RESEARCH ARTICLE

Assessing the Application of the Reference Lung Age Equations on the Jordanian Population

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Abstract: **Background**: Smoking is a major health-related problem in Jordan due to which an effective smoking cessation program is needed. *Lung Age* which emphasizes the concept of a premature aging of the lungs, is a simple notion that smokers can grasp. Employing reference lung age equations can help health care providers convince smokers to quit. In this study the applicability of reference equations was assessed in estimating lung age for the Jordanian population, to aid smoking cessation.

Methods: Adult Jordanians were recruited from Al-Zaytoonah University of Jordan and from several community pharmacies, polyclinics and hospitals located in different areas in Jordan. Overall, 1767 participants of both genders from different age groups were recruited to evaluate the applicability of different reference lung equations for the Estimated Lung Age (ELA). SPSS was used to conduct all statistical analysis.

Results: A paired t-test showed a significant difference ($p<0.05$) between the Chronological Lung Age (CLA) and the ELA among the non-smokers. Similarly, some reference equations including Hansen and Morris and Temple FEF25-75 equations failed to show significant differences in ELA-CLA between different smoking status groups for women.

Conclusions: Our results suggest that the current lung age equations are not reliable in predicting lung age among the Jordanian population, and thus cannot be used in smoking cessation programs.

1. INTRODUCTION

Smoking in Jordan has a high prevalence; according to a study conducted in 2014, 32.3% of the respondents reported being current smokers (54.9% of males and 8.3% of females), and only 2.9% were ex-smokers [1]. In addition there has been a recent increase in the use of water pipes [2, 3], particularly among young people and females. A survey that enrolled 1000 students (grades 6, 8, 10 and 12) found that 36% of the youth have tried water-pipe smoking, 36% of them were males and 64% were female [2]. In addition, there has also been a recent increase in the use of electronic cigarettes [4]. Therefore, it is for healthcare professionals to help persuade cigarette smokers to quit smoking and so reduce ill health and premature deaths [5]. Indeed, promoting behavioral changes, including smoking cessation should be an essential component of strategies that reduce disease risk factors.

Motivating people to quit smoking is the major goal in smoking cessation programs. Quit rates were shown to be improved within participants that were given feedback using biomarkers related to the detrimental effect of smoking, including arterial damage, spirometry and exhaled carbon monoxide [6]. Spirometry is a noninvasive, easily performed and inexpensive test that offers diagnostic insight into the type and the extent of lung function impairment [7]. Spirometry also helps monitor the prognosis of lung disease and response to treatments [7]. The forced expiratory volume in one second (FEV1), due to its ease of measurement and good reproducibility, is the most widely used lung function parameter in clinical practice and research [8]. In 1977, the landmark study by Fletcher and Peto [9] found an annual decline in FEV1 with age which increased in the presence of other factors including smoking. Later, several studies showed that a low value of FEV1 is an independent predictor and a powerful marker of increased risk of Chronic

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Obstruction Pulmonary Disease (COPD), lung cancer, cardiovascular disease, and premature death [9, 10]. Smoking has been shown to accelerate the decline in FEV1, but this effect is modifiable, and can be attenuated by smoking cessation [11].

In the light of increased prevalence of smoking, and as smoking is the most important preventable risk factor in mortality rates, several smoking cessation programs were initiated. One approach used the 'accelerated decline in FEV1' as a tool to help smokers quit, thus the 'lung age concept' was coined [12]. Taking into account patient age, sex, height and ethnicity, the 'estimated lung age' of an individual can be defined as the age at which the measured FEV1 matches the predicted value of a healthy non-smoker [12]. Morris and Temple (1985) were the first to introduce this concept. They estimated lung age from the equations formulated to predict FEV1 [12]. This was made to simplify interpretation of the spirometric test results and turn it into a powerful and clear message that can be delivered to patients. The key message is that smokers' lung age is older than their chronological age. This information could therefore be used to warn smokers of the pulmonary impairment caused by smoking, with the intention of generating a strong stimulus to quit smoking [12]. Indeed, previous work has found that informing smokers of their lung age, obtained from the spirometric lung function, led to increased quit rates [12, 13]. A study that compared participants enrolled in a smoking cessation program who were informed of their lung age and those enrolled in the same program but were not informed found significant differences in the number who remained as nonsmokers after 12 months (13.6% versus 6.4% respectively). However, before considering utility of the lung age concept in Jordan, and in accordance with the American Thoracic Society guidelines which recommend that predictive equations should be derived from a 'relevant' population and should be updated every 10 years [14], it is required to test its applicability to the Jordanian population. Furthermore, several different reference equations are used to predict lung age in the worldwide population [7, 12, 15-19]. The aim of this study is to test the applicability of the previously published Estimated Lung Age (ELA) reference equations in the Jordanian population.

2. MATERIAL AND METHODS

2.1. Participants and Recruitment

This multi-center cross-sectional study examined the applicability of the ELA equation on Jordanian adults. Participants were approached in AlZaytoonah University of Jordan (including students, teaching and administrative staff) and in several pharmacies and polyclinics and hospitals from different locations in Jordan, including the capital city Amman as well as Madaba, Zarqa, Fuheis and Irbid (major cities in Jordan). Advertisements were placed on the internet and in places where participant recruitment was conducted, then a personal approach was used to invite healthy participants who accompanied the patients were asked to consider participation. If they agreed, they were presented with study information and then if they wished to proceed were consented (more detailed information about centers and number of recruits at each city is located in the appendix,

Fig. **1** and Table **8**). Following recruitment, a unique identifier code was used for each participant. Participants were divided into three groups: current smokers, ex-smokers and lifelong nonsmokers.

The inclusion criteria consisted of Jordanian adult, no history of respiratory chronic diseases or recent respiratory infections, and no cardiovascular diseases. Exclusion criteria consisted of inability to perform the spirometry tests successfully (shallow or short inhalation, short exhalation, or coughing during the test led to participate exclusion), aged younger than 18, presence of respiratory or cardiovascular problems. In addition, participants with body mass index (BMI) equal to or higher than 35 were excluded due to the effect of accumulated fat in the abdominal region on ventilatory mechanics [20, 21].

2.2. Data Collection

The weight and height of each participant was measured before the participants performed the test. An explanation of the spirometry test was conveyed by a certified technician.

Participants were given a questionnaire and a consent form. The consent form included a short summary of the study and its objectives, and the questionnaire included questions about medication use, smoking habits and health status, including incidence of asthma and other pulmonary diseases. Ethical approval was granted by the Ethical Committee of AlZaytoonah University, Amman, Jordan.

2.3. Measurement of Pulmonary Function

Spirometry tests were performed according to the European Respiratory Society (ERS) guidelines [22]. The tests were performed using a computer-based spirometer (MIR-Minispir New). The same spirometer was used by the same researcher for all tests and participants. Each participant was asked to take a deep breath and then blow out as strong and as fast as they could for at least 6 seconds until complete exhalation was performed. Nose clips were used to avoid any air leakage and a disposable turbine was used for each participant to preserve high hygiene conditions and prevent any spread of infection. Participants were told to seal their lips around the turbine before blowing out. The test was repeated three times and the best reading was recorded. Forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), forced expiratory rate (FEV1/FVC* 100), forced expiratory flow over the middle one half of the FVC(FEF25-75) and peak expiratory flow rate (PEFR) were measured. Acceptable maneuvers were achieved when the difference between the largest and the next largest FVC was ≤ 0.15L and the difference between the largest and next largest FEV1 was ≤ 0.15L. In PEFR, in adherence with ERS guidelines, the highest reading out of three acceptable readings was recorded.

2.4. Measurement of Anthropometric Parameters

Weight was measured to the nearest 0.1 kg using a standardized electronic weighing machine, with the participants standing without footwear and wearing light clothes. The height of participants was measured with a stadiometer, to the nearest centimeter. Body mass index

Table 1.   Demographics of the participants.

Variable	n	(%)
Gender		
Male	973	(55.1)
Female	794	(44.9)
Obesity Status*		
Underweight	26	(1.5)
Normal weight	714	(40.4)
Overweight	661	(37.4)
Obese	366	(20.7)
Age Group		
18-24	593	(33.6)
25-29	209	(11.8)
30-39	330	(18.7)
40-49	318	(18.0)
50-59	188	(10.6)
60-69	101	(5.7)
Above 70	28	(1.58)
Smoking Status		
Smoker	684	38.7
Ex-smoker	169	9.6
Non-Smoker	914	51.7

*Body mass index (BMI) was calculated by using Quetlet's index (body weight in kg/height in m^2 [21].

(BMI) was calculated by using Quetlet's index (body weight in kg/height in m^2 [23].

2.5. Statistical Analysis

ELA was calculated for each participant using different reference equations including those developed by Morris and Temple [12], Hansen. [19], Newbury [18], and Ben Saad [17]. Equations that were based on a non-Caucasian population were excluded. The frequency and percentage of participants whose ELA was below zero or over 110 years for each lung age equation for each group were evaluated according to a previous work [15]. The frequency and percentage of participants with ELA "clinically and significantly" [15] higher than the CLA for each lung age equation for each group was determined.

ELA was considered as "clinically and significantly" higher than the CLA, when the difference between the ELA and CLA is higher than the 13.4 years in men or 15.0 in women [15]. Paired t-tests were performed to compare the mean of the CLA with the mean of the ELA produced by each lung age equation for each group. Delta ELA-CLA was compared between the three smoking groups using analysis of covariance (ANCOVA). If the assumptions for running ANCOVA were not met Quade's rank analysis of covariance was used. Data were analysed with the Statistical Package for Social Sciences (SPSS) version 23 (Chicago, Illinois) [24].

3. RESULTS

3.1. Demographic Characteristics

A total of 1863 participants met the inclusion criteria and agreed to enroll. Of these, 96 were unable to complete the pulmonary function test (PFT) successfully; therefore, the studied participants included 1767 who were qualified for the study analysis. The demographics of the study participants are displayed in Table **1**. Just over half of the participants were male (55.1%). Most participants were classified as having normal weight or were overweight (40.4% and 37.4% respectively), although the inclusion criteria included BMI to be equal to or less than 35. About a third of the study sample was between the ages of 18 to 24, however, all age groups above 18 were represented in the study (Table **1**). Around half of the participants were lifelong non-smokers (51.7%); only 9.6% were ex-smokers.

Table 2.   Pulmonary function test (PFT) and Estimated Lung Age (ELA) calculated by different equations (numbers represent mean ± SD).

	Male			Female			All Participants		
	Smoker N=536	Ex-smoker N=127	Nonsmoker N=310	Smoker N=148	Ex-Smoker N=42	Non-Smoker N=604	Smoker N=684	Ex-Smoker N=169	Non-Smoker N=914
FEV1	4.05 ± 0.83	3.84 ± 0.69	4.10 ± 0.84	2.90 ± 0.59	3.00 ± 0.43	2.79 ± 0.54	3.80 ± 0.92	3.64 ± 0.73	3.23 ± 0.93
FVC	4.87 ± 0.91	4.69 ± 0.82	4.91 ± 0.97	3.42 ± 0.65	3.42 ± 0.59	3.31 ± 0.59	4.56 ± 1.05	4.38 ± 0.95	3.85 ± 1.099
FEF2575	3.98 ± 1.25	3.79 ± 1.34	3.95 ± 1.20	3.13 ± 0.90	3.68 ± 0.81	2.81 ± 0.91	3.79 ± 1.24	3.76 ± 1.22	3.17 ± 1.22
FEV1/FVC%	83.14 ± 7.18	81.94 ± 6.0	83.51 ± 6.63	84.82 ± 6.38	88.39 ± 7.13	84.33 ± 6.41	83.51 ± 7.04	83.54 ± 6.88	84.05 ± 6.75
Height	1.75 ± 0.07	1.74 ± 0.058	1.74 ± 0.07	1.61 ± 0.06	1.62 ± 0.06	1.59 ± 0.05	1.72 ± 0.09	1.71 ± 0.08	1.64 ± 0.09
BMI	27.53 ± 4.95	26.58 ± 5.13	26.43 ± 5.34	27.58 ± 5.38	24.04 ± 5.03	25.01 ± 4.33	27.55 ± 5.04	25.95 ± 5.21	25.49 ± 4.74
Chronological Age	33.91 ± 12.32	36.79 ± 17.46	36.51 ± 14.85	33.03 ± 10.77	25.05 ± 8.18	36.87 ± 14.96	33.72 ± 12	33.87 ± 16.46	36.75 ± 14.92
Morris & Temple FVC	43.47 ± 32.30	47.81 ± 30.47	40.35 ± 33.29	42.12 ± 22.76	43.61 ± 21.12	43.71 ± 21.41	43.18 ± 30.48	46.76 ± 28.43	42.57 ± 26.08
Morris & Temple FEV1	31.80 ± 23.91	36.97 ± 20.63	29.68 ± 23.41	31.95 ± 20.29	29.03 ± 14.59	34.09 ± 19.01	31.83 ± 23.16	34.99 ± 19.58	32.6 ± 20.7
Morris & Temple FEF2575	39.27 ± 27.27	43.03 ± 29.65	39.71 ± 26.07	40.51 ± 28.15	22.87 ± 27.14	49.7 ± 28.87	39.55 ± 27.45	37.65 ± 30.26	46.51 ± 28.37
Newbary	50.91 ± 25.59	56.09 ± 22.30	48.43 ± 24.89	46.43 ± 15.93	44.25 ± 11.64	47.81 ± 15.06	49.94 ± 23.89	53.15 ± 20.8	48.02 ± 18.96
Hansen	32.09 ± 23.22	35.42 ± 35.37	32.54 ± 22.63	32.30 ± 20.60	20.04 ± 21.79	35.75 ± 25.56	32.14 ± 22.66	31.6 ± 33.15	34.66 ± 24.64
Ben Saad	36.27 ± 16.84	38.75 ± 14.74	33.85 ± 16.09	50.93 ± 4.23	50.78 ± 3.1	52.09 ± 4.13	39.44 ± 16.2	41.74 ± 13.88	45.9 ± 13.17

3.2. Lung Age

PFT chronological age (CLA) as well as estimated lung age (ELA) were calculated by different reference equations for all participants (see Table **2**). Results are shown for the smokers, ex- smokers and non-smokers. Furthermore, results are presented for male and female participants.

3.3. Number of Participants with Abnormal ELA

Number and proportion of healthy Jordanian participants with ELA "clinically and significantly" higher than CLA (Table **3**) which was calculated based on previous work [15]. The percentage within male smokers, ex-smokers and non-smokers varied from as high as 60.4%, 63.0% and 48.4 % respectively (when using Newbury. equation) to 18.5%, 17.3% and 9.35 % respectively (using Morris and Temple

equation using FEV1) for the smoker and ex-smoker, and (Ben Saad for the non-smoker). For female participants the percentage for smokers, ex-smokers and non-smokers who had ELA "clinically and significantly" higher than CLA reached as high as 64.2%, 92.9% and 54.6% respectively (using Ben Saad. equation), while the lowest percentages were 13.5%, 14.3% and 8.4% respectively (using Hansen. equation for smokers and ex-smokers and Morris and Temple equation using FEV1 for the non-smoker).

Combining all participants, the highest percentages of participants who had ELA "clinically and significantly" higher than CLA in the smokers, ex-smokers and non-smokers were 58.0%, 63.9% and 39.3% respectively (using Newbury. equation for smokers and ex-smokers and Ben Saad. equation for non-smokers), while the lowest percentages were 17.7%, 14.8% and 9.7% respectively (using Morris and Temple equation using FEV1 for the

Table 3. Estimated Lung Age "clinically and significantly" higher, numbers represent mean (SD).

Equation	Male			Female			All Participants		
	Smoker N=536	Ex-smoker N=127	Nonsmoker N=310	Smoker N=148	Ex-Smoker N=42	Non-Smoker N=604	Smoker N=684	Ex-Smoker N=169	Non-Smoker N=914
Hansen.	105 (0.2)	19 (0.15)	35 (0.11)	20 (0.14)	6 (0.14)	58 (0.1)	125 (0.18)	25 (0.15)	93 (0.1)
Newbury.	324 (60.4)	80 (63)	150 (48.4)	73 (49.3)	28 (66.7)	192 (31.8)	397 (58)	108 (63.9)	342 (37.4)
Morris & Temple FEV1	99 (18.5)	22 (17.3)	38 (12.3)	22 (14.9)	22 (14.9)	51 (8.4)	121 (17.7)	34 (20.1)	89 (9.7)
Morris & Temple FVC	258 (48.20)	60 (47.20)	128 (41.30)	63 (42.60)	27 (64.30)	188 (31.10)	321 (47.00)	87 (51.50)	316 (34.60)
Morris & Temple FEF2527	122 (54.50)	45 (20.10)	57 (25.40)	43 (15.10)	11 (3.90)	231 (81.10)	165 (32.40)	56 (11.00)	288 (56.60)
Ben Saad	107 (66.90)	24 (15.00)	29 (18.100)	95 (20.50)	39 (8.40)	330 (71.10)	202 (32.40)	63 (10.10)	359 (57.50)

Table 4. Paired t test comparing estimated lung age calculated from different equations with chronological lung age in all smoking status in males.

Equation	Smoker			Ex-Smoker			Non-Smoker		
	Mean ± SD	t	P	Mean ± SD	t	P	Mean ± SD	t	P value
Morris & Temple FVC	-9.56 ± 28.29	-7.82	<0.01	-11.02 ± 26.96	-4.61	<0.01	-3.84 ± 9.32	-2.306	0.022
Morris & Temple FEV1	2.12 ± 19.96	2.46	0.01	-0.18 ± 16.73	-0.12	0.904	6.82 ± 19.20	6.26	<0.01
Morris & Temple FEF2575	-4.32 ± 22.75	-3.58	<0.01	-6.01 ± 21.59	-2.92	0.004	-1.58 ± 20.18	-1.149	0.25
Newbury10	-17 ± 21.56	-18.25	<0.01	-19.30 ± 17.77	-12.24	<0.01	-11.91 ± 20.49	-10.237	<0.01
Hansen	1.82 ± 20.53	2.06	0.04	1.364 ± 30.96	0.50	0.62	3.97 ± 18.81	3.715	<0.01
Ben Saad	-2.35 ± 14.46	-3.77	<0.01	-1.95 ± 15.05	-1.47	0.145	2.66 ± 14.034	3.341	0.001

smoker and non-smoker and Hansen. equation for the ex-smoker).

3.4. Comparing ELA Calculated from Different Reference Equations with CLA

Paired sample t-tests were conducted to compare ELA data calculated from different equations with CLA in males and females (see Tables **4** and **5**, respectively). In the males, statistically significant differences were shown between ELA and CLA when using Morris and Temple equation using FVC, Morris and Temple equation using FEF2575, Newbury and Ben Saad in smokers, while statistical significant differences were shown using Morris and Temple equation using FVC and Newbury in ex-smokers. Moreover,

significant differences were shown between Morris and Temple equation using FVC, Morris and Temple equation using FEV1, Newbury, Hansen and Ben Saad in non-smokers (Table **4**).

In females, significant differences were shown between Morris and Temple equation using FVC, Morris and Temple equation using FEF2575, Newbury, Hansen and Ben Saad in smokers. In addition, significant differences were shown between Morris and Temple equation using FVC, Newbury, and Ben Saad in ex-smokers. Furthermore, significant differences were shown between Morris and Temple equation using FVC, Morris and Temple equation using FEV1, Morris and Temple equation using FEF2575, Newbury and Ben Saad in non-smokers (Table **5**).

Table 5. Paired t test comparing estimated lung age calculated from different equations with chronological lung age in all smoking status in females.

Equation	Smoker			Ex-Smoker			Non-Smoker		
	Mean ± SD	t	P	Mean ± SD	t	P	Mean ± SD	t	P value
Morris & Temple FVC	-9.09 ± 20.92	-5.288	<0.01	-18.55 ± 21.87	-5.499	<0.01	-6.84 ± 19.50	-8.62	<0.01
Morris & Temple FEV1	1.07 ± 16.91	0.77	0.443	-3.98 ± 14.43	-1.787	0.081	2.78 ± 14.67	4.66	<0.01
Morris& Temple FEF2575	-6.57 ± 24.10	-2.82	0.006	1.60 ± 26.83	0.377	0.708	-10.35 ± 19.68	-11.23	<0.01
Newbury10	-13.40 ± 13.40	-12.169	<0.01	-19.20 ± 12.10	-10.278	<0.01	-10.94 ± 12.76	-21.06	<0.01
Hansen	0.72 ± 17.288	0.512	0.61	5.00 ± 20.48	1.583	0.121	1.12 ± 20.21	1.36	0.173
Ben Saad	-17.90 ± 9.58	-22.729	<0.01	-25.74 ± 8.355	-19.971	<0.01	-15.21 ± 12.71	-29.42	<0.01

Table 6. ANCOVA comparing the CLA-ELA between different smoking status (numbers represent mean (SD)).

	Males*		
	Smoker	Ex-Smoker	Non-Smoker
Morris & Temple FEV1 Adjusted Mean (SE) Mean Differences	-2.18(0.84) 4.56#	0.274(1.716) 7.02#	-6.748(1.1)
Newbury Adjusted Mean (SE) Mean Differences	16.9(0.9) 5.07#	19.3(1.8) 7.39#	11.9(1.18)
Ben Saad Adjusted Mean (SE) Mean Differences	1.94(0.59) 4.12#	2.54(1.21) 4.72#	-2.18(0.77)

*Age adjusted for 35.11,. # Significant at P<0.05

3.5. Comparing ELA- CLA among Different Smoking Status

ANCOVA assumptions were only met in the Newbury, Ben Saad, Morris & Temple FEV1 equations in males. Therefore, Quade's rank analysis of covariance was used in females and the rest of the equations in males. The one-way ANCOVA was conducted to determine differences between CLA and ELA on smoking status (controlling for age) where assumptions were met. The Morris and Temple equation using FEV1 showed a statistical significance within smokers, ex-smokers in males. Using the Newbury et al equation showed a statistical significance difference within smokers, ex-smokers in males. Finally, the Ben Saad *et al*. equation showed a statistical significance within smokers and ex-smokers in males (Table **6**). Quade's rank analysis of covariance showed significant differences between the different smoking status groups in all the studies equations except for Hansen and Morris and Temple FEF2575 equations on Females (Table **7**).

4. DISCUSSION

To the best of our knowledge, this study is the first to evaluate the applicability of the published spirometric reference equations for the ELA [12, 17-19] among the Jordanian population. It represents novel results comparing the CLA with ELA; results that may play a crucial role in convincing smokers of the importance of quitting this harmful habit.

Pulmonary function is known to vary between ethnic groups [15, 22, 25]. Hence, ELA reference equations based on American [12, 19], South Australian [18], or North African populations [17] cannot readily be used on the Jordanian population and potentially throughout the middle east. Additionally, one of the drawbacks of Morris and Temple equation is having 20-45% of non-Caucasian origin [12].

The reference equations should be derived from a "representative" population and should be updated at least every 10 years as per spirometry guidelines [22]. Similarly, these recommendations should be based on ELA reference equations. Morris and Temple's ELA reference equations [12] were formulated depending on the predictive equations [26] that are more than 40 years old. Furthermore, Hansen.'s equation [19] was developed from the results of the Third National Health and Nutrition Examination Survey (NHANES-3) data that were collected between 1988 and

Table 7.   Quade's rank analysis of covariance comparing the CLA-ELA between different smoking status.

Equation	Males		Females	
	F	**P Value**	**F**	**P Value**
Morris & Temple FVC	4.9	<0.01	6.53	<0.01
Morris & Temple FEV1`	N/A	N/A	5.51	<0.01
Newberry	N/A	N/A	6.90	<0.01
Ben SAAD	N/A	N/A	3.93	<0.01
Morris & Temple fef2527	3.85	0.02	0.74	0.48
Hansen	3.43	0.03	2.06	0.13

1994, and are now more than 20 years old. Demographic and environmental differences can result in a difference between a 30-year-old today and someone of the same age 40 years ago according to a cohort effect [14]. In addition, the use of outdated technology can produce inaccurate results. The use of old data for the creation of the ELA reference equation may be in part the reason for the diverging results found in our study.

It is noteworthy that the methods of ELA reference equations generation proposed in the previous studies may have some mathematical and statistical flaws. The need to establish a reliable method to estimate spirometric lung age is essential [27, 28]. Morris and Temple did not provide full detail of their method which may be a cause of some of the difference between their results and ours [12]. Additionally, Newbury. used the population mean to estimate lung age [18], which might lead to difficulty in estimating the lung age in individuals. Indeed, in our study, a large standard deviation was found in ELA for non-smokers and for smokers when analysis was based on from Newbury.'s equation [18]. Conversely, Hansen. presented a logical fallacy in which the equations proposed predict the actual mean age of the participants from whom they were derived [19].

In this report, ELA was estimated for 1767 healthy adult participants using equations developed by Morris and Temple [12], Newbury. [18], Hansen. [19] and Ben Saad [17]. Statistically significant differences in ELA results were demonstrated for the same participant when different reference equations were applied, both for males and females. Differences in results appeared even amongst the different equations of Morris and Temple's equations [12]. A significant difference between the CLA and the ELA among non-smokers in both males and females was also evident. Indeed, some of the equations calculated lung ages significantly higher than the chronological one among the healthy group; including the Morris and Temple FVC-based equation [12] and Newbury equation [18] in males, as well as the Morris and Temple FVC-based and FEF2527 based equations [12], and the Ben Saad. [17] and Newbury equations [18] in females. Moreover, upon calculating lung age for the participants, a discrepancy in the results was shown with a lung age above 110 or below zero; this was evident across the different reference equations. Indeed, within male participants, the highest percentage for above 110 years in smokers, ex-smokers and non- smokers was

given using the Morris and Temple equation using FEV1. While, in female participants the highest percentage above 110 years for ex-smokers and non- smokers was given through Morris and Temple equation using FEV1 and Hansen 's equation. Combining all participants, the highest percentage for above 110 years was shown in Morris and Temple's equation using FEV1. The highest percentage for below zero in male smokers, ex-smokers and non- smokers was given using Hansen. for the smokers and ex-smokers and Morris and Temple equation using FEV1 for ex-smokers and non-smokers. In female participants the highest percentage for below zero in smokers, ex-smokers and non- smokers was given using Hansen. Combining all participants, the highest percentage for below zero was shown in Hansen. No equation gave a zero percentage for above 110 years and the lower than zero in male and female participants simultaneously.

Results also showed that ELA predicted by Morris and Temple [12], Ben Saad [17], and Newbury [18] equations was significantly higher in ELA-CLA in smokers when compared with the non-smoker and the lifelong non-smoker groups in males. Conversely, significant differences were not found between smokers, ex-smokers and non-smoker groups in females using Morris and Temple FEF25-75 [12] and Hansen [19] equations. Thus, these results contradict previous findings related to the deteriorating effect of smoking on lungs. These findings also suggest that the existing lung age equations are not reliable in predicting lung age among a Jordanian population and thus cannot be used accurately in order to promote smoking cessation.

The discrepancy in the ELA among the non-smokers shown in this study might be due to the limited methodology used in previous studies [17]. A large percentage of the population sourced for this study was selected from participants that underwent general health screening examination as well as any participants accompanying them in various clinics and hospitals spread all around Jordan, parallel to study design in previous reports [15, 17]. In contrast to previous studies that published ELA reference equations, in which the sample selection was not a random population [12, 17-19], ours was a random sample. Several pharmacies, clinics and hospitals in different locations in Jordan were involved in recruiting volunteers which providing a reasonable degree of confidence in the generalizability of the results to the wider Jordanian population. The sample size required to confirm the

reference equations and to minimize the possibility of Type I or Type II errors due to sampling was found to be at least 150 male and 150 female participants [29]. In this study, the sample size that was used in the calculations was 1767 participants divided according to gender into 973 males and 794 females. Consequently, the sample size involved in this study is adequate and is actually higher than the sample size of numerous previous similar studies that were conducted in more populated countries (*e.g.* n= 125 (18), n= 540 [17] and n= 988 [12]). Furthermore, the calculations Similar percentages for male and female participants were used in this study (55.1% and 44.9% respectively) analogous to previously published studies [12, 18, 19] but in contrast to the studies that had the females dominating the sample [15, 17]. Furthermore, Hansen. formulated one equation [19] for both genders which contradicts prior work that has found a variation between the two genders in FEV1/FVC [30].

The sample used in this study was stratified into ages 18-24, 25-29 and then 10-year age bands for older ages. The sample showed a skewed to the right in age with over 30% of the participants being in the youngest age bracket. Although this was equivalent to previous work [18], the sample size of this study is much larger (1767 versus 988). The differences in the sample size and the gender representation among the samples may explain in part some of the differences in the results.

A representative sample of healthy volunteers was recruited into this study to perform the spirometric measurement, which is essential for the success of these types of studies [15]. This was not the case in previously conducted research. More than half of the participants in a previous study were selected from two church groups in rural USA, within those groups tobacco smoking, alcohol and caffeine intake were prohibited while a vegetarian diet was supported [12]. Another study chose the population from a broad rural community in South Australia focusing on the non-smokers with no history of lung disease [31]. A more recent study from South Australia recruited participants from three locations: in the first location, participants maintained a high level of fitness and had frequent occupational exposure to smoke while fighting fires and in the second location, participants were exposed to industrial and traffic pollution. The latter location revealed a significantly higher prevalence of COPD and lung cancer compared to other towns in Australia (which the authors associated with elevated levels of air pollution) [18]. Another study recruited participants from those undergoing general health screening examination in only one part of the country, such as at a Japanese health care center [15] or at a single hospital in Tunisia [17]. These samples [12, 15, 17, 18, 29] cannot be described as representative of a "normal" population or even a representative of a normal population within the ethnic group.

Another point of strength of the current study is that international guidelines definitions were used to identify the inclusion and non-inclusion criteria in this study for "healthy" adults [14, 26] as recommended in epidemiological studies. Moreover, recent international guidelines [15, 20, 25] for spirometry measurements were also employed [15, 17].

Recommendations for respiratory testing equipment and procedures have been frequently updated by the ATS/ERS [15, 22, 25]. Accordingly, spirometer reference equations should be implemented for a population using equivalent instruments and testing procedures [15, 22, 25]. Previous studies used different equipment, including Stead-Wells and dry rolling-seal spirometers [12, 19] or outdated testing procedures [18] that can provide different results to those currently recommended by the ATS [32]. This study followed the most recent ATS/ERS spirometry guidelines [15, 22, 25] as recommended by previous reports [15].

Previous studies have included only one lung function test, mainly the FEV1, in reference equations with a different model for each gender [12, 18]. Another study used FEV1/FVC [19], which was found to be independent of ethnicity [33, 34]. In addition, within the American population FEV1/FVC showed less variation than other absolute measures of spirometric volume or flow [35]. An ELA reference equation generated using only one spirometric parameter, namely FEV1, raises concerns over the reliability of its predictive capacity [27]. The variability of the spirometry results in healthy adults shown in previous work has created wide variation in ELA [18]. These factors that are associated with the construction of the ELA reference equations might in part explain the discrepancy between the results reported in previous work and those shown in our results.

5. LIMITATIONS

Being the first study of its type in Jordan, several limitations were found. First, this study was not a longitudinal one, therefore may not reveal possible changes connected to age. However most of the previous published equations were also not based on a longitudinal study. Furthermore, the aim of ELA is to give a strong message for smokers to encourage them to quit smoking at the time point of examination regardless of possible future changes. Second, the age of the study population was positively skewed. However, the large sample size that was incorporated into the study and the diversity of the locations gives credibility to our results. In addition, the message for encouragement to quit smoking is best utilized among young age smokers as the earlier a participant quits smoking the less damage smoking will have on health: quitting smoking at the age of 30 increases life expectancy by 10 years while quitting at the age of 60 adds only 3 years to the life expectancy [5]. Finally, data on some potential risk factors were unavailable, including birthweight, indoor air pollution from fuel used for cooking, or outdoor air pollution exposure. However, the objective of this study was to analyze the applicability of ELA equations on the Jordanian population and their ability to differentiate between smokers and nonsmokers who were living in the same geographical areas.

CONCLUSION

In conclusion, results of this study strongly suggest that the existing ELA reference equations are not a reliable source to estimate ELA within the Jordanian population. New regression equations should be formulated that depend on a more relevant population sample and to base the equations on current data. Future studies should follow the

ATS/ERS spirometry guidelines and numerous spirometric parameters should be included as explanatory variables.

LIST OF ABBREVIATIONS

ANCOVA	=	Analysis of Covariance
BMI	=	Body Mass Index
COPD	=	Chronic Obstruction Pulmonary Disease
ELA	=	Estimated Lung Age
ERS	=	European Respiratory Society
FEF	=	Forced Expiratory Flow
FEV1	=	Expiratory Volume in one second
FVC	=	Forced Vital Capacity
NHANES-3	=	Third National Health and Nutrition Examination Survey
PEFR	=	Peak Expiratory Flow Rate
PFT	=	Pulmonary Function Test
SPSS	=	Statistical Package for Social Sciences

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was granted by the Ethical Committee of AlZaytoonah University, Amman, Jordan.

HUMAN AND ANIMAL RIGHTS

No Animals were used for studies that are base of this research. All human procedures were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the participants.

CONFLICT OF INTEREST

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

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Declared none.

APPENDIX

Participants where approached in the following locations:

I) Amman the capital, and the most populated city, we recruited **812** subjects from four sites

1. **AlZaytoonah University of Jordan** which has over 10000 students and about 1000 teaching staff from different areas of Jordan, Amman, Zarqa, Irbid, Madaba and Fuheis.

2. **The Al Husain Medical City**, which is the largest medical compound in Jordan composed of five hospitals that provide health care for the patients coming from different regions of the Kingdome (All the recruited subjects are those who were accompanying the patients.

3. **Zamzam polyclinic** which is a large polyclinic in Khalda near the city center.

4. **Pharmacy one** which is a chain pharmacy which has branches distributed all over the country, were we offered the service of measuring the pulmonary function tests free for the customers.

II) Zarqa: The second largest city 3 sites were included (n= 423).

1. Zarqa Governmental Hospital.
2. ultan Clinic
3. Pharmacy One.

III) Irbid: The third largest city, two sites were included (n=233).

1. **Irbid specialized hospital.**
2. **Zamzam polyclinic of Irbid**

IV) Madaba: one site was included (n=176)

1. **Madaba Health Center.**

V) Fuheis: the least populated city were one site was included (n= 123).

1. **Al-Fuheis Comprehensive Health Center**

Advertisements were placed on the internet and in places were participant recruitment was conducted, then a personal approach was used to invite people to read about the study and consider participation.
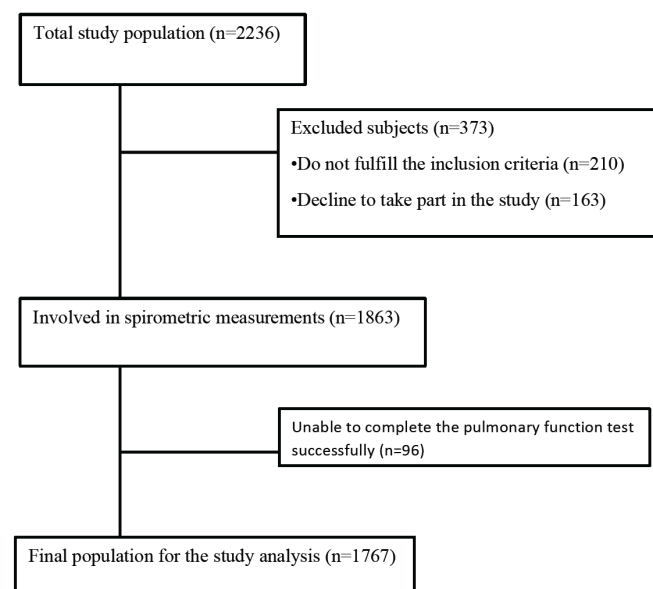

Fig. (1). Recruitment time frame.

Table 8.   Recruits' distribution.

City	Sites	Population	No of Subjects
Amman	4	1,275,857	812
Zarqa	3	792,665	423
Irbid	2	307,480	233
Madaba	1	82,335	176
Fuheis	1	18916	123

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