)				**63**			*75 - 100*
PaCO2 (mmHg)				40			*35 - 45*
HCO3- (meq/liter)				**29**			*22 - 26*
SaO2 (%)				97			*95 - 100*
Aldosterone post SLST (ng/liter)						**114**	*<50*
Cortisol post ODST (µg/L) Hospital day 7						<10	*<50*
24-h urinary cortisol (µg/day)					222		*70 - 320*
24-h urinary metanephrine (µg/day)					107		*< 280*
24-h urinary normetanephrine (µg/day)					**534**		*< 500*
Diagnosis	Hypertensive crisis		Acute cholecystitis	PA with Dilated CMP and CPF			

Figure 1. Timeline. ER — emergency room; US — ultrasound; CT — computed tomography; PA — primary aldosteronism; CMP — cardiomyopathy; CPF — coronary-pulmonary artery fistula; SBP/DBP — systolic and diastolic blood pressure; TSH — thyroid-stimulating hormone; NT pro-BNP — N-terminal pro-B-type natriuretic peptide; ABG — arterial blood gas; ODST — overnight dexamethasone suppression test; SLST — saline load suppression test performed with calcium channel blocker and serum potassium 4 mmol/liter. To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for creatinine to micromoles per liter, multiply by 88.4. †Reference values are affected by many variables, including the patient population and the laboratory methods used. The ranges used at our Hospital are for adults who are not pregnant and do not have medical conditions that could affect the results. They may therefore not be appropriate for all patients

Treatment

Current guidelines suggest the use of unilateral adrenalectomy or mineralocorticoid receptor antagonists (MRA) for the treatment of PA with or without lateralized aldosterone secretion, respectively [1].

Due to the complex and delicate COVID-19 pandemic and in compliance with the WHO declaration and the United States Surgeon General's recommendations [2] suggesting to cancel elective surgeries at hospitals with the concern that elective procedures within facilities may contribute to the spreading of the coronavirus so as to utilize the medical resources needed to manage a potential increase of coronavirus cases, we discharged the patient with a MRA therapy, waiting to perform the adrenal venous sampling in order to confirm the lateralization of overproduction of aldosterone for the best treatment.

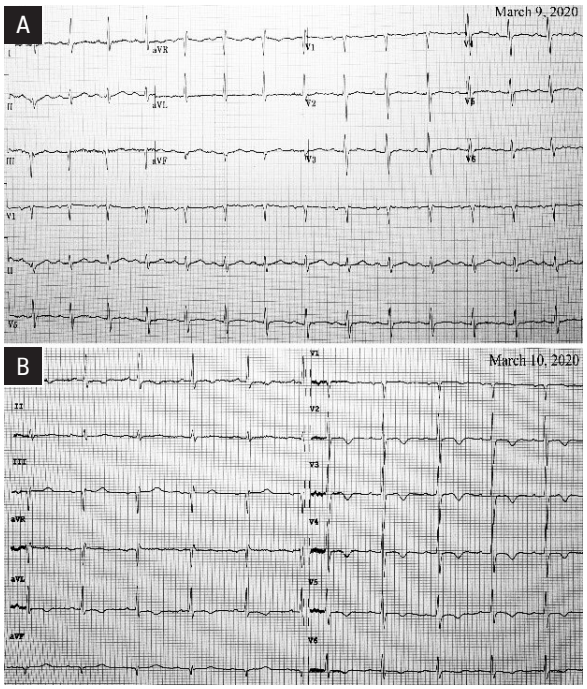

Figure 2. Twelve-lead ECG tracing done upon the arrival of the patient at the emergency room (**A**). ECG tracing showing negative T wave in the precordial leads V2-6 after 18 hours from admission and oral supplement of slow-release potassium chloride (600 mg or 8 mmol per tablet), 2 tablets every 6 hours (**B**)

Outcome and follow-up

About two weeks after discharge, we achieved the normalization of plasma potassium levels and a sufficient BP control with spironolactone 100 mg, amlodipine 5 mg and bisoprolol 2.5 mg a day. Currently in medical treatment the patient is still under follow-up in our clinic with good clinical and echocardiographic outcomes [3, 4] (Tab. 1) and no complications.

Discussion

Hypertensive patients with PA showed the increased risk of coronary artery disease, heart failure, atrial fibrillation and stroke, compared with patients with essential hypertension [5]. The classic presenting signs of PA are resistant or difficult-to-control hypertension and hypokalemia, but less than half of the cases show these two conditions [1] and also because of this the unsuppressible (primary) hypersecretion of aldosterone is often an underdiagnosed cause of hypertension.

A wide body of experimental and clinical researches has demonstrated that inappropriately increased aldosterone secretion induces a significant inflammation, remodeling and fibrosis on cardiovascular system, including endothelial proliferation and vascular remodeling, which cannot be explained only by the increased degree of blood pressure [6–10]. Yet a link between primary aldosterone hypersecretion and coronary artery fistulas has never been reported.

In 1964, James O. Davis et al. have investigated on animal models the aldosterone secretion and the mechanisms which lead to hypersecretion of aldosterone in experimental high-output heart failure secondary to an aortic-caval fistula [11]. Higher aldosterone concentrations in patients free from overt cardiovascular disease are associated with the increased risk of subclinical coronary atherosclerosis and all-cause mortality particularly among individuals with renin suppression [12]. In patients with PA, complex mechanisms may lead to functional and structural abnormalities of the blood vessels walls. Clinical evidence indicates that PA patients may have immune cell activation, increased oxidative stress, endothelial

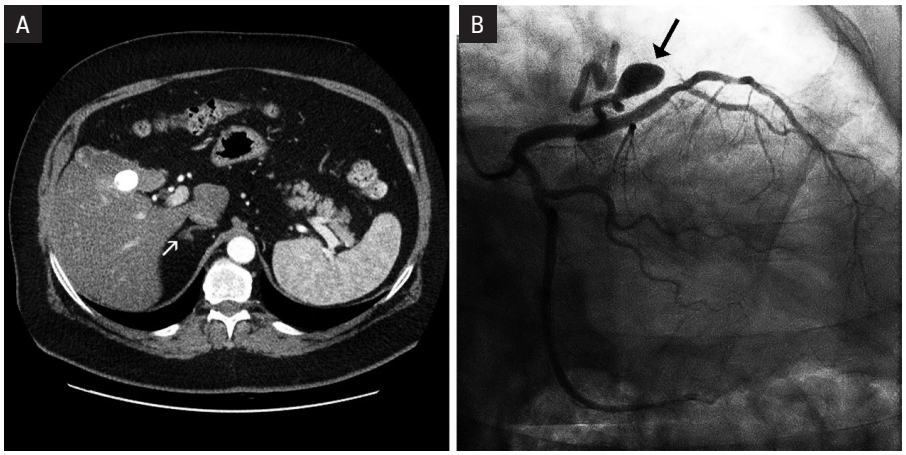

Figure 3. Transverse CT scan of the abdomen showing a nodule in the lateral limb of the right adrenal gland (*Arrow*) compatible with the adenoma measuring 7 mm (**A**). Coronary angiogram clearly showing a coronary artery fistula (*Arrow*) between the proximal left anterior descending artery and the pulmonary artery (**B**)

dysfunction and proliferation with vascular remodeling [13]. There is evidence that aldosterone via the mineralocorticoid receptor (MR) promotes angiogenesis. In vitro findings, aldosterone exacerbated retinal neovascularization in ischemic retinopathy which was attenuated with MR antagonist (MRA). The aldosterone infusion increased VEGF mRNA levels and neovascularization in mice with hindlimb ischemia, and these events were reduced with spironolactone [14]. It was also noticed that in a mouse model, a modest increase in plasma aldosterone concentration can modulate cardiac genes expression and induce the proliferation of cardiac endothelial cells in vivo. Aldosterone-induced MR-dependent proliferation was confirmed ex vivo in human endothelial cells and the pharmacological-specific blockade of the MR by eplerenone inhibited endothelial proliferation in a rat model of heart failure [7]. Recently, Rho-associated kinase (ROCK) activity has drawn attention for its crucial role in angiogenesis [15] and the treatment with eplerenone for 12 weeks improves arterial stiffness, vascular smooth muscle function and microvascular endothelial function, and inhibits ROCK activity in patients with PA [16].

Thus far, however, PA has not been reported as a clear cause for dilated cardiomyopathy [17] or coronary arteriovenous fistulas.

Coronary-pulmonary fistulas (CPFs) are rare congenital or acquired coronary artery anomalies that can originate from any of the three major coronary arteries and drain into any of the cardiac chambers and great vessels. According to a literature review, the published epidemiological data may underestimate the real prevalence of the coronary artery fistulas in the general population, while the incidence in the echocardiographic series was 0.06 to 0.2%, in the necropsy series 14%, and in the angiographic series was 0.1% to 0.67% [18]. Furthermore, with the increasing use of the coronary artery angiography and multidetector computed tomography for the analysis of chest pain, the number of incidentally detected unilateral and multilateral CPFs has been gradually rising [18]. Although they are often incidental findings, patients with CPFs may have arrhythmias, angina, dyspnea, congestive heart failure, pulmonary hypertension, endocarditis, and sudden cardiac death. Therefore, their potential clinical consequences require further investigation in order to adopt either interventional (percutaneous transcatheter closure/surgical ligation) or conservative treatment, preventing any complications such as aneurysm creation, vessel dissection, pericardial effusion, coronary arterial steal phenomenon, thrombosis and myocardial infarction [19]. The American Heart Association/American College of Cardiology guidelines suggest the transcatheter occlusion for symptomatic fistulas and for moderate or large fistulas without clinical manifestations [18]. Nevertheless, the best approach to the asymptomatic CPFs is still controversial.

The excellent echocardiographic results observed after 2 months of MRA therapy support the hypothesis that PA was the primary cause of dilated cardiomyopathy in our patient and his mild-moderate CPF currently deserves a careful follow-up for the appearance of symptoms.

Both PA and CPFs might be associated with ECG changes and arrhythmias. However, a paradoxical T-wave inversion in the precordial leads was observed in our patient after oral potassium supplementation. Changes in electrolyte levels can affect depolarization and/or repolarization and can cause ECG changes. In a recent prospective study on hospitalized hypokalemic patients, it was found that 85% of them experienced ECG changes after potassium correction and 36% of patients who showed a T-flat or T-inverted might have a T-wave inversion after potassium supplementation. In addition to this, there was no significant relationship between post-correction potassium levels and ECG changes [20].

Conclusions

In summary, the presented case highlights novel aspects related to PA and its diagnostic algorithms. Therefore, we strongly recommend cardiologists and clinicians to maintain a high level of suspicion of PA in hypertensive patients with finding of coronary artery fistulas (Tab. 2). Further studies will be needed to clarify this association.

Conflict of interests

The author has no conflict of interest that is relevant to the subject matter included in this work.

Patient consent

Obtained.

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Table 2. Learning points and take-home messages

- Hypertensive crises in the emergency room are often underestimated. Patients, particularly if young and presenting spontaneous hypokalemia, should be referred initially to the Hypertension Reference Centers for screening and/or early diagnosis of secondary forms of hypertension.
- The finding of CPFs in hypertensive patients should lead to a high suspicion of underlying PA.
- In patients with PA and chronic hypokalemia, the correction of serum potassium levels might be associated with transient T-wave inversion on the ECG.
- PA should be excluded as a potential cause of dilated cardiomyopathy, because evidence suggests that there may be a probable causal relationship between dilated cardiomyopathy and PA [17].
- The clinical practice guidelines for case detection, diagnosis, and treatment of PA are an essential part of quality medical practice, though the flexibility in diagnostic and therapeutic decision-making is sometimes the only alternative available, such as during the CoViD-19 pandemic crisis.

CPFs — coronary-pulmonary fistulas; PA — primary aldosteronism

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