Effect of *Cornus Mas* L. Fruit Extract on Blood Pressure, Anthropometric and Body Composition Indices in Patients with Non-Alcoholic Fatty Liver Disease: A Randomized Controlled Trial

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Research

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Abstract

Background:

Obesity is an important factor in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Patients with NAFLD are at increased risk for hypertension. Some investigations have hypothesized that *Cornus mas L.* fruit can improve blood pressure and obesity. We investigated the effect of *Cornus mas L.* fruit extract on blood pressure variables, anthropometric and body composition indices in patients with NAFLD.

Methods:

This double-blind, randomized controlled trial was conducted on fifty patients with NAFLD. Patients received 20 cc/d *Cornus mas L.* fruit extract or placebo for 12 weeks. We measured diastolic blood pressure (DBP), systolic blood pressure (SBP), weight, waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), body fat mass (BFM), body fat percent (BFP) and fat free mass (FFM) at the baseline and after intervention.

Results:

The treatment group compared to the control group showed a significant reduction in DBP (-8.62 ± 11.86 vs. 0.53 ± 8.53 ; $P = 0.009$), SBP (-8.63 ± 14.37 vs. 0.0 ± 12.67 ; $P = 0.04$), BFM (-0.2 ± 3.9 vs. 0.7 ± 2.4 ; $P = 0.02$) and BFP (-0.2 ± 4.9 vs. 0.8 ± 2.8 ; $P = 0.05$) after adjusting for confounding factors. However, we found no significant difference between groups in weight, WC, HC, WHR and FFM ($P > 0.05$).

Conclusions:

Cornus mas L. can improve blood pressure. Further studies with higher dosages of extract are needed to clarify its effects on obesity. Trial registration: Registered on 30 September 2018 at Iranian Registry of Clinical Trials IRCT20180419039359N1 (<https://www.irct.ir/trial/30707>).

Introduction

Non-alcoholic fatty liver disease (NAFLD) is known as the accumulation of fat (mainly triglyceride) in the liver (more than 5% of liver weight) without evidence of alcohol consumption (1, 2). The prevalence of NAFLD is high in both developed and developing countries (3–5). It is the most common chronic liver disease worldwide and the main cause of liver transplantation (6, 7). Obesity, disrupted lipid metabolism, inflammation and insulin resistance are the most important factors that contribute to the development and progression of NAFLD (8–10). A strong association between NAFLD with both metabolic syndrome and cardiovascular disease (CVD) has been confirmed (11, 12). In addition, patients suffering from NAFLD compared to the healthy subjects are at higher risk for hypertension (13, 14). Modifiable lifestyle factors such as weight loss and adherence to the healthy plant-based dietary patterns are the important strategies to manage NAFLD (15–17).

Cornus mas L. (cornelian cherry) fruit is a rich source of compounds with anti-inflammatory and antioxidant properties such as anthocyanins, flavonoids and polyphenols (18–20). It has been reported that *Cornus mas L.* fruit/anthocyanins can improve obesity and hypertension through several mechanisms (21–24). The effects of *Cornus mas L.* fruit on some factors involved in the pathogenesis of NAFLD such as insulin resistance, dyslipidemia and inflammation have been examined (25–27). A few number of studies have examined the effect of *Cornus mas L.* on anthropometric and body composition indices (25, 26). The studies that investigated the effect of *Cornus mas L.* fruit in patients with NAFLD are rare (28). To the best of our knowledge, there is no study evaluating the effect of *Cornus mas L.* fruit on anthropometric and body composition indices in patients with NAFLD. In addition, there was no clinical trial that investigated the effect of *Cornus mas L.* fruit on blood pressure. Accordingly, we designed a clinical trial to examine the effect of *Cornus mas L.* fruit extract blood pressure variables, anthropometric and body composition indices in patients with NAFLD.

Methods

Recruitment and eligibility screening

We recruited the subjects from gastroenterology clinics affiliated with Diabetes Research Center of Shahid Sadoughi University of Medical Sciences, Yazd, Iran, between May 2019 to August 2019. A total of 50 subjects met the inclusion criteria including age 25–65 years, diagnosis of NAFLD by ultrasonography, alanine aminotransferase (ALT) serum concentrations ≥ 30 U/L in men and ≥ 19 U/L in women (29–31), and resident of Yazd city. The exclusion criteria included history of alcohol abuse, viral hepatitis, liver cancer, Wilson, type 2 diabetes mellitus (T2DM), mental diseases, pregnancy, lactation, taking corticosteroids, non-steroidal anti-inflammatory drugs, hypoglycemic drugs, tamoxifen, sodium valproate, methotrexate, amiodarone, anti-retroviral agents for HIV, probiotics, antioxidant and anti-inflammatory supplements such as vitamin D, vitamin E, omega-3 and resveratrol, poor compliance and unwillingness to continue the study.

Trial design

We designed a double-blind randomized, placebo-controlled clinical trial (RCT) evaluating the effect of *Cornus mas L.* fruit extract intake for 12 weeks on blood pressure, anthropometric and body composition indices in patients with NAFLD. at the beginning, the participants signed a written informed consent that was confirmed by the ethical committee of Shahid Sadoughi University of Medical Sciences and Health Services in Yazd (IR.SSU.SPH.REC.1399.019). We registered the protocol at the Iranian clinical trials website (<http://www.irct.ir>) under code number IRCT20180419039359N1 (<https://www.irct.ir/trial/30707>). We stratified the participants according to their age (25–45; 45–65 years) and gender (male/female) and the participants were divided into the treatment group (n = 25) and the control group (n = 25) by a person who did not contribute to the study. To perform randomization, we used random number table that was produced by random allocation software (32). The patients and investigators were blinded until the end of the trial.

Extract preparation

We provided the *Cornus mas L.* fresh fruits from the forests of Ghazvin, Iran. Preparation of the *Cornus mas L.* fruit extract and placebo was performed in the Pharmacy Faculty of Shahid Sadoughi University of Medical Sciences. In addition, to determine total anthocyanins content of final extract we used pH differential method (33). We reported the details of *Cornus mas L.* fruit extract preparation as well as total anthocyanins content determination in our previous article (28).

Intervention

Based on previous clinical trials (25, 27), we considered 20 cc/d *Cornus mas L.* fruit extract as the optimal dosage for treatment group. This amount provided 32 mg/d total anthocyanin. 20 cc liquid extract was equivalent to 2800 mg dried extract. The safety of this dosage was confirmed by previous studies (25, 26, 34, 35). The control group received 20 cc/d placebo without any anthocyanin. The *Cornus mas L.* fruit extract and the placebo that had similar appearance were packed in bottles with the same color, shape and size. The participants received the bottles every 2 weeks. We assessed the rate of compliance every 2 weeks based on extract consumption. Consumption of extracts less than 80% of the prescribed amount was considered as poor compliance. Adverse events during follow-up were checked every 2 weeks.

Dietary intake and physical activity assessment

We evaluated the dietary intake of participants at the baseline and after intervention utilizing a 3-day (1 weekend day and 2 nonconsecutive weekdays) food record. Energy and macronutrients intakes were calculated using nutritionist IV software (version 7.0; N-Squared Computing, Salem, OR, USA) (36). In addition, we used a short form of International physical activity questionnaire (IPAQ) (37, 38) to assess physical activity at the baseline and after intervention.

Blood pressure measurement

We measured diastolic blood pressure (DBP) and systolic blood pressure (SBP) under the standard protocol at the baseline and after intervention, in the nondominant arm after 10 min of rest. Measurements were performed by a trained person using a mercury sphygmomanometer device (MicrolifeBP AG1-10).

Anthropometric and body composition evaluations

Measuring height, weight, waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), body fat mass (BFM), body fat percent (BFP) and fat free mass (FFM) was performed at the baseline and after intervention by stadiometer, scale, and bioelectrical impedance analyzer (BIA) (In Body 770, Korea) based on the standard instructions.

Statistical analysis

We calculated the sample size (25 per group) based on fibrosis score, with 95% confidence interval, $\alpha = 0.05$, power = 80% in our previous article (28). We performed the power analysis, and power = 80% was estimated. Kolmogorov–Smirnov test was utilized to evaluate the parameters distribution. We used independent t-test and chi-square test for continuous and categorical variables, respectively to compare differences between groups. Independent t-test was utilized to compare the means of variables at the end of the study, as well as the mean changes between groups. Parameters with abnormal distribution were compared between groups utilizing Mann-Whitney U test. In addition, we used paired t-test (for parameters with normal distribution), and Wilcoxon test (for parameters with abnormal distribution) to perform within group comparisons. Univariate ANCOVA was used to control confounding factors. The primary outcomes were reported based on intention-to-treat (ITT) approach. Statistical package for social science (SPSS) software (Chicago, Illinois, USA) version 24 was utilized for statistical analyses.

Results

Characteristics and anthropometric variables

A total of 40 patients completed the study. Ten patients were excluded from the trial for reasons such as flatulence (n = 1), immigration (n = 2), unwillingness to continue the study (n = 3), surgery (n = 1), and corona virus pandemic (n = 3) (Figure 1). There was no difference between the treatment group and the control groups in the baseline variables (Table 1). In addition, we found no difference between the treatment group and the control groups in dietary intakes and physical activity at the baseline and after intervention (Table 2). The patients reported no serious adverse event related to extracts during follow-up. One patient with history of gastrointestinal disorders, had flatulence during intervention, and was excluded.

Table 1
Baseline characteristics of patients with NAFLD

Variables	<i>Cornus mas L.</i> (n = 25)	Placebo (n = 25)
Age*, y	41.4 ± 9.5	42.6 ± 9.9
Gender*		
Male, n (%)	12 (48)	11 (44)
Female, n (%)	13 (52)	14 (56)
History of other chronic diseases*		
Yes, n (%)	3 (12)	3 (12)
No, n (%)	22 (88)	22 (88)
DBP*, mmHg	80.16 ± 12.06	78.0 ± 8.57
SBP*, mmHg	121.08 ± 13.43	121.16 ± 15.96
Height*, cm	168.0 ± 11.2	164.4 ± 9.6
Weight, kg	79.7 ± 12.5	80.9 ± 12.8
WC*, cm	97.6 ± 9.1	101.1 ± 9.3
HC*, cm	102.5 ± 5.7	104.9 ± 4.7
WHR*	0.95 ± 0.09	0.96 ± 0.08
BFM*, kg	27.4 ± 6.5	29.9 ± 8.1
BFP*, %	34.5 ± 7.3	36.8 ± 7.6
FFM*, kg	52.3 ± 11.1	51.0 ± 10.5
P values are computed by independent t-test and data are expressed as mean ± standard deviation (SD), but for gender and history of other chronic diseases are computed by chi-square and data are expressed as numbers (percentage).		

Table 2
Dietary intakes and physical activity in patients with NAFLD

variables	<i>Cornus mas L.</i> (n = 25)	Placebo (n = 25)	<i>p</i> [†]
Energy intake, kcal/d			
Baseline	2960.80 ± 885.15	2576.24 ± 617.81	0.11
Week 12	2943.50 ± 758.81	2635.78 ± 621.91	0.16
P	0.85	0.31	
Carbohydrates, g/d			
Baseline	425.08 ± 185.33	375.33 ± 128.80	0.32
Week 12	460.46 ± 172.58	379.39 ± 132.36	0.10
P	0.32	0.80	
Proteins, g/d			
Baseline	95.25 (83.30 to 120.22)	88.36 (67.44 to 99.17)	0.14*
Week 12	93.99 (81.58 to 134.23)	96.34 (74.94 to 113.18)	0.48*
P	0.83**	0.19**	
Fats, g/d			
Baseline	93.18 (54.68 to 156.09)	69.37 (53.85 to 106.85)	0.23*
Week 12	83.46 (59.56 to 99.97)	72.12 (61.25 to 100.25)	0.76*
P	0.30**	0.45**	
Physical activity, (MET/hr/week)			
Baseline	660.45 (307.12 to 791.25)	341.5 (0 to 1705.50)	0.75*
Week 12	850.50 (111.37 to 881.29)	283.75 (0 to 1234.12)	0.49*
P	0.31**	0.28**	
Values of total energy and carbohydrates are presented as mean ± standard deviation (SD), while for proteins, fats and physical activity are presented as median and quartile range.			

Outcomes

At the baseline, there was no significant difference between the treatment and the control groups in terms of DBP (P = 0.46) and SBP (P = 0.98) (Table 3). After intervention, a significant difference between two groups was observed in DBP (P = 0.01), but, SBP remained without significant difference (P = 0.07). The treatment group compared to the control group showed a significant reduction in DBP (-8.62 ± 11.86 vs.

0.53 ± 8.53; P = 0.009), and SBP (-8.63 ± 14.37 vs. 0.0 ± 12.67; P = 0.04) after adjusting for confounding variables (Table 3).

Table 3
Effect of *Cornus mas L.* fruit extract on blood pressure in patients with NAFLD*

Indices	<i>Cornus mas L.</i> (n = 25)	Placebo (n = 25)	<i>P</i> [†]	<i>P</i> ^{††}
DBP (mmHg)				0.009
Baseline	80.16 ± 12.06	78.0 ± 8.57	0.46	
Week 12	71.54 ± 7.82	78.53 ± 10.67	0.01	
P	0.001	0.75		
Mean change of DBP	-8.62 ± 11.86	0.53 ± 8.53	0.003	
SBP (mmHg)				0.04
Baseline	121.08 ± 13.43	121.16 ± 15.96	0.98	
Week 12	112.45 ± 16.63	121.16 ± 16.87	0.07	
P	0.006	0.99		
Mean change of SBP	-8.63 ± 14.37	0.0 ± 12.67	0.02	
Values are presented as mean ± standard deviation (SD).				

In addition, there was no significant difference between the two groups in weight (P = 0.73), WC (P = 0.18), HC (P = 0.10), WHR (P = 0.71), BFM (P = 0.25), BFP (P = 0.29) and FFM (P = 0.67) at the baseline (Table 4). In addition, we found no significant difference between two groups in weight (P = 0.73), WC (P = 0.20), WHR (P = 0.82), BFM (P = 0.14), BFP (P = 0.14) and FFM (P = 0.49) after intervention; but, level of HC (P = 0.03) was significantly higher in the control group compared to the treatment group (Table 4). After adjusting for confounding factors, there was a significant difference between the treatment and the control groups in the mean change of BFM (-0.2 ± 3.9 vs. 0.7 ± 2.4; P = 0.02) and BFP (-0.2 ± 4.9 vs. 0.8 ± 2.8; P = 0.05); however, we found no significant difference between two groups in mean change of weight (0.5 ± 2.3 vs. 0.5 ± 1.5; P = 0.06), WC (0.8 ± 3.3 vs. 1.0 ± 2.7; P = 0.74), HC (0.1 ± 1.3 vs. 0.9 ± 2.5; P = 0.10), WHR (0.01 ± 0.03 vs. 0.00 ± 0.03; P = 0.51) and FFM (0.7 ± 3.7 vs. -0.2 ± 2.4; P = 0.40) (Table 4).

Table 4
Effect of *Cornus mas* L. fruit extract on body composition in patients with NAFLD*

Indices	<i>Cornus mas</i> L. (n = 25)	Placebo (n = 25)	<i>p</i> [†]	<i>p</i> ^{††}
Weight (kg)				0.06
Baseline	79.7 ± 12.5	80.9 ± 12.8	0.73	
Week 12	80.2 ± 12.5	81.4 ± 13.0	0.73	
P	0.32	0.13		
Mean change of weight	0.5 ± 2.3	0.5 ± 1.5	0.99	
WC (cm)				0.74
Baseline	97.6 ± 9.1	101.1 ± 9.3	0.18	
Week 12	98.4 ± 10.2	102.1 ± 10.5	0.20	
P	0.29	0.08		
Mean change of WC	0.8 ± 3.3	1.0 ± 2.7	0.74	
HC (cm)				0.10
Baseline	102.5 ± 5.7	104.9 ± 4.7	0.10	
Week 12	102.6 ± 5.3	105.8 ± 4.9	0.03	
P	0.54	0.11		
Mean change of HC	0.1 ± 1.3	0.9 ± 2.5	0.24	
WHR				0.51
Baseline	0.95 ± 0.09	0.96 ± 0.08	0.71	
Week 12	0.96 ± 0.10	0.96 ± 0.09	0.82	
P	0.44	0.82		
Mean change of WHR	0.01 ± 0.03	0.00 ± 0.03	0.73	
BFM (kg)				0.02
Baseline	27.4 ± 6.5	29.9 ± 8.1	0.25	
Week 12	27.2 ± 6.9	30.6 ± 8.3	0.14	
P	0.94	0.10		
Mean change of BFM	-0.2 ± 3.9	0.7 ± 2.4	0.36	

Values are presented as mean ± standard deviation (SD).

Indices	<i>Cornus mas</i> L. (n = 25)	Placebo (n = 25)	<i>p</i> [†]	<i>p</i> ^{††}
BFP (%)				0.05
Baseline	34.5 ± 7.3	36.8 ± 7.6	0.29	
Week 12	34.3 ± 7.6	37.6 ± 7.8	0.14	
P	0.82	0.16		
Mean change of BFP	-0.2 ± 4.9	0.8 ± 2.8	0.37	
FFM (kg)				0.40
Baseline	52.3 ± 11.1	51.0 ± 10.5	0.67	
Week 12	53.0 ± 11.7	50.8 ± 10.4	0.49	
P	0.30	0.82		
Mean change of FFM	0.7 ± 3.7	-0.2 ± 2.4	0.32	
Values are presented as mean ± standard deviation (SD).				

Discussion

Based on our knowledge, our study was the first study that examined the effect of *Cornus mas* L. fruit extract on blood pressure variable, anthropometric and body composition indices in patients with NAFLD. *Cornus mas* L. fruit extract (20 cc/d) for 12 weeks could improve blood pressure, BFM and BFP. However, weight, WC, HC, WHR and FFM remained without significant change.

Cornus mas L. fruit and its biological compounds through various mechanisms and pathways such as inducing endothelial nitric oxide gene expression, regulating nitric oxide synthase and increasing endogenous production of nitric oxide, modulating nuclear factor-κ B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways, reducing pro-inflammatory cytokines, decreasing peroxynitrate and reactive oxygen species (ROS) levels, and attenuating vasoconstriction by regulating angiotensin-converting enzyme (ACE) and angiotensin II receptor activity can improve hypertension (22, 39–42). There is no study investigating the effect of *Cornus mas* L. fruit on blood pressure in subjects in NAFLD. Johnson et al. (43) reported that daily consumption of blueberry, a rich source of anthocyanins, for 8 weeks in postmenopausal women with pre- and stage 1-hypertension can reduce DBP and SBP. In addition, the study of Broncel et al. (44) found that intake of 300 mg/d *Aronia melanocarpa* extract (another rich source of anthocyanins) for 2 months reduces DBP and SBP in subjects with metabolic syndrome. Moreover, the study conducted by Basu et al. (45) suggested that freeze-dried blueberry beverage (50 g freeze-dried blueberries) daily for 8 weeks can reduced DBP and SBP in participants with metabolic syndrome. However, a clinical study conducted by Hassellund et al. (46) demonstrated that anthocyanins supplementation (320 mg twice per day) after 4 weeks did not reduce blood pressure variables in subjects with hypertension. A meta-analysis found that anthocyanins supplementation has

no effect on DBP and SBP (47). Health status of participants, duration of follow-up, type of supplement and dosage of supplement are the most important differences between these studies. It seems, difference in type of supplements is the main factor explaining discrepancies between findings of mentioned studies. In general, anthocyanins supplementation did not show promising results in this field, while receiving sources of anthocyanins reported the beneficial effects on blood pressure. We used *Cornus mas L.* fruit extract containing several biological compounds that probably have synergistic effects.

On the other hand, the evidence suggested that *Cornus mas L.* fruit and its main compounds by inhibiting hepatic lipogenesis, increasing hepatic lipid oxidation and clearance, regulating the expression of peroxisome proliferator-activated receptors (PPARs), increasing the activity of AMP-activated protein kinase (AMPK) pathway in the white adipose tissue, decreasing adiponectin levels and activating adiponectin signaling, decreasing levels of adipocytokines, reducing the activity of pancreatic lipase and absorption of lipids can reduce obesity (21, 48–52). We found no similar study investigating the effect of *Cornus mas L.* fruit on obesity in patients with NAFLD. Gholamrezayi et al. (26) have examined the effect of 8-week *Cornus mas L.* fruit extract intake (900 mg/d) on anthropometric variables of postmenopausal women, and found a significant decrease in weight and WC. The dosage of *Cornus mas L.* fruit extract in the mentioned study was higher than our study. However, the study of Asgary et al. (25) showed no beneficial effect of *Cornus mas L.* fruit (100 g/d) for 6 weeks in dyslipidemic children and adolescents. Some studies have examined the effect of other rich sources of anthocyanins on body composition. It has been reported that 12-week cranberry extract intake (1500 mg/d) did not change the mean of WC in subjects with T2DM (53). Likewise, Basu et al. (45) did not find the beneficial effect of blueberry intake on weight and WC among subjects suffering from metabolic syndrome. The pilot trial of Zhang et al. (54) reported that purified anthocyanins supplementation (320 mg/d) derived from bilberry and black currant for 12 weeks has no effect on weight, WC, HC and WHR in patients with NAFLD. It seems, dosage and type of extract are important in this field, and probably higher dosages of *Cornus mas L.* fruit extract can improve the indicators of obesity.

To comply with principals of ethics in research, we declare that our research group reported the findings of liver function (28). We used the same data for the present article, and figure 1, sample size information, some baseline characteristics, dietary intakes and physical activity of our previous article were added to the present article.

The present study had some important advantages. This was the first RCT that examined the effect of *Cornus mas L.* fruit extract on blood pressure variables and body composition indices in patients with NAFLD. In addition, the extract was standardized according to total anthocyanin content. Similar to other RCTs, this study had some limitations. Liver ultrasonography was used for diagnosis of NAFLD, while Fibroscan has higher accuracy than ultrasonography (55, 56). As another important limitation, we did not measure the serum levels of anthocyanins to evaluate the bioavailability of anthocyanins.

Conclusion

Overall, 12-week *Cornus mas L.* fruit extract intake (20 cc/d) could improve blood pressure and body fat. However, further studies utilizing higher dosages of *Cornus mas L.* fruit extract are required to clarify the real effects of *Cornus mas L.* fruit extract on obesity.

Abbreviations

ACE

angiotensin-converting enzyme

AMPK

adenosine monophosphate activated protein kinase

ALT

alanine aminotransferase

BFM

body fat mass

BFP

body fat percent

CVD

cardiovascular disease

DBP

diastolic blood pressure

FFM

fat free mass

FPG

fasting plasma glucose

HC

hip circumference

ITT

intention-to-treat

IPAQ

international physical activity questionnaire

MAPK

mitogen-activated protein kinase

MET-h

metabolic equivalent task hours

NAFLD

nonalcoholic fatty liver disease

NF- κ B

nuclear factor- κ B

PPARs

peroxisome proliferator-activated receptors

RCT
randomized controlled trial
ROS
reactive oxygen species
SBP
systolic blood pressure
SPSS
statistical package for social science
T2DM
type 2 diabetes mellitus
WC
waist circumference
WHR
waist-to-hip ratio.

Declarations

Ethics approval and consent to participate

The research council of Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences and Health Services approved the study protocol. The methods were performed in accordance with the Helsinki Declaration. The ethical committee of Shahid Sadoughi University of Medical Sciences and Health Services in Yazd approved the written informed consent (code number: IR.SSU.SPH.REC.1399.019). The written informed consent was obtained from all participants before the data collection.

Consent for publication

Not applicable.

Availability of data and materials

The data and materials of the current study is available from the corresponding author on reasonable request.

Competing interests

The authors have declared no competing interests.

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Authors' contributions

H.M-Kh, M.H, F.Y and Z.S: conducted the study; H.M-Kh, M.A-M and A.R: provided material and technical support, H.F, A.S and F.Y: carried out the statistical analysis, and interpreted the finding; A.S: drafted the manuscript; H.M-Kh: critically revised the manuscript; and H.M-Kh: supervised the study. All authors reviewed the manuscript.

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Figures

Figure 1

eligibility, screening, and follow-up.

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