Homocysteine: A risk factor for the development of cardiovascular events in chronic respiratory diseases.

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Abstract

Respiratory viruses lead to exacerbations of chronic respiratory diseases such as chronic obstructive pulmonary disease and asthma. Many risk factors play a role in the development of asthma and COPD. The homocysteine is thought to play a role in the pathogenesis of asthma and COPD. In this research we aimed to determine homocysteine levels in individuals with asthma and COPD presenting adenovirus and respiratory syncytial virus infections in a period of exacerbated respiratory disease to predict the risk factors that could lead to the development of cardiovascular diseases. Viral agents were determined using ELISA methods. Measurement of homocysteine level was performed with high-performance liquid chromatography. According to the results, the rate of RSV was 41.9% (36/86) and the rate of adenovirus was 34.9% (30/86) in patients with asthma, while the rate of RSV was 40.5% (30/74) and the rate of adenovirus was 39.2% (29/74) in patients with COPD disease. While the homocysteine levels ranged from 10.39 to 16.66 μmol/l in the control group, homocysteine levels of asthma patients were found to be between 13.12-25.21 μmol/l, and in COPD patients, these were found between 12.96-25.96 μmol/l. The homocysteine levels in COPD patient were found statistically significantly higher than control group data ($p<0.05$). The determination of the homocysteine levels and viral respiratory agents in patients with asthma or COPD reduce the risk of mortality and morbidity and help to predict the risk factors that could lead to development of cardiovascular diseases.

Introduction

Viruses spread following quite different ways to host and find settlement in specific organs and tissues. Bacterial, viral and fungal agents take place in the etiology of respiratory tract infections, but it is known that the virus is in the foreground [1]. The main respiratory viruses associated with respiratory diseases are rhinovirus, corona virus, influenza, parainfluenza, adenovirus and respiratory syncytial virus (RSV). These viruses lead to the exacerbation of chronic respiratory diseases [2,3]. Chronic respiratory diseases affect mainly the lungs and airways due to variety factors such as tobacco use, air pollutants, allergens, bad-diet and obesity and environmental stress [4]. Asthma and COPD are the most common chronic respiratory diseases and both have similar properties such as risk factors, pathogenic processes, diagnosis, treatment and protection [5]. Asthma is a chronic inflammatory disease of the airways characterized by variety of recurring symptoms. Individual (genetics, gender and obesity) and environmental (infections, smoking, dust, pollutants and allergens) risk factors play an important role in the development of asthma [5,6].

COPD is an inflammatory disease which includes lung diseases such as chronic bronchitis, airways disease and emphysema, and recently there has been an increase in the prevalence of the disease worldwide [7,8]. COPD has a certain risk factors, but smoking has been conventionally known to be the most important one of these risk factors. Other factors are indoor air pollution caused by biomass fuel, all kinds of organic and inorganic pollutants and outdoor air pollution [9,10]. COPD associated with an abnormal inflammatory response occur via these risks factor.

Homocysteine is a non-essential amino acid formed by the demethylation of methionine [11]. It is one of systemic inflammatory markers and is also an important marker for cardiovascular diseases [12]. Homocysteine which is thought to play a role in the pathogenesis of COPD is prominent in the development of atherosclerosis [13]. Homocysteine is quite common in the elderly and decreased renal function and is a known risk factor for vascular diseases [14-16]. Besides, homocysteine levels are crucial in severe diseases like diabetic nephropathy, renal failure, hyperlipidemia [17]. Therefore,

assessment of homocysteine levels may be an important marker in terms of predetermination of cardiovascular diseases in patients. In this research we aimed to determine homocysteine levels in individuals with asthma and COPD who have adenovirus and respiratory syncytial virus infections in a period of exacerbated respiratory disease to predict the risk factors that could lead to the development of cardiovascular diseases.

Material and Methods

Selection criteria of asthma, COPD individuals and control group

Serum samples of one hundred sixty patients were used in the exacerbated period of asthma and COPD in our study. The criteria for the control group were having no known disease or acute/chronic symptoms and respiratory viral agents. This group was created with 25 healthy individuals. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Kırıkkale University Hospital Ethics Committee.

Determination of adenovirus and respiratory syncytial virus

The ELISA kits (DRG Instruments GmbH, Germany) and serological methods were utilized for the determination of adenovirus and RSV positivity in serum samples of 25 healthy individuals, 86 asthma and 74 COPD patients, (Measuring wavelength: 450 nm; reference wavelength: 620 nm). The tests were performed exactly according to manufacturer's instructions. The control group individuals were checked in terms of whether they had these viral agents.

Measurement of homocysteine levels

Measurement of homocysteine levels was performed with HPLC (Agilent Technologies 1200 series, USA; kits: Immuchrom GmbH, Germany). This study was done according to the manufacturer's instructions: Firstly, the samples were reduced and derivatised for the determination of homocysteine. Then, albumin bound and the oxidized homocysteine were reduced and converted into a fluorescent probe. During a precipitation step high molecular substances were removed. The samples were respectively cooled, centrifuged and injected into the HPLC system. The isocratic separation was done through HPLC at 30°C using a "reversed phase" column. One run lasted 5 minutes. The quantifications were performed by the delivered plasma calibrator; the concentrations were calculated by the "internal standard method". Calculation;

Conc. Sample (μmol/l)=peak area patient × conc. calibrator (μmol/l)/Peak area IS patient × F
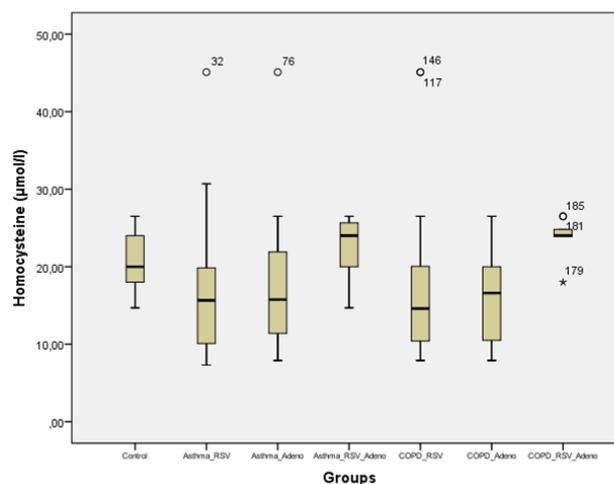
F=Peak area IS calibrator/Peak area homocysteine of the calibrator

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS Inc., USA). All measurements were taken in triplicate. Besides, all data were reported as means ± standard distribution (SD). The critical significance level for the statistical tests performed was set at 0.05. After assessing the normality distribution (Shapiro-Wilk test) and homogeneity of variances, non-parametric Mann Whitney-U and Kruskal Wallis H-tests were used for the comparison of differences between means.

Results

Our study examined serum samples of 160 patients diagnosed with asthma (n=86) and COPD (n=74) as well as 25 healthy individuals. Demographic data of all groups are presented in Table 1 and were evaluated statistically. While the mean age of asthma and COPD patients was 58.4 ± 4.0 and 61.4 ± 6.0 years, mean age of control group was 56.6 ± 8.2 years. No-statistically significant relationship was determined in terms of two groups' age (p>0.05).

Figure 1: *Homocysteine levels in control group and Asthma and COPD patients with RSV and Adenovirus.*

Table 1: *Demographic data of Asthma, COPD and control group.*

	Control (n=25)	Asthma[*] (n=86)	COPD[*] (n=74)
Age (years)	56.6 ± 8.2	58.4 ± 4.0	61.4 ± 6.0
Sex (male/female)	18/7	69/17	59/15
Smoking	21	62	68

Results were expressed as mean ± standard deviation.
[*]Compared to control group values, 0.05.

Our study examined positivity of respiratory syncytial virus (RSV) and adenovirus in serum samples of 160 patients with asthma and COPD (Table 2). According to the results, the rate of RSV was 41.9% (36/86) and the rate of adenovirus was 34.9% (30/86) in patients with asthma, while the rate of RSV was 40.5% (30/74) and adenovirus was 39.2% (29/74) in patients with COPD. Serum samples of asthma, COPD and

healthy control group were used in homocysteine study. Values of homocysteine (μmol/l) of patients with COPD, asthma and control group and their 95% confidence intervals are shown in Table 3. While the homocysteine levels ranged from 10.39 to 16.66 μmol/l in the control group, homocysteine levels of asthma patients were found to be between 13.12 - 25.21 μmol/l and between 12.96 - 25.96 μmol/l in COPD patients. A statistically significant difference between homocysteine levels of asthma and COPD patients was not observed (p>0.05) (Figure 1). However, homocysteine levels of patients were detected to have a statistically significant difference compared with the control group (p<0.05).

Table 2: The ratio of respiratory Syncytial Virus (RSV), Adenovirus and RSV/Adenovirus in both Asthma and COPD.

Respiratory viruses	Asthma (n=86)		COPD (n=74)	
	n	%	n	%
RSV	36	41.9	30	40.5
Adenovirus	30	34.9	29	39.2
RSV/Adenovirus	12	13.9	9	12.2
Viral negative	8	9.3	6	8.1

Viral negative: negative in terms of both adenovirus and RSV

Table 3: Values of homocysteine (μmol/l) of patients with COPD, asthma and control group and their 95% confidence intervals.

Groups	n	Mean ± S.D.	95% Confidence Interval for Mean
			Min.-Max.
Control	25	12.03 ± 3.96	10.39-16.66
Asthma	86		
RSV	40	16.25 ± 7.90	13.72-18.78
Adenovirus	34	17.57 ± 7.50	14.95-20.19
RSV/Adenovirus	12	22.80 ± 3.78	20.40-25.21
Viral negative	8	14.21 ± 2.42	13.12-16.21
COPD	74		
RSV	36	16.92 ± 8.87	13.92-19.92
Adenovirus	29	15.94 ± 5.89	13.70-18.18
RSV/Adenovirus	9	24.04 ± 2.49	22.12-25.96
Viral negative	6	13.86 ± 4.62	12.96-16.73

Values are expressed as mean ± S.D.

Discussion

Respiratory disease caused by viral infections is common in humans. In patients with respiratory diseases such as asthma and COPD, the morbidity caused by respiratory virus infection is very important [18]. Asthma and COPD are significant causes of growing morbidity and mortality in all countries of the world. COPD is the fourth leading cause of death in the world [19]. However, it is expected to rise to third rank in 2030 and to rank fifth in 2020, due to the increase of smokers' addiction levels. Therefore, respiratory diseases are a major socio-economic problem for both developed and developing countries today [20]. The exacerbations of asthma and COPD are major causes of morbidity for all ages. In these periods, it is very likely that an increase in the rate of morbidity could occur via with viral agents. Sigurs et al. explored whether a relationship existed between a viral infection and development of asthma. The data by Sigurs et al. suggest that severe viral infections with asthma increase the likelihood of development of asthma [21]. On the other hand, cardiovascular disease is frequently a cause of morbidity encountered in asthma and COPD patients. The most important evidence of this situation is the endothelial dysfunction in the pathogenesis of respiratory disease [13]. Moreover, endothelial dysfunction is stimulated by increased homocysteine [22]. Hence, we aimed to determine homocysteine levels in individuals with asthma and COPD to predict the risk factors that could lead to the development of cardiovascular diseases. In our study, the homocysteine levels in asthma and COPD patients were found statistically significantly higher than the control group data (p<0.05).

Andersson et al. examined the plasma from nineteen patients with COPD and twenty nine healthy individuals [23]. Similarly, they found that homocysteine levels were higher in COPD group than the control. At the same time, in our study, the relationship between sex and homocysteine levels in individuals with asthma and COPD was evaluated and the importance of sex was revealed in these patients. When the relationship between homocysteine levels with male gender was evaluated in our study, a statistically significant relationship was determined between these two parameters (p<0.05). Besides, the highest homocysteine level in male gender is stated in many studies as in our study [13,24]. The comparison of our results with the literature is limited because parameters of our study were less than the parameters of other studies. But our data is sufficient in terms of conducting statistical calculates and obtaining results.

In conclusion, the respiratory viruses such as RSV and adenovirus lead to the exacerbations of chronic respiratory diseases. These chronic respiratory diseases have led to increased morbidity and mortality by affecting cardiovascular events. It is known that there is a strong link between cardiac disease and homocysteine levels. The determination of the homocysteine levels reduces the risk of mortality and morbidity in individuals with asthma or COPD with viral respiratory agents and helps to predict the risk factors that could lead to development of cardiovascular diseases.

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References

1. Bayrakdar F, Altaş AB, Korukluoğlu G. Solunum Yolu Virüslerinin 2009-2012 Yılları Arasında Ülkemizdeki Mevsimsel Dağılımı. Türk Mikrobiyol Cem Derg 2013; 43: 56-66.

2. Varkeya JB, Varkey B. Viral infections in patients with chronic obstructive pulmonary disease. Curr Opin Pulm Med 2008; 14: 89-94.

3. Busse WW, Lemanske RF, Gern JE. The Role of Viral Respiratory Infections in Asthma and Asthma Exacerbations. Lancet 2010; 4: 826-834.

4. Ait-Khaled N, Enarson D, Bousquet J. Chronic respiratory diseases in developing countries: the burden and strategies for prevention and management. Bulletin of the World Health Organization 2001; 79: 971-979.

5. Wenzel SE. Asthma phenotypes: The evolution from clinical to molecular approaches. Nat Med 2012; 18: 716-25.

6. O'Byrne PM. Global guidelines for asthma management: Summary of the current status and future challenges. Pol Arch Med Wewn 2010; 12: 511-517.

7. Ko FWS, Huı DSC. Air pollution and chronic obstructive pulmonary disease. Respirology 2012; 17: 395-401.

8. Brashier BB, Kodgule RR. Risk Factors and Pathophysiology of Chronic Obstructive Pulmonary Disease (COPD). Supplement To Japı 2012; 60: 17-21.

9. Lundbäck B, Lindberg A, Lindström M, Rönmark E, Jonsson AC, Jönsson E, Larsson LG, Andersson S, Sandström T, Larsson K; Obstructive Lung Disease in Northern Sweden Studies. Not 15 but 50% of smokers develop COPD? Report from the Obstructive Lung Disease in Northern Sweden Studies. Respir Med 2003; 97: 115-122.

10. Mannino DM, Watt G, Hole D, Gillis C, Hart A, McConnachie G, Quinlivan E, Sanyal AJ. The natural history of chronic obstructive pulmonary disease. Eur Respir J 2006; 27: 627-43

11. Pacana T, Cazanave S, Verdianelli A, Patel V, Min HK, Mirshahi F, Quinlivan E, Sanyal AJ. Dysregulated Hepatic Methionine Metabolism Drives Homocysteine Elevation in Diet-Induced Nonalcoholic Fatty Liver Disease. PLoS One 2015; 10: e0136822.

12. Seemungal TA, Lun JC, Davis G, Neblett C, Chinyepi N, Dookhan C, Drakes S, Mandeville E, Nana F, Setlhake S, King CP, Pinto Pereira L, Delisle J, Wilkinson TM, Wedzicha JA. Plasma homocysteine is elevated in COPD patients and is related to COPD severity. Int J Chron Obstruct Pulmon Dis 2007; 2: 313-321.

13. Nunomiya K, Shibata Y, Abe S, Inoue S, Igarashi A, Yamauchi K, Aida Y, Kishi H, Sato M, Watanabe T, Konta T, Ueno Y, Kato T, Yamashita H, Kayama T, Kubota I. Hyperhomocysteinaemia predicts the decline in pulmonary function in healthy male smokers. Eur Respir J 2013; 42: 18-27.

14. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002; 325: 1202-1208.

15. Vasan RS, Beiser A, D'Agostino RB, Levy D, Selhub J, Jacques PF, Rosenberg IH, Wilson PW. Plasma homocysteine and risk for congestive heart failure in adults without prior myocardial infarction. JAMA 2003; 289: 1251–1257.

16. Ravaglia G, Forti P, Maioli F, Servadei L, Martelli M, Arnone G, Talerico T, Zoli M, Mariani E. Plasma homocysteine and inflammation in elderly patients with cardiovascular disease and dementia. Exp Gerontol 2004; 39: 443–450.

17. Audelin MC, Genest J. Homocysteine and cardiovascular disease in diabetes mellitus. Atherosclerosis 2001; 59: 497-511.

18. Mallia P, Johnston SL. Influenza infection and COPD. Int J Chron Obstruct Pulmon Dis 2007; 2: 55–64.

19. Zwaans WAR, Mallia P, vanWinden MEC, Rohde GGU. The relevance of respiratory viral infections in the exacerbations of chronic obstructive pulmonary disease-A Systematic review. J Clin Virol 2014; 61: 181-188.

20. Akgun D. Prevalence of COPD prevention and related factors in Isparta provincial center. Turk Thorac J 2013; 14: 43-47.

21. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med 2000; 161: 1501-1507.

22. D'Angelo A, Selhub J. Homocysteine and thrombotic disease. Blood 1997; 90: 1-11.

23. Andersson A, Ankerst J, Lindgren A, Larsson K, Hultberg B. Hyperhomocysteinemia and changed plasma thiol redox status in chronic obstructive pulmonary disease. Clin Chem Lab Med 2001; 39: 229-233.

24. Graham IM, Daly LE, Refsum HM, Robinson K, Brattström LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheahan RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Lúis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garcon D, Andria G. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA 1997; 277: 1775-1781.

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