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Turkish Thoracic Journal
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AIMS AND SCOPE

Turkish Thoracic Journal is the conceptually scientific, open access and official publication of the Turkish Thoracic Society. The publication language is both Turkish and English and it is an international journal based on independent, unbiased, and double-blind peer-review principles.

Turkish Thoracic Journal started its publication life following the mergence of two seperate journals which are published under the titles "Turkish Respiratory Journal" and "Toraks Journal" until 2007. Archives of both journals were passed on to the Turkish Thoracic Journal.

The aim of Turkish Thoracic Journal is to publish pulmonary disease-related clinical, experimental and epidemiologic studies that are scientifically highly qualified. Additionally, reviews, editorials, letters to the editor, and case reports are also accepted. Reports presented in meetings organized by the Turkish Thoracic Society Head Office or national and international consensus reports are published as supplements. The journal is published 4 times annually, in January, April, July and October. The target-groups are chest diseases physicians, thoracic surgeons, internal medicine doctors and practitioners interested in pulmonary diseases.

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INFORMATION FOR THE AUTHORS

1. The Turkish Thoracic Journal is a periodical of the Turkish Thoracic Society and 4 issues are published annually.

2. The aim of the journal is to convey scientific developments in thoracic diseases and surgery, and to create a dynamic discussion platform about pulmonary diseases. With this intent, the journal accepts articles from all related scientific areas that address thoracic diseases and cell biology, epidemiology, immunology, pathophysiology, thoracic imaging, pediatric chest diseases, environmental and occupational disorders, intensive care, sleep disorders and thoracic surgery. Clinical and research articles, reviews, statements of agreement or disagreement on controversial issues, national and international consensus reports, abstracts and comments of important international articles, interesting case reports, puzzling cases, writings related to clinical and practical applications, letters to the editor, and editorials are accepted.

Presentations and reports of meetings organized by Turkish Thoracic Society Head Office and its branches can be published as supplements.

3. The publication language of the journal is English.

4. The Editorial Committee has the right of not publishing a manuscript that is not in compliance with the authors' instructions, request revisions from the authors and reediting. Submitted manuscripts are published following the evaluation by at least two reviewers, and approval of the Publication Committee.

5. The submitted manuscripts should not been submitted for publication or published elsewhere. Studies previously announced in the congresses are accepted if this condition is stated. Those who want to withdraw their manuscripts from the journal due to delays or any other reason should submit a written application. No royalties or remuneration will be provided to the author(s) and the author agrees that all publication rights belong to the Turkish Thoracic Society. Scientific and legal responsibilities of the published manuscripts belong to the authors.

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7. The content of the submitted manuscripts should conform to the criteria stated in *ICMJE-Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (updated in December 2014-http://www.icmje.org/icmje-recommendations.pdf*).

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10. Research articles should not exceed 3500 words and 35 references. Case reports should not exceed 1500 words and 10 references.

11. Simultaneously with the submission of manuscripts, the "Author Agreement Form" signed by all contributing authors should be sent to the Turkish Thoracic Journal Editorial Office via fax or e-mail. Otherwise, submitted manuscripts will not be taken into consideration.

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a. Articles should be typed double-spaced using Times New Roman style and 12 fonts and should have 3 cm margins on the sides, top and bottom of each page. Page numbers should be placed at the mid-bottom of each page.

b. Articles and reviews should be prepared in accordance with the instructions below:

The first page should include the title of the article in English (should not exceed 90 characters) and the running title in English (should not exceed 45 characters).

The second page should include English abstract that do not exceed 250 words. A structured abstract with Objectives, Material and Methods, Results, and Conclusion sections should contain the aim of the study, main results of the study, and a brief conclusion. The above mentioned structure does not apply to the case reports and reviews; a short abstract of no more than 200 words is required.

At least three key words in English should be placed right after the abstract. Key words should comply with the Medical Subject Headings: MeSH. Medical Subject Headings (MeSH) which can be found at www.nlm.nih.gov/mesh/MBrowser.html.

Third page and the subsequent pages should include the main text.
In review articles, subtitles should be used in order to provide a better understanding on the subject. In a review article, it would be beneficial to provide different sections such as the context of the problem, historical information, basic knowledge, methodology, animal and human experiments, discussion, conclusion, suggestions and future studies.

Research articles should include separate sections for Introduction, Material and Methods, Results, Discussion. Pharmaceutical products can be mentioned either with their generic or commercial names (generic names are preferred). Commercial names should be written with capital letters, followed by the company and its city in parenthesis. Acknowledgements, references, tables and figure legends should follow the main text. Tables should be presented at the end of the text and each on a separate page.

c. The "Acknowledgements" section should be placed at the end of the text before the references and should not exceed one paragraph.

d. References, tables and figures should be placed in the order of appearance in the text. References should be mentioned in brackets and at the end of the sentences. The titles of journals must be abbreviated according to the style used in Index Medicus. Full titles should be used for those that are not cited in Index Medicus. When more than two consecutive references are used, only the first and last reference numbers should be written [such as: 3-9]. When there is more than four authors within the identification of the referred article, only the names of the first three authors should be used followed by "et al.". If an article has four or less authors, all names should be used. Research articles and reviews should not exceed 35 references. Case reports should not exceed 10 references. References should be written according to the Index Medicus and in Vancouver Style as illustrated below.

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Vega KJ, Pina I, Krevsky B. Transplantation is associated with an increased risk for pancreatobiliary disease. Ann Intern Med 1996;124:980-3.

Supplementary
QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994;102 (Suppl 1): 2755-82.

Summary Format (Letter, Summary and Editorial)
Ennzensberger W, Fischer PA. Metronume in Parkinson's disease (Letter). Lancet 1996;347:1337.

Books and Other Monographs
Book
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With author
Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany, NY: Delmar, 1996:56.

With editor
Norman IJ, Redfem SJ, eds. Mental Health Care for Elderly People. New York: Churchill Livingstone,1996: 67-9.

Book chapter
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Phillips SJ, Whistant JP. Hypertension and stroke. In: Laragh JH, Brenner BM; eds. Hypertension: Pathophysiology, diagnosis and management. 2nd ed. New York: Raven Pr, 1995:466-78.

Congress Abstract Book
Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-5.

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Smith J. New agents for cancer chemotherapy. Presented at the Third Annual Meeting of the American Cancer Society, 13 June 1983, New York.

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Online Reports
World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects. www.wma.net/e/policy/pdf/17c.pdf. Updated September 10, 2004. Accessed July 9, 2008.

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e. Tables: Each table should be typed on a separate page and table's entries should be double-spaced. Tables should be numbered with arabic numeral(s) and cited in the order of appearance in the text. A brief title for the table should be written above the table.

f. Figures: All figures should be high-quality (at least 300 dpi resolutions) in .jpeg or .jpg format, and should be provided in black and white. If providing a better understanding of the topic, colored figures will be accepted in limited number. For each manuscript, six figures at most will be accepted. Figures should be numbered with Arabic numeral(s) in order of appearance in the text. The type of the dye that was used, magnification scales, and internal scale bar should be stated for microscopic photographs. A centimetric template should be added for pathologic specimens. Ethical values should be protected in any patient-related photograph or graphs. If the identity of the patient can be revealed by the provided photographs and graphs, a written consent should be requested from the patient. The figures should be cited in parenthesis with their respective numbers within the main text. All figure legends should be on a separate page after references and tables. A written permission is required for reproduced figures.

g. Video: Videos submitted for online broadcasting purposes, on the internet site of Turkish Thoracic Journal, are accepted. The video dossier should be maximum 3MB in size and in .mpeg or .vmf format.

h. Case reports should contain sections for English title, English running title, English abstract, keywords, Introduction, Case Presentation and Discussion. They should include new cases or imply clear messages. All submitted case reports will be first reviewed by the editorial committee and those that do not include new cases and/or do not imply clear messages could be rejected without sending it for arbitration.

i. In puzzling case reports, a short introduction should be followed by the description of the problem, presentation of clue photos and figures, definite diagnosis, and a discussion section where the diagnosis is discussed and educational messages are emphasized.

j. Disagreement/agreement articles should not exceed three pages, and clinical practice articles should not exceed three pages including text, figures, images and references.

k. The section for the "Letters to the Editor" should be formatted shortly and concisely, without any summary, and should be restricted in the number of references since it is mainly written to provide support or criticism over previously published articles.

l. Abbreviations should be written in the accepted international format and under parenthesis on the first mention and this abbreviation should be used throughout the text.

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Information such as the type of the article, institutions, authors, title, abstract, keywords, and cover letter is entered in the first eight steps.

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Step-6: This is the step where the abstract is entered. Abstract should not exceed 200 words for case reports and reviews, and 250 words for research articles. Abstracts for research articles should include the following sections: Introduction, Material and Methods, Results and Conclusion.

Step-7: This is the step where the keywords are entered. English keywords should be selected by connecting the MeSH link provided in this window.

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Part 2

Step-9: From hereon, the identification of the manuscript has been completed. The main text, video and figures of the article should be submitted in this step. There should be no figures within the text file, except for the tables. For instance, three files should be submitted in this step for a manuscript containing one figure and one graph in the body (a file for text, a file for figure, and a file for graph). Figure and video files should be uploaded first. No figures should be placed in the text file. All images, graphs, and other figures within the manuscript should be uploaded with the names used in the manuscript (such as Fig 1 or Graph 1).

Any of the writing editors can be used for the text file (such as Microsoft Word, Notepad, and WordPad). However, MS Word will be necessary if the text contains a table. **Since all identification details were provided in former steps, the authors' names, institutions, and correspondence address are not required herein.**

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- Running title (English)
- Abstract (English)
- Keywords (English)
- Main text
- References
- Tables with titles
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EDITORIAL

The Future Plans of Turkish Thoracic Journal

Oğuz Kılınç[1,2], Metin Akgün[1,3]

[1]Chief Editor
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[3]Department of Chest Diseases, Atatürk University Faculty of Medicine, Erzurum, Turkey

In this issue Yazıcıoğlu A, et al. (1) analyze 58 massive hemoptysis cases, introduce changing etiology of hemoptysis with time, and emphasize the importance of bronchial arterial embolization because of its low morbidity and mortality rate in the management of such cases while keeping surgery in reserve. According the article, tuberculosis was the most common etiology in the past; however, it is the fifth cause in their series. Currently, bronchiectasis is the most common causes of hemoptysis. The other research studies indicates that use of fluorescence in situ hybridization assay in detecting cancer in bronchial lavage specimen was fruitful, investigation of sleep quality and sleep disorders among medical students determines higher Epworth sleepiness score and Pittsburgh score in female students compared to male students, and the study evaluating of heat shock proteins (HSP) role in COPD suggests that HSP27 stepped forward as a diagnostic marker for COPD because of its higher specificity and sensitivity.

The Turkish Thoracic Journal, which is the official journal of Turkish Thoracic Society (TTS), aims to be more visible not only regionally but also globally. For this reason, its publication language was set as English a short while ago. Also, TTS has provided free translation service for Turkish researchers during this transitional period. We believe that rapid, qualified and unbiased evaluation of submitted manuscripts by editors and reviewers also important in maintaining respect of the journal (2). Luckily we, as TTS, have considerable number of MECOR, the American Thoracic Society Methods in Epidemiologic, Clinical, and Operations Research, Program (3) graduates among the editorial board members and reviewers.

We are working hard to increase our visibility in the near future and looking forward to share some good news next year.

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ORIGINAL INVESTIGATION

Investigation of Sleep Quality and Sleep Disorders in Students of Medicine

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Abstract

OBJECTIVES: This study was performed on Suleyman Demirel University medical students to determine the quality of sleep and to investigate factors that affect of sleep quality.

MATERIAL AND METHODS: Suleyman Demirel University Medical students at 1, 2, 3, 4, 5 and 6 classes included to this cross-sectional analytical study (n= 720). Refused to fill to the survey (188), and students were not come to faculty (195), applied survey to 337 students (46.8%). Epworth sleepiness scale (ESS), Pittsburgh (PSQI) and Berlin sleep questionnaires, and 13 pieces closed and open-ended socio-demographic questions were conduct a questionnare under observation. The collected data were analyzed by using descriptive statistics, chi-square, two independent groups t test, Pearson and Spearman's correlation, Mann-Whitney U, Kruskal-Wallis and ANOVA tests.

RESULTS: 337 students participated in the study, 42.1% were male, 57.9% were female, mean age was 21.3 ± 2.1 years. Depending on Body mass index (BMI) 31 were poor, 212 normal, 53 overweight, and 4 obese students. In 118 students (35.3%), and these students have a chronic disease associated with 15.6% used the drug because of illness and 38 percent of students (11.6%) were smokers. 18.1 ± 16.1 min for pupils in times of falling asleep, sleep duration per night. 6.6 ± 1.3h, the mean departure time was 7.7 ± 1.8. Scale with a total score of Pittsburgh class (p= 0.000), age (p= 0.003), BMI (p= 0.015) had a significant correlation between. Pittsburgh PUKI scores and without a significant difference in gender (p= 0.054), the use of stimulant substances (p= 0.032), weight (p= 0.021) and snoring (p= 0.002) with no significant difference were found. ESS total score and gender (p= 0.025), drug use (p= 0.035) and sports activities (p= 0.038). Ten students had snoring (3.0%), 5 students (1.5%) had witnessed apnea. Snoring 17.2% to in ESS > 10 points on it. Pittsburgh, the mean scores of those who witnessed apnea (14.0 ± 5.3), witnessed apnea, according to non-students (10.2 ± 6.4) were higher (p= 0.191).

The effects PSQI and ESS results on the term were statistically significant by the multivariate regression analysis [F(10.602)= 4.56; p< 0.05; Wilkis Lamda 0.864, partial n^2= 0.07]. To estimate of the value of PSQI by the stepwise regression analysis was performed; age and fall asleep properties has been included of the model (R2= 89%, p< 0.05). To estimate of the value of PSQI by the stepwise regression analysis was performed; fall asleep property has been included of the model in the the male gender (R2= 80%, p< 0.05). To estimate of the value of ESS by the stepwise regression analysis was performed; term property has been included of the model (R2= 65%, p< 0.05).

CONCLUSION: Medical school students participating in our study, although female-male ratio close to each other, we found that higher ESS and Pittsburgh scores in female more than male. In this case may be related to physiological, genetic, environmental, cultural and psychological differences.

KEYWORDS: Medical students, sleep quality, sleep disorders, Epworth, Pitssburg

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INTRODUCTION

Sleep is the state, in which the organism temporarily, partially and periodically loses its interaction with surroundings at different intensities and which can be reversed with stimuli, comprising approximately one-third of human life [1,2]. Sleep is not only a time frame left out of daily life but it also is a vital necessity for a long and healthy life [3]. In human life, sleeping is an essential necessity as important as breathing, eating and voiding, and it is the basic condition to be healthy either physically or mentally [4,5]. Sufficient sleep is necessary for the healthy functioning of the body. Sleep affects learning and memory. Sleep is a critical factor for health, body weight and energy level. Properties that affect the quality and quantity of sleep are the length of the elapsed time to fall asleep, the number of times awakening from sleep and sleep duration [6]. Diseases related to sleep disorders are diabetes mellitus, hypertension, stroke, and coronary artery disease. Hypertension, stroke, coronary artery disease, and arrhythmia are more frequently encountered in patients with sleep disorders. It is thought that insufficient sleep in childhood and puberty can negatively affect appetite and energy consumption by having an effect on the hypothalamus. It has been reported that depression symptoms decrease with sufficient sleep durations [7]. In a study conducted in 19 countries in Europe, frequency of insomnia has been detected as 17% [8]. Sleep disorder is a clinical condition that manifests itself due to medical, psychological, environmental, and work reasons, and that shows itself with insomnia as a result of restriction, disruption or loss of sleeping pattern [9]. Sleep is necessary to protect the normal functioning of MSS. Natural communication and balance between neuronal centers is protected by sleep. Glycogen storages of the brain is renewed in sleep.The best indicator of the importance of sleep for the central nervous system is the deceleration of mental activity and the manifestation of psychic disorders (hyperactivity, emotional lability, etc.) in insomnia [10].

Obstructive Sleep Apnea Syndrome (OSAS) was defined in 1997 by ASDA (American Sleep Disorders Association) as a syndrome characterized with repetitive upper respiratory tract obstruction,and it is typically seen together with a reduction in blood oxygen saturation. According to a study, OSAS prevelance has been detected as 4% in male and 2% in female patients [11]. According to a study by Köktürk et al., OSAS prevelance has been found as 0.9-1.9% in patients with habitual snoring [12]. Furthermore, OSAS is the most frequently encountered disease among all 85 sleep disorders that have been defined until today [13]. Major signs of OSAS are snoring, witnessed apnea and daytime sleepiness. Daytime sleepiness is the most frequent cause of application to sleep centers. This study aimed at investigating the factors affecting quality and healthy sleep in students of medicine by assessing the sleep states of these students in a long and difficult education period.

MATERIALS and METHOD

After having received necessary oral and written consents from the Suleyman Demirel University Medical Faculty Advisory Board of Scientific Research Projects (12.01.2011 XIX Meeting, n: 8), informed consent was taken from the students who agreed to participate in the study. The population of across-sectional-analytic study carried out between January and May 2011 was composed of all students of Suleyman Demirel University Medical Faculty (n: 720). Suleyman Demirel University Medical Faculty students attending preparatory school (n: 8) were not included into the study. Sample size was determined as 720 students by having reached all classroom sizes in the faculty. The students who could not participate in the questionnaire for any reason were tried to be reached three times more. As a result, 337 students out of 720 were reached (46.8%) since there were 188 students who did not accept to fill out the questionnaire and 195 students who could not come or were on leave of absence. The students participating in the study were asked a total of 13 open-ended and closed-ended questions oriented at designating their socio-demographic characteristics.

Epworth sleepiness scale, whose validity and reliability has been conducted in Turkey, was used in order to evaluate excessive daytime sleepiness, Pittsburgh sleep quality index (PSQI) was used to evaluate sleep quality and questionnaire forms of Berlin sleep apnea screening tool (BSAST) were performed under supervision.

Items on the Epworth scale comprise a total of 8 likert type questions on a 4-point scale. The measurement is performed separately for each sub-dimension of the scale. The students who receive 10 points and over from a total of 0-24 points are in need to be examined in a sleep laboratory and suffer from excessive daytime sleepiness [14].

Pittsburg Sleep Quality Index is a self-report scale including 19 items. Each item of the scale is equally given 0-3 points. The questions are given 0-3 points and high grades reflect bad sleep quality. The scale consists 7 sub-scales evaluating sleep quality, sleep latency, sleep disorders, sleeping pill use, and loss of functionality during daytime. Total PSQI grade, which ranges between 0-21, is obtained with the addition of sub-scales. A total PSQI grade over 5 indicates, with 89.6% sensitivity and 86.5% specifity, that the individual's sleep quality is insufficient and shows that there is either severe deterioration in two scopes the least or moderate deterioration in three scopes mentioned above [15].

BSAST is comprised of a total of 10 questions in 3 categories, which are as follows: Category I: snoring, witnessed apnea (5 questions); Category II: daytime sleepiness (4 questions); and Category III: hypertension or obesity (1 question). Each category is evaluated in itself, and high risk is established if 2 or more categories are significant. In a study conducted for the reliability of this questionnaire, its sensitivity and specifity have been found as 62% and 43% respectively [16].

Moreover, degree of obesity of the students was found using body mass index (BMI). BMI limit values were calculated as follows: 18.4 kg/m^2 and below: underweight; 18.5-24.9 kg/m^2: normal or healthy weight; 25-29.9 kg/m^2: overweight; 30-39.9 kg/m^2: obese; 40 kg/m^2 and over: morbid obese. The

collected data were analyzed on SPSS 15.0 package program by descriptive statistics, Chi-square, independent two-samples t-test, Pearson and Spearman's correlation, independent samples t-test, Mann-Whitney U test, Kruskall-Wallis and ANOVA tests.

RESULTS

Out of the 337 students participating in the study, 141 of the students (42.1%) were male and 194 (57.6%) were female. Mean age of the students was 21.3 ± 2.1 (the youngest: 18 years of age; the oldest: 30 years of age). Mean sleep duration of the students at night was 6.6 ± 1.3 (3 hours the least; 12 hours the most). 6 of the students (1.8%) were using pleasure-inducing substance. An obese individual was present in the families of 55 students (16.6%) participating in the study. 38 of the participating students (11.6%) were smoking. A vast

Table 1. Socio-demographic data of the students

		n (Number)	% (Percentage)
Gender	Female	194	57.6
	Male	141	41.8
Distribution of the students as regards terms	Term I	57	17
	Term II	67	20
	Term III	68	20.3
	Term IV	42	12.5
	Term V	58	17.3
	Term VI	43	12.8
BMI distribution	Underweight	31	10.3
	Normal	212	70.7
	Overweight	53	17.7
	Obese	4	1.3
Cigarette smoking	Non-smoker	27	71.1
	1-2 daily	2	5.3
	3-5 daily	2	5.3
	5-10 daily	3	7.9
	10-20 daily	2	5.3
	20+ daily	2	5.3
Alcohol consumption	Non-consumer	239	70.9
	1-2 annually	40	11.9
	1-2 monthly	43	12.8
	1-2 weekly	12	3.6
	Regularly everyday	3	0.9
Exercise	Do not exercise	107	31.8
	1-2 hours monthly	100	29.7
	1-2 hours weekly	95	28.2
	At least 3 days in a week	27	8.0
	Regularly everyday	8	2.4
Distribution of the students' level of income	101-600 TL	11	3.3
	601-900 TL	32	9.7
	901-1200 TL	53	16
	1201-1500 TL	64	19.3
	Over 1500 TL	171	51.7
Distribution of students' health insurance	State retirement fund	140	47.1
	Social security Institution	100	33.7
	Pension fund for the self-employed	49	16.5
	Private	1	3
	Other	7	2.3
Pleasure-giving substance use	No	328	97.3
	Yes	6	1.8
Cigarette smoking	No	300	89.0
	Yes	37	11.0

majority of the students participating in the questionnaire had social security (Table 1).

Evaluation of the Data Obtained from the Questionnaire

54 (17.9%) of the participating students were detected by the Epworth sleep scale to have excessive daytime sleepiness. Snoring was present in 10 of the participating students (3.0%). Frequency of excessive daytime sleepiness of the snorers was found as 17.2% and as 12.4% in non-snorers. This difference was not detected to be statistically significant (p= 0.494). Mean dozing off duration of the students who suffered from excessive daytime sleepiness (18.3 ± 15.1 min) was found lower when compared to the ones who did not suffer from excessive daytime sleepiness (16.9 ± 21.4 min); however, the difference was not statistically significant (p= 0.584). Mean sleep duration of the students who suffered from excessive daytime sleepiness (6.4 ± 1.7 h) was found lower when compared to the ones who did not suffer from excessive daytime sleepiness (6.6 ± 1.2 h); however, the difference was not statistically significant (p= 0.277) (Tables 2,3).

Mean PSQI grade of the students was found as 9.03 ± 4.21 by the evaluation of Pitssburg sleep quality questionnaire. The rate of the students whose sleep quality was bad (PSQI > 5) was 79.62%. As regards gender, mean PSQI for female and male students were respectively as 9.27 ± 4.36 and 8.69 ± 3.98. Mean sleep duration, mean awakening time and mean dozing off duration were found respectively as 6.6 ± 1.3 h (3 h the least, 12 h the most), 7.7 ± 1.8 (06:00 the earliest, 17:00 the latest), 18.1 ± 16.1 min (0 min the least, 120 min the most) (Tables 4,5).

Five individuals (1.5%) were found to suffer witnessed apnea with the evaluation of BSAST. Out of the 5 individuals with witnessed apnea, 20.0% was found to have excessive daytime sleepiness by the Epworth scale, and the ones with excessive daytime sleepiness without witnessed apnea was found as 15.5%. This difference was not regarded as statistically significant (p= 0.786). Again, mean Pittsburgh grades of the students with witnessed apnea detected by BSAST (14.0 ± 5.3) was found higher when compared with the students without witnessed apnea (10.2 ± 6.4). However,

Table 2. Comparison of students with excessive daytime sleepiness detected by the EPWORTH scale with some variables

Variables		PSG end %	p
Gender	Female	22	0.025*
	Male	12	
Marital status of the family	Divorced/Widower	22.2	0.425*
	Living separately	50	
Pleasure-giving substance use	No	17.9	0.904*
	Yes	20	
Obesity in the family	Present	16.9	0.232*
	None	24	
BMI	Underweight	25.9	0.079**
	Normal	19.7	
	Overweight	12.5	
	Obese	0	
History of chronic disease	Present	15.9	0.225*
	None	21.5	
Drug use	Present	16.3	0.035*
	None	29.2	
Cigarette smoking	Present	18.8	0.317*
	None	11.8	
Social Security	Present	24	0.363*
	None	16.8	

* Chi-squared test,
** Trend chi-squared test.

Table 3. Comparison of the association between some variables and the scores of the Epworth scale

Epworth cale	Term/Class*	Financial status*	Age**	BMI**	Exercise*	Alcohol consumption*
Tal score	r= -0.072	r= 0.029	r= -0.084	r= -0.110	r= -0.120	r= -0.045
	p= 0.214	p= 0.612	p= 0.146	p= 0.068	p= 0.038	p= 0.440

* Spearman's correlation,
** Pearson correlation.

Table 4. Comparison of the association between some variables and Pittsburgh scores

Variables		PSQI (mean ± std deviation)	p
Gender	Female	10.8 ± 6.1	0.054*
	Male	9.2 ± 6.1	
Social Security	Yes	10 ± 5.9	0.683**
	No	11.8 ± 8.6	
History of chronic disease	Present	10.9 ± 5.5	0.192*
	None	9.7 ± 6.5	
Drug use	Yes	11.7 ± 5.4	0.057**
	No	9.9 ± 6.3	
Cigarette smoking	Yes	9.7 ± 7.1	0.384**
	No	10.2 ± 6.1	
Pleasure-giving substance use	Yes	2 ± 1.4	0.032*
	No	10.2 ± 6.2	
BMI	Underweight	10.1 ± 6.5	0.021***
	Normal	10.8 ± 6.1	
	Overweight-Obese	8.1 ± 5.9	
Snoring	Present	12.1 ± 5.2	0.002**
	None	8.4 ± 5.7	

* Independent samples t test,
** Mann-Whitney U test,
*** Kruskall-Wallis test.

Table 5. Comparison of the association between some variables and scores of the Pittsburgh scale

Pittsburgh scale	Term*	Financial status*	Age**	BMI**	Exercise*	Alcohol consumption*
Total score	r= -0.255	r= -0.059	r= -0.203	r= -0.174	r= -0.098	r= 0.114
p	p= 0.000	p= 0.396	p= 0.003	p= 0.015	p= 0.157	p= 0.101

* Spearman's correlation,
** Pea's correlation.

a significant difference was not observed between the two grades (p= 0.191).

Figure 1 and Figure 2 show Epworth, PSQI and BSAST scares as regards the terms.

For BSAST, the number of students with an elevated risk was found high in Term III and Term IV students.

Multivariate regression analysis found the effect of terms or classes of medical faculty students to have a statistical significance on PSQI and ESS results (F(10.602)= 4.56; p< 0.05; Wilkis Lamda 0.864, partial n2= 0.07).

The effect of terms or classes of the students on PSQI and ESS results was statistically significant (PSQI: F(5.302)= 6.5, p< 0.05; partial n2= 0.09) ESS: F(5.302)= 3.0 p< 0.05; partial n2= 0.05).

In terms of PSQI results, there is a statistically significant difference between 1st year and 5th year students (p< 0.05) and 1st year and 6th year students (p< 0.05); and between 2nd year and 5th year students (p< 0.05) and 2nd year and 6th year students (p< 0.05).

In terms of ESS results, there is a statistically significant difference between 3rd and 5th year students (p< 0.05).

The characteristics of age and dozing off were added into the model in the Stepwise regression analysis conducted to predict PSQI value (R2= %89, p< 0.05).

In the male gender; the characteristic of dozing off was added into the model in the Stepwise regression analysis conducted to predict PSQI value (R2= 80%, p< 0.05). The characteristic of term was added into the model in the Stepwise regression analysis conducted to predict ESS value (R2= 65%, p< 0.05).

DISCUSSION

This is the first epidemiological study on sleep disorders in students of Faculty of Medicine in our country. Since the education given in the Faculty of Medicine is long and difficult, the students are required to be active constantly. Responsibilities brought upon students of medicine, including studying, preparation to frequent exam programs, internship preparations, patient organization, and being on call at the emergency service or the clinic, set forth this situation. Within this cycle, the circadian rhythm of normal sleep is naturally broken, and irregular and less sleep is perceived as normal. If this process continues, there will be deterioration in sleep quality and reduction in sleep duration, leading to

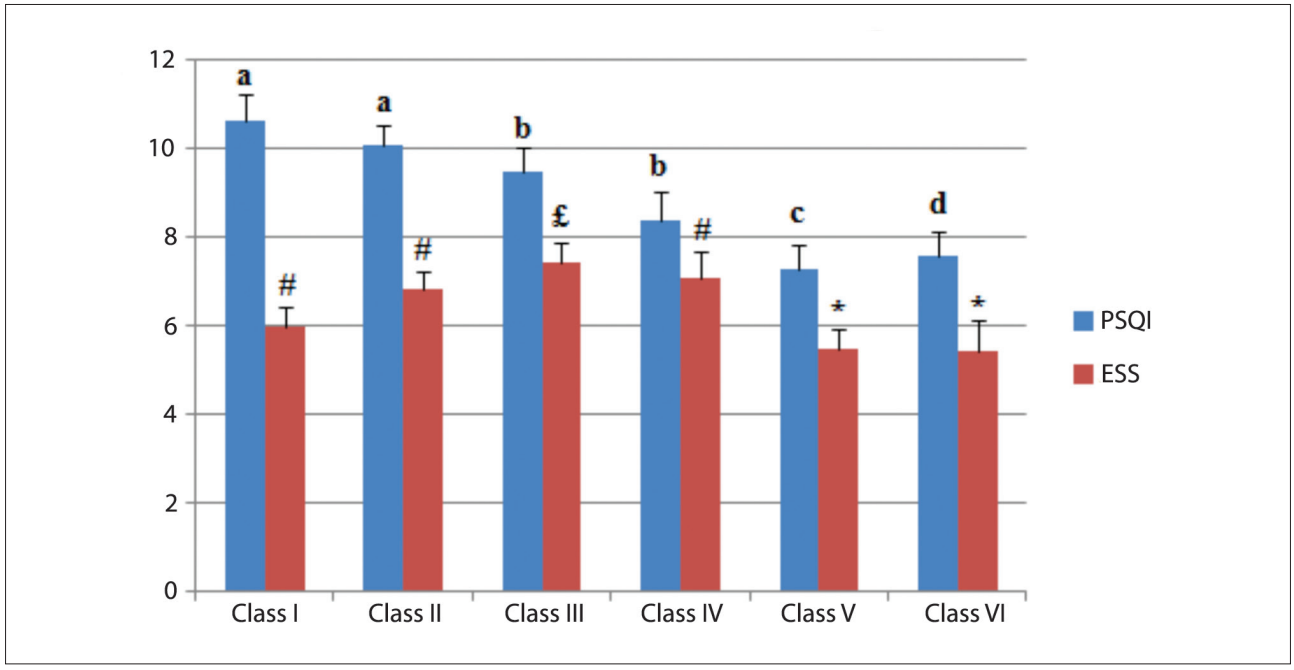

Figure 1. ESS and PSQI scores as regards terms (There is a statistically significant difference between averages carrying different characters (PSQI) and symbols (ESS).

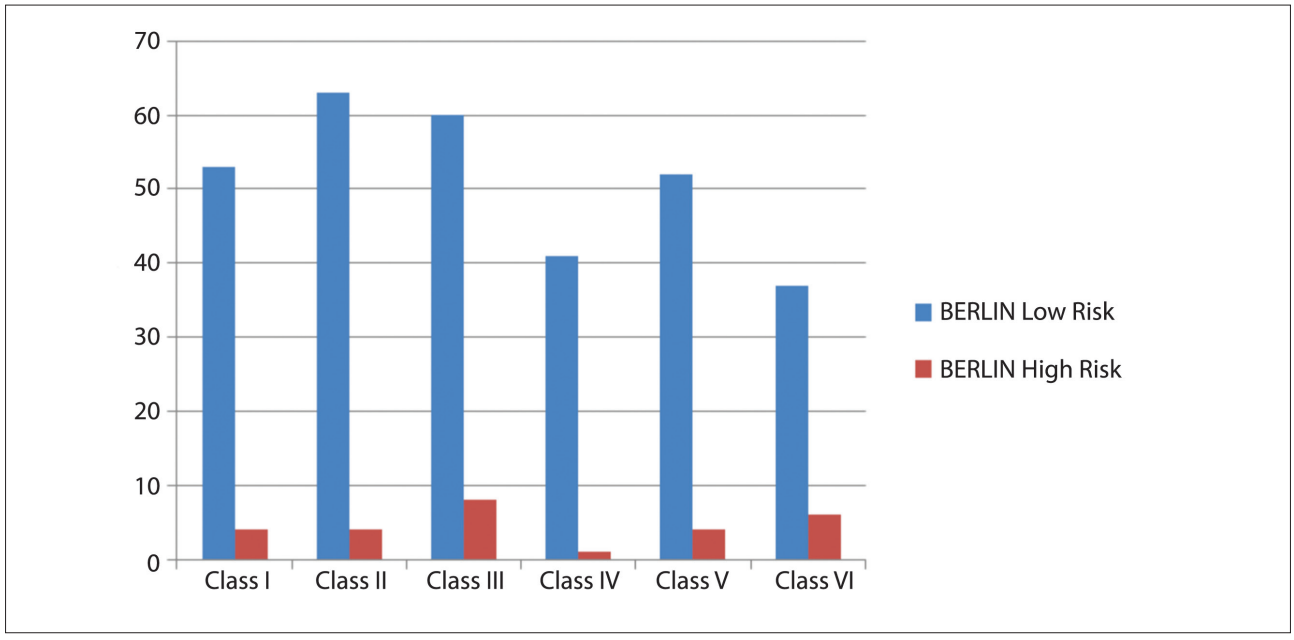

Figure 2. Results of Berlin Sleep Apnea scanning questionnaire as regards terms.

chronic sleep disorders. When long-term effects are considered, this can be related to many systemic chronic diseases.

In this study where we investigated sleep quality and sleep disorders, 54 of the participating students were found by the Epworth scale to suffer from excessive daytime sleepiness (Epworth score > 10 = 17.9%). Epworth scale has been applied to 412 students of medicine (69%) in a study conducted at Gülhane Military Medical Academy (GMMA). It has been determined that 34.5% of the students suffer from excessive daytime sleepiness, and the number of students statingthat they cannot get enough sleep, are tired and not rested (61.8%) are higher than expected [17]. These data were found higher than ours, which could be associated with the fact that GMMA is a military school. Generally, the frequency of individuals with excessive daytime sleepiness show variability [18,19]. Along with the fact that female:male ratio of the students of the Faculty of Medicine participating in our study was close to one another, it was seen that the age range varied between 18-30 and female students suffered from excessive daytime sleepiness more than male students. It was established by our study that 12% of the males and 22% of the females participating in our study suffered from excessive daytime sleepiness. Even though sleep disorder is generally seen more in females as parallel to our study, this rate can vary as shown by epidemiological studies carried

out in different groups. It was seen that excessive daytime sleepiness was more frequently encountered in individuals using drugs when in aggrement with to the one that do not. This situation shows that sleep hygiene and hence sleep rhythm is deteriorated by drug use. It has been reported in studies that drug and substance use lead to insufficient sleep [20]. Our study also shows in aggrement with to these results since sleep quality deteriorated in drug users in our study. Moreover, it was determined that the scores in Epworth scale decreased with increasing age and class term. While Epworth score was higher in normal weight students when compared to overweight and obese students, it was not statistically significant. It has been demonstrated in a study by Başoğlu et al. that excessive daytime sleepiness is seen more in the morbid obese than the obese and that as BMI increases so does daytime sleepiness. We are of the opinion that this difference could occur due to the fact that there were no obese and morbid obese students in our study group [21]. When terms or class years were compared, excessive daytime sleepiness was found higher in the basic training period and lower in the clinical training period, which can be associated with the intense education period and exam stress in the basic training period.

Along with the low number of students smoking and consuming alcohol, 1.8% of them used pleasure-giving substance. Alongside affecting normal life, these habits can deteriorate sleep, sleep hygiene and circadian rhythm, and thus lead to chronic sleep problems. It was observed that as the frequency of exercising increased, Epworth scores of the students decreased significantly. Excessive daytime sleepiness diminished in students who exercised, and hence, these individuals led more quality lives. It was determined that as the financial situation of the students improved, their Epworth scores increased. Circadian rhythm deteriorates with improvement in welfare level, sedentary life, obesity and night life, and as a result, excessive daytime sleepiness can be seen more frequently in these individuals. This situation demonstrates that prevalence of sleep disorders will increase with environmental and genetic factors unless measure is taken later on.

Pittsburgh sleep quality index was found high in individuals that snored in our study. However, it was found lower in overweight and obese students when compared to normal weight students. Epidemiological studies have reported a relation between short sleep duration and excessive body weight [22,23]. Sleep duration has been defined among risks for being overweight in childhood and adolescent periods. It has been put forth that the reduction in leptin and ghrelin levels in short sleep duration is the mechanism that causes this [24]. In our study group, BMI values were found within normal limits and the frequency of overweight and obese students were very low. Therefore, the relation between obesity and the increase in PSQI is not similar to the literature. When PSQI scores between class terms were taken into consideration, it was seen that the scores decreased from basic training towards clinical training, which suggests the difference between courses and applied training. The students are somewhat better during their internship period.

The frequency of snoring has been found as 3.2-12.1% in various epidemiological studies [25]. Mean Pittsburg score of snorers was found significantly higher when compared to non-snorers. The frequency of snorers in our study was 3.0% (10 students). Preisegolaviciute et al. [26] have evaluated sleep quality and life style of the students in the departments of medicine, law and economy in Lithuania and found that the Pittsburg scores of more than half of the students (59.4%) were over 5. They have emphasized the significant difference between students of medicine and students of law and economy and have associated this with the fact that students of medicine spend more time studying, are more worried and are not satisfied with their results, and hence, they study more frequently before going to bed.In a study by Sweileh et al. [27] conducted on sleep habits and sleep problems among Palestinian students, sleep quality has been found "bad" with 9.8%, and a significant relation was found between sleep latency, the frequency of awakening at night and nightmares regarding academic success. Rocha et al. [28] have investigated sleep disorders in high school and university students and applied the PSQI questionnaire to 529 students (m: 241; f: 288), between the ages of 16 and 19, attending to three state schools, private schools and two university preparation schools, in a middle-class neighborhood in Sao Paolo. They have shown that sleep disorders and weak sleep quality were present in the study group. Suen et al. [29] have investigated factors related to sleep quality and sleep hygiene between university students in Hong Kong and found that age, gender, duration of studentship, and the place they lived in are related when gradual choice relation with regression analysis is compared to sleep hygiene and PSQI. In a study by Kang et al. [30] investigating the effects of daytime sleepiness and irregular sleeping program on sleep quality among university students in Taiwan, PSQI and ESS have been implemented on 160 students by semi-structured interview. It has been found that there is a significant relation between program irregularity before going to bed and daily reduction in sleep duration. In multivariate analysis, a significant relation was found between the frequency of irregular sleep and mean daily sleep duration and Pittsburg score. Mayda et al. [31] have aimed at designating the frequency of sleep disorder and the elements affecting it in 4^{th}, 5^{th} and 6^{th} year students of medicine (84 students) at Düzce University and found that mean PSQI score is 5.2 ± 2.7 and the rate of students with a score over 5 is 46.4%. It has been shown in the study that the Pittsburg score of more than half of the students are over 5. Mean PSQI score has been found as 6.15 ± 1.90 and the rate of the students with bad sleep quality has been determined as 59% in a study by Aysan et al. [32] conducted on 300 students attending the faculties of nursing, medicine and pharmaceutics in Izmir. Saygılı et al. [33] have found mean PSQI score of 558 students attending Kırklareli University as 6.9 + 2.4 and 9.5% of the students received a PSQI score of 5 and below. Assaad et al. [34] have applied PSQI to 735 university students between the ages of 18-25 at 6 universities

in Lebanon. In the study, the rate of the students with a PSQI score of < 5 has been determined as 47.3%. It has been established by bivariate analysis that male students had more sleep difficulty than female students (%57.8, %40.8). In our study, female students were detected to experience sleep disorders more. Giri et al. [35] have applied ESS and PSQI to 150 individuals including students of medicine, interns and graduates. ESS score of the students has been found as 26/150 (17.3%), and female individuals have been found to have better sleep quality when compared to male individuals. PSQI scores have been determined as 5.28 ± 2.39 in students, 4.76 ± 2.36 in interns and 7.88 ± 2.5 in graduates. ESS and PSQI have been applied in a study by Pagnin et al. [36] investigating burnout and sleep disorders in 127 pre-clinic med students. Excessive daytime sleepiness by ESS and bad sleep quality by PSQI have been found respectively as 63% and 65%. It has been shown that academic efficiency diminishes with increased excessive daytime sleepiness (OR= 0.86, 95% CI= 0.75 and 0.98). Kabrita et al. [37] have investigated sleep quality on 540 students in state and private universities in Lebanon. Mean night sleep has been found as 7.95 ± 1.34. They have argued that more than half of the students had bad sleep quality with a PSQI of 6.5 h and going to bed and awakening on the weekends are delayed 1.51 h and 2.43 h respectively, which has also led to deterioration in sleep quality. Cheng et al. [38] have conducted a research on 4318 university students in Taiwan and established that the PSQI scores of 2360 (54.7%) students were ≥ 6 and had bad sleep quality and they have also determined that bad sleep quality in university students is associated with the female gender. The results of this study overlap the results of our study. When the relation between sleep quality and physical and mental health is considered, they have emphasized the importance of sleep disorder intervention programs for this population.

Nojomi et al. [39] have conducted a study regarding sleeping pattern on 400 students of medicine and demonstrated that sleep disorder is an important problem among students of medicine and age, gender, life conditions, exercising, and work load are associated with having sleep disorders.

These studies are generally oriented at designating sleep disorders and sleep quality and most of these studies have been conducted over university students. Generally, PSQI scores have been found high in groups receiving long and intense education, just like medicine. In addition, more than half of the study groups in nearly all studies have been found to have bad sleep quality, which has been associated with academic performance. Moreover, it is seen that the concept of sleep hygiene has become more important. The association between sleep quality and sleep hygiene have been put forth with studies. Besides, sleep hygiene comes to prominence as the uppermost factor in increasing sleep quality.

Mean PSQI scores of students with witnessed apnea put forth by BSAST have been detected higher when compared to students without witnessed apnea. A significant difference was not seen when these mean scores were compared. When BSAST scores in terms of class terms were considered, high risk groups were the ones at the third term, which is the end of basic training, and the internship period, which is the end of medical education. Both basic and clinical training periods can be argued to have influence over sleep quality. According to these results, most risk factors of OSAS are not present in our study group since our population is made up of young individuals. We could not detect significant differences in our results as regards BSAST. Limitations to our study were as follows: having difficulty in reaching all students in our study, the redundancy of questionnaire data, factors preventing to reach the general.

The interaction between sleep and disease processes should be considered on epidemiological, behavioral, physiological and neurobiological levels. It is important to train healthcare providers regarding healthy sleeping habits and sleep disorders. Advancements and approaches in technology should be implemented systematically in sleep science. The importance of a regular sleep should be specified under the leadership of media organs and particularly institutions giving healthcare services. It is obvious that this matter should be addressed with utmost importance due to the fact that sleep disorder is an important element to improve health in the field of public health and to prevent chronic diseases.

Sleep disorder is an underlying factor of many psychological problems. Therefore, we are of the opinion that studies aimed at increasing awareness in individuals with sleep disorder complaints should be intensified since the provision of sleep hygiene and comfort can prevent many diseases.

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ORIGINAL INVESTIGATION

Analysis of Chromosome 3, 7 and 8 Centromeric Regions in Bronchial Lavage Specimens by FISH

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Abstract

OBJECTIVES: Multiple genetic changes are observed in malignant tumors but are rare or absent in benign conditions. Aneuploidy is the most common feature of solid tumors including lung cancer and diagnosis of malignant tumors is possible through detection of aneuploidy. The aim of this study was to investigate chromosomal abnormalities in cells from non-small cell lung cancer patients obtained bronchoscopically and to evaluate the suitability of fluorescence in situ hybridization (FISH).

MATERIAL AND METHODS: Bronchial lavage samples of 17 non-small cell lung cancer (NSCLC) patients were evaluated with four-color FISH using deoxyribonucleic acid (DNA) probes specific for the centromere regions of chromosomes 3, 7 and 8. tested specimens were first hybridized with probes, then visualized under fluorescence microscobeand captured with device's camera.

RESULTS: High number of aneuploidic cells were detected in all the samples. Increased or decreased abnormal copies or chromosomes 3, 7 and 8 were obserced in all the 17 patients. Aneuploidy of chromosome 3 (21.35%) was higher than those of chromosome 7 (9.06%) and chromosome 8 (15.47%). Moreover, our results were significant for monosomy and trisomy of chromosome 3, trisomy of chromosome 7, nullisomy, monosomy and trisomy of, chromosome 8 ($p< 0.05$).

CONCLUSION: It has been observed that FISH is a useful technique for detection of aneuploidy in bronchial lavage samples obtained by bronchoscopy. Interphase cells were evaluated without cell culturing with this method and high number of tumor cells were enumerated rapidly. Our study has demonstrated that, FISH technique may be used successfully in detection of chromosome number abnormalities in NSCLC patients and may facilitate evaluation of genetic abnormalities.

KEYWORDS: Lung cancer, bronchial lavage, cytogenetic, fluorescence in situ hybridization, aneuploidy

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INTRODUCTION

Lung cancer, the most frequent malignant tumor worldwide, is a complex disease occurring in one or both lungs as a result of a progressive accumulation of abnormal cells [1]. Lung cancers are divided into two main groups based on how the cells look under the microscope; small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) which accounts for 80% of all lung cancer cases.

Prognosis of lung cancer depends on the stage of the cancer, its size and position in the lung. The early stages associates with few specific symptoms and as a consequence most of the lung cancer cases are in the advanced stage when they are diagnosed and has cancer spread other parts of the body.With early stages, there is a chance of well providing control and relieving the symptoms of lung cancer, with reference to that, several researchers look for early and rapid diagnosis of the lung cancer in the recent years [2,3].

In the recent years, several chromosomal and molecular abnormalities have been found in NSCLC, even its early stages. Most of the studies show that lung cancer occurs gradually with the genetic and cellular changes accumulates in the bronchial epithelial, not with an immediate effect. While early stage tumors show relatively fewer genetic changes, numerous genetic changes are observed in the advanced stages.

Including lung cancer, aneuploidy is the most common feature of solid tumors and as a result, malignant tumors can be diagnosed by detecting aneuploidy. A number of molecular studies have been showed that lung carcinomas are characterized by both low level and high level widespread gains and losses of genetic material at various chromosomes [2-6]. Based on the hypothesis that chromosome aneuploidies contribute to tumorigenesis, numerical chromosomal

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abnormalities in cancer cells can be detected by the FISHmethod [3]. Fluorescence in situ hybridization analysis is a strong method for detecting chromosomal changes in tumor cells. In our study we targeted chromosome 3, 7 and 8 for possible three-probe combination for detecting NSCLC whether these abnormalities could provide an additional prognostic factor. We also aimed to visualize chromosomal abnormalities in bronchoscopically gained cells from NSCLC patients and tested suitability of an interphase FISH assay for detecting cancer cells in bronchial lavage specimens.

MATERIAL AND METHODS

Patients

This study was approved by the Research Ethics Committee of our hospital and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Clinically suspected patients who underwent bronchoscopy with abnormal chest radiography or computed tomography (CT) findings at İstanbul University, Cerrahpaşa Medical Faculty, Chest Diseases Department between March 2013 - September 2014 were enrolled. Collected specimens were fixed in Carnoy's fixative and were kept at 4°C until the final histopathological diagnosis. After the definitive diagnosis was confirmed by cytologic examination, a total of 17 NSCLC patients were included in this study. All of the samples were residual specimens after diagnostic sampling.

The patients included 15 men and 2 women, with an average age of 65 years; range from 55 to 75 years. Clinical information (age, gender, job, family history and smoking status) was obtained from patient or family members. The final diagnosis of samples were made as follows; 13 squamous cell carcinomas and 4 adenocarcinomas (Table 1). To distinguish true aneuploidy levels for chromosome 3, 7 and 8 FISH was performed to peripheral blood cells from healthy individuals. Control group selected from healthy, non-smoking 11 donors. Specimens from patients and cultured peripheral blood cells from control donors were collected and FISH analyse was performed.

Table 1. Patients demographics

Characteristic	n
Sex	
Male	15
Female	2
Age, years	
Mean	64.71
Range	55-75
Smoking Status	
Non-smoker	1
Smoking	16
Family history of cancer	
Yes	9
No	8
Type of NSCLC	
Squamous	13
Adenocarcinoma	4

NSCLC: Non-small cell lung cancer.

Sample Preparation

Washing specimens were collected in 10 mL tubes and centrifuged 400 g for 10 mins. After removing supernatant, fresh Carnoy's fixative was added and this process was repeated 2 times. Following that step, cell pellet washed with 1 x PBS, centrifuged and permeabilized with hypotonic 0.075 mol/L KCl solution at 37°C for 20 mins. Lastly cell pellet was fixed in fresh Carnoy's fixative and stored at 4°C until they were used. Fixed cell samples were spotted on wet, cleaned microscobe slide and allowed to air dry overnight.

FISH Hybridization

Satellite probes used in this study were chromosome specific sequences generated from highly repeated human satellite DNA located in centromeric region of chromosomes. Satellite probes labelled with red fluorophore, green fluorophore and aqua fluorophore used respectively for centromeric regions of chromosomes 3, 7 and 8 were purchased (Cytocell Ltd UK) and four-color FISH was performed using 4', 6-diamidino-2-phenylindole (DAPI), fluorescein isothiocyanate (FITC), sulforhodamine 101 acid chloride (texas red) and aqua filters. The slides were immersed in 40 mL 10 mM HCl and 500 µL 0.5 mg/mL pepsin solution at 37°C for 8-10 mins, followed by soaking in 1 x PBS at room temperature (RT) for 5 mins. The slides were denaturated in %1 neutral buffered formalin solution at RT for 5 mins and soaked again in 1 x PBS for 5 mins. Slides were dehydrated in ethanol series (70%, 85% and 100%), each for 5 mins and allowed to air dry. Using fresh pipette tips, 3 µL of each probe and 1 µL of hybridisation solution were put in a microcentrifuge tube per test and gently vortex to mix. A total of 10 µL probe were placed on the slide, covered with coverslip, and sealed with rubber solution glue. After denatured at 74°C for 1.5-2 mins, slides were incubated 4 hours at 37°C in a humid, lightproof container. After removing coverslip and glue, slides were washed in 0.4 x SSC at 65°C for 2 mins and 2 x SSC, 0.05% Tween 20 for 30 seconds. 20 µL of the DAPI antifade was applied and allowed colour to develop in the dark for 1 hour at 4°C.

Slides were analyzed under the fluorescence microscope (Nikon Eclipse E600, Japan) with fluorescence filter sets and captured with device's camera (COHU Cooled CCD Camera Applied Imaging, Newcastle, UK). Captured images were monitorized with image analyser (MAC Probe 4.3 Applied Imaging, Ne castle, UK).

FISH Scoring

A total of 500 cells for control group and 100 interphase cell for patients were counted. FISH signals are seen as red (centromere region of chromosome 3), green (centromere region of chromosome 7), aqua (centromere region of chromosome 8) and nuclear counterstain are seen as blue.

To avoid bias, FISH slides were scored blindly by two independent observers. Cells did not count if they touch or overlap and counted ones had smooth, well-rounded borders. Cells with signals located on the extreme periphery of the nucleus did not evaluate. Two signals that were connected by a strand were counted as one signal.

Statistical Analysis

Data were analyzed by using the statistical package for thesocial sciences (SPSS v16.0) (Chicago, IL, USA) statistical software. Association between probe aneuploidy for chromosomes 3, 7 and 8 was defined with Mann-Whitney U test. All p values were two-sided and values less than 0.05 were considered statistically significant.

RESULTS

Disomic (normal) cells-the expected FISH result- for control group was detected in a large majority. FISH evidence for different levels of aneuploidy was observed in all 17 NSCLC specimens. For every case, each chromosome signals were counted independently from each other. Control groups were scored 500 consecutive nuclei from each sample by two analysts, each scorer was analyzed about 250 nuclei from a given sample. Typically, a normal cell FISH signals were appeared as 2 red, 2 green and 2 aqua signal each. For related chromosome probe, a cell was considered abnormal if it showed more than two signals which indicates chromosome gain and showed one or no signal which indicates chromosome lose (Figure 1).

Using satellite DNA probes for chromosomes 3, 7, and 8 simultaneous four-color FISH were performed to evaluate the frequency of nullisomic, monosomic, disomic, trisomic, tetrasomic, pentasomic, hexasomic, heptasomic, octasomic and nonasomic cells in specimens. Nullisomic cell lacks both representatives of a pair of homologous chromosomes. Monosomic cell has the homologous chromosomes are represented only once in a cell. Disomic or normal cell has the two homologous chromosomes. Trisomic cell has a single extra homologous chromosome. Tetrasomic cell has four homologues of the same chromosome. Pentasomic cell has five homologues of the same chromosome. Hexasomic cell has six homologues of the same chromosome. Heptasomic cell has seven homologues of the same chromosome. Octasomic cell has eight homologues of the same chromosome and nonasomic cell has nine homologues of the same chromosome.

Analyses of 17 cases by FISH showed normal, nullisomic, monosomic, trisomic, tetrasomic and polysomic (pentasomic, hexasomic, heptasomic and octasomic) cells for chromosome 3. heptasomic and octasomic cells found only in 1 case, hexasomic cells found only in 2 cases and pentasomic cells found only in 7 cases (Table 2). For the control group only normal, monosomic and trisomic cells were spotted for chromosome 3 (Table 3). For chromosome 7, 17 cases showed normal, nullisomic, monosomic, trisomic, tetrasomic and polysomic (pentasomic, hexasomic and nonasomic) cells. Pentasomic, hexasomic and nonasomic cells found only in 1 case (Table 4). For the control group only normal, monosomic and trisomic cells were spotted, 1 control showed tetrasomic cells for chromosome 7 (Table 5). Lastly for chromosome 8 we detected normal, nullisomic, monosomic, trisomic, tetrasomic and polysomic (pentasomic and hexasomic) cells. Pentasomic and hexasomic cells found only in 2 case (Table 6). For the control group only normal, nullisomic, monosomic and trisomic cells were spotted for chromosome 8 (Table 7).

Aneuploidy of chromosome 3 was especially high (21.35%) than the chromosome 7 (9.06%) and chromosome 8 (15.47%). Also our results are significantly different ($p< 0.05$) for monozomy 3, trizomy 3, trizomy 7, nullizomy 8, monozomy 8 and trizomy 8.

DISCUSSION

In the present study, the usefulness of FISH with centromeric DNA probes for bronchoscopically gained bronchial lavage samples and successful analysis of numerical chromosome changes were demonstrated. To visualize evaluation of

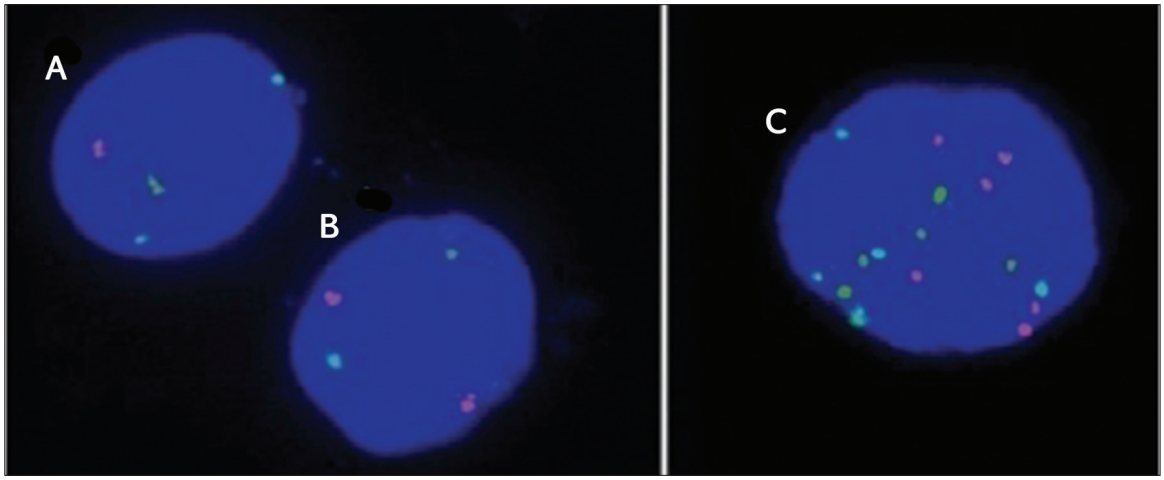

Figure 1. Examples of fluorescence in situ hybridization (FISH) abnormal cells found in patients. FISH clearly shows that cells are not disomic, demonstrating monozomy 3 and monozomy 7 **(A)**, monozomy 7 and monozomy 8, **(B)** and hexasomi 3, pentasomi 7 and pentasomi 8 **(C)**. FISH signals are seen as red (centromere region of chromosome 3), green (centromere region of chromosome 7), aqua (centromere region of chromosome 8) and nuclear counterstain are seen as blue.

Table 2. Result of CEP 3 probe in NSCLC patients

Case no	% of normal cells	% of nullisomic cells	% of monosomic cells	% of trisomic cells	% of tetrasomic cells	% of polisomic cells (> 4 signals)
1	64.62	0.77	8.46	17.69	7.69	0.77
2	72.30	-	3.08	21.54	3.08	-
3	69.12	-	4.41	14.70	7.35	4.41
4	91	-	2	3	3	1
5	91	-	3	4	2	-
6	90	2	5	2	1	-
7	88	-	2	7	3	-
8	69	-	-	5	10	16
9	77.97	-	6.78	13.56	1.69	-
10	71	1	7	16	4	1
11	85	-	7	5	3	-
12	83	1	4	8	4	-
13	89	-	6	3	2	-
14	71	-	3	19	5	2
15	81	-	4	11	4	-
16	81	-	4	11	4	-
17	79	-	5	13	1	2

CEP: Centromere enumeration probes, NSCLC: Non-small cell lung cancer.

Table 3. Result of CEP 3 probe in controls

Case no	% of normal cells	% of nullisomic cells	% of monosomic cells	% of trisomic cells	% of tetrasomic cells
1	98	-	0.60	1.40	-
2	97.20	-	1.12	1.68	-
3	96.80	-	1	2.20	-
4	95.40	-	1	3.60	-
5	98.20	-	1.80	-	-
6	96.60	-	1.40	2	-
7	96.80	-	3.20	-	-
8	99.60	-	-	0.40	-
9	97.20	-	2	0.80	-
10	96.20	-	2.40	1.40	-
11	98	-	1	1	-

CEP: Centromere enumeration probes.

aneuploidic abnormalities in NSCLC patients and provide an additional prognostic factor, we performed enumeration of three-probe FISH and demonstrated that four-color FISH is a feasible assay with an optimal probe combination for investigation of lung carcinomas. We examined interphase cells directly, without prior cell culture and rapidly counted a large number of tumor cells. Our molecular approach served beneficial technique fordetecting aneuploidy and has the potential for improving lung cancer detection. All of our cases showed chromosomal aneuploidy whichincreases mutation rate, gene amplification and/or genomic instability and accompanies tumor progression. Our findings are consistent with previous studies that also found FISH to be more useful and sensitive.

Studies showed that specimens not found to be positive for cancer with routine cytology (RC) were forwarded on for FISH analysis detected more peripheral lung cancers than RC alone [2,7-12]. Halling et al. showed that detecting peripheral tumors of lung cancer by FISH was able to increase the diagnostic sensitivity over routine cytology [8]. A subsequent study by Bubendorf and colleagues noted similar results in which the combination of routine cytology and FISH was increase the sensitivity compared with cytology alone [7]. In a study of 48 selected patients, Sokolova et al. found evidence

Table 4. Result of CEP 7 probe in NSCLC patients

Case no	% of normal cells	% of nullisomic cells	% of monosomic cells	% of trisomic cells	% of tetrasomic cells	% of polisomic cells (> 4 signals)
1	86.90	0.77	1.54	8.46	2.30	-
2	93.08	-	3.85	2.3	0.77	-
3	91.18	-	8.82	-	-	-
4	97	-	1	2	-	-
5	96	-	2	2	-	-
6	93	-	3	4	-	-
7	97	-	1	1	1	-
8	72	-	-	4	14	10
9	89.83	-	6.78	1.69	1.69	-
10	89	-	9	2	-	-
11	88	1	9	1	1	-
12	95	-	2	3	-	-
13	93	1	3	3	-	-
14	89	-	7	2	2	-
15	94	-	5	1	-	-
16	93	-	3	2	2	-
17	89	-	7	3	-	1

CEP: Centromere enumeration probes, NSCLC: Non-small cell lung cancer.

Table 5. Result of CEP 7 probe in controls

Case no	% of normal cells	% of nullisomic cells	% of monosomic cells	% of trisomic cells	% of tetrasomic cells
1	96.80	-	2.40	0.80	-
2	98.50	-	0.93	0.56	-
3	96.80	-	1.60	1.60	-
4	95.20	-	2.80	2	-
5	97.40	-	2.60	-	-
6	97.20	-	2.80	-	-
7	96	-	3.60	0.40	-
8	94.60	-	5.20	0.20	-
9	95.40	-	2.80	1.60	0.2
10	95.20	-	3	1.80	-
11	98.80	-	1	0.20	-

CEP: Centromere enumeration probes.

suggesting that the multitarget FISH test for simultaneous analysis of chromosome 1 and the 5p15, 7p12 and 8q24 loci might improve sensitivity for lung cancer detection [9]. In a study by Schenk et al. using FISH with DNA centromeric probes for chromosomes 3, 8, 11, 12, 17, and 18 aneusomy was present in all 10 primary tumors and 10 malignant effusions from 18 patients with lung cancer [13]. They also found FISH for chromosomes 7, 11, 17, and 18 to be positive in bronchial brushings from 5 patients with lung cancer.

Liu et al. developed a FISH assay to detect lung cancer by DNA centromeric probes for chromosomes 2, 3, 6, 7, 8, 9, 11, 12, 17 on 74 surgically resected NSCLC tissues, 32 operating margin tissue specimens without tumor and 174 bronchial brushing specimens [14]. Aneuploidy rates ranged from 62%-93% in tumor tissues and 29%- 70% in bronchial brushings of lung cancer patients based on individual probe data. The investigators determined that probes targeting chromosomes 3, 7 and 8 provided the optimal three-probe combination for detecting lung cancer. The three FISH probe combination had a similar sensitivity as RC. Based on this research, we tested chromosomes 3, 7 and 8 probe set for genetic abnormalities in NSCLC patients. In our study, the highest aneuploidy rate found was 21.35% for chromosome 3. chromosome 3 monosomy and trisomy were significantly higher ($p< 0.05$). Previous studies demonstrated copy number

Table 6. Result of CEP 8 probe in NSCLC patients

Case no	% of normal cells	% of nullisomic cells	% of monosomic cells	% of trisomic cells	% of tetrasomic cells	% of polisomic cells (> 4 signals)
1	91.54	3.08	2.30	2.30	0.77	-
2	91.54	3.85	3.85	-	0.77	-
3	91.18	-	5.88	2.94	-	-
4	95	-	4	1	-	-
5	93	1	6	-	-	-
6	86	-	12	2	-	-
7	78	1	16	4	1	-
8	71	1	5	16	5	2
9	79.66	1.69	11.86	6.78	-	-
10	77	2	22	-	1	-
11	73	2	22	2	-	1
12	71	4	22	3	-	-
13	91	-	9	2	-	-
14	87	1	9	3	-	-
15	87	-	10	3	-	-
16	92	-	3	5	-	-
17	84	-	12	2	-	2

CEP: Centromere enumeration probes, NSCLC: Non-small cell lung cancer.

Table 7. Result of CEP 8 probe in controls

Case no	% of normal cells	% of nullisomic cells	% of monosomic cells	% of trisomic cells	% of tetrasomic cells
1	98	0.20	0.8	1	-
2	98	-	1.86	0.19	-
3	98	-	2.8	0.2	-
4	97.40	0.20	2	0.4	-
5	97.60	-	2.4	-	-
6	95.80	-	2.4	1.8	-
7	95.80	-	4.2	-	-
8	99.80	-	0.2	-	-
9	96.40	0.20	2.6	0.8	-
10	96.20	-	3.2	0.6	-
11	96	-	4	-	-

CEP: Centromere enumeration probes.

changes on chromosome 3 are observed frequently and chromosomal gain of 3q associated with cancer formation from premalignant to invasive cancer. Also researchers suggested chromosome 3 probably contain many genes that could play oncogenic role [15-17]. Based on these findings, abnormalities of chromosome 3 are important for the development of lung cancer.

The role of epidermal growth factor receptor (EGFR) gene which locates at chromosome 7 in the pathogenesis and progression of various malignant tumors has long been known. The EGFR protein expression has an impact on prognosis, i.e. a higher expression correlates with a higher nuclear grade and larger tumors, and strong continuous membrane immunostaining is significantly associated with shorter survival [18-20]. Dordevic et al. showed EGFR overexpression is not associated with gene amplification but most likely with polysomy of chromosome 7 [18]. In our study the aneuploidy rate for chromosome 7 was %9.06. We hypothesized that increased expression of EGFR correlates with responsiveness to chemotoxic drugs in NSCLC, increased copy numbers of chromosome 7 could be the source of overall EGFR expression at the protein level. Waldman and colleagues demonstrated that the centromeric copy number of chromosome 7 is strongly correlated with tumor grade and labeling index in bladder cancer [21].

Clones with trisomy 7 as the sole abnormality have been reported in cultured malignant cells from NSCLC specimens.

Kubokura et al. investigated the c-myc gene amplification and the numerical abnormalities of chromosome 8 by FISH in NSCLC patients and obtained numerical chromosome 8 abnormalities correlated significantly with a poor prognosis [22]. The c-myc gene localized to 8q24 amplification seemed to be associated with tumor progression, and an overexpression of the c-myc gene protein may be related to metastasis of lung cancer [23,24]. Abnormalities of chromosome 8 was the second most numerical change in this investigation, being observed in 15.47% of the cells. Although there was no significant link between the amplification of the c-myc gene and clinical outcome, we hypothesized that the numerical chromosome 8 aberration might be a factor for survival.

This study is limited due to number of patients, therefore numerical chromosome aberation influences on clinical outcome could not be explained. Additional studies may be performed to explore utility of FISH in patients at an increased risk for developing lung cancer and a testing algorithm that includes other bronchoscopic techniques. For determining an optimal probe set of lung cancer diagnosis, studies should be conducted in representative patient populations.

As a result, our data indicate that FISH is a useful technique for successful detection of aneuploidy without cell culture in bronchial lavage samples obtained by bronchoscopy.

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ORIGINAL INVESTIGATION

Management of Massive Hemoptysis: Analyses of 58 Patients

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Abstract

OBJECTIVES: The objective was to describe changing patterns of etiological factors and treatment modalities for massive hemoptysis.

MATERIAL AND METHODS: From January 2008-December 2012, the medical records of 58 massive hemoptysis patients were reviewed.

RESULTS: Fifty-eight patients, 44 were men (75.9%) and 14 were women (24.1%), with a mean age of 51.4 years (range= 19-84 years), were divided into three groups; surgical management (n= 37, 63.8%), conservative management (n= 14, 24.1%) and bronchial artery embolization (n= 6, 10.4%). One case (1.7%) had combined treatment modality; bronchial artery embolization was followed by surgical resection. Anatomical lung resections were the most preferred resection type in the surgical management group (n= 34, 91.9%). The most common etiological factor was bronchiectasis (n= 19, 32.8%); followed by bronchial cancer (n= 14, 24.1%). The duration of hospitalization in the surgical management group was 11.4 days (range= 4-24); whereas in the bronchial artery embolization group, hospitalization was only four days (range= 2-7) ($p< 0.01$). Prolonged air leak (n= 7; 18.9%) was the most common complication in the surgical management group.

CONCLUSION: We emphasize that bronchiectasis was leading cause of massive hemoptysis. Surgical treatment remains the definitive therapy in the management of massive hemoptysis with decreased mortality rates over decades; whereas bronchial artery embolization is an effective therapeutic tool.

KEYWORDS: Hemoptysis, bronchiectasis, tuberculosis, surgical management

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INTRODUCTION

Hemoptysis is phtisis of blood due to various kinds of lung pathologies or systemic diseases and syndromes [1]. Massive bleeding in the airway is potentially a serious and life-threatening condition because of asphyxiation by blood and can cause sudden airway obstruction and hemodynamic instability [2]. Death due to bleeding is a rare cause of mortality. Massive hemoptysis has a mortality rate of 25-50% in patients who are not treated adequately [2,3]. The anatomical dead space of the tracheobronchial tree is only 200 mL or less. Therefore, expectoration of 200 mL or more of blood over a 24-hour period or bronchial blood loss causing hemodynamic or respiratory compromise can be defined as massive or major hemoptysis [4].

Treatment modalities published for massive hemoptysis include conservative medical therapy, surgical therapy (pulmonary resection), endobronchial control measures (balloon tamponade, endobronchial iced saline lavage) and bronchial artery embolization [2,3]. In the late 1970s and at the beginning of 1980s, the most common etiological factor for massive hemoptysis was tuberculosis and surgical management was the prime therapeutic approach with a mortality rate of over 18% [5,6]. However, over decades, conservative treatment modalities play a major role in the management of hemoptysis [1,3]. The objectives of this study are to determine the changing patterns of etiological factors of massive hemoptysis and evaluate the effectiveness of treatment modalities and outcomes.

This study has been presented as an oral presentation in Turkish Thoracic Surgery Society, 7th National Congress, Wich held at Antalya-Turkey, between 25-28 April 2013.

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MATERIAL AND METHODS

Selection of Patients

From January 2008 to December 2012, 471 patients [302 male (64.1%); 169 female (35.9%)] were admitted to the emergency department of Ataturk Chest Disease and Thoracic Surgery Training and Research Hospital with a complaint of different degrees of hemoptysis. We considered the hemoptysis minor if there was expectoration of blood less than 200 mL per 24 hours; massive if there was expectoration of blood over 200 mL per 24 hours. As anatomical death space of the tracheobronchial tree is only 200 mL and most patients have mortality due to asphyxiation rather than exsanguinations, evaluating the upper bleeding limit as 200 mL for massive hemoptysis is a better approach.

Of those 471 patients, 37 (7.9%) had a previous lung resection and were excluded from the study. 18 patients (3.8%) all of whom had hemoptysis less than 200 mL, refused further treatment modalities after cessation of the hemoptysis with basic treatment modalities; nevertheless, they were also excluded from the study. Out of the 471 patients, 413 (87.7%) had minor hemoptysis and were excluded from the study. There were 58 patients (12.3%) who had an expectoration of blood over 200 mL per day and consequently, were included in the study.

All of the patients who had expectoration of blood over 200 mL per day and did not refuse further treatment were admitted to the intensive care unit (ICU) of the Thoracic Surgery Department and received basic management modalities include strict bed rest, lateral decubitus toward the bleeding site if known, monitoring vital signs and nasal oxygen inhalation. After insertion of intravenous line, appropriate broad-spectrum antibiotics, coagulants (K vitamin), cough suppressants and sedative drugs (diazepam) were given to all patients. Baseline hematological, biochemistry, clotting tests and analysis of blood type were performed and a chest X-Ray was subsequently performed.

All patients underwent either flexible and/or rigid bronchoscopy as soon as possible, for identification of bleeding source, airway toilet and performing possible endobronchial interventions such as adrenaline flush, cold-saline lavage and balloon tamponade. Postero-anterior chest X-Ray and computed tomography of the thorax was performed for the patients who were hemodynamically stable. Pulmonary isolation with a double-lumen endobronchial tube was used as the last choice in refractory massive hemoptysis cases.

Statistical Analysis

Data were analyzed using SPSS (version 17.0, Chicago, IL, USA with the help of a statistician. Differences were considered significant when the probability value of Fisher's Exact Test was less than 0.05.

RESULTS

58 patients were divided into three groups due to the management of a massive hemoptysis, which can be classified as surgical management (n= 37, 63.8%), conservative management (n= 14; 24.1%) and bronchial artery embolization (BAE) (n= 6; 10.4%). The final case had a combined treatment modality; BAE was followed by surgical resection (n= 1; 1.7%). Surgical treatment option has been performed to patients whom had a definitive prior pathology such as lung cancer (including stage, status of metastasis, and resectability), bronchiectasis, arteriovenous malformations and tuberculosis. The patients who had an insufficient general condition, hemodynamic instability, and definitive decision of inoperability for lung tumors have been managed with conservative treatment modalities. BAE was a new approach in our center; patients who managed via BAE has been selected through hemodynamic stability, patients acceptance, and for selected cases only.

Age and Gender

Of the 58 patients who had an expectoration of blood over 200 mL per day, 44 were male (75.9%) and 14 were female (24.1%) with a mean age of 51.4 years (range= 19-84 years). The surgical management group (n= 37) included 28 males (75.7%) and nine females (24.3%) with a mean age of 49.7 years (range= 29-67 years), the conservative management group (n= 14) included 11 males (78.6%) and three females (21.4%) with a mean age of 53.5 years (range= 19-84 years), and the BAE group (n= 6) included four males (66.7%) and two females (33.3%) with a mean age of 55.3 years (range= 35-78 years).

Management

Of those 58 patients, 37 (63.8%) were treated with surgical management, 14 patients (24.1%) were managed with conservative treatment modalities, six patients (10.4%) underwent endovascular control of bleeding with BAE, and one patient (1.7%) was treated by a combination of both BAE and surgical management.

Surgical Management

A total of 37 patients (63.8%) had surgical management for hemoptysis. Of those 37 patients 19 patients (51.3%) underwent lobectomy, three patients (8.1%) underwent pneumonectomy, one patient (2.7%) underwent inferior bilobectomy, three patients (8.1%) underwent lobectomy including another anatomical resection such as segmentectomy (n= 1) or lingulectomy (n= 2), three patients (8.1%) underwent lobectomy including non-anatomical wedge resection and five patients (13.5%) underwent anatomical sublobar resection (lingulectomy n= 4; 10.8% and segmentectomy n= 1; 2.7%). Only three patients (8.1%) underwent wedge resection (Table 1). All resections were performed via thoracotomy; 18 patients (48.6%) were approached by right thoracotomy and 19 patients (51.4%) were approached by left thoracotomy. Chest tube removal time changed between 3-19 days with a mean of 6.6 days (median= 6 days).

For massive hemoptysis cases, during the period of preparing the case for surgical resection, routine blood sample analyses such as complete blood counting (CBC), basic biochemical

Table 1. Surgical management procedures for massive hemoptysis

Surgery	n	%
Lobectomy	19	51.3
Pneumonectomy	3	8.1
Bilobectomy inferior	1	2.7
Lobectomy + sublobar resection	3	8.1
Lobectomy + non-anatomical resection	3	8.1
Sublobar resection	5	13.5
Wedge resection	3	8.1
Total	37	100

examinations, bleeding parameters, and confirmation of blood type were standard basic laboratory tests. Subsequently, an immediate cross-match of blood for a possible transfusion and basic radio-diagnostic evaluations were necessary. The radio-diagnostic techniques included a portable postero-anterior chest X-Ray, and computed tomography of thorax (CT) dependent onto hemodynamic stability of the patient. The pathological changes of the lungs were carefully evaluated by help of a radiologist. After radiological techniques, a bronchoscopy was the accepted diagnostic tool for confirmation of thoracic CT and planning of surgery. After bronchoscopic confirmation of bleeding lobe or segment, a surgical treatment was the preferred treatment option for operable lung diseases.

Every lung cancer patients (n= 4, 10.8%), who had surgical resection in the series had a previously diagnosed lung cancer pathology. Their pathological diagnosis, status of metastasis, TNM classification, bronchoscopic evaluation and scanning tests were all known. Those patients either had a rendezvous for a surgery or has been referred to a thoracic surgery department.

After the operation, all of the patients extubated immediately after the operation and have been welcomed to thoracic surgery intensive care unit (ICU). During the post-operative care of hemoptysis patients, same protocols as other operated patients were followed. These management strategies included routinely control of CBC, and biochemical parameters, appropriate replacements of declining parameters, pain control with analgesics and following-up of drainage from chest tubes. Pulmonary rehabilitation after the operation was our indispensable. After ICU period, patients were followed-up in wards and routine protocols were administered till discharge.

Conservative Management

A total of 14 patients (24.1%) received conservative management for hemoptysis. Conservative management administered high-stage and inoperable lung cancer patients (n= 8, 57.1%), antiphospolipid antibody syndrome patients (n= 2, 14.3%) and patients whom refused surgical management (n= 4, 28.6%). Conservative management includes strict bed rest, lateral decubitus toward the bleeding site if known and nasal oxygen inhalation. Pulmonary isolation with a double-lumen endobronchial tube was used in five patients (35.7%), as the last choice for refractory massive hemoptysis. Other conservative management procedures included rigid bronchoscopy and balloon tamponade (n= 3, 21.4%) and fiberoptic bronchoscopy (n= 6, 42.9%). Both bronchoscopic procedures include airway toilet and performing endobronchial interventions such as adrenaline flush and cold-saline lavage.

Endovascular Management

Of the patients, six patients (10.4%) underwent BAE; 1 patient (1.7%) was treated with BAE followed by lung resection (right lower lobectomy).

Etiological Factors

Of all 58 patients bronchiectasis was the most common pathology (n= 19, 32.8%), followed by bronchial cancer (n= 14, 24.2%), arteriovenous malformation (n= 10, 17.32%) and aspergillosis (n= 5, 8, 6%). Only three patients (5.2%) had hemoptysis due to tuberculosis.

Bronchiectasis was also the most common etiological factor for surgical managed patients (n= 14, 37.8%), followed by arteriovenous malformation (n= 10, 27.0%), aspergillosis (n= 4, 10.8%) and lung cancer (n= 4, 10.8%). In the conservative management group, lung cancers were the most common etiological factor (n= 8, 57.1%), followed by bronchiectasis (n= 2, 14.3%) and antiphospolipid antibody syndrome (n= 2, 14.3%). In BAE group bronchiectasis (n= 2, 33.3%), lung cancer (n= 2, 33.3%) and tuberculosis (n= 2, 33.3%) were the etiological pathologies.

Other etiological factors for massive hemoptysis included actinomycosis (n= 1, 1.7%), pulmonary tromboembolism (n= 1, 1.7%), trauma (n= 1, 1.7%), benign cavity (n= 1, 1.7%), aortic aneurism (n= 1, 1.7%), pneumonia (n= 1, 1.7%) and, idiopathic pulmonary hemoptysis (n= 1, 1.7%) (Table 2).

Hospitalization

The duration of hospitalization ranged between 2 and 29 days (median: 8 days; mean: 9.43 days). Mean hospitalization time for the surgical management group was 11.4 days (range: 4-24; median: 11 days), for the conservative management group was 6.2 days (range: 2-29; median: 4 days); whereas, for the BAE group was 4 days (range: 2-7; median: 3.5 days). The duration difference of hospitalization between the surgical management group and the BAE group was considered as statistically significant by Fisher's Exact Test ($p< 0.01$).

Table 2. Etiological factors of massive hemoptysis

Etiology	n	%
Bronchiectasis	19	32.8
Neoplasm	14	24.2
Arteriovenous malformation	10	17.3
Aspergillosis	5	8.6
Tuberculosis	3	5.2
Other	7	11.9
Total	58	100

Morbidity

In the surgical management group, prolonged air leak more than seven days was the most common complication (n= 7, 18.9%) followed by empyema due to bronchopleural fistula (n= 2, 5.4%). Bronchopleural fistula cases underwent a second operation using the re-thoracotomy approach. The preferred surgical procedure for both cases was bronchopleural fistula repair with myoplasty. In the conservative management group, prolonged mechanical ventilation more than seven days was the most common complication (n= 6, 42.9%). A total of seven cases underwent BAE; however, re-bleeding after BAE was observed in one patient (14.2%) and he underwent surgical resection of the right lower lobectomy.

Mortality

Mortality was seen in three patients (8.1%) in the surgical management group (n= 37), and in 5 patients (35.7%) in the conservative management group (n= 14). Of the 58 patients, a total of eight patients died and mortality of the study was calculated as 13.8%. The mortality difference between the surgical management group and the conservative management group was considered as statistically significant by Fisher's Exact Test ($p< 0.05$).

DISCUSSION

The definition of massive hemoptysis varies widely in the literature (over 200 or 600 mL per day); however, hemoptysis should be evaluated not in terms of the volume of bleeding but from the standpoint of life-threatening airway obstruction and asphyxiation [1,4]. The amount of the bleeding is a significant criteria. But it is also important how long the blood loss continued. Patient may lose 600 mL or more of blood. If such a blood loss occurs gradually and the replacement treatments that the patients need are applied in a timely fashion, blood loss should not generally lead to death. However, if the patient loses 200 mL in an instant, this can lead to death due to acute asphyxia, as anatomical death space of the tracheobronchial tree is only 200 mL. Asphyxiation is the leading cause of mortality, evaluating the upper bleeding limit as 200 mL would be a better approach.

Endo et al. defined massive hemoptysis by one or more of the following: bleeding of over 200 mL per day, bronchial blood loss causing hemodynamic or respiratory compromise, or bleeding resulting in a hematocrit of less than 30% [4]. Hemoptysis patients generally have poor respiratory functions due to underlying lung pathologies [7]. Bleeding and aspiration pneumonia due to clots (even minimal amount) can compromise the pulmonary reserve [4,8]. As the anatomical death space of tracheobronchial tree is only 200 mL, and most of the patients had mortality due to asphyxiation, taking the upper bleeding limit as 200 mL for massive hemoptysis is appropriate.

Etiological factors of hemoptysis changed over decades. In the late 1970s and early 1980s, the most common etiological factors of massive hemoptysis were tuberculosis. Garzon et al. published their ten year experiences about massive hemoptysis in 1977, and showed that tuberculosis was the most common etiological factor (70.5%) followed by bronchiectasis (11%) [5]. In their series both Conlan et al. and Knott-Craig et al. mentioned tuberculosis was still most common etiological factor by 38% and 73.3% respectively [6,9]. However, during the 2000s, tuberculosis was not leading etiological factor in the literature. In their retrospective review, Lee et al. mentioned that bronchiectasis was the common etiological factor (57.4%) followed by tuberculosis (16.7%) [10]. Joughon et al. published a retrospective review of 43 massive hemoptysis cases with bronchiectasis and neoplasms as the primary pathology for massive hemoptysis (both n= 12, 27.9%) followed by tuberculosis (n= 4, 9.3%) [1]. Ong et al. and Ayed et al. also found similar results in which bronchiectasis was the primary etiological factor (65.5% and 58.5% respectively) [2,11]. In our series it has been documented that tuberculosis was neither first, second nor the third pathology for massive hemoptysis. Bronchiectasis was the most common pathology but tuberculosis was the fifth most common pathology for the reasons causing a massive hemoptysis.

Other common etiological factors for massive hemoptysis are arteriovenous malformations and aspergillosis. Aspergillosis is caused by a fungus, *Aspergillus fumigatus*, which is inhaled routinely from atmosphere. The majority of cases occur in people with underlying illness such as tuberculosis, but also with healthy immune systems. Signs and severity vary greatly; hemoptysis due to necrotizing destruction of fungal toxins, is uncommon and surgical resection is treatment option for patients whom had massive hemoptysis.

Surgical treatment remains the definitive therapy in the management of massive hemoptysis when the pathology is localized by bronchoscopy or radiological diagnostic techniques [2]. Garzon et al. reported 90% of surgical manipulation with only 10% of conservative approaches [5]. For decades, the surgical management ratios decreased with an improvement of conservative management and BAE. Conlan et al. published the surgical management rate for massive hemoptysis as 27.6% [6]. Knott-Craig et al. and Joughon et al. published similar ratios for surgical management as 35% and 37.2% respectively [1,9]. However, Ong et al. revealed a rate of surgical management of only 12.9%; yet a BAE rate of 74.2% [11]. In our series most of the patients were treated by surgical procedures; however, non-surgical management procedures play an important role and 34.5% of our series were managed with non-surgical protocols.

A major surgical procedure is an anatomical resection procedure that includes lobectomies, bilobectomies, pneumonectomies and segmentectomies. Metin et al. surgically treated more than 96% of their massive hemoptysis patients with anatomical lung resections [12]. Non-anatomical wedge resections could be performed due to insufficient respiratory functions. In our series we also preferred anatomical resections. We performed anatomical resections to 91.9% of cases; however, 8.1% of cases surgically treated by non-anatomical resections due to their insufficient respiratory functions.

After the surgical procedures, significant postoperative complications such as bronchopleural fistula and empyema,

respiratory insufficiency due to poor respiratory functions, wound infection and postoperative hemorrhage could be seen [2]. Ayed et al.mentioned surgical complications after resection for massive hemoptysis and calculated this as 24.5% [2]. The surgical morbidity in our series was calculated as 24.3% and is very similar to Ayed and colleagues' published series. The most common morbidity in our series was prolonged air leak (n= 7, 18, 9%), which was followed by a bronchopleural fistula (n= 2, 5.4%).

Nevertheless, lung resection due to massive hemoptysis is associated with a high mortality rate. Garzon et al. and Conlan et al. treated massive hemoptysis by surgical management with a mortality rate of 17.6% [5,6]. Knott-Craig et al. published mortality rates as 7.1% in a surgical management group; whereas Joughon et al. mentioned mortality rate as 19% [1,9]. Lee et al. published an in-hospital mortality rate of 15% after various kinds of anatomical lung resections [10]. In our series, the mortality rate in surgical management group was lower than most of the published series. Our surgical mortality rate was 8.1%; a little higher than the rate mentioned by Knott-Craig et al.

Bronchial artery embolization (BAE) has entered the paradigm for treatment of massive hemoptysis and has played an increasingly important role in controlling life-threatening hemoptysis, which was first introduced in 1974 by Remy et al [2,11,13]. The English literature revealed a significant increase in the percentage of BAE with a great success [11]. Ong et al. managed massive hemoptysis mostly with BAE (74.2% of their patients) with a success rate of 77% [11]. Swanson et al. published the effectiveness of BAE and immediate termination of bleeding was achieved with embolization with a success rate of 85.1% [14]. Similarly, Mal et al. discussed the results of 56 massive hemoptysis patients who had been embolized and revealed that immediate control was successful in 43 patients (76.8%) [15]. We performed BAE with a success rate of 85.7%; similar to the above mentioned series.

BAE is associated with low duration hospitalization. Samara et al. prepared a case report in which three cases were presented [16]. All three patients were discharged three to four days after embolization [16]. In our series, the mean duration of hospitalization for the surgical management group was calculated as 11.4 days; whereas in BAE group it was only four days. The difference was considered as statistically significant ($p< 0.01$). Thus, it was concluded that BAE is associated with low morbidity and hospitalization.

CONCLUSION

Bronchiectasis was the leading cause of massive hemoptysis; whereas, tuberculosis, the leading cause in the 1970s and 1980s, was the fifth common pathology. Surgical management remains the definitive therapy for patients with massive hemoptysis with acceptable morbidity and mortality rates. Preferable surgical procedures were anatomical resections for the patients with sufficient pulmonary reserve. Bronchial artery embolization is an effective therapeutic tool and plays an important role in the management of massive hemoptysis with low complication and mortality rates; this procedure also decreases hospitalization duration.

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ORIGINAL INVESTIGATION

Serum Heat Shock Protein Levels and the Relationship of Heat Shock Proteins with Various Parameters in Chronic Obstructive Pulmonary Disease Patients

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Abstract

OBJECTIVES: Chronic Obstructive Pulmonary Disease (COPD) is accompanied by increased cellular stress and inflammation. Most of the Heat Shock Proteins (HSPs) have strong cytoprotective effects. The role of HSPs in COPD pathogenesis has not determined completely. We investigated the serum level of HSPs in COPD patients, smokers without COPD and healthy non-smoking controls. Also, we evaluated the relationship of HSPs with various parameters (inflammatory, oxidative, functional status, quality of life) in COPD patients.

MATERIAL AND METHODS: The levels of stress protein (HSP27, HSP70, HSP60, HSP90, CyPA), interleukin-6, C-reactive protein and malondialdehyde were measured in 16 healthy non-smoker, 14 smokers without COPD and 50 patients with stable COPD. Pulmonary function tests (PFT) and arterial blood gases parameters were measured. Health Related Quality of Life was evaluated and exercise capacity was measured with 6 minute walking test.

RESULTS: Only HSP27 levels was significantly higher in COPD patients when compared with both healthy non-smoker and smokers without COPD (for both, $p< 0.001$). There was a weak-moderate negative correlation between serum levels of HSP27 and PFT parameters and between HSP27 levels and PaO_2. Serum levels of HSP27 showed a weak-moderate positive correlation with symptom, activity and total scores. Subjects evaluated only smokers without COPD and patients with COPD; HSP27 had an area under the curve (AUC) in the receiver operating characteristic (ROC) curve of 0.819 (0.702-0.935; 95% CI; $p= 0.000$).

CONCLUSION: Increased serum levels of HSP27 was found in COPD patients and our results showed sensitivity and specificity of serum HSP27 as diagnostic markers for COPD.

KEYWORDS: COPD, heat shock protein, oxidative stress, hypoxia

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INTRODUCTION

There is prominent inflammatory response and oxidant-antioxidant imbalance in Choronic Obstructive Pulmonary Disease (COPD). Also, persisting inflammatory reactions continue in COPD patients despite cessation of smoking. Cigarette smoking is the major risk factor for COPD. Cigarette smoke contains multipl free radicals and these toxic substances are believed to induce an inflammatory response by adversely affecting oxidant/antioxidant and protease/anti-protease balance in the lung. In fact, COPD don't develop all smokers. The majority of long-term smokers do not develop COPD suggests that failure of compensatory mechanisms that protect the lung from reactive oxygen species (ROS) or xenobiotic materials contributes to development of the disease. The expression of antioxidant genes believed to be important in protection of the lung from cigarette smoke-induced injury. Recent studies indicate that a complex molecular cascade termed the ''unfolded protein response'' (UPR) plays an important role in the regulation of expression of a variety of antioxidant, xenobiotic metabolizing and pro- and anti-inflammatory genes [1].

Heat shock proteins (HSPs) are chaperones that catalyze the proper folding of nascent proteins and the refolding of denatured proteins. HSPs have a role either the renaturation or the destruction of damaged proteins under stressful conditions such as heat, bacterial or viral infections [2]. HSP27 was first reported to contribute to heat shock resistance; subsequently, its involvement in diverse protective mechanisms against toxicity mediated by aberrantly folded proteins or oxidative-inflammatory conditions has also been confirmed [3]. Under normal physiological conditions the synthesis of most HSPs is low. However, when organisms endure stress such as heat shock and inflammation, where protein damage is increased, certain HSP are induced and expressed at high levels [4]. Increased HSPs levels showed in COPD patients. HSPs, especially HSP60 may have a role in COPD pathogenesis and some HSPs might be used as possible

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serum markers for determining COPD in the smoking subjects [5,6]. To our knowledge, the role of HSPs in pathogenesis and diagnosis of COPD has been investigated in few studies.

The aim of our study was to investigate whether the serum levels of various HSPs are elevated in smokers without COPD and COPD patients and to determine the relationship between HSPs and several parameters (inflammatory, oxidative, functional status and quality of life) in COPD patients.

PATIENTS and METHODS

Subjects

This study was done between September 2012 and April 2013. This case control study included 80 patients with COPD and controls. Healthy non-smoker volunteers (n= 16), smokers without COPD (n= 14), patients with COPD (n= 50) were evaluated.

Control group, consisted of 16 healthy non-smoking subjects and 14 smokers without COPD, had normal pulmonary function parameters and they had not any lung disease. All subjects were selected with Stratified Random Sampling Method from amongst the hospital staff. The age and sex of the control subjects were similar to COPD patients.

Fifty stable COPD patients enrolled into the study and they were taken from a hospital respiratory out-patient clinic. COPD was diagnosed according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [7]. In addition, the classification of airflow limitation severity was evaluated according to GOLD guidelines [7]. Patients with no evidence of an exacerbation for one month before study were accepted as clinically stable. Acute exacerbation as defined by GOLD, use of systemic steroids within the past 14 days, asthma, autoimmune diseases, lung cancer, known 1-antitrypsin deficiency and known cardiopulmonary co-morbidity were considered as exclusion criteria.

Ethical approval was obtained by the institutional review board (31.05.2012-09) and informed consent was obtained from each subject.

Age, gender and smoking history were asked and the body mass index (BMI) and pulmonary function tests (PFTs) was detected in all subjects. Six minute walking test (mwt), arterial blood gases (ABG) analysis were done in COPD patients and Health Related Quality of Life (HRQL) also evaluated in COPD patients.

Pulmonary Function Testing

The pulmonary function tests were done using a spirometry device (Ultima CPX 790705-205, St. Paul, MN, USA). The standard spirometric examination was conducted according to European Respiratory Society (ERS) criteria [8]. Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) are expressed as percentages of predicted values (FEV_1% pred and FVC% pred) according to the prediction equations of the ERS [8].

Health Status Measurement

HRQL was assessed in COPD patients using the Turkish version of St. Georges Respiratory Questionnaire (SGRQ) [9,10]. The questionnaire was applied to COPD patients by the same interviewers. The SGRQ has been used extensively for assessing quality of life in patients with COPD and several other chronic lung diseases [11]. It contains 50 items with 76 weighted responses that cover three domains: symptoms-distress due to respiratory symptoms, activity-disturbances of physical activity and impact-overall impact on daily life and well-being. In addition to the domain scores, there is also a total score [9]. The SGRQ is scaled from zero to 100 (with zero representing the best health-related quality of life).

Exercise Performance

Exercise performance was evaluated by the 6 mwt according to the American Thoracic Society Guideline [12].

Arterial blood gas measurement

Arterial blood gas samples of COPD patients were taken at rest, in a sitting position and in room air at the room temperature. Samples were measured by a blood gas analyse device (Rapid lab 348. Biobak., Chiron, Bayer Diagnostic, UK).

Measurement of serum HSPs, CRP, IL-6, CRP and MDA levels

Blood samples were collected between at 8.30-9.30 following 10-hours starvation. Serum was acquired after centrifugation and aliquots were kept frozen at -20°C until further testing. HSP27, HSP70, HSP60, HSP90, CyPA and interleukin-6 (IL-6) were determined using adapted enzyme-linked immunosorbent assay (ELISA) kits according to kits protocol. Levels of HSP27, HSP60, HSP70, HSP90, CyPA, and IL-6 were determined using adapted ELISA kits [(Boster Biological technology., Ltd. (Catalog no: EK0881), assaypro (Catalog no: EH5505-1), Hangzhou eastbiopharm co. ltd. (Catalog no: CK-E11197), Hangzhou eastbiopharm co. ltd. (Catalog no: CK-E11190), Hangzhou eastbiopharm co. ltd. (Catalog no: CK-E90142), Boster immumoleader (Catalog no: EK0410), respectively] according to kits protocol.

The concentration of serum malondialdehyde (MDA) was determined by High-performance liquid chromatography (HPLC) using Immuchrom commercial kit (ImmuChrom GmbH, Munich, Germany) according to kit protocol.

Serum levels of C-reactive protein (CRP) were routinely analyzed by the Central Laboratory at the hospital.

Statistics

Data were analyzed using the statistical package for the social sciences (SPSS) software statistical program. Results were given as median and 95% CI. A p value of < 0.05 was considered statistically significant. Statistical analysis was performed using Kruskal-Wallis test for multiple-group comparisons; Mann-Whitney U test was performed to test any observed differences for significance and results were interpreted according to Benferroni correction. Chi-square test was performed to compare gender distribution between

groups. Spearman's correlation was used to assess non-parametric data. Receiver operating characteristic (ROC) curves were plotted to show sensitivity and specificity of the evaluated HSPs.

RESULTS

Age, gender and BMI were found similar between the patient population and control subjects (p> 0.05). Patient characteristics and PFT parameters were shown in Table 1.

There was no statistically significant difference in the levels of HSP70, HSP90, HSP60 and CyPA between groups (p> 0.05) (Table 2). The serum levels of HSP27 were statistically higher in COPD patients than in both healthy non-smoker and smokers without COPD (for both p< 0.001) (Table 2, Figure 1A). There was no statistically significant difference in the levels of HSP27 between healthy non-smoker and smokers without COPD (p> 0.05).

When the HSPs evaluated according to classification of airflow limitation severity; 29 (58%) COPD subjects were GOLD I-II and 21 COPD subjects (42%) were GOLD III-IV. Statistically significant difference only were found for HSP27 between healthy non-smoker and COPD GOLD I-II (p< 0.01), healthy non-smoker and COPD GOLD III-IV (p< 0.001), smokers without COPD and COPD GOLD I-II (p< 0.05), smokers without COPD and COPD GOLD III-IV (p< 0.001), COPD GOLD I-II and COPD GOLD III-IV (p< 0.05) (Figure 1B).

There was no statistically significant difference in the IL-6 levels between groups (p> 0.05). The levels of CRP were statistically higher in COPD patients than in both healthy non-smoker and smokers without COPD (p< 0.001 for both) and the levels of MDA were significantly higher in COPD patients when compared to healthy non-smoker (p< 0.001) and smokers without COPD (p< 0.01) (Table 2).

The mean duration of disease was 6.00 ± 6.25 year, the mean PaO_2 was 63.35 ± 9.71 mmHg, $PaCO_2$ was 37.94 ± 5.90 mmHg, and SaO_2 was 91.27 ± 4.40%, the mean 6 mwt was 368.36 ± 112.10 m and the mean symptom score was 53.47 ± 24.51, activity score was 50.59 ± 22.37, impact score was 38.23 ± 22.89 and total score was 44.50 ± 22.03 in COPD patients (Table 3). The mean PaO_2 levels was significantly higher in COPD GOLD I-II patients (68.38 ± 7.17 mmHg) than COPD GOLD III-IV patients (56.41 ± 8.49 mmHg) (p< 0.001).

Table 1. Demographic characteristics of all subjects

	Healthy non-smoker	Smokers without COPD	COPD patients
n	16	14	50
Age (Year)	65.18 ± 4.72	66.00 ± 6.43	66.84 ± 7.39
Male /Female	14/2	14/0	46/4
BMI (kg/m^2)	24.46 ± 2.17	24.70 ± 2.02	24.73 ± 4.37
Smoking history			
Never smoker	16 (100%)	0	7 (14%)
Ex-smoker	0	0	23 (46%)
Current smoker	0	14 (100%)	20 (40%)
Pack-years	0	24.85 ± 5.64[b]	35.76 ± 10.73
Lung function			
FEV_1 (pred %)	96.43 ± 5.09[a, b]	90.42 ± 4.43[b]	54.70 ± 1.98
FVC (pred %)	92.93 ± 6.44[b]	87.78 ± 6.77[b]	69.18 ± 17.26
FEV_1/FVC (%)	87.25 ± 2.46[b,c]	81.78 ± 2.96[b]	56.72 ± 1.08

COPD: Chronic obstructive pulmonary disease, FEV_1: Forced expiratory volume in one second, FVC: Forced vital capacity, BMI: Body mass index.
Compared with group II; [a] p< 0.01; [c] p< 0.001.
Compared with group III; [b] p< 0.001.

Table 2. Serum levels of heat shock proteins, interleukin-6, malondialdehyde, C-reactive protein in all groups

	Healthy non-smoker	Smokers without COPD	COPD patients
HSP 27 (pg/mL)	983 (662.67-2749.85)[a]	1370 (1042.65-1963.34)[a]	2679.50 (2474.63-3230.44)
HSP 70 (ng/mL)	151 (111.17-170.5)	101.55 (101.12-195.96)	92.05 (105.18-150.53)
HSP 90 (ng/mL)	3.41 (2.97-6.02)	3.1 (2.79-9.39)	3.06 (3.33-6.09)
HSP 60 (ng/mL)	1.71 (1.41-2.54)	2.31 (1.81-2.9)	2.02 (2.19-3.93)
CyPA (ng/mL)	0.87 (0.74-1.97)	0.8 (0.71-2.45)	0.84 (0.91-1.48)
IL-6 (pg/mL)	65.4 (45.4-117.14)	66.25 (59.98-106.14)	60.9 (63.3-81.54)
CRP (mg/L)	0.1 (0.05-0.4)[a]	0.04 (0.05-0.36)[a]	0.9 (0.84-1.42)
MDA (µmol/L)	1 (0.67-1.07)[a]	1.02 (0.96-1.04)[b]	1.06 (1.04-1.07)

Compared with group III; [a] p< 0.001; [b] p< 0.01.
Results were given as median and 95% CI.

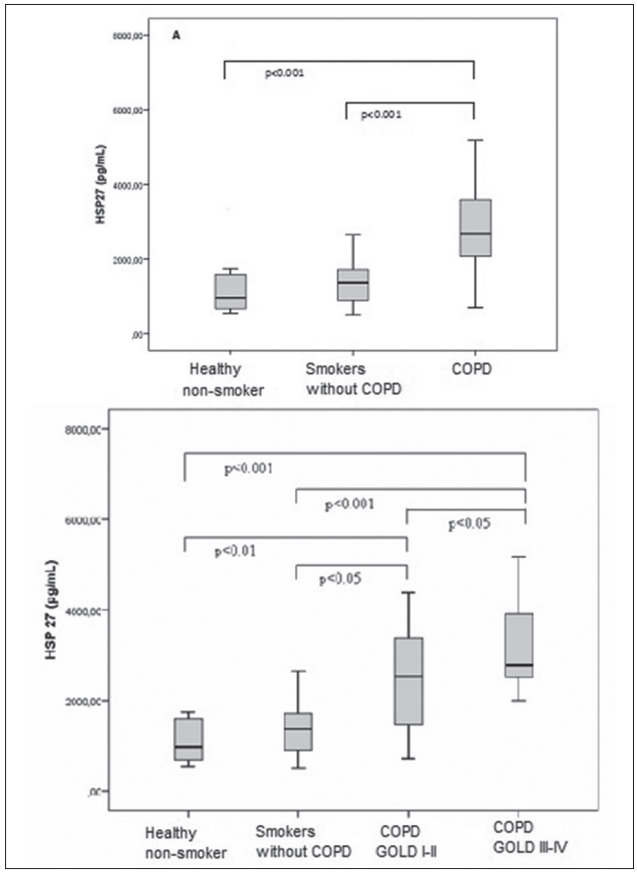

Figure 1. (A) The comparison of the serum levels of HSP27 in healthy non-smokers, smokers without COPD and COPD patients, **(B)** The comparison of serum HSP27 levels between groups when COPD evaluated according to severity of airflow obstruction in COPD.

Table 3. The duration of disease, arterial blood gases levels, 6 minute walking test and SGRQ scores in Chronic Obstructive Pulmonary Disease patients

	COPD patients (n= 50)
Duration of disease (year)	6.00 ± 6.25
6 mwt (m)	368.36 ± 112.10
pH (mmHg)	7.41 ± 0.03
PaO_2 (mmHg)	63.35 ± 9.71
$PaCO_2$ (mmHg)	37.94 ± 5.90
SaO_2 (%)	91.27 ± 4.40
SGRQ (Score)	
Symptom	53.47 ± 24.51
Activity	50.59 ± 22.37
Impact	38.23 ± 22.89
Total	44.50 ± 22.03

COPD: Choronic obstructive pulmonary disease, SGRQ: St. Georges respiratory questionnaire.

Serum levels of HSP27 showed a weak to moderate negative correlation with FEV_1, FVC and FEV_1/FVC values (Respectively, r= -0.428, p< 0.01, r= -0.389, p< 0.01, r= - 0.383, p< 0.01). Only weak to moderate positive correlation were found between serum levels of HSP60 and IL-6 levels (r= 0.327, p< 0.05). Serum levels of HSP27 showed a weak to moderate positive correlation with symptom, activity and total scores and duration of disease (respectively, r= 0.351, p< 0.05, r= 0.294, p< 0.05, r= 0.316, p< 0.05). There was a weak to moderate negative correlation between HSP27 and PaO_2 (r= -0.367, p< 0.01). There was a weak to moderate positive correlation between HSP27 and duration of disease (r= 0.399, p< 0.01) (Table 4).

Table 4. "r" values determined with correlation analysis in COPD group

	HSP27 r	HSP60 r
FEV_1 (%p)	-0.428**	
FVC (%p)	-0.389**	
FEV_1/FVC (%p)	-0.383**	
pH	0.380**	
PAO_2 (mmHg)	-0.367**	
$PACO_2$ (%)		-0.311*
IL-6		0.327*
SGRQ (score)		
Symptom	0.351*	
Activity	0.294*	
Total	0.316*	
Duration of disease (Year)	0.399**	

* p< 0.05
** p< 0.01.
COPD: Choronic obstructive pulmonary disease, SGRQ: St. Georges respiratory questionnaire.

In addition, we evaluated diagnostic value of HSP27 because of increased HSP27 levels was found in COPD patients. Subjects evaluated only smokers without COPD and patients with COPD; HSP27 had an area under the curve (AUC) in the receiver operating characteristic (ROC) curve of 0.819 (0.702-0.935; 95% CI; p= 0.000). A HSP27 level of 2260 pg/mL was taken as the cut-off between smokers without COPD and COPD patients, HSP27 had a sensitivity of 78% and specificity of 70% (ROC curve) (Figure 2A,B).

DISCUSSION

Our study shows increased levels of HSP27 in COPD patients. But the levels of HSP27 levels were not significantly different between non-smokers and smokers without COPD. Also, the levels of serum HSP27 are significantly increased in both COPD GOLD I-II and COPD GOLD III-IV patients than control subjects. When the patient's general health status was deteriorated, increased levels of HSP27 determined. The negative relationship was found between HSP27 levels and ABG and PFT parameters.

The reasons of increased release of HSPs into the extracellular environment are; the constant induction of inflammatory signals and upregulation of intracellular HSPs due to increased cellular turnover [5]. HSPs may be increased in several inflammatory disease. Elevated serum levels of HSP27 were reported in inflammatory disorders including acute coronary syndrome and chronic allograft nephropathy and increased HSP90 immunostaining was found in inflammatory regions of human atherosclerotic plaque [13-15]. They are highly conserved chaperone proteins that regulate the folding and processing of damaged proteins and

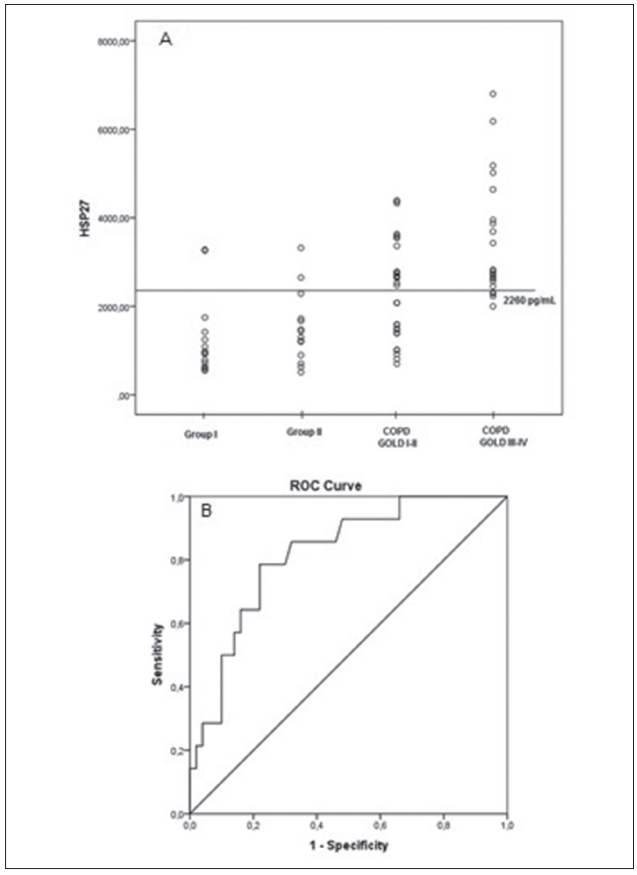

Figure 2. (A) A cut-off level of HSP27 in smokers without COPD and COPD patients, **(B)** Receiver operating characteristic (ROC) curve indicating sensitivity and specificity of HSP27 to diagnose COPD in the Smoking study population.

therefore exert significant anti-inflammatory action and they can modulate inflammation through several mechanisms [16]. In one study, it is found that exercise modulates oxidative stress and inflammation in Aging and Cardiovascular Diseases by suppressing inflammatory pathways and also upregulates repair proteins such as HSPs [17]. Another study showed that HSP27 expression level is associated with the degree of chronic inflammation in benign prostat hypertrophy. In this study it is showed that the expression of HSP27 increased with more inflammation and this suggests that elevated inflammatory stimulation induces HSP27 expression [18]. HSP27 is also required for IL-1-induced expression of the pro-inflammatory mediators IL-6 and IL-8, and the function of HSP27 may sensitize these cells against pro-inflammatory stimuli by augmenting pro-inflammatory signaling [19]. The constant induction of inflammatory signals and increased cellular turnover result in upregulation of intracellular heat shock proteins and augmented release into the extracellular environment in COPD [5]. COPD is an inflammatory disease and the progressive inflammation in COPD continues despite cessation of smoking. For this reason, we expect that the HSPs levels increase in COPD patients. Indeed, some authors showed the increased HSPs levels in COPD patients [5,6,20].

HSP27 and HSP90 behaviors as a defensive factor against unfavorable stimuli such as heat shock and oxidative stress and it can modulate ROS and increases glutathione levels [21]. This ability results cytoprotective affects of HSP27. It is showed that HSP27 and 90 have a protective against oxidative stress [22]. The facilitator effect in the antioxidant defenses of increased HSP expression was already shown in healthy sedentary subjects [23]. HSP27 expression in smokers with or without COPD may be predominantly attributed to hypoxia and inflammation and they have protective effect in the lung cells against oxidative stress in smokers and COPD patients [20]. Increased levels of HSP27 in the lungs of smokers and especially smokers with COPD showed that increased levels of HSP27 is related with primarily oxidative stress and partly inflammation [20]. Increased serum HSP27 levels were found in subjectively healthy smokers who determined emphysema with HRCT without spirometric impairment [24]. These results shows that immune response caused by inhaled toxins in smokers' make pulmonary changes in HRCT and cause decreased HSP27 levels into the pulmonary vascular network in COPD sensitive subjects and HSP27 increases after the development of radiological COPD even thought there was no functional impairment. We found increased serum HSP27 levels in COPD patients. There was no significantly difference in HSP27 levels between healthy non-smokers and smokers without COPD subjects. Therefore we think that increased serum HSP27 levels may not be directly associated with smoking and it can only be detected increased when COPD develops. But COPD patients had higher smoking index than smokers without COPD in our study. This may affect our data. However, oxidative stress due to smoking causes the secretion of proteins but increased serum HSP27 levels in COPD patients may ascribed to other contributing factors such as hypoxia and inflammation. Also, the mean MDA levels were significantly higher in COPD patients compared with healthy non-smokers and smokers without COPD, but we did not show any relationship between HSP27 and MDA levels as an indicator of oxidant system. Further studies must be done for determining the exact antioxidant role of HSP27 in COPD patients.

Elevated HSP27 levels were reported in inflammatory disorders and HSPs expression is low under physiological conditions [14]. But HSP27 levels are temporarily increased when stress events developed and later their concentrations are decreased by termination of the acute triggering. HSP27 levels increase only when its cytoprotective effects are necessary [25]. Contrary, a continuous increase in serum HSP27 levels parallel with disease severity was shown previously [5]. Augmentation of tissue destruction in late stages of COPD and systemic inflammation of COPD may cause a systemic spillage of HSP27 into the vascular bed. Similarly, we found a continuous enhancement in serum HSP levels with severity of airflow limitation and there was an increase in HSP27 levels when respiratory function decreased and duration of disease increased. It also supports the idea that HSP27 is related with the increased tissue destruction and systemic inflammation in COPD. The relationship between serum HSP27 levels and PFT parameters as well as duration of disease interpreted that serum levels of HSP27 may be useful predictor of severity of airflow limitation in COPD stages and evaluation of response to treatment. Furthermore, we think that in addition to the systemic inflammation of COPD, hypoxia can be a contributing factor on continuous increases of HSP27, because there was a prominent hypoxia in COPD GOLD III-IV patients.

Previous experimental studies have showed increased production of HSPs in response to anoxia, presumably to help stabilize/protect protein structure/function [26,27]. Responses of HSP are organ specific [26,28]. There are a little data for the production of HSPs in the lung airway cells response to chronic hypoxia [29]. Increased HSP70 and HSP90 and unchanged HSP70 levels in lung tissue against chronic hypoxia were shown [29-31]. Consequently, the activation of heat shock response is important in stress-responsive pathways to long-term anoxic survival [32]. In our study, PaO_2 negatively correlated with serum HSP27 levels. This interpreted that hypoxia is a prominent contrubuting factor on HSP27 levels in COPD patients.

The HSP levels are related with circulating levels of CRP and cytokines. Cytokines may increase the induction of HSPs and contrarily HSPs may decrease the release of cytokines [33]. Serum CRP and IL-6 levels were positively correlated with serum HSP levels [34]. The mechanisms of increased HSP expression due to inflammation are still not understood. In nuclear factor-IL-6 may have regulatory roles in HSP expression [35]. Different responses may be seen in HSP expression against cytokines, for example IL-6 levels increased the HSP90 levels but decreased HSP70 levels in peripheral blood mononuclear cells [36,37]. We only found a positive correlation between HSP60 and IL-6 but there was no correlation between HSPs and CRP levels. Because some of the HSP cover more than one gene, their inducible expression may be changed according to comment.

Previous studies showed that in generally increased HSP levels in COPD patients [6,20]. They may originate peripheral airways, lung interstitial cells or in other organs [5,6]. The role of extracellular HSP60 is unclear but some studies showed their pro-inflammatory effects in atherosclerosis [38,39]. Similar to Hacker's et al study, we did not find that HSP60 have a role in the pathogenesis of COPD [5]. Furthermore, we did not find any difference in serum HSP70 and HSP90 levels between the groups. The differences between study results can be due to methodological differences and differences of subject characteristics (age, gender, smoking index and respiratory function). On the other hand, HSPs are paradoxical molecules. Intracellular HSPs have beneficial and protective roles but extracellular HSPs are signal molecules for the immune system and extracellular HSPs have a modulating effect to the secretion of pro-inflammatory cytokines [40]. The exact role of serum HSPs in COPD, determines of endogenous and exogenous trigger mechanisms has to be addressed in further studies.

To our knowledge, effect of HSPs on quality of life in COPD patients has not been examined until now. Molecular chaperone expression may induce with psychological stress and psychological stress induced HSP expression was shown in rats [41,42]. For this reason, we wanted to evaluate the relationship between SGRQ scores and HSPs. SGRQ symptom, activity and total scores were significantly associated with higher serum concentrations of HSP27 in COPD patients. These findings implicate that HSP27 may be a related factor on quality of life in COPD patients. On the other hand, several factors such as duration of the disease, disease severity and hypoxia were closely related to quality of life in COPD patients [43-45]. For this reason increased HSP27 levels may be related with decreased respiratory functions and hypoxemia on quality of life in COPD. Moreover, HSP27 may be used for the evaluation of functional status and prediction of disease severity because of HSP27 levels correlated with PFT parameters.

Proteomic analysis for determining of disease markers in COPD patients have been done previously. Serum levels of HSP27 and HSP70 may be a potential diagnostic marker and they can show disease severity [5]. Similarly these results, serum contents of HSP27 showed high sensitivity and specificity for diagnosis of COPD in our study. Because of the high sensitivity and specifity, HSP27 might be a suitable marker for diagnosis of disease according to our results.

In conclusion, the level of HSP27 was increased in COPD patients. Because smokers without COPD subjects had normal levels of HSP27 one can suppose that hypoxia is the effective factor rather than oxidant stress on serum levels of HSP27. In addition, HSP27 may be a marker of quality of life and functional status. Further investigations enrolling higher numbers of patients are needed to establish the role of HSP27 on COPD pathogenesis.

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CASE REPORT

Right Sided Aortic Arch Resembling Asthma

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Abstract

Exertional dyspnoea and shortness of breath at rest are common complaints in asthmatic patients. However, symptoms sometimes do not resolve under optimal medical treatment. In such cases infrequent causes of dyspnoea may be the underlying basis. We present a 38-year-old patient who suffered from shortness of breath not amenable to medical treatment for asthma for five years. In her medical history, the patient was on salbutamol inhalation as well as budesonide/formoterol inhalation for 5 years and the symptoms did not ameliorate. We diagnosed a right sided aortic arch after investigations. In this rare anomaly, both trachea and oesophagus might be encircled and compressed by large vessels as well as the aortic arch. Although some signs of right sided aortic arch can be recognized in chest radiograph and spirometry, accurate diagnosis is made by contrast enhanced computed tomography or angiography. Delay in diagnosis of right sided aortic arch may result in unnecessary investigations and prolonged periods of ineffective treatment. Diagnosis of right sided aortic arch leads to improvement in symptoms and withdrawal of unnecessary treatment.

KEYWORDS: Right sided aortic arch, asthma, dyspnoea, shortness of breath, spirometry

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INTRODUCTION

Shortness of breath, particularly during exercise, is a common complaint in everyday practice of physicians. Although treatment of most of the patients might be considered as straightforward, some cases remain challenging and symptoms of the patient continue. In such cases, infrequent causes of dyspnoea may be researched. We present a patient who suffered from shortness of breath not amenable to medical treatment for asthma for five years and diagnosed with right sided aortic arch.

CASE PRESENTATION

A 38-year-old female was admitted to our clinic suffering from cough and shortness of breath during both exercise and cough. She was on salbutamol inhalation as well as budesonide/formoterol inhalation for 5 years and the symptoms did not ameliorate. Chest radiograph did not reveal any information except indistinct shadow of aortic knob at the left side of the mediastinum (Figure 1). Spirometry revealed a peak expiratory flow of 55% of predicted. Expiratory flow volume loop was flattened so we proceeded to carry out a contrast enhanced computed tomography of thorax in order to investigate a large airway obstruction (Figure 2). Contrast enhanced computed tomography (CT) disclosed a right sided aortic arch and Kommerell's diverticulum, both of them were compressing the trachea (Figure 3,4). The patient was referred to cardiovascular surgery and an informed consent was obtained.

DISCUSSION

Treatment of asthma is frequently undemanding, although symptoms of some patients persist under optimal medical treatment. After excluding inappropriate inhaler devices, poor adherence to treatment, or persistent provoking factors, other causes of exertional dyspnoea should be researched in these patients and diagnostic tests should be assessed meticulously [1,2].

Several pathologies can result in compression to trachea and right sided aortic arch is one of them [3]. Both trachea and oesophagus might be encircled and compressed by large vessels as well as the aortic arch. In the chest radiography, absence of the shadow of the arcus aorta on the left side of the mediastinum might indicate a right sided aortic arch [2]. However, the sensitivity and the specificity of chest radiograph for right sided aortic arch are not well established [1].

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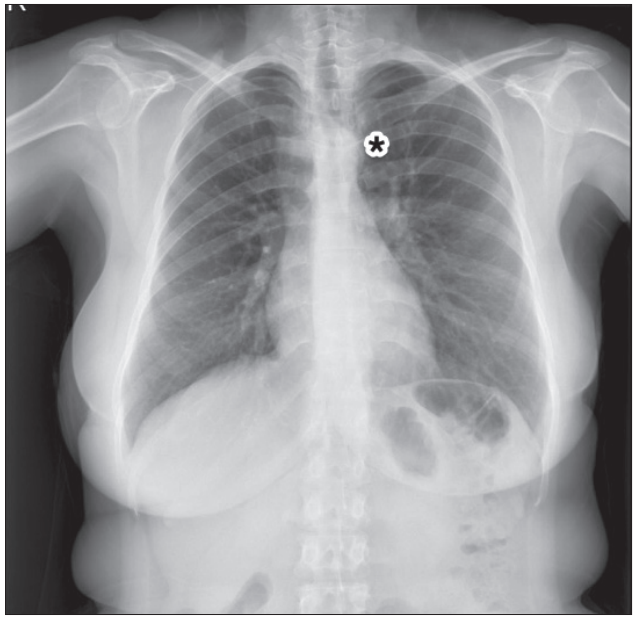

Figure 1. Chest radiograph, asterisk shows the indistinct shadow of the aortic knob.

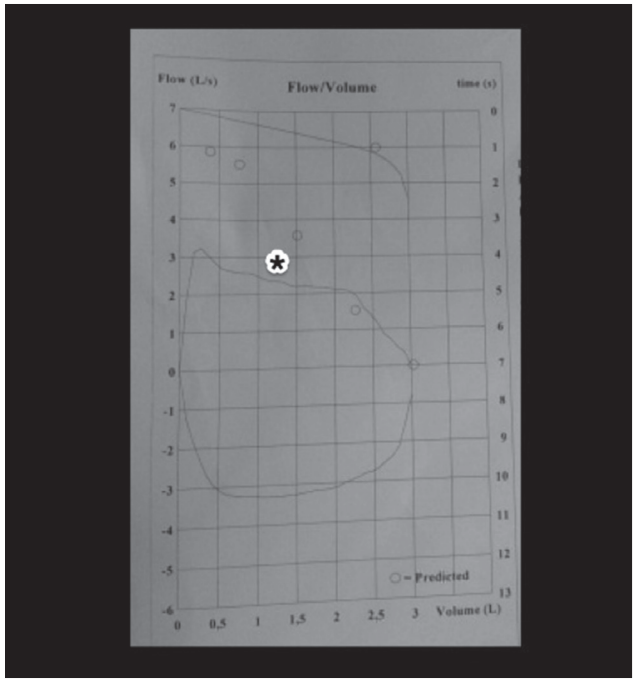

Figure 2. Spirometry flow volume loop of the patient, asterisk shows the flattened expiratory flow volume loop.

Not only measured parameters but also flow volume loop should be analysed in spirometry results. An extrinsic tracheal compression may be suspected from flattened expiratory flow volume loop in the spirometry [1,2]. Thoracic CT and magnetic resonance imaging (MRI) are the best methods to diagnose right sided aortic arch [2]. An unrecognized right sided aortic arch may result in unnecessary investigations and prolonged periods of ineffective treatment [1]. Diagnosis of right sided aortic arch without delay leads to improvement in symptoms and withdrawal of unnecessary treatment [1].

If the compression of the trachea and oesophagus is severe, the symptoms are likely to occur in infancy and early childhood [4,5]. Otherwise, symptoms such as cough, dysphagia, exertional dyspnoea, and chest pain usually appear in adulthood [4,5]. In the presented case, dyspnoea with cough might be due to tracheal compression by large vessels during forced expiration [4,5]. Similar symptoms may manifest in most asthmatic patients in relation to airway hyperreactvity [4,5].

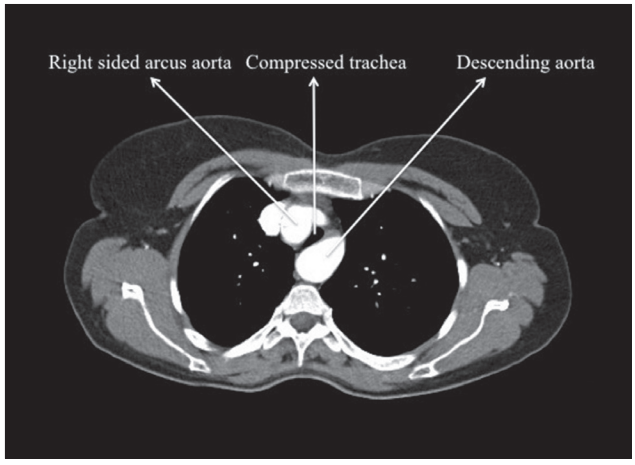

Figure 3. Computed tomography of the thorax, compressed tracheal lumen is shown.

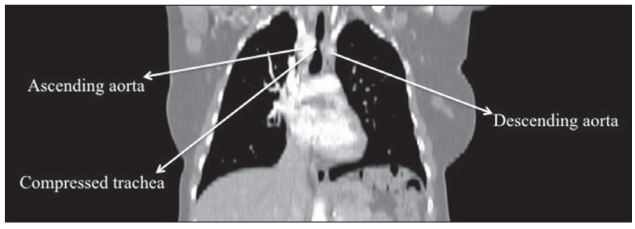

Figure 4. Reformatted image in coronal plane, compression on the tracheal lumen can be seen.

In conclusion, right sided aortic arch might be considered in differential diagnosis of asthmatic patients with persisting symptoms in order to avoid unnecessary treatment and prolonged airway compression. Flattening of spirometry flow volume curve might play a role in the diagnosis of this rare anomaly.

Informed Consent: Written informed consent was obtained from patient who participated in this case.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.İ., A.B., A.C.T.; Design - S.İ., A.B., A.C.T.; Supervision - S.İ., R.G., A.B.; Resources - S.İ., A.B., R.G.; Materials - S.İ., A.C.T.; Data Collection and/or Processing - S.İ., A.B., A.C.T.; Analysis and/or Interpretation - S.İ., A.B., A.C.T., R.G.; Literature Search - S.İ., R.G.; Writing Manuscript - S.İ., A.B., A.C.T.; Critical Review - S.İ., A.B., R.G., A.C.T.

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CASE REPORT

Two Chronic Granulomatous Disease Diagnosed in Adult Age

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Abstract

Chronic granulomatous disease (CGD) is a rare, inherited primary immunodeficiency that is usually diagnosed at adulthood and is presented with recurrent bacterial and fungal infections. In this case report, two adult cases of CGD have been presented. A 29-year-old woman was referred to our clinic with hypoxic respiratory failure, with a pre-diagnosis of multidrug resistant tuberculosis (TB). Her lung biopsy had been reported as granulomatous inflammation, she hadnot responded to a 5 month anti-TB treatment. A complete medical history consisted of 4 occasions of treatment with anti-TB drugs and that her sister and brother had undergone TB therapy. However, since childhood, a TB bacilli had never been isolated microbiologically in the siblings. Patient's parents were third degree consanguineous. The patient still had a purulent drainage around the operation site. Microbiological studies of the wound drainage and respiratory tract have not encountered any specific microorganism. Investigation of an immunodeficiency has proved CGD through nitroblue tetrazolium test. Her siblings has been diagnosed as CGD as well.

Second case, a 19-year-old male, has been admitted to our clinic with complaints of fever, chest pain and an abscess lesion in the anterior chest wall. His medical history comprised 3 recurrences of pneumonia within last 2 years. In physical examination, a 3 x 5 cm fluctuant swelling lesion on the anterior chest wall. Radiologically, new pneumonic consolidations were detected. Sputum specimens did not provide any specific microorganism, cultures of the chest-wall abscess fluid grew aspergillus. His parents had been living in the same village but no consanguinity was known. Due to recurrent infections, immunodeficiency tests had been investigated. He was diagnosed as CGD due to dihydrorhodamin test. These two cases signify that, in our country where consanguinity is common, etiology of recurrent unexplained infections, abscesses and granulomas should be investigated and CGD should be in differantial diagnosis list.

KEYWORDS: Abscess, chronic granulomatous disease, granuloma, immunodeficiency, tuberculosis

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INTRODUCTION

Chronic granulomatous disease (CGD) is a genetic primary immunodeficiency characterized by the formation of granuloma, where defense mechanisms against bacteria and fungi infections are weakened. The reported incidence from the United States of America is 1/200.000: however, there is no incidence data reported from our country. The disease may show X-linked or autosomal recessive heredity, and autosomal recessive forms are mostly encountered in consanguineous marriages [1].

The disease manifests itself with serious bacterial and fungal infections recurring since childhood [2]. The most frequently affected organ in CGD is the lungs. Skin involvement that progress with skin infections and subcutaneous abscess, suppurative adenitis, sinusitis, liver abscess, osteomyelitis, and necrotizing fungal infections can be seen. Granulomatous infection, autoimmune events and rheumatologic diseases can be detected.

Diagnosis is made under the age of 3 in X-linked inherited form and under the age of 8 in autosomal recessively inherited form [3-5]. There are reported cases where diagnosis is rarely made late and CGD is detected after the third or fourth decades of life [6,7].

In company with the literature, this case report aimed to present two CGD cases diagnosed in adult ages by having received their informed consent.

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CASE PRESENTATION

Case 1

The 26-year-old female patient was referred to our clinic with respiratory failure and a preliminary diagnosis of multidrug-resistant (MDR) tuberculosis (TB). The patient presented with complaints such as dyspnea, cough, sputum, fever, weight loss and night sweating. It was found out from her history that the patient had applied to another hospital with the same complaints five months prior and that she had been examined with suspected tuberculosis (Figure 1). Acid-fast bacillus (AFB) had been detected negative in her three sputum samples and bronchial lavage. Standard antituberculosis treatment had been started with isoniaside, rifampicin and ethambutol upon having detected granulomatous inflammation on lung biopsy. Due to the development of hypoxemic respiratory failure in the third month of treatment, corticosteroid was added to the treatment regimen. The patient had been referred to our clinic with a preliminary diagnosis of MDR-TB by having detected clinical and radiological progression in the fifth month of treatment. The posteroanterior (PA) chest radiography during this period is seen on Figure 2.

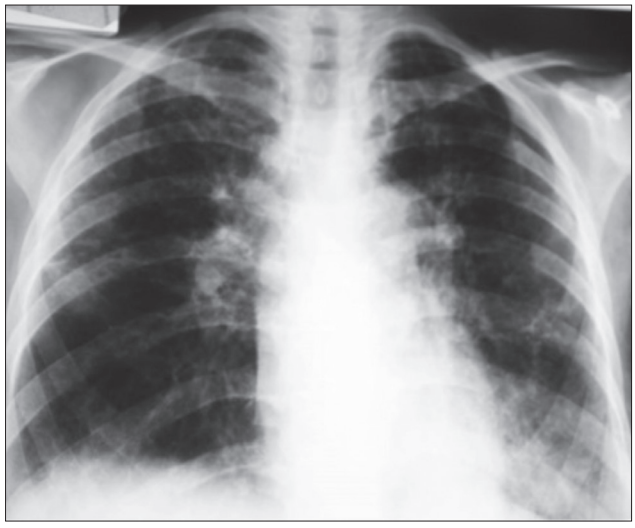

Figure 1. (**Case 1)** Before TB treatment, another center.

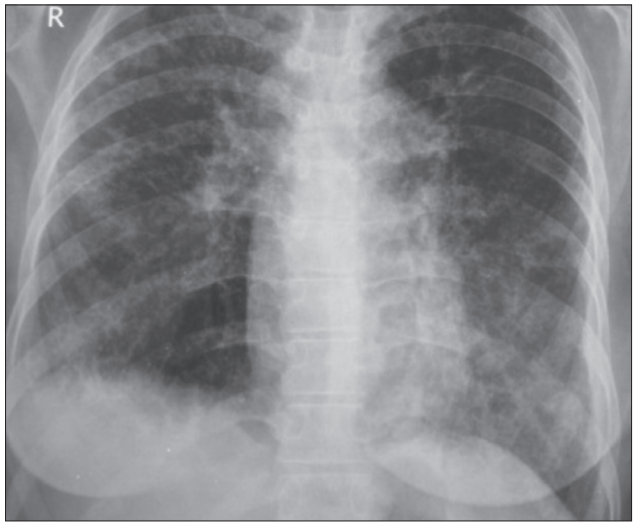

Figure 2. (Case 1) Fifth month of TB treatment.

The patient had a history of receiving treatment for tuberculosis four times before. Her sister was hospitalized in the pediatric service of our hospital with a preliminary diagnosis of MDR-TB. Her brother had also received TB treatment.

The patient was weak and fatigued. Her body temperature was 37.2°C and respiratory rate per minute was 16. Many skin scars were present on her knees and elbows from childhood. On respiratory examination, rales were heard in bilateral lower regions in the inspirium. Purulent leakage still continued from her previous operation site. Micronodular infiltrations were seen in all zones on chest graphy. On high resolution computed tomography (HRCT), an image concordant with small-airway disease accompanied by micronodules, nodules, interseptal thickening and reticular markings (Figure 3A,B) was seen. White blood cell count was 12.25 K/L and hemoglobin was 11 g/dL on complete blood count. Biochemical tests were normal except for a slight albumin fall (3.3 g/dL). On arterial blood gas test, partial oxygen pressure was 54 mmHg and oxygen saturation was 86% in room temperature. C-reactive protein was found as 88 g/L.

Antituberculosis treatment was continued. ARB was (-) on three sputum samples. Growth was not detected in sputum nonspecific culture and fungus culture. There was no growth in the cultures of the leakage from the operation site.

Three detailed histories were taken from the family and patient. First of all, the patient had received tuberculosis

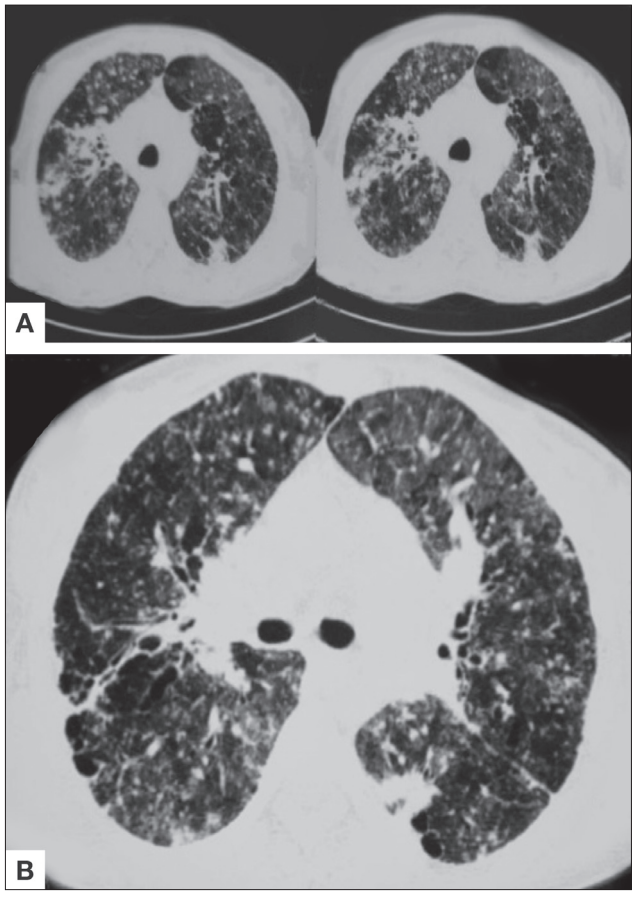

Figure 3. (Case 1) (A) Presenting HRCT, **(B)** presenting HRCT.

treatment for six months with TB diagnosis as a result of scars with abscess on her body at the age of ten. At the age of eleven, the patient had been reported to have granulomatous inflammation with liver biopsy and had received TB treatment for six months. The patient had been treated with a diagnosis of pulmonary TB ate the ages of twenty-one and twenty-four. The 23-year-old brother of the patient had received TB treatment at the age of nine. Patient's sister had also received TB treatment twice and by having been accepted as clinically unresponsive, she had been referred to the pediatric service of our hospital with a preliminary diagnosis of MDR-TB.

The Tuberculosis Control Dispensary with which the patient is affiliated, was contacted and it was found out that tuberculosis bacilli were detected in the sputum samples of neither our patient nor her siblings. The parents of the patient were third-degree relatives, and their twins died when they were 9-months-old. Deaths at childhood ages were present on the siblings and cousins of the parents.

In the light of this information, our patient was investigated in terms of immunodeficiency; IgG: 21.5 (7-16) g/L, IgA: 10.1 (0.7-3.1) g/L, IgM: 1.1 (0.05-0.3) g/L. Upon contacting the immunology clinic, the patient was asked for nitroblue tetrazolium test (NBT) which was reported as unresponsive.

The patient, who was diagnosed with chronic granulomatous disease, was referred to the immunology department. Her siblings were also diagnosed with CGD. The patient who was started on interferon gamma, trimethoprim-sulfamethoxazole (TMP-SMX) and itraconazole treatment is followed without complications and is in a clinically stable condition in the third year of treatment.

Case 2

The 19-year-old male patient applied to our clinic with fever and chest pain ongoing for a week and swelling on the anterior thoracic wall ongoing for two months. The patient had lost 7-8 kg in the last one year. The patient had suffered pneumonia three times for the last two years, but he did not have any additional diseases. Nothing was detected in family history. On physical examination, the patient was weak and he was 1.55 cm tall. His body temperature was 37.4°C. A swollen, rubescent, fluctuating soft tissue lesion, 3 x 5 cm in diameter, which had an increase in temperature was present on the sternum on the anterior wall of the right chest. Other system examinations were normal. White blood cell count was 10.20 K/L and hemoglobin was 8.7 g/dL on complete blood count. Biochemical tests were within normal limits and C-reactive protein was detected as 122 g/L.

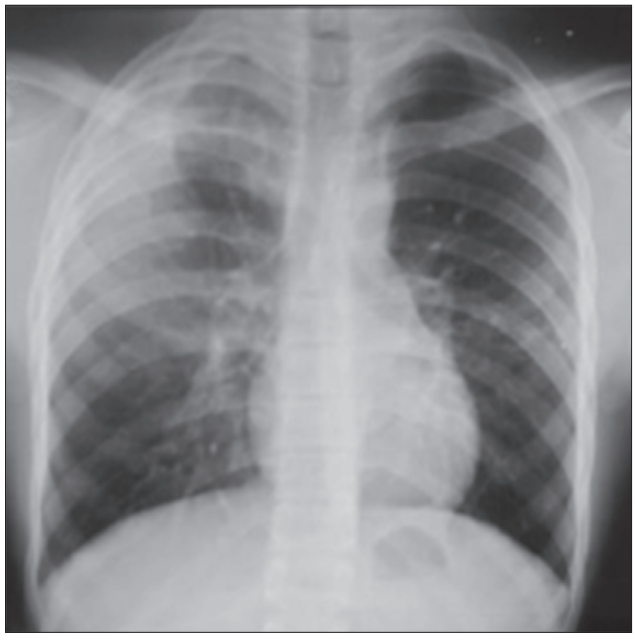

Figure 4. (Case 2) Presenting PA chest radiography.

On PA chest radiography, peripheral nonhomogeneous density increase was detected on upper right and middle left zone (Figure 4). On thoracic CT, homogenous opacity located on the right upper lobe pleura and fibrotic shrinkage with parenchymal infiltration in its surrounding, which stretched out to the parenchyma from the lower left lobe pleura were monitored (Figure 5A,B). The patient had been treated with a diagnosis of bilateral pneumonia two years prior and had developed pneumonia in different locations six months later. The case was investigated for TB and ARB was detected (-).

Antibiotherapy was started on the patient. Sputum cultures and ARB were (-). ARB was not detected on the lesion taken form the anterior chest wall; there was no growth in nonspecific culture. Dense neutrophils were reported in cytological investigation. *Aspergillus* grew in fungus culture.

The parents of the patient lived in the same village and did not know about any history of consanguineous marriage. Human immunodeficiency virus was negative in the patient who suffered frequent infections. Immunoglobulin values of the patient tested for immunodeficiency were normal. Dihidrorhodamin test was found concordant with CGD.

The patient, who was referred to the immunology department, was started on interferon gamma, voriconazole, cefixime, and TMP-SMX treatment. The clinically responsive patient is followed without complications in the second year of treatment. The 12-year-old sister of our patient, who did not have any complaints, was also diagnosed with CGD and is now under follow-up.

DISCUSSION

History of suffering frequent infections was present in our first case and granulomas were detected histopathologically. TB bacilli had never been proven for the patient and her siblings but treatment had been received many times. Our second case had suffered from infections frequently in recent years and presented to us with skin abscess. These cases emphasize the necessity to question hereditary immunodeficiency in inexplicable resistant infections which the person suffers frequently especially in a country, like ours, where consanguineous marriages are often seen. Moreover, although tuberculosis is the first thing that comes to mind in granulomatous lesions, differential diagnosis should be definitely made.

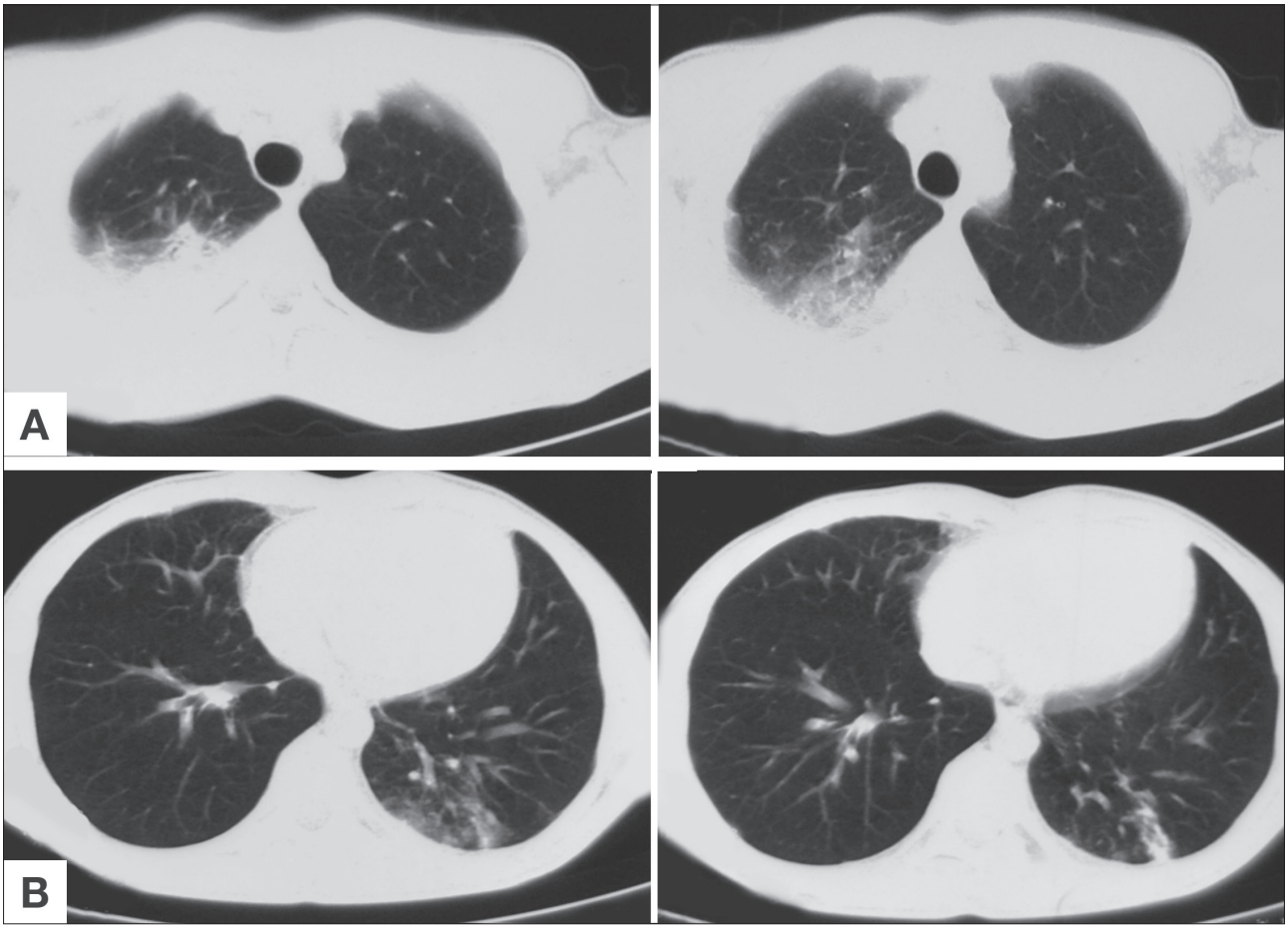

Figure 5. (Case 2) (A) Presenting thoracic CT, **(B)** presenting thoracic CT.

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system plays the basic part in killing catalase-positive microorganisms after phagocytosis of neutrophilia and monocytes. Due to the mutation in the genes coding the proteins in NADPH oxidase enzyme complex in CGD, superoxide radicals are not formed and respiratory burst does not happen [1]. Fever and leucocyte response occur during infection; however, the microorganism is not killed [8,9]. The most frequently determined agents are microorganisms that produce catalase, such as *Staphylococcus aureus* and *Aspergillus* types. In addition, infection can develop with *Serratia marcescens*, *Burkholderia cepacia* kcomplex and *Nocardia* types [5].

Growth and developmental delay can be seen in patients and increase in length can be late [10]. The most frequent involvement region in CGD is the lungs; recurrent pneumonia and pulmonary abscess can be seen. Due to recurrent infections, bronchiectasis, obliterative bronchiolitis, hypoxia and respiratory failure can develop [11]. Since granulomatous lesions can be detected in the lungs histopathologically, sarcoidosis or tuberculosis can also be considered. Skin is the second most involved organ. Although staphylococcus is the most frequently isolated pathogen, the agent cannot be isolated all the time [5]. The first case that we reported had been started on TB treatment as a result of widespread skin abscesses and having detected granulomas in liver biopsy afterwards. Finally, the case had been using TB medication upon detecting granulomas in the lungs. Pulmonary failure was present during her application to our clinic. This patient is important in terms of emphasizing microbiologic diagnosis and culture positivity as golden standards in TB diagnosis when every granulomatous inflammation may not be TB or sarcoidosis.

The other case did not have a history of infection or any other disease until two years prior. It has been reported in the literature that a history of infection may not be present until early adult period and these patients have been accepted as mild forms of CGD according to mutation type [2]. This case had also suffered frequent pulmonary infections in recent years and applied to our clinic due to skin abscess.

Apart from infections, granuloma formation, autoimmune events and rheumatologic disease may manifest in chronic granulomatous disease (CGD). Inflammatory intestinal disease, bladder granulomas and genitourinary system complications have been reported most frequently in X-linked heredity. Increase in liver enzymes, splenomegaly, hepatomegaly, portal hypertension and thrombocytopenia may also develop [7,12]. Depending upon recurrent infections, hypergammaglobulenemia, chronic disease anemia and high C-reactive protein levels are frequently observed [11,13]. Granulomas were detected in the liver

when our first case was eleven years old. Both cases had anemia and C-reactive protein elevation.

The most important point for diagnosis is that the disease should come to mind. CGD should come to mind in a history of recurrent, serious and unusual infection and in individuals suffering from hepatosplenomegaly, diarrhea and delay in wound healing. Exclusion of infectious diseases, other immunodeficiencies, and diseases like cystic fibrosis, hyperimmunoglobulin E syndrome and Crohn's disease is of grave importance for differential diagnosis. The siblings should also be scanned when CGD diagnosis is made [11,13]. Neutrophil functions and superoxide production are evaluated for diagnosis. To this end, direct superoxide production measurement, ferrocytochrome C reduction test, nitroblue tetrazolium (NBT) reduction test and dihidrodamin-123 (DHR) oxidation methods are used [1]. CGD diagnosis was made with NBT test in our first case and DHR-123 test in our second case.

When diagnosis is made, TMP-SMX, itraconazole and prophylaxis, interferon-gamma as immunomodulatory (IFN-γ) treatments are applied. The patients should be vaccined for measles, chicken pox, and annual flu, and BCG should be performed due to the risk of disseminated disease [14]. Rapid and aggressive treatment is necessary once the infection develops. Surgical treatment may be necessary in persistent and recurrent cases [5]. Most frequent cause of mortality has been reported as pneumonia and sepsis due to *Aspergillus* or *Burkholderia cepacia* [15]. Both cases are followed with IFN, TMP-SMX and itraconazole treatments without complications.

In conclusion, patients with inexplicable frequent infection attacks, abscess, and granuloma presence should be examined and tested for chronic granulomatous disease. It is important in terms of prognosis if this disease is listed in differential diagnosis in adult age in our country where consanguineous marriages are frequently observed.

Author Contributions: Concept - F.T.A., T.S., E.A., S.B., D.A.T., Y.B., O.A.; Design - F.T.A., T.S., E.A., S.B., D.A.T., Y.B., O.A.; Supervision - F.T.A., T.S., E.A., S.B., D.A.T., Y.B., O.A.; Data Collection and/or Processing - F.T.A., T.S., Y.B., D.A.T.; Analysis and/or Interpretation - F.T.A., T.S.; Literature Review - F.T.A., T.S.; Writer - F.T.A., T.S.; Critical Review - F.T.A., T.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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