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Correspondence to:

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The burden of pancreatic cancer is rising in Brazil

Paulo Andrade Lotufo[I]

Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo (SP), Brazil

[I]MD, DrPH. Full Professor, Department of Internal Medicine, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo (SP), Brazil.
orcid.org/0000-0002-4856-8450

In a recent Editorial in this Journal, we reported that in Brazil among the middle-aged population stratum, the mortality rates due to pancreatic cancer are increasing at a pace of 2% per year for both sexes.[1] For this trend to be better understood, we have analyzed the data relating to incidence, mortality and case-fatality rates for pancreatic cancer published up to 2019.

It is important to emphasize two points concerning the methods and conclusions relating to pancreatic cancer epidemiology. Firstly, there are inconsistencies in the information published worldwide. Secondly, despite the discrepancies between the methods, there is no doubt that the burden of pancreatic cancer is increasing in Brazil.

Geographical and temporal comparisons of incidence and mortality rates for pancreatic cancer are not easy to estimate. The reasons for this are that the symptoms of pancreatic cancer are only rarely identified in the early stage, and that the disease is usually at an advanced stage when diagnosed.[2] Pancreatic cancer is the most fatal of all major cancers, with a median survival time of only approximately six months and five-year relative survival of only 4%.[3]

From 1980 to 2013, all-age pancreatic cancer mortality rates among men increased in Europe, Brazil, Japan and South Korea but declined in Australia, Canada, Mexico and the United States. On the other hand, among women, mortality increased in Europe, Brazil, the United States, Japan and South Korea but decreased in Canada and Mexico. The highest pancreatic cancer death rates for both sexes in 2012 were observed in eastern European countries and Japan.[4]

The age-standardized rates due to pancreatic cancer from 2000 and 2014 showed that the highest rates were recorded for the states of the central-western region, for both genders. The temporal trends of the age-adjusted rates were different for women, with a non-significant annual percentage change of 0.4% (95% confidence interval: -0.2 to 1.0) from 2000 to 2014, without any inflection of the curve. On the other hand, for men, a significant upward trend was observed from 2000 to 2004, with an annual percentage change of 3.7% (95% confidence interval: 0.6 to 7.0), followed by a period of stability, but increasing again from 2010 to 2014, with an annual percentage change of 2.7% (95% confidence interval: 0.2 to 5.2).[5]

A comparison of incident rates for pancreatic cancer among 43 countries revealed that in Brazil, there were non-statistically significant annual percentage increases in age-adjusted incident rates for pancreatic cancer, of 2.4% for men, and 0.1 % for women from 1988 to 2007 (the most recent data available). For men, the increase in the rate was constant over the period of observation and it occurred at the same pace for all birth cohorts analyzed.[6] Another analysis on age-standardized incidence rates, using the GloboCan database, revealed that the highest incidence rate rises among men in Brazil were between 1998 and 2007, with an average annual percentage change of 10.4% (95% confidence interval: 0.8 to 21).[7] The number of hospitalizations due to pancreatic cancer (not the incidence rate) within the Brazilian National Health System doubled from 2005 to 2012 for both sexes, from 2.4/100,000 to 4.5/100,000, with the highest increase in the states of the northeastern region.[8]

The importance of this demonstration of the increase in the burden of pancreatic cancer is that there are no detectable risk factors for this neoplasm.[9] People with pancreatic cancer have not benefited from recent improvements in overall survival relating to genetic profiling ("precision medicine"),[10] and no screening tool has been proven to be effective for early diagnosis of pancreatic cancer.[11]

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Address for correspondence:
Centro de Pesquisa Clínica e Epidemiologia, Hospital Universitário (HU),
Universidade de São Paulo (USP)
Av. Prof. Lineu Prestes, 2.565
Butantã — São Paulo (SP) — Brasil
Tel. (+55 11) 3091-9300
E-mail: palotufo@usp.br

Fetal structural anomalies diagnosed during the first, second and third trimesters of pregnancy using ultrasonography: a retrospective cohort study

Fernando Felix Dulgheroff[I], Alberto Borges Peixoto[II], Caetano Galvão Petrini[III], Taciana Mara Rodrigues da Cunha Caldas[IV], Daniela Rocha Ramos[V], Fernanda Oliveira Magalhães[VI], Edward Araujo Júnior[VII]

Mário Palmério Hospital Universitário (MPHU), School of Medicine, Universidade de Uberaba (UNIUBE), Uberaba (MG), Brazil

[I]MD. Physician, Department of Obstetrics and Gynecology, Mário Palmério Hospital Universitário (MPHU), School of Medicine, Universidade de Uberaba (UNIUBE), Uberaba (MG), Brazil.
orcid.org/0000-0002-2949-4601

[II]MD, PhD. Adjunct Professor, Department of Obstetrics and Gynecology, Mário Palmério Hospital Universitário (MPHU), School of Medicine, Universidade de Uberaba (UNIUBE), Uberaba (MG); and Adjunct Professor, Department of Obstetrics and Gynecology, Universidade Federal do Triângulo Mineiro (UFTM), Uberaba (MG), Brazil.
orcid.org/0000-0002-1655-3609

[III]MD. Physician, Department of Obstetrics and Gynecology Mário Palmério Hospital Universitário (MPHU), School of Medicine, Universidade de Uberaba (UNIUBE), Uberaba (MG), Brazil.
orcid.org/0000-0002-9589-0004

[IV]MD. Physician, Department of Obstetrics and Gynecology, Mário Palmério Hospital Universitário (MPHU), School of Medicine, Universidade de Uberaba (UNIUBE), Uberaba (MG), Brazil.
orcid.org/0000-0001-5330-5260

[V]MD. Medical Resident, Department of Obstetrics and Gynecology, Mário Palmério Hospital Universitário (MPHU), School of Medicine, Universidade de Uberaba (UNIUBE), Uberaba (MG), Brazil.
orcid.org/0000-0002-0833-537X

[VI]MD, PhD. Adjunct Professor, Department of Internal Medicine, Mário Palmério Hospital Universitário (MPHU), School of Medicine, Universidade de Uberaba (UNIUBE), Uberaba (MG), Brazil.
orcid.org/0000-0002-0581-4279

[VII]MD, PhD. Associate Professor, Department of Obstetrics, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), São Paulo (SP), Brazil.
orcid.org/0000-0002-6145-2532

KEY WORDS:
Ultrasonography.
Prenatal.
Prevalence.
Fetal anomalies.

ABSTRACT

BACKGROUND: The prevalence of congenital abnormalities in general populations is approximately 3-5%. One of the most important applications of obstetric ultrasound is in detection of fetal structural defects.

OBJECTIVE: To assess fetal structural anomalies diagnosed using ultrasound in the three trimesters of pregnancy.

DESIGN AND SETTING: Retrospective cohort study at the Mário Palmério University Hospital of the University of Uberaba (Universidade de Uberaba, UNIUBE), from March 2014 to December 2016.

METHODS: Ultrasound data at gestational weeks 11-13 + 6, 20-24 and 32-36 were recorded to identify fetal anomalies in each trimester and in the postnatal period. The primary outcome measurements were sensitivity, specificity, positive predictive value and negative predictive value for detection of fetal anomalies and their prevalence.

RESULTS: The prevalence of anomalies detected using ultrasound was 2.95% in the prenatal period and 7.24% in the postnatal period. The fetal anomalies most frequently diagnosed using ultrasound in the three trimesters were genitourinary tract anomalies, with a prevalence of 27.8%. Cardiac anomalies were diagnosed more often in the postnatal period, accounting for 51.0% of all cases. High specificity, negative predictive value and accuracy of ultrasound were observed in all three trimesters of pregnancy.

CONCLUSION: Ultrasound is safe and has utility for detecting fetal anomalies that are associated with high rates of morbidity and mortality. However, the low sensitivity of ultrasound for detecting fetal anomalies in unselected populations limits its utility for providing reassurance to examiners and to pregnant women with normal results.

INTRODUCTION

The prevalence of congenital abnormalities in the general population is approximately 3%-5%.[1] Since the first report of the use of ultrasound in obstetrics,[2] this has become an important tool for detection of fetal structural defects.[3]

According to the International Society of Ultrasound in Obstetrics and Gynecology, the ideal period for screening for structural defects is the second trimester of pregnancy (weeks 18 to 22).[4] In Brazil, the preferred period for performing ultrasound screening in the second trimester is between 20 and 24 weeks of gestation. In developed countries, the number of referrals for ultrasound examinations in the first trimester (11-13 + 6 weeks) has increased, while second-trimester ultrasound is considered to be the gold standard for detecting structural anomalies.[5] First-trimester ultrasound has utility for confirming fetal viability and gestational age, evaluating the risk of chromosomal disorders and fetal anomalies and detecting twin pregnancy and chronicity.[4]

Evaluation of nuchal translucency in the first trimester has emerged as a tool for screening for fetal structural anomalies.[6,7] There is an association between increased nuchal translucency and chromosomal abnormalities, particularly trisomy 21 and structural anomalies. Greater nuchal translucency has been correlated with increased risk of trisomy 21 and fetal anomalies, especially cardiac abnormalities.[6,7]

More than 80% of fetal anomalies develop before 12 weeks of gestation. Therefore, good visualization of the fetus at this stage enables early detection of structural anomalies.[8] The ultrasound detection rates for major structural anomalies in the first and second trimesters range from 13.0% to 43.6% and from 21% to 85%, respectively.[9-12] The overall sensitivity increases to 93% when first and second trimester ultrasound examinations are combined.[13]

Detection of structural anomalies within the gestational period makes it possible to plan interventions during pregnancy or during the immediate and early postpartum period, thereby reducing perinatal and infant morbidity and mortality.[14,15] In addition, early detection facilitates multidisciplinary planning for maternal-fetal interventions that may be required during the gestational period and provides greater information for parents and relatives.[16]

OBJECTIVE

The objective of the present study was to assess the fetal anomalies diagnosed using ultrasound in the first, second and third trimesters of pregnancy.

METHODS

Study design, setting and ethics

This retrospective cohort study evaluated prenatal ultrasound examinations performed at the Mário Palmério University Hospital of the University of Uberaba (Universidade de Uberaba, UNIUBE) from March 2014 to December 2016.

The present study was approved by the Research Ethics Committee of UNIUBE (August 24, 2017; CAAE: 73231517.9.0000.5145). The need for informed consent was waived due to the retrospective nature of the present study.

Ultrasound evaluations and measurements

Ultrasound data were extracted using the Astraia software (Astraia Software GmbH, 2000-2015, Munich, Germany) and were divided into three groups: first trimester (11-13 + 6 weeks), second trimester (20-24 weeks) and third trimester (32-36 weeks).

The ultrasound findings were confirmed during the postnatal period through physical examination of the newborn or imaging examinations, or via necropsy in cases of death. The objective of the present study was to detect structural abnormalities using prenatal ultrasound examination; however, such examinations are unable to confirm syndromic diagnoses.

Participants and anomaly detection

Women with singleton pregnancies in which gestational age had been established using the date of the last menstrual period and was confirmed through ultrasound in the first trimester were included in this study. Cases of major and minor anomalies identified through ultrasound, together with clinical evaluation or complementary imaging tests in the postnatal period, were included. Participants who underwent first-trimester ultrasound examinations but not second-trimester ultrasound examinations were not excluded from the present study: such participants were reevaluated during the third trimester of pregnancy. The exclusion criteria for the present study were the following: (1) cases of fetal death; (2) ultrasound examinations performed after a diagnosis of fetal anomaly was made, if no other fetal anomalies had been diagnosed during previous examinations; (3) cases of pregnant women who underwent second trimester scans only, without a third trimester scan; and (4) twin pregnancies.

Major anomalies were defined as those that were considered to be lethal, severe or moderate. Minor anomalies were defined as abnormalities that would be excluded from the European Surveillance of Congenital Anomalies registry given that their medical, functional and esthetic consequences would be minor.[17]

Ultrasound examinations

Ultrasound examinations were performed by three experienced examiners with at least five years of experience of using a Voluson E6 device (General Electric Healthcare, Zipf, Austria) equipped with a convex volumetric transducer (RAB4-6L) and operated via the abdominal route. First, second and third-trimester scans were performed in accordance with the guidelines of the International Society of Ultrasound in Obstetrics and Gynecology.[4,18] When required, a complementary examination via the transvaginal route was performed using a volumetric endocavitary transducer (RIC5-9-D).

In accordance with our department's protocol, second-trimester examinations (20-24 weeks) and third-trimester examinations (32-36 weeks) were offered to all pregnant women after an initial first-trimester ultrasound examination (11-13 + 6 weeks). The follow-up for fetuses that were found to have structural defects was individualized according to the structural defect identified. The structural anomalies thus detected were divided into eight groups according to the following body systems: central nervous system; face and nape of neck; thorax; heart; gastrointestinal tract; genitourinary tract; skeleton; and others. This last group included anomalies of body systems other than those listed above.

Sample size and statistical analysis

Case recruitment was guided by an expected 3% prevalence of fetal structural anomalies, in a screening cohort with an estimated 50% sensitivity for fetal anomaly detection. We planned to recruit approximately 600 participants in each group, to achieve a sampling error of approximately 4% for sensitivity. Thus, this sample would have 80% power to detect fetal anomalies.

The data were transferred to Excel 2010 spreadsheets (Microsoft Corp., Redmond, WA, USA) and were analyzed using the Statistical

Package for the Social Sciences (SPSS) software, version 14.0 (SPSS Inc., Chicago, IL, USA). The following variables were evaluated: maternal age, weight, height, body mass index, number of pregnancies, parity, number of miscarriages, newborn weight, race, history of smoking and alcohol use, history of folic acid use before and during pregnancy, chronic diseases, diseases that started during gestation, history of consanguinity, history of structural abnormalities in previous gestations and/or in the family, type of childbirth and type and location of the structural defect.

Quantitative variables were evaluated using the Kolmogorov-Smirnov test and were presented as means and standard deviations. Categorical variables were evaluated using absolute and percentage frequencies and were presented in tables. The primary outcome measurements were the sensitivity, specificity, positive predictive value and negative predictive value for detection of fetal anomalies and their prevalence. To perform the calculations, comparison was made between the findings from the ultrasound examination and the clinical and imaging examinations on the neonate after delivery.

RESULTS

A total of 3,377 ultrasound examinations were performed in the prenatal period. A total of 699 examinations were excluded: 44 due to twin pregnancies and 655 because the delivery did not take place in our service and/or the patient was lost to follow-up. Thus, a total of 2,678 examinations were included in the present statistical analysis. These were divided into three groups, performed at 11-13 + 6 weeks (n = 1,102), 20-24 weeks (n = 683) and 32-36 weeks (n = 893) (**Figure 1**).

After application of our inclusion and exclusion criteria, it was found that only 18.6% (498/2,678) of these women underwent ultrasound examination in all three trimesters. The percentages of the women who underwent ultrasound examination in the first trimester alone, second trimester alone and third trimester alone were: 17.5% (469/2,678), 8.7% (233/2,678) and 21.2% (568/2,678), respectively. However, 8.5% (228/2,678) underwent ultrasound examination in the first and second trimesters; 11.5% (308/2,678) in the first and third trimesters; and 14.0% (375/2,678) in the second and third trimesters.

The clinical and epidemiological characteristics of the study population are shown in **Tables 1 and 2**.

The total rates of structural abnormalities detected in the prenatal period and at birth were 2.95% (79/2,678) and 7.24% (194/2,678), respectively. The rates of structural defects diagnosed using ultrasound in the first, second and third trimesters were 1.2% (13/1,102), 4.4% (30/683) and 4.0% (36/893), respectively.

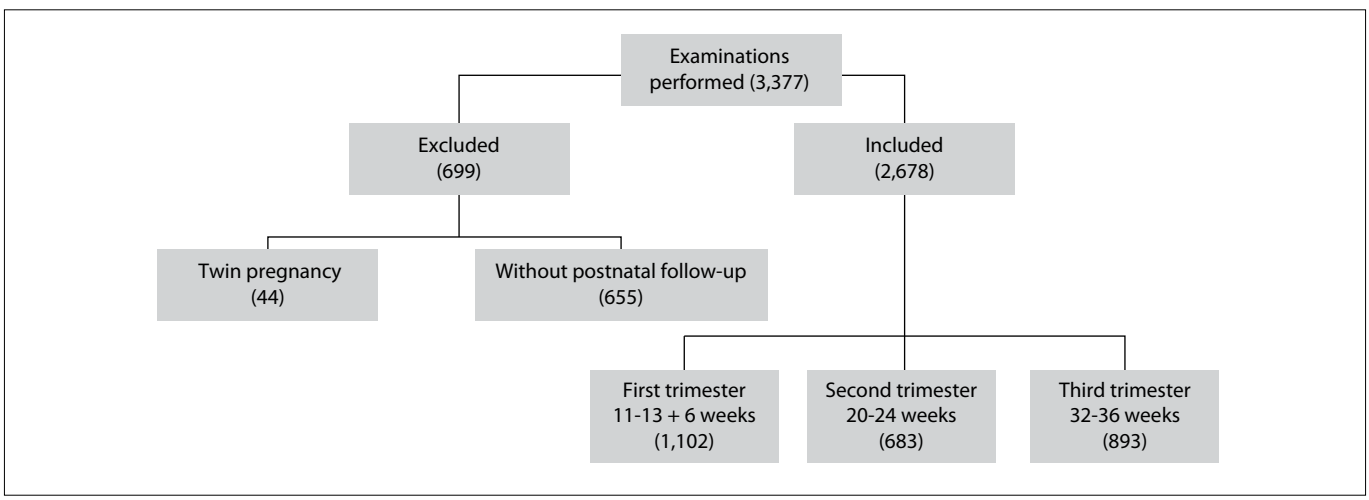

Figure 1. Flowchart of cases included and excluded during the study period.

Table 1. Clinical characteristics of the maternal population studied

Characteristic	First trimester			Second trimester			Third trimester		
	Mean	SD	(Min-Max)	Mean	SD	(Min-Max)	Mean	SD	(Min-Max)
Age (years)	27.14	± 6.55	(14-45)	26.79	± 6.72	(14-45)	26.97	± 6.79	(13-45)
Weight (kg)	72.41	± 16.58	(40.0-141.7)	71.89	± 17.27	(40-148)	73.81	± 16.80	(40-148)
Height (m)	161.79	± 6.75	(106-180)	161.88	± 6.52	(145-180)	161.57	± 6.79	(106-180)
BMI (kg/m^2)	27.75	± 6.32	(15.6-89.0)	27.48	± 6.32	(15.6-55.7)	28.55	± 6.98	(17.1-96.3)
Number of pregnancies	3.26	± 1.26	(2-8)	3.53	± 1.67	(2-11)	3.41	± 1.57	(2-11)
Parity	0.97	± 1.08	(0-5)	1.12	± 1.17	(0-5)	1.14	± 1.23	(0-6)
Number of abortions	1.34	± 0.75	(0.6)	1.38	± 0.96	(0-6)	1.25	± 0.79	(0-6)
Newborn weight (g)	3,035.88	± 550.7	(820-4,044)	3,081.57	± 578	(820-4,525)	3, 016.6	± 609.1	(1,410-4,525)

SD = standard deviation; Min-Max = minimum-maximum; BMI = body mass index.

Table 2. Ethnicity, personal antecedents, pre-existing diseases and type of delivery of the study population

Characteristics	First trimester		Second trimester		Third trimester	
	n/N	(%)	n/N	(%)	n/N	(%)
Ethnicity						
White	514/1,102	(46.6)	308/683	(45.1)	386/893	(43.2)
Black	155/1,102	(14.1)	106/683	(15.5)	137/893	(15.3)
East Asian	8/1,102	(0.7)	5/683	(0.7)	3/893	(0.3)
South Asian	0/1,102	(0)	1/683	(0.1)	1/893	(0.1)
Mixed	298/1,102	(27.0)	215/683	(31.5)	275/893	(30.8)
Not informed	127/1,102	(11.5)	48/683	(7.0)	91/893	(10.2)
Smoking						
Yes	52/1,102	(4.7)	46/683	(6.7)	83/893	(9.3)
No	997/1,102	(90.5)	630/683	(92.2)	787/893	(88.1)
Quit	1/1,102	(0.1)	1/683	(0.1)	2/893	(0.2)
Not informed	52/1,102	(4.7)	6/683	(0.9)	21/893	(2.4)
Alcohol						
Yes	10/1,102	(0.9)	13/683	(1.9)	19/893	(2.1)
No	1033/1,102	(93.7)	660/683	(96.6)	849/893	(95.1)
Quit	4/1,102	(0.4)	3/683	(0.4)	2/893	(0.2)
Not informed	55/1,102	(5.0)	7/683	(1.0)	23/893	(2.6)
Folic acid (before pregnancy)						
Yes	92/1,102	(8.3)	42/683	(6.1)	54/893	(6.0)
No or not informed	1010/1,102	(91.7)	641/683	(93.9)	839/893	(94.0)
Folic acid (during pregnancy)						
Yes	666/1,102	(60.4)	383/683	(56.1)	429/893	(48.0)
No or not informed	436/1,102	(39.6)	300/683	(43.9)	464/893	(52.0)
Diabetes mellitus						
Type 1	3/1,102	(0.3)	1/683	(0.1)	2/893	(0.2)
Type 2	4/1,102	(0.4)	3/683	(0.4)	3/893	(0.3)
GDM	59/1,102	(5.4)	38/683	(5.6)	64/893	(7.2)
Others	14/1,102	(1.3)	7/683	(1.0)	5/893	(0.6)
No or not informed	1022/1,102	(92.7)	634/683	(92.8)	819/893	(91.7)
Hypertension						
CAH	52/1,102	(4.7)	37/683	(5.4)	55/893	(6.2)
GH	7/1,102	(0.6)	8/683	(1.2)	20/893	(2.2)
Hypertensive peak	1/1,102	(0.1)	0/683	(0)	0/893	(0)
No or not informed	1042/1,102	(94.6)	638/683	(93.4)	818/893	(91.6)
Thyroid diseases						
Gestational hypothyroidism	22/1,102	(2.0)	14/683	(2.0)	15/893	(1.7)
Previous hypothyroidism	1/1,102	(0.1)	0/683	(0)	1/893	(0.1)
Hypothyroidism	96/1,102	(8.7)	66/683	(9.7)	80/893	(9.0)
Hyperthyroidism	3/1,102	(0.3)	1/683	(0.1)	1/893	(0.1)
No or not informed	980/1,102	88.9	602/683	(88.1)	796/893	(89.1)
Consanguinity						
Yes	15/1,102	(1.4)	5/683	(0.7)	8/893	(0.9)
No	874/1,102	(79.3)	575/683	(84.2)	703/893	(78.7)
Not informed	213/1,102	(19.3)	103/683	(15.1)	182/893	(20.4)
Familial or previous fetal abnormalities						
Yes	19/1,102	(1.7)	13/683	(1.9)	15/893	(1.7)
No or not informed	1083/1,102	(98.3)	670/683	(98.1)	878/893	(98.3)
Type of delivery						
Caesarean	752/1,102	(68.2)	415/683	(60.8)	557/893	(62.4)
Forceps	3/1,102	(0.3)	0/683	(0)	2/893	(0.2)
Vaginal	321/1,102	(29.1)	248/683	(36.3)	318/893	(35.6)
Not informed	26/1,102	(2.4)	20/683	(2.9)	16/893	(1.8)

GDM = gestational diabetes mellitus; CAH = chronic arterial hypertension; GH = gestational hypertension; n/N = ratio between number of participants analyzed with complete outcome and the total number of participants in each trimester of pregnancy.

The most frequently diagnosed fetal anomalies in the three trimesters were in the genitourinary tract (27.9%, 22/79), heart (17.7%, 14/79), gastrointestinal tract (14.0%, 11/79), skeleton (11.4%, 9/79), single umbilical artery (11.4%, 9/79), central nervous system (7.6%, 6/79), face and neck (7.6%, 6/79) and thorax (2.5%, 2/79). In the postnatal period, cardiac abnormalities were the most common anomalies identified, accounting for 51.0% (99/194) of all cases diagnosed. The cardiac septal defects observed included atrial septal defect, ventricular septal defect and aneurysmal interatrial septum. The valve defects diagnosed included single atrioventricular valve, tricuspid insufficiency, mitral insufficiency, pulmonary insufficiency and pulmonary valve stenosis. The cardiac chamber defects included right ventricular dilatation, left ventricular enlargement, dilatation of the right chambers, right ventricular hypertrophy and hypoplastic right chambers (**Table 3**, **Figure 2**).

After cardiac abnormalities, the structural defects next most frequently identified in the postnatal period were in the genitourinary tract (11.8%, 23/194), central nervous system (8.8%, 17/194), face and nape of the neck (8.8, 17/194), skeleton (7.7%, 15/194), single umbilical artery (6.2%, 12/194), gastrointestinal tract (4.6%, 9/194) and thorax (1.0%, 2/194) (**Table 3**). The most common structural defects identified in each body system were hydronephrosis, congenital clubfoot, asymmetrical skull, left pulmonary sequestration, ovarian cyst, atrial dimorphism and single umbilical artery, respectively.

Among the 2,678 ultrasound examinations included in the present study, 79 structural abnormalities were diagnosed during the prenatal period. Twenty-five anomalies were identified through ultrasound examination and confirmed during the postnatal period, while 54 anomalies were identified through ultrasound examination but not confirmed during the postnatal period. Of the 2,678 ultrasound examinations, 2,599 were unremarkable in the prenatal period. Of these, 2,430 were unremarkable in both the prenatal and postnatal periods. In contrast, 169 anomalies were not detected through ultrasound but were identified in the postnatal period (**Table 4**).

The sensitivity, specificity, positive predictive value, negative predictive value, accuracy, positive likelihood ratio and negative likelihood ratio of ultrasound examinations for diagnosing structural defects in the first trimester were 14.06%, 98.65%, 39.13%, 94.90%, 93.73%, 10.0 and 0.87, respectively. The sensitivity, specificity, positive predictive value, negative predictive value, accuracy, positive likelihood ratio and negative likelihood ratio of ultrasound examinations for diagnosing structural defects in the second trimester were 27.78%, 98.14%, 45.45%, 96.06%, 94.43%, 14.6 and 0.73, respectively. The sensitivity, specificity, positive predictive value, negative predictive value, accuracy, positive likelihood ratio and negative likelihood ratio of ultrasound examinations for diagnosing structural defects in the third trimester were 23.91%, 97.76%, 36.67%, 95.94%, 93.95%, 10.9 and 0.78, respectively (**Table 5**).

DISCUSSION

The present study evaluated the fetal structural abnormalities in an unselected population, using prenatal ultrasound in the first, second and third trimesters of pregnancy. The international classification of diseases of the World Health Organization aims to classify these structural defects according to etiology or pathogenetic mechanism.[19] However, in the present study, structural abnormalities were classified according to the body systems in which they occurred, in order to determine the systems that were most frequently affected in an unselected population.

The prevalence of structural defects was 2.95% during the prenatal period and 7.24% during the postnatal period. Oakley et al.[1] reported that the prevalence of fetal anomalies at birth ranged from 2% to 5% in general populations. In the United States and Europe, the rates of fetal anomalies at birth have been reported to be 3.0% and 2.4%, respectively.[20,21] In Saudi Arabia, the prevalence of fetal anomalies at birth was reported to be 4.6%.[22]

The primary factors that affect the prevalence of fetal anomalies in different populations are consanguinity, use of assisted reproduction techniques, tobacco exposure, air pollution, water contamination and pesticide and agrochemical exposure.[23-28] The rates of consanguinity and smoking described in Brazilian populations have been lower than those in other countries.[22,29] We postulate that the higher prevalence of structural defects observed in the present study (higher than observed in North America, Europe and Saudi Arabia) may have arisen through assessment of cases referred to a tertiary-level healthcare center, instead of cases within a general population.

The prevalences of structural abnormality types vary according to the population assessed and the time of diagnosis. Molina-Giraldo et al.[30] conducted a study in Bogota, Colombia, and found that the most common fetal anomalies at birth were those of the central nervous system. Sallout et al.[22] reported that genitourinary tract anomalies were the most frequently diagnosed fetal anomalies during the prenatal period and at birth in Saudi Arabia. In the present study, genitourinary tract and cardiac anomalies were the most frequently diagnosed anomalies during the prenatal period and at birth, respectively.

The rate of detection of fetal cardiac defects has been reported to be low and dependent on the study population.[31,32] The sensitivity of fetal echocardiography for diagnosing cardiac anomalies was found to be 33.9% in low-risk populations and 68.8% in high-risk populations,[31] with septal defects accounting for the majority of cases of diagnostic failure.[32] The high frequency of genitourinary tract anomalies observed in the present study may have been due to their greater ease of detection through ultrasound, in comparison with defects of other body systems such as the heart and central nervous system.

During the first trimester of pregnancy, the sensitivity of ultrasound for detecting fetal anomalies in the present study ranged

Table 3. Structural abnormalities detected using ultrasound in the three trimesters of pregnancy, according to fetal body system

Body systems/malformations	Detection rate - n (%)							Postnatal n (%)
	First trimester		Second trimester		Third trimester		Total number of US examinations	
CNS	0	(0)	3	(10.3)	3	(8.4)	6 (7.6)	17 (8.8)
Macrocrania	0	(0)	1	(3.4)	0	(0)	1 (1.3)	3 (1.6)
Ventriculomegaly	0	(0)	2	(6.9)	2	(5.6)	4 (5.0)	2 (1.0)
Hyperechoic lesions	0	(0)	0	(0)	0	(0)	0 (0)	2 (1.0)
Choroid plexus cyst	0	(0)	0	(0)	0	(0)	0 (0)	4 (2.1)
Cerebellar hypoplasia	0	(0)	0	(0)	1	2.8	1 (1.3)	0 (0)
Craniotabes/asymmetric skull	0	(0)	0	(0)	0	(0)	0 (0)	6 (3.1)
Face and nape of neck	2	(14.2)	0	(0)	4	(11.2)	6 (7.6)	17 (8.8)
Ocular hypertelorism	0	(0)	0	(0)	0	(0)	0 (0)	2 (1.0)
Dysmorphic ear	0	(0)	0	(0)	0	(0)	0 (0)	5 (2.6)
Micrognathia	0	(0)	0	(0)	1	(2.8)	1 (1.3)	2 (1.0)
Flat face	0	(0)	0	(0)	1	(2.8)	1 (1.3)	0 (0)
Increased nuchal fold	1	(7.1)	0	(0)	1	(2.8)	2 (2.5)	5 (2.6)
Cystic hygroma	1	(7.1)	0	(0)	1	(2.8)	2 (2.5)	0 (0)
Ogival palate	0	(0)	0	(0)	0	(0)	0 (0)	1 (0.5)
Cleft lip	0	(0)	0	(0)	0	(0)	0 (0)	1 (0.5)
Cleft palate	0	(0)	0	(0)	0	(0)	0 (0)	1 (0.5)
Chest	0	(0)	0	(0)	2	(5.6)	2 (2.5)	2 (1.0)
Congenital diaphragmatic hernia	0	(0)	0	(0)	1	(2.8)	1 (1.3)	0 (0)
Lung sequestration	0	(0)	0	(0)	1	(2.8)	1 (1.3)	2 (1.0)
Heart	0	0	9	(31.0)	5	(14.0)	14 (17.7)	99 (51.0)
Septal defect	0	(0)	2	(6.9)	2	(5.6)	4 (5.0)	76 (39.2)
Valve defects	0	(0)	1	(3.4)	1	(2.8)	2 (2.5)	12 (6.2)
Coarctation of the aorta	0	(0)	0	(0)	0	(0)	0 (0)	2 (1.0)
Heart chamber defect	0	(0)	3	(10.3)	1	(2.8)	4 (5.0)	7 (3.6)
Double-outlet right ventricle	0	(0)	1	(3.4)	0	(0)	1 (1.3)	1 (0.5)
Pericardial effusion	0	(0)	2	(6.9)	1	(2.8)	3 (3.8)	1 (0.5)
GIT	2	(14.2)	4	(13.7)	5	(14.0)	11 (14.0)	9 (4.7)
Gastroschisis	0	(0)	0	(0)	1	(2.8)	1 (1.3)	1 (0.5)
Omphalocele	0	(0)	0	(0)	1	(2.8)	1 (1.3)	3 (1.6)
Ascites	1	(7.1)	0	(0)	0	(0)	1 (1.3)	0 (0)
Abdominal cyst	0	(0)	0	(0)	1	(2.8)	1 (1.3)	0 (0)
Ovarian cyst	0	(0)	1	(3.4)	0	(0)	1 (1.3)	5 (2.6)
Duodenal atresia (double bubble sign)	1	(7.1)	0	(0)	0	(0)	1 (1.3)	0 (0)
Esophageal atresia (collapsed stomach)	0	(0)	2	(6.9)	2	(5.6)	4 (5.0)	0 (0)
Hepatic calcifications	0	(0)	1	(3.4)	0	(0)	1 (1.3)	0 (0)
GUT	3	(21.4)	9	(39.1)	10	(27.8)	22 (27.9)	23 (11.8)
Renal cyst(s)	0	(0)	1	(3.4)	0	(0)	1 (1.3)	3 (1.6)
Renal dysplasia	1	(7.1)	1	(3.4)	0	(0)	2 (2.5)	0 (0)
Hydronephrosis	1	(7.1)	6	(42.9)	9	(25.0)	16 (20.3)	14 (7.2)
Pelvic kidney	0	(0)	1	(3.33)	1	(2.8)	2 (2.5)	2 (1.0)
Multicystic kidneys	0	(0)	0	(0)	0	(0)	0 (0)	2 (1.0)
Enlarged kidneys	0	(0)	0	(0)	0	(0)	0 (0)	2 (1.0)
Unilateral renal agenesis	0	(0)	1	(3.4)	0	(0)	1 (1.3)	0 (0)
Skeleton	3	(21.4)	1	(3.4)	5	(14.0)	9 (11.4)	15 (7.7)
Polydactyly	0	(0)	0	(0)	1	(2.8)	1 (1.3)	2 (1.0)
Congenital clubfoot	1	(7.1)	1	(3.4)	3	(8.4)	5 (6.3)	11 (5.7)
Short long bones	0	(0)	0	(0)	1	(2.8)	1 (1.3)	0 (0)
Hand agenesis	2	(14.2)	0	(0)	0	(0)	2 (2.5)	0 (0)
Clubhand	0	(0)	0	(0)	0	(0)	0 (0)	1 (0.5)
Congenital dislocation of the knee	0	(0)	0	(0)	0	(0)	0 (0)	1 (0.5)
Others	4	(28.6)	3	(10.3)	2	(5.6)	9 (11.4)	12 (6.2)
Single umbilical artery	4	(28.6)	3	(10.3)	2	(5.6)	9 (11.4)	12 (6.2)
Total	**13**	**(100)**	**30**	**(100)**	**36**	**(100)**	**79 (100)**	**194 (100)**

US = ultrasound; CNS = central nervous system; GIT = gastrointestinal tract; GUT = genitourinary tract.

from 13.0% to 43.6%. The detection rate was much higher when a detailed morphological protocol was adopted, such that up to 76.3% of major structural defects were detected. A previous study in a general population reported a first-trimester detection rate of 90% for complex congenital heart disease (either alone or in association with extracardiac abnormalities) and 69.5% for complex central nervous system anomalies.[33] The sensitivity of ultrasound has been found to be higher in the second trimester, ranging from 21% to 85%.[9-12]

Detection of fetal anomalies in the third trimester is technically more challenging due to fetal growth, poor imaging with static ultrasound and decreased quantities of amniotic fluid.[34] To date, few studies have evaluated the sensitivity of ultrasound for diagnosing fetal anomalies in the third trimester.[34,35] However, this

Table 4. Number of cases diagnosed and not diagnosed through ultrasound, relative to the number of cases diagnosed in the postnatal period

	Postnatal examination (+)	Postnatal examination (–)
Ultrasound (+)	25	54
Ultrasound (–)	169	2,430

Postnatal examination (+): with fetal anomaly; Postnatal examination (-): without fetal anomaly; Ultrasound (+): with fetal anomaly; Ultrasound (-): without fetal anomaly.

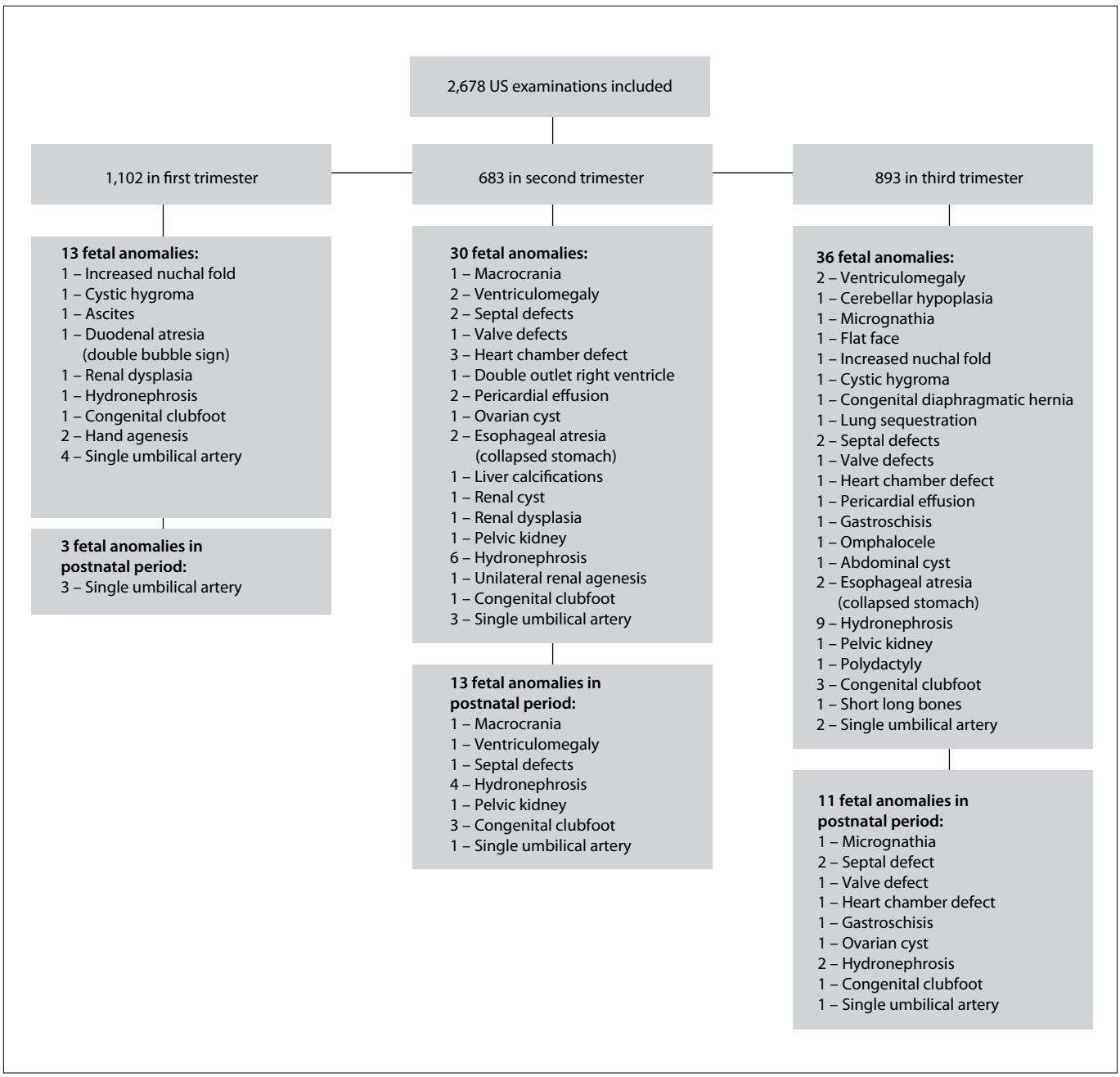

Figure 2. Flowchart of ultrasound (US) examinations among the cases included, according to trimester.

examination has an important role in identifying defects, particularly those of the central nervous system and genitourinary tract that do not develop or become evident before the third trimester.[36,37] Manegold et al.[34] evaluated 8,074 ultrasound examinations in a prospective study over the three trimesters of pregnancy. They found an additional 15% of fetal defects in the third trimester, especially in the genitourinary tract, heart and gastrointestinal tract. In the present study, the sensitivity of ultrasound in the first, second and third trimesters for detecting structural abnormalities was 14.06%, 27.78% and 23.91%, respectively.

According to Eureniuns et al.,[38] variability in the reported sensitivity of ultrasound may be due to differences in the study design, type of clinical center involved, examiners' experience and definitions used to classify the anomalies. The low sensitivity of ultrasound observed in the present study may have resulted from the inclusion of fetal anomalies such as septal and valve defects and the absence of 11 complex cardiac abnormalities, which were excluded due to a lack of postnatal results in our center's database. These defects are often small and/or transitory.[39] Chitty et al.[39] indicated that only clinically significant cardiac defects should be included when assessing the ability of ultrasound to detect structural defects. Despite this recommendation, simple cardiac defects and other defects such as congenital dislocation of the knee and ogival palate were present in the present study to evaluate the limitations of ultrasound for diagnosing fetal anomalies.

The specificity, negative predictive value and accuracy of ultrasound for identifying fetal structural anomalies were high in all three trimesters of pregnancy, despite low sensitivity and positive predictive value values. These results demonstrate that ultrasound is a reliable method for confirming structural defects and for reassuring examiners and pregnant women with normal results. The low positive predictive value may be explained by the low prevalence of fetal anomalies in the present study sample.

The limitations of the present study were its exclusion of a large number of cases due to loss of follow-up and lack of postnatal results, its small number of cases with fetal anomalies and its retrospective nature. It was not possible to determine the cumulative accuracy of ultrasound, since not all the cases included had a first-trimester scan. Furthermore, first-trimester scans have not been established as routine by the Brazilian Ministry of Health.[40] We carefully excluded from the analysis all fetal anomalies that were repeatedly reported in subsequent scans, in order to avoid inconsistencies in the accuracy of the first, second and third trimester scans.

The strength of the present study was its inclusion of an unselected population in a single center, which resulted in a high follow-up rate during pregnancy.

Table 5. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of ultrasound in the first, second and third trimesters of pregnancy

Trimester	Sensitivity	Specificity	PPV	NPV	Accuracy	+ LH	- LH
First	14.06%	98.65%	39.13%	94.90%	93.73%	10.0	0.87
Second	27.78%	98.14%	45.45%	96.06%	94.43%	14.6	0.73
Third	23.91%	97.76%	36.67%	95.94%	93.95%	10.9	0.78

PPV = positive predictive value; NPV = negative predictive value; +LH = positive likelihood ratio; -LH = negative likelihood ratio.

CONCLUSION

In summary, ultrasound is a reliable tool for counseling the parents of children with severe fetal anomalies that are associated with high rates of morbidity and mortality. However, the low sensitivity of ultrasound in detecting fetal anomalies in unselected populations limits its utility for providing reassurance to examiners and pregnant women with normal results.

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Address for correspondence:
Edward Araujo Júnior
Departamento de Obstetrícia, Escola Paulista de Medicina - Universidade Federal de São Paulo (EPM-UNIFESP)
Rua Napoleão de Barros, 875
Vila Clementino
São Paulo – SP, Brasil
CEP 04024-002
Telephone/fax: +55-11-5571-0761
E-mail: araujojred@terra.com.br

Is rectus abdominis thickness associated with survival among patients with liver cirrhosis? A prospective cohort study

Maria Ciocîrlan[I], Mircea Mănuc[II], Mircea Diculescu[III], Mihai Ciocîrlan[IV]

Department of Gastroenterology and Hepatology, Fundeni Clinical Institute, Bucharest, Romania

[I]MD. Physician and Assistant Professor, Department of Gastroenterology and Hepatology, Fundeni Clinical Institute, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.
orcid.org/0000-0001-7697-6366

[II]MD. Physician and Associate Professor, Department of Gastroenterology and Hepatology, Fundeni Clinical Institute, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.
orcid.org/0000-0003-1142-5859

[III]MD. Physician and Professor, Department of Gastroenterology and Hepatology, Fundeni Clinical Institute, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.
orcid.org/0000-0003-0073-5990

[IV]MD. Physician and Senior Lecturer, Department of Gastroenterology and Hepatology, University of Medicine and Pharmacy, Agrippa Ionescu Clinical Emergency Hospital, Bucharest, Romania.
orcid.org/0000-0002-6363-0320

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ABSTRACT

BACKGROUND: Sarcopenia may affect patients with liver cirrhosis and worsen disease outcomes.

OBJECTIVES: To evaluate ultrasound-measured psoas major (PM) and rectus abdominis (RA) thickness for predicting survival among patients with liver cirrhosis.

DESIGN AND SETTING: Prospective cohort study in a tertiary-level hospital.

METHODS: 61 patients with liver cirrhosis were prospectively included during a 15-month period and followed up for at least six months. Cirrhosis was classified using the Child-Pugh score. Sarcopenia was assessed using surrogate parameters: handgrip strength (HGS), mid-arm muscle circumference (MAMC) and SGA (subjective global assessment). We used ultrasound to measure RA and PM thickness at admission.

RESULTS: There were 41 men. The patients' mean age was 58.03 ± 10.8 years. 26.22% of them were Child-Pugh A, 45.9% B and 27.86% C. The patients were followed up for 11.9 ± 5.63 months. RA thickness correlated moderately with MAMC ($r = 0.596$; $P < 0.0001$) and HGS ($r = 0.515$; $P < 0.0001$) and decreased with increasing SGA class (A, 10.6 ± 2.8 mm; B, 8.3 ± 1.9 mm; C, 6.5 ± 1.9 mm; $P < 0.0001$). Survival at six months was independently predicted by using the model for end-stage liver disease-serum sodium score (odds ratio, OR 1.305; 95% OR confidence interval 1.083-1.572; $P = 0.005$). Survival during follow-up was independently predicted by RA thickness (hazard ratio, HR 0.701; 95% HR confidence interval 0.533-0.922; $P = 0.011$) and ascites (HR 1.876; 95% HR confidence interval 1.078-3.267; $P = 0.026$). PM thickness did not have any predictive value.

CONCLUSIONS: As a surrogate marker of sarcopenia, RA thickness may predict survival among patients with liver cirrhosis.

INTRODUCTION

Nutritional status is often impaired among patients with liver cirrhosis, and this results in malnutrition in more than 50% of the cases.[1] The main feature of malnutrition comprises loss of muscle mass and altered functionality, i.e. sarcopenia.[2] It has been reported that about 40% of patients with liver cirrhosis are sarcopenic and the percentage of such patients increases along with the severity of the disease.[3] Studies have shown that sarcopenic cirrhotic patients are at higher risk of developing complications (refractory ascites, hepatorenal syndrome, spontaneous bacterial peritonitis or hepatic encephalopathy) and death.[3-6]

The current "gold standard" in evaluating sarcopenia is skeletal muscle mass estimation by means of computed tomography (CT) scans or magnetic resonance imaging (MRI). The "skeletal muscle index", which takes into account all skeletal muscle groups at the level of the L3-L4 vertebrae,[3,6,7] the "psoas muscle thickness by height"[5,7] or the "psoas muscle index" can be measured.[8-10] However, CT scans and MRI are of limited use as screening tools, as they may be expensive, time consuming (MRI) and prone to artifacts in patients with ascites (MRI)[11] or irradiating (CT scans).[12]

Surrogate markers of sarcopenia include the Subjective Global Assessment (SGA) score and anthropometric measurements such as mid-arm muscle circumference (MAMC) and hand grip strength (HGS). SGA, MAMC and HGS show correlations with the skeletal muscle index in cirrhotic patients and in other populations.[13,14]

The prognosis for patients with liver cirrhosis is determined using several scores. The Child-Pugh score was the first one to be proposed.[15] The model for end-stage liver disease (MELD) was implemented as a prognostic score for cirrhotic patients undergoing trans-jugular intrahepatic

portal-systemic shunts, but it is currently used for prioritization of liver transplantation patients.[16] MELD-serum sodium (MELD-Na) was subsequently developed because hyponatremia proved to be an independent factor for liver disease that correlated with complications and death.[17]

Newer prognostic models encompass sarcopenia as an independent risk factor. These models include the "MELD-sarcopenia"[3,18] and "MELD-psoas"[7] scores.

OBJECTIVE

The aim of our study was to estimate the presence of sarcopenia by means of abdominal muscle ultrasound and use this measurement to predict survival among patients with liver cirrhosis.

METHODS

Study design, setting and ethics

This was a prospective cohort study in a tertiary-level hospital in Bucharest, Romania.

The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Fundeni Clinical Institute (no. 5906/3.03.2015; date of approval: March 5, 2015). Informed consent was obtained from all participants.

Participants and setting

We evaluated all patients with liver cirrhosis who had been consecutively admitted to the 2nd Department of Gastroenterology and Hepatology, Fundeni Clinical Institute, over a one-year period between March 2015 and May 2016. These patients were seen either as outpatients during scheduled control visits or as inpatients who had been admitted due to complications of liver disease (ascites, hepatorenal syndrome, spontaneous bacterial peritonitis, encephalopathy or gastrointestinal bleeding).

The exclusion criteria were the following: age < 18 years; presence of acute alcoholic hepatitis, decompensated cardiac or pulmonary disease, chronic renal failure, hepatocellular carcinoma or other malignancies, neuromuscular disorders or musculoskeletal disorders; or refusal to participate in the study.

Variables and data collection

Demographic data were collected (age and gender), along with information on the etiology of cirrhosis and its complications. We calculated the following prognostic scores for all patients upon admission: Child-Pugh, MELD and MELD-Na.

We performed nutritional assessments on all the patients, using SGA[19] and anthropometric measurements: triceps skinfold (TS) and mid-arm circumference (MAC). We calculated the MAMC based on TS and MAC using the formula MAMC = MAC – 3.14*(TS).[8] We determined the HGS of the dominant hand in all patients, using a Jamar dynamometer.

We measured the body mass index (BMI), but we did not report it because it may be overestimated in patients with ascites and edema.[1] We did not use the CT scan or MRI examinations to estimate sarcopenia. Instead, for the skeletal muscle mass evaluation, we performed ultrasound (SonoAce X8, Samsung Medison, Seoul, Korea) to measure the thickness of two skeletal muscles: the rectus abdominis (RA), with a linear 7.5 MHz probe; and the psoas major (PM), with a 3.5 MHz convex probe.

For the RA measurement, the patients were asked to lie down in a supine position, with their legs straight, in a relaxed manner. A pillow was placed under the patient's head. We identified the *linea alba* approximately 2 cm above the level of the umbilicus; from there, by moving the probe paramedially, we were able to view the right and left RA.[20,21] We obtained transverse sections of the RA. We measured the thickness of each RA at about 3 cm laterally from the umbilicus, between the anterior and posterior fascial borders. Minimal pressure was applied when performing the examination and making the measurements, so that we would not compress the muscle. Measurements were made at the end of a normal expiration. We performed three consecutive measurements and recorded the highest value. Both right and left-side measurements were obtained and we used their mean value for comparison purposes (mRA).

For the PM measurement, the patients were asked to lie on each side, in an oblique position. We scanned each psoas muscle (in coronal sections) from the origin, going to the lower pole of the kidney and towards the iliac crest.[22] The iliac crest corresponds to the L4-L5 vertebrae and is the level at which the cross-sectional area of the psoas muscle is largest.[23] We made three measurements on each side and recorded the maximum distance that we obtained between the anterior and the posterior borders of the muscle, perpendicular to the longitudinal fibers, at a level slightly above the iliac crest. We defined this as the PM thickness. Both right-side and left-side measurements were obtained and we used their mean value for comparison purposes (mPM).

All nutritional measurements and sonographic examinations were performed by the same investigator (Maria Ciocîrlan), within 24 hours of admission.

Patients were followed up until liver transplantation, until death or for at least six months after their initial assessment. Patients who did not have subsequent admissions or check-ups at the hospital were contacted by telephone, in order to gather data on complications, survival or cause of death.

Statistical analysis

Categorical variables were presented as absolute numbers and, in some cases, as percentages. Differences among categorical variables were tested using Fisher's exact test for two groups.

Continuous variables were presented as means and standard deviations (SD) and, in some cases, as ranges. Differences in the means of continuous variables were tested using the Mann-Whitney U test for two groups or the Kruskal-Wallis test for more than two groups. A stem-and-leaf chart was used to comparatively present the means and their 95% confidence intervals. Correlations between two continuous variables were explored using the Pearson r coefficient.

Logistic regression was used to identify independent odds ratios (OR) and their 95% confidence intervals for the likelihood of death at six months.

A multivariate Cox's proportional hazards regression model was used to predict survival. Hazard ratios (HR) were presented as absolute numbers and 95% confidence intervals.

P-values less than 0.05 were considered statistically significant.

The IBM SPSS Statistics 25 software was used for statistical analysis.

RESULTS

The data on demographics, disease severity, complications, nutritional status and follow-up are presented in **Table 1**.

We did not find any significant differences in mean thickness between the right and left PM (P = 0.70), or between the right and left RA (P = 0.93). There was a moderate correlation between mRA and mPM (r = 0.46, P = 0.001).

The mRA and mPM thicknesses were significantly greater in men than in women (mRA men 9.1 ± 2.5 mm versus mRA women 7.8 ± 2 mm, P = 0.021; and mPM men 27.7 ± 5.7 mm versus mPM women 24.8 ± 3.5 mm, P = 0.049). There were no sex-related differences in disease severity distribution (mean MELD score, mean MELD-Na score and Child-Pugh class) or mortality during the follow-up.

Mean muscle thickness correlated moderately with MAMC and HGS values (for mRA and MAMC, r = 0.596, P < 0.0001; for mRA and HGS, r = 0.515, P < 0.0001; for mPM and MAMC, r = 0.323, P = 0.013; and for mPM and HGS, r = 0.496, P < 0.0001). mRA decreased significantly with progression of malnutrition, as estimated using SGA class (P = 0.001) (**Figure 1**). There were no significant differences in mPM among SGA classes (P = 0.071).

Out of the 61 patients, 18 died during follow-up, after a mean period of 7.2 months (range: 2-13 months). Death during follow-up occurred solely as a consequence of complications from liver cirrhosis.

Death at six months was predicted in the univariate analysis by presence of ascites (P = 0.045), mRA (P = 0.028), MELD score (P = 0.008) and MELD-Na score (P = 0.006). In the multivariate analysis, only the MELD-Na score predicted death at six months (OR 1.305; 95% OR confidence interval 1.083-1.572; P = 0.005).

Occurrence of death during the follow-up was similarly predicted in the univariate analysis by presence of ascites (P = 0.002),

Table 1. Demographic, severity, nutritional status and survival data of the patients included (n = 61)

	Number of patients
Sex ratio	
males/females	41/20
Age, mean ± SD, years	58.03 ± 10.8
Cirrhosis etiology	
alcoholic	28
viral	24
mixed	6
other*	3
Ascites	
absent	11
mild	14
moderate	20
severe†	16
Spontaneous bacterial peritonitis	5
Hepatorenal syndrome	2
Variceal hemorrhage	7
Encephalopathy grade	
0	36
1	17
2	8
3	0
4	0
Child-Pugh score	
A	16
B	28
C	17
SGA class	
A	20
B	32
C	9
	mean ± SD
Follow-up (months)	11.9 ± 5.63 (range: 2-28)
MELD score	12.23 ± 3.53
MELD-Na score	13.72 ± 4.54

SD = standard deviation; MELD = Model For End-Stage Liver Disease; SGA = subjective global assessment.
*one patient with autoimmune disease, one patient with hemochromatosis and one patient with Budd-Chiari syndrome. †ascites as in SGA classification (0 – absent, 1 – mild, 2 – moderate, 3 – severe).

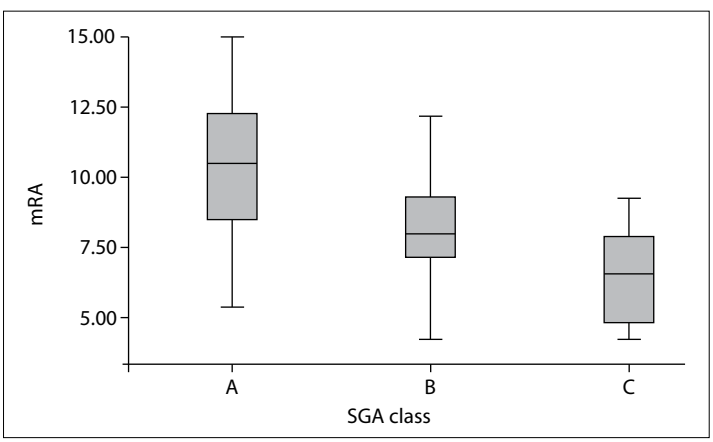

Figure 1. Mean rectus abdominis (mRA) measurements and their 95% confidence intervals for each subjective global assessment (SGA) class (P = 0.001).

mRA (P = 0.001), MELD score (P = 0.015) and MELD-Na score (P = 0.013). In the multivariate analysis, only mRA (HR 0.701; 95% HR confidence interval 0.533 - 0.922; P = 0.011) and ascites (HR 1.876; 95% HR confidence interval 1.078-3.267; P = 0.026) predicted occurrence of death during the follow-up.

DISCUSSION

Our aim was to find a simple, reproducible, noninvasive method for evaluating sarcopenia in patients with liver cirrhosis. Thus, we proposed measurement of muscle mass through ultrasound examination. Ultrasound is easy to perform, even among patients presenting difficulty in mobilization, and it is reproducible and non-irradiating. For critically ill patients, medical teams have implemented bedside ultrasound to evaluate skeletal muscle mass, with measurement of the thickness of the diaphragm or the quadriceps.[24,25]

There are no current nomograms for RA thickness in relation to age, gender, BMI and body composition. Previous studies have estimated the mean RA thickness in healthy populations, using ultrasound.[20,21] Rankin et al. examined 123 healthy subjects (55 males) with a mean age of 40.6 ± 14.1 years. The mean values for RA thickness were significantly higher in men, with 12.5 mm ± 2.2 mm (right RA) and 12.4 ± 2.4 mm (left RA), than in women, with 10.2 ± 1.6 mm (right RA) and 10.2 ± 1.5 mm (left RA); P < 0.001.[20] Tahan et al. conducted a study on 156 healthy subjects (75 males), with a mean age of 24.3 ± 7.2 years. They measured the thickness of RA muscles at a level similar to the level that we did. There was no significant difference between the left and right RA (P = 0.16). The mean values for RA thickness in men were 10.3 ± 1.8 mm (right RA) and 10.4 ± 1.9 mm (left RA), which were significantly greater than those in women: 8.7 ± 1.2 mm (right RA) and 8.3 ±1.3 mm (left RA); P < 0.001.[21] We obtained similar results, but at different muscle thickness values, given that our patients had advanced liver disease and 67.2% of them presented malnutrition graded as SGA B or C.

Similarly, there are no current recommendations regarding the use of ultrasound in evaluating muscle mass and sarcopenia in patients with liver cirrhosis. Tandon et al. evaluated 159 patients with liver cirrhosis (56% male; mean age 58 ± 10 years; mean MELD 10 ± 3; 60% Child-Pugh class A) and developed a model to identify sarcopenia, using a combination of BMI and the thigh muscle thickness (AUROC 0.78 for men and 0.89 for women).[13]

Sarcopenia has already been described as an independent predictor of survival in cases of liver cirrhosis.[3,7,18,26] Montano-Loza et al. proved that sarcopenia is an independent predictor of mortality in situations of cirrhosis and that it does not correlate with the severity of the disease as evaluated by prognostic scores (Child-Pugh and MELD).[3] Another study conducted on a larger population (669 cirrhotic patients),[18] proposed adding sarcopenia to MELD score (MELD-sarcopenia) for better prediction of mortality, especially among patients with MELD scores lower than 15. Sarcopenia alone would be equivalent to adding 10 points to the calculated MELD score. Similarly, Durand et al. developed a "MELD-psoas" score that could better predict mortality among patients with MELD score lower than 25 or with refractory ascites.[7]

Among our patients, although sarcopenia estimated from the RA thickness significantly predicted death at six months in univariate analysis (P = 0.028), only MELD-Na score remained an independent risk factor in multivariate analysis. However, death during follow-up (at a mean time of 11.9 months) was independently predicted from the RA thickness (HR 0.701; 95% HR confidence interval 0.533 - 0.922; P = 0.011).

PM thickness did not have any predictive value. We felt that it was more difficult to identify and accurately scan the PM muscle than the RA, using ultrasound, due to interposition of bowel gas and ascites and presence of truncal edema.

This study has several limitations. Firstly, we did not perform CT or MRI to evaluate sarcopenia. Nevertheless, very high correlations between ultrasound and MRI-measured thicknesses of RA have been reported: r = 0.932 to 0.963 in non- athletes (P < 0.001);[28] and r = 0.847 to 0.926 in athletes (P < 0.001).[29] Moreover, in several reports on liver cirrhosis, sarcopenia evaluated using CT or MRI has been fairly well predicted by the same surrogate markers that we used: MAMC (AUROC 0.84, P = 0.001;[13] and r = 0.385, P < 0.001[8]), HGS (r = 0.382, P < 0.001[8]) and SGA class (in men only marginally P = 0.051;[8] or a modified score in all patients, P < 0.001[13]).

Secondly, we did not perform multiple operator measurements or multiple measurements at different time points. Studies published previously by Tahan et al.[21] and Wachi et al.[28,29] also used a single operator. However, since our method for RA measurement is simple and straightforward, the expected intra-operator measurement variability would be low.[30] In healthy adults, the standard error of measurement for RA thickness is 0.2 mm, which represents 2% of our corresponding mRA values.[31,32]

Thirdly, during follow-up, in our study cohort, only four patients were evaluated to be listed for liver transplantation. One of these was subsequently listed and successfully underwent liver transplantation. Although there are almost 6000 liver-related deaths annually in Romania and 500 to 600 patients with liver cirrhosis are listed per year, only 852 liver transplantations were performed over the last 17 years, after a mean of 20 months of waiting time on the list.[33,34]

CONCLUSION

To the best of our knowledge, this was the first study that aimed to evaluate sarcopenia in patients with liver cirrhosis, using sonographic measurements of the RA muscles. Simple RA measurement might prove to be a useful marker for sarcopenia and prediction of

survival among liver cirrhosis patients, after validation of the present results through a comparative study making correlations with the gold standard (CT or MRI), and after further refinement.

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Address for correspondence:
Mircea Mănuc
Fundeni Clinical Institute
Department of Gastroenterology and Hepatology
258 Fundeni Street, 2nd District
022328 – Bucharest – Romania
Tel. 0040746102477
E-mail: gastrohepbuc@yahoo.com

Different effects of heating and freezing treatments on the antioxidant properties of broccoli, cauliflower, garlic and onion. An experimental in vitro study

Hikmet Can Çubukçu[I], Nazlı Seda Durak Kılıçaslan[II], İlker Durak[III]

Department of Medical Biochemistry, Ankara Üniversitesi Tıp Fakültesi, Ankara, Turkey

[I]MD. Medical Biochemistry Specialist, Department of Medical Biochemistry, Mareşal Çakmak Devlet Hastanesi, Erzurum, Turkey.
orcid.org/0000-0001-5321-9354

[II]MSc. Research Assistant, Department of Field Crops, Ankara Üniversitesi Ziraat Fakültesi, Ankara, Turkey.
orcid.org/0000-0001-7221-3572

[III]PhD. Professor, Department of Medical Biochemistry, Ankara Üniversitesi Tıp Fakültesi, Ankara, Turkey.
orcid.org/0000-0003-2962-4370

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ABSTRACT

BACKGROUND: Vegetables have some beneficial effects on human health due to their antioxidant compounds, like polyphenols. Cooking leads to many physical and chemical changes to plant structure that can alter the phytochemical compounds of vegetables.

OBJECTIVES: To investigate the effects of heat treatment and freezing on the antioxidant properties of garlic, onion, broccoli and cauliflower.

DESIGN AND SETTING: Experimental in vitro study in a university laboratory.

METHODS: Fresh broccoli (*Brassica oleracea* var. italica), cauliflower (*Brassica oleracea* var. botrytis), garlic (*Allium sativum*) and onion (*Allium cepa*) were obtained from a local store. These vegetables were divided into three treatment groups: raw, heated and frozen. The heat treatment consisted of heating them in a drying oven at 150 °C for 20 minutes. The freezing treatment consisted of keeping them frozen at -20 °C until analysis. The total phenolic content, antioxidant activity and malondialdehyde levels of the vegetables were measured using the Folin-Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and thiobarbituric acid reactive substances, respectively.

RESULTS: Heat treatment had deleterious effects on the antioxidant properties of onion and garlic; and it decreased the antioxidant activity of broccoli. Freezing improved the antioxidant activity of broccoli and garlic, but had detrimental effects for cauliflower and onion.

CONCLUSIONS: Heat treatment and freezing exhibit different effects on the antioxidant properties of broccoli, cauliflower, garlic and onion. Convenient cooking and storage patterns should be identified for each vegetable, to obtain the best nutritional benefit from the antioxidant compounds of vegetables.

INTRODUCTION

Consumption of fruits and vegetables is associated with reduced risks of hypertension, stroke, coronary heart disease, cancer, dementia and type 2 diabetes mellitus.[1] The beneficial effects of vegetables and fruits are attributed to presence of certain bioactive compounds, like polyphenols. Flavonoids, phenolic acids, lignins and stilbenes are polyphenols that exhibit antioxidant effects.[2]

Broccoli (*Brassica oleracea* var. italica) is a member of the Cruciferous family.[3] Glucosinolates, flavonoids, cinnamic acid derivatives, carotenoids, ascorbic acid, xanthophylls and minerals are among the substances that broccoli contains. The anticarcinogenic, antimutagenic and antioxidant properties of these compounds contribute to the health benefits from broccoli.[4]

Cauliflower (*Brassica oleracea* var. botrytis L.) is also included in the Cruciferous family. The components of cauliflower include glucosinolates, ascorbic acid, carotenoids, phenolic compounds and vitamin E.[3] It has anticarcinogenic and antioxidant effects, like other cruciferous vegetables.[5,6]

Garlic (*Allium sativum*) has been consumed as a vegetable and natural remedy for centuries.[7] In addition to organosulfur compounds, garlic contains high amounts of vitamins, minerals and phenolic compounds. So far, garlic has been reported to have anticancer, antimicrobial, antioxidant, anti-inflammatory, immunomodulatory and cardioprotective properties.[8]

Onion (*Allium cepa* L.) is cultivated in many parts of the world, given its adaptable nature. Flavonoids and alk(en)yl cysteine sulfoxides are the main bioactive groups in onion, and these compounds are responsible for antithrombotic, antiasthmatic, anticarcinogenic, antioxidant, antifungal and antibacterial effects.[9]

Most vegetables are consumed after a cooking procedure that may involve boiling, microwaving, steaming or baking. Cooking can lead to many physical and chemical changes in plant structure. The concentration of phytochemical compounds in vegetables can be increased through the matrix softening effect and improved extractability. Conversely, heat treatment can cause thermal degradation of these nutrients.[10] The antioxidant activity of vegetables is mainly based on their phytochemical compounds, like polyphenols. Cooking can trigger not only oxidation of these compounds, but also leakage of water-soluble compounds. Nevertheless, inactivation of prooxidant enzymes through heat treatment can result in enhanced antioxidant activity.[11] Additionally, partially oxidized polyphenols can have higher antioxidant activity than the non-oxidized form. Moreover, heat treatment can generate more potent antioxidant products called Maillard reaction products.[12]

Fresh vegetables are preferably stored in a refrigerator or freezer because they only remain fresh for a short time. However, freezing can also impair the nutritional quality of some vegetables.[13]

OBJECTIVE

The aim of this study was to investigate the effects of heat treatment and freezing on the antioxidant properties of garlic, onion, broccoli and cauliflower by analyzing total phenolic content, antioxidant activity and malondialdehyde levels.

METHODS

Chemicals used

Methanol, ethanol, trichloroacetic acid, disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium carbonate were purchased from Merck (Darmstadt, Germany). 2-thiobarbituric acid, 2,2-diphenyl-1-picrylhydrazyl, Folin-Ciocalteu phenol reagent and gallic acid were purchased from Sigma-Aldrich (Steinheim, Germany).

Vegetables and sample preparation

Fresh broccoli (*Brassica oleracea* var. italica), cauliflower (*Brassica oleracea* var. botrytis), garlic (*Allium sativum*) and onion (*Allium cepa*) were obtained from a local store. Each vegetable was divided into three treatment groups: raw, heated and frozen.

The heat treatment consisted of heating the vegetables in a drying oven at 150 °C for 20 minutes. After the heat treatment, the vegetables were stored at 4 °C for 12 hours in contact with air until analysis. The freezing treatment consisted of keeping the vegetables frozen at -20 °C until the time of analysis. The raw vegetables consisted of fresh vegetables stored at 4 °C in a sterile container in the refrigerator. A sample of 10 grams of each vegetable was used for analysis.

After heating and freezing treatment, the vegetables were homogenized in distilled water using the DIAX 900 homogenizer (Heidolph, Kelheim, Germany) just before analysis, to make an aqueous extract at a concentration of 10% (w/v). After centrifugation at 4000 g for 10 minutes, the supernatant fractions of the homogenates were isolated for analysis.

Measurement of malondialdehyde levels

Malondialdehyde (MDA) levels were measured spectrophotometrically by using the thiobarbituric acid reactive substances (TBARS) method. This is based on the interaction between thiobarbituric acid (TBA) and MDA in an acidic solution to yield a pink-colored dye.[14] One ml of a solution of ethanol (95%; v/v), phosphate buffer, trichloroacetic acid (20%; w/v) and thiobarbituric acid (2%; w/v) was added to each 0.1 ml of the sample in a tube. After 30 minutes of incubation in boiling water, the tubes were centrifuged at 4 °C in a Harrier 18/80 centrifuge (MSE, London, UK). The absorbances of the clear supernatant fractions were measured spectrophotometrically at 532 nm by means of a Helios Alpha ultraviolet/visible spectrophotometer (Unicam, Cambridge, UK).

Analysis of antioxidant activity

The antioxidant activity levels of the vegetables were determined according to their 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.[15] 975 µl of methanolic DPPH solution was added to each 25 µl sample in a tube. Following incubation for 30 minutes in the dark, the decline in absorbance was measured at 517 nm spectrophotometrically, against a negative control. Antioxidant activity was expressed as the percentage DPPH depletion.[16]

Determination of total phenolic content

The total phenolic content of the vegetables was determined using the Folin-Ciocalteu phenol reagent, as described by Obanda and Owuor.[17] Briefly, 0.5 ml of Folin-Ciocalteu phenol reagent was mixed with 0.5 ml of the aliquot. After incubation for 5 minutes, 1 ml of Na_2CO_3 and 1 ml of water were added to the mixture. Following a further 30 minutes of incubation, the blue-colored end product was measured spectrophotometrically at 700 nm.

Statistical analysis

The data analyzed in this study were obtained from ten repetitions and were evaluated using the Statistical Package for the Social Sciences (SPSS), version 11.5. Multiple comparisons of the experimental groups were analyzed using the ANOVA and Kruskal-Wallis tests. Tukey's honestly significant difference (HSD), Tamhane's and Dunn's tests were used for post-hoc evaluation of subgroups, where suitable. The relationships between biochemical parameters were assessed using Spearman and Pearson correlation tests. The statistical significance level was taken to be P values less than 0.05.

RESULTS

The freezing and heat treatments had different effects on the antioxidant activity, phenolic content and MDA levels of the vegetables, as shown in **Table 1** and Figures 1-4. All treatments were compared with raw vegetables.

Although the freezing treatment increased the antioxidant activity levels of broccoli and garlic, those of cauliflower and onion declined after this treatment. The phenolic contents of broccoli and cauliflower were enhanced through freezing, while the phenolic content of onion was reduced. Nevertheless, the MDA levels of frozen vegetables were found to be higher than those of raw vegetables.

Even though the heat treatment increased the antioxidant activity of broccoli, the antioxidant activity levels of heated garlic and onion were found to be lower than those of raw garlic and onion. Furthermore, the phenolic content of garlic, onion and cauliflower decreased through the heat treatment. Although the MDA concentration in onion was increased through the heat treatment, there were no statistically significant differences between raw and heated broccoli, cauliflower and garlic.

In addition to the low positive correlation between the phenolic content and antioxidant activity levels of onion ($r = 0.467$, $P < 0.01$) and broccoli ($r = 0.412$, $P < 0.05$), a moderate positive

Table 1. Comparisons of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, phenolic compounds and malondialdehyde levels of raw, frozen and heated vegetables

Vegetable	Test	Group	n	Mean	SD	ANOVA - KW P-values	Post-hoc comparison P-values			Tests used
							raw	frozen	heated	
Broccoli	DPPH radical scavenging activity (%)	raw	10	15.47	1.25	< 0.001		< 0.001	0.002	ANOVA-Tukey's HSD
		frozen	10	19.93	1.43		< 0.001		< 0.001	
		heated	10	17.52	0.72		0.002	< 0.001		
	Phenolic compounds (mg/dl)	raw	10	6.41	0.13	< 0.001		0.017	0.112	KW-Dunn's Test
		frozen	10	8.12	0.10		0.017		< 0.001	
		heated	10	6.21	0.13		0.112	< 0.001		
	Malondialdehyde (nmol/g)	raw	10	47.74	11.54	0.018		0.015	0.152	ANOVA-Tukey's HSD
		frozen	10	60.65	10.12		0.015		0.526	
		heated	10	55.97	6.24		0.152	0.526		
Cauliflower	DPPH radical scavenging activity (%)	raw	10	4.38	0.71	0.048		0.042	0.687	ANOVA-Tukey's HSD
		frozen	10	3.73	0.49		0.042		0.214	
		heated	10	4.17	0.47		0.687	0.214		
	Phenolic compounds (mg/dl)	raw	10	3.87	0.08	< 0.001		0.072	< 0.001	ANOVA-Tukey's HSD
		frozen	10	4.00	0.15		0.072		< 0.001	
		heated	10	2.91	0.12		< 0.001	<0.001		
	Malondialdehyde (nmol/g)	raw	10	56.46	7.40	0.022		0.021	0.110	ANOVA-Tukey's HSD
		frozen	10	69.20	9.35		0.021		0.717	
		heated	10	65.73	12.35		0.110	0.717		
Garlic	DPPH radical scavenging activity (%)	raw	10	30.59	2.15	< 0.001		< 0.001	< 0.001	ANOVA-Tukey's HSD
		frozen	10	39.72	2.15		< 0.001		< 0.001	
		heated	10	17.69	2.24		<0.001	< 0.001		
	Phenolic compounds (mg/dl)	raw	10	12.96	0.20	< 0.001		0.996	< 0.001	ANOVA-Tukey's HSD
		frozen	10	12.95	0.20		0.996		< 0.001	
		heated	10	9.11	0.21		< 0.001	< 0.001		
	Malondialdehyde (nmol/g)	raw	10	739.40	95.87	0.595		na	na	ANOVA-Tukey's HSD
		frozen	10	753.27	142.26		na		na	
		heated	10	703.51	89.11		na	na		
Onion	DPPH radical scavenging activity (%)	raw	10	13.72	0.75	< 0.001		< 0.001	< 0.001	ANOVA-Tamhane's Test
		frozen	10	9.55	0.74		< 0.001		< 0.001	
		heated	10	6.04	0.19		< 0.001	< 0.001		
	Phenolic compounds (mg/dl)	raw	10	7.18	0.13	< 0.001		< 0.001	< 0.001	ANOVA-Tamhane's Test
		frozen	10	4.50	0.05		< 0.001		< 0.001	
		heated	10	4.72	0.04		< 0.001	< 0.001		
	Malondialdehyde (nmol/g)	raw	10	51.78	13.55	< 0.001		0.362	< 0.001	ANOVA-Tukey's HSD
		frozen	10	60.97	15.17		0.362		< 0.001	
		heated	10	91.05	15.67		< 0.001	< 0.001		

SD = standard deviation; ANOVA = analysis of variance; KW = Kruskal-Wallis test; HSD = honestly significant difference; na = not available.

correlation was observed between the phenolic content and antioxidant activity of garlic (r = 0.639, P < 0.01). Even though the antioxidant activity levels of onion (r = -0.644, P < 0.01) and cauliflower (r = -0.381, P < 0.05) showed negative correlations with their MDA levels, the antioxidant activity of broccoli showed a significantly low positive correlation with MDA level (r = 0.422, P < 0.05), as shown in **Table 2**.

DISCUSSION

The effects of heat treatment on broccoli, cauliflower, onion and garlic were investigated in the present study. The heat treatment that was applied was analogous to baking.

The present study revealed that heat treatment led to an increase in the antioxidant activity of broccoli, while its total phenolic content was stable during this process (P = 0.002 and P = 0.112 respectively). Additionally, the malondialdehyde levels remained unchanged during the treatment.

The cooking methods on this topic that were previously studied have mainly approximated boiling, microwaving and steaming. Pellegrini et al. reported that boiling and oven-steaming caused increases in the total phenolic content and antioxidant capacity of fresh broccoli, while microwaving did not.[18] In contrast to these findings, Zhang and Hamauzu indicated that microwaving and boiling both led to reduction of the total phenolic content, total carotenoids, ascorbic acid levels and antioxidant activity of fresh broccoli.[19] To our knowledge, the impact of baking on broccoli had not previously been investigated. The heat treatment used in the present study is commonly used in baking. The increased antioxidant activity established in the present study may have been due to enhanced extractability of antioxidants other than phenolics, like ß-carotene. This is in line with data from a previous study that demonstrated that the ß-carotene isomer was released through cooking.[20]

The contradictory data in the literature regarding cauliflower mainly focused on certain cooking methods, including boiling, steaming, microwaving and sous vide (cooking under vacuum). Volden et al. noticed that boiling, blanching and steaming had all detrimental effects on total phenolic content, ascorbic acid levels and antioxidant activity.[21] Reis et al. stated that while microwaving had no effect on the phenolic content of cauliflower, boiling, steaming and sous vide led to reductions of phenolic compounds. Additionally, antioxidant activity was only increased through microwaving.[22] Girgin and El demonstrated that steaming caused increases in total phenolic content and antioxidant activity, but that boiling caused reduced levels.[23]

As far as we know, the present study is the first to examine the effects of baking on cauliflowers' antioxidant properties. Heated cauliflower showed lower total phenolic content than raw cauliflower, as shown in **Table 1**. However, the antioxidant activity of cauliflower seems to be more stable, possibly because of great retention of antioxidant compounds other than phenolic compounds.

We demonstrated that heat treatment had a reducing effect on the phenolic content and antioxidant activity of garlic. Likewise, Park et al. noticed that the DPPH radical scavenging activity and total phenolic content of raw garlic extract were higher than those of heated garlic extract.[24] A positive relationship between the phenolic content and antioxidant activity of garlic was found in our study (r = 0.639, P < 0.01). Hence, it could be hypothesized that deterioration of antioxidants, including phenolic compounds, led to the decrease in antioxidant activity.

In our study, the total phenolic content of onion was decreased through heat treatment. To date, contradictory results regarding

Table 2. Correlations between the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, phenolic compounds and malondialdehyde levels of vegetables

Vegetable	Test	Correlation coefficient (r)		
		DPPH radical scavenging activity (%)	Phenolic compounds (mg/dl)	Malondialdehyde (nmol/g)
Broccoli	DPPH radical scavenging activity (%)		0.412*	0.422*
	Phenolic compounds (mg/dl)	0.412*		0.309
	Malondialdehyde (nmol/g)	0.422*	0.309	
Cauliflower	DPPH radical scavenging activity (%)		-0.200	-0.381*
	Phenolic compounds (mg/dl)	-0.200		-0.022
	Malondialdehyde (nmol/g)	-0.381*	-0.022	
Garlic	DPPH radical scavenging activity (%)		0.639**	0.115
	Phenolic compounds (mg/dl)	0.639**		0.112
	Malondialdehyde (nmol/g)	0.115	0.112	
Onion	DPPH radical scavenging activity (%)		0.467**	-0.644**
	Phenolic compounds (mg/dl)	0.467**		-0.254
	Malondialdehyde (nmol/g)	-0.644**	-0.254	

*P < 0.05; **P < 0.01.

alteration of phenolic content have been reported in the literature. Although microwave heating was found to increase the concentration of quercetin, possibly through facilitating extractability, reduced flavonoid content was observed in boiled onion, possibly due to transition to water.[25] Quercetin glycosides, which are the main flavonoids of onion, can undergo thermal degradation. Rohn et al. showed that quercetin diglucosides were converted to quercetin monoglucosides and quercetin aglycone through roasting.[26]

Reduced flavonoid content in onion was detected when frying, sautéing, boiling, steaming and microwaving treatments were applied. However, baking for 5 minutes at 200 °C did not show any effects on the flavonoid content of onion, as indicated in the same study.[27] On the contrary, the quercetin diglucoside and quercetin glucoside concentrations in onion were decreased through heat treatment that was maintained for longer than 10 minutes.[28] The discrepancy might be attributable to the variety of heat treatment, the degree of applied heat and its duration. Our results are in agreement with the study by Harris et al.[28] Not only the phenolic content but also the antioxidant activity of onion decreased through heat treatment (**Table 1**). Furthermore, a positive correlation was observed between the phenolic content and antioxidant activity (**Table 2**). These results might be explained by the fact that the antioxidant activity of onion is attributable to its phenolic content. Additionally, the increase in malondialdehyde levels showed that the oxidation process induced by heat treatment led to degradation of antioxidant ingredients.

The effects of consumer storage conditions on broccoli, cauliflower, garlic and onion were investigated in the current study. It should be borne in mind that the group of raw vegetables was stored in a refrigerator at 4 °C to mimic consumer routines.

Frozen broccoli was found to contain higher antioxidant activity, total phenolic content and malondialdehyde levels, compared with raw broccoli. According to our findings, frozen storage preserves the antioxidant compounds of broccoli better than does raw storage.

Divergent results have been reported in the literature. Li et al. stated that the trans beta-carotene and folate levels of broccoli decreased through freezing, while the ascorbic acid levels did not change.[29] Patras et al. indicated that the antioxidant activity and ascorbic acid levels of unblanched frozen broccoli were lower than those of fresh and blanched frozen broccoli. No significant difference in phenol concentration was observed between the study groups.[30] Conversely, slightly increased phenolic content was reported for two broccoli cultivars. The total carotenoid concentrations of frozen broccoli cultivars were higher than those of raw ones.[31] These conflicting findings may have been due to variation in the cultivars studied. Furthermore, vegetables were blanched prior to freezing in some studies.

Frozen cauliflower showed lower antioxidant activity in the present study. Moreover, its malondialdehyde levels increased with freezing. However, no significant change was detected in its total phenolic content. These findings might be explained by the reduction in antioxidants through freezing. These decreased antioxidant levels would thus be due to decrease in the levels of some compounds other than phenolics. Murcia et al. showed that freezing did not significantly affect the antioxidant capacity of cauliflower.[32] Additionally, Li et al. did not find any statistically significant differences in trans beta-carotene and ascorbic acid levels between frozen and raw cauliflower.[29] In contrast with previous studies, Volden et al. reported that total phenolic content, L-ascorbic acid levels and antioxidant capacities of some cauliflower cultivars were reduced through freezing.[33] Our results are partially in agreement with the latter study. In addition to decreased antioxidant activity and increased malondialdehyde levels, we also observed a negative correlation between these two parameters ($r = -0.381$). It can be inferred from our findings that freezing may somehow lead to an oxidation process in cauliflower.

Frozen storage maintained the antioxidant activity of garlic better than raw storage. However, the freezing process did not give rise to any change in total phenolic content or malondialdehyde levels. Thus, the increased antioxidant activity could be attributable to preservation of antioxidants other than phenolic compounds. Murcia et al. claimed that freezing had a detrimental effect on the antioxidant activity of garlic. However, in Murcia's study, frozen vegetables were separately purchased from a supermarket as commercially processed vegetables.[32] Therefore, the low quality of the study design makes it hard to come to an accurate conclusion.

In the present study, the phenolic content and antioxidant activity of frozen onion were found to be lower than those of raw onion. On the other hand, the malondialdehyde concentration did not change through freezing, as shown in **Table 1**. Nimfali and Bacchiocca found that the oxygen radical absorbance capacity and phenolic content were higher in frozen onion than in raw onion. Nevertheless, according to those authors, addition of ascorbic acid for preservation of the packaged product interfered with the analysis.[34] In contrast to the previous study, our results showed that frozen storage did not possess the essential conditions for preserving the antioxidant properties of onion.

CONCLUSION

Heat treatment showed deleterious effects on the antioxidant properties of onion and garlic. Conversely, the antioxidant activity of broccoli was enhanced through heat treatment. These findings suggest that thermal degradation of antioxidants may occur in some vegetables, while the antioxidant activity can increase through the improved extractability and matrix softening effects of heat treatment.

Freezing can improve the extractability of the antioxidant compounds of some vegetables like broccoli and garlic. On the other hand, frozen storage did not exhibit convenient conditions

for preserving the antioxidant properties of cauliflower and onion. Additionally, the increased malondialdehyde levels of cauliflower may somehow have been a result caused by the increased oxidation process due to freezing.

Further research should be undertaken to establish what the most favorable vegetable consumption and storage patterns would be, to obtain the best benefit from the antioxidant compounds of vegetables.

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Authors' contributions:
Experimental design: İlker Durak, Nazlı Seda Durak Kılıçaslan
Biochemical analysis and performing the experiment: Hikmet Can Çubukçu, Nazlı Seda Kılıçaslan, İlker Durak
Statistical analysis: Hikmet Can Çubukçu
Writing the manuscript: Hikmet Can Çubukçu, İlker Durak

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Address for correspondence:
Hikmet Can Çubukçu
Maresal Cakmak State Hospital
Department of Medical Biochemistry, Biochemistry Laboratory
25070 Palandöken, Erzurum, Turkey
Tel. + 904423172264
E-mail: hikmetcancubukcu@gmail.com

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Effects of curcumin on cardiovascular risk factors in obese and overweight adolescent girls: a randomized clinical trial

Sahar Saraf-Bank[I], Alireza Ahmadi[II], Zamzam Paknahad[III], Mohammadreza Maracy[IV], Mojgan Nourian[V]

Pediatric Cardiovascular Research Center, Isfahan, Iran

[I]MSc. Doctoral Student and Dietitian, Students' Research Committee, Isfahan University of Medical Sciences; Doctoral Student and Dietitian, Food Security Research Center, Isfahan University of Medical Sciences; and Doctoral Student and Dietitian, Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran.
orcid.org/0000-0003-3443-9559

[II]MD. Associate Professor and Cardiologist, Pediatric Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran.
orcid.org/0000-0002-2462-0162

[III]PhD. Full Professor and Dietitian, Food Security Research Center, Isfahan University of Medical Sciences, and Full Professor and Dietitian, Department of Clinical Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran.
orcid.org/0000-0002-1864-2576

[IV]PhD. Full Professor and Statistician, Department of Epidemiology and Biostatistics, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran.
orcid.org/0000-0002-3695-0863

[V]PhD. Assistant Professor and Dietitian, Food Security Research Center, Isfahan University of Medical Sciences, and Assistant Professor and Dietitian, Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran.
orcid.org/0000-0003-2181-6474

KEY WORDS:
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Diet, reducing.
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ABSTRACT

BACKGROUND: Obese adolescents are at higher risk of development of cardiovascular risk factors and obesity in later life. Dietary intake of antioxidants, particularly curcumin, as an active ingredient of turmeric extract, may have noticeable effects on obesity and its important complications such as cardiovascular risk factors. Therefore, the aim of this study was to assess the effects of curcumin supplementation on cardiovascular risk factors among overweight and obese female adolescents.

DESIGN AND SETTING: Randomized placebo-controlled clinical trial; Pediatric Cardiovascular Research Center, Isfahan, Iran.

METHODS: 60 adolescent girls (aged 13-18 years) were randomly assigned to receive either placebo or intervention. The adolescents were asked to consume one 500 mg tablet per day, containing either standardized 95% turmeric extract or placebo, and to undergo a weight maintenance or a mild weight loss diet for 10 weeks. Anthropometric and biochemical indices were assessed at the baseline and the end of the intervention.

RESULTS: Curcumin supplementation had beneficial effects on body mass index ($P = 0.019$), waist circumference ($P = 0.008$), hip circumference ($P = 0.030$), high-density lipoprotein levels ($P = 0.042$) and triglyceride/high-density lipoprotein ratio ($P = 0.021$). However, in univariate analysis of covariance, no significant differences were found between the intervention and placebo groups after 10 weeks of supplementation ($P > 0.05$).

CONCLUSIONS: Prescription of curcumin supplementation along with use of a slight weight loss diet might have beneficial effects on some cardiovascular risk factors among overweight and obese female adolescents. Larger clinical trials with higher curcumin doses and longer duration are needed to confirm the results from the current study.

CLINICAL TRIAL REGISTRATION: IRCT20171107037302N1

INTRODUCTION

Today, childhood obesity is becoming a serious problem worldwide.[1] It has been shown that overweight adolescents are at greater risk of comorbidities relating to obesity in adulthood, like ischemic heart disease.[2] The prevalence of overweight and obesity have been rapidly increasing in developing countries. These are around 5%-13.5% and 3.2%-11.9%, respectively, among Iranian adolescents.[3] It seems that childhood obesity (i.e. at ages less than 18 years) may be an important predictor for comorbidities in adulthood, such as hypertension and dyslipidemia.[4] Therefore, finding a practical solution for this problem is of utmost importance.

It has been reported that several lifestyle factors, including duration of nocturnal sleeping, practicing of physical activity and the length of time spent watching television can be classified as possible causes of childhood obesity.[1] Moreover, children's dietary intake is a noticeable factor relating to development of central and general obesity among children and adolescents.[5,6]

One study has shown that some types of foods, like spices, may play an important role in combating obesity and its associated complications.[7] In this context, interest has increasingly been focused on turmeric extract, given that curcumin is its active ingredient. Several studies have reported on the possible effects of curcumin against obesity, inflammation, diabetes and cardiovascular disease and related risk factors.[7-9] However, the results from the available studies assessing the effect of curcumin on lipid profiles have been contradictory.[10,11] One meta-analysis showed that curcumin supplementation did not have any beneficial effect on lipid fractions.[9] In addition, the existing results regarding glycemic indices have been insufficient and inconsistent.[11,12]

Based on our review of the literature, we did not find any study assessing the potential effects of curcumin supplementation on cardiovascular risk factors among adolescents. Therefore, selection of this target group to determine the possible effects of curcumin is of great importance.

OBJECTIVE

The aim of this study was to assess the effects of curcumin supplementation on cardiovascular risk factors, including anthropometric measurements, glycemic indices and lipid profile among overweight and obese female adolescents in Iran.

METHODS

Study design and setting

This was a randomized parallel clinical trial conducted at the Pediatric Cardiovascular Research Center, Isfahan University of Medical Sciences, Isfahan, Iran. The present clinical trial report followed the CONSORT checklist.

Ethical issues

This study was approved by the ethics committee of Isfahan University of Medical Sciences, Isfahan, Iran (no. 396160; July 2017). The registration number of this study in the Iranian Registry of Clinical Trials (http://www.irct.ir/) is IRCT20171107037302N1. Before the start of the study, oral and written informed consents were obtained from the adolescents and from one of their parents, respectively.

Participants

Overweight or obese female adolescents were recruited for this trial, which was conducted over a 10-week period. These adolescent girls (aged between 13-18 years) were included in this randomized placebo-controlled clinical trial between September 2017 and January 2018, at the Pediatric Cardiovascular Research Center, Isfahan, Iran. This research center has a clinic for overweight and obese children and adolescents aged 6-18 years, and the medical records available at this center go back as far as 1992. From all over the city, general practitioners and some relevant specialists such as endocrinologists refer overweight and obese children to this center to be enrolled in its weight management program. At this clinic, children are encouraged to increase their physical activity, reduce their empty calorie intake (calories from solid fats and added sugar), select healthier foods and improve their lifestyle (through measures such as reducing their television time).

From 1,050 medical records that were screened by the present researchers, 227 adolescents were deemed to be potentially eligible participants (for being overweight or obese). Out of these 227 potentially eligible adolescents, 105 of them were not available, 27 were unwilling to participate in the study, 17 were suffering from chronic diseases or were consuming drugs, 14 were on a specific diet in other weight management clinics and 4 had not yet started to have a menstrual cycle. In total, 60 post-pubescent overweight or obese adolescent girls were included in accordance with the inclusion criteria described below. Body mass index percentile for age between the 85th and 95th was considered to represent overweight and more than the 95th was considered to represent obesity, in accordance with the World Health Organization criteria.[13]

Participants were selected based on the following inclusion criteria: having had a menstruation cycle for more than six months; absence of any history of chronic diseases (diabetes, hypo and hyperthyroidism, liver, renal and cardiovascular disease and polycystic ovary syndrome); absence of medication use (drugs affecting the metabolism of lipids and carbohydrates, such as statins, metformin, hormone therapy drugs, multivitamin minerals, corticosteroids and non-steroidal anti-inflammatory drugs); and absence of any history of weight reduction over the past three months. Participants would be excluded if they had poor compliance with the diet and supplements that were administered (use of less than 80% of the supplements) and if they were not willing to continue in the study.

Study conduction

Random sequence generation and allocation concealment

Random permuted blocks (dual blocks) of participants based on BMI percentile (85th < BMI percentile < 95th; and BMI percentile ≥ 95th), according to the data obtained from the files available in the pediatric cardiovascular research center were formed by the researchers. A trained employee of the pediatric cardiovascular research center who was blinded to the random allocation sequence assigned adolescents to the curcumin and placebo groups using SPSS software-generated random numbers.

Blinding procedures

To maintain blinding, the curcumin supplement and its quite similar placebo (shape, color, odor and label) were produced and encoded as A and B by Karen Critical Pharmaceutical and Nutritional Supplements Company, Tehran, Iran. Thereafter, supplements A and B were randomly assigned to each arm of the study. Accordingly, the researcher, participants and statistics analyst were completely unaware of the assigned intervention.

Intervention

A weight maintenance diet was prescribed for those with BMI between the 85th and 95th percentile and a weight reduction diet (maximum of one pound, or 454 grams, per month) for those with BMI more than the 95th percentile.[16] The prescribed diet

was designed based on healthy eating advice.[17] The distribution of macronutrients in the prescribed diets was 50%-60% for carbohydrates, 15%-20% for protein and 30% for total fat, which is similar to the regular Iranian pattern.[18] The participants received the precise amount of each food group along with a sample food menu. In addition, a comprehensive exchange list was given to these adolescents. Before the start of the study, group sessions were held for the adolescents and their mothers. In these sessions, the proper way of using the sample food menu and exchange list was taught to both the adolescents and their mothers.

The adolescents were asked to consume one 500 mg tablet per day, containing either standardized 95% turmeric extract or placebo, with their meal for 10 weeks. During the study period (at week 5), the participants completed a physical activity questionnaire and a three-day dietary record (one weekend day and two weekdays).

At the end of the study period, fasting blood samples were collected. In addition, anthropometric indices and the blood pressure of all subjects were measured. The physical activity questionnaire and three-day dietary records were completed by all participants.

To check the participants' compliance with curcumin supplement use, phone calls were made to both the adolescents and their mothers every fortnight. Furthermore, the adolescents were asked to complete a checklist and to return the supplement containers at the end of week 10. The adolescents' adherence to the diet was checked from the dietary records that they completed during the study.

Outcomes

We made preliminary and final assessments of the outcomes. The primary outcomes were anthropometric measurements and glycemic indices and the secondary outcome was the lipid profile.

At the beginning of the study, fasting blood samples were collected and the anthropometric indices and blood pressure of the adolescents were measured. General information was obtained from all the participants, including the parents' education level and occupation, family income, smoking history, past medical history and medication use. Furthermore, a validated physical activity questionnaire[14] and a three-day dietary record (one weekend day and two weekdays) were completed by the adolescents. Total energy expenditure for each adolescent was calculated in accordance with the recommendations from the Institute of Medicine.[15]

Daily dietary intakes and physical activity assessment

Dietary intakes were assessed using a three-day dietary record (one weekend day and two weekdays) at the beginning (week 1), middle (week 5) and end (week 10) of the study period. All participants were instructed on how to record their dietary intakes before the intervention started. After the adolescents had completed the dietary records, the precise weight in grams of each food item was determined by the researchers through household measurements. The daily dietary intake of each food item was entered into the Nutritionist IV software (First Databank Division, The Hearst Corporation, San Bruno, CA, USA) for analysis on total energy intake and macro and micronutrients.

The adolescents' physical activity levels were assessed through a validated physical activity questionnaire[14] at baseline and at weeks 5 and 10. This questionnaire included nine physical activity levels that were graded based on their respective metabolic equivalent of task (MET).

Blood pressure and anthropometric measurements

Anthropometric indices were measured at baseline and at the end of the study period. Weight was measured using a Seca scale, to the nearest 0.1 kg, with the subjects barefoot and wearing light clothing. Height was measured using a wall-mounted tape measure, to the nearest 0.5 cm, without shoes. Body mass index was calculated by dividing weight (kg) by height squared (m^2). Waist circumference and hip circumference were measured using an inelastic tape with an accuracy of 0.1 cm, at the midpoint between the lower rib and top of the iliac crest and at the largest hip circumference, respectively.

After 10 minutes of resting in a sitting position, blood pressure was measured twice using a digital sphygmomanometer (OMRON, M3, HEM-7134-E), and the average of these two measurements was used for the statistical analysis.

Biochemical assessment

Blood samples of 10 ml were taken after overnight fasting (12-14 hours), at baseline and after 10 weeks of the intervention. These were centrifuged at 3000 rpm for 15 minutes and were stored at -70 °C until use. The lipid profile, including total cholesterol, low-density lipoprotein, high-density lipoprotein and triglycerides, was assessed using commercially available enzyme assay kits (Pars Azmoon, Tehran, Iran). Colorimetric assay kits were used to determine fasting blood sugar (Pars Azmoon, Tehran, Iran). Insulin levels were measured by means of the enzyme-linked immunosorbent assay (ELISA) method (Hangzhou Eastbiopharm Co. Ltd., Hangzhou, China). The inter and intra-assay variations of the insulin assessment were less than 10%. The homeostasis model assessment for insulin resistance was calculated based on the following formula: [Fasting plasma insulin (U/ml) × Fasting blood glucose (mg/dl)]/405.[19]

Statistical analysis

The sample size was calculated based on a formula that is available for parallel trials.[20] The type 1 error (α) was considered to be 0.05; the type 2 error (β) was 0.1 (i.e. power = 90); d (the significant difference in fasting plasma glucose levels) was 0.38;

S_1 (the standard deviation of the fasting plasma glucose level in the control group) was 0.32; and S_2 (the standard deviation of the fasting plasma glucose level in the intervention group) was 0.45.[21] Based on these data, 22 participants were required for each arm of the study. To account for possible withdrawals during the study, we included 30 adolescents in each group, i.e. in the intervention and placebo groups.

Presence of normal distribution of variables was assessed using Q-Q plots and the Shapiro-Wilk test. Logarithmic transferred means were used for variables with an unusual distribution. Linear regression was applied to predict the approximate values of missing data. In the current study, there were only five withdrawals (less than 10% of the study population). In addition, there was a high correlation between the baseline and endpoint values. Therefore, linear regression had the capacity to predict missing data with the right precision. In this method, endpoint values of variables were considered to be dependent variables and baseline values were considered to be independent variables. In the output from the linear regression analysis, there were constant values in the coefficient table. Through using the formula "y (endpoint) = x (baseline)+b (constant value)", the endpoint values of the variables could be predicted.

The general features of the participants were compared by means of the independent-sample t test and the chi-square test. In addition, dietary intakes, physical activity levels and between-group comparisons were checked using an independent-sample t test. For within-group analyses, a paired-sample t test was applied. Univariate analysis of covariance (ANCOVA) was used to compare between-group differences. Confounding factors were detected by conducting a correlation test between potential confounding factors and the baseline values of the variables. Variables with r correlation ≥ 0.2 were considered to be confounding factor. In this regard, the potential effects of physical activity level, turmeric powder intake and intakes of some dietary antioxidants such as vitamin C, vitamin E, selenium and beta-carotene were controlled for, in the adjusted model.

All variables were reported as the mean ± standard deviation (SD). The Statistical Package for the Social Sciences, version 18, was used for the statistical analyses (SPSS Inc., Chicago, IL, USA). P-values less than 0.05 were considered to be significant in this study.

RESULTS

Over the course of the study period, five adolescents withdrew from the study based on personal reasons, educational problems including conflicting school appointments, fear of blood sampling and moving to another city (**Figure 1**). Nevertheless, through prediction of the missing values, the data of all 60 participants were entered into the statistical analysis (30 in each group).

There were no substantial side effects among the participants. However, some adolescents had mild headaches and nausea, which were resolved through continual use of curcumin capsules.

Approximately seven months were spent on sampling, subject enrollment, completion of the intervention and data collection. The baseline characteristics of the participants are presented in **Table 1**. As shown in this table, there were no significant baseline differences in age, overweight/obesity distribution or socioeconomic status among the participants.

Table 2 shows the participants' daily dietary intakes during the study period. There were no substantial differences in total energy or micro and macronutrients between the two groups. In addition, no significant difference in the physical activity levels of the participants

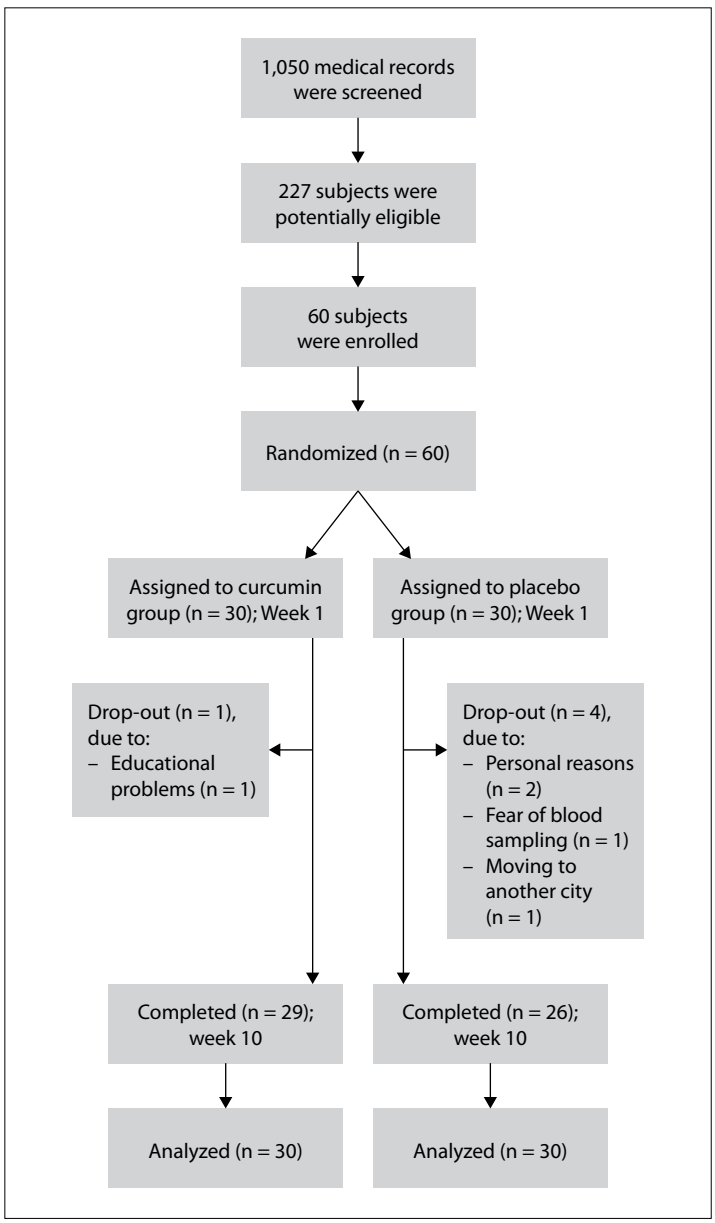

Figure 1. Flowchart of the study.

was detected between the two groups (34.70 ± 6.40 MET-hour/day in the curcumin group and 36.82 ± 8.00 MET-hour/day in the placebo group; P-value = 0.262).

The crude within and between comparisons of anthropometric and biochemical values between the curcumin and placebo group are illustrated in **Table 3**. According to the results available, there was a significant reduction in anthropometric measurements among the adolescents in the curcumin group. In addition, a desirable improvement in lipid profile was detected in the intervention group. The results from the paired-sample t test showed that the participants in the placebo group only had significant reductions in weight and body mass index. There were no differences in the baseline and the end values for the variables between the two groups.

Table 4 presents the comparison of differences in the mean changes to anthropometric and biochemical indices after 10 weeks of supplementation. Regarding the results obtained, there were no significant differences between the two groups in the crude or adjusted models.

Table 1. General baseline characteristics of the female adolescents

Variables	Curcumin (n = 30)	Placebo (n = 30)	P-value[1]
Age (years); mean ± standard deviation	16.03 ± 1.56	15.98 ± 1.72	0.907
Overweight/obese status; n (%)			
Overweight	9 (30.0)	11 (36.7)	0.584
Obese	21 (70.0)	19 (63.3)	
Father's education level; n (%)			
Illiterate	1 (3.3)	0 (0.0)	0.493
< 12 years	10 (33.3)	13 (43.3)	
12 years	13 (43.3)	9 (30.0)	
> 12 years	6 (20.0)	8 (26.7)	
Mother's education level; n (%)			
Illiterate	0 (0.0)	0 (0.0)	0.955
< 12 years	9 (30.0)	8 (26.7)	
12 years	15 (50.0)	16 (53.3)	
> 12 years	6 (20.0)	6 (20.0)	
Father's job; n (%)			
Employee at non-executive level	7 (23.3)	12 (40.0)	0.345
Self-employed	21 (70.0)	15 (50.0)	
Worker at executive level	2 (6.7)	2 (6.7)	
Dead	0 (0.0)	1 (3.3)	
Mother's job; n (%)			
Employee	3 (10.0)	3 (10.0)	1.000
Self-employed	1 (3.3)	1 (3.3)	
Housewife	26 (86.7)	26 (86.7)	
Family income; n (%)			
Low	2 (6.7)	6 (20.0)	0.252
Moderate	24 (80.0)	22 (73.3)	
high	4 (13.3)	2 (6.7)	
Smoking in family, n (yes%)	5 (16.7)	5 (16.7)	1.000

[1]P-values are from the chi-square test and the independent-sample t test.

DISCUSSION

The results from this parallel randomized trial study showed that curcumin supplementation for overweight and obese female adolescents over a 10-week period had a reducing effect on body mass index, waist circumference, hip circumference, high-density lipoprotein levels and triglyceride/high-density lipoprotein ratio and an increasing effect on insulin levels, in within-group analyses. Participants in the placebo group had significant reduction in weight and body mass index. Although we could not detect any significant differences between the intervention and placebo groups, the within-group results were considerable. This was the first time that the effects of curcumin supplementation had been studied among post-pubescent overweight or obese female adolescents in a developing country.

Previous studies have shown that childhood obesity and central obesity can be strong predictors for the presence of obesity and cardiovascular risk factors later in life.[22,23] However, body mass index presents some limitations as a marker for obesity. It does not show individuals' fat distribution or their degree of muscularity.[24] It seems that measurements of waist circumference and central obesity are better than body mass index as indicators for cardiovascular risk factors among children and adolescents.[25]

In the present study, although we could not detect any significant difference between the intervention and placebo groups,

Table 2. Daily dietary intakes of the female adolescents during the study[1]

Variables	Curcumin (n = 30)	Placebo (n = 30)	P-value[2]
Energy (kcal)	1930.93 ± 365.16	1832.67 ± 330.20	0.279
Carbohydrate (g)	285.24 ± 58.78	271.52 ± 59.83	0.374
Protein (g)	54.34 ± 1.20	57.79 ± 1.25	0.199
Fat (g)	66.95 ± 21.96	61.38 ± 20.73	0.317
Saturated fat (g)	18.82 ± 1.54	17.03 ± 1.56	0.358
Polyunsaturated fat (g)	16.16 ± 5.42	15.15 ± 5.25	0.465
Monounsaturated fat (g)	21.83 ± 9.38	19.66 ± 8.59	0.354
Zinc (mg)	6.73 ± 1.28	6.77 ± 1.26	0.968
Potassium (mg)	2739.27 ± 638.48	2516.01 ± 544.54	0.150
Calcium (mg)	759.98 ± 1.30	757.93 ± 1.28	0.908
Selenium (mg)	0.075 ± 1.36	0.069 ± 1.35	0.271
Folate (mcg)	255.85 ± 1.40	241.26 ± 1.44	0.649
Vitamin E (mg)	19.57 ± 1.71	17.58 ± 1.95	0.519
Vitamin C (mg)	125.62 ± 1.75	97.06 ± 1.72	0.052
β-carotene (mcg)	580.79 ± 2.68	443.36 ± 2.19	0.118
α-tocopherol (mg)	7.75 ± 1.78	6.71 ± 1.65	0.279
Dietary fiber (g)	18.13 ± 1.40	16.10 ± 1.57	0.506
Turmeric powder (teaspoon/day)	2.13 ± 0.81	2.36 ± 0.85	0.284

[1]Data are logarithmic-transformed mean ± standard deviation, except for energy, carbohydrate, fat, polyunsaturated fat, monounsaturated fat, potassium and turmeric, which are presented as mean ± standard deviation; [2]P-values are from the independent-sample t test.

Table 3. The effect of curcumin supplementation on anthropometric and cardiovascular risk factors after 10 weeks of intervention[1]

Variable	Curcumin (n = 30)		Placebo (n = 30)		Within group		Between groups	
	Before	After	Before	After	P-value[2]	P-value[3]	P-value[4]	P-value[5]
Weight (kg)	83.55 ± 1.21	82.87 ± 1.21	81.52 ± 1.18	80.79 ± 1.17	0.060	0.042	0.599	0.581
BMI (kg/m²)	31.43 ± 2.84	31.00 ± 2.85	30.27 ± 2.83	30.00 ± 2.82	0.019	0.046	0.318	0.380
WC (cm)	100.31 ± 1.14	97.86 ± 1.13	97.07 ± 1.08	96.96 ± 1.09	0.008	0.918	0.245	0.738
HC (cm)	114.18 ± 2.77	113.17 ± 2.77	111.16 ± 2.76	110.53 ± 2.75	0.030	0.316	0.190	0.233
WHR	0.88 ± 1.06	0.86 ± 1.07	0.87 ± 1.07	0.87 ± 1.06	0.071	0.684	0.791	0.371
SBP (mmHg)	111.66 ± 7.85	111.36 ± 12.01	106.80 ± 11.66	109.56 ± 9.79	0.866	0.116	0.063	0.528
DBP (mmHg)	75.83 ± 9.05	75.14 ± 9.09	72.83 ± 7.62	73.72 ± 7.27	0.660	0.571	0.170	0.505
FBS (mg/dl)	87.23 ± 7.04	85.97 ± 7.02	86.23 ± 6.00	86.91 ± 4.88	0.205	0.335	0.556	0.546
Insulin (U/ml)	12.29 ± 1.71	14.81 ± 1.51	12.58 ± 1.55	13.58 ± 1.52	0.009	0.298	0.852	0.428
HOMA-IR	3.11 ± 1.93	3.41 ± 1.49	2.93 ± 1.19	3.17 ± 1.32	0.158	0.283	0.670	0.505
T-chol (mg/dl)	159.80 ± 27.72	161.41 ± 30.99	164.70 ± 27.78	162.32 ± 28.02	0.453	0.238	0.497	0.905
HDL (mg/dl)	48.93 ± 1.17	50.77 ± 1.18	50.96 ± 1.17	50.73 ± 1.20	0.042	0.826	0.325	0.985
LDL (mg/dl)	83.56 ± 1.33	85.12 ± 1.32	86.73 ± 1.27	85.41 ± 1.28	0.444	0.206	0.588	0.960
TG (mg/dl)	109.57 ± 1.48	102.16 ± 1.46	107.21 ± 1.57	106.30 ± 1.42	0.068	0.847	0.843	0.675
TG/HDL	2.49 ± 1.16	2.19 ± 0.95	2.39 ± 1.27	2.30 ± 0.98	0.021	0.499	0.765	0.659

BMI = body mass index; WC = waist circumference; HC = hip circumference; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; FBS = fasting blood sugar; HOMA-IR = homeostasis model assessment of insulin resistance; T-chol = total cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TG = triglycerides.
[1]Data are log-transformed mean ± standard deviation, except for SBP, DBP, FBS, HOMA-IR, TC and TG/HDL ratio, which are presented as mean ± standard deviation; [2]within curcumin group, obtained from paired t test; [3]within placebo group, obtained from paired t test; [4]comparison of baseline values between curcumin and placebo groups using independent-sample t test; [5]comparison of endpoint values between curcumin and placebo groups using independent-sample t test.

Table 4. Comparison of differences in means changes in anthropometric and biochemical indices among female adolescents between the curcumin and placebo groups[1]

Variable	Curcumin (n = 30)	Placebo (n = 30)	P-value[2]	P-value[3]
Weight (kg)	-0.71 ± 2.16	-0.81 ± 1.96	0.860	0.965
BMI (kg/m²)	-0.42 ± 0.91	-0.30 ± 0.74	0.567	0.426
WC (cm)	-2.54 ± 4.89	-0.05 ± 5.60	0.073	0.070
HC (cm)	-1.11 ± 2.68	-0.69 ± 3.26	0.592	0.833
WHR	-0.01 ± 0.03	0.003 ± 0.05	0.194	0.115
SBP (mmHg)	-0.30 ± 9.63	2.76 ± 9.36	0.216	0.418
DBP (mmHg)	-0.68 ± 8.44	0.88 ± 8.48	0.474	0.796
FBS (mg/dl)	-1.26 ± 5.33	0.68 ± 3.83	0.109	0.126
Insulin (U/ml)	1.94 ± 5.06	1.05 ± 5.28	0.506	0.619
HOMA-IR	0.30 ± 1.14	0.23 ± 1.17	0.823	0.909
T-chol (mg/dl)	1.61 ± 11.61	-2.37 ± 10.78	0.174	0.059
HDL (mg/dl)	1.91 ± 4.91	-0.04 ± 5.99	0.170	0.062
LDL (mg/dl)	1.57 ± 10.73	-1.24 ± 5.71	0.211	0.166
TG (mg/dl)	-8.34 ± 25.28	-5.57 ± 31.62	0.710	0.668
TG/HDL	-0.29 ± 0.66	-0.08 ± 0.70	0.246	0.191

BMI = body mass index; WC = waist circumference; HC = hip circumference; WHR = waist to hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; FBS = fasting blood sugar; HOMA-IR = homeostasis model assessment of insulin resistance; T-chol = total cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TG = triglycerides.
[1]Data are mean ± standard deviation; [2]obtained from independent-sample t test; [3]obtained from univariate analysis of covariance (ANCOVA) adjusted for turmeric intake, vitamin C, vitamin E, selenium, β-carotene intake and physical activity.

curcumin supplementation significantly reduced body mass index, waist circumference and hip circumference in the intervention group after 10 weeks. Moreover, a trend towards significant reduction was detected in relation to body weight and waist-hip ratio (WHR) among the participants in the curcumin group. Participants in the placebo group had a significant reduction in weight and body mass index. The reduction in weight and body mass index in both groups can be attributed to prescribed diets. However, participants in the curcumin group had a significant reduction in their body size (i.e. waist circumference and hip circumference). The results from some previous studies are in line with those of the present study.[12,26]

It has been proposed that curcumin supplementation reduces body weight and body mass index by increasing the underlying metabolic rate,[27] with downregulation of adipocytic transcriptional factors such as peroxisome proliferator-activated receptor γ and, therefore, suppression of preadipocyte differentiation.[27,28] The results from one meta-analysis showed that curcumin supplementation had the capacity to reduce waist circumference, as seen in studies on subjects with body mass index less than 30 kg/m² and studies with durations of more than eight weeks.[29] However, further studies are needed to determine the potential mechanism of curcumin for reducing abdominal obesity and visceral fat.

In this study, we documented a significant increase in insulin levels after 10 weeks of curcumin supplementation. However, fasting blood sugar levels and the homeostasis model assessment of

insulin resistance did not change significantly. A previous study was unable to show any beneficial effect from a hypocaloric diet on fasting insulin and blood glucose levels in obese children, even after reducing the calorie intake required for weight maintenance by 30%.[29] Therefore, it seems that this significant increase in insulin levels is mediated through curcumin intake.

Some studies have shown that curcumin supplementation is effective in reducing fasting glucose levels and insulin resistance in diabetic patients.[8,30] However, other surveys on non-diabetic patients failed to prove that curcumin produced any significant reducing effect on glycemic indices.[11,31] In the current study, a significant increase in insulin levels was observed among the participants in the curcumin group.

Curcumin consumption may increase insulin secretion from pancreatic cells and improve pancreatic function over the course of time.[32] The results from a study on animals suggested that curcumin intake might increase insulin secretion by increasing stimulation of glucagon-like peptide-1 secretion.[33] The results from an *in-vitro* study also confirmed the insulin-releasing effect and stimulating action of turmeric in cell cultures from the pancreas and muscle tissues of adult mice.[34] However, for more precise interpretation of such results, larger clinical trials with higher doses of curcumin are needed.

In the present study, there were significant improvements in high-density lipoprotein levels and the triglyceride/high-density lipoprotein ratio among the adolescents in the curcumin group. In addition, a marginally significant reduction in triglyceride levels was observed. When differences in mean change were compared, a marginally significant effect was detected in relation to total cholesterol and low-density lipoprotein levels. In this context, the results from previous studies are similar to those of the current study.[12,26]

It has been shown that the prevalence of some cardiovascular risk factors in Middle Eastern countries are completely different from those in other parts of the world (i.e. low levels of high-density lipoprotein cholesterol and high levels of triglycerides).[35] Documenting this result among Iranian people is of great importance, given that consumption of refined carbohydrate is more prevalent among this population and since one of the obvious features of this population is the presence of a hypertriglyceridemic waist phenotype.[36]

The results from the present study also showed that curcumin intake could improve this specific dyslipidemia (i.e. low levels of high-density lipoprotein cholesterol and high levels of triglycerides) among adolescents, without the need for any significant restriction of carbohydrate consumption. A previous study showed that curcumin supplementation might increase total cholesterol slightly.[10] Administration of curcumin can improve the lipid profile through increasing fatty acid β-oxidation and lipoprotein lipase, suppressing fatty acid synthase and downregulating lipogenic genes and enzymes such as sterol-regulatory element-binding protein-1, acetyl-CoA carboxylase and peroxisome proliferator-activated receptor-α.[11,37] In addition, through increasing the expression of ABCG1 (ATP-binding cassette subfamily G member 1), curcumin may increase serum high-density lipoprotein levels by enhancing high-density lipoprotein-dependent lipid efflux.[38]

To the best of our knowledge, this was the first time that the effects of curcumin supplementation in parallel with a diet leading to slight weight loss were assessed in relation to cardiometabolic risk factors among post-pubescent overweight or obese female adolescents in a developing country. The participants received similar diets for slight weight loss with specific macronutrient distribution, as a basic intervention to control for the confounding effect of dietary intakes. Moreover, the effect of potential confounders was taken into consideration in the statistical analysis. In the current study, we included otherwise healthy overweight and obese girls. Therefore, it would be possible to generalize the results from this study to similar populations of adolescents.

However, several limitations might affect the final results. Due to financial limitations, we were unable to determine the serum levels of curcumin in order to confirm the participants' adherence. Therefore, their compliance with the prescribed supplements was checked through other possible ways. Furthermore, for better interpretation of the findings, particularly glycemic indices and insulin resistance status, we would have needed to assess the serum free fatty acid levels. However, we were unable to do this due to budget limitations. Although the number of withdrawals among the subjects during the study period was negligible, the missing data from these subjects could have affected the final results from this study. However, we tried to reduce this effect by predicting the missing values through linear regression analysis.

CONCLUSIONS

Prescription of curcumin supplementation along with a diet leading to mild weight loss may have beneficial effects on some cardiovascular risk factors in overweight and obese female adolescents. Larger clinical trials with higher doses of curcumin and longer duration are needed to confirm the results from the current study.

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Address for correspondence:
Mojgan Nourian
Department of Community Nutrition, School of Nutrition & Food Science, Isfahan University of Medical Sciences
Isfahan, Iran
Phone: 03137923172
Fax: 03136681378
E-mail: nourian@hlth.mui.ac.ir

Lung metastases at the initial diagnosis of high-grade osteosarcoma: prevalence, risk factors and prognostic factors. A large population-based cohort study

Chao Zhang[I], Xu Guo[II], Yao Xu[III], Xiuxin Han[IV], Jun Cai[V], Xin Wang[VI], Guowen Wang[VII]

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

[I]MD, MSc. Surgeon, Department of Bone and Soft Tissue Tumors, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, China.
orcid.org/0000-0001-7096-8488

[II]MD, MSc. Surgeon, Department of Bone and Soft Tissue Tumors, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, China.
orcid.org/0000-0001-5942-325X

[III]MBBS. Doctoral Student, Department of Bone and Soft Tissue Tumors, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, China.
orcid.org/0000-0003-4601-665X

[IV]MD, MSc. Surgeon, Department of Bone and Soft Tissue Tumors, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, China.
orcid.org/0000-0001-6745-8794

[V]PhD. Assistant Professor, Centre for Research and Development of Anti-Tumor Drugs, Tianjin Institute of Medical and Pharmaceutical Sciences, Tianjin, China.
orcid.org/0000-0003-1321-0317

[VI]PhD. Associate Professor, Department of Epidemiology and Biostatistics, First Affiliated Hospital, Army Medical University, Chongqing, China.
orcid.org/0000-0001-9325-3194

[VII]MD, MSc. Surgeon, Department of Bone and Soft Tissue Tumors, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, China.
orcid.org/0000-0001-6549-1144

KEY WORDS:
Survival analysis.
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Neoplasm metastasis.
Retrospective studies.

ABSTRACT

BACKGROUND: Osteosarcoma is the most prevalent malignant bone tumor in children and adolescents. Lung metastases are associated with poor prognosis.

OBJECTIVE: The aim here was to explore the prevalence of and risk and prognostic factors for lung metastases in high-grade osteosarcoma patients.

DESIGN AND SETTING: Retrospective cohort study based on the Surveillance, Epidemiology and End Results (SEER) database in the United States.

METHODS: Data on 1,408 high-grade osteosarcoma patients registered in the SEER database between 2010 and 2015 were extracted. From these, all patients with high-grade osteosarcoma and initial lung metastasis were selected for analysis on risk and prognostic factors for lung metastases. Overall survival was estimated.

RESULTS: There were 238 patients (16.90%) with lung metastases at diagnosis. Axial location, tumor size > 10 cm (odds ratio, OR 3.19; 95% confidence interval, CI: 1.58-6.45), higher N stage (OR 4.84; 95% CI: 1.94-12.13) and presence of bone metastases (OR 8.73; 95% CI: 4.37-17.48) or brain metastases (OR 25.63; 95% CI: 1.55-422.86) were significantly associated with lung metastases. Younger age and surgical treatment (hazard ratio, HR 0.46; 95% CI: 0.30-0.71) favored survival. Median survival was prolonged through primary tumor surgery.

CONCLUSIONS: The factors revealed here may guide lung metastasis screening and prophylactic treatment for osteosarcoma patients. A primary tumor in an axial location, greater primary tumor size, higher lymph node stage and presence of bone or brain metastases were significantly correlated with lung metastases. The elderly group (≥ 60 years) showed significant correlation with poor overall survival. For improved survival among high-grade osteosarcoma patients with lung metastases, aggressive surgery on the primary tumor site should be encouraged.

INTRODUCTION

Osteosarcoma is the most prevalent malignant bone tumor in children and adolescents. An overall annual prevalence of 0.2-3 cases per 100,000 population has been reported.[1] Despite the rarity of osteosarcoma, it remains one of the deadliest cancers during the pubertal growth spurt. Lung metastases have been reported to be one of the challenging factors associated with a poor prognosis.[2]

Approximately 20% of osteosarcoma patients present with metastatic disease at the time of the initial diagnosis. The most prevalent metastatic type is lung metastasis, which occurs in more than 80% of the cases.[3,4] Despite the development of novel treatments for osteosarcoma, 30-40% of these patients still relapse and the long-term post-relapse survival among these individuals has been reported to be less than 20%.[5,6]

Undoubtedly, osteosarcoma patients can benefit from early diagnosis and treatment of metastases. Thus, a reasonable degree of lung metastasis screening for osteosarcoma patients at diagnosis is important. Male sex and the site involved (femur and tibia) were confirmed to be associate with greater occurrence of metastasis in a Mexican clinical trial.[7] The primary tumor size was reported to be a risk factor for lung metastasis among patients with osteosarcoma.[8,9]

Currently, radiography is one of the most widely applied clinical screening strategies. However, radiography barely captures metastases until they physically form. Therefore, studies looking into the risk factors for lung metastasis occurrence among patients with osteosarcoma are warranted.

The prognostic factors for osteosarcoma patients with lung metastases have been drawing the attention of researchers around the world. In previous studies, a series of prognostic factors for lung metastases in osteosarcoma patients were reported, including sex,[10] non-necrotic metastases,[11] number of pulmonary nodules,[3,5-6] late relapse,[12,13] unilateral lung involvement,[6,12] completeness of surgical resection of all tumor sites detected[5,6] and histological response to chemotherapy.[13] There is a need for studies on large populations, to analyze the prognostic factors for lung metastases among osteosarcoma patients. Furthermore, there is a need to investigate factors associated with survival among patients with high-grade osteosarcoma with lung metastases.

OBJECTIVE

In the present study, in which information from the Surveillance, Epidemiology, and End Results (SEER) database was analyzed, we aimed to investigate the prevalence of and risk factors for lung metastases among high-grade osteosarcoma patients. Furthermore, survival analysis was conducted to evaluate the prognostic factors for high-grade osteosarcoma with lung metastases.

METHODS

Study design, ethics and setting

This was a retrospective cohort study based on a database of patients with cancer in the United States. This study complied with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Tianjin Medical University Cancer Institute & Hospital Ethical Review Board (Ek2018022).

Study population

The National Cancer Institute's open public database, Surveillance, Epidemiology and End Results (SEER), provides cancer incidence and survival data from 18 established cancer registries across the United States. SEER is a particularly useful tool for assessing the epidemiological characteristics of cancer.

Data on cancer patients were obtained from the SEER database. The SEER*Stat 8.3.5 software (https://seer.cancer.gov/data/) was used to generate the case listing. Since the details of metastases were not available before 2010, data on high-grade osteosarcoma patients diagnosed between 2010 and 2015 were collected. Patients diagnosed as having "9192/3: parosteal osteosarcoma" or "9187/3: intraosseous well-differentiated osteosarcoma" were excluded from the analyses, given that these diagnoses present a less aggressive clinical course, compared with other high-grade subtypes. Such patients are treated differently: they are diagnosed at autopsy or via the death certificate, and any presence of lung metastases or implementation of follow-up remains unknown.[14]

From this database, 1,408 patients who had been identified as presenting high-grade osteosarcoma between January 1, 2010, and December 31, 2015, were selected for analysis on the prevalence of lung metastases and their risk factors. Data on those diagnosed as presenting high-grade osteosarcoma with lung metastases between 2010 and 2014 (i.e. with at least one year of follow-up) were used to conduct survival analysis and to investigate the prognostic factors for lung metastases.

Variables and statistical analysis

The patients' demographic and clinical characteristics were included and categorized as follows: age (≤ 24, 25-59 or ≥ 60 years); sex (female or male); race [white, black, AI (American Indian/Alaska Native) or API (Asian or Pacific Islander)]; marital status (married or unmarried); insurance status (insured or uninsured); location (extremities: long and short bones of the upper and lower extremities; axial skeleton: pelvis, spine and ribs; or others: mandible, skull and other atypical locations); tumor size (≤ 5, 5-10 or > 10 cm); regional lymph node stage (N0 or N1); histology (osteosarcoma and not otherwise specified (NOS) or others); and presence or absence of bone metastases, liver metastases or brain metastases.

The differences in prevalence of lung metastases between the categorical variables were analyzed using Pearson's chi-square test or the rank-sum test. The risk factors for high-grade osteosarcoma patients with initial lung metastases were determined primarily through univariable logistic regression. Moreover, factors that achieved significant levels were incorporated into the multivariable logistic regression model to control the potential confounding factors.

The primary outcome from the survival analysis was the overall survival, which was defined as the length of time from when the high-grade osteosarcoma was first diagnosed to the occurrence of all causes of death. Kaplan-Meier curves and log-rank tests were used to analyze survival differences. At the same time, multivariable Cox proportional-hazards regression was conducted based on the aforementioned factors, with P-values < 0.05 taken to be significant, and taking into account the surgical treatments applied to the primary site (not applied or applied).

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 23.0 (IBM Corporation, Armonk, NY, USA) and all charts on survival were produced using MedCalc 15.2.2. Two-tailed $P < 0.05$ was considered to be statistically significant.

RESULTS

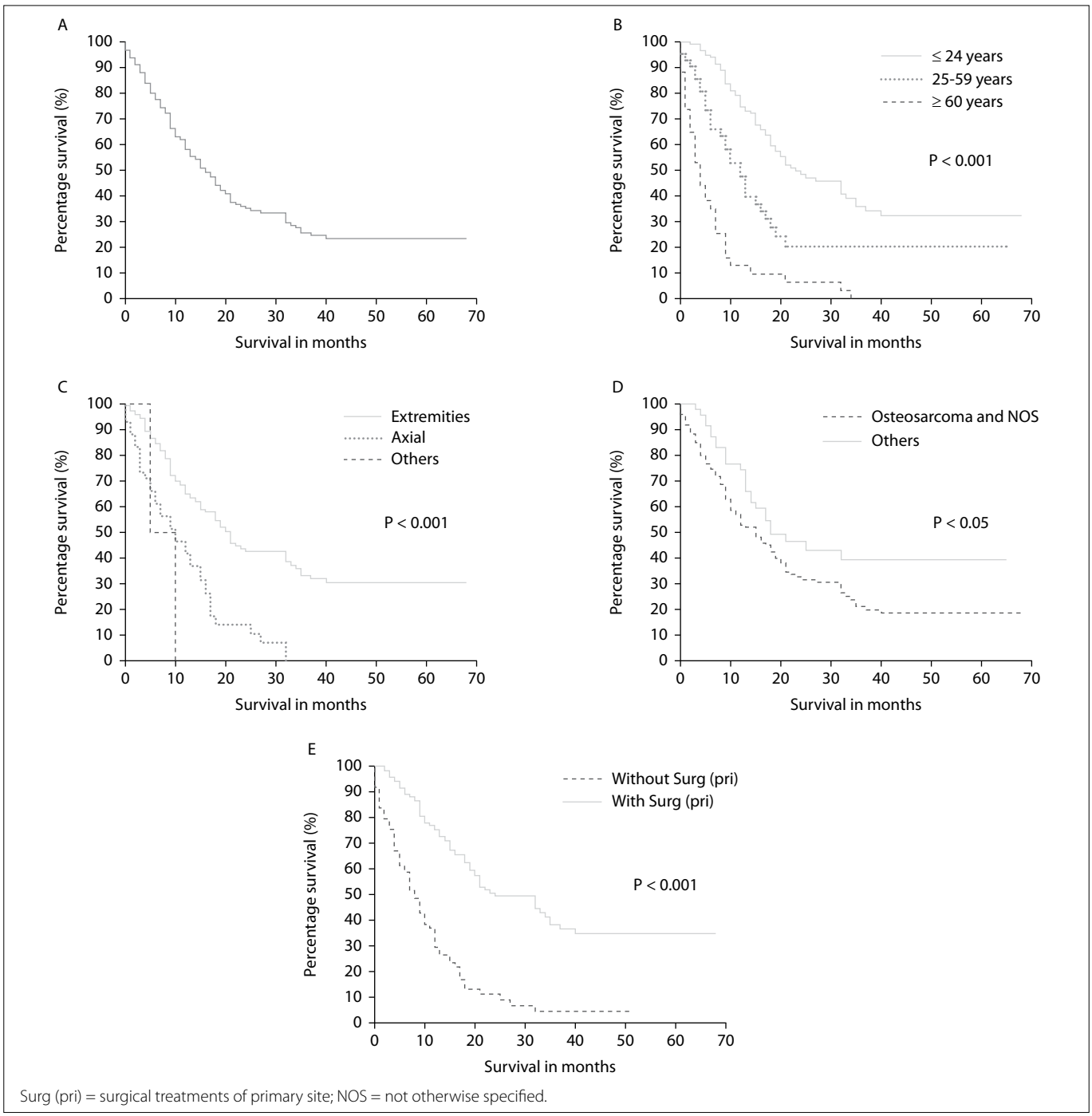
Demographic and clinical characteristics

A total of 1,408 high-grade osteosarcoma patients were included in the current study. Among them, 623 (44.2%) were male and

785 were female (55.8%). Their mean age was 29.95 ± 22.10 years. Most of the participants involved were white (N = 1,056; 75.0%). Among the participants, there were 195 high-grade osteosarcoma patients with lung metastases [69 males (35.4%) and 126 females (64.6%); mean age of 30.16 ± 24.09 years] who had been followed up for at least one year (**Figure 1**, **Table 1**).

Prevalence of lung metastases

Among the 1,408 eligible patients with high-grade osteosarcoma, an initial lung metastasis was diagnosed in 16.90% of the entire cohort (238/1,408). Patients in the age group ≥ 60 years presented significantly higher prevalence of lung metastases than did the younger age groups (χ^2 = 8.05; P = 0.018). The prevalences of



Surg (pri) = surgical treatments of primary site; NOS = not otherwise specified.

Figure 1. Kaplan-Meier analyses on overall survival among high-grade osteosarcoma patients. The high-grade osteosarcoma patients with lung metastases are shown together (A, overall) and stratified according to age (B), location (C), histology (D) and surgical treatments of the primary site (E).

Table 1. Baseline of the demographic and related clinical characteristics among patients diagnosed with high-grade osteosarcoma with and without lung metastases (LM)

Subject characteristics	Number of osteosarcoma patients (2010-2015)		P-value	Number of osteosarcoma patients (2010-2014)		P-value
	With LM (n, %)	Without LM (n, %)		With LM (n, %)	Without LM (n, %)	
Age, in years						
≤ 24	147 (17.78)	680 (82.22)		119 (17.40)	565 (82.60)	
25-59	48 (12.66)	331 (87.34)	0.02	42 (13.46)	270 (86.54)	0.09
≥ 60	43 (21.29)	159 (78.71)		34 (21.12)	127 (78.88)	
Sex						
Female	92 (14.77)	531 (85.23)	0.06	69 (13.48)	443 (86.52)	0.01
Male	146 (18.60)	639 (81.40)		126 (19.53)	519 (80.47)	
Race						
White	176 (16.67)	880 (83.33)		145 (16.67)	725 (83.33)	
Black	40 (18.02)	182 (81.98)		31 (17.42)	147 (82.58)	
AI	3 (21.43)	11 (78.57)	0.92	3 (23.08)	10 (76.92)	0.90
API	18 (16.07)	94 (83.93)		15 (16.13)	78 (83.87)	
Unknown	1 (25.00)	3 (75.00)		1 (33.33)	2 (66.67)	
Marital status						
Unmarried	181 (17.22)	870 (82.78)		150 (17.30)	717 (82.70)	
Married	49 (14.98)	278 (85.02)	0.34	38 (14.56)	223 (85.44)	0.33
Unknown	8 (26.67)	22 (73.33)		7 (24.14)	21 (75.86)	
Insurance status						
Insured	228 (17.09)	1,106 (82.91)		185 (16.88)	911 (83.12)	
Uninsured	7 (15.22)	39 (84.78)	0.74	7 (17.95)	32 (82.05)	0.91
Unknown	3 (10.71)	25 (89.29)		3 (13.64)	19 (86.36)	
Location						
Extremities	174 (17.42)	825 (82.58)		144 (17.50)	679 (82.50)	
Axial	54 (23.08)	180 (76.92)	< 0.001	42 (22.58)	144 (77.42)	< 0.001
Others	2 (1.28)	154 (98.72)		2 (1.50)	131 (98.50)	
Unknown	8 (42.11)	11 (57.89)		7 (46.67)	8 (53.33)	
Tumor size (cm)						
≤ 5	12 (5.63)	201 (94.37)		12 (6.63)	169 (93.37)	
5-10	69 (13.22)	453 (86.78)	< 0.001	57 (13.60)	362 (86.40)	< 0.001
> 10	108 (22.78)	366 (77.22)		87 (22.54)	299 (77.46)	
Unknown	49 (24.62)	150 (75.38)		39 (22.81)	132 (77.19)	
N stage						
N0	199 (15.06)	1,122 (84.94)		163 (15.02)	922 (84.98)	
N1	16 (50.00)	16 (50.00)	< 0.001	13 (54.17)	11 (45.83)	< 0.001
Unknown	23 (41.82)	32 (58.18)		19 (39.58)	29 (60.42)	
Histology						
Osteosarcoma and NOS	179 (18.19)	805 (81.81)	0.05	147 (18.26)	658 (81.74)	0.05
Others	59 (13.92)	365 (86.08)		48 (13.64)	304 (86.36)	
Bone metastasis						
None	193 (14.41)	1,146 (85.59)		158 (14.39)	940 (85.61)	
Yes	39 (63.93)	22 (36.07)	< 0.001	31 (60.78)	20 (39.22)	< 0.001
Unknown	6 (75.00)	2 (25.00)		6 (75.00)	2 (25.00)	
Liver metastasis						
None	230 (16.49)	1,165 (83.51)		188 (16.42)	957 (83.58)	
Yes	4 (44.44)	5 (55.56)	0.03	3 (37.50)	5 (62.50)	< 0.001
Unknown	4 (100.00)	0 (0.00)		4 (100.00)	0 (0.00)	
Brain metastasis						
None	229 (16.39)	1,168 (83.61)		186 (16.23)	960 (83.77)	
Yes	4 (66.67)	2 (33.33)	0.001	4 (66.67)	2 (33.33)	< 0.001
Unknown	5 (100.00)	0 (0.00)		5 (100.00)	0 (0.00)	
Surg (pri)						
None	91 (37.60)	151 (62.40)		74 (36.27)	130 (63.73)	
Yes	147 (12.65)	1,015 (87.35)	< 0.001	121 (12.74)	829 (87.26)	< 0.001
Unknown	0 (0.00)	4 (100.00)		0 (0.00)	3 (100.00)	
Total	**238 (16.90)**	**1,170 (83.10)**		**195 (16.85)**	**962 (83.15)**	

LM = lung metastases; AI = American Indian/Alaska Native; API = Asian or Pacific Islander; NOS = not otherwise specified; Surg (pri) = surgical treatments of primary site.

lung metastases in males and females were 14.77% and 18.60%, respectively, without any significant difference (χ^2 = 3.63; P = 0.057). Moreover, the prevalence of lung metastases did not show any significant difference with regard to different racial groups (χ^2 = 0.50; P = 0.92) (**Table 1**).

Risk factors for developing lung metastases

Univariable analysis showed that the factors of axial location (odds ratio, OR = 1.42; 95% confidence interval, CI = 1.01-2.01; P = 0.045), greater primary tumor size (OR = 4.94; 95% CI = 1.01-2.01; P < 0.001), higher regional lymph node (N) stage (OR = 5.64; 95% CI = 2.77-11.46; P < 0.001), presence of bone metastasis (OR = 10.53; 95% CI = 6.11-18.14; P < 0.001), presence of liver metastasis (OR = 4.05; 95% CI = 1.08-15.21, P = 0.04) and presence of brain metastasis (OR = 10.20; 95% CI = 1.86-56.02; P = 0.01) were positively associated with presence of lung metastases.

Multivariable logistic regression suggested that axial location, greater tumor size, higher N stage and presence of bone metastases or brain metastases were all significantly associated with lung metastases seen at the initial diagnosis (**Table 2**).

Survival analysis and prognostic factors for lung metastases

Among the 195 high-grade osteosarcoma patients with lung metastases included in the one-year survival analysis, the end of the follow-up was marked by death in the cases of 128 of the patients (65.64%). The median overall survival time was 16.00 months (95% CI = 12.81-19.19 months; **Figure 1A**). Kaplan-Meier analysis showed the overall survival among subjects with older age (**Figure 1B**; P < 0.001), axial location (**Figure 1C**; P < 0.001) and osteosarcoma and NOS (**Figure 1D**; P < 0.05) was lower than that of their counterparts. Conversely, patients with surgical treatment of the primary site presented markedly higher overall survival than did the subjects without surgery (**Figure 1E**; P < 0.001).

In the multivariable Cox regression model, the results showed that elderly patients (≥ 60 years; hazard ratio, HR = 3.48; 95% CI = 2.14-5.66; P < 0.001) were associated with poor overall survival, with a median survival time of four months. However, surgery at the primary site was positively associated with better overall survival (HR = 0.46; 95% CI = 0.30-0.71; P < 0.001). The median survival time could be prolonged from 8 months to 24 months through surgery at the primary site (**Table 3**).

DISCUSSION

To the best of our knowledge, this investigation was the largest population-based study to estimate the prevalence, risk and prognostic factors for initial lung metastases in cases of high-grade osteosarcoma. Based on our results, lung metastases were found in 16.9% of the high-grade osteosarcoma patients at the initial diagnosis. The prevalence of initial lung metastases estimated in the present study was less than was seen in another study based on a single-center database, in which the prevalence was 29.5%.[11]

Because of the deleterious effect on survival generated through lung metastases among high-grade osteosarcoma patients, a predictive system for determining whether high-grade osteosarcoma patients have lung metastases and/or for choosing radiographic scanning needs to be delineated. Our results suggested that high-grade osteosarcoma patients were at significantly higher risk of developing lung metastases at diagnosis if their osteosarcomas were characterized by the primary tumor in an axial location, greater primary tumor size, higher N stage and presence of bone metastases or brain metastases. Thus, radiographic scanning and/or further screening should be considered at diagnosis for high-risk high-grade osteosarcoma patients. We therefore recommend that physicians should construct risk assessment tools using the aforementioned risk factors and should provide different screening strategies for patients with different risk levels.

In terms of prognostic determinants, with three age groups analyzed among our osteosarcoma patients with lung metastases, multivariable Cox regression analyses showed that younger age (< 60 years) was one of the favorable prognostic factors among high-grade osteosarcoma patients with lung metastases. Our results were further strengthened by many previous studies that have revealed that a

Table 2. Univariable and multivariable logistic regression for analysis on the associated factors for development of lung metastases among patients diagnosed with high-grade osteosarcoma (diagnosed between 2010 and 2015)

Subject characteristics	Univariable		Multivariable	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Location				
Extremities	1 (Reference)	1.00	1 (Reference)	1.00
Axial	1.42 (1.01-2.01)	0.045	1.15 (0.72-1.83)	0.56
Others	0.06 (0.02-0.25)	< 0.001	0.04 (0.00-0.37)	0.01
Unknown	NA	NA	NA	NA
Tumor size (cm)				
≤ 5	1 (Reference)	1.00	1 (Reference)	1.00
5-10	2.55 (1.35-4.82)	0.004	1.73 (0.84-3.56)	0.14
> 10	4.94 (2.66-9.20)	< 0.001	3.19 (1.58-6.45)	0.001
Unknown	NA	NA	NA	NA
N stage				
N0	1 (Reference)	1.00	1 (Reference)	1.00
N1	5.64 (2.77-11.46)	< 0.001	4.84 (1.94-12.13)	0.001
Unknown	NA	NA	NA	NA
Bone metastasis				
None	1 (Reference)	1.00	1 (Reference)	1.00
Yes	10.53 (6.11-18.14)	< 0.001	8.73 (4.37-17.48)	< 0.001
Unknown	NA	NA	NA	NA
Liver metastasis				
None	1 (Reference)	1.00	1 (Reference)	1.00
Yes	4.05 (1.08-15.21)	0.04	0.00 (0.00- NA)	1.00
Unknown	NA	NA	NA	NA
Brain metastasis				
None	1 (Reference)	1.00	1 (Reference)	1.00
Yes	10.20 (1.86-56.02)	0.01	25.63 (1.55-422.86)	0.02
Unknown	NA	NA	NA	NA

OR = odds ratio; CI = confidence interval; NA = not available.

Table 3. Multivariable Cox regression for analysis on the prognostic factors for high-grade osteosarcoma with lung metastases (diagnosed between 2010 and 2014)

Subject characteristics	No. of osteosarcoma patients with LM		Survival, Median (IQR), mo	HR (95% CI)	P-value
	Overall	Deceased (n, %)			
Age, years					
≤ 24	119	65 (54.62)	23.00 (15.02-30.98)	1 (Reference)	1.00
25-59	42	30 (71.43)	12.00 (8.63-15.37)	1.87 (1.17-2.99)	0.01
≥ 60	34	33 (97.06)	4.00 (2.38-5.62)	3.48 (2.14-5.66)	< 0.001
Location					
Extremities	144	84 (58.33)	21.00 (17.09-24.91)	1 (Reference)	1.00
Axial	42	37 (88.10)	10.00 (4.80-15.20)	1.32 (0.83-2.10)	0.24
Others	2	2 (100.0)	5 (NA)	3.96 (0.93-16.81)	0.06
Unknown	7	5 (71.43)	NA	NA	NA
Histology					
Osteosarcoma and NOS	147	102 (69.39)	15.00 (11.03-18.97)	1 (Reference)	1.00
Others	48	26 (54.17)	18.00 (8.89-27.11)	0.68 (0.44-1.05)	0.08
Surg (pri)					
None	74	64 (86.49)	8.00 (5.82-10.19)	1 (Reference)	1.00
Yes	121	64 (52.89)	24.00 (14.89-33.11)	0.46 (0.30-0.71)	< 0.001

LM = lung metastases; IQR = interquartile range; mo = months; HR = hazards ratio; CI = confidence interval; NA = not available; NOS = not otherwise specified; Surg (pri) = surgical treatments of primary site.

correlation exists between increasing age and poorer prognosis among osteosarcoma patients.[15-18] Thus, more attention needs to be paid to elderly high-grade osteosarcoma patients with lung metastases.

In addition, the results showed that surgical treatment was a factor that favored greater survival among high-grade osteosarcoma patients with lung metastases. Accordingly, we would recommend aggressive surgical removal of the primary site tumor, in order to improve the survival of high-grade osteosarcoma patients with lung metastases. However, no information on the detailed surgical approaches adopted among osteosarcoma patients was recorded in the public SEER database.[19] Therefore, no comparison of surgical approaches can be made, and surgical subgroup analyses cannot be conducted. Thus, we are unable to accurately recommend the type of surgery that should be used for treating osteosarcoma patients with lung metastases. Hence, further studies should be conducted in order to confirm the results in the future.

Inevitably, there are some limitations in the present study. Firstly, the SEER database does not gather information about local recurrence or metastases during the follow-up, which may affect the prognosis. Secondly, the detailed diagnostic approach for determining situations of an initial lung metastases and the surgical details of treatments implemented among osteosarcoma patients were not recorded in the public SEER database. Accordingly, no comparison of diagnostic approaches could be made, and surgical subgroup analyses could not be undertaken. Thirdly, among the osteosarcoma patients with lung metastases, asymptomatic cases and metachronous lung metastases cases were not recorded in the public SEER database. Hence, the real occurrence rate of lung metastases among osteosarcoma patients may have been underestimated. Lastly, due to the limited sample size, we could not maintain enough statistical power to get a more stable result and, consequently, further studies are needed in order to confirm these results.

The prevalence of initial lung metastases among our high-grade osteosarcoma patients was 16.9%. High-grade osteosarcoma patients with the primary tumor in an axial location, greater primary tumor size, higher N stage and presence of bone metastases or brain metastases were more likely to have lung metastases at diagnosis. Younger patients (< 60 years) and surgical treatment were factors that favored better survival among high-grade osteosarcoma patients with lung metastases.

CONCLUSIONS

The associated factors, including primary tumor in an axial location, greater primary tumor size, higher lymph node stage and presence of bone metastases or brain metastases, were significantly correlated with lung metastases that were detected through screening among high-grade osteosarcoma patients. These factors can potentially be used for lung metastasis screening. The elderly age group (≥ 60 years) was found to be significantly correlated with poor overall survival among the high-grade osteosarcoma patients with lung metastases. To improve the survival of osteosarcoma patients with lung metastases, aggressive surgery on the primary tumor site should be encouraged.

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Address for correspondence:
Dr. Xin Wang
Department of Epidemiology and Biostatistics, First Affiliated Hospital, Army Medical University, 30 Gaotanyan Street, Shapingba District, Chongqing 400038, China
Tel. +86 17830912151
E-mail: wangxinmarine@126.com

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Assessment of risk factors associated with falls among the elderly in a municipality in the state of Paraíba, Brazil. A cross-sectional study

Alba Rejane Gomes de Moura Rodrigues[I], José Cesar Assef[II], Carlos Bezerra de Lima[III]

Primary Healthcare and Family Health Strategies, Patos (PB), Brazil

[I]BSN. Nurse and Doctoral Student of Surgical Research, Faculdade de Ciências Médicas da Santa Casa de São Paulo (FCMSCSP), São Paulo (SP); and Instructor, Unidade Acadêmica de Enfermagem (UAENF), Universidade Federal de Campina Grande (UFCG), Cajazeiras (PB), Brazil.
orcid.org/0000-0003-1451-2114

[II]MD. Associate Professor, Department of Surgery, and Director of Emergency Services, Faculdade de Ciências Médicas da Santa Casa de São Paulo (FCMSCSP), São Paulo (SP), Brazil.
orcid.org/0000-0001-7341-9003

[III]BSN, MD. Nurse, Nursing Department, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro (RJ); Teacher of Postgraduate Program, Faculdade Brasileira de Ensino Pesquisa e Extensão (FABEX), João Pessoa (PB), Brazil.
orcid.org/0000-0001-6711-4676.

KEY WORDS:
Risk factors.
Aged.
Accidental falls.

ABSTRACT

BACKGROUND: Falls among the elderly are one of the main public health problems that have direct consequences for their health. They reduce these individuals' autonomy and functional independence.

OBJECTIVE: The objective of this study was to evaluate the risk factors associated with falls among elderly people enrolled within primary healthcare.

DESIGN AND SETTING: Cross-sectional study conducted at primary healthcare units in the municipality of Patos, state of Paraíba, Brazil.

METHODS: The Fall Risk Score and Timed Up and Go (TUG) test were used for evaluating the risk of falling among 316 elderly individuals. The independent variables used were sociodemographic and health conditions, while the dependent variable was the frequency of falls on the same level, over the course of previous years. The descriptive statistical tests used were the chi-square and Mann-Whitney tests.

RESULTS: Occurrence of falls was reported by 211 of the 316 participants, representing a prevalence of 66.8% with confidence interval 61.6-72.0. The logistic regression results showed, after adjusting for all variables included in the model, that only the variables of vestibular disorders, self-assessed health status and dizziness/vertigo (trend) were significant ($P \leq 0.05$). Most of the elderly participants had two or more associated pathological conditions. The participants were predominantly female (68.4%).

CONCLUSIONS: Higher occurrence of falls was observed among female elderly individuals who suffered recurrent falls, had had low levels of schooling, presented comorbidities, had comorbidities and made use of drugs. These conditions predisposed these individuals to greater vulnerability to the risk of falls.

INTRODUCTION

The numbers of elderly people are growing rapidly. Both the community collectively and healthcare professionals, family members and caregivers individually need to reflect on their commitment towards dealing with the changes that occur as people become older. Aging is a natural, progressive and irreversible process that directly interferes with biological and functional conditions. Hence, one-off actions aimed at promoting healing and/or rehabilitation are insufficient.[1]

Statistical data show that Brazil has 20.6 million elderly people, representing 10.8% of its total population. By 2060, this country's elderly population is expected to reach 58.4 million (26.7% of the total population).[2] Accordingly, guidance for actions to diminish the risk factors for falls in home settings will become very relevant.

The incidence of falls varies across countries. Studies conducted in Latin America and the Caribbean region have identified that, on an annual basis, the proportion of older adults suffering falls ranges from 21.6% in Barbados to 34% in Chile.[3] These data emphasize that there is a need to take a more accurate look into the settings within which elderly people live. This is a major challenge, with regard both to identifying the elderly people who are at risk and to planning preventive strategies, irrespective of geographical location.

Falls are the sixth leading cause of death among people over 65 years old. They make these individuals fragile, insecure and unable to perform their daily activities for fear of falling again.[4] Both intrinsic factors (physiological changes stemming from aging itself; presence of morbidities; and deficits in balance, vision, hearing or gait) and extrinsic factors (environmental risks due to poor lighting or inadequate or slippery floors; risk-prone behaviors, such as going upstairs and downstairs; and routine activities of daily living) play important roles.

The multifactorial circumstances of this interaction of factors may predispose this group of vulnerable individuals to falls.[5,6]

Occurrences of falling have recently become a public health problem due to the complications resulting from them. They place a burden on the healthcare system and bring about physical mobility limitations. According to the Brazilian Ministry of Health, about 30% of people aged over 65 fall at least once a year.[7] In a study conducted in Australia among elderly people living in the community, it was reported that 2-6% of falls were associated with fractures and approximately 1% of falls were associated with hip fractures.[8]

The municipality of Patos, in the state of Paraíba, was the scenario for the present study. A total of 426 hospital admissions due to falls were registered between January 2008 and December 2015, which accounted for 6.9% of all hospitalizations.[9] In addition to this high percentage of hospital admissions due to falls, this municipality does not have resources for rehabilitation, and no study on falls among the elderly had previously been conducted in this municipality. Recognizing the most vulnerable groups and acting so as to prevent falls by engaging the efforts of an interdisciplinary team can contribute towards minimizing these events.[9]

OBJECTIVE

The objective of this study was to assess the risk factors associated with falls among elderly people enrolled within primary healthcare in the municipality of Patos, Paraíba, Brazil.

METHODS

This was a quantitative cross-sectional study carried out between April and December 2016 at 40 family health units (Unidades de Saúde da Família, USF), among which 38 were located in the urban area and two in the rural area of the municipality of Patos, in the state of Paraíba, Brazil. The population sample size was calculated by considering a prevalence of falls of 30%. This was in accordance with the national prevalence of falls in Brazil[1,8] for a finite population of 13,453 elderly people. It was thus determined that a sample of 316 individuals would be needed.

A posteriori power calculations indicated that all the variables that were shown to be significantly associated in the logistic regression model had a statistical power of at least 94.8% for the sample size used in our study and the effect size we found (odds ratio-based).

The elderly participants were selected across the healthcare districts of the municipality by means of systematic sampling and by organizing a single listing of these districts. There are four healthcare districts in the municipality of Patos, each with ten healthcare units. The average number of elderly people per district was 3,200. The proportion of the sample across each of the four districts was calculated on this basis.

The sample selection process was then performed according to areas and micro-areas where community health agents acted, which, in turn, belonged to various census tracts (districts). Each community health agent had a spreadsheet containing data on their corresponding microarea, regarding street names, numbers of households per street with their respective full address and the estimated number of elderly people per household.

A draw was conducted among each five elderly people who had been selected to participate, based on the spreadsheet data. Thus, it was possible for more than one elderly person in the same household to participate in this study, given that individuals were selected, rather than their household. There were occurrences of situations in which two or more elderly people from the same household were selected to participate in the study, but only one of them was actually interviewed. Also, there were cases in which all the elderly people selected from a single household were interviewed. Overall, an average of 8-10 elderly people were selected from each healthcare unit. We did not have any dropouts or refusals.

The inclusion criteria were that the participants needed to be ≥ 60 years of age and be resident in an area assigned to the units in question. Elderly people presenting some cognitive deficit, according to information provided by the community health agents, and those making use of any kind of device to aid walking, or a wheelchair, were excluded.

For data collection, two types of instruments were used. Firstly, a questionnaire was applied to gather sociodemographic data, with questions on gender, age, marital status, level of schooling, income and current occupation, among other data; and to gather self-reported information on the elderly participants' morbidities. Secondly, two fall risk assessment instruments were used: the Fall Risk Score, adapted from a study by Shiaveto,[10] which uses five criteria to evaluate the risk of falls among the elderly population; and the TUG (Timed Up and Go) test, which assesses gait and balance.

In the TUG test, the time spent (in seconds) for the elderly individual to stand up from a seated position in a chair, walk a 3-meter distance, turn around, walk back towards the chair and sit down again was recorded. The individuals underwent this test once beforehand so that they could become familiar with it. They were given no assistance whatsoever as they took the test.

The Fall Risk Score evaluates the following: 1- Whether the elderly individuals had had any falls previously; 2- Whether they were using any medication; 3- Whether they presented any sensory deficits; 4- Their mental state; and 5- Their gait.

The following independent variables were selected for this study: sociodemographic variables: (age, gender, marital status and schooling level); and health status variables: self-perception of health status, living alone, walking without difficulty, presence of changes (comorbidities), types of changes (comorbidities),

medications (quantity and type) and other health hazards (dizziness/vertigo). The dependent variable was the frequency of falls from the same level over recent years.

The participants' cognitive ability was evaluated with the help of the Functional Activities Questionnaire (FAQ), devised by Pfeiffer.[11] This scale was used in its version validated for use in Brazilian Portuguese. Cognitive ability was categorized as follows: absence of cognitive decline, or mild, moderate or severe critical decline. Elderly people with severe cognitive decline were excluded.

The interviews to administer the questionnaires were conducted by one author of the present study (ARGMR) and by three interviewers from the nursing undergraduate course who were enrolled in a course module on elderly people's healthcare and were trained and instructed on how to administer the questionnaire and its assessment grading scales. The interviews were conducted by means of visits to the elderly people's homes, which had previously been scheduled by the health agent in charge of the area where these elderly people lived.

Following data collection, a database was prepared in an Excel spreadsheet. To carry out the analysis, the database was exported from the Excel spreadsheet to the Statistical Package for the Social Sciences (SPSS) software, version 17.0. The data were then subjected to descriptive analysis (absolute and percentage frequencies), and the normality of age distribution was checked by means of the Shapiro-Wilk test, which showed that this variable did not follow normal distribution ($P < 0.05$).

Subsequently, in order to check the association between occurrences of falls and each of the categorical variables, chi-square tests or Fisher's exact tests, as appropriate, were performed. To check whether there was an association between occurrences of falls and age, the non-parametric Mann-Whitney test was used. The variables that were found to be significantly associated with occurrences of falls were then fed into a logistic regression model to check the independent predictors of occurrences of falls, after adjustment for the other variables of the model. The significance level adopted for the statistical analysis was $P < 0.05$.

This study was approved by our institution's research ethics committee, through decision no. 962,318 (CAAE 38956414.00000-5181), on February 25, 2015, in accordance with Resolution No. 466/12 of Brazil's National Health Council (Conselho Nacional de Saúde).[12] All study participants were guaranteed that their participation would be voluntary, of their own free will, and were only included in the study after they had read and signed an informed consent statement. After informing illiterate elderly people about the objectives of the study, the person responsible for each individual in this situation who agreed to participate in the study was then asked to sign the voluntary consent form in lieu of this elderly individual.

RESULTS

Altogether, 316 elderly people were evaluated. Out of the total number of elderly people interviewed, 68.4% were females. The most prevalent age group was from 60 to 69 years old (40.5%). The minimum age was 60 years, and the maximum age was 99 years, with a mean of 73, median of 72 and standard deviation of 9. The majority of the elderly people (64.7%) had completed primary education, while 19.9% were illiterate. Regarding their marital status, 47.5% were married, 22.8% widowed and 15.8% were divorced.

Occurrence of falls in the period between 2015 and 2016 was reported by 211 of these 316 participants, thus representing a prevalence of 66.77%, with a confidence interval (CI) of 61.6-72.0. **Table 1** shows the distribution of the elderly people studied according to their sociodemographic variables and histories of falls. A significant association was found between occurrences of falls and schooling level ($P = 0.042$). Among the individuals who sustained falls, there was a greater proportion who were illiterate or who had only completed primary education. No significant differences were found in relation to the other demographic variables.

With regard to the possible risk factors for occurrences of falls, comorbidities and self-assessed health status were evaluated (**Table 2**). Among the self-reported pre-existing chronic

Table 1. Distribution of the elderly people studied, according to sociodemographic variables and history of falls. Patos (PB), 2016

Sociodemographic variables	Categories	No falls	With falls	P
n		n = 105	n = 211	
Gender	Male	35 (33.3)	65 (30.8)	0.744
	Female	70 (66.7)	146 (69.2)	
Marital status	Single	10 (9.5)	34 (16.1)	0.335
	Married	55 (52.4)	95 (45.0)	
	Divorced	18 (17.1)	32 (15.2)	
	Widowed	22 (21.0)	50 (23.7)	
Schooling level	Illiterate	13 (12.4)	50 (23.7)	0.042
	Incomplete primary education	55 (52.4)	101 (47.9)	
	Completed primary education	12 (11.4)	32 (15.2)	
	Incomplete secondary education	2 (1.9)	4 (1.9)	
	Completed secondary education	13 (12.4)	11 (5.2)	
	Incomplete higher education	0 (0.0)	1 (0.5)	
	Completed higher education	10 (9.5)	12 (5.7)	

comorbidities or diseases, the proportions of these that showed associations with other pathological conditions were as follows: systemic arterial hypertension (81.5%), arthrosis (37.9%) and vestibular disorders (33.2%).

Self-assessed health status showed a statistically significant association with occurrences of falls among the elderly people who participated in our study. When asked about how they assessed their own health status, 45.6% considered it good; 26.6%, normal; and 26.3%, very good. A significant association was found between occurrences of falls and self-assessed health status (P < 0.001). Among the individuals who suffered falls, there were higher proportions with good and normal health status, whereas among individuals who reported not falling, there was a higher proportion with very good health status (**Table 3**).

Table 2. Association between comorbidities and presence of falls. Patos (PB), 2016

Comorbidities	Categories	No falls	With falls	P
		n = 105	n = 211	
Diabetes	No	76 (72.4)	129 (61.1)	0.065
	Yes	29 (27.6)	82 (38.9)	
Hypertension	No	33 (31.4)	39 (18.5)	0.015
	Yes	72 (68.6)	172 (81.5)	
Stroke	No	104 (99.0)	201 (95.3)	0.108
	Yes	1 (1.0)	10 (4.7)	
Low blood pressure	No	100 (95.2)	206 (97.6)	0.309
	Yes	5 (4.8)	5 2.4)	
Heart disease	No	91 (86.7)	164 (77.7)	0.081
	Yes	14 (13.3)	47 (22.3)	
Neurological disease	No	103 (98.1)	201 (95.3)	0.349
	Yes	2 (1.9)	10 (4.7)	
Insomnia	No	90 (85.7)	174 (82.5)	0.567
	Yes	15 (14.3)	37 (17.5)	
Hypothyroidism	No	103 (98.1)	194 (91.9)	0.055
	Yes	2 (1.9)	17 (8.1)	
Depression	No	93 (88.6)	184 (87.2)	0.868
	Yes	12 (11.4)	27 (12.8)	
Arthritis	No	86 (81.9)	159 (75.4)	0.242
	Yes	19 (18.1)	52 (24.6)	
Arthrosis	No	82 (78.1)	131 (62.1)	0.006
	Yes	23 (21.9)	80 (37.9)	
Vestibular disorders	No	92 (87.6)	141 (66.8)	< 0.001
	Yes	13 (12.4)	70 (33.2)	
Incontinence	No	104 (99.0)	199 (94.3)	0.067
	Yes	1 (1.0)	12 (5.7)	
Anxiety	No	85 (81.0)	168 (79.6)	0.897
	Yes	20 (19.0)	43 (20.4)	
Osteoporosis	No	70 (66.7)	113 (53.6)	0.035
	Yes	35 (33.3)	98 (46.4)	

Because the elderly participants still had a fairly independent and autonomous lifestyle, they self-identified as independent. However, they suffered falls more often probably because they enjoyed walking around, given that they felt more self-confident and did not fear being exposed to risk factors (**Table 3**). In this study, the prevalence of falls was higher among the elderly people who self-assessed their health status as good.

Among the individuals evaluated, significant associations were found between occurrences of falls and occurrences of comorbidities and use of medications. Use of several medications was another trait found in our study among the elderly people who sustained falls (**Table 4**).

Significant associations were found between occurrences of falls and use of medications, quantity of medications, use of antihypertensive drugs and use of analgesics. Among individuals who suffered falls, there were higher proportions of use of medications, quantity of medications and use of antihypertensives and analgesics.

Logistic regression analysis

The variables that were significantly associated with occurrences of falls in the univariate analysis underwent logistic regression analysis, in which occurrence of falls was used as a dependent variable. The aim of this analysis was to identify which variables were independent predictors of occurrence of falls, with adjustment for all other variables included in the model. Because of the strong association between the variables of use of medications and dosages of medications, only the variable of use of medications was included in the final model. This decision was

Table 3. Distribution of the elderly people according to the occurrence of falls, in relation to the variable of health status assessment in the municipality of Patos (PB), 2016

Health status assessment	Overall		No falls		With falls		P[1]
	n	%	n	%	n	%	
Very good	83	26.3	53	63.9	30	36.1	
Good	144	45.6	38	26.4	106	73.6	
Normal	84	26.6	13	15.5	71	84.5	< 0.001
Poor	3	0.9	1	33.3	2	66.7	
Very poor	2	0.6	0	0.0	2	100	

[1]Chi-square test.

necessary in order to avoid multicollinearity in the model, thus allowing for thorough implementation (**Table 5**).

From the logistic regression analysis, it could be seen that, after adjustment for all variables included in the model, only the variables of vestibular disorders, self-assessed health status and dizziness/vertigo (trend) were significant. Interpretation of odds ratios (OR) showed that individuals with vestibular disorders presented a 2.23-fold greater risk of falling than those without vestibular disorders. Furthermore, in relation to individuals with very good health status, individuals with good health status had a 3.53-fold higher risk of falling; and individuals with normal health status had a 5.45-fold greater risk of falling. Lastly, individuals with dizziness/vertigo had a 1.87-fold greater risk of falling, in relation to individuals without dizziness/vertigo.

The presence of vestibular disorders, imperfect self-assessed health status and presence of dizziness/vertigo were independent predictors for occurrence of falls. The other predictors, while being statistically significant when evaluated separately through logistic regression, were not statistically relevant.

Table 4. Distribution of medication-related factors, in relation to occurrences of falls. Patos (PB), 2016

Medications	Categories	No falls	With falls	P
n		105	211	
Use of medications	No	17 (16.2)	8 (3.8)	< 0.001
	Yes	88 (83.8)	203 (96.2)	
Number of medications	1 to 2	74 (84.1)	130 (64.0)	0.002
	3 to 4	12 (13.6)	52 (25.6)	
	5	2 (2.3)	21 (10.3)	
Use of antihypertensives	No	33 (31.4)	39 (18.5)	0.015
	Yes	72 (68.6)	172 (81.5)	
Use of hypoglycemic agents/ insulin agents	No	77 (73.3)	133 (63.0)	0.089
	Yes	28 (26.7)	78 (37.0)	
Use of diuretics	No	99 (94.3)	189 (89.6)	0.239
	Yes	6 (5.7)	22 (10.4)	
Use of analgesics	No	70 (66.7)	108 (51.2)	0.013
	Yes	35 (33.3)	103 (48.8)	
Use of sedatives	No	102 (97.1)	202 (95.7)	0.757
	Yes	3 (2.9)	9 (4.3)	
Use of antidepressants	No	105 (100.0)	207 (98.1)	0.306
	Yes	0 (0.0)	4 (1.9)	

DISCUSSION

In this study, the prevalence of the occurrence of falls in the period between 2015 and 2016 was 66.77%. We found that 91.2% of the sample was using some sort of medication, mainly for controlling systemic arterial hypertension, diabetes mellitus or pain. Use of drugs and presence of diseases are two risk factors for occurrences of falls.[10] The prevalence of falls was higher among females and older individuals with lower schooling levels.

The prevalence of falls last year (66.77%), as found in this study, can be considered high, in comparison with other Brazilian studies, in which the reported prevalence has ranged from 28% to 37.5%.[13-15] In a systematic review of the literature, Sandoval

Table 5. Logistic regression analysis using occurrence of falls as a dependent variable. Patos (PB), 2016

Variable	Beta	SE beta	OR	95% CI lower limit	95% CI upper limit	P
(Intercept)	-3.677	1.522	0.03	0.00	0.48	0.016
Schooling level: incomplete primary education	-0.081	0.420	0.92	0.40	2.08	0.846
Schooling level: completed primary education	0.300	0.535	1.35	0.47	3.91	0.575
Schooling level: incomplete secondary education	-0.245	1.091	0.78	0.10	8.16	0.822
Schooling level: completed secondary education	-0.427	0.606	0.65	0.20	2.14	0.481
Schooling level: incomplete higher education	14.473	1455.398	Inf	0.00	NA	0.992
Schooling level: completed higher education	-0.157	0.671	0.85	0.23	3.21	0.815
Age	0.031	0.019	1.03	0.99	1.07	0.096
Systemic arterial hypertension (yes)	-0.298	1.043	0.74	0.09	6.07	0.775
Arthrosis (yes)	0.362	0.319	1.44	0.77	2.71	0.256
Vestibular disorder (yes)	0.801	0.400	2.23	1.04	5.03	0.045
Osteoporosis (yes)	0.244	0.297	1.28	0.71	2.29	0.410
Use of medications (yes)	0.570	0.640	1.77	0.51	6.38	0.373
Use of antihypertensives (yes)	0.338	1.051	1.40	0.16	11.27	0.748
Use of analgesics (yes)	-0.054	0.318	0.95	0.51	1.77	0.866
Health status assessment: good	1.261	0.348	3.53	1.80	7.06	< 0.001
Health status assessment: normal	1.696	0.438	5.45	2.35	13.19	< 0.001
Health status assessment: poor	0.634	1.400	1.89	0.13	50.31	0.650
Health status assessment: terrible	15.668	1023.691	Inf	0.00	NA	0.988
Dizziness/vertigo (yes)	0.625	0.318	1.87	1.00	3.51	0.050

SE = standard error; OR = odds ratio; CI = confidence interval.

et al.[16] found that the prevalence of falls among elderly residents living in the community ranged from 15.9% to 56.3%. A lower prevalence was found in the United States, while the highest prevalence was found in Brazil. In Europe, and more specifically in Spain and Italy, the prevalence was found to be 30.5% to 31.8%.[17] In Africa, a study conducted in Nigeria showed a prevalence of 23%.[18] In Asia, a study conducted in China showed a prevalence of 26.4%.[19] Differences in prevalence between studies have not been analyzed in depth and may have been caused by different study designs and varying methodologies.

With regard to self-reported pre-existing chronic comorbidities or diseases, the proportions of these that showed associations with other pathological conditions were as follows: systemic arterial hypertension (77.2%), osteoporosis (42.1%), diabetes (35.1%), arthrosis (32.6%), vestibular disorders (26.3%), anxiety (22.5%) and urinary incontinence (92.3%). It is important to emphasize that the elderly participants could choose to report the presence of more than one comorbidity. Among those that we evaluated, systemic arterial hypertension, osteoporosis, diabetes, arthrosis, vestibular disorders, thyroid problems and urinary incontinence were statistically significant for the risk of falls.

In the present study, we found that the elderly participants had low levels of schooling, and that 49.4% had not finished elementary school. This was found to be relevant regarding occurrences of falls. Lopes,[20] in Uberaba, in the state of Minas Gerais, Brazil, also found a high percentage of low levels of schooling among elderly people living in the community, and that 43.1% of them were illiterate. The percentage in their study was slightly lower than what we found in our study, but it was still a significant predictor of falls in that population. In a cohort study conducted on 1,415 elderly people in the city of São Paulo, Brazil, Perracini and Ramos[21] found that most of the elderly people who had had falls were illiterate and fell recurrently. Their finding was similar to our own in this study. Marin et al.[22] and Freitas et al.[23] also considered that the level of schooling was an important factor that deserved to be highlighted, since it might have made it more difficult to provide care services, given that such clients would be less involved in their own care and in the risk prevention process.

Development of multiple chronic pathological conditions and comorbidities are among the consequences that accompany aging, along with intake of various medications. Chronic diseases can most often cause systemic problems that require regular use of more than one medication, and this contributes towards occurrences of falls.

The presence of comorbidities is a risk factor, and systemic arterial hypertension is an aggravating factor for the risk of falls and fractures. Hence, given the high prevalence of systemic arterial hypertension in the population of the present study, there is a need for hypertensive patients to be better monitored for the pharmacological interactions among antihypertensive drugs, by medical and healthcare professionals. The aim in doing this is both to improve their health status and to reduce the occurrence of falls.[24]

Tests to assess the decline in visual function with age were not used in the present study because most of the elderly participants did not report any complaints regarding their vision. We consider this to be a weak point in the present study.

In assessing patients using Dowton's Fall Risk Score scale,[10] we found that among the elderly people who had reported previous falls, such reports were more frequent among those who had been using hypertensive medications. Regarding the time that these individuals took to do the TUG test, their average time was found to be 11.4 s and the standard deviation was 3.5 s. Upon applying the TUG cutoffs, most of these elderly people, i.e. 177 (56.0%) were classified as being at medium risk of falls.

Several studies have addressed medications as a major risk factor for falls. In a study carried out in Ribeirão Preto, in the state of Sao Paulo, Brazil, Fabrício et al.[25] observed that 70% of the elderly participants were using some type of medication before falling and that 42% of them used polypharmacy. This corroborates our findings.

The main causes of falls that were self-reported by the elderly participants were the following: dizziness (42.7%), carelessness (24.2%), slipping (14.7%), imbalance (11.8%) and alcohol consumption (3.8%). We were able to group these causes into extrinsic and intrinsic factors that led to falls. The intrinsic factors of dizziness and (to a smaller but significant extent) imbalance predominated and were more prevalent than the extrinsic factors. This corroborate the findings of Fhon,[26] who also observed higher prevalence of intrinsic risk factors. Among the extrinsic factors, carelessness and slipping can, according to Guimarães,[27] be triggered by the aging process. This process alters gait patterns, limits the amplitude of dorsiflexion of the ankles and reduces strength.

Despite the significant aspects of falls among the elderly and their associated factors that were identified in our study, memory bias may have interfered in the results from this evaluation, given that the occurrences of falls recorded here were based on self-reports. The cross-sectional design was also one of the limitations of the study, since this design does not allow for direct assessment of the possible cause and effect relationship between the predictors of falls. Nevertheless, the direction and magnitude of these associations may contribute towards identifying important risk factors that are useful for preventing occurrences of these falls among the elderly at home.

CONCLUSION

Falls and fractures among elderly people are multifactorial. This study showed that the prevalence of falls was high and associated with comorbidities. High blood pressure was found to be a preponderant risk factor. Therefore, interdisciplinary actions to meet the needs of the elderly are essential for preventing falls.

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Address for correspondence:
Alba Rejane Gomes de Moura Rodrigues
Faculdade de Ciências Médicas da Santa Casa de São Paulo (FCMSCSP)
Rua Dr. Cesário Motta Jr., 61
Vila Buarque - São Paulo (SP) — Brasil
CEP 01221-020
Tel. (+55 11) 3367-7700
E-mail: rejanegomesmoura@gmail.com

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Integrated care pathway for rectal cancer treatment: cross-sectional post-implementation study using a logic model framework

Silvia Takanohashi Kobayashi[I], Alessandro Gonçalves Campolina[II], Maria del Pilar Estevez Diz[III], Patrícia Coelho de Soárez[IV]

Instituto do Câncer do Estado de São Paulo (ICESP), São Paulo (SP), Brazil

[I]MD, MSc. Ophthalmologist, Centro de Investigação Translacional em Oncologia, Instituto do Câncer do Estado de São Paulo (ICESP), Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, BR.
orcid.org/0000-0001-8864-051X

[II]MD, MSc, PhD. Scientific Researcher, Centro de Investigação Translacional em Oncologia, Instituto do Câncer do Estado de São Paulo (ICESP), Faculdade de Medicina FMUSP, Universidade de Sao Paulo (FMUSP), Sao Paulo, SP, BR.
orcid.org/0000-0002-0233-0797

[III]MD, PhD. Attending Physician, Centro de Investigação Translacional em Oncologia, Instituto do Câncer do Estado de São Paulo (ICESP), Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, BR.
orcid.org/0000-0003-3176-9747

[IV]MPH, PhD. Associate Professor, Department of Preventive Medicine, Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, BR.
orcid.org/0000-0001-8383-0728

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ABSTRACT

BACKGROUND: Management of rectal cancer has become more complex with multimodality therapy (neoadjuvant chemoradiotherapy and surgery) and this has led to the need to organize multidisciplinary teams. The aim of this study was to report on the planning, implementation and evaluation of an integrated care pathway for neoadjuvant treatment of middle and lower rectal cancer.

DESIGN AND SETTING: This was a cross-sectional post-implementation study that was carried out at a public university cancer center.

METHODS: The Framework for Program Evaluation in Public Health of the Centers for Disease Control and Prevention (CDC) was used to identify resources and activities; link results from activities and outcomes with expected goals; and originate indicators and outcome measurements.

RESULTS: The logic model identified four activities: stakeholders' engagement, clinical pathway development, information technology improvements and training programs; and three categories of outcomes: access to care, effectiveness and organizational outcomes. The measurements involved 218 patients, among whom 66.3% had their first consultation within 15 days after admission; 75.2% underwent surgery < 14 weeks after the end of neoadjuvant treatment and 72.7% completed the treatment in < 189 days. There was 100% adherence to the protocol for the regimen of 5-fluorouracil and leucovorin.

CONCLUSIONS: The logic model was useful for evaluating the implementation of the integrated care pathways and for identifying measurements to be made in future outcome studies.

INTRODUCTION

Colorectal cancer is the third leading type of cancer worldwide, accounting for about 1,200,000 new cases and 600,000 deaths annually.[1] According to the National Cancer Institute of Brazil,[2] approximately 34,280 new cases of colorectal cancer were expected to occur in this country in 2016.

About 25% of occurrences of colorectal cancer are located in the rectum. Over the last few decades, there have been major achievements in rectal cancer treatments, with the introduction of neoadjuvant therapy and total mesorectal excision for surgical removal of the tumor. Today, the treatment for middle and lower rectal cancer consists of three phases: first, the staging phase based on colonoscopy, computed tomography (CT) and magnetic resonance imaging (MRI) scans; followed by a second phase of neoadjuvant therapy with concomitant chemotherapy and radiotherapy (nCRT). The last phase is the surgery, including total mesorectal excision.

Integrated care pathways (ICPs) have been adopted into oncology practice as a tool for enhancing both quality and value by limiting undesirable variability and reducing cost, while providing the optimal course of care for a patient's specific diagnosis.[3] ICPs are structured multidisciplinary care plans that detail essential steps in the care for patients with a specific clinical problem. They support translation of clinical guidelines into local protocols and their subsequent application to clinical practice.[4] ICPs have been implemented worldwide, but the reporting of the implementation processes is frequently poor and there is a lack of evidence about their impact.

In the present study, an ICP for neoadjuvant treatment of middle and lower rectal cancer was implemented at a public university cancer center with about 10,000 new cancer patients per year, across the state of São Paulo, Brazil. The planning and implantation of the ICP involved participation by medical oncologists, gastrointestinal surgeons, radiation oncologists, endoscopists,

radiologists, pathologists, anesthesiologists, physicians, nurses, nutritionists, social workers, psychologists and physiotherapists. This multidisciplinary team standardized practices and constructed a flowchart outlining the sequence and timing of consultations, staging procedures, nCRT and surgery (**Figure 1**).

In order to report on the experience of implementing this ICP, a program logic model was used to inform the planning and development of the evaluation process. Logic models are defined as pictures of the way in which planners think their program is going to work. They comprise the theory and assumptions underlying the program.[5]

Logic models originate from the field of program evaluation and are diagrams that convey relationships between contextual factors, inputs, processes, program activities and intended outcomes.[6-8] They may depict all or some of the following basic components: inputs, activities, outputs and outcomes (**Figure 2**). Inputs refer to the resources that go into the program, for it to perform its planned activities, and these can include human, financial, organizational and community resources. Activities refer to processes, tools, events, technology and actions that are implemented through the program and by its staff, in relation to the target population. Outputs are the direct products of program activities, usually measured in countable terms (e.g. the number of multidisciplinary meetings held or the number of first medical consultations booked). Outcomes are the changes that result from the activities and outputs of the program. They describe specific changes to the behavior, knowledge, skills, status and level of functioning of the target population for the program. In summary, logic models are flowcharts that display a logical sequence of steps in program implementation and achievement of desired outcomes.[8]

They have been used in a variety of fields,[9] and there is growing recognition of their importance in the planning, implementation and evaluation of funded programs. For example, the United States Centers for Disease Control and Prevention (CDC) have used logic models[8] to evaluate the effectiveness of public healthcare programs and show the success of these programs in achieving intended outcomes, to key stakeholders.

As far as we know, no studies on ICPs for neoadjuvant treatment of middle and lower rectal cancer, with analysis using program logic modelling, had previously been conducted. Furthermore, standardization of treatment for this type of cancer at our institution was not an easy task: there had been complaints about delays in radiotherapy, examinations and surgery; time interval measurements between the phases of treatment were unknown; and there were difficulties in managing all the steps of the forms of rectal cancer care that were in use.

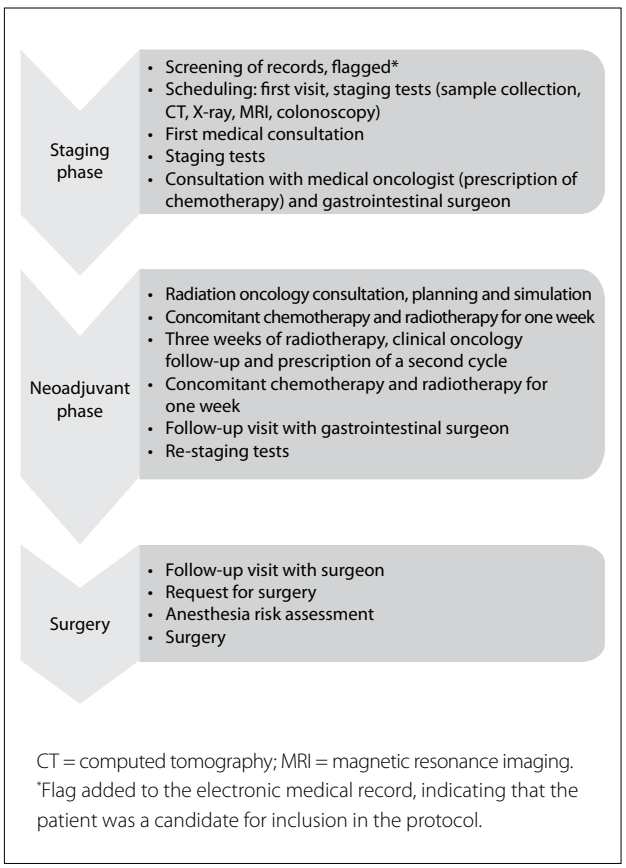


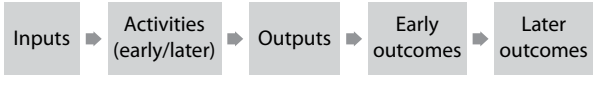
Figure 1. Flowchart of the integrated care pathway for rectal cancer that was started at the Instituto do Câncer do Estado de São Paulo, São Paulo, Brazil, in 2011.

OBJECTIVE

The aim of this study was to report on the planning, implementation and evaluation of an ICP for rectal cancer treatment, using a logic model.

METHODS

This is a cross-sectional post-implementation study reporting the implementation of an administrative and healthcare program of cancer care at the Cancer Institute of the State of São Paulo (Instituto do Câncer do Estado de São Paulo, ICESP), São Paulo, Brazil, 2011-2013. A program logic model approach was adopted with the aim of designing an evaluation that would focus on relevant healthcare outcomes (access to care, effectiveness of care and organizational outcomes) and factors that were involved in achieving these outcomes, using the CDC's Framework for Program Evaluation in Public Health.[8] Thus, a set of flow charts



Source: Adapted from: Centers for Disease Control and Prevention. Introduction to program evaluation for public health programs: A self-study guide. Atlanta, 2011.

Figure 2. Logic model.

that displayed the sequence of logical steps and desired outcomes was used to link the key elements of the model: inputs, activities, outputs, early outcomes and later outcomes.

In the present study, the development of the logic model began with a review of the literature. This identified thinking, policy and research relating to colorectal cancer treatment and the role of ICPs in the delivery of care, both in Brazil and in other countries. It also involved a review of policy and program documents and one-to-one interviews with a sample of six managers and thirteen healthcare professionals involved in the development and delivery of the ICP. This phase resulted in identification of program goals, objectives and inputs.

The inputs were listed as the service users (patients included in the care pathway) and the resources (human resources and facilities) that were needed to carry out activities (**Table 1**).

Table 1. Logic model – Inputs and activities

Background	**Period prior to implementation of the integrated care pathway:** A public teaching hospital specializing in oncology opened its doors in May 2008, to treat public healthcare system patients who had been diagnosed with cancer. Patients were admitted by medical oncologists or surgeons. Although the established multimodal treatment for middle or lower rectal cancer consisted of neoadjuvant chemoradiotherapy followed by surgical resection, there was no coordination between the phases, which harmed the continuity of care. Until 2010, radiotherapy was done in a different service.
Goal	To implement an integrated care pathway for neoadjuvant treatment of rectal cancer, consisting of radiotherapy with 5040 cGy delivered in 28 fractions (540 cGy in the boost phase and 4500 cGy in the pelvic phase), over a five-week period. Concomitant chemotherapy (FULV regimen[12] with 350 mg/m^2 of 5-fluorouracil and 20 mg/m^2 of leucovorin) was delivered as two five-day courses during the first and fifth weeks of radiotherapy. Surgery with total mesorectal excision consisted of open rectosigmoid resection (ORR), laparoscopic rectosigmoid resection (LRR) or abdominoperineal resection (APR).
Objectives	To manage all steps of the treatment for middle and lower rectal cancer and provide multidisciplinary continuity of care.
Inputs	
1. Service users	**Inclusion Criteria**: · Patients with rectal cancer **Exclusion Criteria**: · Patients with metastatic disease at diagnosis · Patients who were unable to undergo neoadjuvant treatment: clinical condition precluded the use of nCRT; or immediate surgery was indicated; or a rapid course of neoadjuvant radiotherapy was indicated · Patients who had previously been treated for cancer · Patients who had not adhered to the nCRT regimen
2. Resources	**Human Resources**: medical oncologists, gastrointestinal surgeons, radiation oncologists, endoscopists, radiologists, pathologists, anesthesiologists, physicians, nurses, dieticians, social workers, psychologists, physiotherapists, hospital administrators and data managers
	Facilities: chemotherapy sector, radiotherapy sector, operating rooms, inpatient units, consultation rooms, imaging service and electronic medical records
Activities	
1. Stakeholder engagement	· Clinical staff engagement: Multidisciplinary meetings were held under the leadership of a board of directors. Medical oncologists, surgeons, radiation oncologists, radiologists, pathologists, clinicians and anesthesiologists reviewed the neoadjuvant treatment protocol for middle and lower rectal cancer and defined the intervals between the phases of the treatment.
2. Clinical pathway development	· An integrated care pathway was designed as a flowchart by the administrative group. · Identification of patients' input into the clinical pathway · Definition of the time interval between record screening and the first medical consultation · Booking first medical consultations on the pathway · Staging test standardization · Definition of term reports · Sharing of chemotherapy and radiotherapy session schedules · Active monitoring of surgery requests · Definition of time interval between neoadjuvant treatment and surgery
3. Information technology improvements	· Enablement of pathway patient identification using a flag added to the electronic patient charts · Development of a report to identify pathway patients who have consultations and tests scheduled · Development of a report to identify pathway patients who do not have any scheduling · Development of a report to calculate dates of future steps on the pathway, to help in reception sector scheduling · Development of the flag deactivation process
4. Training program	· Training program for outpatient reception workers to enable schedule tests and consultations in accordance with the flowchart · Training program to enable use of the reports that have been developed · Training program to activate flags: regulation sector · Training program to deactivate flags: physicians

The next phase involved identification of all the activities (services or interventions) that were developed as requirements for fulfilling the implementation goals. The direct results from the activities (outputs) were linked to expected goals, which originated indicators and measurements for evaluating pathway outcomes (**Table 2**). The results from the activities (outputs) were evaluated in accordance with the goals and were expressed as the percentage of patients who achieved the goal.

All consecutive patients with middle or lower rectal cancer who were admitted to the public university cancer center between May 2011 and December 2013 were evaluated. These patients were named the ICP group (ICPg). Patients who had undergone prior treatment, those who had not undergone nCRT treatment and those who presented metastatic disease at diagnosis were excluded.

A single database in Microsoft Excel was built up, using information extracted from the following electronic healthcare records: Tasy system (Philips Clinical Informatics, Blumenau, Brazil), Laserfiche document scanning system (Long Beach, CA, USA), Mosaiq radiation therapy system (Elekta AB, Stockholm, Sweden) and the hospital cancer registry (HCR). The following information

Table 2. Pathway implementation – outputs, outcomes and measurements/indicators

Concept and evaluation criteria	Measurements/indicators	Goal	Description	Source
Outputs				
1. Pathway implantation	Time interval between electronic medical record flagging and first consultation (days)	≤ 15 days	mean, % patients within the target	electronic health records
	Time interval between first medical consultation and start of neoadjuvant treatment (days)	≤ 46 days	mean, % patients within the target	electronic health records, radiation therapy system
	Time interval between start and end of neoadjuvant treatment (days)	≤ 45 days	mean, % patients within the target	radiation therapy system
	Time interval between end of neoadjuvant treatment and surgery (weeks)	≤ 14 weeks	mean, % patients within the target	electronic health records
	Time interval between first medical consultation and surgery (days)	≤ 189 days	mean, % patients within the target	electronic health records
	Adherence to FULV regimen protocol for concomitant chemotherapy	100%	% adherence	electronic health records
Early outcomes				
1. Access to care	Time interval between admission and first consultation (days)	Comparison of the measurements of pathway implementation with the period before ICP implementation	mean, % patients within the target	electronic health records
	Time interval between first medical consultation and start of neoadjuvant treatment (days)	Comparison of the measurements of pathway implementation with the period before ICP implementation	mean, % patients within the target	electronic health records
	Time interval between end of neoadjuvant treatment and surgery (weeks)	Comparison of the measurements of pathway implementation with the period before ICP implementation	mean, % patients within the target	electronic health records
2. Effectiveness of care	Time interval between first medical consultation and surgery (days)	Comparison of the measurements of pathway implementation with the period before ICP implementation	mean, % patients within the target	electronic health records
3. Organizational outcomes	Resource use	Comparison of the measurements of pathway implementation with the period before ICP implementation	numbers of consultations, CT scans, MRI scans, colonoscopies and radiotherapy sessions	administrative database
Later outcomes				
1. Effectiveness of care	Overall survival time	Comparison of the measurements of pathway implementation with the period before ICP implementation	survival curves	retrospective cohort study
2. Organizational outcomes	Cost evaluation	Comparison of the measurements of pathway implementation with the period before ICP implementation	cost of treatment	retrospective cost analysis

FULV: 350 mg/m^2 of 5-fluorouracil and 20 mg/m^2 of leucovorin, which was used as the regimen protocol for concomitant chemotherapy.
CT = computed tomography; MRI = magnetic resonance imaging; ICP = integrated care pathway.

was obtained: the date when the patient was included in the ICPg, the date of the first medical consultation, the start and end dates of nCRT and the date of the surgery.

Statistical analyses were performed using the Statistical Package for the Social Sciences, version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Qualitative variables were shown as counts and percentages. Means, medians, standard deviations and 95% confidence intervals (95% CI) were calculated for quantitative variables. The significance level adopted for all statistical tests was 5%.

This study was approved by the Research Ethics Committee of the University of São Paulo (São Paulo, Brazil), under protocol no. 126/14, on May 9, 2014.

RESULTS

The clinical staff and the administrative team were the stakeholders in developing the ICP. They were the people with an interest in the results from the evaluation and were the intended users of its findings. A board of medical oncologists, surgeons, radiation oncologists, radiologists, pathologists, clinicians and anesthesiologists was assembled with the aim of reviewing the neoadjuvant treatment protocol for middle and lower rectal cancer. Multidisciplinary meetings were held under the leadership of the board to discuss treatment steps and intervals between phases.

The clinical protocol consisted of radiotherapy, with 5,040 cGy delivered in 28 fractions (540 cGy in the boost phase and 4,500 cGy in the pelvic phase), over a five-week period. Concomitant chemotherapy (FULV regimen,[10] comprising 350 mg/m^2 of 5-fluorouracil and 20 mg/m^2 of leucovorin, intravenously, on days D1-D5) was delivered in two courses during the first and fifth weeks of radiotherapy. The surgery, with total mesorectal excision, consisted of open resection (ORR), laparoscopic resection (LRR) or abdominoperineal resection (APR) of the rectum and sigmoid.

The team of medical oncologists, surgeons and regulators established a record screening system in order to include patients in the ICPg. In this, a stamp placed on the patient's admission chart was used to signal and identify new patients for the regulation sector.

Based on the ideal 15-day interval for the first consultation that had been established by the medical team, the outpatient reception sector reserved vacancies within the medical schedules to guarantee slots for first consultations with the medical oncologist and gastrointestinal surgeon for ICPg patients.

The medical oncologists and surgeons defined the types and quantities of laboratory tests, imaging scans and colonoscopies for staging. In accordance with the opinions of radiologists, pathologists, endoscopists and reception staff, they defined a desirable range of 15 days between the tests and the follow-up medical consultation.

The radiotherapy and chemotherapy sectors, nurses and reception staff developed a shared spreadsheet for concomitant sessions of chemotherapy and radiotherapy. Their aim was to ensure mutual real-time viewing of both sectors in the first and fifth weeks of neoadjuvant treatment.

The surgery scheduling sector developed a worksheet to monitor ICPg patients. This contained the following information: start and end dates of the nCRT, expected date for surgery, expected date for clinical and anesthesia risk assessment, date of clinical and anesthesia risk assessment, expected date for surgical scheduling request and date of the surgery. The goal of the worksheet was to monitor and advise patients based on the expected dates, in order to schedule the procedure at a time close to the ideal for performing the surgery after nCRT.

Improvements to the Tasy electronic health record system needed to be developed by the information technology sector in order to accomplish the ICP. An alert in the patients' medical chart, called a flag, was created to allow both the administrative and the care team to identify each ICPg patient. This flagging would appear on the initial screen for patient chart users, as the following information: "Integrated care pathway for neoadjuvant treatment of rectal cancer". It was decided through reaching a consensus that the regulation sector would be responsible for inserting the flagging.

Several reports were developed within the Tasy electronic health record system. A report was created to make it possible to see which patients had been flagged but did not have any consultations scheduled. This was used by the administrative team to recall patients who were in this situation. Another report was developed to allow outpatient scheduling by the reception sector without the need for the flowchart steps and deadlines to be memorized. This report calculates the dates of future steps from the first medical consultation, according to the schedules available.

It was also necessary to develop a process to deactivate the flagging in the cases of patients who should not be in the pathway (due to metastatic disease, for example) or patients who had completed the pathway: doctors and/or the regulation sector staff could proceed with deactivation of the flagging.

A training program was developed in order to introduce the ICP step-by-step to the administrative sectors. Outpatient reception sectors were systematically trained to use the reports, with the aim of ensuring correct scheduling of medical consultations and tests.

The regulation sector staff was trained to flag eligible patients who had been identified by the medical teams through screening the documentation of patients who had been referred to the hospital.

The information technology sector developed a training manual for flag deactivation. This manual was presented at medical meetings and has been made available via e-mail to medical oncologists and surgeons.

Table 1 shows the inputs and activities of the logic model for ICP implementation.

Table 2 shows the outputs, outcomes and indicators selected for evaluating pathway implementation: five relating to time interval

measurements between the phases of treatment and one relating to adherence to the FULV regimen protocol for concomitant chemotherapy. An indicator target was established by the multidisciplinary team during the development of the ICP. Measurements of access to care, effectiveness of care and organizational outcomes were selected for evaluating the early and late outcomes (**Table 2**).

Table 3 presents the results from the measurements and indicators relating to ICP implementation. A total of 413 patients who had been diagnosed with rectal cancer were admitted to the service between May 2011 and December 2013. Among these, 195 were excluded (92 whose clinical condition precluded the use of nCRT or who required immediate surgery; 21 who had previously been treated for cancer; 74 who presented metastatic disease at the time of the diagnosis; and eight who had not adhered to the nCRT regimen). Therefore, the measurements involved 218 patients, who were named the ICP group (ICPg): 66.3% had their first consultation within 15 days after admission; 67.9 started the nCRT within 46 days after their first consultation; 89.9% completed the nCRT regimen within 45 days; 75.2% underwent surgery within 14 weeks after the end of neoadjuvant treatment; and 72.7% completed the treatment within 189 days. The rate of adherence to the FULV regimen protocol was 100%.

Table 3. Measurements/indicators of pathway implementation among patients treated for rectal cancer at the Instituto do Câncer do Estado de São Paulo, São Paulo, Brazil, 2011-2013

Measures/indicators	Indicator goal	% ICP within goal	ICP (n = 218)
Δ EMR flagging - first consultation (days)			
mean (SD)	≤ 15 days	66.3	12.7 (8.8)
95% CI			(11.5-14.1)
median			13.0
Δ first consultation - start of nCRT (days)			
mean (SD)	≤ 46 days	67.9	48.4 (29.8)
95% CI			(44.3-52.3)
median			39.0
Δ first - last nCRT session (days)			
mean (SD)	≤ 45 days	89.9	40.1 (6.7)
95% CI			(39.2-41.0)
median			39.0
Δ last nCRT session - surgery (weeks)			
mean (SD)	≤ 14 weeks	75.2	14.8 (4.6)
95% CI			(14.2-15.4)
median			13.2
Δ first consultation - surgery (days)			
mean (SD)	≤ 189 days	72.7	192.0 (45.8)
95% CI			(185.9-198.1)
median			177.0
Adherence to FULV1 regimen protocol	100%	NA	100%

ICP = integrated care pathway: patients were admitted between May 12, 2011, and December 31, 2013 (after implementation of the ICP); EMR = electronic medical record; NA = not applicable; nCRT = neoadjuvant chemoradiotherapy; Δ = time interval between; [1]FULV = 350 mg/m^2 of 5-fluorouracil and 20 mg/m^2 of leucovorin.

DISCUSSION

ICP is an administrative and care milestone that combines administrative support with care needs in order to ensure multidisciplinary care. Implementation of a clinical pathway within daily practice is challenging, especially in public hospitals with high demand and limited resources.

Regarding pathway implantation, the initial activity of engaging stakeholders showed that there was a need to standardize and disseminate the clinical pathway between the various medical specialties and find solutions to ensure that the treatment steps were achieved. Previously, referral to another team or to the next stage was done only after the end of the preceding stage. Some adaptations were made because of a lack of time resources: for example, the medical oncologists prescribed chemotherapy until chemoradiotherapy sessions started to be scheduled, because of difficulties in coordinating the sessions. Scheduling the surgery at the right time after neoadjuvant treatment was also a challenge.

In this regard, the National Comprehensive Cancer Network, through its clinical practice guidelines for oncology, advocates a multidisciplinary approach involving oncologists, gastroenterologists, surgeons, radiation oncologists and radiologists.[11] Some institutions have organized their multidisciplinary teams through systematic meetings, in the form of "tumor boards".[12] However, there is a lack of research demonstrating the effectiveness of the multidisciplinary approach.[13-16]

To develop the clinical pathway, administrative support was necessary to ensure that the flowchart design defined by the medical teams within daily practice was implemented. The care teams (multiprofessional and medical) raised any critical issues and needs that had to be resolved.

Regarding inputs and activities, communication problems between the teams were a barrier that needed to be overcome. The gap between the care team and the administrative team is an aggravating factor: on one hand, the care team perceives the administrative team to be a bureaucratic control sector focused exclusively on productivity; on the other hand, the administrative team perceives the care professionals to be technical experts who excessively request supplementary tests and resources without having any management experience. Data in the literature have demonstrated that there is a need for evaluation studies on clinical pathways, in order to check the proposed interventions, behavioral changes and context, and to identify the critical success factors.[17]

Flagging (i.e. stamps that were placed on the regulatory documentation) and shared spreadsheets were simple solutions that were developed to enable communication between the stakeholders. Another critical point was the need to rationalize resources and processes. Previously, a diversity of diagnostic tests had been requested by doctors and there had been delays in issuing imaging examination reports. Through defining staging tests and interval deadlines, rational use of resources became possible.

The training program was especially necessary because of the high turnover rate in the outpatient reception sector. Several meetings were held during the implementation of the ICP: between medical teams, between medical and care teams and between care and administrative teams. These interactions brought the various professionals together and facilitated mutual understanding of their respective attributions, thus placing value on the importance of each professional within the strands of the clinical pathway.

Finally, regarding outputs and outcomes, it has been pointed out in the literature that there is a lack of indicator descriptions for colorectal cancer protocols. Ludt et al.[18] developed a list of 52 quality indicators to cover relevant aspects of the treatment of colorectal cancer, among which 11 related to diagnostic procedures, 28 to therapeutic management, six to follow-up and seven to the patient's perspective. These authors noted that there was some difficulty in putting the indicators into operation because of a lack of data source specification and collection methods. They also showed that indicators focusing on the surgical treatment predominated and pointed out that there was a need to measure the quality of care.

In this study, the indicators showed opportunities for improvement. Specific studies and actions are needed in order to increase the percentage of patients with ranges of values for these indicators that are within the targets.

Management of middle and lower rectal cancer has become complex with the multimodality therapy of nCRT and surgery. This has led to a need to monitor access to all phases of the treatment. Eldin et al. showed that there were difficulties in relation to adherence to treatment guidelines among stage II/III rectal cancer patients in Alberta, Canada, because of lack of access to medical oncologists among patients, and the distance from these patients' homes.[19] Gallego-Plazas et al. evaluated rectal cancer treatment in a tertiary-level hospital and pointed out that delays in the intervals between the different phases of treatment and lack of coordination were critical factors.[20]

In relation to effectiveness of care, there is no agreement regarding the impact on overall survival of multimodal treatment for rectal cancer. Wiegering et al. reported that increased use of neoadjuvant therapy and total mesorectal excision led to improvement of overall survival.[21] Chang et al. also reported that use of neoadjuvant treatment was increasing but did not find any differences in five-year overall survival.[14]

The organizational outcome indicators selected in the present study were related to resource use and cost evaluation. Although use of integrated care pathways has been correlated with improvement to the quality of care, cost reduction and optimization of resource allocation,[22] few studies have quantified their effectiveness.

Some limitations of the present study can be highlighted. Firstly, early and later outcomes (**Figure 4**) were not evaluated separately. Furthermore, since the purpose of the study was to analyze an ICP implementation process, outcomes before and after the intervention were not compared. Secondly, it might be argued that a wider group of participants could have been included to reflect differences in views among participants from similar backgrounds. Although the proposal to evaluate ICPs for rectal cancer treatment came from a hospital/university joint research network and members of this network formed the research team, inclusion of a wider group of stakeholders in the process generated further ownership and support for the subsequent evaluation. Thus, we believe that this exercise was conducted among a reasonably coherent group of stakeholders, across the range of roles involved in rectal cancer treatment.

We found that the logic model was an effective planning and evaluation tool and a useful project management resource that greatly increases the likelihood that ICP goals would be reached, consistently with these aims. However, some of the difficulties in developing a logic model were significant, including the availability of time among the stakeholders, the requirement for trained staff to conduct the evaluation process and the need for institutional commitment to the project.

Future studies should provide comparisons with the period before the implementation of the ICP, in order to evaluate early outcomes relating to access to care (reduction of the time intervals of the treatment), effectiveness of care (reduction of the total duration of the treatment) and organizational outcomes (resource use).

CONCLUSIONS

Implementation of an ICP for rectal cancer treatment, analyzed by means of a logic model approach, was feasible and informed the design of this complex intervention for evaluation of rectal cancer care.

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Address for correspondence:
Silvia Takanohashi Kobayashi
Instituto do Câncer do Estado de São Paulo
Av. Dr Arnaldo 251
Cerqueira Cesar — São Paulo (SP) — Brasil
CEP 01246-000
Tel. (+55 11) 3893-3550
E-mail: silvia.kobayashi@uol.com.br

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Association between benign prostate enlargement-related storage and voiding symptoms and systolic blood pressure: a single-center cross-sectional study

Eşref Oğuz Güven[I], Ismail Selvi[II], Eda Karaismailoğlu[III]

Department of Urology, Ankara Oncology Training and Research Hospital, Ankara, Turkey

[I]MD. Associate Professor, Department of Urology, Ankara Oncology Training and Research Hospital, Ankara, Turkey.
orcid.org/0000-0003-4919-9383

[II]MD. Urologist, Department of Urology, Karabük Üniversitesi Eğitim ve Araştırma Hastanesi, Karabük, Turkey.
orcid.org/0000-0003-3578-0732

[III]PhD. Research Assistant, Department of Biostatistics, Kastamonu Üniversitesi Tıp Fakültesi, Kastamonu, Turkey.
orcid.org/0000-0003-3085-7809

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ABSTRACT

BACKGROUND: Lower urinary tract symptoms significantly worsen quality of life. The hypothesis that they might lead to serious systolic blood pressure alterations through inducing sympathetic nervous activity has not been studied so far.

OBJECTIVES: To investigate the relationship between benign prostate enlargement-related storage and voiding symptoms and systolic blood pressure.

DESIGN AND SETTING: Cross-sectional single-center study on data from a hospital patient record system.

METHODS: We evaluated the medical records of all consecutive patients with benign prostate enlargement-related lower urinary tract symptoms admitted between January 2012 and December 2017. Storage and voiding symptoms were assessed separately. International Prostate Symptom Score, uroflowmetry, postvoiding residual urine volume and systolic blood pressure were recorded. Pearson correlation and linear regression analysis were used.

RESULTS: Positive correlations were found between systolic blood pressure and all of the storage symptoms. Among these, urgency had the most significant effect. There were 166 patients (41.4%) with urgency for urination, which increased mean systolic blood pressure from 124.88 mmHg (average value in elevated blood pressure group) to 132.28 mmHg (average value in stage-1 hypertension group). Hesitancy in urinating and feeling of incomplete bladder emptying had weak positive correlations with systolic blood pressure. There was a negative correlation between systolic blood pressure and intermittency of urination.

CONCLUSIONS: With increasing numbers of urine storage symptoms, systolic blood pressure also increases, while the opposite occurs for voiding symptoms in patients with benign prostate enlargement. We conjecture that storage symptoms may lead to this increase through inducing sympathetic hyperactivity. Further prospective studies with larger groups are needed to confirm these findings.

INTRODUCTION

Two types of lower urinary tract symptoms are associated with symptomatic benign prostate enlargement: storage symptoms and voiding symptoms. The detrimental effects of storage symptoms on patients' quality of life, which have been recognized as a major burden on healthcare resources, are more significant than voiding symptoms.[1] An association between lower urinary tract symptoms and cardiovascular hyperstimulus based on an overactive sympathetic nervous system has been reported.[2] The filling and voiding cycles of the bladder trigger sympathetic activity and, thus, benign prostate enlargement-related storage and voiding symptoms stimulate autonomic hyperactivity.[3-5]

Since blood pressure elevation indicates sympathetic hyperactivity, this subject has become a focus of interest over recent years.[3-5] The presence of hypertension as a component of metabolic syndrome has been recognized to play a role in the development of severe lower urinary tract symptoms.[6] It has been shown that bladder dysfunction may occur in the presence of endothelial dysfunction in the pelvic vascular system. The mechanism is based on increased sympathetic activity, especially α1-adrenoreceptor activity. This pathway is common for hypertension and severe lower urinary tract symptoms.[6,7] Other studies have demonstrated that there is an association between benign prostate hyperplasia and hypertension via activation of insulin-like growth factor and increased sympathetic nervous system activity.[8-10]

So far, published studies have mainly focused on the International Prostate Symptom Score or, specifically, on the effects of nocturia on the blood pressure.[4,5] On the other hand, to the best of our knowledge, the relationship between each lower urinary tract symptom and systolic blood pressure (SBP) has not been studied. There is a serious lack of information about this topic.

OBJECTIVES

Our aim was to investigate the association between SBP (especially stage-1 hypertension) and each benign prostate enlargement-related storage or voiding symptom, separately.

METHODS

Study design and ethics

Our study was designed as a cross-sectional evaluation of data extracted from our hospital's patient record system. It was conducted after obtaining approval from the local ethics committee at our hospital (protocol number: 2018-05/58; date of approval: May 2, 2018), and in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Checklists compiled using the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) recommendations were also used.

Participants

The data of all 732 patients aged 50 years and over who presented benign prostate enlargement-related lower urinary tract symptoms and were admitted to our urology clinic between January 2012 and December 2017 were assessed.

Patients who presented stage-2 hypertension, diabetes, hyperlipidemia, uncontrolled hypothyroidism, obesity or metabolic syndrome; renal, cardiac, pulmonary, vascular, hepatic or psychiatric diseases; or sleep disorders or active urinary tract infections were excluded in order to eliminate other etiologies that might trigger sympathetic activity. Except for benign prostate enlargement, patients with histories of other urinary diseases, urethral manipulations and strictures or pelvic or cardiovascular surgery were excluded. Patients who were using antihypertensive drugs or alpha-blocker medications that might affect the autonomic nervous system were also excluded from the study in order to avoid the presence of misleading lower urinary tract symptoms and blood pressure measurements.

Blood pressure assessment and hypertension classification

We used the 2017 guidelines of the American College of Cardiology/American Heart Association for blood pressure classification.[11,12] Through these guidelines, all participants were firstly classified into four main groups based on their SBP and diastolic blood pressure (DBP) measurements: normotension (SBP < 120 mmHg and DBP ≤ 80 mmHg), elevated blood pressure (120 ≤ SBP < 130 mmHg and DBP > 80 mmHg), stage-1 hypertension (130 ≤ SBP < 140 mmHg or 80 ≤ DBP < 90 mmHg) and stage-2 hypertension (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg.) Patients with stage-2 hypertension were excluded from the present study because these patients were using antihypertensive medication.

The patients thus selected were divided into three groups with regard to their SBP values. Patients with SBP of 110-119 mmHg (normotension), SBP of 120-129 mmHg (elevated blood pressure) and SBP of 130-139 mmHg (stage-1 hypertension). These were named the first, second and third group, respectively.

Basic vital signs and blood pressure measurements were part of our routine for every patient and these values were systematically recorded. During each visit, measurements were made after the patient had rested for at least five minutes in a warm room while sitting in a back-supported position.[12] Systolic and diastolic blood pressure from the left brachial artery were measured using an automated blood pressure monitor (Tango, SunTech Medical, USA). Because blood pressure changes over time, it was measured at least twice on the same day with an interval of three minutes.[12] After completion of the ultrasound, uroflowmetry and blood tests, the patients made a second visit approximately two weeks later. The average values for systolic blood pressure were recorded. No medication was given during this two-week period, so that the possible effects of alpha-blocker medications on systolic blood pressure were removed.

Lower urinary tract symptom assessment and flow analysis

Following the International Continence Society's classification scheme, patients who experienced urgency for urination, nocturia and high frequency of urination were classified as those with storage symptoms and patients who experienced intermittency of urination, hesitancy in urinating, feeling of incomplete bladder emptying and straining to urinate were classified as those with voiding symptoms. A validated Turkish-language version of the seven-item International Prostate Symptom Score was applied to assess subjective urinary symptoms. Each question was scored from 0 to 5. Total scores in the ranges of 0-7, 8-19 and 20-35 were classified as mild, moderate and severe, respectively.

Uroflowmetric analysis, including peak urinary flow rate, was recorded for every patient, followed by physical examination, digital rectal examination, urinalysis and prostate-specific antigen (PSA) measurement. Uroflowmetric measurements were performed using the Flowmaster Wireless Uroflowmeter (MMS-Medical Measurement Systems, Enschede, Netherlands). Prostate volume and postvoiding residual volume were measured using a 3.5-MHz

transabdominal ultrasound probe (Acuson Sequoia 512; Siemens Medical Solution, Mountain View, CA, USA). This was positioned suprapubically in accordance with the ellipsoid formula, which consisted of multiplication together of the largest anteroposterior diameter (height, H), transverse diameter (width, W) and cephalocaudal diameter (length, L) with 0.524 ($H \times W \times L \times \pi/6$).[13] Among the patients with benign signs from rectal examination and a PSA value < 2.5 ng/ml, those with prostate sizes over 25 g or peak urinary flow rate values under 13 ml/s were classified as patients with benign prostate enlargement.

Sample size calculation and statistical analysis

In order to achieve a power of 80.4% with 92.5% confidence interval for the statistical analysis, the sample size was designed to include at least 83 individuals in each of the three groups of SBP. The calculation yielded a total of 249 individuals.

Statistical analyses were performed using the IBM Statistical Package for the Social Sciences (SPSS), version 21 (IBM, Armonk, NY, USA). Continuous variables were presented as the mean ± standard deviation. The normality of continuous variables was evaluated using the Shapiro-Wilks test. Comparisons of continuous variables were performed using the independent-sample t test and one-way analysis of variance (ANOVA). Comparisons between pairs of categorical variables were made using chi-square analysis. The Pearson correlation coefficient was used to analyze associations between pairs of continuous variables. Multiple linear regression analysis was used to assess multivariate relationships between SBP and other variables. Candidate independent variables for multiple linear regression analysis were identified using univariate analysis. All tests were two-tailed and P-values of less than 0.05 were considered to be statistically significant.

RESULTS

It was determined that 401 patients were eligible for inclusion in this study. **Figure 1** shows the flowchart for our study.

The mean age of all the patients was 57.26 ± 9.36 years. In the presence of urgency of urination, nocturia and high frequency of urination, the SBP values increased and the classification of the patients changed from "elevated blood pressure" to "stage-1 hypertension". However, voiding symptoms did not lead to a similar effect on the systolic blood pressure (**Table 1**).

When the cutoff value was set at 40 g, the increase in prostate volume did not lead to a significant change in SBP (P = 0.305). Similarly, no statistically significant relationship between postvoiding residual volume and SBP was found (P = 0.174) (**Table 1**). Among the seven symptoms, urgency for urination was found to have the most significant effect on SBP with an increase of 6.55 mmHg (P < 0.001). That effect was followed by nocturia, high frequency of urination, hesitancy in urinating and feeling of incomplete bladder emptying with increases of 4.63, 3.33, 2.37 and 1.67 mmHg, respectively. While straining to urinate had no effect on SBP, intermittency of urination led to a decrease in SBP of 1.78 mmHg (**Table 2**). The distributions of each group were presented in relation to the presence of lower urinary tract symptoms and the severity of the International Prostate Symptom Score (**Table 3**). This analysis did not identify any significant effects on SBP from the severity of the International Prostate Symptom Score and the peak urinary flow rate (**Tables 2** and **3**). As the number of storage symptoms increased, the SBP value also increased (P < 0.001, r = 0.536). On the contrary, as the number of voiding symptoms increased, the SBP value decreased (P < 0.001, r = -0.327).

In a subgroup analysis that was composed of 33 patients who only had storage symptoms and 27 patients who only had voiding symptoms, SBP was found to be significantly higher in the patients who only had storage symptoms (135.21 ± 6.10 versus 128.04 ± 7.62 mmHg) (P < 0.001).

DISCUSSION

Many studies have investigated factors such as age and health-related, physical, psychiatric, lifestyle, socioeconomic and metabolic factors that might show associations with storage and voiding symptoms.[3,14-18] Some studies have found divergent results regarding the factors associated with storage and voiding symptoms.[4,5] Previous epidemiological studies have investigated links between sympathetic overactivity and lower urinary tract symptoms.[4,5,19-22] Recently, the effects of lower urinary tract symptoms on sympathetic nervous system activity have been drawing attention.

Some studies have demonstrated that pathophysiological similarities and common pathways exist between lower urinary tract symptoms and autonomic nervous system hyperactivity.[20,21] Animal models have demonstrated that autonomic nervous system activity is an important determinant of prostate growth and, thus, that it is associated with lower urinary tract symptoms.[20,21] McVary et al. reported that changes to the American Urological Association Symptom score, Benign Prostatic Hyperplasia Impact Index score and Quality of Life score had significant effects on systolic and diastolic blood pressure.[21] They found a positive correlation between total symptom scores and blood pressure, although they did not evaluate the effect of each symptom separately. Similarly, in another study, International Prostate Symptom Score and peak urinary flow rate values were identified as positively related variables for diastolic blood pressure, and this finding was associated with increased sympathetic activity.[22]

Systolic or diastolic blood pressure has been presented as a determinant of nocturia.[23] The circadian rhythm of blood pressure has been investigated among patients with benign prostate enlargement, and presence of nocturia has been found to be an

independent risk factor for non-dipper hypertension. Its presence has also been reported to be a poor prognostic factor for cardiovascular morbidity and mortality.[24]

Martin et al. evaluated many factors associated with uncomplicated storage and voiding symptoms. None of the storage and voiding symptoms were found to be significant in terms of SBP and DBP.[3] Based on the effects of thyroid dysfunction on autonomic nervous system activity, they noted that increased modulation of beta-2 adrenergic receptors may lead to voiding symptoms.[25] On the other hand, if their conjecture were correct, this reasoning should also explain a similar relationship between SBP and both voiding and storage symptoms.

According to a rodent model, chronic intermittent hypoxia causes increased HIF-1 alpha synthesis. This alerts the sympathetic neurons and stimulates the release of epinephrine, norepinephrine and other catecholamines. As a result, blood pressure increases.[26] This mechanism is very similar to benign prostate enlargement. Prostate hyperplasia gives rise to inadequate blood flow to prostate cells. It activates chronic hypoxia and ischemia, and many mediators such as HIF-1 alpha, VEGF and TGF beta start to be released. The association between sympathetic systemic activity and lower urinary tract symptoms can be explained by this mechanism.[27] In another animal model, it was shown that hypertensive rats exhibited symptoms of voiding dysfunction and had increased presence of sympathetic neurotransmitters. Alpha-1 sympathetic hyperactivity is a well-known dynamic component of benign prostate enlargement because it increases the smooth muscle tone of the prostate and causes bladder outlet obstruction.[22]

According to Jang et al., in patients with sympathetic hyperactivity, alpha blockers were less effective.[28] They commented that sympathetic hyperactivity was a negative prognostic factor for progression of benign prostate enlargement. However, this correlation was only observed in relation to the total International Prostate Symptom Score. They measured the patients' heart rate variability to determine sympathetic activity, although all of the patients were normotensive.

Our study differs from the previous studies because we investigated each voiding and storage symptom separately, in terms of their association with SBP. We found that storage symptoms were more significant determinants than voiding symptoms, in terms of stage-1 systolic hypertension. Even if these patients have blood pressure values that do not require any medication, they still could experience cardiac problems in their future lives.

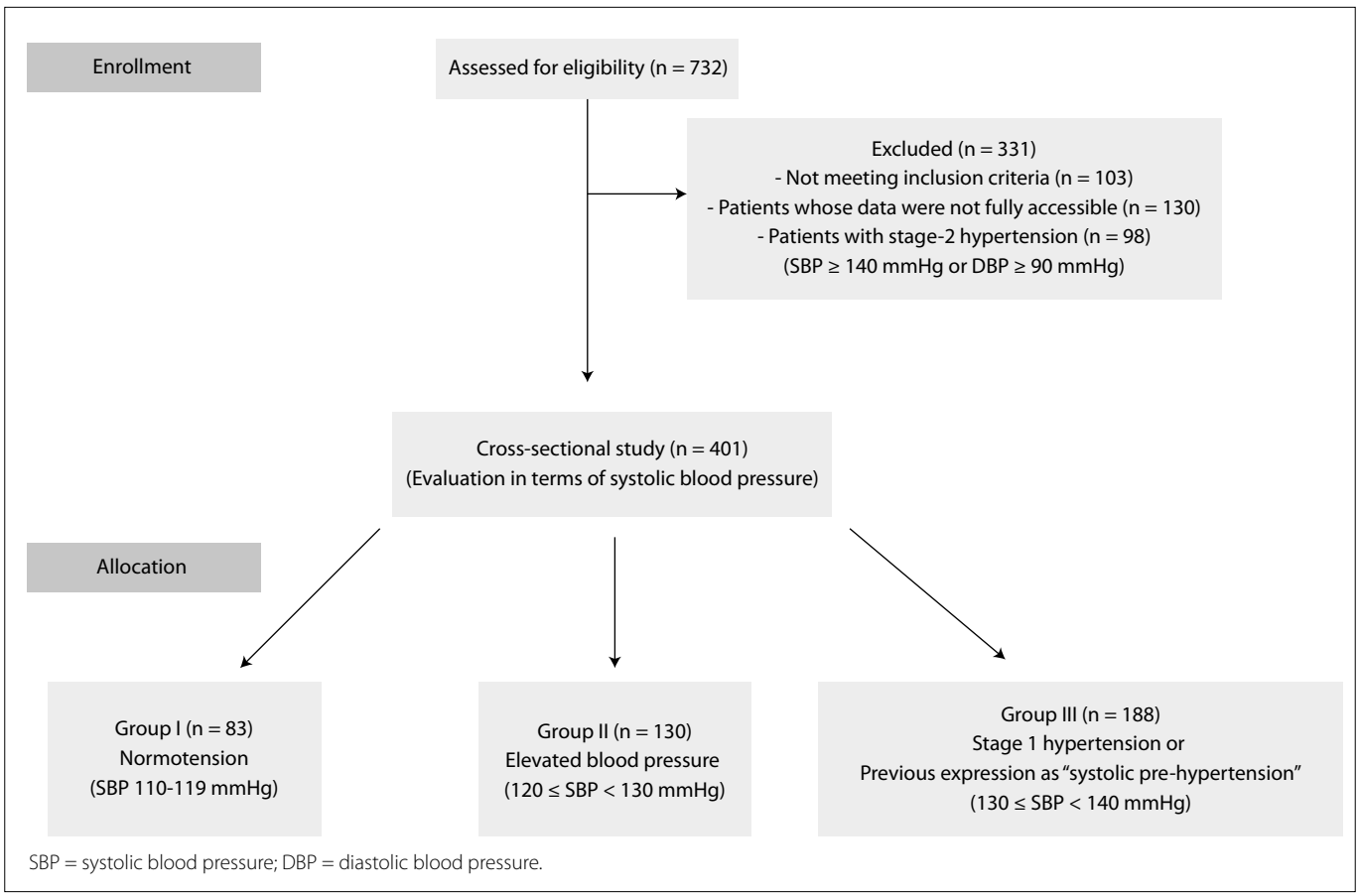

Figure 1. Flowchart of the study population.

According to the 2007 guidelines of the European Society of Cardiology/European Society of Hypertension, and the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, prehypertension was classified as 130 ≤ SBP < 140 mmHg and/or 80 ≤ DBP < 90 mmHg.[12,29] It was recommended that cardiologists should start administering antihypertensive medication when prehypertension is detected. Since then, systolic prehypertension has been the focus of discussion and research. In the 2014 guidelines, the recommendation was that cardiologists should not start treatment with medication based on the presence of systolic prehypertension; instead, very close follow-up was recommended, given that these patients were at very high risk of harboring or developing serious cardiovascular diseases.[12]

The 2017 guidelines of the American College of Cardiology/American Heart Association changed the definition of prehypertension, to create two different stages: elevated blood pressure (120 ≤ SBP < 130 mmHg and DBP > 80 mmHg) and stage-1

Table 1. Changes to systolic blood pressure according to lower urinary tract symptoms, post-voiding residual urine, prostate volume and age

Variable		Mean value of systolic blood pressure ± standard deviation	P-value
Urgency of urination	Absent (n = 235)	124.88 ± 7.50	< 0.001
	Present (n = 166)	132.28 ± 6.32	
Nocturia	Absent (n = 213)	125.06 ± 7.15	< 0.001
	Present (n = 188)	131.22 ± 7.48	
Urgency and nocturia	Absent (n = 296)	125.74 ± 7.26	< 0.001
	Present (n = 105)	134.16 ± 6.21	
High frequency of urination	Absent (n = 281)	126.84 ± 8.01	< 0.001
	Present (n = 120)	130.51 ± 7.08	
Intermittency of urination	Absent (n = 292)	129.02 ± 7.72	< 0.001
	Present (n = 109)	125.06 ± 7.75	
Hesitancy in urinating	Absent (n = 271)	129.04 ± 7.89	< 0.001
	Present (n = 130)	125.66 ± 7.50	
Feeling of incomplete bladder emptying	Absent (n = 306)	128.30 ± 7.96	0.104
	Present (n = 95)	126.79 ± 7.73	
Straining to urinate	Absent (n = 222)	130.10 ± 7.59	< 0.001
	Present (n = 179)	125.27 ± 7.51	
PVR (ml)	< 20 (n = 298)	128.24 ± 7.91	0.174
	21-50 (n = 55)	126.09 ± 8.12	
	> 50 (n = 48)	128.23 ± 7.64	
Prostate volume (g)	25-40 (n = 188)	128.38 ± 8.08	0.305
	> 40 (n = 213)	127.56 ± 7.77	
Age		R = -0.068	0.174

PVR = post-voiding residual urine.
Continuous variables are given as mean ± standard deviation (independent-sample t test). Correlation between age and systolic blood pressure was analyzed using Pearson correlation analysis.

Table 2. Effects of urinary symptoms on systolic blood pressure (linear regression analysis)

Covariates	t-value	β (95% confidence interval)	P-value
Constant	71.38	124.42 (120.99 to 127.85)	< 0.001
Urgency of urination	8.49	6.55 (5.03 to 8.06)	< 0.001
Nocturia	6.03	4.63 (3.12 to 6.14)	< 0.001
High frequency of urination	4.46	3.33 (1.86 to 4.80)	< 0.001
Intermittency of urination	-2.47	-1.78 (-3.75 to -0.43)	0.014
Hesitancy in urinating	2.57	2.37 (0.55 to 4.18)	0.011
Feeling of incomplete bladder emptying	1.80	1.67 (-0.15 to 3.49)	0.016
Straining to urinate	-1.67	-1.71 (-3.73 to 0.31)	0.096
Qmax	-1.91	-0.14 (-0.27 to 0.01)	0.057
IPSS-moderate	-0.54	-0.44 (-2.05 to 1.16)	0.588
IPSS-severe	0.75	-0.76 (-1.23 to 2.75)	0.454

Qmax = peak urinary flow rate; IPSS = International Prostate Symptom Score.
"IPSS-mild" was taken as the reference category, and so the categories of "IPSS-moderate" and "IPSS-severe" were evaluated in relation to the reference value.

hypertension (130 ≤ SBP < 140 mmHg or 80 ≤ DBP < 90 mmHg).[11] Thus, the term "systolic prehypertension" was replaced by "stage-1 systolic hypertension". The alert value requiring follow-up became 130 ≤ SBP < 140 mmHg.

Patients with benign prostate enlargement experiencing lower urinary tract symptoms, who did not need antihypertensive medication, were included in our study. We divided them into subgroups in terms of SBP. Although we did not detect any statistically significant correlation between the International Prostate Symptom score, peak urinary flow rate and SBP when lower urinary tract symptoms were examined individually, the other six symptoms except for straining to urinate were found to have significant effects on SBP. Among these six symptoms, urgency of urination, nocturia and high frequency of urination had the strongest effects on SBP and shifted the blood pressure stage from "elevated blood pressure" to "stage-1 hypertension". The contributions of these symptoms to increased SBP were 6.55, 4.63 and 3.33 mmHg, respectively and these increases were statistically significant. Considering that, according to the new American College of Cardiology/American Heart Association classification, the differences between the groups are only 10 mmHg, and that such differences in SBP (especially in the case of increases) change the therapeutic approach that physicians take, we believe that these results are clinically significant as well.

Hypertension and benign prostatic hyperplasia (BPH) are common age-related diseases. These two pathophysiological conditions coincide in about 25-30% of men over 60 years old.[30] Hypertension increases the risk of moderate-to-severe lower urinary symptoms by 1.5-fold.[31] Conversely, we observed that severe lower urinary symptoms induced higher systolic blood pressure. Because all the patients in our study had blood pressure values under 140 mmHg, there was no need for medication.

We demonstrated that as the number of storage symptoms increased, the SBP value also increased. As the number of voiding symptoms increased, the SBP value decreased. Sympathetic activity was not directly measured in this study, but our findings were compatible with those of other studies that measured sympathetic activity and suggested that sympathetic activity could be triggered via storage symptoms.[4,20,21,28]

When the cutoff value was set at 40 g, we observed that the increase in prostate volume did not lead to a significant change in SBP. This was expected, because there is no direct correlation between prostate volume and the severity of lower urinary tract symptoms. Prostatic hyperplasia is a histological phenomenon and does not always have to cause obstruction, whereas lower urinary tract symptoms are a mixture of neuromuscular changes that are assumed to interact with the autonomic nervous system. Prostate enlargement of solely non-obstructive nature has no direct connection with the neuronal changes that play a major role in lower urinary tract symptoms. It is assumed that prostate enlargement alone does not have an impact on the autonomic nervous system unless it is symptomatic.

We did not find any relationship between postvoiding residual volume and SBP. This may be explained by the low residual urine volumes. Most of the patients were within the normal range and hardly any were above the generally well-accepted limit of 50 ml. At values under 50 ml, there are no SBP-related changes, possibly

Table 3. Distribution of groups in terms of presence of urinary symptoms and IPSS severity

Variable		Systolic blood pressure			P-value
		110-119 mmHg (n = 83)	120-129 mmHg (n = 130)	130-139 mmHg (n = 188)	
Urgency of urination	Absent, n (%)	71 (85.8)	99 (76.2)	65 (34.6)	< 0.001*
	Present, n (%)	12 (14.5)	31 (23.8)	123 (65.4)	
Nocturia	Absent, n (%)	59 (71.1)	90 (69.2)	64 (34.0)	< 0.001*
	Present, n (%)	24 (28.9)	40 (30.8)	124 (66.0)	
High frequency of urination	Absent, n (%)	75 (90.4)	91 (70.0)	115 (61.2)	< 0.001*
	Present, n (%)	8 (9.6)	39 (30.0)	73 (38.8)	
Intermittency of urination	Absent, n (%)	49 (59.0)	90 (69.2)	153 (81.4)	< 0.001*
	Present, n (%)	34 (41.0)	40 (30.8)	35 (18.6)	
Hesitancy in urinating	Absent, n (%)	47 (56.6)	77 (59.2)	147 (78.2)	< 0.001*
	Present, n (%)	36 (43.4)	53 (40.8)	41 (21.8)	
Feeling of incomplete bladder emptying	Absent, n (%)	62 (74.7)	89 (68.5)	155 (82.4)	0.015*
	Present, n (%)	21 (25.3)	41 (31.5)	33 (17.6)	
Straining to urinate	Absent, n (%)	32 (38.6)	56 (43.1)	134 (71.3)	< 0.001*
	Present, n (%)	51 (61.4)	74 (56.9)	54 (28.7)	
IPSS	Mild, n (%)	28 (33.7)	34 (26.2)	78 (41.5)	0.065
	Moderate, n (%)	38 (45.8)	67 (51.5)	71 (37.8)	
	Severe, n (%)	17 (20.5)	29 (22.3)	39 (20.7)	

IPSS = International Prostate Symptom Score.
*Chi-square test.

because there is no irritation effect on the bladder. At values above 50 ml, compensation due to continuous sympathetic stimulation develops and there is inhibition of nerve conduction.[19,32]

Because heart rate variability can be assessed as a non-invasive indicator of sympathetic nervous system function, some researchers have used it to analyze sympathetic activity.[4,28] Thus, they synchronously evaluated the relationship between heart rate variability and lower urinary tract symptoms because heart rate variability reflected spontaneous changes in autonomic activity. In another study, changes to SBP and DBP were recorded one and five minutes after a tilt table test, respectively, to assess alterations in autonomic activity. Thus, the response to circulatory stress was evaluated via the tilt table.[21]

One limitation of our study was that we did not measure the variability of sympathetic activity via heart rate variability or a stress tilt test. We defend our hypothesis based on the findings in the literature. If we had been able to prove that variability in sympathetic activity was present, the power of our study would have become higher, but this was not possible because of our study design. Another limitation of our study was the small number of patients.

To our knowledge, this was the first study to evaluate voiding and storage symptoms separately in terms of their associations with SBP, in patients with benign prostate enlargement-related lower urinary tract symptoms.

CONCLUSIONS

We presume that storage symptoms are directly related to systolic blood pressure levels. When each symptom was analyzed individually, urgency of urination, nocturia and high frequency of urination were found to be correlated with the most significant rises in systolic blood pressure. We also found that the combination of urgency of urination and nocturia had an adjuvant effect and together increased the systolic blood pressure more than each symptom did alone. In accordance with previous studies, we hypothesize that storage symptoms provoke sympathetic system activity and may manifest as stage-1 hypertension, which is an important precursor for cardiovascular diseases. Further prospective studies with larger patient groups are needed to prove this relationship and to show whether treatment of storage symptoms may help in treating stage-1 systolic hypertension.

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Address for correspondence:
Ismail Selvi
Department of Urology, Karabük University Training and Research Hospital
78200 Karabük, Turkey
Tel. +90 0370 415 80 00
Fax. +90 0370 412 56 28
E-mail: ismselvi33@hotmail.com

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Validation of a new tool for evaluating subjects' satisfaction with medicine package leaflets: a cross-sectional descriptive study

Carla Pires[I], Pedro Joel Rosa[II], Marina Vigário[III], Afonso Cavaco[IV]

School of Pharmacy, University of Lisbon, Lisbon and Centre Regions, Portugal

[I]PhD. Pharmacist and Invited Professor, Department of Pharmacotherapy, Research Center for Biosciences and Health Technologies (CBIOS), Universidade Lusófona, Campo Grande, Lisbon, Portugal.
orcid.org/0000-0003-4526-0271

[II]PhD. Psychologist, Statistician and Assistant Professor, Psychology Department, School of Psychology and Life Sciences, Lusophone University of Humanities and Technologies (ULHT), Lisbon, Portugal; Human Environment Interaction Lab (HEI-lab), ULHT, Lisbon, Portugal; Instituto Universitário de Lisboa (ISCTE-IUL), Cis-IUL, Lisbon, Portugal.
orcid.org/0000-0001-8432-1802

[III]PhD. Linguist and Associate Professor, Department of General and Romance Linguistics, School of Arts and Humanities & Centre of Linguistics, University of Lisbon, Lisbon, Portugal.
orcid.org/0000-0001-7640-4798

[IV]PhD. Pharmacist and Associate Professor, Department of Social Pharmacy, School of Pharmacy, Research Institute for Medicines and Pharmaceutical Sciences, University of Lisbon, Lisbon, Portugal.
orcid.org/0000-0001-8466-0484

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ABSTRACT

BACKGROUND: Package leaflets of medicines need to be intelligible, but tools for their evaluation are scarce.
OBJECTIVE: To validate a new tool for assessing subjects' satisfaction with medicine package leaflets (LiS-RPL).
DESIGN AND SETTING: Cross-sectional descriptive study conducted in two regions of Portugal (Lisbon and Centre).
METHODS: 503 participants (53.1% male) were selected according to convenience and homogenously distributed into three groups: 1 to 6; 7 to 12; and > 12 years of schooling. LiS-RPL was developed based on international regulation guidelines and was initially composed of 14 items. Twelve package leaflets were tested. Dimensionality calculations included: exploratory factor analysis and minimum rank factor analysis; Kaiser-Meyer-Olkin index and Bartlett's sphericity test to assess matrix adequacy for exploratory factor analysis; exploratory bifactor analysis with Schmid-Leiman solution to detect possible existence of a broad second-order factor; and Bentler's Simplicity Index and Loading Simplicity Index to assess factor simplicity. Diverse coefficients were calculated to assess reliability.
RESULTS: Minimum rank factor analysis detected a two-factor or single-factor structure. Exploratory factor analysis with 12 items showed a two-factor structure, explaining 69.11% of the variance. These items were strongly correlated with each other (r = 0.80). Schmid-Leiman: all items seemed to represent the general factor (loadings above 0.50), which was 76.4% of the extracted variance. Simplicity indices were good (percentile 99): Bentler's Simplicity Index of 0.99 and Loading Simplicity Index of 0.48. Internal consistency indexes indicated good reliability. LiS-RPL was shown to be homogenous.
CONCLUSION: LiS-RPL is a validated tool for evaluating subjects' satisfaction with medicine package leaflets.

INTRODUCTION

According to the European Guideline on the Readability of the Labelling and Package Leaflet of Medicinal Products for Human Use, package leaflets of medicines (from here on, referred to simply as package leaflets) need to be simple, clear and comprehensible. This is why legibility tests involving groups of patients need to be conducted, so as to ensure that package leaflets are readable.[1] Additionally, in designing package leaflets, special focus should be given to elements such as use of a simple writing style, adequate font size and layout (e.g. line spacing, use of bullet points and consistent headings) and adequate contrast.[2]

Questionnaires using Likert scales (i.e. a psychometric scale for questionnaires comprising an intermediate value and an odd number of alternatives, usually five or seven) are commonly used in readability/legibility tests to evaluate the readability of package leaflets.[3-6] In these tests, the scores of the Likert scales are often used to check readability after package leaflets have been optimized, i.e. test-retest methodology is usually applied.[3,4,6]

These scales are easy to apply and often present high response rates (84-91%) and completion rates (70-85%).[4,7] Another advantage is that 20-item questionnaires in which responses are assessed using a Likert scale can be completed fast: in less than five minutes if the questionnaire is simple. Lastly, these tools give rise to good internal consistency, reliability and construct validity.[7]

Although to our knowledge no Likert scale-based tool has been specifically developed by the European Medicine Agency to specifically evaluate package leaflets in a given language,[1-2] some studies have reported on this type of tool for other materials in general. For instance, DISCERN,

which includes 16 items, and EQUIP, which contains 20 items, can be applied in English, German or Portuguese. DISCERN and EQUIP are used to measure the perceived quality of written health-related information, including its graphical presentation (for EQUIP), as seen by patients and/or by healthcare professionals, but they are not specific for evaluating package leaflets.[8,9]

In contrast, other tools such as the Consumer Information Rating Form were specifically developed to evaluate consumers' perceptions of the comprehensibility, utility and design quality of written information relating to medicines, such as package leaflets. This tool is composed of the following evaluations: perceptions of comprehensibility (five items), intended future use, perceived usefulness of information (eight items) and design quality (seven items). Only the perceived comprehensibility and usefulness of the information are assessed on a five-point scale, from 1 (very hard) to 5 (very easy).[6]

Tools using Likert scales for these purposes have also not been reported for Portuguese package leaflets.[4] Pires et al. found that sociodemographic characteristics explained Portuguese users' opinions of package leaflets: lower socioeconomic status or higher frequency of taking medicines positively influenced participants' overall opinion and/or perception regarding package leaflets.[5] Worryingly, previous studies detected that package leaflets of authorized medicines were too complex, and were difficult to understand and use, thus confirming that even approved package leaflets need to be optimized.[5,10-12]

OBJECTIVE

The aim of this study was to validate a questionnaire (LiS-RPL) that had been designed to evaluate subjects' satisfaction with the readability of package leaflets for medicines.

METHODS

Setting and ethical approval

This study formed part of a larger research project that had previously been communicated to INFARMED, I.P. (the Portuguese medicines agency), and to the National Data Protection Commission (CNPD) of Portugal.[11] Subjects were informed of the nature and general goals of the study and voluntarily agreed to answer the questionnaires. They were also informed that they could leave the study at any moment. Furthermore, this study did not involve administration of medicines or other substances to humans or animals.

It was conducted within the development of a PhD thesis on Pharmacy (Socio-Pharmacy; School of Pharmacy of the University of Lisbon), which was announced in official statement no. 4719/2016 in the Official Gazette of the Portuguese Republic (https://dre.pt/application/file/a/74059402).

Participants

Overall, 503 participants were selected according to convenience, in two Portuguese regions (49.3% of the participants were living in urban areas and 50.7% in rural areas), during 2014. Public and private schools, municipalities, the army or other institutions with a considerable number of collaborators/employees were contacted by email or telephone. In case of acceptance, a day was defined for administering the questionnaires. Around half of the participants were male (53.1%), and the participants' education level was stratified into three groups: 32% had had 1-6 years of schooling; 37%, 7-12 years of schooling; and 31%, more than 12 years of schooling (or higher education in Portugal). More details on the study design can be found in previous studies.[5,10,11]

Instrument

The tool for evaluating the subjects' perceptions of or satisfaction with the readability of package leaflets (LiS-RPL) was developed based on the criteria of the European Guideline on the Readability of Package Leaflets, and on the Consumer Information Rating Form, which is used to evaluate information for consumers, such as package leaflets.[1,6]

Among the items considered in the Consumer Information Rating Form, those referring to intended future use and perceived usefulness of information were not included in our tool, because these issues are not specifically described in the European Guideline on the Readability of Package Leaflets.[1] Furthermore, these issues may be considered to be more subjective and dependent on patients' previous knowledge of their health condition.

Our questionnaire was composed of 14 items, since the European Guideline recommends the development of questionnaires comprising 12 to 15 questions in order not to tire out the participants.

The LiS-RPL analysis is presented in **Tables 1** and **2**, and the Portuguese version in **Table 3**. Each item was classified using a Likert scale of 1-5 to rate the level of satisfaction or perception according to the following labels: 1 = poor; 2 = not very satisfied; 3 = no opinion; 4 = satisfied; and 5 = good (**Table 3**).[3,13] A similar tool was pretested in a previous study (n = 63 participants), in which the opinions of physicians, pharmacists and potential users of medicines regarding the readability of the package insert of an over-the-counter medicine were collected.[3]

In the present study, the labels of satisfaction or perception were redesigned based on the supposition that the use of two labels of quality (poor and good), two labels of satisfaction (not very satisfied and satisfied) plus a neutral point (no opinion) might contribute towards improving participants' understanding. Also, LiS-RPL was pretested on the first 50 study participants. All of the participants used this tool correctly, i.e. no usability issues were identified.

Package leaflets selected for evaluation

The package leaflets that were tested were randomized using the MS Excel software function from a large database used in a previous study (with approximately 500 Portuguese package leaflets).[14] A total of 12 randomized package leaflets were tested: six package leaflets from over-the-counter medicines (three package leaflets comprising more than 1500 words and another three with less than 1500 words) and six package leaflets from prescription medicines (also with either more than or less than 1500 words). The cutoff of 1500 words was defined because previous studies had concluded that package leaflets with fewer than 1500 words tended to be easily read and understood.

These package leaflets (grouped as described above) were distributed equally among three groups of participants that were defined according to their numbers of years of schooling (1-6, 7-12 or > 12).

More details on the selection and type of package leaflets can be consulted in **Table 4** and in the study by Pires et al.[5,10,11] All the package leaflets that were tested were organized in accordance

Table 1. Descriptive statistics for the items that were designed to evaluate subjects' satisfaction with the readability of package leaflets for medicines (LiS-RPL) (n = 469)

Items of LiS-RPL*	Mean	SD	Skewness	Kurtosis
Item 1 - Font size	3.47	1.44	-0.55	-1.14
Item 2 - Font type	4.13	1.11	-1.38	1.13
Item 3 - Layout of the titles of the sections	4.07	1.02	-1.11	0.62
Item 4 - Color of the text	4.30	0.95	-1.53	2.20
Item 5 - Line spacing	3.85	1.23	-0.86	-0.45
Item 6 - Use of the en-dash throughout the text	4.14	0.98	-1.22	1.21
Item 7 - Clarity of the text	3.84	1.16	-0.91	-0.90
Item 8 - Length of the sentences	4.03	1.08	-1.05	0.33
Item 9 - Number of sentences in each paragraph	4.02	1.13	-1.07	0.30
Item 10 - Description of possible side effects	3.90	1.10	-0.87	-0.17
Item 11 - Comprehensibility of medical terms	3.66	1.17	-0.56	-0.74
Item 12 - Clarity of the instructions for the user	3.96	1.07	-0.99	0.24
Item 13 - Use of abbreviations throughout the text	3.45	1.18	-0.31	-0.78
Item 14 - Repetition of the brand name of the medicine throughout the text	4.01	1.01	-0.98	0.46

SD = standard deviation.

*Each item was classified using a Likert scale of 1-5, to assess the level of satisfaction/perception: 1 = poor; 2 = not very satisfied; 3 = no opinion; 4 = satisfied; and 5 = good.

Table 2. Pattern matrix from exploratory factor analysis on the questionnaire that was designed to evaluate subjects' satisfaction with the readability of package leaflets for medicines (LiS-RPL) (n = 469)

Items of LiS-RPL*	Factor 1 Clarity and comprehension of text	Factor 2 Format	h^2
Item 12 - Clarity of the instructions for the user	1.01		0.74
Item 11 - Comprehensibility of medical terms	0.90		0.62
Item 7 - Clarity of the text	0.81		0.61
Item 10 - Description of possible side effects	0.76		0.66
Item 2 - Font type		1.10	0.67
Item 5 - Line spacing		0.86	0.61
Item 4 - Color of the text		0.80	0.61
Item 1 - Font size		0.78	0.34
Item 3 - Layout of the title of the sections		0.70	0.55
Item 9 - Number of sentences in each paragraph		0.57	0.68
Item 8 - Length of the sentences		0.57	0.62
Item 6 - Use of the en-dash throughout the text		0.52	0.55
Eigenvalue	6.74	1.29	-
Total variance explained (%)	56.10	10.76	-

*Each item was classified using a Likert scale of 1-5, to assess the level of satisfaction/perception: 1 = poor; 2 = not very satisfied; 3 = no opinion; 4 = satisfied; and 5 = good.

Table 3. Responda ao questionário assinalando com um número de 1 a 5 a opção que melhor se adequa à sua opinião:

		1 - Mau **2 - Pouco satisfeito** **3 - Sem opinião** **4 - Satisfeito** **5 - Bom**
	Como classifica:	**Responda de 1 a 5**
1.	O tamanho da letra	
2.	O tipo de letra	
3.	A apresentação dos títulos	
4.	A cor do texto	
5.	Os espaços entre as linhas	
6.	A utilização de listas de informações no texto	
7.	A simplicidade da linguagem	
8.	O tamanho das frases	
9.	O tamanho dos parágrafos	
10.	A forma como é dada a informação sobre os efeitos secundários	
11.	A simplicidade dos termos médicos	
12.	A forma de dar instruções ao doente	
13.	A repetição do nome do medicamento ao longo do folheto	
14.	A sua satisfação geral com a forma como a informação é dada	

Table 4. Selection of the leaflets among participants according to education level groups and the features of the package leaflets tested.

Education level	Features of package leaflets - part A						
	Active substances	Therapeutic groups	Pharmaceutical presentations	Type of dispensing	Length (words)**	Font size	Font type
0-6 years of schooling	Gelatin (78 mg or 5532 mg) + glycerol (6.5 g)	Drugs altering gut motility	Rectal gel	Over-the-counter	≤ 1,500 (966 words)	7	Arial
	Minoxidil (50 mg/ml)	Topical products for hair loss	Solution	Over-the-counter	> 1,500 (2,220 words)	8	Arial
	Cefatrizine (50 mg/ml)	Antibacterial drugs	Oral suspension powder	Prescription medicine	≤ 1,500 (1,465 words)	8	Arial
	Ipratropium bromide (0.52 mg/2.5 ml) + salbutamol (3 mg/2.5 ml)	Antiasthmatics	Aerosol	Prescription medicine	> 1,500 (1,683 words)	8	Arial
7-12 years of schooling	Povidone-iodine (100 mg/ml)*	Vaginal disinfectant	Solution	Over-the-counter	≤ 1,500 (1,075 words)	6	Arial
	Oxymetazoline (0.5 mg/ml)	Nasal decongestants	Nasal spray	Over-the-counter	> 1,500 (1,714 words)	8	Arial
	Ofloxacin (3 mg/ml)	Topical antibacterials	Ophthalmic drops	Prescription medicine	≤ 1,500 (1,345 words)	8	Other sans serif
	Clomipramine (10/25/75 mg)	Antidepressants	Tablets	Prescription medicine	> 1,500 (2,699 words)	10	Arial
> 12 years of schooling	Choline salicylate (87 mg/g)	Anti-ulcerants	Oral gel	Over-the-counter	≤ 1,500 (924 words)	6	Arial
	Acetylsalicylic acid (400 mg) + ascorbic acid (240 mg)	Analgesic and antipyretics	Effervescent tablets	Over-the-counter	> 1,500 (2,346 words)	7	Arial
	Dexamethasone (1 mg/ml) + neomycin (10 mg/ml) + polymyxin B (10000 IU/ml)	Topical corticosteroids	Optical drops	Prescription medicine	≤ 1,500 (1,056 words)	8	Arial
	Methylprednisolone (40 mg/ml) + lidocaine (10 mg/ml)	Corticosteroids	Parenteral injection	Prescription medicine	> 1,500 (3,487 words)	7	Arial

*Only female participants received these questionnaires (A and B); ** Package leaflets with ≤ 1,500 words (average = 1,138.5 words, standard deviation = 198.3) and package leaflets with > 1,500 words (average = 2,358.2; standard deviation = 616.5).

Education level	Features of package leaflets - part B								
	Active substances	Layout of the title	Color	Line Spacing	En-dash‡	Length of sentences (average no. of words)	No. of paragraphs	Abbreviations	Repetition of brand names
0-6 years of schooling	Gelatin + glycerol	Capitalized	Black	≤ 1 pt	1	13.8	15	5	1 ‡‡
	Minoxidil	Capitalized	Black	> 1 pt	6	14.9	54	12	1
	Cefatrizine	Capitalized	Black	> 1 pt	5	14.2	39	12	1
	Ipratropium bromide	Capitalized	Black	> 1 pt	8	17.2	38	4	1
7-12 years of schooling	Povidone-iodine	Not capitalized	Black	> 1 pt	1	12.4	29	1	1
	Oxymetazoline	Not capitalized	Yes	> 1 pt	6	13.5	57	15	0 ‡
	Ofloxacin	Capitalized	Black	> 1 pt	5	14.5	30	1	1
	Clomipramine	Capitalized	Black	> 1 pt	4	17.6	28	16	1
> 12 years of schooling	Choline salicylate	Capitalized	Black	> 1 pt	1	14.7	6	3	1
	Acetylsalicylic acid + ascorbic acid	Capitalized	Yes	> 1 pt	8	14.8	31	16	0
	Dexamethasone + neomycin	Not capitalized	Black	> 1 pt	3	14.7	25	5	1
	Methylprednisolone + lidocaine	Capitalized	Black	> 1 pt	4	16.5	45	5	0

‡ = Number of groups of items with en-dash; ‡‡ = The name of medicine appears at least once per more than 50 words.

with the template of the European Medicine Agency and were composed of the following sections: 1. What X is and what it is used for; 2. What you need to know before you <take> <use> X; 3. How to <take> <use> X; 4. Possible side effects; 5. How to store X; and 6. Contents of the pack.[15]

Procedure

A day was previously scheduled in all institutions that accepted the invitation to participate in the present study: participants were invited to be present, but they were free to quit. A colored printed version of the package leaflets and the LiS-RPL were distributed. The participants were told not to consult the package leaflet before all the instructions had been issued. A researcher explained aloud how to use LiS-RPL, and all participants were invited to clarify any queries that they might have regarding how to use the scale, before starting to complete the questionnaire.

The participants were required to classify their satisfaction with or perception of aspects of the package leaflet that was being tested, with regard to clarity, simplicity and comprehensibility of the text, and any typographic or printing issues. The LiS-RPL was self-administered.[3] All participants completed the task in less than 15 minutes.

Data analysis

Descriptive statistics

For the present study, questionnaires in which any of the values on any of the scale items had not been filled out were excluded. Thus, the final sample comprised 469 completed forms. The first objective was to describe the central trend, dispersion and distribution of the ratings of all the items. Multivariate tests for skewness and kurtosis, as proposed by Mardia, were also examined. Significant results in the Mardia test supported our decision to use polychoric correlations.[16]

Dimensionality

To examine dimensionality (which was our second objective), identification and fine-tuning of the instrument factor structure was conducted through an exploratory factor analysis. The number of factors was decided upon based on the results from the minimum average partial method in conjunction with the results from the parallel analysis using minimum rank factor analysis. This was based on random permutation of the sample data and comparison with the percentage of common variance that was extracted via minimum rank factor analysis.[17-18]

Number of factors to retain

An in-depth analysis based on the percentage of explained variance and on root-mean-square residuals was performed to select the number of factors to retain.[19] Root-mean-square residuals summarize the residual covariance matrix and the model fit, such that lower values represent a better model fit. Since the two-factor solution provided a higher percentage of explained variance and lower root-mean-square residuals, compared with the single-factor solution, the exploratory factor analysis was forced into two factors.

An exploratory factor analysis with a unweighted least-squares extraction method using a polychoric matrix was performed. The unweighted least-squares extraction method was chosen because it produces inter-factor correlation estimates of greater accuracy.[20] The promin oblique rotation method was applied to gain better solutions in the ordinal dataset, thereby allowing factors to be oblique so that factor simplicity could be maximized.

Kaiser-Meyer-Olkin index and Bartlett's sphericity test

The adequacy of the matrix for exploratory factor analysis was examined by assessing the determinant, the Kaiser-Meyer-Olkin index and Bartlett's sphericity test. Only factor loadings ≥ 0.40 were considered substantive.[21] Items with low communalities ($h^2 < 0.30$) or cross-loading items (item loading at 0.40 or higher in two or more factors) were eliminated. Whenever low factor loadings, low communalities or cross-loadings were found, any such items were removed and exploratory factor analysis was performed again until a stable structural solution was found.[19,22]

Exploratory bifactor analysis

An exploratory bifactor analysis with the Schmid-Leiman solution was performed to examine the possible existence of a broad second-order factor that would directly influence the observed variables.[23] Performing this exploratory bifactor analysis allowed us to determine whether a measurement could be treated as a single factor, or whether it would be best represented as separate but related factors.[24]

In a bifactor model, an overall factor accounts for relationships between individual items (akin to a single-factor model) and is labelled as a general factor.[25] Additionally, an IBM-SPSS syntax written by Wolf and Preising was used to calculate the total extracted variance accounted for by the general factor and first-order factors.[26]

Bentler's Simplicity Index and Loading Simplicity Index

The factor simplicity was assessed through Bentler's Simplicity Index and Loading Simplicity Index, in which greater values represent simpler and more interpretable solutions.[27,28]

Reliability of the instrument

Lastly, the reliability of the instrument was assessed using tests of internal consistency and homogeneity for each of the subscales and the overall score. Cronbach's alpha, ordinal alpha and inter-item correlation coefficients were used for the subscales. Ordinal alpha was computed manually, since it has been shown

to estimate reliability more accurately than Cronbach's alpha for ordinal response scales.[29-30]

The overall scale score was assessed using McDonald's total omega coefficient (ωt), which represents the proportion of total common variance in the instrument.[31] Lastly, McDonald's hierarchical omega coefficient (ωh) was computed manually for the bifactor structure, following the recommendations of Widhiarso and Ravand (2014).[31-32] McDonald's hierarchical omega coefficient can be seen as an estimate of the general factor saturation of an instrument, thus enabling examination of the extent to which a overall score is interpretable as a measurement of a single common factor.[33-35]

Cronbach's alpha, ordinal alpha and McDonald's omega (ωt and ωh) with values higher than 0.7 and mean item correlation between 0.15 and 0.50 were regarded as acceptable.[30,31] Correlation coefficients were interpreted in accordance with the criteria described by Cohen.[33]

Statistical software

Descriptive and reliability analyses were carried out using the IBM-SPSS statistics software, version 25.0. Exploratory factor analysis and exploratory bifactor analysis were both conducted via the Factor software, version 10.8.[34]

RESULTS

Descriptive statistics

Descriptive statistics for each item are presented in **Table 1**. Item 4 (color of the text) showed the highest values for the mean and dispersion. Skewness and kurtosis values were within ranges that were adequate for univariate normal distribution. However, Mardia's test showed the presence of excessive multivariate kurtosis ($K_2 = 331.12$; $P < 0.001$).[16]

Dimensionality examination

The minimum average partial method and minimum rank factor analysis indicated a two-factor structure and a single-factor structure, respectively. The matrix determinant was > 0.001 and the Kaiser-Meyer-Olkin value was 0.90, which confirmed the adequacy of the sample. The significance of the result from Bartlett's sphericity test, i.e. $\chi^2(98) = 2851.70$; $P < 0.001$, meant that the polychoric correlations between the items were large enough to conduct exploratory factor analysis.

Number of factors to retain

The exploratory factor analysis on the 14 items showed a two-factor structure that explained 60.0% of the variance. However, item 13 (use of abbreviations throughout the text) and item 14 (repetition of the brand name of the medicine throughout the text) were removed due to low communalities (< 0.3) and factor loadings (< 0.4), and the exploratory factor analysis was performed again. The pattern matrix from the exploratory factor analysis on 12 items and the communalities are shown in **Table 2**.

Together, the two factors explained 69.11% of the variance. Factor 1 (clarity and comprehension of text) comprised four items and accounted for 56.10% of the variance, while factor 2 (format) consisted of the remaining eight items and accounted for 10.76% of the variance.

No cross-loadings were found in the pattern matrix, and all significant item loadings were greater than 0.4. Similarly, the factor simplicity indices were also adequate, such that Bentler's Simplicity Index was 0.98 (percentile 99) and the Loading Simplicity Index was 0.48 (percentile 98).[27] The two factors were strongly correlated with each other (r = 0.80), thus supporting non-orthogonality.

Exploratory bifactor analysis with Schmid-Leiman solution

According to the Schmid-Leiman solution, all items seemed to represent the general factor because they showed loadings above 0.50.[23] The range of factor loadings was between 0.51 (item 1: font size) and 0.79 (item 9: number of sentences in each paragraph).

The loadings of the two first-order factors on the second-order factor were 0.84 and 0.95 for factor 1 (clarity and comprehension of text) and factor 2 (format), respectively. The results from the Schmid-Leiman solution for the present instrument are shown in **Table 5**.

Table 5. Results from Schmid-Leiman solution produced through a questionnaire that was designed to evaluate subjects' satisfaction with the readability of package leaflets for medicines (LiS-RPL), in a sample of 469 participants

Item	F1 First-order factor	F2 First-order factor	G Second-order factor
Item 1 - Font size	-0.15	0.24	0.51
Item 2 - Font type	-0.19	0.34	0.72
Item 3 - Layout of the title of the sections	0.03	0.22	0.71
Item 4 - Color of the text	-0.15	0.25	0.74
Item 5 - Line spacing	-0.06	0.27	0.73
Item 6 - Use of the en-dash throughout the text	0.14	0.16	0.71
Item 7 - Clarity of the text	0.43	-0.01	0.65
Item 8 - Length of the sentences	0.14	0.18	0.76
Item 9 - Number of sentences in each paragraph	0.16	0.18	0.79
Item 10 - Description of possible side effects	0.41	0.02	0.70
Item 11 - Comprehensibility of medical terms	0.48	-0.04	0.62
Item 12 - Clarity of the instructions for the user	0.55	-0.07	0.66
Variance explained (%)	76.40	18.10	5.50

The general factor accounted for 76.4% of the extracted variance, a proportion that was evidently above the range that would be considered to be indicative of the presence of a general factor (40-50%), whereas the two first-order factors explained 18.10% (factor 1: clarity and comprehension of text) and 5.50% (factor 2: format) of the variance.[22]

Hierarchical solution

The hierarchical solution according to two first-order factors and one second-order factor (G) exhibited good simplicity indices, such that Bentler's Simplicity Index was 0.99 and the Loading Simplicity Index was 0.48 (percentile 99).[27]

Reliability

All the internal consistency indexes indicated good reliability of measurement (**Table 6**). The mean inter-item correlation of factor 1 was only slightly above 0.5, which can be considered satisfactory. The overall scale score showed a mean inter-item correlation of 0.43, indicating that the items forming the scale were homogenous.[31,35]

DISCUSSION

Participants

Overall, 469 (93.2%) out of 503 (100%) sets of results from LiS-RPL were included (**Tables 1** and **2**), which is in line with the response rates in other studies involving Likert scales, i.e. response rates of 84%-91%.[7] Since the participants were selected according to convenience in only two regions of Portugal, the data collected is possibly not representative of the entire Portuguese population.

Tool evaluated (LiS-RPL)

Items removed

Two items were removed: item 13 (use of abbreviations throughout the text) and item 14 (repetition of the brand name of the medicine throughout the text), since they did not contribute significantly to the construct of the LiS-RPL. Interestingly, like the remaining purposed items, numbers 13 and 14 were based on the recommendations of the European Guideline on the Readability of Package Leaflets: abbreviations and acronyms should not usually be used, and in general a reference to "your medicine, this medicine, etc." is considered more suitable than repeating the name of the product, respectively.[1]

These items (13 and 14) were removed because their contribution was not statistically significant. The reasons for this may have been the following: readers are accustomed to consulting texts containing abbreviations within their daily routine, such as in the texts of newspapers or the internet; and, furthermore, the names of medicines are heterogeneously distributed in package leaflets.

Items selected

The scale was composed of 12 items that presented a stable structure (high communalities, high factor loadings and explanation of almost 70% of the variance). The variance explained was above the acceptable level (50%).

It was possible to identify two dimensions in factor 1 (clarity and comprehension of text) and in factor 2 (format). Factor 1 was composed of four items: clarity of the patients' instructions (item 12); comprehensibility of the medical terms (item 11); clarity of the text (item 7); and description of the possible side effects (item 10). Factor 2 (format) was composed of eight items: font type (item 2); line spacing (item 5); color of the text (item 4); font size (item 1); layout of the title of the sections (item 3); number of sentences in each paragraph (item 9); length of the sentences (item 8); and use of the en-dash throughout the text (item 6).

In addition to being stable and valid, this structure seemed to be coherent, since factors 1 and 2 contributed to the same types of issues: one relating to content and the other to form. Out of the 14 items thus selected, the color of the text was the most variable factor in participants' responses. This may have been related to the diversity of social and educational factors in our sample.

Regarding the results from the bifactor analysis, we cannot rule out the possibility that the scale, as a whole, might be considered unidimensional. McDonald's omega coefficient indicated that this total score was very strongly correlated with the hypothetical domain in which the items formed a subset, thus supporting the computation of the total score for the scale.[31] This scenario may be explained by the fact that the clarity and comprehension (Factor 1) of the text and format (Factor 2) issues are strongly interrelated

Table 6. Internal consistency and homogeneity measurements for the questionnaire that was designed to evaluate subjects' satisfaction with the readability of package leaflets for medicines (LiS-RPL)

	Cronbach's alpha	Ordinal alpha	Mean inter-item correlation	McDonald's total omega	McDonald's hierarchical omega
Factor 1 – Clarity and comprehension of text	0.85	0.92	0.58	-	
Factor 2 – Format	0.86	0.90	0.45	-	
Overall scale score	-	-	0.43	0.93	0.85

with regard to the readability and intelligibility of package leaflets, since both dimensions contribute towards readers' comprehension.[4]

Acceptability rate and recommendations

LiS-RPL is a reliable and validated tool for evaluating participants' satisfaction with or perception of package leaflets, for the European Portuguese-speaking population. LiS-RPL addresses two dimensions relating to package leaflets: the clarity and comprehension of the text and format issues.

It seems that government policy and/or health promotion interventions should include specific measures to ensure that package leaflets are truly comprehensible and usable, such as application of validated tools. These matters are scarcely represented in regulations.

Limitations

The Likert scale used did not follow the usual label pattern, i.e. very satisfied, fairly satisfied, neutral, not very satisfied or not at all satisfied.[3,36] This may have introduced the possibility of linguistic constraints, such as (i) the differences between the labels *satisfeito* (satisfied) and *bom* (good) may not have been clear to all participants; and (ii) comparison of the labels *pouco satisfeito* (not very satisfied) versus *bastante satisfeito* (very satisfied) may be considered more suitable than *pouco satisfeito* (not very satisfied) versus *satisfeito* (satisfied). Nevertheless, the ordinal nature of the scale from a completely negative to a completely positive response can be assumed to have been preserved. In this regard, additional linguistic and statistical evaluations are recommended in future studies.[36]

CONCLUSION

LiS-RPL is a reliable and validated tool for evaluating participants' satisfaction with or perception of package leaflets for the European Portuguese-speaking population. LiS-RPL addresses two dimensions of package leaflets: the clarity and comprehension of the text and format issues.

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Address for correspondence:
Carla Pires
Research Center for Biosciences and Health Technologies (CBIOS), Universidade Lusófona, Campo Grande 376, 1749-024 Lisbon, Portugal
Tel. +351 217 515 500
E-mail: p5558@ulusofona.pt

Frailty-associated factors among Brazilian community-dwelling elderly people: longitudinal study

Maycon Sousa Pegorari[I], Darlene Mara dos Santos Tavares[II]

Universidade Federal do Triângulo Mineiro (UFTM), Uberaba (MG), Brazil

[I]PhD. Physiotherapist and Adjunct Professor, Physiotherapy Course, Universidade Federal do Amapá (UNIFAP), Macapá (AP), Brazil.
orcid.org/0000-0003-4015-9895

[II]PhD. Nurse and Associate Professor, Department of Nursing Education and Community Health Nursing Graduate Program, Universidade Federal do Triângulo Mineiro (UFTM), Uberaba (MG), Brazil.
orcid.org/0000-0001-9565-0476

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Frailty assessment.
Frailty syndrome.
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ABSTRACT

BACKGROUND: Frailty among elderly people is associated with negative health outcomes. Through gaining better understanding of this syndrome over different time periods, healthcare actions that take predictive factors into consideration may be facilitated.

OBJECTIVE: To identify factors associated with frailty syndrome among community-dwelling elderly people over a two-year follow-up.

DESIGN AND SETTING: Longitudinal study on elderly people living in Uberaba (MG), Brazil.

METHODS: Elderly individuals were selected through multiple-stage conglomerate sampling from a national database. Participants were interviewed and evaluated in 2014 and again in 2016. Predictors were considered at the baseline, and frailty categories (frail, pre-frail or non-frail) at the follow-up. Frailty was identified based on the Fried criteria. Associations with socioeconomic factors, health status and physical performance were investigated using multinomial logistic regression.

RESULTS: 353 individuals participated in both assessments. The final model showed that age over 80 years was predictive of both pre-frailty and frailty (odds ratio, OR 4.92; 95% confidence interval, CI: 1.57-15.38; OR 8.64; 95% CI: 2.05-36.35, respectively), while dependency regarding basic activities of daily living (OR 3.66; 95% CI: 1.22-11.02) and poor lower-limb physical performance (OR 7.87; 95% CI: 1.97-31.39) predicted frailty. A one-unit increased score for advanced activities of daily living decreased the frailty rate by 15% (OR 0.85; 95% CI: 0.74-0.99).

CONCLUSION: Age over 80 years was predictive of pre-frailty and frailty, while dependency in basic activities of daily living and poor physical performance predicted frailty. A one-unit increased score for advanced activities of daily living decreased the frailty rate by 15%.

INTRODUCTION

Frailty among elderly people is considered to be a priority within public health. One reason for this is that presence of this syndrome predicts occurrences of adverse events that threaten the long-term sustainability of healthcare actions and systems. Moreover, frailty presents a negative influence on elderly people's quality of life.[1]

Physical frailty is "a medical syndrome with multiple causes and contributing factors" that is characterized by impairment of "strength, endurance and physiological functions", thus leading to "greater individual vulnerability in developing functional dependency and/or death".[2] From an operational point of view, the two measurements of frailty that have been most used (with high validity and reliability) are Fried's frailty phenotype and Rockwood and Mitnitski's frailty index.[3]

In a systematic review, frailty was found to be associated with several sociodemographic, physical, biological, lifestyle and psychological factors.[4] Moreover, some risk factors for frailty were identified, such as advanced age, female gender, black race, lower income, lower educational level, cardiovascular diseases, multimorbidity, functional impairment, poor self-rated health, depressive symptoms, cognitive impairment, obesity, undernutrition, smoking and alcohol use.[5]

In Brazil, however, the available evidence is only recent and there is a lack of longitudinal studies analyzing the factors that determine frailty.[6,7] In a study on 207 community-dwelling elderly people who were followed up for 12 months, the factors associated with frailty that predicted worsening of frailty status were histories of cancer, urinary incontinence and reduced capacity to perform advanced activities of daily living.[6] Another one-year investigation conducted among 129 elderly people after hospital discharge did not identify any variables that were predictive of change (improvement or worsening) to frailty condition.[7]

Feng et al.[5] considered that it was essential to determine the factors associated with frailty when developing interventions to prevent or reduce the frailty-associated burden among community-dwelling elderly people.

OBJECTIVE

Given the low number of studies within the elderly population of Brazil and the need to understand the factors that determine frailty, the aim of this study was to identify frailty-associated factors among community-dwelling elderly people over a two-year follow-up.

METHODS

Ethics

This study was approved (protocol no. 493,211, dated December 13, 2013, and protocol no. 573,833, dated March 28, 2014) by the human-research ethics committee of the Federal University of the Triângulo Mineiro (Universidade Federal do Triângulo Mineiro, UFTM).

Study design, participants and sample size

This was a longitudinal study, conducted among elderly people living in the urban area of Uberaba, state of Minas Gerais (MG), over a two-year follow-up (2014-2016). Uberaba is the main municipality of the area known as the "Triângulo Sul" of Minas Gerais, which is composed of 27 municipalities in the Triângulo Mineiro region of this state. In 2010, the estimated population of Uberaba was 328,272 citizens, its human development index (HDI) was 0.772 and life expectancy was 75.7 years.[8] According to data from the Brazilian Institute for Geography and Statistics (Instituto Brasileiro de Geografia e Estatística, IBGE), Uberaba had an elderly population (i.e. of age greater than or equal to 60 years) of 37,365 people in 2010, which represented 12.62% of the total population.[8]

Population definition was done using a multiple-stage conglomerate sampling process. This process took into consideration the sectors defined by the Brazilian National Household Survey, with information from neighborhoods and streets that was made available by IBGE. Random household selection was conducted to identify elderly people in their homes.

The sample for the present study was composed of individuals who met the following inclusion criteria: (a) they participated at both times (2014 and 2016); (b) they did not present any cognitive deficit, as identified using the translated and validated Brazilian version of the Mini-Mental State Examination (MMSE), with cut-off points defined according to their educational level;[9] (c) they were able to walk, with or without the use of walking aids (cane, crutches or walkers); and (d) they agreed to participate in the survey through signing a free and informed consent statement. Participants were excluded in the following situations: (a) inability to reach the participant, even after three attempts; (b) moving to another city; (c) occurrence of hospitalization at the time of the visit; and (d) presence of diseases that prevented the assessments. In the 2014 baseline assessment, 710 elderly people were interviewed.

In 2016, attempts were made to reach all the elderly people who had participated in the first stage of the survey (n = 710), in their homes. After the eligibility criteria and the losses had been taken into consideration (detailed in **Figure 1**; other reasons could be insufficient address or incomplete data), 353 elderly people were considered in the present investigation. Thus, these 353 individuals were evaluated both in 2014 and in 2016.

Because of the possibility of reading and comprehension problems, the interviews with the elderly people were conducted face-to-face in their homes. Therefore, interviewers (who were undergraduate and postgraduate students) were selected and trained regarding ethical issues within research and, additionally, they were accompanied by field supervisors (senior researchers).

Dependent variable

The presence of frailty syndrome was investigated using the five items that Fried et al. described as components of the frailty phenotype.[10] These were the following: (1) Presence of non-intentional weight loss, as assessed through the question "In the past year, did you lose 4.5 kg without intention?"; (2) Muscle strength loss verified based on handgrip strength, using a manual hydraulic dynamometer; the mean value from three measurements was obtained and the cutoff points proposed by Fried et al.[10] were used; (3) Self-reported exhaustion and/or fatigue, as measured through two questions: "Did you feel that you had to make an effort to take care of your habitual tasks?" and "Were you unable to move forward with your things?"; (4) Presentation of

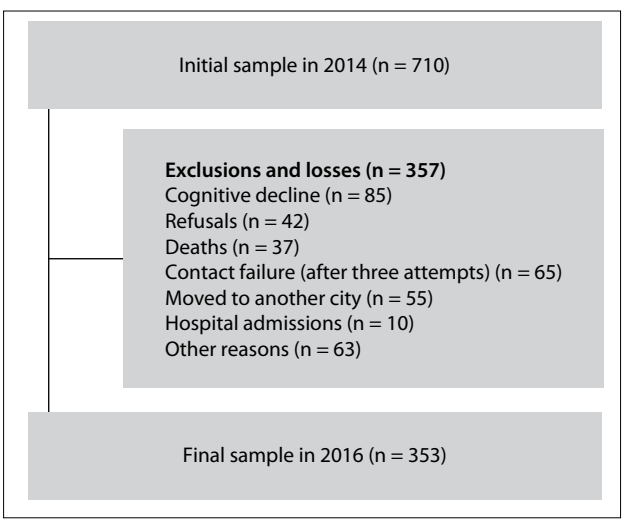

Figure 1. Study sample flow diagram.

slow walking speed, considering the time (in seconds) that was taken to walk a distance of 4.6 m, with the cutoff points as proposed by Fried et al.;[10] and (5) Poor physical activity level, as ascertained using the long version of the International Physical Activity Questionnaire (IPAQ). Elderly people presenting three or more of these items were classified as frail; those with one or two of these items were classified as pre-frail; and those with none of these items were considered to be robust or non-frail.[10] A detailed description of the components can be accessed in previous publications.[10-12]

Exploratory variables

The following were considered to be exploratory (independent) variables:

1. Socioeconomic characteristics – age range in years (60 to 69, 70 to 79 or 80 or over), sex (male or female), marital status (with or without a companion), living arrangements (alone or with company), schooling in years (none, 1 to 4 or 5 or more) and individual monthly income in minimum wages (no income, less than or equal to 1 minimum wage, or 2 or more minimum wages);
2. Clinical health indicators – number of diseases, number of regular medications, health self-perception (very poor, poor, fair, good or very good), hospital admissions in the past 12 months (yes or no) and falls in the past 12 months (yes or no);
3. Functional incapacity – measured using patient-reported outcomes such as the Katz scale[13] for basic activities of daily living (BADL); the Lawton and Brody scale for instrumental activities of daily living (IADL),[14] categorized as dependent (total or partial dependency) or independent (without incapacity for BADL and IADL); and 13 questions of a social nature for advanced activities of daily living (AADL),[15] in which the response alternatives were "never did", "stopped doing" or "still doing", with scoring in the range of 1-3 points, a minimum score of 13 points and a maximum of 39 points;
4. Fear of falling – measured using the Falls Efficacy Scale International - Brazil (FES-I Brazil), which was analyzed as a continuous variable, with scores ranging from 16 to 64;[16] and
5. Physical performance – assessed using the Brazilian version of the Short Physical Performance Battery (SPPB), which was categorized as follows: 0-3 points, very poor performance; 4-6 points, poor performance; 7-9 points, moderate performance; and 10-12 points, good performance.[17]

Statistical analysis

Statistical analysis was done using the absolute and percentage frequency distribution for categorical variables and central trend (mean) and dispersion (standard deviation) measurements for quantitative variables. Univariate and multivariate analyses were done using logistic multinomial regression analysis, in order to investigate associations between the exploratory variables and the dependent variable (frailty status). Thus, the exploratory variables (predictors) were obtained from the baseline (2014) and the frailty status (frail, pre-frail or non-frail) was obtained from the follow-up assessment (2016). The variables of interest were chosen in accordance with the criterion established ($P < 0.20$) and were included in the multivariate regression model. Predictors associated with pre-frailty and frailty were identified using odds ratios, through multinomial logistic regression, considering a significance level of 5% ($P < 0.05$) and a 95% confidence interval (CI). The data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 21.0.

RESULTS

In 2014, the majority of the 353 elderly people who were interviewed were women, in the age range of 60-69 years, and were living with a companion. **Table 1** presents the distribution of the socioeconomic variables according to frailty status at the baseline.

Table 2 presents the univariate analysis on the frailty-associated factors during the follow-up. Predictors were considered at the baseline, and frailty categories (frail, pre-frail or non-frail) in the follow-up assessment.

The variables included in the multivariate model of the multinomial logistic regression are presented in **Table 3**. Age in the range of 80 years or over was a predictor of both frailty (OR = 8.64; 95% CI: 2.05-36.35) and pre-frailty (OR = 4.92; CI: 1.57-15.38), while dependency in basic activities of daily living (OR = 3.66; 95% CI: 1.22-11.02) and poor physical performance (OR = 7.87; 95% CI: 1.97-31.39) were predictors of frailty. Additionally, the results indicated that an increase of one point in the score for advanced activities of daily living decreased the rate of occurrence of the condition of frailty among these elderly individuals by 15% (OR = 0.85; 95% CI: 0.74-0.99) (**Table 3**).

DISCUSSION

The present study identified frailty predictors over a two-year follow-up period. These included advanced age, dependency relating to BADL and poor physical performance. On the other hand, ability to perform AADL provided a protective effect.

The results indicated that advanced age (80 years or over) was an independent predictor for both pre-frailty and frailty. Other investigations have also found that age was a frailty marker,[18-20] including two systematic reviews.[4,5]

Age is an important indicator of the association between frailty categories and mortality.[21] A systematic review indicated that the numbers of pre-frail and frail elderly people become greater at advanced ages, which suggests that frailty is a progressive condition and, hence, that it may appear more frequently among elderly people older than 80 years.[5] Moreover, Fulop et al.[22] discussed the existence

of common but non-identical pathways of frailty and aging; they suggested that the characteristics of frailty syndrome were more accentuated than those of regular ageing. Thus, all individuals older than 70 years would need to be screened for frailty syndrome, in order to improve the management of individuals with this condition.[2,23]

The association of BADL dependency as a frailty predictor seen in the present study is divergent from the findings of other Brazilian studies.[6,7] Nevertheless, an investigation in Italy, with a 4.4-year follow-up, found that worsening of the condition presented by non-frail individuals was associated with dependency

Table 1. Socioeconomic, clinical and health variable distribution among the elderly people, according to the condition of frailty at the baseline. Uberaba (MG), Brazil, 2014 (n = 353)

Variables	Frailty syndrome							
	Frail (n = 34)		Pre-frail (n = 196)		Non-frail (n = 123)		Total	
	n	%	n	%	n	%	n	%
Age range (in years)								
60-69	12	35.3	92	46.9	68	55.3	172	48.7
70-79	12	35.3	78	39.8	40	32.5	130	36.8
80 or over	10	29.4	26	13.3	15	12.2	51	14.4
Sex								
Male	9	26.5	60	30.6	51	41.5	120	34.0
Female	25	73.5	136	69.4	72	58.5	233	66.0
Marital status								
With a companion	15	44.1	88	44.9	65	52.8	168	47.6
Without a companion	19	55.9	108	55.1	58	47.2	185	52.4
Living arrangements								
Alone	5	14.7	43	21.9	27	22	75	21,2
Accompanied	29	85.3	153	78.1	96	78	278	78.8
Educational level (in years)								
None	9	26.5	34	17.3	17	13.8	60	17.0
1-4	20	58.8	100	51	65	52.8	185	52.4
5 or more	5	14.7	62	31.6	41	33.3	108	30.6
Income								
No income	-	-	22	11.2	12	9.8	34	9.6
1 minimum wage* or lower	29	85.3	82	41.8	50	40.7	161	45.6
2 or more minimum wages	5	14.7	92	46.9	61	49.6	158	44.8
Health perception								
Positive	3	8.8	75	38.3	74	60,2	152	43.1
Negative	31	91.2	121	61.7	49	39.8	201	56.9
Hospital admission (past year)								
Yes	11	32.4	32	16.3	15	12.2	58	16.4
No	23	67.6	164	83.7	108	87.8	295	83.6
Falls								
Yes	16	47.1	46	23.5	24	19.5	86	24.4
No	18	52.9	150	76.5	99	80.5	267	75.6
Number of diseases (mean ± SD)	7.97 ± 3.91		6.14 ± 3.42		4.69 ± 3.23		5.81 ± 3.53	
Number of medications (mean ± SD)	4.97 ± 3.11		3.61 ± 2.63		2.48 ± 2.37		3.34 ± 2.69	
BADL								
Dependent	15	44.1	35	17.9	13	10.6	63	17.8
Independent	19	55.9	161	82.1	110	89.4	290	82.2
IADL								
Dependent	30	88.2	103	52.6	58	47.2	191	54.1
Independent	4	11.8	93	47.4	65	52.8	162	45.9
AADL (mean ± SD)	25.2 ± 2.86		27.53 ± 2.92		28.1 ± 3.46		27.51 ± 3.20	
FES-I-Brazil (mean ± SD)	35.26 ± 14.91		26.14 ± 12.33		22.88 ± 9.83		25.88 ± 12.26	
SPPB (mean ± SD)	5.21 ± 2.23		8.61±2.12		9.93 ± 1.63		8.74 ± 2.36	

BADL = basic activities of daily living; IADL = instrumental activities of daily living; AADL = advanced activities of daily living; FES-I-Brazil = Falls Efficacy Scale International - Brazil; SPPB = Short Physical Performance Battery; SD = standard deviation.

*Minimum wage in Brazil in 2014: R$ 724.00/month (US$ 175.50); and in 2016: R$ 880.00/month (US$ 213.32).

in relation to activities of daily living.[18] Furthermore, according to Fried et al.,[24] functional incapacity may cause difficulty in accessing healthcare services or actions from healthcare professionals, which would lead to increases in unrecognized and unaddressed healthcare needs.[24] Thus, implementation of monitoring actions and control over functional incapacity factors are strategies not

Table 2. Socioeconomic, clinical and health variables associated with the condition of frailty, using univariate analysis. Uberaba (MG), Brazil, 2014-2016 (n = 353)

Variables	Frailty syndrome					
	Pre-frail			Frail		
	OR	95% CI	P	OR	95% CI	P
Age range (in years)						
60-69		1			1	
70-79	1.68	1.01-2.78	0.045	2.17	0.97-4.85	0.058
80 or over	6.47	2.19-19.13	0.001	16.10	4.64-55.84	< 0.001
Sex						
Male		1			1	
Female	0.86	0.53-1.42	0.567	0.98	0.47-2.04	0.952
Marital status						
With a companion		1			1	
Without a companion	0.88	0.55-1.41	0.601	1.01	0.50-2.01	0.980
Living arrangements						
Alone	0.92	0.52-1.63	0.788	0.98	0.43-2.27	0.984
Accompanied		1			1	
Educational level (in years)						
None	2.04	0.94-4.41	0.071	2.71	0.99-7.44	0.052
1-4	1.22	0.72-2.05	0.456	0.92	0.41-2.04	0.837
5 or more		1			1	
Income						
No income	0.86	0.39-1.89	0.714	0.44	0.09-2.16	0.315
1 minimum wage or lower	1.22	0.74-2.01	0.429	1.71	0.83-3.52	0.147
2 or more minimum wages		1			1	
Health perception						
Positive		1			1	
Negative	1.38	0.86-2.21	0.177	2.58	1.23-5.44	0.012
Hospital admission (past year)						
Yes	1.25	0.65-2.43	0.503	1.74	0.72-4.23	0.221
No		1			1	
Falls						
Yes	1.71	0.94-3.11	0.080	3.25	1.49-7.08	0.003
No		1			1	
Number of diseases (mean ± SD)	1.05	0.98-1.13	0.133	1.15	1.05-1.27	0.004
Number of medications (mean ± SD)	1.05	0.96-1.15	0.271	1.19	1.06-1.36	0.004
BADL						
Dependent	1.62	0.79-3.28	0.183	5.19	2.24-12.07	< 0.001
Independent		1			1	
IADL						
Dependent	2.26	1.39-3.64	0.001	4.05	1.91-8.56	< 0.001
Independent		1			1	
AADL	0.94	0.87-1.01	0.111	0.78	0.71-0.89	< 0.001
FES-I-Brazil (mean ± SD)	1.01	0.99-1.03	0.246	1.04	1.01-1.07	0.003
Physical performance (SPPB)						
Very poor	3.82	0.44-33.52	0.226	56.87	6.18-523.79	< 0.001
Poor	2.80	1.07-7.31	0.035	23.02	7.03-75.33	< 0.001
Moderate	1.78	1.07-2.94	0.025	3.07	1.18-8.01	0.022
Good		1			1	

OR = odds ratio; 95% CI = 95% confidence interval; P < 0.20; 1 = reference category – non-frail group; BADL = basic activities of daily living; IADL = instrumental activities of daily living; AADL = advanced activities of daily living; FES-I-Brazil = Falls Efficacy Scale International - Brazil; SPPB = Short Physical Performance Battery.

only for maintaining functional capacity among elderly people,[25-26] but also for prevention of consequent conditions of frailty.

The present study found that an increase of one unit in the AADL score may have a protective effect against occurrences of frailty. These results are corroborated by an investigation among Brazilian elderly people that identified that the chance that frailty would worsen within 12 months was smaller (20%) when the elderly individual was categorized as "still doing" an AADL.[6]

AADLs are complex activities involving social interaction, such as work or participation in community groups, meetings, cultural events, trips and other activities.[15] Hence, they represent integrity of physical function, social function and performance in social roles.[27] In addition, they are predictors of frailty.[28] Therefore, elderly people with active social networks are likely to be less frail than those with less social engagement.[29] Moreover, social participation and factors such as security, strong social cohesion and neighborhood belongingness[29] are protective and provide balance in community frailty levels.[30]

Another frailty predictor is poor physical performance (4-6 points), as assessed using the SPPB. An Italian study with a mean follow-up period of 4.4 years found that poor physical performance (score lower than 8 points) was significantly associated with increased risk of becoming frail and with worsening frailty status.[18]

Previous cross-sectional studies identified the feasibility of using the SPPB to detect frailty among elderly people (score lower than 9 points),[31] including detection of early signs of frailty before occurrence of slow walking speed among very old people (score of 8 points).[32] Cesari et al.[33,34] highlighted that the SPPB identified elderly people with greater vulnerability to stressors and elevated risk of negative health-related events, which are matters related to frailty syndrome. Therefore, these findings may explain the results from the present study.

The SPPB provides a simple measurement of physical performance that is easy to carry out, without any need for special equipment or extensive training for evaluators.[32] Furthermore, it is one of the clinical tools most used for identifying frailty.[35] Additionally, it provides a viable and objective definition for the complex concept of frailty, both in clinical practice and in research.[33,34]

Among the limitations of the present study, there were considerable losses of follow-up. A further limitation was that absence of cognitive decline was considered to be an inclusion criterion in the present study, given that presence of cognitive decline could have interfered with comprehension of the variables analyzed (especially considering the self-reported nature of some of the data). Moreover, it needs to be acknowledged that a relationship between frailty and cognitive decline exists.

In the light of the results from the present study and the fact that frailty is a highly prevalent syndrome in aging populations,[1] it is imperative to identify and manage this condition properly.[23] In this regard, knowledge of frailty-associated factors and the complexity of their determinants aids construction of early preventive and intervention actions.[5,12]

Table 3. Final multinomial logistic regression model including the variables associated with the condition of frailty in a population of community-dwelling elderly people. Uberaba (MG), Brazil, 2014-2016 (n = 353)

Variables	Frailty syndrome					
	Pre-frail			Frail		
	OR	95% CI	P	OR	95% CI	P
Age range (in years)						
60-69		1			1	
70-79		-			-	
80 or over	4.92	1.57-15.38	0.006	8.64	2.05-36.35	0.003
BADL						
Dependent		-		3.66	1.22-11.02	0.021
Independent		1			1	
AADL		-		0.85	0.74-0.99	0.037
Physical performance (SPPB)						
Very poor		-			-	
Poor		-		7.87	1.97-31.39	0.003
Moderate		-			-	
Good		1			1	

OR = odds ratio; 95% CI = 95% confidence interval; P < 0.05; 1 = reference category - non-frail group; BADL = basic activities of daily living; AADL = advanced activities of daily living; SPPB = Short Physical Performance Battery.

CONCLUSION

Being 80 years of age or older was a predictor for conditions of pre-frailty and frailty, while dependency in basic activities of daily living and poor physical performance were predictive of frailty. An increase of one unit in the score for advanced activities of daily living decreased the rate of occurrence of the condition of frailty among these elderly people by 15%.

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Address for correspondence:
Darlene Mara dos Santos Tavares
Departamento de Enfermagem em Educação e Saúde Comunitária do Curso de Graduação em Enfermagem da Universidade Federal do Triangulo Mineiro (UFTM)
Av. Frei Paulino, 30
Abadia — Uberaba (MG) — Brasil
CEP 38025-180
Tel. (+55 34) 3700-6154
E-mail: darlene.tavares@uftm.edu.br

Is it really a duplication cyst? Hypothesizing with insufficient data

Dimitrios Sfoungaris[I]

Aristotelion University of Thessaloniki, Greece

[I]MD, PhD. Assistant Professor, First Department of Pediatric Surgery, Aristotelion University of Thessaloniki, Thessaloniki, Greece.
orcid.org/ 0000-0001-5567-5118

Dear Editor,

The majority of digestive tract duplications that cause symptoms do so during early infancy.[1,2] Cases with adult onset are very rare and present with a combination of signs and symptoms that include abdominal pain, intestinal obstruction and gastrointestinal bleeding.[3] One such case was described by Huang et al.,[3] and they followed on with a survey of the literature that revealed 20 additional adult cases, published up to July 2017. Furthermore, the feature of their case that is even more interesting is that the lesion developed on the antimesenteric border of the ileum, thus constituting an absolute rarity among cases of intestinal duplication, since these lesions have been reported to develop on the mesenteric border of the intestine.[1,2,4]

However, from carefully reviewing the authors' findings, we have serious doubts about whether the lesion described is indeed a digestive tract duplication. The size of the lesion was not mentioned, but we can assume from the pictures that it is about 15 cm long in its dilated and edematous condition. Its location was reported to be 150 cm distally to the Treitz ligament, but its distance from the ileocecal valve was not mentioned. Assuming that the length of the jejunoileum in adults while alive is 258 cm on average,[4] it would be possible for the lesion to arise around 100 cm proximally to the ileocecal valve. Interestingly, both the size and site of the lesion conform well with the characteristics of Meckel's diverticulum, which may be as long as 56 cm,[5] and which in 28% of the cases is located between 91 and 167 cm proximally to the ileocecal valve.[4]

From the pictures, we perceive that the lesion did not share a common wall with the native intestine, and that it emerged from its antimesenteric border as a true diverticulum, surrounded by a seromuscular layer. The authors did not mention anything about its communication with the native intestine's lumen, or about its blood supply, even though both of these are important features for characterization of the lesion.[4] Both cystic and tubular duplications shared a common wall with the native intestine and, regarding the type of tube, a communication would usually exist at one or both ends of the duplication.[1,2] As an exception to this rule, a type of duplication cyst called the neurenteric cyst also exists: this is separate from the intestine but is attached in some way to the vertebrae and/or is accompanied by vertebral anomalies.[1,2] However, no such anomalies were mentioned.

In conclusion, the imaging and operative data that are presented in this report do not support the diagnosis of an intestinal duplication. Instead, they fit very well with the much more common Meckel's diverticulum, which in this case was infected, a complication more commonly occurring in adults,[5] like the patient described in the report.

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Address for correspondence:
Dimitrios Sfoungaris
Aristotelion University of Thessaloniki, General Hospital 'G. Gennimatas'
Ethnikis aminis, 41
546 35 Thessaloniki – Greece
Tel. +30 2310 992516
Cel. +30 6944356358
Fax. +30 2310 992529
E-mail: dsfounga@auth.gr

Response to: Is it really a duplication cyst? Hypothesizing with insufficient data

Zhi-Hao Huang[I]

Wuhan University Zhongnan Hospital, Wuhan, Hubei Province, China

[I]MD, MSc. Surgeon, Department of Colorectal and Anal Surgery, Wuhan University Zhongnan Hospital, Wuhan, Hubei Province, China.
orcid.org/0000-0002-4277-1626

Dear Editor,

I would like to provide some clarifications regarding the questions raised in the letter about our article.[1]

Enteric duplications are rare but can occur anywhere along the digestive tract from the oral cavity to the rectum.[2,3] The letter mentions that it would be possible for the lesion to arise around 100 cm proximally to the ileocecal valve. Although the majority of enteric duplications occurs intra-abdominally and over half of them are ileal duplications,[4-7] it cannot be denied that they may also occur at other possible distances or locations in the digestive tract.

As stated in our paper,[1] it is difficult to diagnose enteric duplications because of the non-specificity of symptoms and presentation. However, ultrasound, computed tomography scans and magnetic resonance imaging have been useful. Ultrasound can depict the characteristic location adjacent to the bowel and the two-layered wall of enteric duplications and can show the clear blood supply between the native tissue and the lesion.

The letter states that we did not mention anything about the communication of the lesion with the native intestinal lumen, or about the blood supply for the lesion, even though both of these are important features for its characterization.

However, the requirements set forth by the author of the letter were described in **Figure 2** of our paper.[1] According to Ladd (1940), these congenital malformations involve the mesenteric

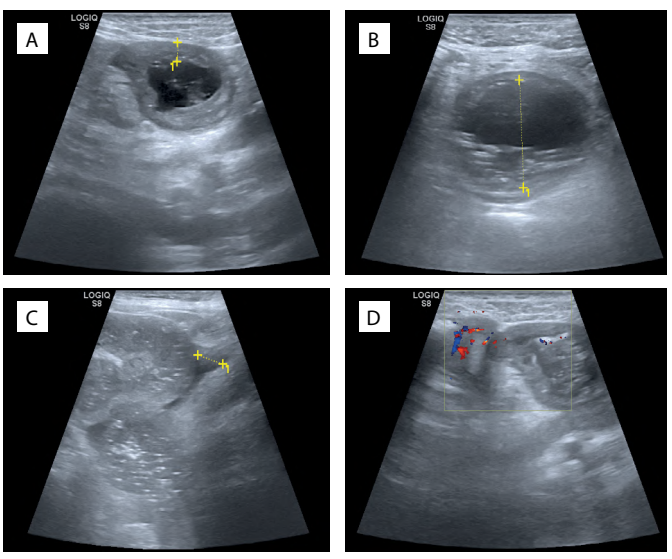

Figure 2. Abdominal ultrasonography: (A) A distended bowel can be seen below the umbilicus, and peristalsis is not apparent. The wall of the tube is thickened, and the lumen at the beginning of its expansion is compressed. (B) A dark area is seen between the intestines. (C) The intestinal wall is raised into the lumen, and the two intestinal tubes share the same wall of the bowel canal. (D) A small amount of colored blood flow signal can be seen on the wall of the dilated intestinal tube.

side of the associated alimentary tract and share a common blood supply with the native bowel.[8]

This description conforms with the blood flow signals of Figure 2 (D) of our paper.[1] The legend of this figure states: "A small amount of colored blood flow signal can be seen on the wall of the dilated intestinal tube". It can also be seen in Figure 2 (D) of our paper that the native intestine and abnormal lesion share a common wall, which consists of a thick low-echo area.[1]

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Address for correspondence:
Zhi-Hao Huang
First Hospital of Peking University, Beijing, China
East Area of Second Floor, Research Center Building, First Hospital of Peking University, Xicheng District, Beijing, China
Tel. +86 17812128901
E-mail: 616967250@qq.com

At the request of the authors, we report that in the paper published in the Sao Paulo Medical Journal, volume 137, issue number 4, DOI: 10.1590/1516-3180.2018.0458220719, pages 349-55 (in the title):

Where it read:

"Procalcitonin levels among patients with fever secondary to severe intracerebral infection. A cross-sectional study"

It should read:

"Procalcitonin levels among patients with fever secondary to intracerebral hemorrhage and severe infection. A cross-sectional study"

INSTRUCTIONS FOR AUTHORS

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Acknowledgements and funding

Grants, bursaries and any other financial support for studies must be mentioned separately, after the references, in a section named "Acknowledgements." Any financial support should be acknowledged, always with the funding agency name, and with the protocol number whenever possible. Donation of materials used in the research can and should be acknowledged too.

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Case reports, case series, narrative reviews and letters to the editor

Starting in June 2018, only individual case reports dealing with situations of public health emergencies will be accepted by *São Paulo Medical Journal*. Case reports that had already been accepted for publication up to May 2018 will still be published in a timely manner.

After initial evaluation of scope by the editor-in-chief, case reports, case series and narrative reviews will be considered for peer-review evaluation only when accompanied by a systematic search of the literature, in which relevant studies found (based on their level of evidence) are presented and discussed.[12] The search strategy for each database and the number of articles obtained from each database should be shown in a table. This is mandatory for all case reports, case series and narrative reviews submitted for publication. Failure to provide the search description will lead to rejection before peer review.

The access route to the electronic databases used should be stated (for example, PubMed, OVID, Elsevier or Bireme). For the search strategies, MeSH terms must be used for Medline, LILACS, and Cochrane Library. DeCS terms must be used for LILACS. EMTREE terms must be used for Embase. Also, for LILACS, the search strategy must be conducted using English (MeSH), Spanish (DeCS) and Portuguese (DeCS) terms concomitantly. The search strategies must be presented exactly as they were used during the search, including parentheses, quotation marks and Boolean operators (AND, OR, and NOT). The search dates should be indicated in the text or in the table.

Patients have the right to privacy. Submission of case reports and case series must contain a declaration that all patients gave their consent to have their cases reported (even for patients cared for in public institutions), in text and images (photographs or imaging examination reproductions). The Journal will take care to cover any anatomical part or examination section that might allow patient identification. For deceased patients whose relatives cannot be contacted, the authors should consult the Editor-in-Chief. All case reports and case series must be evaluated and approved by an ethics committee.

Case reports should be reported in accordance with the CARE Statement,[7] including a timeline of interventions. They should be structured in the same way as original articles.

Case reports must not be submitted as letters. Letters to the editor address articles that have been published in the *São Paulo Medical Journal* or may deal with health issues of interest. In the category of letters to the editor, the text has a free format, but must not exceed 500 words and five references.

FORMAT: FOR ALL TYPES OF ARTICLES

Title page

The title page must contain the following items:

1. Type of paper (original article, review or updating article, short communication or letter to the editor);
2. Title of the paper in English, which should be brief but informative, and should mention the study design.[14] Clinical trial, cohort, cross-sectional or case-control study, and systematic review are the most common study designs. Note: the study design declared in the title should be the same in the methods and in the abstract;
3. Full name of each author. The editorial policy of the *São Paulo Medical Journal* is that abbreviations of authors' names must not be used; therefore, we ask that names be stated in full, without using abbreviations;
4. Each author should present his/her ORCID identification number (as obtained from www.orcid.org);
5. Each author should indicate the way his/her name should be used in indexing. For example: for "João Costa Andrade", the indexed name could be "Costa-Andrade J." or "Andrade JC", as preferred;
6. Each author should indicate a valid, up-to-date email address for contact;
7. The author's professional background (Physician, Pharmacist, Nurse, Dietitian or another professional description, or Undergraduate Student); and his/her position currently held (for example, Master's or Doctoral Student, Assistant Professor, Associate Professor or Professor), in the department and institution where he/she works, and the city and country (affiliations);
8. Place or institution where the work was developed, city and country.
9. Date and venue of the event at which the paper was presented, if applicable, such as congresses, seminars or dissertation or thesis presentations.
10. Sources of financial support for the study, bursaries or funding for purchasing or donation of equipment or drugs. The protocol number for the funding must be presented with the name of the issuing institution. For Brazilian authors, all grants that can be considered to be related to production of the study must be declared, such as fellowships for undergraduate, master's and doctoral students; along with possible support for postgraduate programs (such as CAPES) and for the authors individually, such as awards for established investigators (productivity; CNPq), accompanied by the respective grant numbers.
11. Description of any conflicts of interest held by the authors (see above).
12. Complete postal address, e-mail address and telephone number of the author to be contacted about the publication process in the Journal (the "corresponding author"). This author should also indicate a postal address, e-mail address and telephone number that can be published together with the article. *São Paulo Medical Journal* recommends that an office address (rather than a residential address) should be informed for publication.

Second page: abstract and keywords

The second page must include the title and a structured abstract in English with a maximum of 250 words. References must not be cited in the abstract.

The following headings must be used in the structured abstract:

- Background – Describe the context and rationale for the study;
- Objectives - Describe the study aims. These aims need to be concordant with the study objectives in the main text of the article, and with the conclusions;
- Design and setting – Declare the study design correctly, and the setting (type of institution or center and geographical location);
- Methods – Describe the methods briefly. It is not necessary to give all the details on statistics in the abstract;
- Results – Report the primary results;
- Conclusions – Make a succinct statement about data interpretation, answering the research question presented previously. Check that this is concordant with the conclusions in the main text of the article;
- Clinical Trial or Systematic Review Registration – Mandatory for clinical trials and systematic reviews; optional for observational studies. List the URL, as well as the Unique Identifier, on the publicly accessible website on which the trial is registered.
- MeSH Terms - Three to five keywords in English must be chosen from the Medical Subject Headings (MeSH) list of Index Medicus, which is available at http://www.ncbi.nlm.nih.gov/sites/entrez?db=mesh. These terms will help librarians to quickly index the article.
- Author keywords - The authors should also add three to six "author keywords" that they think express the main article themes. These keywords should be different from the MeSH terms and preferably different from words already used in the title and abstract, so as to improve the discoverability of the article by readers doing a search in PubMed. They provide an additional chance for the article to be retrieved, read and cited. Combinations of words and variations (different wording or plurals, for example) are encouraged.

References

For any manuscript, all statements in the text that do not result from the study presented for publication in the *São Paulo Medical Journal* but from other studies must be accompanied by a quotation of the source of the data. All statements regarding health statistics and epidemiological data should generally be followed by references to the sources that generated this information, even if the data are only available electronically.

São Paulo Medical Journal uses the reference style known as the "Vancouver style," as recommended by the International Committee of Medical Journal Editors (ICMJE). Follow the instructions and examples at www.icmje.org, item "References", for the format.

In the text, the references must be numbered in the order of citation. The citation numbers must be inserted after periods/full stops or commas in sentences, and in superscript (without parentheses or square brackets). References cited in the legends of tables and figures must maintain sequence with the references mentioned in the text.

In the list of references, all the authors must be listed if there are up to and including five authors; if there are six or more, the first three should be cited, followed by the expression "et al." For books, the city of publication and the name of the publishing house are mandatory. For texts published on the internet, the complete uniform resource locator (URL) or address is necessary (not only the main home page of a website or link), so that by copying the complete address into a computer internet browser, the Journal's readers will be taken to the exact document cited, and not to a general website.

At the end of each reference, please insert the "PMID" number (for papers indexed in PubMed) and the "doi" number if available.

Authors are responsible for providing a complete and accurate list of references. All references cited in the text must appear in the reference list, and every item in the reference list must be cited in the text. Also, citations must be in the correct sequence.

Manuscripts that do not follow these guidelines for references will be returned to the authors for adjustments.

The reference list should be inserted after the conclusions and before the tables and figures.

Figures and tables

Images must be submitted at a minimum size that is reproducible in the printed edition. Figures should be sent at a resolution of 300 DPI and minimum size of 2,500 pixels (width) and be recorded in ".jpg" or ".tif" format. Images submitted in inadequate formats will not be accepted.

Images must not be embedded inside Microsoft PowerPoint or Microsoft Word documents, because this reduces the image size. Authors must send the images separately, outside of .doc or .ppt documents. Failure to send the original images at appropriate sizes leads to paper rejection before peer review.

Flowcharts are an exception: these must be drawn in an editable document (such as Microsoft Word or PowerPoint), and should not be sent as an image that can't be changed.

Figures such as bars of line graphs should be accompanied by the tables of data from which they have been generated (for example, sending them in the Microsoft Excel spreadsheets, and not as image files). This allows the Journal to correct legends and titles if necessary, and to format the graphs according to the Journal's style. Graphs generated from software such as SPSS or RevMan must be generated at the appropriate size, so that they can be printed (see above). Authors must provide internal legends/captions in correct English.

All the figures and tables should be cited in the text. All figures and tables must contain legends or titles that precisely describe their content and the context or sample from which the information was obtained (i.e. what the results presented are and what the kind of

sample or setting was). The reader should be able to understand the content of the figures and tables simply by reading the titles (without the need to consult the text), i.e. titles should be complete. Acronyms or abbreviations in figure and table titles are not acceptable. If it is necessary to use acronyms or abbreviations inside a table or figure (for better formatting), they must be spelled out in a legend below the table or figure.

For figures relating to microscopic findings (i.e. histopathological results), a scale must be embedded in the image to indicate the magnification used (just like in a map scale). The staining agents (in histology or immunohistochemistry evaluations) should be specified in the figure legend.

DOCUMENTS CITED

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