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Vakuf's Hospital in Sarajevo. The first hospital facility in Bosnia and Herzegovina. Recorded around 1898. Taken from the book: Valerijan Žujo "Doktor Karel Bayer". Courtesy of the author.

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Sex Differences in the Use of Cardiovascular Drugs: A Survey of Patients in a Single Center

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Abstract

Objective. Patients and medical professionals have a common misconception that cardiovascular diseases (CVD) predominantly affect men, which can lead to less prescribing of cardiovascular drugs to women. This study examined whether there were sex differences in the administration of cardiovascular (CV) drugs in patients admitted to the intensive care unit of the Internal Medicine Clinic of Foča University Hospital (ICFUH). **Materials and Methods.** The study comprised 332 patients hospitalized at the ICFUH from January 1st to June 30th, 2019. The following data on leading CVD and risks related to CV drug administration were collected: age, hyperlipidemia (HLD), diabetes mellitus (DM), chronic kidney disease (CKD), liver disease (LD), heart failure (HF), hypertension (HTN), myocardial infarction (MI), and stroke (S). The amount of the CV drugs of interest (statins, antiplatelet drugs, calcium channel blockers, ACE inhibitors, beta blockers, diuretics) administered during hospitalization was expressed as the Defined Daily Dose (DDD)/100 bed-days (BD) for patients of both sexes separately. **Results.** During hospitalization in the intensive care unit of ICFUH, female patients were less likely to be treated with statins than male patients (30.1 vs. 57.5 DDD/100 BD, P<0.05). There was no difference between sexes regarding the use of antihypertensive drugs. Women were less likely to be treated by antiplatelet therapy, more precisely by acetylsalicylic acid (30.4 vs. 36.9 DDD/100 BD, P<0.05). **Conclusion.** Our study indicates that there were sex differences in CV drug administration in ICFUH. Presuming that drugs used during hospitalization were at least partially a continuation of the previous therapy prescribed by the family doctor, it is possible that such differences exist in primary care.

Key Words: Sex Differences ▪ Cardiovascular Diseases ▪ Cardiovascular Drugs ▪ Statins.

Introduction

The leading cause of death worldwide, as well as in Bosnia and Herzegovina (B&H), is cardiovascular disease (CVD). Globally, it is estimated that about one third of deaths in both men and women are caused by CVD (1). In 2018, CVD caused 51.5% and 47.2% of overall deaths in the Federation of Bosnia and Herzegovina (FB&H) and the Republika Srpska (RS), respectively (2, 3). In FB&H in 2019, 4 of the 5 leading causes of death in both sexes were forms of CVD. Mortality rates from CVD were similar, but even higher in the female population: 325.3 deaths in women and 319.0 deaths in men per 100,000 (2). In the same period in the RS, it was found that 12% more women than men died of CVD (3). However, patients and medical professionals have a common misconception that CVD predominantly affects men, which can lead to suboptimal management and treatment of CVD in women (1). There are studies about sex differences in medical treatment of CVD that point out that women with CVD may receive less aggressive care than men (4, 5). Most studies come from high-income countries, most of all from the United Kingdom and the United States of America. Economic status definitely is not the only factor, but together with societal and physiological

factors it can significantly lead to sex differences in the use of cardiovascular (CV) medications. B&H is a middle-income country which indicates possible differences in this field compared to well-developed countries. Greater sex polarization in terms of economic status is expected in countries with lower GDP than in high-income countries. Furthermore, citizens of B&H may have poorer access to medicines compared to countries with a higher GDP (6). We do not have any data on whether sex differences in the administration of CV medications exist in our country. The aim of this study was to examine whether there were sex differences in the administration of the most commonly used groups of cardiovascular medication in patients hospitalized in the intensive care unit of the Internal Medicine Clinic of Foča University Hospital (ICFUH).

Material and Methods

A descriptive cross-sectional drug use study was performed using the administrative health-related databases of the Intensive Care Unit of ICFUH from January 1st to June 30th, 2019. The hospital databases were used to extract data of interest on the medical condition of patients and the drugs administrated. All data were taken using a coding system to prevent patient identification during the further process of analysis. Patients with decompensated cirrhosis and patients with stage 5 chronic kidney disease (eGFR <15 mL/min/1.73 m2 or dialysis) were previously excluded from the study, as well as patients with acute kidney injury.

Comparison of the Patients' Basic Characteristics between Sexes

The following data on the presence of leading CVD and risks with a strong influence on therapy for CVD were observed for each patient: age, hyperlipidemia (HLD), diabetes mellitus (DM), chronic kidney disease (CKD), liver disease (LD), heart failure (HF), hypertension (HTN), myocardial infarction (MI), and stroke (S). All data were expressed as the percentage of affected patients in the overall number, for both sexes and for each of the six months analyzed. Patients with HLD, CKD, LD and HF were divided into subgroups on the basis of the stage of their condition, but there were not enough data for statistical analysis. Due to the lack of data in the documentation, it was not possible to calculate the total cardiovascular risk for most patients.

Comparison of Drug Administration to Different Sexes

Data on administration of several classes of CV medication during hospitalization, in terms of drug type, dosage, dosing interval, and duration of administration, were extracted for each patient. The classes of drugs of interest were statins, antiplatelet drugs, calcium channel blockers (CCB), ACE- inhibitors (ACEI), beta blockers (BB) and diuretics. Drug administration (consumption) was expressed as the number of DDD/100 BD (defined daily dose per 100 bed days), and as the percentage of patients treated with each medication group. DDD/100 BD represents the standardized unit for measuring drug consumption for inpatients, allowing comparisons across various time periods and patient groups. DDD is the average maintenance daily dose per its main indication in adults, defined by the World Health Organization. A "bed day" is a day during which a patient stays overnight in a hospital. Patients admitted to the hospital in the morning and released before the night were excluded from the study (7).

Statistical Analysis

All results are given as the mean ± SD or mean (95% CI). The patient's observed characteristics were compared between men and women by calculating the odds ratio (with female gender used as the reference category). The comparisons between the quantity of drugs administrated to women and men were performed using the Wilcoxon rank-sum test. A 2-sided $P<0.05$ was considered statistically significant. All data analyses were conducted using the SPSS statistical software (version 15 for Windows).

Results

The study included 332 patients hospitalized in the intensive care unit of ICFUH from January 1st to June 30th, 2019. 59.9% of them were men (N=199) and 40.1 were women (N=133). The women were older (67.1±34.3) than the men (63.6±33.1), but the difference was not statistically significant. All patients were stratified into three groups in terms of age as follows: not old (<65), old (65-84) and very old (≥85). We only found sex differences in the group of very old people, where there were more women than men (Figure 1). There was no difference in duration of hospitalization between women and men (5.01±0.85 days for men vs. 4.95±0.74) days for women. Since reliable data on the presence/absence of atherosclerotic cardiovascular disease (ASCVD) were not available for most patients, it was necessary to limit the analysis of ASCVD to MI and S. Both sexes had similar rates of HLD, DM, CKD, LD, HF, HTN, and S, but men were more likely to suffer from MI (Figure 2). Regarding HLD, CKD and LD, the lack of relevant data made it impossible to classify most patients into groups according to the severity of their condition. Regarding HF, it was found that, out of 89 male patients with confirmed HF, 9 of them were in stage C/D. In female patients, we found a very similar frequency of the most severe stage of HF. Of the 61 female patients who were suffering from HF, there were 7 with stage C/D of the disease.

Comparison of Drug Consumption

The percentage of patients treated was determined for each type of medication studied. 28.6% of men were treated by statins, in relation to 20.3% of women, but the difference was not statistically significant. However, when comparing the consumption of statins expressed as DDD/100 BD, we found that these drugs were administered to men to a higher extent than to women (Table 1). We found no difference between men and women regarding consumption of antihypertensive drugs (diuretics, ACEI, BB and CCB). However, we found that women were less likely to be treated by antiplatelet therapy, more precisely by acetylsalicylic acid (ASA), while there was no difference regarding the frequency and quantity of use of ADP antagonist drugs (Table 1).

We further analyzed statin therapy in order to learn if it was carried out in accordance with the current guidelines for statin prevention of cardiovascular diseases, and therapy (8-10). All male patients with hypercholesterolemia and/or established ASCVD were on high intensity statin therapy. On the other hand, we found 7 women with ACSVD and unregulated blood cholesterol levels who were on medium intensity statin therapy, although we could not find a reason for the lower dose. We also found that 7 women with hyperlipidemia were not treated by statins nor any other type of antilipemic medication. Statins were prescribed for primary prevention of CVD in at least 10 men and only 2 women.

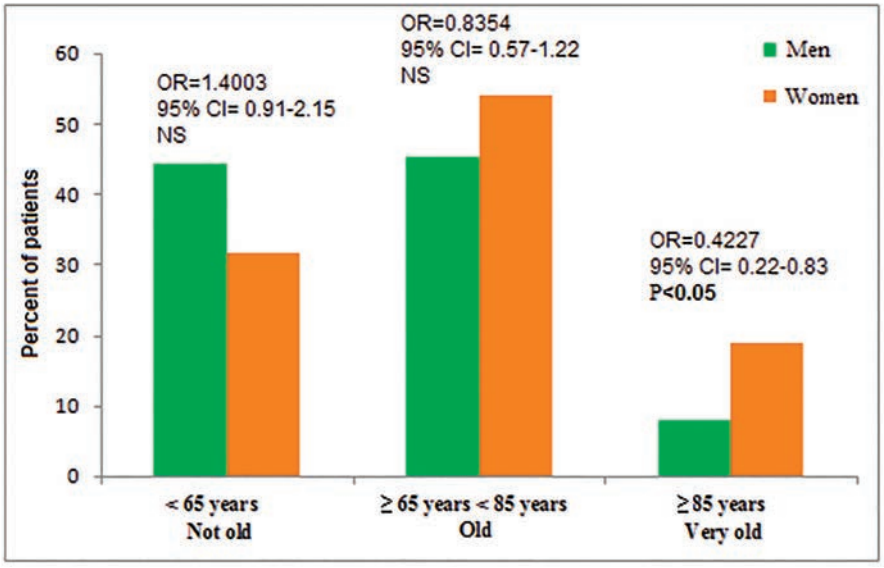

Figure 1. Sex distribution in three age groups of patients.

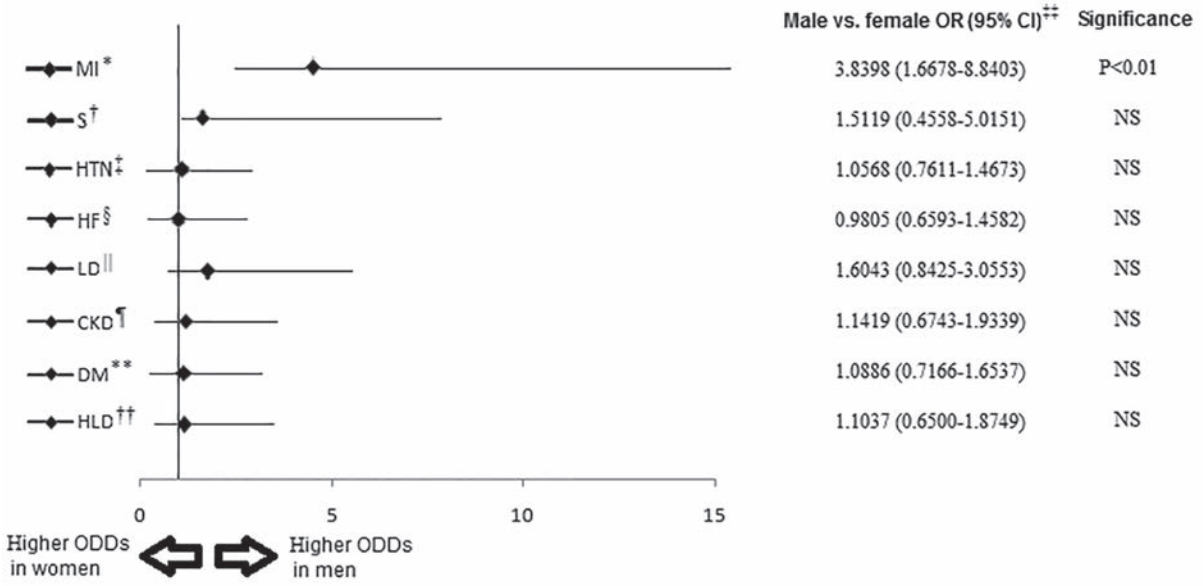

Figure 2. Sex differences in the frequency of medical conditions with a strong influence on the administration of cardiovascular medications. *Myocardial infarction; †Stroke; ‡Hypertension; §Heart failure; ||Liver disease; ¶Chronic kidney disease; **Diabetes mellitus; ††Hyperlipidemia; ‡‡Odds ratio for the prevalence of medical conditions in male and female sex (female sex used as referent category) CI Confidence interval

Table 1. Sex Differences in the Consumption of Cardiovascular Medication

Drug class	Women DDD/100BD* Mean (95% CI)	Men DDD/100BD* Mean (95% CI)	Significance
Statins	30.1 (2.8-80.0)	57.5 (13.4-85.9)	P<0.05
Diuretics	85.5 (55.2-118.3)	89.4 (50.9-136.0)	NS
ACE I†	74.3 (38.3-114.4)	91.5 (61.1-129.1)	NS
BB‡	16.2 (8.4-21.7)	20.0 (11.9-28.3)	NS
CCB§	44.6 (19.0-93.4)	40.5 (8.4-62.9)	NS
ASA‖	30.4 (21.3-54.5)	36.9 (35.1-103.9)	P<0.05
CLOP¶	24.6 (15.5-50.2)	24.4 (19.7-65.2)	NS

*Defined Daily Doses per 100 Bed Days; †ACE inhibitors; ‡Beta blockers, §Calcium channel blockers, ‖Acetylsalicylic acid, ¶Clopidogrel.

Discussion

During hospitalization in the intensive care unit of ICFUH, female patients were less likely to be treated by statins and ASA than male patients. CVD is the leading cause of death of women worldwide. Only in underdeveloped countries is CVD not in first place, because of the large number of deaths from neonatal and infective diseases, such as respiratory infections, diarrhea and malaria (5).

According to the Institute for Public Health of FB&H and the Institute of Statistics of the RS, the leading cause of death in women in B&H in 2019 were diseases of the circulatory system. In our study, the incidence of leading CVD other than MI was the same for both sexes. Taking into account the average age of our patients, this result is expected and in accordance with data from B&H and other high- and middle-income countries (2, 3, 5). However, patients and medical professional share the common opinion that CVD is mostly found in male patients. This is based on the fact that men generally suffer from CVD at a younger age than women, but it is obvious that women are not im-

mune to CVD, and this problem must be taken seriously. We should be worried because there is evidence that women receive less treatment than men. This may be one of the reasons why women have worse outcomes and higher mortality from CVD than men. On the other hand, the recommendations for prevention and therapy for CVD do not differ for men and women in the latest clinical guidelines (8).

Globally, it is recognized that more medications are given to women than to men, but also the therapy given to women is more often not in accordance with the current therapeutic guidelines than therapy given to men (11, 12). In terms of the adult population of the United States, it has been proven that it is less probable that women will receive guideline-recommended statin therapy (13). It has been found that women more commonly refuse and discontinue statins, but also statins are less often offered to women (14, 15). The situation in Foča University Hospital seems to be similar regarding sex differences in statin use. Beside patients from Foča, patients from the entire eastern part of the RS gravitate to Foča University Hospital. This means that the same pattern can be expected throughout RS or at least its eastern part. The percentage of FB&H patients treated in this hospital is too small to be representative. Although in this study there was no difference in the percentage of patients treated with statins, the DDD/100 BD was higher in men, suggesting that women receive less aggressive statin therapy. In other words, men are more likely to be on intensive statin therapy than women. In the further step, it was shown that all men with conditions indicating high intensity statin were treated in accordance with the recommendations. In contrast, some women with an indication for high-intensity statins were treated by medium intensity statin therapy, and some women with hyperlipidemia were not even treated with statins, nor any other form of antilipemic medication. Statins were prescribed more often in men than in women for primary prevention of CVD, but this finding should be taken with caution, because we were not able to find enough data to evaluate total cardiovascular risk in most of our patients. In addition, according to the percentage of our patients treated by statins, we could assume that statins were not prescribed to the recommended extent according to the current recommendations (8-10, 16). Although most authors studied sex differences in the primary prevention of CVD, there are findings about sex differences in pharmacotherapy in survivors of acute MI, one year after acute MI. It was shown that women under the age of 55 years were less likely to be on optimal therapy by the end of 1 year after discharge (17).

Statins are the most commonly used antilipemic drugs. They can be prescribed for both primary and secondary prevention of CVD. Statin use in inpatients was the continuation of previous therapy in around two-thirds of patients. Less than a third of patients were statin- naive. In addition, once statin therapy has been initiated in the hospital setting it should be continued after the patient is discharged, if there are no limiting factors. This implies that sex differences in statin use could exist in primary care, and not only in secondary and tertiary care, and that a large number of female patients may be potentially undertreated by statins.

Several studies reviewed differences in prescription of, but also in the pharmacology and pharmacokinetics of the main classes of antihypertensive drugs (18, 19). It was noticed that ACEI, and BB were more often prescribed to men, but in contrast, women were more likely to receive diuretics and CCB (18). The reasons for less prescription of ACEI and ARB in women are found in the fact that young women are at risk of pregnancy (18). Women older than 65 years used 33% more antihypertensive drugs than men of the same age, although women adhered more closely to the prescribed therapy (19). Although our study did not find any differences in the use of antihypertensive drugs, it is not possible to conclude that no differences exist in the entire population. The fact that inpatients are regularly monitored for blood pressure does not leave the possibility of unrecognized hypertension. In addition, the small number of patients may be the reason for not noticing sex differences in the use of antihypertensive drugs.

There are some findings showing that women are less likely to receive ASA and ACE I than men (1, 10). This partly agrees with our findings, because we also noticed less ASA was given to women, but both genders have the same probability of being treated with ACEI. According to our findings, there was no difference in the administration of other antihypertensive drugs between women and men. The sex difference in ASA administration may be based on the fact that ASA is no longer recommended for primary prevention of CVD at all (8), or in people older than 70 years (16). Furthermore, female patients had less chance of being evaluated concerning total CV risk.

In addition to the differences in therapy administered, it is possible that there are some gender differences in adherence to medication regimens (20). Also, the fact that women have a higher risk of experiencing adverse drug reactions than men may contribute to the worse therapeutic management of female patients (21). The reasons for sex differences in CV drug consumption could be at least partly based on the fact that women are more likely to experience some adverse reactions to CV drugs, especially to statins and ASA.

Limitations of the Study

The main limitation of this study is the relatively small number of patients observed. Also, because of the retrospective study design, it was not always possible to determine the reasons for prescribing/not prescribing cardiovascular medication.

Conclusion

Our study indicates that there are sex differences in cardiovascular medication administration in tertiary care. It probably also suggests that such differences persist throughout the primary care of the entire population, because the therapy administered to hospitalized patients to some extent reflects the chronic therapy prescribed by family doctors in primary care. CVD is definitely the most frequent cause of death of women in B&H, but its importance in women is possibly underestimated.

What Is Already Known on This Topic:
Current recommendations for the prevention and therapy of CVD do not recognize sex differences. There is evidence from several countries that women may be treated less well than men, although the prevalence of CVD in both sexes is similar. This problem has not been studied so far in our country or the region.

What This Study Adds:
This study provides insight, for the first time to our knowledge, into the existence of sex differences in the administration of cardiovascular drugs in a hospital in Bosnia and Herzegovina. This study revealed the need to conduct population research on this topic.

Authors' Contributions: Conception and design: DD, DS, ND and RM; Acquisition, analysis and interpretation of data: DD, DS, MC, MK, BC, and DP; Drafting the article: DD, MC, ND, MK and BC; Revising it critically for important intellectual content: DD, ND, MK, DP, and RM; Approved final version of the manuscript: all authors.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Article
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The Value of Stress Echocardiography Imaging and Functional Parameters in Patients with aVR Lead ST-Segment Elevation during an Exercise Stress Test to Detect Significant Left Main Stenosis

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Abstract

Objective. To evaluate the role of functional and imaging parameters during exercise stress echocardiography (SE) in the presence of ST-segment elevation (ST-E) in aVR leads to predict significant left main/left main equivalent/or ostial left anterior descending (LAD) stenosis (LM+). **Methods.** The study population included 548 patients with ECG and echo markers of myocardial ischemia, in whom diagnostic coronary angiography was performed. We analyzed the patients' clinical characteristics, ECG changes, wall motion score index (WMSI) by stress echocardiography (SE), as well as functional capacity during exercise (METs) and Duke treadmill score. **Results.** aVR ST-segment elevation was found in 60/548 (11%) patients, whereas aVR ST-E was found in 23/57 patients with left main LM stenosis (Sn 40%, Sp 92%, PPV 38%, NPV 93%). When aVR ST-E was combined with other functional/imaging parameters, patients with aVR ST-E and LM+ had significantly worse functional capacity in METs (5.0±2.2 vs. 6.7±2.3, P=0.005), lower Duke score (-6.8±6.8 vs. -3.6±4.1, P=0.049), and higher deterioration of WMSI (0.51±0.24 vs. 0.39±0.24, P=0.046). Significant multivariable predictors of the left main (LM) stenosis were aVR ST-E and positive SE in LAD territory in the whole group of patients, and Delta WMSI, Duke score and METs achieved in patients presented with aVR ST-E during exercise. **Conclusion.** The aVR ST-segment alone has intermediate sensitivity in detecting significant LM stenosis in patients referred to SE testing for chest pain. When combined with other functional and imaging parameters, including poor exercise functional capacity in METs, lower Duke score or greater WMA in the territory of LAD, its diagnostic power to detect LM significantly increases.

Key Words: aVR Lead ST-Segment Elevation ▪ Left Main Stenosis ▪ Stress Echocardiography.

Introduction

The augmented unipolar right arm (aVR) lead, also known as the orphan lead, was originally constructed to detect electrical changes from the right ventricular outflow tract and basal inter-ventricular ischemia (1-4). The aVR lead provides electrical information about the left ventricular basal septum as well as global ischemia. Many studies have shown that ST-segment elevation (ST-E) in the aVR lead is an important predictor of acute severe stenosis or obstruction of the left main (LM) or proximal left anterior descending (LAD) coronary artery. Due to the limited specificity of electrocardiographic (ECG) ST- segment changes in general, ST-E in the aVR lead during exercise tests was ignored for a long time by the practice guidelines (1, 5, 6). However, the latest recommendations on exercise testing again emphasize the importance of the aVR lead ST-E as a marker of significant inducible myocardial ischemia (7). The stress echocardiography (SE) testing that reveals regional wall motion abnormalities (WMA) is spe-

cific for coronary artery disease (CAD). The significance of ST-E in the aVR lead combined with wall motion abnormalities (WMA) during exercise treadmill SE in the prediction of significant LM or LM equivalent stenosis has not been fully evaluated. We hypothesized that exercise-induced aVR lead ST-E, combined with other functional parameters obtained during exercise, and imaging by SE, would improve the predictive value of aVR lead ST-E alone to detect significant and life-threatening LM stenosis.

The aim of our study was to evaluate the role of exercise-induced aVR lead ST-E as a predictor of significant LM or LM equivalent, or ostial LAD stenosis (LM+) in patients referred to exercise SE testing, in combination with other functional and imaging parameters obtained during SE.

Materials and Methods

This was a retrospective study including all the patients undergoing exercise SE at the Stress Echo Lab at the Clinical Center of Serbia, from 2012-2017. Out of 14,529 patients in whom SE was performed, 548 patients were included in the further analysis, who had both ECG signs and echo WMA suggestive of myocardial ischemia, and in whom diagnostic coronary angiography was also performed. Patients with non-interpretable ECG, such as those with pacemaker rhythm, left bundle branch block, baseline ECG ST-segment abnormalities, and patients with coronary artery bypass grafts (CABG) were not included in the analysis.

All patients underwent exercise tests on a Quinton 5500 treadmill (Quinton Cardiology, Inc. Bothell WA, USA), with standard Bruce protocol and continuous 12-lead ECG monitoring. The endpoints were: target heart rate (85% of maximum heart rate for age), severe chest pain requiring termination of exercise, and/or ST segment changes (ST-E of 1mm or more in any lead, without q wave, or ST-segment depression of at least 1 mm in at least two contiguous leads in three consecutive beats). We calculated the Duke treadmill score as an index combining treadmill exercise time using the standard Bruce protocol, maximum ST-segment deviation and exercise-induced angina, and presented metabolic equivalent (MET), describing the functional capacity or exercise tolerance of the patients during exercise testing. All patients provided informed consent for performing SE. Two-dimensional echocardiography monitoring was performed at baseline and immediately after treadmill exercise (peak stress), on a Vivid E9 ultrasound machine (General Electric Healthcare, Wauwatosa, USA). Regional wall-motion analysis was evaluated at baseline and at peak stress, with side by side analysis and semi-quantitative assessment of the Wall-Motion Score Index (WMSI). According to the Recommendations of the American Society of Echocardiography, a 17-segment model of the left ventricle (LV) was used (8-11). The WMSI was derived by dividing the sum of individual segment scores (ranging from 1-normal to 4 dyskinetic) by the number of interpretable segments. Left anterior descending artery (LAD) positivity was defined as the occurrence of new or the worsening of pre-existing WMA in at least 3 adjacent segments in the LAD-vessel territory. Two experienced observers independently reviewed the echo images, with inter-observer concordance of 95% (K=0.948). The extension and severity of induced ischemia was expressed as Delta WMSI (a difference between the resting WMSI and peak WMSI).

All 548 patients were referred for coronary angiography. Significant coronary artery stenosis was defined as ≥70% narrowing of the diameter stenosis of the coronary artery. As the left main equivalent disease, we considered significant ≥50% narrowing of the diameter stenosis of the ostial/proximal LAD artery and the ostial/proximal circumflex artery.

Statistical Analysis

Data were analyzed by descriptive and analytical statistics using SPSS statistical software (IBM SPSS statistics, Version 21.0, SPSS Inc. Chicago, IL), and expressed as mean ± standard deviation for normally distributed data, or as frequency and percentages for categorical data. The student's t-test and chi-square test were used to compare data

between the patients with and without significant LM stenosis (LM+). The Spearman correlation was applied to investigate the relationship between the SE parameters and aVR ST-E with LM stenosis. Calculations (classic reliability calculations) of sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were performed according to the standard definitions. Moreover we determined the positive likelihood ratio (LR+). ROC analyses and logistic regression analysis (uni and multivariate) were used to assess predictors of significant LM stenosis. A P value <0.05 was considered statistically significant.

Results

The study included 548 patients (mean age 61±9 years, 389 males and 159 females). aVR lead ST-E was present in 60/548 (11%) patients, whereas significant LM stenosis by angiography was found in 57 patients (10%). Both aVR ST-E and LM+ were present in 23 patients (Sn 40%, Sp 92%, PPV 38%, and NPV 93%). There was no significant difference between patients with or without aVR ST-E, except for body mass index that was higher in patients with aVR ST-E, and previous myocardial infarction, that was more prevalent in patients without aVR ST-E (Table 1).

Patients with aVR ST-E and LM had significantly worse functional and imaging parameters during stress echo, including worse Duke score and functional capacity as expressed in METs, as well as more severe echocardiographic signs for myocardial ischemia, particularly in LAD territory. Hemodynamic compromise and angina were more prevalent in patients with LM, but they did not reach statistical significance. Interestingly, the concomitant ST depression in contralateral leads was similar in patients with and without LM disease (Table 2). The coronary angiography findings in patients with and without aVR ST-E during SE testing are presented in Table 3. The aVR ST-E was significantly associated with stenosis in LM, with borderline significance for the proximal part of LAD (Table 3).

Out of general patients' data BMI was positively correlated with AVR STE findings (P=0.025)

Table 1. Baseline Clinical Parameters of Patients in Relation to aVR Lead ST-E during Exercise Testing

Variables	aVR ST-E +; (N=60)	aVR ST-E -; (N=488)	P*
Age (mean, yrs)	60.3±7.6	61.6±8.9	0.243
Sex male, N (%)	43 (72)	343 (70)	0.898
BMI (Mean ±SD)	29.6±3	26.7±3.8	0.039
Family history, N (%)	28 (46.7)	239 (48.9)	0.703
Smokers, N (%)	10 (16)	108 (22)	0.317
Diabetes mellitus type 2, N (%)	17 (28.3)	139 (28.4)	0.958
Diabetes mellitus insulin-dependent, N (%)	19 (31.6)	105 (21.5)	0.081
Hypertension, N (%)	50 (83.3)	399 (81.2)	0.813
Hyperlipidemia, N (%)	46 (76.7)	343 (70.2)	0.372
Previous myocardial infarction, N (%)	12 (20)	168 (34.4)	0.024
Beta-blockers, N (%)	48 (80)	364 (74.6)	0.627
ACE inhibitors, N (%)	40 (66.7)	308 (63)	0.880
Nitrates, N (%)	20 (33.3)	191 (39.1)	0.262
Aspirin, N (%)	45 (75)	372 (76.20	0.403
Clopidogrel, N (%)	11 (18.30)	136 (27.9)	0.080
Statins, N (%)	37 (61.7)	297 (60.9)	0.763

*Student's t-test and Chi-square test; BMI=Body mass index; ST-E=ST-segment elevation; aVR=Augmented unipolar right arm.

Table 2. SE Data in Patients with aVR ST-E in Relation to the Presence of Significant LM stenosis (LM+)

Variables	aVR ST-E+ and LM+; (N=23)	aVR ST-E+ and LM-; (N=37)	P*
Blood pressure drop, N (%)	6 (26)	4 (10.8)	0.123
Test angina, N (%)	10 (43.5)	11 (29.7)	0.278
Target SMF, N (%)	9 (39)	19 (51.3)	0.356
HRR, N (%)	15 (65)	28 (75.7)	0.290
ST-D D2, D3, Avf, N (%)	22 (95.7)	33 (89)	0.379
ST-D in V3/V4-V6, N (%)	20 (87)	30 (81)	0.553
ST-D diffuse, N (%)	20 (87)	27 (73)	0.201
MET (Mean ±SD)	4.95±2.2	6.7±2.3	0.005
Duke score (Mean ±SD)	-6.8±6.8	-3.6±4.1	0.049
Delta WMSI (Mean ±SD)	0.51±0.24	0.39±0.24	0.046
LAD positivity, N (%)	20 (87)	22 (59.5)	0.024

*Student's t-test and Chi-square test; LM=Left main coronary artery; ST-D=ST-segment depression; ST-E=ST-segment elevation; LAD=Left anterior descending coronary artery; WMSI=Wall motion score index; aVR=Augmented unipolar right arm; MET=Metabolic equivalent; HRR=Heart rate recovery: ST-D diffuse=ST segment depression in D2, D3, aVF, V3/4-6 segments.

Table 3. Coronary Angiography Findings in Patients with and without aVR ST-E during SE Testing

Variables	aVR ST-E + (N=60)	aVR ST-E - (N=488)	P*
LM+ group N (%)	23 (38.3)	34 (6.96)	<0.001
LM – group			
One-vessel CAD N (%)	10 (16.7)	157 (32.2)	0.411
LAD, N (%)	7 (70)	61 (38.9)	0.052
LCx, N (%)	1 (10)	20 (12.7)	0.800
RCA, N (%)	2 (20)	76 (48.4)	0.081
Two-vessel CAD, N (%)	13 (21.7)	129 (26.4)	0.481
LAD, N (%)	7 (53.8)	92 (71.3)	0.061
Three-vessel CAD, N (%)	6 (10)	77 (15.8)	0.960
None, N (%)	8 (13.3)	85 (17.4)	0.943

*Chi-square test; CAD=Coronary artery disease; RCA=Right coronary artery: LCx=Left circumflex coronary artery; LAD=left anterior descending coronary artery; LM=Left main coronary artery; ST-E=ST-segment elevation; aVR=Augmented unipolar right arm.

as well as the findings of combined AVR STE and stenosis (P=0.019). Among the investigated cardiological parameters, only ST depression in leads V3 to V6 and V4 to V6 did not correlate with the findings of both stenosis (P=0.657) and AVR STE (P=0.205). Moreover, LAD positivity (P=0.291) and ST depression (P=0.137) were not significantly associated with AVR STE. After confirming the associations of the investigated parameters, we performed regression analysis.

In logistic regression, a significant equation (model) was obtained for prediction of significant LM stenosis (χ^2=79.038; P=0.001; B=2.103; Wald= 208.840; Exp(B)=0.122; R^2 Nagelkerke=0.601; total classification %=90.8). Findings of univariate analysis were used to selected parameters to be tested in multivariate analysis. Significant predictors by multivariate analysis were aVR ST-E and LAD positivity in the whole group of patients. We also constructed an equation for prediction of significant LM stenosis in the group of patients with aVR ST-E (χ^2=76.055; P=0.001; B=3.051; Wald= 195.537; Exp(B)=0.047; R^2 Nagelkerke=0.670; total classification %=96.5). Interestingly, in patients with aVR ST-E, the magnitude of ischemia presenting as Delta WMSI, together with the Duke score and MET categories (good >5, and poor <5) were predictors of significant LM stenosis (Table 4).

Table 4: Regression Analyses for Prediction of Significant LM Stenosis

Variables	OR	95% for CI	P	OR	95% for CI	P
	Univariate analysis			Multivariate analysis		
Whole sample						
Patients' sex	0.542	0.837-4.065	0.188	-	-	-
Patients' age	0.036	0.009-1.077	0.079	-	-	-
BMI	0.034	0.003-1.159	0.494	-	-	-
aVR ST-E	1.554	1.536-11.225	0.001	0.255	0.160-0.350	0.001
LAD positivity	1.333	1.030-4.231	0.001	0.054	0.001-0.106	0.045
Delta WMSI	1.240	0.485-29.765	0.242	-	-	-
ST-D diffuse	0.570	1.225-7.872	0.438	-	-	-
MET score	0.247	0.778-1.138	0.521	-	-	-
Duke score	-0.065	0.855-1.023	0.124	-	-	-
Constant	26.106	-	0.997	-	-	-
aVR ST-E						
Patients' sex	0.307	0.214-4.347	0.634	-	-	-
Patients' age	0.009	0.004-1.149	0.797	-	-	-
BMI	0.048	0.011-1.232	0.540	-	-	-
LAD positivity	0.195	0.251-4.637	0.754	-	-	-
Delta WMSI	5.303	0.367-97.527	0.001	0.420	0.228-0.612	0.001
ST-D diffuse	0.725	0.549-14.824	0.507	-	-	-
MET score	1.399	0.501-1.142	0.027	-	-	-
Duke score	0.174	0.834-1.127	0.014	-0.011	-0.0018-0.004	0.002
Constant	24.422	-	0.997	-	-	-

LM=Left Main; ST-E=ST-Segment elevation; ST-D=ST-Segment depression; ST-D Diffuse=ST segment depression in leads D2, D3, aVF, V3/4-6 segments; LAD=Left anterior descending coronary artery; LM=Left main stenosis; BMI=Body mass index; WMSI=Wall motion score index; MET=Metabolic equivalent; OR=Odds ratio.

A receiver operating characteristic curve (ROC) analysis (Table 5) identified cut-off values for Delta WMSI, MET and Duke score. Finally, when aVR ST-E was combined with other functional and imaging parameters, the predictive value for detection of significant LM stenosis increased significantly (Table 6). In particular, when aVR ST-E was combined with LAD positivity and low MET achieved during SE, its sensitivity to detect significant LM stenosis increased from 40% to 88%.

Table 5. ROC Analysis of Functional and Imaging Parameters in Prediction of Significant LM Stenosis

Variables		AUC (%)	P	Cut-off value	Sensitivity (%)	Specificity (%)	LR+
aVR ST-E +	Delta WMSI	87.5	0.001	0.31	81.8	76.1	3.42
	MET score	25.4	0.001	5.5	73.9	67.6	2.28
	Duke score	18.7	0.001	-4.750	60.9	62.2	1.61

LM=Left Main; WMSI=Wall motion score index; MET=Metabolic equivalent; ST-E=ST-Segment elevation; aVR=Augmented unipolar right arm; AUC=Area under the curve; LR+=Positive likelihood ratio.

Table 6. Reliability of Prediction of Significant LM Stenosis

Variables		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+
Whole sample	aVR ST-E only	40.35	92.46	38.33	93.03	5.35
aVR ST-E +	MET categories	73.91	67.57	58.62	80.65	2.28
	LAD positivity	73.91	45.95	45.95	73.91	1.37
	ST-D diffuse + LAD positivity	70.01	44.44	48.28	66.67	1.26
	ST-D diffuse + MET cat	70.01	66.67	60.87	75.00	2.10
	ST-D diffuse + LAD positivity + MET cat	87.57	60.01	64.71	75.00	2.19

ST-D diffuse=ST segment depression in leads D2, D3, aVF, V3/4-6 segments; LAD=Left anterior descending coronary artery; MET cat=MET score<5; PPV=Positive predictive value; NPV=Negative predictive value; LR+=Positive likelihood ratio.

Discussion

Our results show that aVR ST-E during SE testing exercise has intermediate sensitivity for detection of significant LM stenosis in patients with chest pain who are referred for exercise stress testing. However, when combined with other functional and imaging parameters, including functional capacity in METs, Duke treadmill score, and particularly severe myocardial ischemia in the territory of LAD, as documented by SE, its diagnostic power significantly improves. In addition, aVR ST-E, if not predictive of significant LM stenosis, is associated in the majority of cases with proximal LAD/Cx stenosis and/or multivessel coronary artery disease.

ST-E in the aVR lead is not such a rare finding during treadmill stress testing, with incidence ranging from 10 to 25% (7), which corresponds to 11% of our patients with both ECG and echo markers of myocardial ischemia. ST-E in the aVR lead is thought to result from two possible mechanisms: diffuse sub-endocardial ischemia with ST-D in the lateral leads, producing reciprocal change in the aVR which could explain the extensive ischemia on ECG, or ischemia of the basal septum (12-14).

Nevertheless, the usefulness of aVR in detection of severe LM stenosis is still debatable in the published literature. In our group of patients, 38% of our patients with aVR ST-E during stress echo testing and a positive echo for myocardial ischemia, had angiographically proven significant LM/LM equivalent, or ostial LAD stenosis on coronary angiography. Thus, the presence of exercise-induced aVR ST-E did not always indicate the presence of significant LM stenosis, but also proximal LAD or Cx lesions, or multi-vessel disease, as demonstrated by our study. In comparison to earlier data (1, 15), the sensitivity of aVR ST-E to detect LM in our study was lower than in previous literature, demonstrating high sensitivity, of more than 80%, but low specificity. However, the results of previous studies may be compromised by the methodology where exercise testing follows angiography, not as per routine and a rationale practice, with invasive evaluation of coronary artery disease indicated when the stress test is positive and suggestive of myocardial ischemia.

Previous data regarding the role of myocardial perfusion imaging (MPI) studies in addition to ECG (aVR lead ST-E), as markers of myocardial ischemia during exercise testing, failed to provide incremental information for detection of significant LM stenosis (5, 16). These investigators showed that in some patients aVR ST-E could detect LM or ostial LAD disease when SPECT was negative, although multivariate analysis showed that stress LVEF and the percentage of reversible LAD ischemia were significant predictors of LM/ostial LAD stenosis. In contrast, our study demonstrated that the amount and not only the site of myocardial ischemia, as detected by stress echocardiography, may suggest significant left main stenosis.

The role of ST-depression in prediction of LM stenosis other than aVR ST-E is not consistent in the literature. In a study of 200 patients undergoing ECG exercise testing (16), it was shown that patients would most likely have LM stenosis in the presence of ST depression in at least 5 leads on ECG, but not combined with aVR ST-E. It has been also shown that ST-E in V1 is more indicative of proximal LAD stenosis if accompanied by aVR lead ST-E (16, 17). In addition, in our study there was no specific and incremental role of ST-D accompanying ST-E to predict LM stenosis – the rate of concomitant ST depression was similar in patients with and without LM. Nevertheless, our study did show that aVR ST-E and inferolateral ST-D, present on ECG during SE, may indicate significant LM, especially if exercise is terminated with less than 5 METs achieved. However, even in the absence of imaging, poor functional parameters, including METs and particularly the Duke score during exercise, have an incremental role in detecting significant LM stenosis in the presence of aVR ST-E.

Limitations of the Study

This study only included patients with both positive ECG and echocardiographic signs of myocardial ischemia followed by angiography, so a number of patients with possible LM stenosis without both ECG and echo evidence of myocardial ischemia were not included. However, a larger group would further decrease the sensitivity and positive predictive value of aVR ST-E but not the other exercise functional parameters or imaging signs of myocardial ischemia. Also, during angiography we did not perform invasive evaluation of LM by fractional flow reserve to prove myocardial ischemia, as suggested by the guidelines (18). In the presence of documented ischemia by noninvasive testing, evaluation of LM by FFR is not recommended.

Conclusion

The aVR lead ST-E has intermediate sensitivity to detect LM stenosis, but can also disclose significant stenosis in a high proximal segment of LAD, or multivessel disease. However, when combined with functional parameters during exercise, including MET and Duke score, and particularly imaging parameters during SE testing, including the site and myocardial area at risk, its diagnostic power to detect significant LM coronary stenosis significantly improves, and may help to stratify patients for early coronary angiography and revascularization.

What Is Already Known on This Topic:

ST-segment elevation in the EKG lead aVR is an important predictor of acute obstruction of the left main coronary artery during acute coronary syndrome. To date, its use in predicting significant left main stenosis or the left main equivalent during the treadmill stress test is unclear in the literature. Despite its availability and simplicity for detecting inducible myocardial ischemia, the limited sensitivity and specificity of aVR lead ST-segment elevation suggest the need to combine ECG findings with imaging during the exercise test in order to improve it.

What This Study Adds:

Stress echocardiography testing showed potential to improve the diagnostic power of lead aVR in detection of significant LM stenosis. When we combined lead aVR ST-segment elevation with functional and imaging parameters, including poor exercise functional capacity in METs, lower Duke score or greater wall motion abnormalities in the territory of the left anterior descending artery, its diagnostic power to detect left main stenosis significantly increased.

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Narrative Review
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mcr Genes Conferring Colistin Resistance in *Enterobacterales*; a Five Year Overview

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Abstract

The present review aims to study and detect the global emergence of *mcr* genes in *E. coli, K. pneumoniae* and *Salmonella* spp., isolates from human specimens over the last six years. Nowadays the rise of multidrug-resistant superbugs has made essential the return of drugs that were previously abandoned. A clear example is colistin, which acts against multidrug - resistant gram - negative pathogens, including *Enterobacterales*. Colistin resistance is an unfortunate fact, with the emergence of *mcr* genes conferring resistance to colistin in *Enterobacterales* posing the most recent threat. Literature about *mcr* genes and their spread in *E. coli, K. pneumoniae* and *Salmonella spp.* is cited, focusing on the emergence of *mcr* genes in human specimens since 2015. The data were taken from the *PubMed* and *Scopus* databases. It seems that the *mcr*-1 gene continues to be the protagonist among the three species. *E. coli* is the dominant species harbouring *mcr* genes. Moreover, plasmid - mediated colistin resistance is also conferred upon other species that carry different genes resistant to antibiotics. There are only scarse reports on human *Salmonella spp* isolates harbouring *mcr* genes. Finally, the emergence of the *mcr-9* gene in all of them is quite remarkable. **Conclusion.** Plasmid - mediated colistin resistance in *Enterobacterales* is a global issue and has been worsening over the years. The continuous mutations of *mcr* gene subtypes underline the need for better surveillance, constant investigation and wise use of colistin, especially in countries with high levels of antibiotic resistance.

Key Words: Colistin ▪ Resistance ▪ *mcr* Genes ▪ *Enterobacterales.*

Introduction

Antibiotic resistance is one of the greatest open battle fronts of modern medical science and humanity. In the 1990s, the need to add more antibiotics to the therapeutic quiver resulted in the restoration of outmoded drugs, such as polymyxins. Polymyxins are a group of 15 peptide molecules of similar function and morphology, which are divided into 5 categories (polymyxins A - E), and they generally act against gram - negative microorganisms (1, 2).

Among them, two are used in clinical practice: polymyxin B and colistin (polymyxin E). Both have been administered systematically and in various ways since their discovery. However, in the late 1970s, they were considered responsible for causing nephrotoxicity and neurotoxicity. Therefore, their widespread use was limited to the treatment of *Pseudomonas spp*. lung infections in patients with cystic fibrosis, as well as to ear and eye infections (2). Unfortunately, the development of multidrug-resistant (MDR) superbugs of the *P. aeruginosa, A. baumannii* and *K. pneumoniae* species, gave rise to the need for the return of polymyxins, and particularly colistin, in therapeutic regimens. In recent decades, intravenous administration of colistin has increased, while the incidence of its side effects has significantly decreased. Regarding its local administration, its use continues successfully (3). Nevertheless, it should not be forgotten that nephrotoxicity still remains one of colistin's side effects. Therefore, pharmacokinetics are essential in order to determine the optimal thera-

peutic and non-nephrotoxic concentrations of colistin. Planning the appropriate directed antibiotic therapy could also prevent the emergence and proliferation of new resistant species (4). Specifically, heteroresistance, a phenomenon responsible for the increase of colistin resistance, makes colistin an inadequate antibiotic. Therefore, colistin is recommended to be administrated in combination with other antibiotics, and not as monotherapy (2, 4).

Morphologically, colistin is characterized by a cyclic heptapeptide and a tripeptide side chain. The *N*-terminus of the side chain is acetylated by a fatty acid tail, which is responsible for colistin's side effects. On the basis of structural differences, colistin is classified into two chemical molecules: colistin A (polymyxin E1) and colistin B (polymyxin E2) (2).

Colistin is a narrow - spectrum antibiotic that is ineffective against gram - positive bacteria. It acts against most *Enterobacterales,* including multidrug-resistant *E. coli, Enterobacter spp., Klebsiella spp., Citrobacter spp., Salmonella spp.,* and *Shigella spp*. It also acts against some non - fermenters, such as *A. baumannii, P. aeruginosa* and *Stenotrophomonas maltophilia,* but is inactive against *Burkholderia cepacia* and *Pseudomonas mallei* (3, 5).

Despite the need for colistin due to the proliferation of resistant bacteria, its prolonged use has brought undesirable results. Emergence of colistin resistant isolates in clinical medicine, the agriculture sector and animal husbandry has become frequent in many geographical areas. The most worrisome effects of this phenomenon have become apparent in the increasing infections due to colistin - resistant pathogens (6).

Colistin Resistance

Over the past 10 years, the rate of colistin resistance has increased. For this reason, extensive research and studies are being conducted on the mechanisms of colistin resistance. Initially, resistance was attributed exclusively to intrinsic mechanisms. More specifically, genetic modifications in the chromosomal genome of pathogens were considered to be responsible for their resistance (3).

Paradigms of intrinsic resistance mechanisms have been observed in a variety of colistin target pathogens. *P. aeruginosa* uses a kinase system (PmrA / PmrB and PhoP / PhoQ) which controls the transcription of *pmrHIJKLM* operon, resulting in the production of an enzyme (N4-aminoarabinosis) that modifies the lipid load of the cell wall. *K. pneumoniae* regulates the action of regulatory *PhoPQ* and *PmrAB*, by inactivating a gene, *mgrB* (6). Finally, the *Proteeae* tribe, which includes *Proteus spp*., *Providencia spp.* and *Morganella morganii*, seems to share the same intrinsic mechanisms for colistin resistance (7). Specifically, *P. mirabilis* and *S. marcesens* present resistance based on the coding of its *arnBCADTEF* operon genes and the *eptB* gene, aiming to substitute cations in the lipopolysaccharide (LPS) layer (5).

A notable phenomenon of colistin resistance is heteroresistance. In the case of heteroresistance, different degrees of sensitivity to the antibiotic are observed in subpopulations of the same bacterium, and the resistance is not detectable by conventional susceptibility methods (8). It is attributed to mutations that occur in chromosomal genes, such as the *lpxA, lpxC* and *lpxD* of *A. baumannii*. Heteroresistance is thought in some cases to be responsible for the development of colistin resistance, especially during colistin treatment. Apart from *A. baumannii,* colistin heteroresistance has also been observed in other species, including *K. pneumoniae* (2).

Over recent decades, chromosomal resistance mechanisms have been the only explanation for colistin resistance. However, 2015 was a milestone, as researchers found clear scientific evidence regarding the plasmid - mediated colistin resistance mechanisms. A pioneer study was conducted in China by Liu et al. where the researchers highlighted the existence of a plasmid that carries a colistin resistance gene in *Enterobacterales,* and named it *mcr*-1, although it should not be overlooked that the first reports of *mcr* genes and their enzymes emerged in the 1980s in E. coli isolates. These isolates were from poultry in China (2, 9).

The newly discovered mobile *mcr*-1 gene is responsible for reducing the negative charge of lip-

id A. This action is carried out by the transfer of glucosamine from lipid A through the mediation of the enzyme phosphoethanolamine transferase, which is encoded by *mcr*-1. The reduction in load results in the inability of colistin to adhere to lipid A (6). In subsequent years, reports of the *mcr-1* gene in the family of *Enterobacterales* increased globally. At the same time, various publications demonstrated the evolution of the series of *mcr* genes, including a plethora of variants and subtypes. Particularly, in recent years around 22 variants of *mrc*-1 (*mcr*-1.2 to *mcr*-1.22) have been discovered, which differ only by a few amino acids and they are up to 99% identical. It seems that these similarly structured variants provide the same ratio of colistin resistance to pathogens. Furthermore, in later years, 10 novel *mrc* alleles emerged, from the original *mcr-1* to the novel *mcr*-10 gene, which enhanced *Enterobacteriaceae* resistance to colistin even more (2).

Detecting colistin resistance determinants in gram - bacteria has become a significant laboratory challenge. The disc diffusion method and agar dilution method have proven to be inaccurate in determining colistin susceptibility. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the broth dilution method is more reliable. Nowadays, the broth microdilution method is recommended and performed by the majority of routine laboratories to test colistin susceptibility. Moreover, the innovative rapid polymyxin NP test is similarly effective as the broth dilution method. Nowadays, efforts have been made to expand its use in non-fermenting bacilli as well. Regarding the bacteria's minimum inhibitory concentrations (MICs), they seem to be affected by the plasma colistin concentrations; a concentration of 2μg/mL is the optimal dose, resulting in MICs ≤1μg/mL (2, 4).

Nowadays, the scientific community is focusing on finding new strategies to reverse colistin resistance. Several approaches have been made. The reduction of *mcr* gene expression at the gene level is one of the most popular. Additional approaches are the discovery and use of new antibiotics (eravacycline, plazomicin, and artilysin) and the determination of the optimal colistin concentrations, combined with extra agents (amikacin, aztreonam, rifampin etc.). However, it is important to underline that the reduction in the spread of colistin resistance depends on wise colistin consumption, and perseverance in hospital hygiene measures (4).

mcr Genes and *Enterobacterales*

This study aims to review the current data and bring together the most recent research relating to the detection of *mcr* genes in *Enterobacterales* isolates in human specimens. Specifically, an effort was made to deposit studies over the last six years, which present the progress of *mcr* genes and their subtypes detected in *E. coli, K. pneumoniae* and *Salmonella spp.* Recent research studies detecting *mcr* genes in *Enterobacterales* exclusively from human specimens are listed below. The data were taken from *PubMed* and *Scopus* databases, using the key words: "*mcr*", "genes", "humans", "*Escherichia coli*", "*Klebsiella pneumoniae*" and "*Salmonella*" for the search query. The search limitations included only medical results and full text articles. The timeline was set between 2015 and 2021. Studies which did not include human specimens were excluded.

E. Coli

E. coli was the first pathogen in which a plasmid-mediated *mcr* gene was isolated in 2015 (9). The same year, Carattoli *et al.* monophasic variant of serovar Typhimurium (4,5,12:i:- confirmed the spread of the *mcr*-1 gene in both animals and humans. They also reported the presence of the *mcr*-2 gene, exclusively in *E. coli* strains from animals in Belgium, and highlighted the spread of the *mcr*-3 gene from Asia and the United States. They also predicted the danger of the emergence of *mcr*-4 (10).

During 2016 - 2017, the spread of the *mcr*-1 gene in *E. coli* strains increased dramatically. Several studies reported clinical isolates of colistin - resistant *E. coli* harboring the *mcr*-1 gene and its wide spread in South America (11, 12). In the same

year, Italy and Algeria reported resistant *E. coli* strains carrying the *mcr*-1 gene on a clinical level (13, 14). Similar clinical reports of *mcr*-1 genes in *E. coli* were published in both England and Wales (15), in the USA (16), in Japan (17), in Egypt (18), in Taiwan (19), and Malaysia (20), and expanded globally to numerous countries. Additionally, in December 2017, Liu et al. (21) identified a colistin-resistant *E. coli* clinical isolate carrying two plasmid-borne colistin-resistant genes, *mcr*-1 and the newly identified *mcr*-3. It is noteworthy that many studies reported *E. coli* isolates harbouring *mcr* genes together with multiple resistance mechanisms (22, 23).

Over the past three years, worldwide reports of colistin - resistant *E. coli* clinical isolates have soared. The *mcr*-1 gene has remained the protagonist, with reports from both clinical and community studies (24, 25). However, in 2019 Kieffer *et al.* reported the role of the *mcr*-5 gene in the colistin resistance of *E. coli*. Finally, in the same year, the novel *mcr*-9 gene was identified. According to Kieffer *et al.* the way that the *mcr*-9 gene in *E. coli* confers resistance was rather bizarre. Although the *mcr*-9 gene in wild-type *E. coli* strains seemed only to reduce susceptibility, it led to resistance once induced by small concentrations of colistin (26).

K. Pneumoniae

K. pneumoniae is one of the most clinically important bacteria in terms of resistance to antibiotics and nosocomial infections. However, on a smaller scale, plasmid - mediated colistin resistance manifests a common course with that of *E. coli*. In 2016, many studies identified resistant strains of *K. pneumoniae* carrying the *mcr*-1 gene in various countries, e.g., in European, North American, and southeast Asian areas (27). A similar report was also made by Guetet *et al.* during their study of a stool specimen of an infant with diarrhea in China (28). In Italy, in the same year, the *mcr-1.2* variant of a KPC-3-producing ST512 *K. pneumoniae* isolate from a leukemic child was detected for the first time (29). The *mcr*-1 gene was also reported in France, China, Laos and Lebanon (30-33). It is widely known that strains of *K. pneumoniae* commonly carry various types of resistance genes. A characteristic paradigm was recorded by Dalmolin et al. (34) in 2017 in Southern Brazil, who isolated a clinical strain that harbored both the *mcr*-1 and bla_{KPC-2} genes. In 2020, the *mrc*-8.1 gene was detected in clinical *K. pneumoniae* isolates in various countries, such as Lebanon, Qatar and Morocco (35-37). Finally, the same year, Wang *et al.* isolated 28 *K. pneumoniae* strains which harbored the novel *mcr*-9 gene from patients in Belgium, Denmark, Montenegro, Poland, Romania, Serbia, Slovenia and Spain (38).

Salmonella Spp.

The human records in which *Salmonella* strains were found to bear plasmid - mediated colistin resistance mechanisms, are obviously fewer than those of *E. coli* and *K. pneumonia*e. In Portugal 2016, a 4 - year study detected the presence of the *mcr*-1 gene in *Salmonella* serotypes recovered from human clinical specimens (39). The same year in China, both *mcr*-1 and $bla_{CTX-M-55}$ were detected on a single plasmid in *S. enterica* for the first time (40). Furthermore, Carnevali et al. (41) demonstrated the occurrence of *mcr*-1 in colistin-resistant *S. enterica* isolates gathered from humans and animals, between 2012 and 2015. Similar reports were made from Doumith et al. in England and Wales (15). In 2020, reports indicated the emergence of colistin-resistant *S. enterica* carrying the *mcr*-9 gene (42). Finally, in 2019 a novel *mcr* homologue *mcr-9*, identified in a *Salmonella enterica* serotype *Typhimurium (*S. *Typhimurium*) genome, was reported by Carroll et al., isolated from a human patient in Washington State in 2010 (43).

Discussion

The use of the "formerly abandoned" colistin as a last - line antibiotic to treat gram-negative bacterial infections emphasizes the need to develop new antibiotics and also the necessity for prudent use of the existing ones. Despite the short period of colistin re-use (5-6 years), studies have shown that

Table 1. Molecular Epidemiology of Colistin Resistance in Human Isolates

Pathogen	*Mrc* genes	Year (Area) of detection	References
E. coli	*mrc*-1	2015 (China), 2016 (globally)	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20
	mrc-3	2017 (China)	21
	mrc-5	2019 (Czech Republic)	26
	mrc-9	2019 (Czech Republic)	26
K. pneumoniae	*mrc*-1	2016 (Europe, North America, Asia, China, Brazil)	27, 28, 30, 31, 32, 33, 34
	mrc-1.2	2016 (Italy)	29
	mrc-8.1	2020 (Lebanon, Qatar and Morocco)	35, 36, 37
	mrc-9	2020 (Belgium, Denmark, Montenegro, Poland, Romania, Serbia, Slovenia, Spain)	38
Salmonella spp.	*mrc*-1	2016 (Portugal, China, England and Wales)	15, 39 , 40
	mrc-9	2019-2020 (USA)	42 , 43

resistant bacterial strains against it have increased alarmingly.

Regarding *Enterobacterales,* especially *E. coli* and *K. pneumoniae*, the emergence of novel *mcr* genes has become a global threat (Table 1). Over recent years, *E. coli* and *K. pneumoniae* human isolates which carry the *mcr*-1 gene, have been observed increasingly around the globe. In fact, it seems that plasmid - mediated colistin is acquired by isolates harbouring other resistance traits, such as ESBLs or carbapenemases, resulting in the multidrug or extensively drug resistant phenotype. Even though the most commonly identified *mcr* gene appears to be *mcr*-1, isolates have been recorded to carry other types as well. The emergence of the *mcr*-9 gene is remarkable and its prevalence is increasing significantly.

Although human clinical samples for *Salmonella* spp. have not been extensively recorded and studied, *mcr* genes seem to seem to spread through the food producing chain (41). In fact, the discovery of the *mcr*-9 gene in food routed for human consumption underscores the need for further investigation. Plasmid - mediated colistin resistance in *Enterobacterales* is an issue that has worsened over the years. The latest news about the identification of the novel *mcr*-10 gene in strain 090065 of *Enterobacter roggenkampii* in 2020 underlines this global issue and the need for more strict surveillance of colistin use (24).

Conclusion

To conclude, in this review an effort was made to study and detect the global emergence of *mcr* genes in *E. coli, K. pneumoniae, Salmonella* spp. isolates from human specimens. *E. coli* strains are the most resistant among *Enterobacterales*, since they carry several *mcr* genes and their subtypes. Moreover, *E. coli, K. pneumoniae* and *Salmonella* spp strains which carry the *mcr*-1 gene have been reported simultaneously on different continents around the globe. It is remarkable that a plethora of countries have underlined the increasing threat of colistin resistant *Enterobacterales.* This could sound the alarm for several other areas and countries as well. Particularly, Greece is one of the countries with the highest antibiotic resistance rates in Europe, so it is of paramount importance for Greece to investigate further colistin resistance in *Enterobacterales,* among others (44-46). Surveillance and screening for colistin resistant *Enterobacterales* is highly recommended in livestock, animal farms, imported meat and poultry, along with monitoring antibiotic use. Future work could aim at well-organized universal surveillance programs of colistin resistance, in order to prevent the dangerous spread of the life-threatening superbugs.

What Is Already Known on This Topic:
There is currently a continuous and dangerous augmentation of colistin resistance. Studies have focused on the mechanisms used by pathogens in order to become colistin resistant. The general emergence of mcr genes is a fact.

What This Study Adds:

Collective data were gathered from the global biography, and a review performed about the emergence of different types of mcr genes, specifically focused on E. coli, K. pneumoniae and Salmonella spp, over the last five years. Additionally, this study underlines the severity of the global spread of colistin and multi-resistant Enterobacterales, which is often combined with a lack of surveillance programs.

Conflict of Interest: The authors declare that they have no conflict of interest.

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In Utero Exposure to Antihypertensive Medication during the First Trimester: Is the Risk Worth Taking?

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Abstract
The aim of this study is to evaluate and present the evidence so far, regarding fetal outcomes after in utero exposure to antihypertensive medication. Hypertensive disorders during pregnancy constitute a significant risk factor for maternal and fetal outcomes, necessitating antihypertensive treatment. However, current data concerning the safety of in utero exposure to antihypertensive medication are controversial. While some studies recommend the administration of certain agents, others underline the possible adverse effects on fetal development. This review aims to summarize the outcomes of studies published during the last decade, referring to first trimester in utero exposure to antihypertensive agents. In general, a-methyldopa, β-blockers and calcium channel blockers are the first or second treatment line for hypertension during pregnancy. However, ACEIs, ARBs and diuretics are mostly contraindicated, as the potential risk outweighs the benefits of their administration. Additionally, several drugs should be avoided, due to the lack of data regarding their safety. **Conclusion.** As current studies are restricted for ethical reasons, there is a significant lack of evidence concerning diverse antihypertensive agent use. In utero exposure to antihypertensive medication needs to be carefully evaluated and supported by further research.

Key Words: Drugs ▪ Hypertension ▪ Pregnancy ▪ Safety ▪ Side Effects.

Introduction

Hypertensive disorders are one of the leading causes of maternal and fetal pregnancy-related complications, affecting 5-10% of pregnancies (1). They include four main clinical entities: chronic hypertension, gestational hypertension, pre-eclampsia and pre-eclampsia superimposed on chronic hypertension (2). Of these categories, only chronic hypertension corresponds to the subject of this review, which focuses on pharmaceutical antihypertensive treatment during the first trimester.

Chronic hypertension is defined as blood pressure above 140/90 mmHg, recorded before pregnancy or before 20 weeks of gestation. Since chronic hypertension is diagnosed before or in the first half of pregnancy, therapy should be administrated in early pregnancy (3). During this period, organogenesis occurs and the fetus is more vulnerable to possible drug-induced effects. The existing literature provides controversial findings regarding the safety of antihypertensive drug administration. Some studies underline the potential risk for fetal development, whereas others dispute it, mentioning hypertension itself as a risk factor for fetopathy. Moreover, chronic hypertension is correlated with advanced maternal age and comorbidities such as diabetes mellitus and obesity, which makes the interpretation of the results more challenging (4). Thus, the aim of this review is to clarify the inconsistency of studies already published, by evaluating and presenting evidence of fetal outcomes after in utero exposure to antihypertensive medication.

Literature Search

The studies used in this review were identified by searching MEDLINE data base using PubMed Central NCBI. More specific, the Medical Subject Heading terms were: "congenital abnormalities OR congenital disorder OR congenital abnormality OR congenital anomaly OR congenital malformation OR birth defects pregnancy" AND "mothers OR pregnant OR gestational OR prenatal OR perinatal OR gestation". The main Medical Subject Heading terms regarding antihypertensive medication, were applied in the literature search, based on the previous terms, followed by the name of each drug or drug class, as follows: "antihypertensive agent OR β-adrenergic receptor blocking agent OR β-blocker OR β-antagonist OR adrenergic β-3 receptor antagonists OR adrenergic β-2 receptor antagonists OR adrenergic β-1 receptor antagonists OR antiadrenergic OR atenolol OR bisoprolol OR carvedilol OR esmolol OR metoprolol OR propranolol", "calcium channel blockers OR calcium channel antagonists OR nifedipine OR amlodipine OR CCBs", "diuretics OR loop diuretics OR thiazides OR carbonic anhydrase inhibitors OR furosemide OR hydrochlorothiazide OR bumetanide OR amiloride OR eplerenone OR spironolactone OR acetazolamide", "a2-adrenergic agonists OR clonidine OR methyldopa", "ACE inhibitors OR angiotensin-converting enzyme inhibitor OR captopril OR enalapril OR lisinopril OR ARBs OR angiotensin receptor blockers OR valsartan OR Olmesartan OR losartan OR RAS-inhibiting medication OR renin-angiotensin system blockers OR AT1 blockers".

Inclusion and Exclusion Criteria

The authors performed the following search strategy for each specific drug class, using the previous Medical Subject Heading terms. After adjusting the investigation to the last 11-year period, the research was performed on studies published from January 2010 to June 2021. Further initial inclusion criteria included the use of the English language, the availability of the full text online and a study type referring to the human species. After this initial search the authors collected several papers for further evaluation. Among these, a certain number was excluded by reading the title or the abstract, according to the following exclusion criteria: 1) title not relevant, 2) not referring exclusively to pregnant patients, 3) an indication for the antihypertensive drug different from chronic hypertension, 4) papers not referring to the adverse fetal outcomes of the administered drug. The remaining papers were fully examined and only excluded if they presented perinatal effects or exclusively questioned second or third trimester exposure to antihypertensive agents. Finally, after removing several common papers among the results, the final number of papers included in this review was 48 (Table 1).

The papers included consist of 22 original (cases, cohorts and reposts) and 26 reviews (meta-analyses, systematic reviews, narrative reviews). The chart below presents the aforementioned studies and the number of participants for the original papers (Figure 1).

Table 1. Final number of papers that were included in the study according to the class of drugs

Papers	Drug class						
	B-blockers	RAS blockers*	CCBs†	Diuretics	A2 agonists‡	SADC§	Total
After database searching (N)	6,923	3,297	5,960	727	258	-	-
Excluded with titles and abstracts (N)	6,893	3,273	5945	701	238	-	-
Fully read (N)	30	24	18	26	20	-	-
Finally selected (N)	10	11	2	1	1	23	48

*Renin angiotensin system blockers; †Calcium channel blockers; ‡Alpha 2 adrenergic agonists; §Several Antihypertensive Drug Classes referring to the papers that included several antihypertensive drug classes and were identified from the research of more than one drug class study.

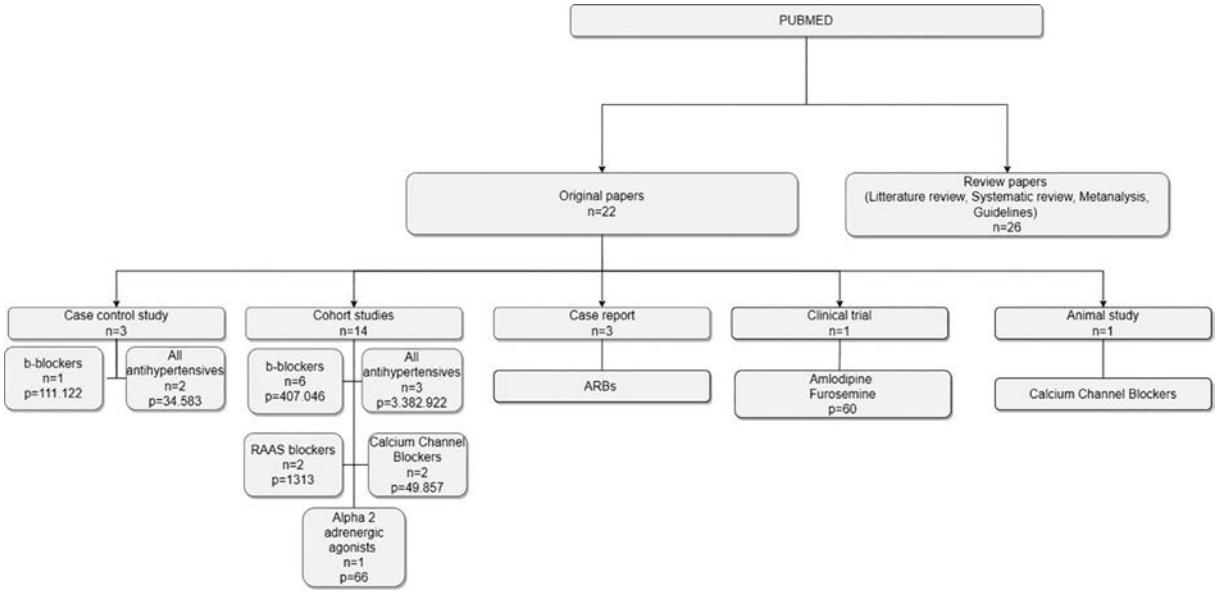

Figure 1. Details regarding the obtained studies.

Definitions

The severity of hypertension is the main factor differentiating the use of each drug class. Mild hypertension is defined as Blood Pressure, BP 140-149/90-99 mmHg, moderate as BP 150-159/100-109 mmHg and severe as BP ≥ 160/≥110 mmHg. In this review, terms such as mild-to-moderate hypertension and non-severe hypertension are considered as equivalent (1). Low birth weight is a term used to describe neonates who are born weighing less than 2.500gr. Preterm birth is defined as the live birth of a neonate prior to 37 weeks of gestation (3).

Following these criteria and definitions, recent existing literature regarding the safety of antihypertensive drug administration was reviewed.

Centrally Acting Alpha-2 Adrenergic Agonists

Alpha-2 adrenergic agonists inhibit vasoconstriction via a central mechanism by decreasing catecholamine release (5). The most frequently administered drugs for controlling chronic hypertension during pregnancy are a-methyldopa and clonidine. A-methyldopa is the drug of choice recommended almost by all guidelines (1, 3, 5). Follow-up studies in the 1980s of children exposed to the drug in utero demonstrate that methyldopa has a record of safety in pregnancy. These findings are also verified by updated data. Specifically, recent studies suggest that treatment with methyldopa does not affect the maternal uterine artery Doppler pulsatility and Resistance Indices, and state that it does not impair placental circulation and subsequent fetal development (5). Clonidine is similar to methyldopa when it comes to safety and efficacy, but has a different mechanism of action as it stimulates a2-adrenergic receptors in the brainstem. It is recommended as a third-line agent for multidrug control of refractory hypertension during pregnancy (5). A retrospective cohort study that took place at the University of Washington Obstetric Hypertension Clinic indicated that clonidine has heterogeneous hemodynamic effects when used in pregnancy. According to this study, women whose response was characterized by a reduction in cardiac output delivered infants with a lower birth weight percentile (6).

B-Adrenergic Receptor Blocking Agents

B-adrenergic receptor blocking agents, commonly referred to as β-blockers or β-antagonists, are a diverse drug class that mainly works by blocking the β-adrenoreceptors in the peripheral circulation, heart, airways, liver, and pancreas. This blockage leads to a decrease in blood pressure following a series of mechanisms, including the reduction of myocardial contractility and of the secretion of angiotensin II (7). Within the drug class, there are different beta-1, beta-2, and alpha blocking activities, resulting in variations in efficacy and adverse effects after exposure to different drugs. Thus, fetal health outcomes after in utero exposure to β-blockers do not always constitute a class effect, as they may vary according to each specific medication used (7).

According to several international guidelines (1), labetalol is the most commonly prescribed β-blocker for the treatment of hypertensive disorders in pregnancy, whereas atenolol is mostly contraindicated due to its potentially higher risk for adverse fetal outcomes (8). Since β-blockers and especially labetalol are considered to be first line agents for the treatment of both chronic and gestational hypertension, their safety for the developing fetus is of great scientific interest (1). The current literature focuses on two main categories of adverse fetal outcomes after first trimester in utero exposure to β-antagonists: major or organ-specific congenital anomalies and other distinct outcomes, such as birth weight and gestational age.

In general, β-blockers are considered to be safe for the developing fetus, as most studies exclude any major risk for overall major congenital anomalies (9-13). However, their association with minor or organ-specific congenital anomalies is less clear in the existing literature (Table 2).

Table 2. Safety of Antihypertensive Drugs during the First Trimester

Administered drugs	Number of studies*	Number of studies†
Centrally acting alpha-2 adrenergic agonists		
A-methyldopa	(1, 3, 5)	None reported
Clonidine	(5)	(6)
B-blockers	(2, 9, 10, 11, 12, 13)	(7, 8, 9, 11, 14, 15, 16, 17,18, 19, 20, 21, 22)
Labetalol	(1)	(7)
Atenolol	Mostly contraindicated (8)	(7, 8, 19)
Metoprolol	(7)	(19)
Propanolol	(7)	(19)
Calcium channel blockers	(13, 18, 24, 25)	(13‡, 15‡, 24‡)
Nifedipine	(1)	-
Amlodipine	Unclear (22)	Unclear (22)
Renin-angiotensin system blockers	(34§, 35§)	(28, 29, 30)
ACEIs	-	(27, 30, 31)
ARBs	-	(4, 26, 32, 33)
Renin inhibitors		
Aliskiren	Unclear (36)	Unclear (36)
Diuretics	(1)	(37, 38, 39)
Thiazide diuretics	(1, 36, 39, 40)	(5, 36, 41, 42)
Furosemide	(44, 45, 46)	(30, 37, 47)
Spironolactone	-	(5, 30, 45, 48)
Eplerenone	Unclear (30, 46)	Unclear (30)

*Studies which report that administration is mostly safe with minor risk; †Studies which report fetal adverse outcomes; ‡Based only on a small number of cases; §When accounting for confounders.

Certain studies (9, 11, 14-16) suggest that an increase in cardiovascular malformations, cleft lip or palate and central nervous system malformations is present or cannot be excluded. J. E. H. Bergman et al. (14), in a recent case-malformed-control study, found that there were significantly increased odds of multi-cystic renal dysplasia occurring after maternal exposure to combined alpha- and beta-blockers. Fisher et al. (17) in the 2017 analysis of National Birth Defects Prevention Study data associated β-blockers' use with an increased risk of Coarctation of the Aorta, Pulmonary Valve Stenosis, Perimembranous Ventricular Septal Defects and Secundum Atrial Defects. Some previous studies are based on a small number of cases, do not include an untreated comparison group, and are characterized by heterogeneity. These outcomes should, therefore, be interpretated with caution, as they could potentially be attributed to the severity of the underlying maternal hypertension. Wu et al. (10) in a recent updated meta-analysis of observational studies found no association between β-blocker exposure and an increased risk of heart malformations, cleft lip or palate. Other authors (2, 12, 13) also supported the absence of an association between drug exposure and congenital cardiac anomalies.

Moreover, β-antagonists have been accused of other adverse fetal health outcomes. Fitton et al. (8), in a recent data linkage cohort study, associated in-utero exposure to β-blockers with an increased risk of preterm birth, low birth weight and being Born Small for Gestational Age, SGA. Other studies (7, 15, 18-22) suggest similar findings. Furthermore, some of them introduce the increased risk of fetal growth restriction (FGR), (7, 19, 22, 23) and perinatal mortality (15). Duan et al. (7) analyzed the effect of diverse β-blocker subtypes on birth weight, suggesting that labetalol and atenolol were associated with the highest rate of low birth weight and an increased risk of being born SGA. On the other hand, after metoprolol and propranolol exposure, the risk of being born SGA was not significantly increased (7). Tanaka K et al. (19) found that the incidence of FGR (Fetal Growth Restriction) was higher in fetuses exposed to propranolol and atenolol, followed by metoprolol exposure, whereas bisoprolol and carvedilol were not strongly associated with FGR.

Calcium Channel Blockers

The Calcium Channel Blocker drug class, also known as CCBs, is classified in two categories: dihydropyridines and non-dihydropyridines. The drugs most commonly administered in pregnancy belong in the first category and, among them, nifedipine is recommended by various guidelines as an alternative for first line and second line therapy in non-severe and severe hypertension during pregnancy (1). The antihypertensive therapeutic effect of nifedipine and other dihydropyridines is mainly due to peripheral arterial vasodilation secondary to inhibition of calcium in vascular smooth muscle cells (24). According to several animal studies (13, 18, 24) high doses of CCBs administered to pregnant rats and rabbits were associated with an increased risk of cardiovascular and skeletal malformations, such as digital and limb defects. This association was not found in human fetuses, thus CCBs are not considered to be teratogenic in humans (13, 18, 24, 25). Only a few studies (13, 15, 24) indicated that CCB use during the first trimester was associated with an increased risk for malformations and congenital anomalies of the upper gastrointestinal tract, but they were based on a small number of cases. Ritchie et al. (24) reported an increase in the miscarriage rate following first trimester in utero exposure to CCBs. Fitton et al. (15), in a recently published systematic review, suggested conflicting results after exposure to CCBs with some evidence of increased perinatal mortality and preterm birth. Moreover, according to a recent review, fetal outcomes after amlodipine exposure varied widely, including normal birth, developmental delay, arm weakness and even loss of cardiac activity at 12 weeks' gestation (22) (Table 2). All in all, CCBs are effective for antihypertension treatment during pregnancy, and they are considered to be safe for the developing fetus (13).

Renin-Angiotensin System Blockers

The renin-angiotensin system, RAS plays a vital role in regulating blood pressure and homeostasis. Angiotensin-converting enzyme inhibitors, ACEIs, and angiotensin receptor blockers, ARBs, interfere with this system and therefore are amongst the first-line medications for the management of hypertension in non-pregnant women (26). They modulate RAS by either inhibiting an enzyme responsible for the conversion of angiotensin I to angiotensin II or by antagonizing the effects of angiotensin II at its receptors (27). Since strong evidence is associated with congenital malformations after second and third trimester exposure, these drugs are contraindicated during late pregnancy (28). However, it remains unclear whether their use is teratogenic if exposure is limited only to the first trimester (29).

Pieper (30) found out that exposure to these drugs during all three trimesters can cause birth defects, while the incidence of congenital malformations after only first trimester exposure is lower. The most common abnormalities amongst them are those of the cardiovascular, central nervous and urogenital systems. Taking into consideration that the fetal RAS develops mainly within the first ninety days of gestational age, exposure to RAS blockers in early pregnancy can lead to renal hypoperfusion and ischemia. As a result, exposure to these drugs has been linked with the need to perform a caesarean section, or with complications such as early miscarriage, stillbirth, elective termination of pregnancy, small for gestational age, preterm birth, low birth weight, low Apgar score and neonatal anuric renal failure (28). However, according to Buawangpong et al, ACEIs prenatal exposure was related to overall congenital malformations, preterm delivery, low birth weight, miscarriage, and elective termination of pregnancy (27). Furthermore, these drugs are known to cause fetal RAS blockage syndrome, which may occur not only following exposure during the second and third trimester, but also after use of these drugs at the beginning of pregnancy (27). This syndrome consists of fetal hypotension and anuria with oligohydramnios, leading to Potter sequence, which includes lung hypoplasia, skull ossification, facial deformations and contractures of limbs (30, 31) (Table 2).

Concerning the ARBs, recent studies have confirmed that there is a higher rate of birth defects in cases with ARBs exposure lasting longer than 6 gestational weeks, and this may reveal a teratogenic vulnerability in the second half of the first trimester (26, 32). Moreover, ARBs are more dangerous than ACEIs with increasing risk for renal impairment, due to their higher blocking effect and longer half-live (4, 33).

On the other hand, some studies conclude that there is no increased risk for congenital malformations after first trimester exposure to RAS blockers, when accounting for the underlying hypertension and confounders, such as advanced maternal age, the presence of diabetes mellitus and obesity (34, 35) (Table 2).

Renin Inhibitors

The renin inhibitors, whose main representative is aliskiren, are another drug class whose use has an effect on the RAS system. Concerning aliskiren, due to lack of data it is unknown whether the placenta is permeable by this drug. Consequently, it is not clear if this drug can cause congenital malformation to the fetus (36) (Table 2).

Diuretics

The use of diuretic treatment during pregnancy remains debatable, mainly due to theoretical concerns about reduced plasma volume (5). While the European Society of Hypertension 2013 guidelines (37) and others (38, 39) contraindicate the use of diuretics in pregnancy, the National Heart, Lung, Blood Institute suggest diuretics as an alternative second line treatment (1).

Thiazide Diuretics

Thiazide diuretics act by inhibiting the sodium/chloride cotransporter located in the distal convoluted tubule of a nephron. According to Hyperten-

sion Canada guidelines (40) and ACOG (41), thiazide diuretics can be used as a second-line treatment for non-severe hypertension. Nevertheless, the European Society of Cardiology and the Society of Obstetric Medicine of Australia and New Zealand do not recommend their use in pregnancy (36, 42). Recent studies (5) have indicated that thiazides may cause volume contraction and electrolyte abnormalities, but this appears to be rare with small doses. Moreover, NICE guidelines (43) have proposed that diuretics, particularly chlorothiazide, should not be used since they might be associated with a high risk of congenital malformations and neonatal complications, such as thrombocytopenia, hypoglycemia and electrolyte disorders. Despite the previous suggestions, there is not sufficient evidence that low-dose thiazide diuretics are harmful for pregnancy (1), so women with pre-existing hypertension may continue their current antihypertensive medication (36).

Loop Diuretics

Loop diuretics act by inhibiting the sodium-potassium-chloride co-transporter in the thick ascending loop of Henle. Paulino Vigil-De et al. (44) and others (45) confirmed that furosemide is not associated with adverse pregnancy outcomes when used in women with mild/moderate chronic hypertension. However, according to the European Society of Cardiology (37) and others (30, 46), bumetanide and furosemide can cause oligohydramnios, as well as fetal electrolyte abnormalities.

Potassium-Sparing Diuretics/ Mineralocorticoid Receptor Antagonists

Spironolactone is contraindicated during the preconception period and throughout pregnancy because of its antiandrogenic effect that can lead to fetal feminization (5, 30, 45, 47). However, an early study on male rats did not indicate any evidence of an antiandrogenic effect (47). The available literature includes only one case report describing ambiguous genitalia in a human newborn of a mother treated with spironolactone until the fifth week of gestation (48). However, eplerenone does not act as an androgen receptor blocker (47). The European Society of Cardiology underlined the deficiency of data regarding the use of eplerenone during pregnancy (36). Consequently, eplerenone should only be used in pregnant women when treatment with other diuretics is ineffective (30) (Table 2).

Discussion

To the best of our knowledge, the current evidence about each antihypertensive drug class, based on already published literature, varies widely. While the use of several drug classes is strongly encouraged due to the existence of sufficient data, the field remains unclear concerning some others. According to the 2018 European Society of Cardiology Guidelines, a-methyldopa, β-blockers, mainly represented by labetalol and calcium channel blockers, primarily represented by nifedipine, are the first-line treatment for hypertension in pregnancy (36). Indeed, we did not find any concrete evidence against those drugs. However, some other drug classes, such as ACEIs, ARBs or spironolactone, should not be routinely prescribed to women of reproductive age, in order to avoid possible adverse fetal outcomes, on the basis of several studies.

Nevertheless, considerable attention must be paid when regulating antihypertensive therapy in a pregnant woman, taking into consideration the need for applying a personalized treatment plan. It is of utmost importance that the benefits of the therapy administered outweigh the potential risks for the developing fetus. Both earlier and recent studies demonstrate that untreated hypertension is associated with possible adverse maternal and fetal outcomes. Specifically, it is associated with a high risk of pre-eclampsia, growth restriction and congenital heart disease (3). Consequently, it is not surprising that the findings of this review should be interpretated with caution. The teratogenicity observed could be attributed to maternal factors and comorbidities coexisting with hypertension during pregnancy, such as obesity, advanced maternal age and diabetes mellitus (26). Furthermore,

it is feasible that several limitations might have impacted the results. Certain studies were designed for a small sample size, while some included heterogenic study groups. Another potential source of error could be the possibility of recall bias and the lack of adjustment for other confounding factors such as maternal age and Body Mass Index. Inevitably, there is limited evidence concerning the safety of the use of several drug classes due to the impossibility of conducting further research because of ethical restrictions.

Conclusion

Hypertensive disorders are one of the most common complications of pregnancy. The effectiveness and relative safety of certain antihypertensive medications makes them suitable for administration during the first trimester. However, specific drugs should be avoided during this gestational period, such as ACEIs, ARBs or spironolactone, since they are supported by deficient data. The necessity of designing further studies, as well as reevaluating the data already assessed appears to be imperative.

What Is Already Known on This Topic:

Antihypertensive treatment is a necessity in cases of hypertensive disorders during pregnancy, as the risk for maternal and fetal outcomes is quite significant. However, the safety of in utero exposure to antihypertensive medication is ambiguous. Some studies recommend the administration of certain agents, while others underline the possible adverse effects on fetal development.

What This Study Adds:

This study summarizes the possible adverse outcomes of antihypertensive agents during the first trimester of gestation. In utero exposure to some of these drugs raises caution regarding their effectiveness and safety. The present review quotes the most recent data, offering a most recent and clear depiction of current antihypertensive drugs effects during the first gestational trimester.

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Intraoperative Myomectomy during Caesarean Section

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Abstract

Objective. The aim of this article is to support intracaesarian myomectomy as an alternative method of treatment. **Case Report.** This article presents a case of myomectomy during a caesarean section at 38+1 weeks' gestation, with a subserosal myoma on the left wall of the uterus. **Conclusion.** The postoperative period was free of complications, lending credit to the possibility that intracaesarean myomectomy can have a favourable outcome.

Key Words: Pregnancy ▪ Myoma ▪ Myomectomy ▪ Caesarean ▪ Fibroid.

Introduction

The most common benign neoplasm in women of reproductive age is a myoma. In women presenting with myomas, especially if multiple, their quality of life is significantly impaired, and the healthcare burden increased (1). It is known that multiparous women have a smaller chance of presenting with myomas in their lifetime (2). While there is definitely a connection between myomas and fertility, the evidence remains controversial. Published studies suffer from dissimilar planning and selection bias (3).

Myomas can have severe adverse effects in pregnancy. Depending on the location of the myoma, pain, bleeding, premature membrane rupture, preterm labour, obstruction of delivery, and even miscarriage present as possible ramifications (4). An elective postnatal myomectomy is commonly performed due to the asymptomatic nature of myomas in 70% of cases (5). However, myomectomy during a caesarean section lowers the risk of many complications, such as post-operative haemorrhage, multiple surgeries and complications of anaesthesia (6).

This article presents a case of myomectomy during a caesarian section, and the patient's healthcare follow-up.

Case Report

The patient, 34 years of age, was admitted to hospital with a diagnosis of G2P2 at 38+1 weeks' gestation. She had had a prior caesarian delivery and a medical history of thalassemia trait minor. In the course of antenatal care, a subserosal uterine fibroid was detected at 4+4 weeks of gestation on the left anterior wall of the uterus (size 3.66×3.38 cm) (Figure 1) with gradual growth, as recorded in subsequent ultrasounds, reaching a size of 7.45×5.48 cm at the B level ultrasound scan (Figure 2).

At the time of admission, the patient's blood pressure was 134/62 mmHg with a heart rate of 100 bpm. Slight oedema in both feet was present.

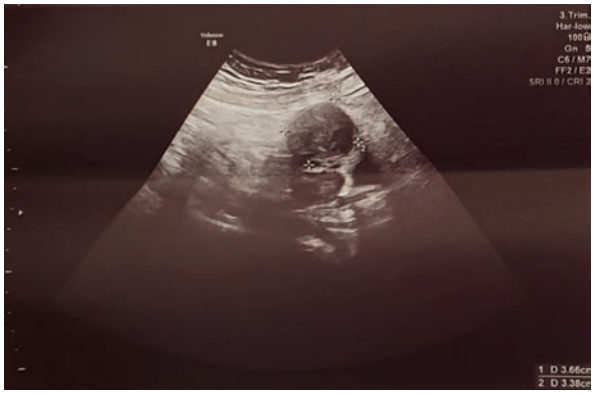

Figure 1. Subserosal Fibroid Size 3.66×3.38 cm.

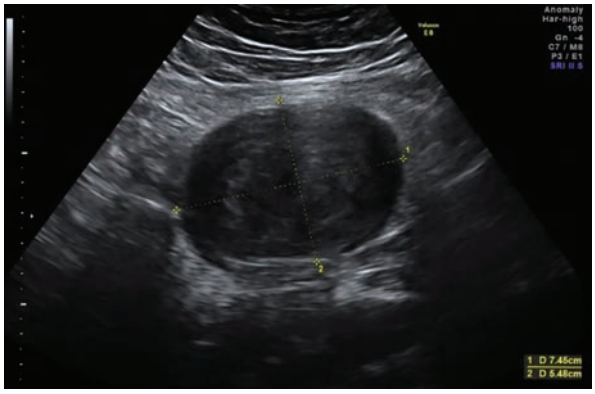

Figure 2. Subserosal Fibroid 7.45×5.48 cm.

Cardiac evaluation did not reveal any abnormalities. The uterus was past 36 weeks in size. The embryo had cephalic presentation. The amniotic sac had not ruptured. The cervix showed no dilation or effacement. The cardiotocography-non stress test (NST) ranged between normal parameters, with FHR at 153 bpm. The patient's hematocrits were 33.1%, INR was 0.83, blood type B positive. Standard virological screening tests were negative. Apart from the fibroid, B level and Doppler ultrasound scans showed no abnormalities.

Taking into account her previous pregnancy, an elective caesarean delivery was planned. A Pfannenstiel incision was performed. During the operation, while entering the peritoneal cavity, the subserosal fibroid was recognized on the left wall of the main body of the uterus. The fibroid presented with multiple adhesions to the peritoneum and the large intestine. The transverse cut to the uterus enabled the extraction of a live and healthy 2730 gr female baby, with Apgar score of 9 at 1 minute and 10 at 5 minutes. The placenta was round, with no abnormalities. The umbilical cord was found free of abnormalities. Intraoperative bleeding of the fibroid occurred due to symphysiolysis, and a myomectomy was performed for immediate hemorrhage assessment. The excision of the fibroid was performed with a minimal increase in surgical time without any further intraoperative complications. Haemostasis was achieved (Figure 3).

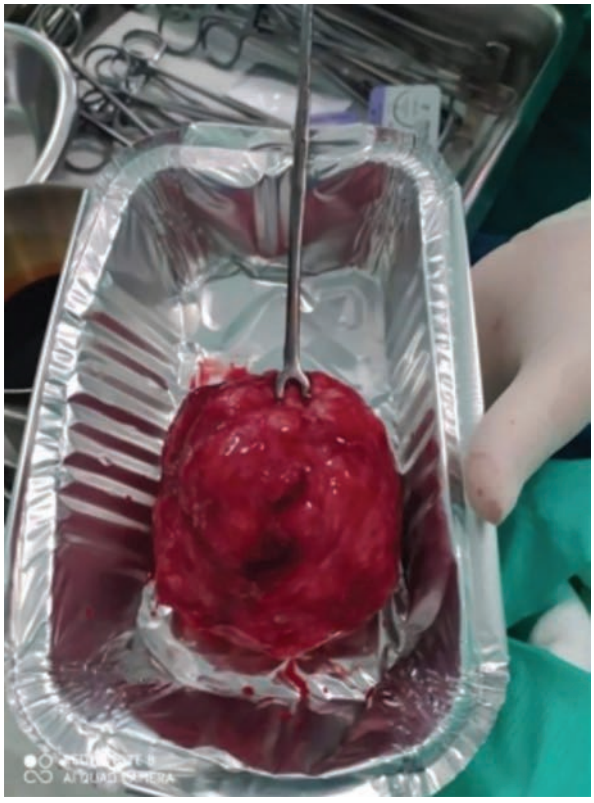

Figure 3. Fibroid after removal from the uterus.

Post-operatively, oxytocin and ergometrin infusion were administered to the patient for 24 hours, along with broad spectrum antibiotics and analgesics. Blood tests were performed after the surgery on postoperative days one and three, with no significant drop in hematocrit levels. The post-operative period was free of complications, and no blood transfusion was deemed necessary. The patient was discharged on the fourth postoperative day.

A month later, the histological examination showed a fibroid of the usual type, with hyaline

necrosis and increased cellularity. At the 4 week follow-up examination the patient was in good health, and on the ultrasound scan there were no signs of the fibroid, uterine rupture or blood in the peritoneal cavity.

Discussion

The percentage of fibroids appearing in pregnant women can be as high as 10%, and may further increase if advanced age or difficulties created by obesity are taken into account (7). It is suggested that fibroids enlarge during pregnancy through various mechanisms. Genetic, hormonal and enviromental factors have been shown to contribute to the development of uterine fibroids. These factors include steroid hormones, growth factors, cytokines, and genetic and epigenetic anomalies (8).

Fibroids tend to double in size up to the seventh week of gestation, reaching their maximum size in the second trimester, while in the third trimester they tend to recede (8). The existence of fibroids can cause complications in pregnancy, with severe repercussions for the fetus, ranging from preterm birth and spontaneous abortion, to placental abruptions and emergency caesarean delivery. Infertility due to a large myoma is not unusual (9).

The published literature presents conflicting reports regarding the procedure of myomectomy during pregnancy. The common method of treating fibroids is expectant and removal by surgery at a later date, after the birth (10). The gravid uterus presents with a higher chance of morbidity and mortality during myomectomy compared to a non-gravid uterus, as well as an increased risk of haemorrhage and the need for blood transfusion, since the gravid uterus is more highly vascularised. There is an 18% to 35% chance of the adverse outcome of abortion due to myomectomy in pregnancy (11).

A recent systematic review and meta-analysis performed by Goyal et al. (12) attempted to gather evidence from the published literature about the safety of caesarean myomectomy. In the reviews presented, the most commonly associated complication in a myomectomy during caesarean delivery was haemorrhage. The analysis performed by the group showed that, even though myomectomy during caesarean delivery increased the need for blood transfusion, evidence indicated no discernible difference in the incidence of haemorrhage. Similar results were shown by a retrospective case control study (13). There was no significant increase in the percentage of haemorrhage between the group that underwent caesarean myomectomy and the control group that underwent only a c-section.

A common indicator for performing myomectomy during a caesarean delivery is the size of the myoma. An assessment plan is considered in cases where the myoma exceeds a diameter of 3 cm (14). Apart from morbidity, fear of excessive blood loss and a postpartum decrease in size advocate against fibroid removal, as well as increased intraoperative time (12).

Myomectomy during caesarian section lowers the risks commonly found in successive pregnancies, while eschewing the necessity for interval myomectomy, as well as enabling vaginal delivery in subsequent births (8). Performed by experienced surgeons, myomectomy during a c-section is a safe procedure. The size and location of the myoma are the main factors that contribute to the decision. Predictably, a myoma that lies at the site of the uterine incision can be safely removed. Intramural myomas close to the fallopian tubes are best left intact (15). Routine myomectomy should be considered only for the safe closure of the uterine breach, or to accommodate the delivery of the fetus (8).

Adhesions are a common complication in operative gynaecology, with possible detrimental effects on the health of the patient. Left intact, bowel obstruction, or inadvertent ureteral, vascular or bowel injury may occur, in addition to obstruction in the operation field due to anatomical changes. Another potential complication is chronic pelvic pain, however the existing literature presents conflicting evidence (16). For these reasons, adhesiolysis is a well-advised practice, albeit one that carries its own intraoperative risks, that include injury to the surrounding organs and vessels (17).

In our case, the removal of the myoma was decided intraoperatively due to the excessive adhesions that resulted in intraoperative haemorrage. The excision of the fibroid was performed with a minimal increase in surgical time, without generating intraoperative complications. The patient's post-operative period showed no complications, and no blood transfusion was required.

Current medical evidence drives our routine practice, and removal of myomas is discouraged during caesarian delivery. It used to be thought of as an operation with potentially disastrous effects on the mother and was used exclusively in cases of pedunculated subserosal fibroids (18). However, there have been cases of intracaesarian myomectomy with no complications (5, 10). According to Kwawukume, enucleation of a myoma can be performed with minimal difficulty in pregnancy, since the tissue is softer (14). The decision to document this case was made in order to highlight the fact that myomectomy can be effective and safe, on a case by case basis, when performed by an experienced surgeon.

Conclusion

The current literature presents conflicting data regarding the necessity of intraoperative myomectomy during a caesarian delivery. Taking into consideration the high prevalence of myomas in women, more studies are needed on the treatment of myomas during pregnancy. To the best of our knowledge, the aforementioned management can be a safe practice when performed by experienced attending physicians.

What Is Already Known on This Topic:
Myomas can have severe adverse effects in pregnancy. Depending on the location of the myoma, pain, bleeding, premature membrane rupture, preterm labour, obstruction of delivery, and even miscarriage present as possible ramifications (4). An elective postnatal myomectomy is commonly employed due to the asymptomatic nature of myomas in 70% of cases (5).

What This Case Adds:
Myomectomy during caesarian section lowers the risks commonly found in successive pregnancies, while eschewing the necessity for interval myomectomy, as well as enabling vaginal delivery in subsequent births (8). This article presents a case of myomectomy during a caesarean section at 38+1 weeks of gestation, with a subserosal myoma on the left wall of the uterus. The post-operative period was free of complications, lending credit to the possibility that intracaesarean myomectomy can have a favourable outcome.

Authors' Contributions: Conception and design: DG; Acquisition, analysis and interpretation of data: DG, NK and CB; Drafting the article: DG, IK, GB, AS and PT; Revising it critically for important intellectual content: NK, GM, KK, IG and IF; Approved final version of the manuscript: DG, NK, CB, IK, GB, GM, KK, IG, AS, PT, IF and AM.

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Embolism of a Piece of Iron after Penetrating Neck Injury: From Right Internal Jugular Vein to Left Lower Lobe of the Lung

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Abstract

Objective. This case report of a 40-year-old male patient describes the embolization of a piece of iron trapped in the jugular vein following an industrial injury. **Case Report.** The 40-year-old male patient was admitted to the emergency service for bleeding from the neck. In contrast to the first X-ray image, no foreign body was observed at the injury site or aspiration of fluid during emergency surgery. Following x-ray radiography, an opacity was observed in the left lower lobe of the lung, consistent with a foreign body. Echocardiography demonstrated no pathological manifestation in the atria, ventricles, or major pulmonary artery. There was no need to intervene because the patient was asymptomatic and had no noticeable symptoms of hemoptysis. The outpatient clinic's radiological and clinical control examinations were normal at the end of six months. **Conclusion.** The movement of the foreign body to the lung within one or two hours may have resulted from our manipulation or may have occurred spontaneously. As a result, any foreign bodies that have invaded the vascular system should be removed as soon as possible.

Key Words: Foreign Bodies ▪ Emergency Service ▪ Pulmonary Arteries ▪ Jugular Veins.

Introductıon

The most fatal complication related to a foreign body penetrating the lung parenchyma via the heart is estimated to be 0.01% (1). When the heart is penetrated by a foreign body, it leads to a life-threatening situation. In the literature, endocarditis, valvular insufficiency or mural thrombus are reported (2, 3). Foreign bodies removed via open heart surgery have been reported (4). There is no standard approach in this regard, since the penetration of foreign substances is a very varied situation. The vascular route is used by 88% of foreign bodies that reach the heart. For 54% of these patients, surgical intervention was performed; in 29% of them, the foreign body was removed percutaneously, and 14% were monitored conservatively (5, 6).

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This report is about the route taken by a piece of iron, trapped in the jugular vein after an industrial injury, in a 40-year-old male patient.

Case Presentation

A 40-year-old male patient was admitted to the emergency service with neck bleeding. Once his case history had been investigated, it was found that a fragment of metal had become caught in his neck by accident while he was cutting iron. He had no history of injury or surgery. During the initial examination, a minor hematoma on the right side of his neck was observed. His vitals were stable. No significant pathology was found in his laboratory values, and chest radiography was normal. CT angiography of the neck was performed to rule out vascular injury. It was detected that a piece of iron sized 1.31×0.6 cm was stuck in the right internal

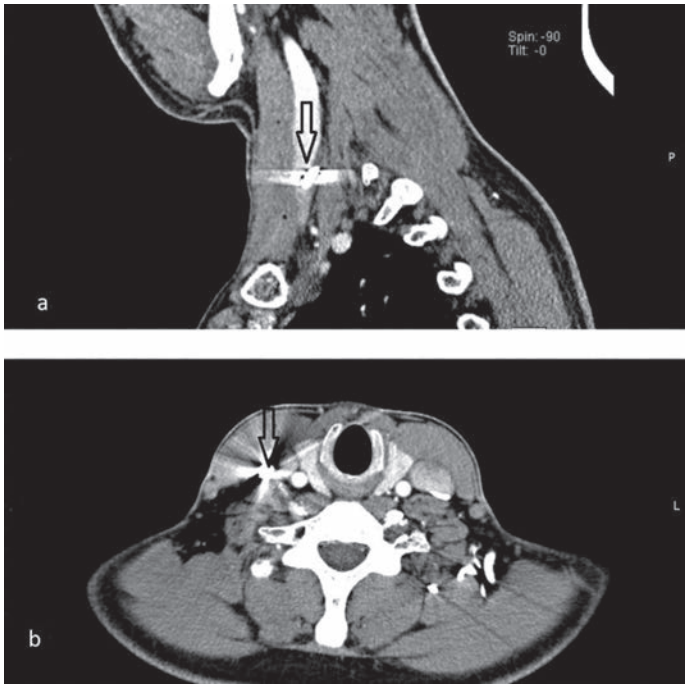

Figure 1. View of the foreign body in the neck: a- Foreign body in the right internal jugular vein on sagittal CT angiography (black arrow); b- Radial image of the metallic foreign body on axial CT angiography of the neck (black arrow).

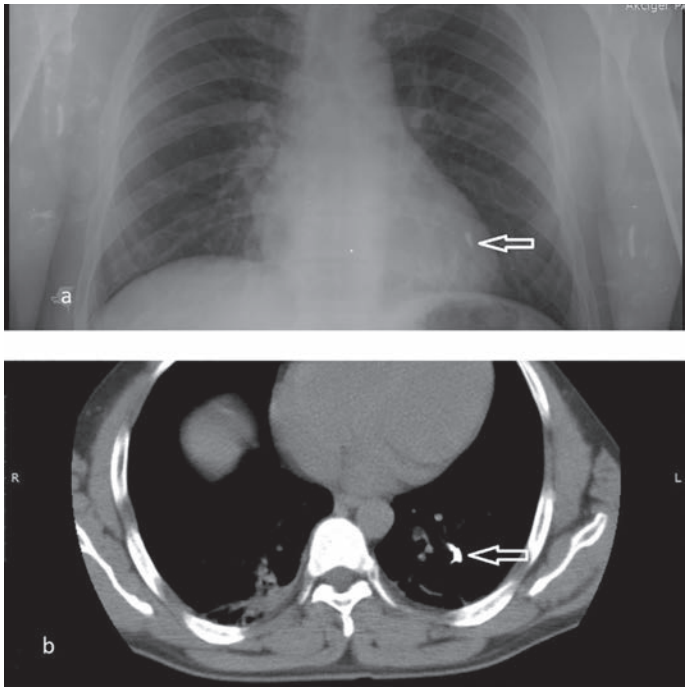

Figure 2. View of the foreign body in the chest: a- Metallic foreign body with smooth, sharp edges on chest radiography of the left lower lobe (white arrow). b- Foreign body in the subsegmental branch of the pulmonary artery on axial CT (white arrow).

jugular vein (Figure 1-a, b). The patient was taken to surgery immediately, and the internal jugular vein was repaired, however, the foreign body was not found. An X-ray image of the neck and aspiration fluid was taken and no foreign body was detected. The patient was transferred for inpatient treatment and echocardiography was performed. No pathological signs were observed in the atria, ventricles or main pulmonary artery. A second radiography examination of the chest was performed immediately thereafter. An opacity consistent with the foreign body was observed in the left lower lobe of the lung (Figure 2-a). On the contrast CT angiography of the thorax, the foreign body was seen in the left lower lobe (Figure 2-b). It was understood that the penetrating foreign body had progressed from the jugular vein to the vena cava superior, the right atrium, the right ventricle, the pulmonary artery and finally, to the left subsegmental branch of the lung. No intervention was undertaken since the patient was stable and there was no sign of hemoptysis. He was discharged on the third day. Radiological and clinical control in the outpatient clinic were normal after 6 months.

Discussion

Treatment for foreign bodies that have reached the subsegmental branches of the pulmonary artery varies according to the case. Although follow-up is recommended for patients with limited life expectancy in most cases, early intervention is recommended for foreign bodies that have reached the heart and main pulmonary artery. Successful results have been achieved with endovascular intervention (7). To the best of our knowledge, this is the first case where a piece of iron travelled from the jugular vein to the pulmonary arterial tree. Similarly, stents that have reached the

segmental branches of the pulmonary artery, and thoracoabdominal injuries due to medical devices and high-energy weapons have been previously reported (8).

The approach to be adopted for small pieces of iron embedded in the pulmonary segmental branches is arguable. Conservative treatment and observation was performed for the vascular migration of a piece of iron that reached the pulmonary artery through the popliteal vein. Venous system-related aneurysms can cause pulmonary embolism (9, 10). However, follow-up is recommended for asymptomatic patients. An operation may be required for symptomatic patients with pulmonary infarction, pulmonary abscess and erosion. If the foreign body is located in the periphery, a wedge resection may be performed (11, 12).

The patient's history of trauma and surgery should certainly be taken and considered. If and when any vascular or thoracic injury is possible due to penetrating foreign bodies, X-ray radiography should be performed first. This will provide significant information about the location of the foreign body before and after migration. Opacities observed on X-rays can suggest the presence of a piece of iron or metal. Computed tomography angiography can provide the best image for vascular injuries. Metallic foreign bodies give a radial image on CT angiography, and extravasation of the opaque substance is always a guiding factor for vascular injury. There is always the possibility that magnetic resonance angiography may trigger migration of metallic foreign bodies and therefore, magnetic resonance angiography should be avoided. Metallic foreign bodies within the human body may be affected by the strong magnetic field, and thereby it may trigger travel to the vascular system and lead to injuries. Our example supports the use of a "wait-and-see" approach in the treatment of such individuals (13-15). Non-iatrogenic pulmonary embolism is characterized as embolisation to the pulmonary circulation of different foreign bodies (Table 1).

Complications can be prevented if early diagnosis and treatment are performed in cases in which a foreign body has penetrated the vascular system (16-18). Early removal of a foreign body prevents fatal consequences, such as serious cardiac injury or pulmonary embolism. Rigid manipulation for exposure should be avoided during surgery and/or

Table 1. Recent Research on Non-thrombotic Pulmonary Embolism Including Examples of Multiple Foreign Agents Affecting the Pulmonary Circulation Intravenously

Study	Age (year)	Sex	Type of foreign body	Entry Site	Final Destination	Management
Fernandez-Ranvie GG et al. 2013 (9)	20	Male	Bullet	Left femoral vein	Right pulmonary artery	Observation alone
Ciarrocchi AP et al. 2021 (14)	46	Male	Wooden	No apparent	The artery of the lateral basal segment of the right lower lobe	Thoracotomy Removal foreign body
Lebon M et al. 2018 (21)	41	Male	Sewing needle	Superficial femoral artery and the femoral vein	Embolism of pulmonary lobar arteries	Sewing needle removal with surgıcally
Gschwind CR et al. 2002 (22)	29	Male	Piece of metal	Subcutaneous veins	Right lung	Followed wıth medıcıne
Desai M et al 2020 (15)	22	Male	Glass fragment	Right internal jugular vein	Left lower lobe pulmonary artery	Endovascular Retrieval
Sakai T et al. 2018 (19)	19	Male	Iron hammer fragment	Femoral vein	Pulmonary artery	Removal with video-assisted thoracoscopy
Nally L et al. 2012 (20)	21	Male	Bullet	Left arm	Right lower pulmonary lobe	Removed (no specific description how)

preparation of patients for surgery. Such forceful manipulations can push the foreign body into the circulatory system. The foreign body should be removed without damaging the surrounding tissues by very gentle exposure and dissection. Foreign bodies can move within an hour or a few days, or even years.

Conclusion

Movement of foreign bodies in the vascular system can occur easily and early. In our case, the arrival of the foreign body in a lung within one or two hours may have resulted from our manipulation or may have occurred spontaneously. Therefore, it is important that patients not be moved or that they are carried to the operating room with minimal movement.

What Is Already Known on This Topic:

Studies on foreign body migration into the pulmonary circulation loop are rare and essentially generally iatrogenic. An earlier case had advanced through the popliteal vein, across the vena cava, into the right atrium and right ventricle, and into the pulmonary artery. The authors describe an unusual instance in which a metal particle moved to the left pulmonary artery after an industrial accident caused penetrating jugular vein damage. The unique feature of this case is that it is both asymptomatic and a rare case with good prognosis.

What This Case Adds:

The authors conclude that metal objects entering the body should be continuously observed and intervention should be avoided in asymptomatic individuals, as a supplementary to the existing research.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Images in Clinical Medicine
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Iris Flocculi in a 28 Year-Old Woman

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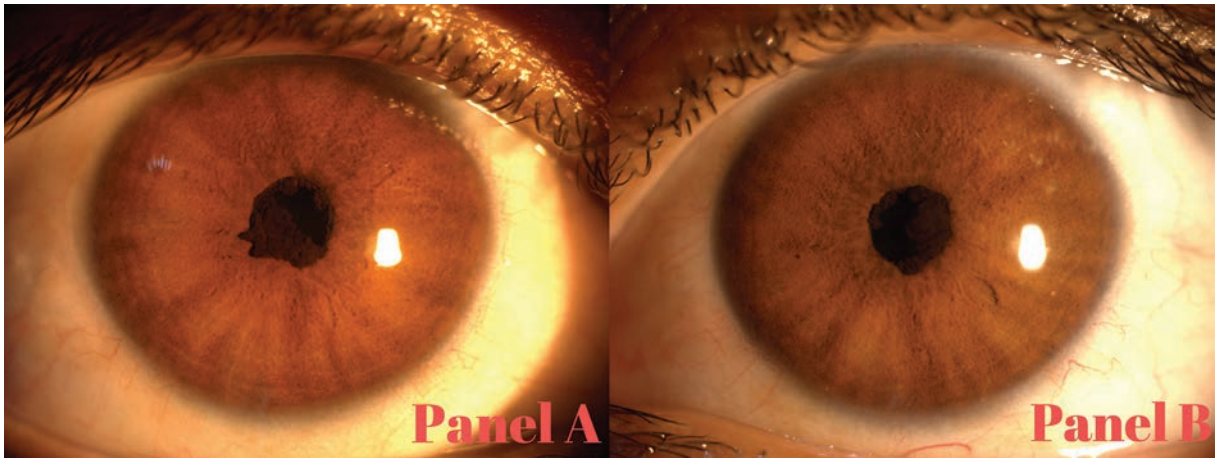
Key Words: Iris Flocculi ▪ Aortic Aneurysm ▪ Visual Acuity.



A 28-year-old asymptomatic woman presented to the Eye clinic for routine examination. Slit-lamp examination showed the presence of multiple wrinkled dark-brown excrescences of the iris along the pupillary border in both eyes compatible with a diagnosis of iris flocculi (Panel A and Panel B). The patient stated she had the lesions for a long time. Visual acuity and intraocular pressure were normal. Iris flocculi are pigmented epithelial cysts along the pupillary margin that can wax and wane overtime. They typically are asymptomatic and do not result in any visual symptoms and are considered as isolated and benign, requiring no treatment. Laser photocoagulation with cyst rupture or surgical excision of the cysts may be considered in symptomatic cases. The condition is important for the occasional association with aortic aneurysms and dissection due to a shared mutation of smooth muscle gene alpha-actin 2 (ACTA-2) and myosin heavy chain (MYH-11). In such cases, congenital mydriatic pupil with loss of accommodation secondary to smooth muscle dysfunction could also occur. It is recommended to screen all affected patients and family members for aortic aneurysm. The patient underwent chest CT which did not show aortic aneurysm.

Conflict of Interest: The authors declare that they have no conflict of interest.

Case Report
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Unusual Axillary Artery Branching Pattern Associated with Multiple Neural Variations

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Abstract

Objective. This report presents a unilateral branching pattern of the axillary artery (AA) represented by an unusual common trunk division, vessel multiplications and concomitant neural variations. **Case Report.** In a Greek male cadaver, the right AA branched into a subscapular trunk and two accessory lateral thoracic arteries of variable origin and course. Concomitantly, a high-level interconnection between the musculocutaneous and median nerves was identified, as an accessory lateral root of the median nerve. More interestingly, a rare innervation of the upper part of the latissimus dorsi muscle by a lower subscapular nerve was also revealed. **Conclusion.** In-depth knowledge of the typical and variant AA branching patterns and coexisting neural variations is of paramount importance for surgeons and interventional physicians, for a safer diagnosis and for performing uneventful procedures in that area.

Key Words: Variation ▪ Subscapular Artery ▪ Thoracodorsal Artery ▪ Angular Branch ▪ Trunk.

Introduction

The axillary artery (AA) branching pattern presents with wide variability, particularly between the genders and different ethnic groups (1). Many of its branches may arise through common trunks (2), and others are multiplied. Clumping of the branches and common origin variants have been identified, with a higher incidence in Africans (3) and females (4). Neural and vascular aberrancies are usually paired. The current cadaveric report highlights an unusual subscapular trunk (SST) division into multiple branches, in combination with a rare variant innervation of the latissimus dorsi muscle (LDM) by the lower subscapular nerve. In addition, two accessory lateral thoracic arteries (LTAs) and a high-level interconnection between the musculocutaneous and median nerves (MCN-MN) were identified, forming an additional lateral root of the MN.

This paper presents a rare case with important surgical and clinical variations of the AA, the MN and LDM innervation.

Case Report

During the right axilla dissection of a 70-year-old formalin-fixed Greek cadaver, in the Department of Anatomy and Surgical Anatomy of the Medical School of the Aristotle University of Thessaloniki, an unusual SST, along with three LTAs were found. Moreover an atypical LDM innervation and a high-level interconnection of the MCN with

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the MN were revealed. At the AA 3rd part, the anterior circumflex humeral artery (ACHA) and an unusual SST were recorded (Figure 1A, 2A). A circumflex accessory LTA supplied the upper part of the subscapularis muscle (SSM). The SST initially trifurcated into the posterior circumflex humeral artery (PCHA), the subscapular artery (SSA) and the second accessory LTA. The second trifurcation constituted from the SSA division into the thoracodorsal artery (TDA) and the circumflex scapular artery (CSA) in common origin with the lower subscapular artery (LSSA) (Figure 1B, 2B). At the upper humeral part, a (MCN-MN) interconnection was noticed. This thick interconnection is equivalent to an additional lateral root (2nd) of the MN, according to Buch-Hansen's (5) description. The brachial plexus posterior cord, typically, gave off the upper subscapular, the thoracodorsal and the lower subscapular nerves. The lower subscapular nerve innervated the SSM and, very interestingly, the LDM upper part (Figure 1C, 2C).

Discussion

The typical AA pattern occurs in only 27% of cases (6), as 23 variant patterns have been described as having a female predilection (4, 6). The most common variations involve the origin of the AA branches via common trunks (2).

No complete embryological theory explains the development of the AA network and its further variability. Rodríguez-Niedenführ et al. (7) supported the notion that upper limb arteries develop by selective enlargement or regression of a capillary micro-network plexus, in a proximal to distal fashion, following the development of muscular and osseous elements. Hypothetically, this could explain the discrepancy between the AA's wide variability in the origin of its branching pattern on the one hand, and the stability in the course of its branches and distribution on the other (laterality). Constancy in axillary muscular and vascular termination exists, while the vessels' origin is sub-

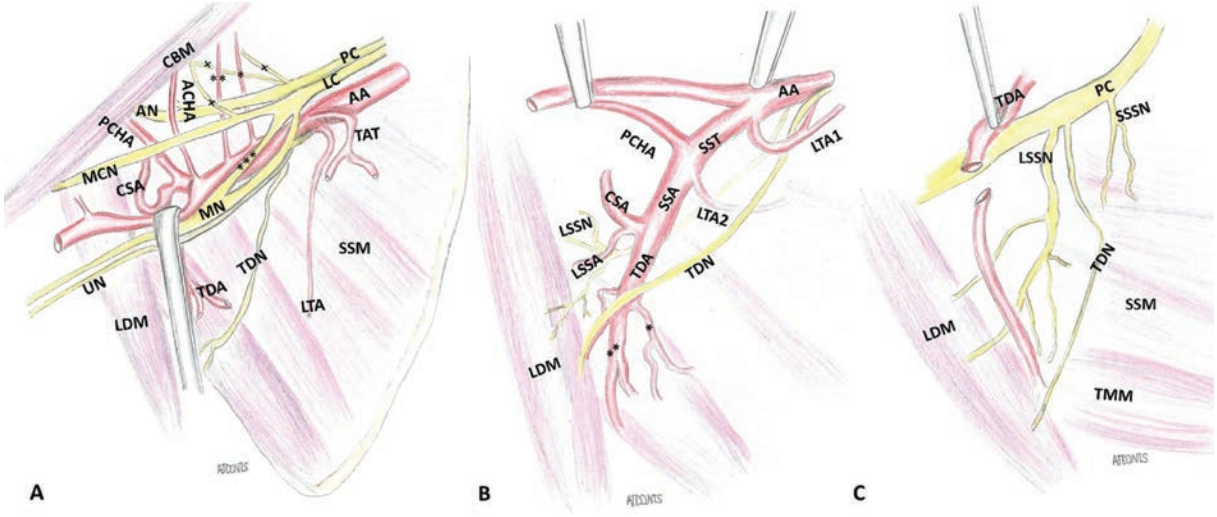

Figure 1. **A.** The axillary artery (AA) division into a thoracoacromial trunk (TAT) and a lateral thoracic artery (LTA). Two small-caliber (* and **) branches to the coracobrachialis muscle (CBM) and the shoulder joint capsule. CBM innervation by the musculocutaneous nerve (MCN) and by three additional thin neural branches (+) (two of them originate from the brachial plexus-BP posterior cord-PC), and the third from the MCN after its typical formation from the BP lateral cord-LC), MCN-MN interconnection (***, the additional MN lateral root), UN-ulnar nerve, LDM-latissimus dorsi muscle, SSM-subscapular muscle. **B.** AA 3rd part: circumflex accessory (LTA_1) loop anterior to thoracodorsal nerve (TDN). Subscapular trunk (SST) trifurcation into the posterior circumflex humeral artery (PCHA), subscapular artery (SSA) and LTA_2. SSA trifurcation into the thoracodorsal artery (TDA), the circumflex scapular artery (CSA) with common origin with the lower subscapular artery (LSSA). TDA division into a common trunk (*of descending course) which bifurcates into a branch for the serratus anterior muscle (SAM) and another for the SSM. A 2nd common trunk (**) along the lateral scapular margin until its lateral inferior angle (TDA angular branch). **C**. BP PC typical division into the superior subscapular, the thoracodorsal and the lower subscapular nerves (SSSN, TDN AND LSSN). Next to the TDN origin, the LSSN, typically innervates SSM and the upper part of the latissimus dorsi muscle (LDM), TMM-teres major muscle.

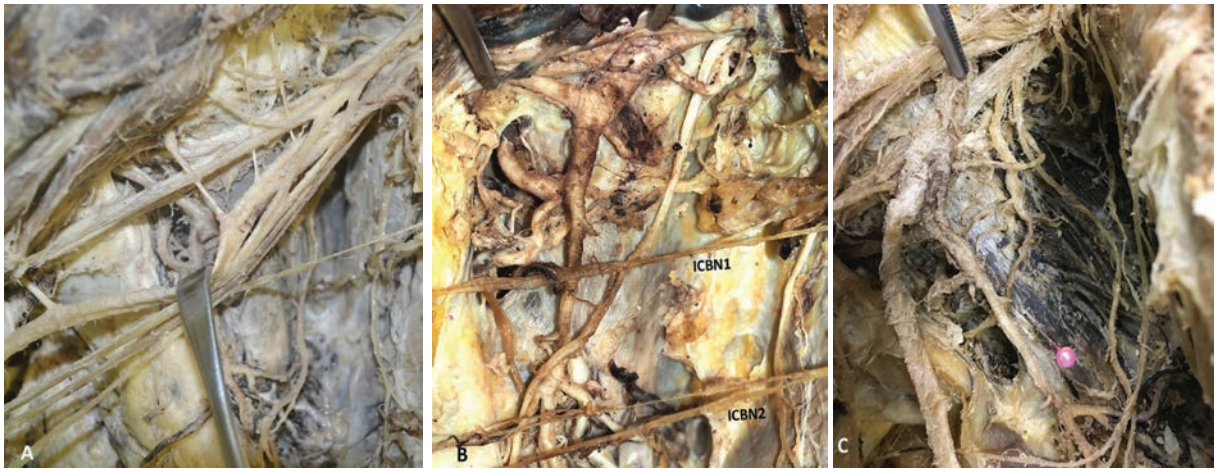

Figure 2. **A.** Right axilla dissection, **B.** ICBN-intercostobrachial nerve, **C.** Dissected area.

ject to environmental or genetic changes, or is directly affected by the brachial plexus development (7). Among the AA branches, the LTA, the SSA and the PCHA have the most variable origin and branching patterns (6). In the present case, two accessory LTAs were identified, thus three LTAs in total supply the lateral thoracic wall. LTA may variably originate from the thoracoacromial trunk (67.2%), less frequently from the AA, SSA and TDA (6), and more rarely from the SSA-PCHA common trunk (8). A high LTA origin (28.9%) (2), subscapular (4.2%) (6) and a low, from the deep brachial artery, origin, have also been described (9). Surgeons should be aware of the LTA variants, since the artery must remain intact during axilla reconstruction.The SSA may originate from the 3rd part (47.2-94.1%), the 2nd part (1.66-52.8%) and the 1st part (0.6%) of the AA. It also may create a common trunk with the PCHA (3.8-20%), the LTA (10%), and the transverse cervical artery in 6% of cases (10). The PCHA may have a highly variable origin from the AA 3rd part (77.1%), from the SSA (12%), from the deep brachial artery (8.4%), and from the LTA (1.2%) (11).

In the current case, great emphasis should be placed on the LDM upper part variant innervation by the LSSN, identified in 2.8% of cases (12). The high-level MCN-MN interconnection is also of high importance. According to Venieratos and Anagnostopoulou (13), the current case is classified as Type I, since the communicating branch originates before the MCN pierces the CBM. It is not clear whether this branch is considered an (MCN-MN) interconnection or a second lateral root of the MN. According to Buch-Hansen, who compare the thickness of the communicating branch with that of the MN lateral root, the MN in our case presents a 2nd lateral root (5). Detailed knowledge of the AA variant branching pattern is of paramount importance, as the variants may pose diagnostic dilemmas during angiography, and intraoperative misinterpretations during lymph node dissections. Thus, preoperative regional blood supply mapping, identified by Doppler ultrasound imaging or angiography (14) is an essential tool for planning and protecting the flaps and for making dissection quicker and easier. The SSA branching pattern may serve as a donor graft for reconstruction of the lateral thoracic wall and oromandibular defects, as well as in mastectomy restoration (6). In-depth knowledge of MCN-MN interconnections is of great importance for the diagnosis and treatment of MN and MCN dysfunction, especially in posttraumatic surgical repair (15).

Conclusion

This case describes an unusual AA branching pattern with a SST formation associated with accessory LTAs. A high level MCN-MN interconnection, as an additional lateral root of the MN was also re-

vealed. A rare innervation of the LDM upper part by the LSSN was also observed. Surgeons should bear such variants in mind when performing muscle flap transfers, thoracic wall reconstruction, and axillary oncology procedures.

What Is Already Known on This Topic:

The typical axillary artery (AA) pattern occurs in only 27% of cases. The lateral thoracic (LTA), the subscapular (SSA) and the posterior circumflex humeral (PCHA) arteries present the widest variability in their origin and branching patterns. High SSA origin was reported in 28.9% of cases, and low origin far more rarely. A common subscapular trunk may give rise to a thoracoacromial trunk, the LTA, the SSA and the PCHA.

What This Case Adds:

The right-sided axillary artery had an unusual branching into a subscapular trunk and two accessory lateral thoracic arteries of variable origin and course. Concomitantly, a high-level interconnection between the musculocutaneous and median nerves was identified (the so-called additional lateral root of the MN). A very rare innervation of the upper part of the right latissimus dorsi muscle by a lower subscapular nerve was also found. The above combination has never been reported before.

Authors' Contributions: Conception and design: NL and MP; Dissection and photographs: NL and KN; Acquisition, analysis, and data interpretation: NL, MP, GP; Schematic drawings: IA; Drafting the article: NL and MP; Revising it critically for important intellectual content: GP, GS and KN; Approved final version of the manuscript: NL, MP, IA, GP, GS and KN.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Research Article
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The Effect of Bleaching on the Basic Colour and Discoloration Susceptibility of Dental Composites

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Abstract

Objectives. To test the influence of a bleaching procedure using 16% carbamide peroxide (CP) on the colour of composite materials and their consecutive subjection to discolouration in beverages. **Materials and Methods.** Nanocomposite Z550 (3M ESPE) and the microhybrid Z250 (3M ESPE) composite materials were selected for the research. 16% CP was applied to composite plates (15 samples each material) for seven hours a day for 14 days simulating at-home nightguard vital bleaching. The test samples were then divided randomly into three sub-groups and submerged in instant coffee, green tea and Coca Cola for 30 days. The control group (N=5) samples were kept in deionized water. Determination of the L*a*b* dimensions of colour of the polymerized discs was performed by calibrated spectrophotometer 24 hours later, after the bleaching procedure, and on days 7, 15 and 30 after immersion in the beverages. **Results.** After application of 16% CP, there were perceptible changes in the colour of both test materials, which did not exceed the boundaries of acceptability ($\Delta E<3.48$). Coca-Cola did not cause discolouration of the tested composites, but coffee and tea changed their colour above the level of perceptibility already seven days after immersion. A statistically significant difference in the change in colour was established between the materials when they were immersed in coffee ($P<0.05$). **Conclusions.** 16% CP does not affect the basic colour of the composites. Immersion in a beverage led to an unacceptable change in the colour of both test materials in coffee and tea, primarily towards black. The greatest discolouration after bleaching was shown by Z550 when immersed in coffee.

Key Words: Composite Resins ▪ Tooth Bleaching Agents ▪ Carbamide Peroxide ▪ Spectrophotometry ▪ Coffee.

Introduction

Treatments to bleach teeth are one of the most frequently requested dental procedures in contemporary dental therapy (1). The bleaching procedure removes internal and external discolouration of teeth, using peroxide-based preparations. Hydrogen peroxide (HP) can be applied directly or may occur by decomposition of carbamide peroxide (CP) in the mouth (2). The home method or nightguard vital teeth bleaching technique, in a custom tray worn overnight, represents the gold standard of whitening (3).

During treatment to bleach teeth, preparations based on HP inevitably come into contact with restorative materials (4). It is thought that preparations used for bleaching teeth may lead to deterioration of the physical and chemical properties of restorations (5). The interaction of composite fillings with bleaching preparations may be of practical significance if, before bleaching, the colour of the restoration completely matches the colour of the teeth, but after the procedure there is a perceptible change in colour. In that case, it will be necessary to change the colour of the filling. The stability of the colour of aesthetic fillings after application of the bleaching gel, depending on the type of composite, has demonstrated inconsistent results in previous research (6). Some studies showed

that preparations based on CP at lower concentrations do not lead to any differences in the colour of the composites before and after bleaching (4, 5, 7), whilst others showed the opposite (8, 9). After completion of bleaching of teeth, the material inevitably comes in contact with coloured liquids. Bleaching of restorative materials may lead to them being more subject to discolouration (7).

The aim of this study was to examine the action of 16% CP bleaching preparations on the colour of two composite materials of different composition, and subjecting them to discolouration in beverages after the bleaching procedure.

Materials and Methods

In this study, two composite materials with the A2 Vita shade of colour were tested (Table 1), a nanocomposite material (Filtek Z550, 3M ESPE, St. Paul, MN, USA) and a microhybrid composite (Z250, 3M ESPE, St. Paul, MN, USA). The bleaching preparation used was 16% CP gel Vivastyle® (Vivadent, Schaan, Liechtenstein). Four immersion liquids were used (deionized water, instant coffee, green tea and Coca Cola), and the changes in colour were judged at various time intervals. Samples distribution of experimental and control groups presented in Figure 1.

Preparation of Samples

Plates made of the two composite materials, 10×2 mm in size, were prepared in a split mould. The material was placed between two microscopic plates, 1 mm thick, covered in celluloid Mylar strips, whereby a flat surface was obtained, and the surplus material was squeezed out without any bubbles forming. The samples were polymerized

Table 1. Composite Materials Tested in This Research

Composite	Manufacturer	Type	Matrix composition	Filler particles	Filler amount (wt/vol)	Lot
Filtek Z550	3M ESPE, St. Paul, MN, USA	Nanohybrid	BIS-GMA, UDMA, BIS-EMA, PEGDMA, TEGDMA	Combination of surface modified zirconia/silica with 0.1-10 μ particles and surface modified silica particles size of 20 nm.	82/68%	N502352
Filtek Z250	3M ESPE, St. Paul, MN, USA	Microhybrid	Bis-GMA, UDMA, Bis-EMA	Zirconia/silica particles 10 –3500 nm	75-85/60	N535897

Bis-GMA=Bisphenol A-glycidyl methacrylate; UDMA=Urethane dimethacrylate; BisEMA=Ethoxylatedbisphenol-A-dimethacrylate; TEGDMA=Triethylene glycol dimethacrylate; PEGDMA=Polyethylene glycol dimethacrylate.

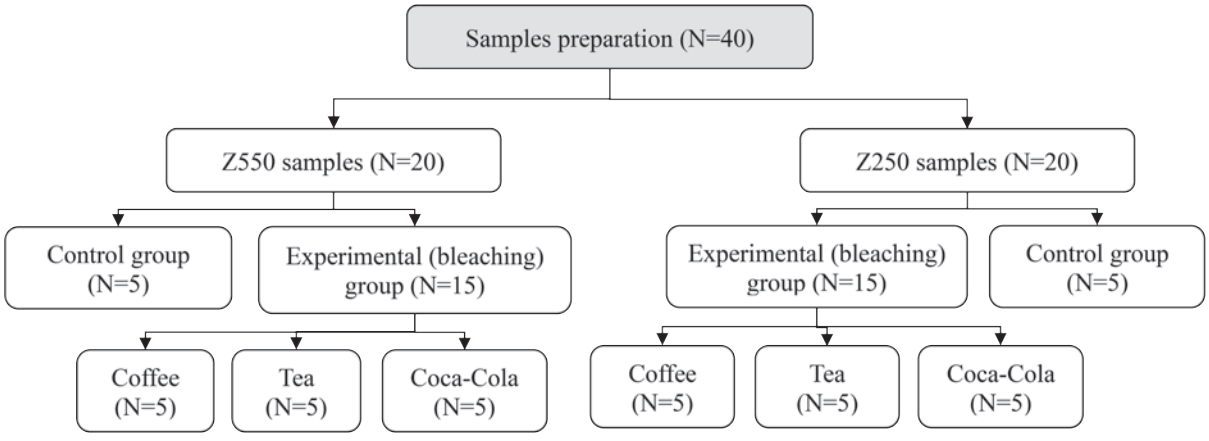

Figure 1. Distribution of samples between experimental and control groups.

through a glass plate using a cordless LED lamp (Elipar™ FreeLight 2 LED Curing Light, 3M ESPE, St. Paul, MN, USA) for 20 s on both sides. Before polymerization, the power of the LED lamp was checked using a radiometer, and it was shown to be more than 1000 mW/cm^2. The thickness of all samples was checked with callipers. In order to standardize the surfaces and create clinical-like conditions, all the samples were polished using fine and superfine paper discs (Sof-Lex™ Contouring and Polishing Discs Kit, 3M Company, St. Paul MN, USA) fixed in a contra-angle handpiece at reduced speed for 10 s, with moderate pressure. After the samples had been prepared, they were placed in distilled water for 24 hours at 37°C to complete the polymerization process.

The Procedure to Bleach the Composites

16% CP was applied to the whole surface of the composite plates in the experimental group (15 samples each) for seven hours a day at 37°C, to imitate what is known as nightguard vital teeth bleaching. The gel was then wiped with cellulose fibre, and the samples were rinsed under running water for one minute, followed by distilled water. For the remainder of the time during the day the samples were placed in de-ionized water at 37°C. This procedure was repeated for 14 days (10).

The Procedure for Discolouration of the Samples

After two weeks' bleaching, five samples of each material were randomly chosen and immersed in different liquids for 30 days. The beverages used were: Nescafe 3 in1 instant coffee Classic (Nestle, Hungaria, Kft. Szerenczi Gyara), Lipton green tea Nature (Unilever, Belgium) and Coca Cola (Coca Cola HBC, Sarajevo, BH). In order to prevent contamination by bacteria, fresh test solutions were prepared every day. The content of the packet of instant coffee (17.5 g) was diluted in 150 ml boiled but off the boil water, according to the manufacturer's instructions. The solution was stirred and left to cool for 10 min (11). 200 ml hot boiled water was poured over a bag of green tea (30 g) and left to cool for 10 min. A hermetically factory sealed 0.5 liter bottle of Coca Cola was used at room temperature. During the experiment, the samples were kept in these liquids for four hours and then rinsed with distilled water, and for the rest of the day they were kept in deionized water. Throughout the experiment the test materials were stored in an incubator at 37°C. Five samples from the control group were kept in the incubator at 37°C in deionized water which was changed every day until the end of the experiment.

Spectrophotometry

For spectrophotometric determination of colour, a previously calibrated Vita Easyshade Compact spectrophotometer was used (Vita Zahnfabrik, Bad Säckingen, Germany). For each measurement the samples were dried with cellular fibre and then the L*a*b* colour parameters were measured according to the CIEL*a*b* three-dimensional colour space. Measurements were taken whereby the samples were placed on a white background so there would not be any absorption of colour parameters. To ensure measurements were taken at the same spot each time, the active part of the spectrophotometer was set to focus on the middle of each sample. Determination of the initial values of L_0*a_0* and b_0* colour dimensions in each group was undertaken 24 hours after the preparation and polymerization of the samples. The measurements were repeated three times for each sample, and the mean value and standard deviation calculated. Measurements were repeated after the bleaching cycles, and on days 7, 15 and 30 after immersion in the beverages. The L*a*b* values of the control groups were determined again after 30 days.

The CIELAB colour space defines each colour through three values, placing each colour at a specific spot on a sphere. The achromatic vertical axis L* relates to the lightness of the object, and has a value from 0 for complete black to 100 for perfect white colour. On the a* coordinate the positive value is red and the negative value is green. On the b* coordinate, the positive b* value is yellow and blue is the negative b* value. By categorizing individual

L*a*b* values measured during and at the end of the experiment (L^*_n, a^*_n, b^*_n) in the CIEL*a*b* formula, the changes in all three parameters of colour were obtained (ΔL*, Δa*, Δb*). When ΔL*, Δa*, Δb* are entered into the given formula, the value ΔE is obtained, that is, the difference in position of two points on the CIEL*a*b* three-dimensional colour space, which represents the total change in colour (ΔE) that took place between two measurements. According to Ghinea et al., a minimal difference between two colours that amounts to more than 1.74 exceeds the threshold of perceptibility, and a value of 3.48 the threshold of acceptability (12).

Statistical Analysis

The statistical analysis was conducted in SPSS v.20 (Statistical Package for Social Sciences), the Windows program package. For description of the data we used arithmetic means, standard deviation and the formula of the International Commission on Illumination (CIE) for measuring chromaticity and determination of small differences in colour:

$$\Delta E_{ab}=[(\Delta L*)^2 + (\Delta b*)^2 + (\Delta a*)^2]½ \quad (13).$$

The statistical analyses used were parametric t-tests for independent samples, multivariate ANOVA to establish the significance of changes in colour, and post-hoc Bonferroni-corrected t-tests for independent samples for appropriate correction of P-values. All analyses were conducted at the level of significance of 5%.

Results

The Effect of 16% Carbamide Peroxide on the Basic Colour of the Tested Composites

After application of 16% CP for 14 days, there was a perceptible change in the colour of the composite materials. The total change in colour (ΔE_w) for Z250 amounted to 1.94 (±0.67), while for Z550 it amounted to 2.34 (±0.65). No statistically significant difference was found in the values of the colour change after bleaching between the tested materials (all samples combined into one group), ΔE_w: t(28)=-1.665; P=0.0535. The total change established that ΔE_w primarily took place in the dimension of lightness due to a rise in the L* values (Table 2).

Table 2. Changes in L*a*b* Colour Parametres and ΔL, Δa and Δb after Application of 16% Carbamide Peroxide

Composite	Chromaticity coordinates	Mean		Mean Δ±SD
		Baseline	After 14 days	
Z250	L*	77.57	79.86	2.29 (±0.12)
	a*	-1.25	-1.31	-0.06 (±0.19)
	b*	17.99	18.24	0.25 (±0.05)
Z550	L*	79.06	80.87	1.81 (±0.38)
	a*	0.37	0.70	0.33 (±0.12)
	b*	24.62	25.93	1.31 (±0.28)

Δ=colour difference of the coordinate; SD=Standard deviation; L*=Light/dark colour coordinate; a*=Red/green colour coordinate; b*=Yellow/blue colour coordinate.

The Susceptibility of the Tested Composites to Discolouration in Beverages

Immersion of the bleached samples of the composite materials in beverages led to perceptible changes in the colour of both tested materials (ΔE>1.74) in tea and coffee even after seven days, and immersion in both beverages for 30 days resulted in an unacceptable change in colour (Table 3).

By multivariate ANOVA for ΔE, a statistically significant interaction was found between composite materials and beverages [F(2.24)=5.21; P=0.013]. Post-hoc testing was performed in order to determine the differences in ΔE values between the two materials (Z250 vs. Z550) according to individual solutions (tea / coffee / Coca-Cola). T-tests for independent samples with Bonferroni correction of the P value [original P-value (0.05) divided by the number of comparisons (total 3): 0.05 / 3 = 0.016] showed a statistically significant difference in the changes in colour between these two materials in coffee (Table 3), where the nano-material Z550 had a statistically significantly higher ΔE value of t(8)=-3.61; (P=0.007). Table 4 shows the values of the changes in L*a*b* colour dimensions

Table 3. Means and Standard Deviations of the Overall Colour Change (ΔE) of Two Bleached Tested Materials after Immersion in Different Beverages for 30 Days

Material	Total colour change (ΔE) over time (days)	Tea M (± SD)	Coffee M (± SD)	Coca-Cola M (±SD)
Z250	1-7	2.68 (±0.43)	4.03 (±0.92)*	0.73 (±0.54)
	1-14	4.28 (±0.43)*	5.60 (±1.27)*	1.14 (±0.51)
	1-30	7.07 (±0.93)* NS	7.44 (±0.98)* †	1.36 (±0.47) NS
Z550	1-7	3.27 (±0.48)	5.24 (±0.60)*	0.69 (±0.28)
	1-14	4.95 (±0.52)*	7.30 (±0.73)*	1.07 (±0.35)
	1-30	7.75 (±1.02)* NS	9.42 (±0.75)* †	1.05 (±0.43) NS

*Indicates clinically unacceptable value (ΔE>3.48); †Indicates statistically significant differences in colour changes between Z250 and Z550 on the thirtieth day of immersion according to T-tests for independent samples with Bonferroni correction (P=0.007); NS= Indicates statistically insignificant differences in colour changes between Z250 and Z550 on the thirtieth day of immersion in tea (P=0.308) and Coca-Cola (P=0.304) according to T-tests for independent samples with Bonferroni correction.

Table 4. Descriptive Values of the L* a* b* Dimensions of Colour according to Material, Solution and Point of Measurement after Bleaching

Chromaticity coordinates (days)		Material					
		Z250			Z550		
		Beverage			Beverage		
		Tea	Coffee	Coca-Cola	Tea	Coffee	Coca-Cola
		M (±SD)	M (±SD)	M (±SD)	M (±SD)	M (±SD)	M (±SD)
L*	First	80.31 (±0.36)	80.18 (±0.60)	79.86 (±0.74)	81.01 (±0.24)	80.69 (±0.39)	80.91 (±0.53)
	Seventh	78.40 (±0.32)	77.20 (±0.61)	79.27 (±0.45)	78.41 (±0.46)	76.91 (±0.62)	80.51 (±0.60)
	Fourteenth	76.85 (±0.31)	75.73 (±0.89)	78.81 (±0.39)	77.21 (±0.58)	75.16 (±0.43)	80.33 (±0.57)
	Thirtieth	74.48 (±0.51)	73.83 (±0.86)	78.62 (±0.38)	74.69 (±0.98)	73.14 (±0.34)	80.46 (±0.60)
a*	First	-1.14 (±0.04)	-1.41 (±0.31)	-1.31 (±0.25)	0.69 (±0.12)	0.69 (±0.06)	0.71 (±0.18)
	Seventh	-0.73 (±0.18)	-0.54 (±0.33)	-1.18 (±0.25)	1.60 (±0.17)	1.87 (±0.18)	0.85 (±0.26)
	Fourteenth	-0.19 (±0.29)	-0.29 (±0.44)	-1.23 (±0.22)	2.07 (±0.21)	2.23 (±0.21)	0.97 (±0.27)
	Thirtieth	0.69 (±0.43)	0.07 (±0.39)	-1.23 (±0.35)	2.80 (±0.24)	2.95 (±0.24)	0.91 (±0.23)
b*	First	18.10 (±0.30)	18.50 (±0.34)	18.24 (±0.40)	26.26 (±0.12)	25.85 (±0.45)	25.67 (±0.29)
	Seventh	19.91 (±0.42)	20.98 (±0.91)	18.05 (±0.29)	27.99 (±0.38)	29.25 (±0.89)	26.17 (±0.48)
	Fourteenth	20.43 (±0.44)	21.63 (±1.17)	18.56 (±0.28)	29.07 (±0.59)	30.30 (±1.14)	26.51 (±0.56)
	Thirtieth	21.66 (±0.66)	21.98 (±1.03)	18.61 (±0.42)	30.18 (±0.55)	30.97 (±1.16)	26.57 (±0.55)

Decrease in the L* parameter indicating that the material became darker; Increase in a* values indicating redness; Increase in b* values indicate that specimens become more yellowish.

of the tested composite materials in coffee, tea and Coca Cola. It was established that both composite materials were discoloured towards black, red and yellow, and that the degree of discolouration rose over time (1-30 days). After 30 days immersed in deionized water, the control group samples did not change colour above the level of perceptibility. The mean value and standard deviation of ΔE for Z250 were 0.94(±0.17), and for Z550 0.91(± 0.30).

Discussion

Changes in Colour of the Tested Composites under the Influence of Carbamide Peroxide

On the basis of the results obtained in this research, it may be said that application of 16% CP to composite materials led to a change in colour which surpasses the threshold of perceptibility of ΔE>1.74 in both composites. However, nei-

ther form of material tested after bleaching (ΔE_w) changed colour beyond the threshold of acceptability of AT>3.48, and the filling did not need to be changed. The action of 16% CP led to slight discolouration of the microhybrid composite, and more of the nanohybrid, although there was no statistically significant difference in colour between the two tested materials (P=0.0535). Although it is possible that this change in colour is partially related to the process of absorption of water by the composite during the time the sample was kept in deionized water at 37°C, it is more likely that the change in the colour of the composite occurred at the time of the application of 16% CP, because the samples in the control group (continually immersed in deionized water) changed colour below the level of perceptibility.

The mechanism of the action of the peroxide from the bleaching preparation is based on the release of extremely reactive and unstable free radicals (14) that seek to bind with organic molecules and thereby achieve stability. The active substances in CP are aggressive oxidants which lead primarily to the release of non-polymerized monomers, but also other substances, from the composites (15). The minimal change in colour can be explained by the breakdown of the bonds of the weakly polymerized resin matrix (11). The free radicals lead to degradation of the organic and inorganic components of the composites and the disruption of the matrix-filler interface (5). In the inorganic part of the composites, the free radicals cause displacement of the inorganic particles and dissolution of the filler ions as a result of the softening of the resin components in the material (5, 16). In the organic part of the composite, the free radicals cause the breakage of the polymer chains and disruption of the double C=C bonds (9). In addition, peroxide leads to the release of monomers from the three-dimensional polymer network of the composite (17). The final result of the action of the free radicals is the occurrence of micro-cracks and diffusion of water into the composite (10). Therefore, it is always necessary to polymerize composite materials, according to the manufacturers' recommendations, and thereby reduce the quantity of residual monomers.

Our results confirm the results of previous research of imperceptible change in the colour of composite material after bleaching (5-7, 15, 18). Previous research also showed that under the action of bleaching preparations the microhybrid material changed colour least of the materials tested (8). In contrast to these results, some research confirmed clinically unacceptable colour values after bleaching (9, 19), which may be explained by the different compositions of the composite materials, the bleaching protocols, the length of application and the concentration of the gel (5, 6, 9). Preparations that contain higher concentrations of HP lead to higher values of colour change, regardless of the fact that the application is shorter in duration (8). With increases in gel concentration and the duration of immersion, the release of monomers also increases (17). Both materials tested in this research were subjected to the same conditions, that is, the action of CP preparations of equal concentration with the same duration of application, and they were prepared in the same conditions of optimum polymerization, verified by radiometric measurement of the strength of the lamplight. Moreover, both materials have a high percentage of filler, almost the same in weight, which reduces the probability of water sorption. The change in the colour of the composite materials may be primarily ascribed to the organic component, that is, the hydrophilic monomers, UDMA and TEGDMA, in line with previous research in which changes in the colour of composites after bleaching were linked to the presence of the monomer TEGDMA (6). This monomer has low molecular weight and is one of the main monomers that are released from resin based composite materials (17).

*Change in the Colour of the Composite on the L*a*b* Axes after Bleaching*

The values of changes on the L*a*b* colour axes after bleaching showed that the greatest changes took place in the dimension of lightness, due to the increase in the L* value in both materials (Table 2). Both tested composites became slightly lighter,

which may be explained by the dissolution of the matrix due to bleaching, which led to the creation of pores on the surface, filled with saliva or air (20). The change in the value of the L* coordinates may be the result of changes in the roughness of the surface due to elution of the monomers (15). These results are in accord with the results of previous research, (9, 14, 18), which also showed an increase in the L* dimension. On the other hand, after bleaching with highly concentrated 35% HP, de Andrede et al. obtained a lighter colour only in the nanocomposite (7), whilst Pecho et al. did not find any change in the L* values (15).

After bleaching with 16% CP the a* parameter of the tested materials remained almost unchanged, as in previous research (9). Regarding the b* axis, a slight change took place in the direction of an increase in yellow colour for both materials tested. This direction of change in the composites' colour on the yellow-blue axis after bleaching with 16% CP was also found in previous studies (9, 10, 21).

Since the change in colour of the composites due to bleaching did not cross the threshold of acceptability, it may be concluded that 16% CP does not act on the basic colour of the composites. That is to say, whitening does not bleach the material in the same way as it bleaches teeth.

Change in Colour after Bleaching and Immersion in Beverages

Immersion of composites in beverages after bleaching led to a gradual increase in ΔE in both materials tested (Table 3). The threshold of acceptability of the bleached samples of both materials was already exceeded after seven days' immersion in coffee, and the strongest change in colour occurred after 30 days' immersion. When the individual L*, a*, b* dimensions of colour are considered, the composite samples immersed in beverages after bleaching showed the same direction of change in colour in both tested materials. The greatest difference was found in the L* dimension, where, after immersion in the beverages, there was a fall in value towards black, and in the chromatic dimensions there was a slight increase on the a* and b* axes (Table 4), as in previous research (21).

The susceptibility of the composites to discolouration is ascribed to the gradual absorption of water and the hydrophilic characteristics of their components (22). Together with the water, pigment is also absorbed into the organic component of the material. This fluid intake is ascribed to the hydrophilic monomers Bis-GMA and TEGDMA (18), and since TEGDMA is only present in the nanocomposite, this may be one of the reasons for the greater discolouration of that material in coffee. Discolouration by coffee occurs as a result of roasting, whereby it decomposes thermally into a brown caramel substance, which reacts with the chlorogenic acids, creating a browny-black pigment (23). Tea leads to discolouration of the composite thanks to tannin (24). The effect of Coca Cola on the change in colour in this experiment was found to be minimal, that is, there was no perceptible change in colour in either of the tested materials, just like in the deionized water in the control group.

Limitations of the Study

As a limitation of this research we can mention that the protocol conducted is not completely equivalent to *in vivo* conditions, because the oral environment is a dynamic medium in which the secretion of saliva takes place continually, and consummation of beverages is intermittent. The discolouration of composite materials in the conditions of the oral environment may have a different pattern, because aesthetic restorations are exposed to the action of various factors in the oral environment, such as moisture, oral hygiene habits, diet etc. In future research, it is necessary to test how different concentrations of hydrogen peroxide act on the physical properties of dental restorative materials, and the use of artificial saliva as a medium between application sessions. Control clinical studies should also test and establish the cumulative effect of all the factors present in the oral cavity on the colour and surface properties of composite materials. Practice and research have

shown that preparations for bleaching teeth effectively change the basic colour of hard dental tissue. However, it is possible that the colour of any composite fillings present in teeth that are bleached by several shades, may not match the new colour of the dental tissue. Therefore, the patient should be informed that it is probable that after the bleaching procedure they will have to change their existing fillings.

Conclusions

According to the methodology used and on the basis of the results obtained from the research, it may be concluded that bleaching with 16% CP did not change the basic colour of microhybrid and nanohybrid composite materials beyond the threshold of acceptability. Imperceptible changes in colour after bleaching may be ascribed to the dimension of lightness, so that both composites became slightly lighter. Between the universal and the nanomaterial there was no statistically significant difference in the change in colour after bleaching ($P>0.05$). Coffee and tea caused perceptible changes in the colour of previously bleached samples of both composite materials already after seven days of immersion, and the degree of discolouration increased over time. The discolouration of the composites in coffee and tea occurred primarily in the lightness dimension in the direction of black.

What Is Already Known on This Topic:
During teeth bleaching treatment, preparations based on hydrogen peroxide inevitably come in contact with restorative materials, and after whitening the aesthetic effect may be compromised. Knowledge of differences in the action of bleaching on individual types of composites may affect the choice of materials for direct fillings.

What This Study Adds:
This research is a scientific contribution to increasing knowledge about the action of carbamide peroxide on modern day composite materials used in everyday clinical practice. On the basis of the results obtained of exposure to beverages after bleaching, recommendations can be made to patients regarding consumption after conducting the procedure to bleach their teeth. The research showed how the dietary habits of patients who wish to bleach their teeth may influence the appropriate choice of composite material.

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The Relationship of Caries Risk and Oral Hygiene Level with Placement and Replacement of Dental Restorations

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Abstract

Objectives. To assess the caries risk and oral hygiene level that affect the placement and replacement of restorations. **Materials and Methods.** A practice-based study performed in private clinics. A total of 76 dentists participated. The sample consisted of 10,076 restorations of >14-year old patients collected by volunteer dentists over a period of two months. Clinicians were asked to record the details of their patients and also the state of oral hygiene and caries risk of each patient. The data were analysed using Microsoft Excel and SPSS 20.00 software. It was considered that a P value less than 0.05 was significant. **Results.** 50.9% restorations replaced were due to secondary caries with moderate oral hygiene, with no significant differences. Of Class II type, 75% were replaced in moderate oral hygiene with a significant difference. There was a significant difference between the use of amalgam and composite according to the state of oral hygiene. 47.7% of the individuals who had their restorations replaced due to secondary caries had high caries risk. According to CI II cavity type, it was shown that 70.5% of the patients had moderate and 23% of the patients had high caries risk. **Conclusion.** Most restorations were replaced due to secondary caries. There is a synergetic relationship between oral hygiene level and caries risk in patients and the possibility of secondary caries development and restoration replacement.

Key Words: Amalgam ▪ Caries Risk ▪ Composite ▪ Dental Restoration ▪ Oral Hygiene.

Introduction

One of the most common and significant features of dental practice is caries treatment. Over all, caries is taken to be a progressive disease that finally damages the tooth unless a dentist intervenes, and the trend in intervention is minimal, either by prevention, fissure sealant or resin infiltration rather than surgical intervention. Understanding of caries has altered significantly, and this alteration needs to be seen in dental practice. The significance of caries risk assessment as a precondition for suitable preventive and treatment interventions needs to be understood, and e practical information provided on how general practitioners can include caries risk assessment in their management of caries (1).

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In the meantime, many risk factors remain undefined. Insufficient salivary flow and structure, high amounts of cariogenic bacteria, insufficient fluoride exposure, gingival recession, and immunological components are the physical and biological risk factors for enamel or root caries which need special health care. Behavioral factors under a person's control are directly related to caries incidence in that person. These factors include: poor oral hygiene, improper dietary habits, frequent consumption of oral medications containing sugar, incorrect methods of feeding infants, and genetic factors (2). Other factors related to caries risk include poverty, deprivation or social status; education level; dental insurance availability; dental sealant application; the presence of orthodontic appliances; and poorly designed partial dentures (3).

According to many studies, it is said that the restoration replacement rate due to secondary caries is higher for composite restorations compared to amalgams and that it is related to the oral hygiene of the individual and increases when caries risk increases (4-6). The risk of restoration failure because of secondary caries increases when the patient has high caries risk. Amalgam replacement due to secondary caries is less frequent than composite, which has been explained in the literature by various factors: I. the metallic structure of amalgam: It has been reported that the antibacterial effect of metal ions released from dental materials may have an effect on secondary caries (7). II. the microbial ecosystem adjacent to the restoration: Svanberg et al. reported that the amount of *Steptococcus mutans* detected in composite restoration margins was significantly higher than in amalgam restorations (8). III. plaque accumulation: In a study by Friedl et al., plaque accumulation between composite restorations and the tooth surface was reported to be higher than between amalgam restorations and tooth structure interface (9).

The aim of this study is to assess the caries risk and oral hygiene level that affect the placement and replacement of restoration

Materials and Methods

This research was conducted after receiving permission from the authorities of Tishk International University (Decree No. 14 on September 4/2020).

Study Design

The study group was formed by dentists working in private dental clinics. The clinicians were general dentists and their experience ranged from one year to 10 years. They were selected by personal contact, which was the first appointment with them. Dentists who specialized in any form of dental specialty were not included in the study. The university from which the dentists graduated was not taken into account. Dentists working in private dental clinics in the city center of Erbil were included in the study in order to be able to represent the entire northern Iraqi region, as well as to maintain contact with the dentists from the beginning to the end of the study. A total of 100 dentists were contacted, but only 80 responded. The number then fell to 76 because two dentists had a lack of data and two withdrew because they did not have time. Information was provided about the aims, method and requirements of the study. After providing the information, volunteer dentists who were open to participate in the study were included.

Collection of Data

A second appointment was made with the dentists who had volunteered to participate in the study so that the details of the study could be explained. The researcher completed a presentation for the dentists who attended, for work in their private practice. The presentation included information, for example, on the criteria for patient selection, the number of restorations to be collected for each dentist, and how to use the data collection form. Each group included two or three dentists for the presentation. The participants asked questions and the researchers clearly answered all their questions. The researchers asked the participant dentists to collect the data within two months, but additional time was given for clinicians who could not achieve a sufficient number of restorations within the time given.

Standardization and Calibration

The clinicians were asked to choose the patients aged over 14 years so that they had complete permanent dentition. Clinical photographs and radiographs of some restorations that needed to be replaced for various reasons were discussed with each clinician. These photographs, used for calibration by clinicians, were taken from books and websites. The criteria of secondary caries detection used in this study included visual, tactile and radiograph methods. Visual detection involved the valuation of discoloration, staining or other

visual alterations. Tactile detection concentrated on evaluation of the presence of any ditching. Radiographic recognition was used either alone or in combination with visual assessment (10). We requested the clinicians to collect data on secondary caries from vital teeth, anterior and posterior, with neither cracked nor broken restorations, and did not specify the tooth location as posterior or anterior. Oral hygiene levels or caries risk status of patients used by general dental practitioners in practice-based studies (11, 12) were used instead of detailed indices. Parameters such as the current active and cavitated carious lesions, the number of restorations present in the mouth, and the frequency of tooth brushing were used when determining caries risk. Patients were classified as low, moderate and high risk according to the parameters indicated above. Plaque accumulation in the mouths of patients was taken into account when oral hygiene level was determined.

Sample

The sample consisted of 14 and >14 year-old patients who had complete permanent dentition. Patients were grouped as: 15-19, 20-29, 30-39, 40-49, 50-59, and >60 years of age. Clinicians were asked to record the following data: the patient's gender, age, the number of restored teeth, cavity type, and material used, restoration placement, and the reasons for replacement of each restoration. Clinicians were asked to collect data such as the number of teeth to be restored, the type of cavity (according to Black's classification), the type of material used and replaced, the age of the restoration being replaced (based on the patients' statements), in addition to oral hygiene and caries risk assessment of the patients. Furthermore, the clinicians' gender and professional experience was also recorded. Forms were designed for each visit, not for each patient. More than one form was completed for patients for whom numerous restorations were placed.

Statistical Analysis

The data were collected in Microsoft Excel, and SPSS 20.00 software for Windows was used to evaluate the findings. The Pearson Chi-Square test was used. It was considered that a P value less than 0.05 was significant.

Results

Sample Distribution

The data were collected from a total of 4,771 patients. Of those patients, 53% (2,528) were male and 47% (2,243) were female. The difference between male and female patients was non-significant (Table 1).

Cavity Type

Operative procedures were predominantly performed in class II cavities (Pearson Chi-Square test P=0.000 for both males and females) in male and female patients (40.2% in males and 40.9% in females). Class I cavities were the second most common type of cavities where operative procedures were performed, after class II in both males and females (Table 2).

Table 1. The Number of Patients According to Age Group

Age groups (years)								
Gender	15-19	20-29	30-39	40-49	50-59	>60	Total	P-value*
Male	239	974	751	375	120	69	2528	0.643
Female	263	742	715	354	121	48	2243	
Total	502	1716	1466	729	241	117	4771	-

*At P<0.05. Pearson Chi-Square test.

Table 2. The Distribution of Classes of Restorations According to Patient Gender

Gender	Restoration class					Total N (%)
	Class I N (%)	Class II N (%)	Class III N (%)	Class IV N (%)	Class V N (%)	
Male	1726 (33)	2101 (40.2)	558 (10.7)	341 (6.5)	498 (9.5)	5224 (100)
Female	1547 (32.2)	1964 (40.9)	517 (10.8)	338 (7)	439 (9.1)	4805 (100)
Total	3273 (32.6)	4065 (40.5)	1075 (10.7)	679 (6.8)	937 (9.3)	10029 (100)

Oral Hygiene

Only 8.2% of all restorations investigated in this study were placed in patients with good oral hygiene (Table 3). Of the individuals who had restorations replaced, 6% had good, 67.2% had moderate, and 26.8% had bad oral hygiene, with significant a difference (P=0.001).

Table 3. Number and Percentage Distribution of Placed and Replaced Restorations According to Oral Hygiene Level

Oral hygiene state	Placed restorations	Replaced restorations	P-value*
	N (%)	N (%)	
Good	823 (8.2)	80 (6.0)	0.001
Moderate	7329 (73.1)	899 (67.2)	
Bad	1877 (18.7)	359 (26.8)	
Total	10029 (100.0)	1338 (100.0)	

*At P<0.05; Pearson Chi-Square test.

It was determined that more than half (50.9%) the individuals who had restorations replaced due to secondary caries had moderate oral hygiene and about 46% had poor oral hygiene levels (P=0.001). Three-quarters (75%) of Class II type cavity restorations were placed in individuals with moderate oral hygiene levels, with a significant difference (P=0.001) (Table 4).

The effect of oral hygiene level on the choice of restorative material: Amalgam was the preferred material in more than half of the patients with poor oral hygiene, and composite restorations were preferred in patients with good oral hygiene (79%). There were very highly significant differences in the choice of restorative materials between all oral hygiene levels (P=0.000) (Table 5).

Table 4. Oral Hygiene Levels in Patients with Replaced Restorations in Class II Cavities

Oral hygiene state	Restorations replaced due to secondary caries	P-value	Restoration replaced in class II cavities	P -value*
	N (%)		N (%)	
Good	11 (2.5)	0.001	214 (5.3)	0.001
Moderate	223 (50.9)		3050 (75.0)	
Bad	204 (46.6)		801 (19.7)	
Total	438 (100.0)		4065 (100.0)	

*At P<0.05; Pearson Chi-Square test.

Table 5. Choice of Restorative Material and Oral Hygiene Level

Material	Oral hygiene level			Total	P-value*
	Good N (%)	Moderate N (%)	Bad N (%)		
Amalgam	54 (5.3)	515 (50.7)	446 (43.9)	1015	0.000
Composite	561 (7.9)	56037 (79)	933 (13.1)	7097	0.000
GIC	20 (28.6)	41 (58.6)	9 (12.9)	70	0.000
Other	108 (21.2)	271 (53.2)	130 (25.5)	509	0.000
Total	743 (100)	6430 (100)	1518 (100)	8691	-

*At P<0.05; Pearson Chi-Square test.

Table 6. Number and Percentage Distribution of Caries Risk in the Studied İndividuals

Caries risk	With restoratio	P-value	With replaced restoration	P-value*
	N (%)		N (%)	
High	2300 (22.9)		434 (32.4)	
Moderate	6883 (68.6)	0.121	826 (61.7)	0.521
Low	846 (8.4)		78 (5.8)	
Total	10029 (100.0)		1338 (100.0)	

*At P<0.05; Pearson Chi-Square test.

Table 7. The Distribution of Class II Cavities According to Caries Risk

Caries risk	Class II restorations were placed		P-value*	With replaced restorations due to secondary caries		P-value*
High	948	23.3		209	47.7	
Moderate	2866	70.5	0.071	214	48.9	0.062
Low	251	6.2		15	3.4	
Total	4065	100.0		438	100.0	

*At P<0.05; Pearson Chi-Square test.

Caries Risk

Table 6 shows the number and percentage distribution of caries risk of the studied individuals. In this study, 68.7% of all restorations were performed in patients with moderate caries risk, and 22.9% in patients with high caries risk. There were no significant difference between the three levels of caries risk in initial restorations (P=0.121). Of the restoration replacements, 60% were performed in patients with moderate caries risk, and 32% in those at high risk for caries, with no significance difference (P=0.521).

The distribution of Class II cavities according to caries risk is shown in Table 7. Of the patients, 70.5% had moderate and 23% of the patients had high caries risk, with no significant difference (P=0.07). Only 47.7% of the individuals who had their restorations replaced due to secondary caries had high caries risk, and 48.9% of them had moderate caries risk (P=0.062).

Discussion

A research design defined in international medical literature as practice-based was used in this study. This type of research depends on data analysis as the clinicians are in daily routine dental work (13). The data reported by clinicians are essential in the estimation and evaluation of new materials and techniques in the biomedical field, including medicine and dentistry. Long-term controlled clinical trials are ideally used to test materials and techniques in dentistry with a small number of patients and clinicians. In addition, long-term controlled clinical trials need to choose a group of patients and expert clinicians to carry out the survey without time limits and under controlled conditions, which is very difficult to apply in day-to-day practice. Thus, controlled clinical trials are more accurate than practice-based studies. (14). The disadvantages of practice-based research have been pointed out as follows (13, 14): differences in clinicians' treatment decisions and assessment of quality, the criteria are not standardized for making treatment decisions in restoration replacement, and there is a possibility of misunderstanding the instructions in the research procedure.

Both clinical experience and scientific research have the possibility of reinforcing the evidence-based groundwork of dental practices (15). Henceforth, creating links between practicing clinicians and academics can initiate some developments in increasing the effectiveness of dental services in daily practice (16).

The effects of oral hygiene and caries risk on individuals' restorative treatments have been addressed in only two studies. In these studies, published by Burke et al. (11) and Tyas (12), the dentists' evaluations were taken into consideration instead of detailed indices to determine patients' oral hygiene levels and caries risk. A similar methodology was used in this study. It is therefore advisable to approach the data obtained in this part of the study cautiously. However, the primary goal of practice-based research is the scientific dissemination of the routine practice of clinicians. Perhaps the most important reason for the willingness of dentists to take part in such studies is that they were not required to spend any extra time doing the work, apart from the time spent to record the data. Therefore, it is not possible to obtain detailed indices of the degree of caries risk or oral hygiene levels from clinicians in such studies. In a study conducted by Burke et al. (2001) (11) with 32 dentists, it was found that 37% of the patients had good oral hygiene, 44% of them had moderate oral hygiene, and 19% had bad oral hygiene. Regarding caries risk, 26% had high and 40% had low caries risk. In this study the results were similar. In this study, restoration placement was present in 8% of those with good oral hygiene, and there was an increase in the possibility of needing restoration with moderate oral hygiene (73%) and in bad oral hygiene (19%), where this low percentage with bad oral hygiene may be related to neglecting regular dental visits. Tyas (12) found these rates as 13.9% and 7.4% for amalgam and composite, respectively. In the current study, composite restorations were found in only 7.9% of the individuals with good oral hygiene. On the other hand, motivating the patient to practice good oral hygiene is also of great importance. Another important point that should be noted in routine patient visits is the control of secondary caries.

Studies conducted to date have shown that secondary caries plays an important role in restoration replacement. As previously stated, secondary caries does not differ histopathologically from primary caries, and similar factors play a role in both types of caries. Studies in the literature have shown that there is a relationship between oral hygiene level and the development of caries (17, 18). In this study, restorations were replaced in only 5.8% of the individuals with low caries risk, and in about half of those with poor oral hygiene. The causes of replacing restorations may be attributed to three major categories (19): clinician factors, material properties, and patient factors. Studies have found a positive correlation between good oral hygiene and restoration lifespan. Especially after inadequate polishing, the environment required for growth of *Steptococcus mutans* in composite materials is improved. This, combined with poor oral hygiene, may increase the formation of secondary caries, creating synergistic effects (20). Occasionally a mixture of factors may be the cause of the failure, even though clinicians seldom register more than one cause for replacement of restorations. The majority of failures happen gradually, but rapid failures can also happen, e.g., restoration fracture. The presence of defects may not be to an extent that it necessities instant restoration replacement (19). Since defects occur gradually there is a chance for repair by minimally invasive dentistry rather than entire restoration removal and replacement. By minimally invasive dentistry the dentist can repair the restoration and refurbish a defect (10).

It is known that oral hygiene practices affect the development of interproximal caries. Particularly in individuals who did not use dental floss, class II type cavities were encountered more frequently (21). In our study, 23.3% of class II type cavities were found in individuals with high caries risk and 19% of them in those with bad oral hygiene. However, in the current study, amalgam and composite restorations were replaced due to secondary caries at similar rates. This may have been affected by the differences in the educational backgrounds of the clinicians, as well as the diagnostic instruments used by the clinicians when deciding to replace the restorations.

Limitations of the Study

Limitations of the study include: differences in the clinicians' treatment decisions and assessment of

quality, in their restoration replacement, criteria that are not standardized for making treatment decisions, and the possibility exists of clinicians misunderstanding the instructions in the research procedure. The involvement of dentists who may have not undertaken any training or continuing education courses in diagnosing restoration failures may be regarded as a limitation of this study. The study did not specify anterior and posterior teeth but included all teeth generally.

Conclusion

There is synergetic relationship between oral hygiene and caries risk in patients with the possibility of development of secondary caries, and restoration replacement, especially in CI II cavity types. This information is important for communicating the experience of clinicians to scientists. Henceforth, establishment of links between experienced clinicians and academics can improve dental services in everyday practice.

What Is Already Known on this Topic:

Physical and biological risk factors for enamel or root caries consist of insufficient salivary flow and structure, high numbers of cariogenic bacteria, insufficient fluoride exposure, gingival recession, immunological components, and the need for special health care. Other factors related to caries risk include poverty, deprivation, or social status; the number of years in education; dental insurance coverage; use of dental sealants; use of orthodontic appliances; and poorly designed or ill-fitting partial dentures. Behavioral factors under a person's control are directly related to caries incidence in that person. These factors include poor oral hygiene; poor dietary habits, frequent use of oral medications that contain sugar; inappropriate methods of feeding infants, and genetic factors.

What this Study Adds:

This study proved that there is a relationship between oral hygiene and caries risk in individuals and the need for restoration, and secondary caries and restoration replacement.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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COVID-19 and Care for the Elderly in Long-Term Care Facilities: The Role of Information Communication Technology

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Abstract

Objective. To review the changes in communication in long-term care facilities (LTCF) during the COVID-19 pandemic. **Methods.** A systematic literature review was conducted through a keyword search of the PubMed and Ovid Embase databases. In accordance with the inclusion and exclusion criteria, 11 articles were selected and analysed qualitatively. **Results.** The use of information communication technology was heterogeneous, and it was used to bring together several different groups of users: LCTF residents' families, hospitals, specialists, and general practitioners. The modes of communication and preferred ways to reduce the social isolation of LTCF residents from their family members were described. Various smartphone applications have been designed for both socializing and the use of telemedical solutions. Opportunities for peer-to-peer social interaction between the elderly through information communication technologies have been neglected. **Conclusion.** Video calls may bring greater satisfaction to residents and their families. Telemedicine and interdisciplinary cooperation between healthcare professionals have increased the quality of medical care in long-term care institutions during the COVID-19 pandemic.

Key Words: COVID-19 ▪ Information Technology ▪ Elderly ▪ Long Term Care Facility ▪ Communication.

Introduction

The COVID-19 pandemic has changed the way work is done at LTCFs and revealed many shortcomings. On the one hand, staff have worked to protect institutionalized elders, whereas on the other hand there has been an outbreak of ageism within society and the media (1, 2). Regions of Italy in which families are more fragmented and inclusion in LTCFs is more accessible were affected more severely in the first wave. At the same time, the official statistics underestimated the number of deaths directly or indirectly related to COVID-19 (3, 4). A high percentage of deaths took place among the elderly in LTCFs. This was also the reason why numerous recommendations and guidelines were accepted to prepare LTCFs for potential further COVID-19 outbreaks (5-7). With the intention of preventing the spread of the virus, many chose a strategy of partially or completely banning physical contact between LTCF residents and their loved ones. Due to concerns that inadequate physical contact and social isolation would cause a deterioration in their physical and psychological health, calls were made to enable residents to maintain active contact with their families, and communication shifted to the virtual domain (8-11).

COVID-19 also brought about some welcome changes, such as stronger communication between different levels of health care, and better connections between different medical disciplines and LTCF staff to provide better quality care for residents in LTCFs (6). The development of communication information technology in recent decades has enabled the rapid development of communication channels. Communication between healthcare workers has moved to smartphones and the

internet, both within and outside institutions. Healthcare workers and LTCF staff have remained in touch through regular phone calls, e-mail, and video conferences, using various platforms (6, 12). Telemedicine has experienced a breakthrough. In France there was a 15-fold increase in the number of teleconsultations in March 2020 compared to February 2020 that were probably made in primary care. A hospital in France, that had already been using teleconsultations in LTCFs since January 2018, did not see any increase in the number of teleconsultation in the same time period (13). However, the truth is that there are very few institutions that have the option of telemedicine. In Switzerland, only 3.9% of 466 LTCFs used teleconsultations (14). Inadequate basic infrastructure and technical equipment remain a problem for LTCFs. A question worth asking is whether internet accessibility will become an essential human right (15).

The aim of this research was to systematically review the changes in long-term care facilities (LTCF) during the COVID-19 pandemic.

Methods

This review article investigates the role of information communication technology in LTCFs for the elderly in different countries around the world during the COVID-19 pandemic. The methods and times of searching PubMed and Ovid Embase are shown (16, 17). The keywords used for searching — long-term care facility, COVID-19, and information communication technology — were expanded using their synonyms. Boxes 1 and 2 present the search strings in full, synonyms included.

A qualitative methodology of analysis and synthesis was employed to evaluate article quality. The articles were defined on the basis of their research design, and the research patterns, goals and results were reviewed. After this qualitative analysis, a quantitative description of the research designs was also carried out.

The PubMed search yielded 34 documents. Ovid Embase returned 198 documents; after applying the additional filters "aged" and "article" this was reduced to 44 articles, which were included in the step-by-step review using the PRISMA 2009 Checklist. After removing duplicates, 70 articles were examined. After examining the titles and abstracts of the articles, inclusion and exclusion criteria were used to further analyse 32 articles:

- Inclusion criteria: elders, elders in institutional care, information technology (IT), social isolation.

Nursing Homes [Mesh] OR Residential Facility OR Assisted Living Facility OR Extended Care Facility OR Skilled Nursing Facility OR Old Age Home OR Home for the Aged OR Long Term Facility OR Long Term Care Facility OR Care Home OR Long Term Care Home) AND (Covid-19 OR coronavirus OR SARS-CoV-2 OR severe acute respiratory syndrome coronavirus 2 OR COVID-19) AND (Cell Phone[Mesh] OR cell phone OR cellular phone OR mobile phone OR computer OR smartphone OR ipad OR gadget OR Apps OR communication OR Information Technology [Mesh] OR IT OR Telemedicine OR device OR iphone.

Box 1. List of keywords used to search PubMed (November 28th, 2020) (16).

Nursing home OR long term care Facility OR long term Facility OR Residential Facility OR Assisted Living Facility OR Extended Care Facility OR Old Age Home OR Skilled Nursing Facility OR Home for the Aged OR Homes for the Aged OR Care Home OR Long Term Care Home) AND (covid-19 OR coronavirus OR Sars-Co-2 OR severe acute respiratory syndrome coronavirus) AND (telemedicine OR information technology OR Cell phone OR cellular phone OR mobile phone OR computer OR smartphone OR ipad OR gadget OR Apps OR communication OR IT OR device OR iphone.

Box 2. List of keywords used to search Ovid Embase (December 1st, 2020) (17).

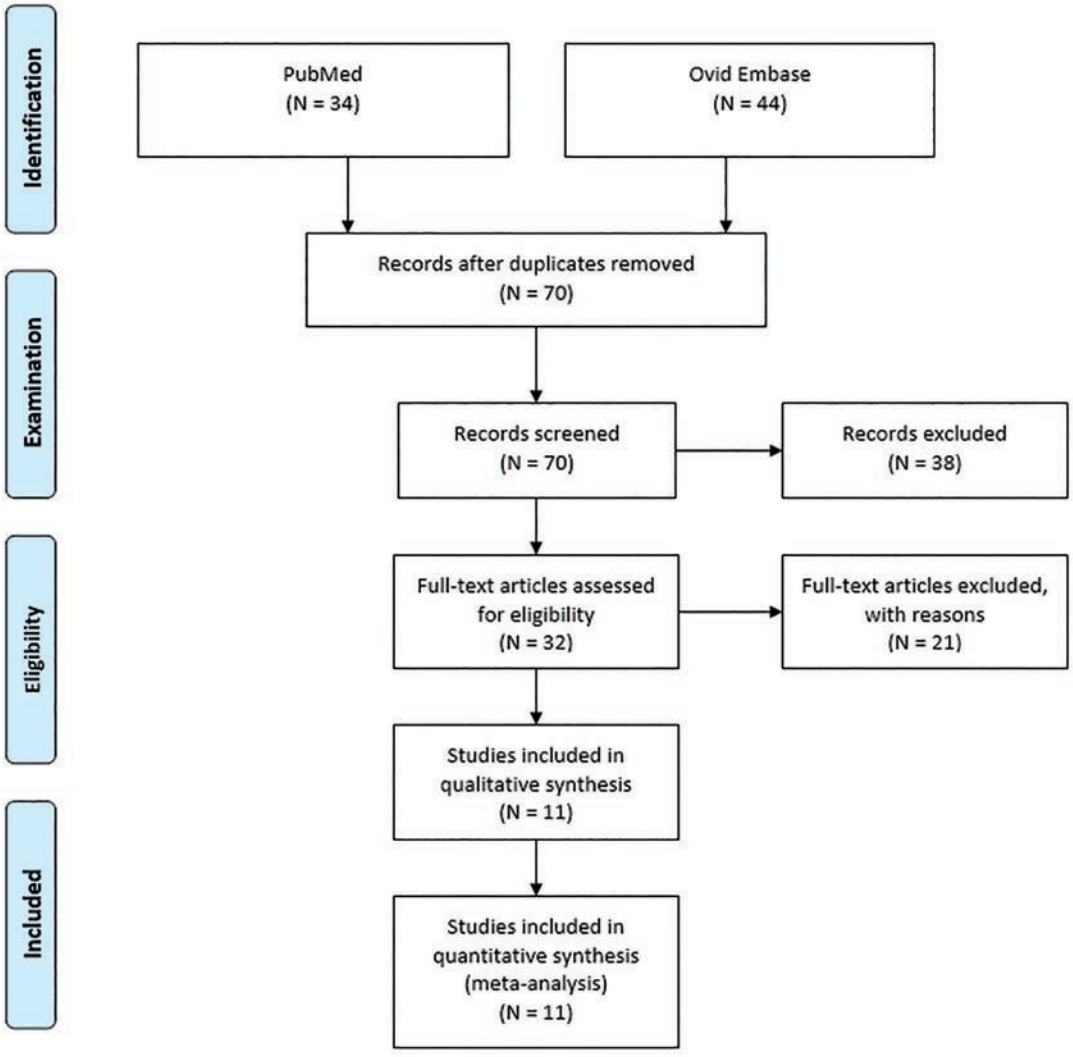

Figure 3. Step-by-step article examination based on PRISMA 2009.

- Exclusion criteria: use of IT for diagnostic purposes, use of IT for data analysis

Thirty-two articles were examined in all. After this step, 11 articles were reviewed in more detail (18-28). Figure 3 shows the decision process.

Results

This systematic review encompassed five population-scale case studies (50%), four cross-sectional studies (40%), one review article (10%), and one qualitative ethnographic study (10%), listed in Table 1. Due to the heterogenous study designs, quantitative analysis was not undertaken.

Table 1. Articles Reviewed

Author	Title, study design, description, place	Sample, goal, result
Archbald-Pannone LR (18)	"COVID-19 collaborative model for an academic hospital and long-term care facilities".	Sample: US region
		Goal: Central support for optimal geriatric and palliative care in LTCFs during the COVID-19 pandemic.
	Population-scale case study: telemedicine consultations played a vital role in connecting hospitals (geriatric-palliative team) with local LTCFs, United States, 2020.	Result: Telemedicine consultations: 12 institutions contacted, five taking part (5/12), outbreak at two institutions, 12% and 19% mortality from COVID-19, respectively.
Harris DA (19)	"Rapid telehealth-centered response to COVID-19 outbreaks in postacute and long-term care facilities".	Sample: One LTCF with 48 residents, 41 of them positive for COVID-19.
		Goal: Managing/overcoming the outbreak of COVID-19 in LTCF with the help of teleconsultations.
	Population-scale case study: the influence of daily teleconsultations on the rate of hospitalization and mortality from COVID-19	Result: Quick implementation of teleconsultations and daily videoconferences between LTCF staff and a multidisciplinary team from the university health center. Hospitalization rate 37.5% (18/41), mortality 12.5% (6/41); in 1 month after the outbreak 13 teleconsultations were made, after which nine residents remained in LTCF care.
Koeberle S (20)	"COVID-19 outbreak: organisation of a geriatric assessment and coordination unit. A French example".	Sample: Region in France.
		Goal: Reorganization and centralization of health care, connecting hospitals, gps and LTCFs through a call center and a centralized data center at a university hospital.
	Population-scale case study: setting up a call center intended for healthcare workers at different levels, evolution of appropriate software, centralization of data, France, 2020.	Results: 235 calls in 16 days, of which 189 calls were for determining level of care, 99 referred to LTCF residents, 18 referred to residents in assisted living facilities, and 72 calls were from the home environment. Rate of hospitalization: 11/99 in the LTCF, 2/18 in assisted living facilities, 38/72 in the home environment.
Shrader CD (21)	"Responding to a COVID-19 outbreak at a long-term care facility".	Sample: LTCF, US.
		Goal: Mastering the COVID-19 outbreak in LTCFs at three levels: containing the spread of the disease, therapy, communication.
	Population-scale case study: Zoom for communication between management and staff with family members, introducing video calls for communication between residents and their family members, United States, 2020.	Result (only communication described): Communication of management with staff and family members of residents, phone calls by loved ones were replaced with Zoom meetings. Weekly calls were set up between a group of loved ones and management/staff. Use of video calls between residents and loved ones with the help of staff.
Ohligs M (22)	"Employment of telemedicine in nursing homes: clinical requirement analysis, system development and first test results".	Sample: One rural LTCF in Germany and one GP, physician–patient relationship already established.
		Goal: Setting up teleconsultations by the GP with the LTCF, lowering the rate of emergency service use.
	Population-scale case study: implementing telemedicine in one rural LTCF with a connection between the LTCF and the GP. To implement telemedicine into the GP's office, regulation changes were necessary.	Result: In 7 months 56 routine and urgent consultations were made, and there was one hospitalization.

Table 1 (continued)

Author	Title, study design, description, place	Sample, goal, result
Sacco G (23)	"Communication modality preferences by frail hospitalized and institutionalized older adults during COVID-19 confinement: a cross-sectional survey".	Sample: 163 elders in isolation on a geriatric ward or in a LTCF.
		Goal: Determining the mode of communication preferred by the elderly (phone call vs. Video call) using a survey. Determining the percentage of elderly that can communicate independently, satisfaction after completed mode of communication, differences in relation to age and place of care.
	Cross-sectional study: Phone call vs. video call in a geriatric ward and a LTCF. Elders in geriatric wards were more independent, it was more likely that a phone call would suffice, in the LTCF there was higher satisfaction with a video call with the assistance of staff, France, 2020.	Result: Elders preferred a phone call (55.3%) to a video call (44.7%), satisfaction after completed communication was similar (87% vs. 89%), age and gender did not influence their preference. Elders on the geriatric ward found it easier to establish communication (22.8%) than LTCF residents (3.8%), phone calls made them more independent than video calls (30.1% vs. 5.1%), hospitalized patients were more satisfied with communication than LTCF residents (92% vs. 74%), and LTCF residents were more satisfied after a video call.
Gallo Marin B (24)	"Experiences of Rhode Island assisted living facilities in connecting residents with families through technology during the COVID-19 dandemic".	Sample: 46 institutions that received 254 tablet computers.
		Goal: Reducing the social isolation of the residents.
	Cross-sectional study: survey, donation of 254 tablet computers to LTCFs from nonprofit organizations, wi-fi is a requirement, Rhode Island, US.	Result: 11 of 46 LTCFs participated (24% response), 63.6% of institutions used tablets for more than one purpose: video calls 90.9%, communication of staff with loved ones 36.4%, telemedicine 36.4%, social care 27.3%, free time 27.3%, other 18.2%, administration 9.1%.
Monin JK (25)	"Family communication in long-term care during a pandemic: lessons for enhancing emotional experiences".	Sample: 168 family members/friends, LTCF residents.
		Goal: To evaluate nine modes of communication (window + phone, Facebook, mail, SMS, letter via staff, videoconference, letter via postal service, sent objects, phone) using the Positive and Negative Affect Scale.
	Cross-sectional study: mode of communication (without personal visits) related to higher positive and lower negative experience of LTCF residents and their loved ones, on-line survey, US, 2020.	Result: Family members assessed their own satisfaction as greater than the residents', the most-used mode of communication was a phone, a higher frequency of phone calls was related to fewer negative emotions, a higher frequency of electronic mail use was related with more positive emotions, letters were related to more negative emotions with family members as well as residents, speed of communication or synchronous communication was related to more positive emotions. Video conferences were not related to either more positive or less negative emotions. Shortcoming: The study was carried out on family members, who assessed their own feelings and the feelings of the resident.
Siu HYH (26)	"A cross-sectional survey assessing the preparedness of the long-term care sector to respond to the COVID-19 pandemic in Ontario, Canada."	Sample: 240 physicians, 54 nurses; those invited were members of expert organizations involved with care at LTCFs.
		Goal: Using a survey to determine how physicians and nurses assess LTCF preparedness for a COVID-19 outbreak.
	Cross-sectional study: physicians at LTCFs assessed how institutions are ready for an epidemic, implementation of guidelines, cooperation between management and LTCF physicians, Ontario, Canada.	Result: 160 out of 294 invited (54% response) took part in the study, none of the returned surveys was excluded. The survey was completed mainly by physicians (80%) from an urban area (87.3%). Five most common measures: 1) isolation protocol for respiratory infections (92.5%), 2) active testing of new admissions (90%), 3) staff education (83.1%), 4) active coordination with local public health service representatives (83.1%), 5) encouraging ill staff to stay home (83.1%). 38 of the participants mentioned the established virtual health solution (5/160, 3.1%).

Table 1 (continued)

Author	Title, study design, description, place	Sample, goal, result
Banskota S (27)	"15 smartphone apps for older adults to use while in isolation during the COVID-19 pandemic".	Sample: 15 top-ranking apps in the field of 1) social networks, 2) medicine, visual and hearing aids, 3) health and fitness, 4) food and drinks.
		Goal: To find cheap and accessible apps that would help elders during a pandemic with lowering social isolation as well as with food supply, maintaining health, and health care.
	Review article: 15 selected Apps on iOS or Android devices most suited for use by the elderly, study made using the PRISMA principle, USA, 2020.	Result: Selected 15 apps, elders can use smartphones with adequate training. Shortcoming: The article refers to the elderly in general, but such apps could be used by elderly people in LTCFS.
Zamir S (28)	"Intergroup 'Skype' quiz sessions in care homes to reduce loneliness and social isolation in older people".	Sample: Three LTCFs, total of 87 residents with dementia and eight staff members.
		Goal: Connecting elders, peer-to-peer communication, socialization inside and outside LTCFs.
	Ethnographic design: Informal feedback, diary and semi-structured interview with resident and staff, researchers used video quiz via Skype in which several LTCF residents participated. Great Britain.	Result: Staff inclusion was mandatory for preparation and technical support, and staff also participated. After three meetings, residents became competitive and each LTCF had a top star that knew the answers to many of the questions. Residents memorized the top star resident. Residents from different LTCFs that had things like place of residence or professions in common reached out to each other. They remembered the answers, not the technology (they did not recognize the equipment after retaking the quiz). Eventually the number of residents participating grew. Residents expressed positive emotions. Cohesion within LTCFs and between them increased. The only negative emotion was uneasiness regarding its outlook. Shortcoming: Equipment difficulties, time strain on the staff.

US=United States; LTCF=Long-Term Care Facility: GP=General practitioner.

To sum up the first five population-scale case studies, three of them used information communication technology to connect hospitals with LTCFs, two used telemedicine solutions (18, 19), and in one case a call centre was set up with centralized data support (20) At one LTCF, information communication technology was used for communication at the LTCF and at home with family members (21). In Germany, a rural GP's office was similarly connected because of prior familiarity

Table 2. Use of Information Communication Technology during the COVID-19 Pandemic

Mode of use	Participants				References
	Healthcare staff	LTCF staff	Residents	Family members	
Professional use					
Interpersonal cooperation among healthcare workers	+	+	–	–	18, 19, 20, 26
Telemedicine services	+	+	+	–	18, 19, 21, 24, 26, 27
Communication between staff and family members	+	+	–	+	21, 24
Reducing social isolation					
Communication between residents and family members	–	-	+*	+*	20, 21, 23, 25, 26
Peer-to-peer social contact	–	+	+*	+	27, 28

LTCF=Long-Term Care Facility; *Communication took place in most cases with the help of staff.

with the residents (22). Three cross-sectional studies describe communication and preferential uses of various communication modes for connecting residents with their loved ones and reducing social isolation in two cases (23-25). One cross-sectional study focuses on LTCF staff assessment of their level of preparedness for the COVID-19 outbreak. This study indirectly reveals that only a small percentage of staff (3.1%) used telemedicine to prepare for the COVID-19 pandemic better (26). The selected articles also include a review article, which lists 15 appropriate apps for use on smartphones intended for elders. Apps are intended to help with socializing as well as telemedicine, everyday needs, or as an aid for visually impaired and hearing impaired elders (27). The ethnographic study was intended for peer social contact among the elderly using a video quiz, which required substantial help from staff (28).

Discussion

All the articles in this review, which explores the impact of information communication technology in LTCFs during the COVID-19 pandemic, can be divided into two parts by intention of use: professional use and reduction of social isolation. Most articles focus on using information communication technology as a tool to improve communication among healthcare workers from various backgrounds and between healthcare workers and non-healthcare workers. Information communication technology enables interpersonal cooperation between professionals from different professions, either by using video conferences, setting up a call centre, or using telemedicine (18-20, 22, 24, 26, 27). Interpersonal cooperation and the use of telemedicine greatly reduced both the hospitalization rate and the mortality of LTCF residents during the first wave of the pandemic. The authors report the mortality numbers as 12%, 12.5%, and 19% respectively (18-20).

The second part of the article concerns social contact, mainly describing communication between residents and their family members, an elder's communication with the environment, or between staff and family members (20, 21, 23, 26, 27). Information communication technology was used to reduce social isolation. Conclusions regarding the various modes of communication are inconsistent. Whereas Cochrane's meta-analysis in Noone et al. concluded that video calls do not reduce elders' feelings of loneliness, Sacco et al. discovered that most elders preferred a regular phone call (55.3%) to a video call (44.7%) (23, 29). The elderly also found it easier to make a phone call independently. Satisfaction after communicating was similar (87% vs. 89%). An important data point is that the elderly on geriatric wards find it easier to establish communication on their own (22.8%), in contrast to LTCF residents (3.8%), who expressed greater satisfaction after a video call (23). In practice, residents suffering from dementia may also decline video calls, and in solitary cases a phone call has proven to be a better solution.

From the viewpoint of care and research, too little emphasis is placed on ways to connect the elderly with one another within LTCFs or other types of institutions. Undoubtedly, the way people chat with someone who shares memories regarding the same profession or living environment is different. This is how various apps either entertain us or offer us a chance to broaden our social circle (27, 28). This article offers a review of various ways to use information communication technology in long-term institutionalized care. The communication solutions most frequently used were detected. The COVID-19 pandemic has set new practices in motion in many different areas, many of which are still being studied. It is possible that some relevant articles or other ways to use information communication technology have been overlooked.

LTCFs are an ideal environment in which to evolve telemedicine. It would make sense to develop telemedical support for LTCFs based on the regional areas and hospitals with which they most commonly cooperate. At the same time, telemedical support could be provided by GP offices due to a shortage of LTCF physicians in some areas. Coordinated action between regional hospitals, LTCF physicians, LTCF staff, and emergency services would enable better quality of care, and reduce hospitalization rates (30, 31).

In recent years, care for elderly individuals with accompanying chronic illnesses has become complex, and LTCF physicians have had to learn more about geriatric care, rehabilitation, and palliative care. The Netherlands saw the development of education in the area of institutionalized medicine, mostly among European physicians that work in LTCFs and who are GPs or family medicine physicians (32). LTCF physicians in Slovenia are mostly family medicine specialists. Slovenia needs to prepare a training program for LTCF physicians, and push to change regulations to enable the development of telemedicine and interdisciplinary cooperation (22, 33). Many training programs should also be prepared for nursing staff. It is known that nurses are often sceptical of vaccinations by medical staff (34). The Hesitancy in the COVID-19 vaccination programme could affects communication strategy in LCTFs.

Conclusion

Video calls at LTCFs performed with the help of staff bring greater satisfaction to residents and family members, but in a hospital environment this connection was not found. The COVID-19 pandemic has accelerated the process of bringing LTCFs into contact with hospitals and cooperation among healthcare workers with the aid of information communication technology. Telemedicine services were used frequently. Institutional lockdown in the COVID-19 pandemic has shaken established procedures in LCTFs. It has indicated the impoverished use of software and audio-visual modes of communication. On the part of users, social isolation stood out. Tensions arose on the part of employees due to differences in professional instructions and doubts posted on social media. We believe that it is therefore necessary to initiate education. Elderly residents should be educated about the use of mobile and electronic applications that could reduce social exclusion. Evidence-based decision-making should be promoted between employees, which can be carried out through video and teleconferences between different experts or webinars. Future quantitative and qualitative studies of quality eldercare in long-term care facilities is needed.

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