Association between obesity and chronic obstructive pulmonary disease in Moroccan adults: Evidence from the BOLD study

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Abstract

Objective: The Moroccan cross-sectional study aimed to investigate obesity in association to chronic obstructive pulmonary disease in Fez city.

Methods: A cross-sectional survey was carried out in Moroccan adults. Anthropometric and spirometry measurements were performed using standardized guidelines.

Results: Among the 744 participants, 53.9% of were women, with a mean age of 55.27 years (SD = 10.29). Nearly all women declared themselves to be never smokers, while 21% of men were current smokers. Overall, chronic obstructive pulmonary disease prevalence decreased with body mass index (p = 0.01) and waist circumference (p < 0.001). The same trends were also observed in women. The adjusted regression analysis showed a significant independent association between chronic obstructive pulmonary disease and decreasing overweight (OR.: 0.66; Classe (0.40-0.98)), obesity (OR.: 0.66 Cl_{95%}: (0.36–0.89)), and a decreasing waist circumference-abdominal obesity (OR_a: 0.58 Cl_{95%}: (0.34–0.99)) in the whole population. The same association remains significant in women for overweight (OR₂: 0.18 Cl_{95%}: (0.06–0.54)) and for waist circumference-abdominal obesity (OR₃: 0.40 Cl_{95%}: (0.19–0.85)). All these associations disappeared for men.

Conclusion: Chronic obstructive pulmonary disease decreases with the increase in body mass index and waist circumference. The effect of waist circumference on the chronic obstructive pulmonary disease was greater among women, regardless of the tobacco factor. A multicenter study would help to confirm the accuracy of these findings in a larger sample of the Moroccan population. Developed lifestyle programs in patients with chronic obstructive pulmonary disease should be considered.

Keywords

BOLD study, chronic obstructive pulmonary disease, obesity, abdominal obesity, Morocco

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Introduction

Obesity and chronic obstructive pulmonary disease (COPD) are a common public health concern and are among the common leading causes of mortality and morbidity worldwide.^{1,2} Moreover, the prevalence reported by the Global Burden of Disease Study reports was about 251 million cases of COPD globally in 2016,³ suggesting a large heterogeneous distribution worldwide. In the MENA (Middle East and North Africa) region, the prevalence of COPD ranged from 4.2% to 13.3%.⁴ In Morocco, the prevalence reported by El Rhazi et al.⁵ was about 12.6% among Fez city adults.

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It is well known that COPD risk increases by exposure to indoor and outdoor air pollution and tobacco smoking.³ But nutritional disorders such as overweight and obesity have become a common concern,⁶ which leads to significant morbidity and complications. In the general population, obesity correlates to decreased life expectancy.^{1,7} Unlike in individuals with COPD,⁶ obesity has a paradoxical protective effect explained by a protective effect of adiposity and composition of muscle.⁸ Obesity may negatively influence respiratory function through the amount and improper distribution of body fat, which worsens COPD results and reduces quality of life.^{9,10}

Most information about the prevalence of obesity in COPD comes from high-income countries. Projection of this prevalence shows that it is varied considerably between developed and developing countries.¹¹ Cross-sectional data carried out in the MENA region suggest that two-third of COPD patients were affected by overweight or obesity.¹² In Morocco, obesity and COPD are major public health problems.¹³ Their prevalence has been mounting alarmingly as a result of the nutritional and epidemiological transition.¹⁴ According to the World Health Organization's (WHO) 2017 Global Nutrition Report (GNR) in Morocco,¹⁵ the prevalence of overweight and obesity in adults were 57% and 22%, respectively. Research suggests that both COPD and obesity will likely increase further in the coming years,^{14,16} and given that the results of the obesity paradox among COPD are conflicting, it is crucial to gain more knowledge on the profile of obese patients with COPD.

To the best of our knowledge, the association between obesity and COPD in the Moroccan population has never been studied before. Thus, this study is the first population-based report in Morocco to investigate the association between obesity with COPD in Moroccan adults, using a strict spirometric protocol and several nutritional objective measures.

Method

Study design and participants

The current study is a cross-sectional survey carried out through the BOLD initiative during the period 2010–2013. Its design and rationale methodology have already been published.¹⁷

The sample size was determined based on a 15% risk factor prevalence, 2% accuracy, 95% confidence intervals (CIs), and a cluster impact of 2; the sample size was measured at 600 individuals and rounded to 800 to accommodate those who did not participate or were absent during the survey.

Only adults aged 40 years old or more were included. Participants who are <40 years old, sick bedridden people, and people with mental illnesses of non-acceptable quality of spirometry were excluded from the study because they were ineligible. The reasons for exclusion are published in detail elsewhere.⁵ A total of 760 subjects with acceptable quality spirometry data were included in the current study. Among them, 16 participants (2%) with the lowest and highest distribution of ratio between energy intake and energy requirement were excluded. Finally, 744 participants were included in the analysis.

Data collection

Anthropometric measurements. Anthropometric measurements were taken twice; the average of the two measurements was used to correctly assess BMI, waist circumference (WC), and hip circumference.

Measurements of the BMI were taken according to the WHO Guidelines,¹⁸ height and weight were measured using a calibrated equipment (stadiometer and weighing scale, respectively), and categorized into four groups, namely, underweight (<18.5 kg/m²), normal (18.5–25 kg/m²), overweight (>25–29.9 kg/m²), and obese (>30 kg/m²).

The abdominal perimeter or WC were quantified using standardized Measurement according to The WHO STEPS protocol, at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. The WC values were then categorized into two groups using the sex-specific cut-off point for abdominal obesity from the WHO¹⁸ and divided into normal WC (WC \leq 102 cm in men \leq 88 cm in women), and abdominal obesity (WC > 102 cm in men and >88 cm in women). The hip circumference measurement was taken around the widest portion of the buttocks.

Waist-to-hip ratio (WHR) was calculated by dividing the WC of the hip after standardizing the measures of both. The sex-specific cutoff points of WHR were ≥ 0.90 in men and ≥ 0.85 in women for abdominal, fat and < 0.90 in men and < 0.85 in women, for normal WHR.¹⁸

Spirometry

Spirometry was performed according to the American Thoracic Society $(ATS)^{19}$ criteria by trained and certified technicians. Separate measurements of forced expiratory volume in the first second (FEV1) and forced vital capacity (FVC) were made before and at least 15 min after two puffs of 200 mg of salbutamol administered using a metered dose inhaler with Volumatic spacer (GlaxoSmithKline; Uxbridge, England). FEV1/FVC ratio and values were expressed as percentage of predicted values. A value less than 70% indicates the COPD subject 70 to be stade1 and above, and a non-COPD subject would have VEMS/CVF ≥ 0.70 .

Each spirogram was reviewed and graded using ATS guidelines and quality-checked by the BOLD Pulmonary Function Reading Centre at Imperial College of London, UK.

Nutritional assessments

The nutrition variables were collected through a Moroccanvalidated food frequency questionnaire (FFQ),²⁰ comprising 32 food sections and 255 food items. The frequency of consumption of different foods reported in the FFQ was estimated by selecting one of the eight consumption levels: never, once to three times a month, once a week, two to four times a week, five to six times a week, once a day, two to three times, and more than four times. To calculate the total energy intake (TEI), we created a syntax using SPSS.20 software. Then food intake was calculated by multiplying the frequency of consumption of each food by its nutrient content (per 100 g) and by the standard local portion size (such as plate, bowl, different-sized spoons (tablespoon, teaspoon), teapot, teacup and glass of water, and using photographs from a booklet), and then adding the contribution of all foods. TEI was calculated by multiplying the frequency of consumption of each food by its relative energy (per 100 g) and specific serving size, and then adding the contribution of all foods.

Statistical analyses

First, the data were described using means and standard deviation (SD) or median and interquartile rate (IQR) for quantitative variable according to their distribution (normal or not), and the qualitative variable were described using percentages.

The mean exposure variable was the BMI. It was treated as an ordinal variable and classified into four categories, namely, underweight, overweight, and obese. WC and WHR were treated as binary variables using the sex-specific cutoff points described above. The bivariate association between COPD and each of these risk factors was examined using the chi-square test and Student's test as appropriate.

Regarding the multivariate analysis, binary logistic regression was used to analyze separately the association between COPD as an independent variable, and BMI (categories: Underweight, Normal weight, Overweight, Obesity), WC (as a categorical: Normal or abdominal obesity) and WHR (as a categorical: Normal or abdominal obesity) as explicative variables. The binary regression models were adjusted on the following potential confounders: age, gender, smoking habit (as categories: Never smoker, Former smoker, and Current smoker), educational level (illiterate, primary, Secondary/High school and University), as well as TEI (quantitative by kcal). However, for women, the smoking habits were excluded from the model (percentage of smokers among women was very low, at 1%).

Effect sizes were interpreted as crude odds ratios (OR_b) and adjusted odds ratios (OR_a) with 95% CIs. All analyses were performed using SPSS V.20 software. The significance threshold was fixed at 0.05.

Ethical approval

The BOLD protocol was conducted with the principles of the GEP (Good epidemiological Practice), after approval by the

Ethics Committee of the University Hospital Center Hassan II Fez, Morocco, as well as The University of Fez, Morocco. Each participant provided written informed and signed consent based on the nature and possible consequences of the study.

Results

Demographic characteristics

The main demographic characteristics of the included participants are presented in Table 1. The mean age was 55.27 years (SD = 10.29), and 53.9% were female. Nearly all women (99%) declared that they had never smoked, while 21% of men were current smokers and most of the population was illiterate (69.1% of females and 39.3% of men (p < 0.001)).

BMI, WC, and WHR categories by gender

The mean BMI in the study population was 27.9 ± 5.3 kg/m², with a higher prevalence of obesity in women compared to men (44.9% vs 14.3%, respectively (p < 0.001)), higher prevalence of WC-abdominal obesity (90.3% vs 24.8%, respectively (p < 0.001)), and higher prevalence of WHR-abdominal obesity (79.3% vs 73.8%, respectively (p = 0.04)). However, the prevalence of overweight was higher in men than in women (41.1% vs 36.2%, respectively, p < 0.001) (Table 1).

COPD prevalence by age, tobacco status, and anthropometric parameters

The overall prevalence of COPD was 14.1%. It was significantly more prevalent in men than women (19.2% vs 9.7%; p < 0.001). The prevalence of COPD increased with age (from 7.2% for 40–49 years to 29.5% for 70 years and above (p < 0.001)) and with smoking status (from 11.8% among never smokers, 18% for former smokers and 24.3% for current smokers (p < 0.01)).

For anthropometric parameters, overall COPD prevalence decreased with increased BMI from 25% for underweight to 10% for obesity (p = 0.01) and with WC from 20.2% for normal WC to 10.1% for abdominal obesity (p < 0.001) (Table 2). The same trends were observed in women; for BMI (from 40% in underweight to 8.9% in obesity (p < 0.001)) and for WC (from 30.8% in normal WC to 7.5% in abdominal obesity; (p < 0.01). Conversely, this association was not significant in men. For both genders, the prevalence of COPD was not associated either with the level of education or with WHR (Table 2).

The age, educational level, and TEI adjusted logistic regression analysis (Table 3) showed a significant independent association between COPD and decreasing overweight (OR_a: 0.66; CI_{95%} (0.40–0.98)), obesity (OR_a: 0.66; CI_{95%}:

	Total <i>N</i> = 744	Women % $N = 401$	Men % N = 343	þ value
Age categories				
40-49	237	36.9	25.9	< 0.005
50–59	295	38.7	40.8	
60–69	134	14.7	21.9	
70+	78	09.7	11.4	
Education level				
Illiterate	414	69.1	39.3	<0.001
Primary	150	15.5	25.7	
Secondary and high school	130	12.5	23.3	
University	50	3.0	11.1	
Smoking habit				
Never smoker	542	99.0	42.3	<0.001
Former smoker	128	0.5	36.7	
Current smoker	74	0.5	21.0	
BMI(kg/m ²)				
Underweight	12	01.2	02.0	<0.001
Normal weight	217	17.7	42.6	
Overweight	286	36.2	41.1	
Obesity	229	44.9	14.3	
WC (cm)				
Normal	297	9.7	75.2	<0.001
Abdominal obesity	447	90.3	24.8	
WHR				
Normal	173	20.7	26.2	0.04
Abdominal fat	571	79.3	73.8	

Table 1. General characteristics of adults from Fez, Morocco, participating in the BOLD Study (N = 744).

BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio.

(0.36–0.89)) and also a decreasing WC-abdominal obesity (OR_a: 0.58; CI_{95%}: (0.34–0.99)) in the whole population. The same association remains significant in women for overweight (OR_a: 0.18; CI_{95%}: (0.06–0.54)), and for WC-abdominal obesity (OR_a: 0.40; CI_{95%}: (0.19–0.85)). All these associations disappeared for men.

Discussion

This cross-sectional study aimed to assess the prevalence of COPD in an adult Moroccan population (age \geq 40 years old) and to analyze its relationship with different aspects of obesity notably the BMI, WC, and WHR as part of the international BOLD study. The main results showed that the COPD decreased significantly with the increase in BMI and WC in the whole study population. After the stratification on gender, these associations disappeared for men, and remained statistically significant in women for overweight and for WC.

The inverse relationship observed between COPD and obesity (BMI) concords with cross-sectional studies that reflect the paradoxical relationship between obesity and COPD.^{12,21}²⁴ The first one was the study conducted in the MENA region in 2010,¹² in 2187 patients, which showed that the COPD risk decreased with the severity of obesity (35.1%)

of COPD subjects were overweight, 20.4% were obese, and 9.2% were morbidly obese, while 35.3% were normal weight). However, these conclusions were done without performing any adjustment on smoking status or other confusing factors. Vanfleteren et al.²⁴ reported that compared to subjects without chronic airflow limitation (CAL), subjects with CAL have more low BMI (adjusted odds ratio (OR): 2.23, $CI_{95\%}$; (1.75, 2.85)) and less obesity (adjusted OR: 0.78, $CI_{95\%}$; (0.65, 0.94)). The same association has been explored in longitudinal studies and it is reported that BMI is an independent prognostic factor for COPD.^{7,25}–²⁸ The Copenhagen City Heart Study reported that the risk of death in COPD increases with weight loss (RR 2.14 $CI_{95\%}$; (1.18–3.89)), but not with weight gain (RR 0.95; $CI_{95\%}$ (0.43–2.08)).²⁵

Depending on the severity and history of the disease, BMI progresses differently in COPD. In some studies, patients tend to lose weight relative to lack of appetite and energy expenditure.²⁹ While others have found the opposite outcome, that COPD patients have an increased risk of developing obesity relative to reduced physical activity and long-term administration of systemic glucocorticoids.³⁰ Marchioro et al. reported that subjects with mild COPD tend to have increases in BMI (Δ BMI = 0.7 ± 2.2), while those with moderate, severe, or very severe COPD tend to have a decrease in BMI (Δ BMI = -0.4 ± 3.0 to -0.8 ± 3.3).³¹

	Total <i>N</i> = 744	COPD %	<i>p</i> -value	Women N = 401	COPD %	p-value	Men <i>N</i> = 343	COPD %	p-value
Age category									
40-49	237	7.2	<0.001	148	4.7	<0.001	89	11.2	<0.00 l
50–59	295	11.2		155	7.7		140	15.0	
60–69	134	23.9		59	23.7		75	24.0	
70 +	78	29.5		39	15.4		39	43.6	
Education									
Illiterate	414	15.9	0.1	277	11.2	0.7	137	25.5	0.07
Primary	150	13.3		62	06.5		88	18.2	
Secondary_highschool	130	10.0		50	06.0		80	12.5	
University	50	12.0		12	8.3		38	13.2	
Smoking habit									
Never smoker	542	11.8	<0.0I	397	09.3	_	145	17.2	0.3
Former smoker	128	18.0		2	0		126	18.3	
Current smoker	74	24.3		2	0		72	25.0	
BMI (kg/m ²)									
Underweight	12	25.0	0.01	5	40.0	0.001	7	14.3	0.6
Normal weight	217	19.8		71	19.7		146	19.9	
Overweight	286	12.6		145	4.8		141	20.6	
Obesity	229	010.0		180	08.9		49	14.3	
WC (cm)									
Normal	297	20.2	<0.001	39	30.8	<0.001	258	18.6	0.3
Abdominal obesity	447	10.1		362	7.5		85	21.2	
WHR									
Normal	173	11.0	0.1	83	7.2	0.2	90	14.4	0.1
Abdominal fat	571	15.1		401	9.7		253	20.9	

Table 2. Estimated prevalence of spirometrically confirmed COPD in a sample of the Moroccan population, by sociodemographic parameters and anthropometric measurement; N = 744.

BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio.

Bold values are significant variables with p-values related to the comparison of COPD prevalence among men and women in the four age categories.

Table 3.	Adjusted logistic	regression mo	dels explaining t	he association o	f COPD witl	h different aspects	of obesity amo	ng subjects with
COPD (n	= 744).							

		All (n = 744)		Women ($n = 401$).		Men ($n = 343$)	
		OR*	CI 95%	OR**	CI 95%	OR***	CI 95%
Model I	BMI (kg/m ²)						
	Normal	I		I		I	
	Underweight	1.39	(0.33;5.83)	3.32	(0.43;25.27)	0.31	(0.03;3.06)
	Overweight	0.66	(0.40;0.98)	0.18	(0.06;0.54)	0.93	(0.50;1.72)
	Obesity	0.66	(0.36;0.89)	0.69	(0.21;1.06)	0.84	(0.32;2.21)
Model 2	WC (cm)						· · · · · ·
	Normal	I		I		I	
	Abdominal obesity	0.58	(0.34;0.99)	0.40	(0.19;0.85)	0.76	(0.37;1.54)
Model 3	WHR						· · · · · ·
	Normal	I		I		I	
	Abdominal fat	1.31	(0.73;2.35)	0.93	(0.36;2.41)	1.58	(0.76;3.30)

OR: odds ratio; CI: confidence interval; BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio.

*Adjusted analysis for age, gender, smoking habit, educational level, and total energy intake.

**Adjusted analysis for age, educational level, and total energy intake.

****Adjusted analysis for age, smoking habit, educational level, and total energy intake.

Although most studies addressing such relationship have used BMI as an anthropometric measure, due to its ease of measuring or reporting, BMI may not be an ideal index of obesity in prediction of pulmonary dysfunction.^{12,32,33} WC and WHR require precise measurements and well-qualified and competent staff to achieve it according to the

recommendations of scientific societies, given the fact that they reflect better the intensity of obesity as well as abdominal and visceral body fat distribution.³⁴ Therefore, WC and WHR are used in many studies,^{32,33,35_37} as a complementary measurement to BMI. Our study showed that WC was more strongly associated with low COPD than BMI. When compared to normal participants, the COPD was halved in abdominal obesity than in normal WC. This conclusion was consistent with the EPIC cross-sectional study,³⁸ and US prospective study,³⁹ but contradicts the ECLIPSE study,⁹ which concluded that there was an increase in the accumulation of extra-uterine fat in COPDs. Thus, In the EPIC Study and in both genders, FEV1 and FVC were linearly and inversely related across the entire range of WHR.³⁸ Prospective data found that abdominal and central obesity measured as waist and hip circumferences are greater determinants of risk of lung function parameters than the BMI measurement and that the risk of COPD increased among overweight or obese participants only if they had a large waist circumference.36,40,41 Leone et al.⁴² found that in metabolic syndrome (MS), abdominal obesity was the main predictor of impaired lung function. Also, in a US prospective study, middle-aged to older women and men with a large WC (\geq 110 cm in women or ≥ 118 cm in men) had a 72% increased risk of COPD.⁴⁰

Conversely, in a Chinese prospective cohort study and after adjustment for WC, overweight, and obesity (BMI) were not associated with an increased risk of COPD compared with normal weight.⁴¹

The comorbidity obesity and COPD is a paradoxical relationship difficult to grasp, associating a mechanical factor with an inflammatory mechanism.³⁸ In fact, the fat stored around the chest and abdomen, surrounding internal organs such as the heart and lung, produce more pro-inflammatory cytokines, which may affect mechanically, and functionally, the respiratory function. That is why WC and WHR appear more strongly related to pulmonary function parameters than BMI. Having said that, exploring the relationship between anthropometric measurements and their effect on lung parameter dysfunction cannot be done without compromising on the confounding role of underweight and the potential effect of smoking. In fact, smoking plays an important role in loss of appetite as well as weight loss.⁸ But after a smoking decrease or complete cessation, signs of weaning appear quickly, such as increase in appetite, weight gain, but not necessarily an improvement in respiratory parameters.³⁸ In our study, underweight was not related to COPD, nether in men after smoking adjustment, nor in women who were predominantly nonsmokers (0.5% were current smokers and 0.5% were ex-smokers). After excluding the effect of smoking and underweight, possible reasons for lower COPD in abdominal obesity could be relative to the chronology of COPD progression as a cause or consequence of abdominal obesity.^{29_31} However, and given the complexity of such association, we cannot support a finding of a chronologic causation or consequence in our cross-sectional context.

In addition, respiratory function is influenced by gender differences.^{39,42} Women have smaller airways, reduced lung volume, and lower maximum respiratory volume than men. Also, and linked to increased levels of female sex hormones, women have a stronger inflammatory response and generate more oxidative stress in the airways than men⁴³ and therefore, are more prone to develop airflow limitation. In our sample, women have more abdominal obesity compared to men, but are predominantly nonsmokers. These conditions may therefore play a role in the lower prevalence of lung disability in obesity and abdominal obesity in women compared to men.

Study's strengths and limitations

This study has some limitations. The first one is related to its cross-sectional design, which made the establishment of a cause-and-effect relationship between different aspects of obesity and COPD difficult and unclear. In fact, exposure and outcomes were measured at the same time, which is why the direction of their association remains uncertain. The second limitation is related to the possible error bias dependent on lung function measurements, such as FEV1 and FVC, which can be affected by the voluntary effort of participants. Moreover, basic disability can affect the performance of lung function tests. To overcome this limitation, individuals with poor quality measurements or missing data on lung function measurements were also excluded from our analyses. Another limitation is due to the absence of physical activity data, and the lack of the potential impact of sedentary on the body composition in COPD. This limitation could be considered as low since most of the participants were retired or homemakers and therefore less active than the rest of the population as shown previously.44 Given that data were collected from a single center, this can compromise on the generalization of these results to the whole population. However, comparing the sociodemographic characteristics (29.2 of women and 29.4% for men have 40 years and above and 9.9% of women and 9.5% of men have more than 60 years old) of the study sample to the Moroccan population, showed no statistical differences and the results could be considered as a representative.⁴⁵

On the other hand, this study has several advantages. To our knowledge, this is the first study that explored the association between BMI, WC, WHR, and COPD in a Moroccan population, while the BREATHE study¹² evaluated only the BMI reported by COPD subjects. The assessment by wellskilled and qualified staff, of respiratory function in our study followed the BOLD protocol standard and a quality control,¹⁷ which provided high-quality measurements of Forced Expiratory Volume in one second (FEV1) and Forced Vital Capacity (FVC). The precision and rigor measurement of waist and hip circumferences which reflect other aspects of obesity in addition to BMI, in accordance with the standard and recommended procedures¹⁸ as well as data concerning smoking, the severity and the duration of exposure to tobacco using standardized questionnaires⁴⁶ could be considered as precise information which make results more reliable and accurate.

Conclusion

In conclusion, this study showed that COPD decreases with increased BMI and WC. We also identified that the effect of WC on the COPD was greater among women compared to men, regardless of the tobacco factor. A multicenter study would help to confirm the accuracy of these findings and to understand better this relationship in a larger sample of the Moroccan population. These results highlight the importance of anthropometric measurements that should be integrated in the COPD care given. Developed programs of lifestyle modification with the objective of improving abdominal fat distribution in patients with COPD should be considered.

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Contributors

A.B. contributed to the data collection, led the analyses and interpretation of the data, and wrote the final manuscript. V.G.-L. conceived the study idea, its design, and led the analyses and interpretation of the data. K.E.K., A.A.C., and Z.H. contributed to the statistical analyses and the manuscript redaction. M.C.B., M.E.B., and C.N. contributed to the study design and data collection. K.E.R. supervised the study design, the data collection, and corrected the manuscript. All authors have read and approved the manuscript. All approved the final version to be published.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The BOLD protocol was conducted with the principles of the GEP (Good epidemiological Practice) after approval by the Ethics Committee of the Medical School of Fez, and in the University Hospital of Fez, Morocco (Serial Number: AR MOR v4_14 Sep 2009).

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Informed consent

Each participant provided written informed and signed consent based on the nature and possible consequences of the study.

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