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Comparison of thiol/disulphide homeostasis parameters in patients with COPD, asthma and ACOS

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Abstract. – OBJECTIVE: Chronic obstructive pulmonary disease (COPD), asthma and asthma-COPD overlap syndrome (ACOS) are obstructive pulmonary disorders with different manifestations. Status of oxidation in tissues is important in obstructive pulmonary disorders. Smoking, acute exacerbations of COPD and asthma were associated with a marked imbalance in oxidant or antioxidant status due to increased oxidative stress in tissues and blood. Oxidative conditions may cause a reversible formation of mixed disulphides among protein thiol groups. The aim of this study was to compare parameters related with thiol/disulphide homeostasis in patients with COPD, asthma and ACOS.

PATIENTS AND METHODS: Patients (n= 135, 69 females, 66 males) who were referred with a diagnosis of COPD, asthma or ACOS were included in the study. Thiol/ disulphide homeostasis parameters in blood were analysed by a newly established method that measures the exact thiol/ disulphide status in the body.

RESULTS: The patients with COPD, asthma or ACOS were similar for demographic parameters other than age and number of cigarettes smoked. Measured thiol/disulphide homeostasis parameters were similar among these patient groups. When these biochemical measurements were adjusted for age and number of cigarettes by using regression analysis, similarity for thiol/disulphide homeostasis parameters among patient groups persisted.

CONCLUSIONS: To best of our knowledge, this is the first study to compare thiol/disulphide homeostasis parameters in COPD, asthma and ACOS patients. Similarity of thiol/disulphide homeostasis parameters among these patient groups supports the current view of Dutch hypothesis that COPD, asthma and ACOS share similar pathophysiological features but display different clinical manifestations.

Key Words:
COPD, Asthma, ACOS, Thiol/disulphide homeostasis, Oxidation.

Introduction

Chronic obstructive pulmonary disease (COPD) and asthma are the most common obstructive pulmonary diseases. Patients with asthma-COPD overlap syndrome (ACOS) have features of both asthma and COPD. The lack of clear features and distinct biomarkers makes the diagnosis of ACOS difficult. COPD, asthma and ACOS are disorders with heterogeneous manifestations. The mechanism and the biochemical features of ACOS are largely unknown[1-4].

The balance of oxidation and anti-oxidation in respiratory tissues is important in the physiopathology of obstructive pulmonary diseases. Reactive oxygen species (ROS) are unstable species and have unpaired electrons, capable of initiating oxidation. Lungs have a high-oxygen environment and are susceptible to injury mediated by ROS[5], which are important in re-modelling of extracellular matrix and blood vessels by stimulating secretion of mucus, inactivating antiproteases and regulation of cell proliferation or apoptosis[6]. Increased levels of ROS are associated with inflammatory reactions, such as alteration of transcription, signal transduction, or gene expression of pro-inflammatory mediators[6]. Smoking, acute exacerbations of COPD and asthma are associated with increased oxidative stress and marked oxidant/antioxidant imbalance that can be detected in blood[7,8].

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Thiols can form disulphide bonds and undergo oxidation reactions when exposed to oxidants[9]. Under oxidative stress, reversible formation of mixed disulphides between protein thiol groups can occur. Disulphide bonds can be reduced to thiol groups and dynamic thiol-disulphide homeostasis is maintained[10].

The aim of this prospective study was to compare blood biomarkers related with thiol/disulphide homeostasis in patients with COPD, asthma and ACOS. To the best of our knowledge, this is the first article about the comparison of thiol/disulphide homeostasis in obstructive pulmonary diseases.

Patients and Methods

In this prospective study, 135 patients (69 females, 66 males) who were referred to the Department of Chest Diseases, Ankara Ataturk Training and Research Hospital, Yildirim Beyazid University between January 2015 and January 2016 were included in the study. The study was approved by the Local Ethics Committee of Yildirim Beyazid University (14.1.2015/21). Informed consent was obtained from all patients. Patients who had COPD or asthma or ACOS according to the ACOS 2015 guideline and had a moderate obstruction ($50 < FEV_1\% < 80$) in pulmonary function tests (PFT) were included in the study[11]. Patients with inflammatory diseases, malignancies, diabetes mellitus, cardiovascular diseases, cerebrovascular diseases, acute-chronic kidney or liver diseases or proteinuria were excluded from the study.

Blood samples were taken after a minimum of eight hours of fasting. Collected samples were centrifuged at 1500 rpm for 10 minutes to separate the plasma and serum, and serum was stored at -80°C until analysis. Thiol/disulphide homeostasis parameters were measured as described previously by Erel and Neselioglu[12]. Briefly, reducible disulphide bonds were first chemically reduced for the formation of free and functional thiol groups. Sodium borohydride was used as reducing agent and it was removed by using formaldehyde. For detection of all of the thiol groups including reduced and native forms, 5,5¢-dithiobis-2-nitrobenzoic acid was used. Half of the difference between total thiols and native thiols yielded the dynamic disulphide amount. After determination of native and total thiols, disulphide amounts, disulphide/total thiol percent ratios, native thiol/total thiol percent ratios and disulphide/native thiol percent ratios were calculated[12].

Statistical Analysis

All analyses were performed by using SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA). It was considered statistically significant when the two-sided *p*-value is lower than 0.05. The normality of each variable was checked by applying Shapiro-Wilk test. Variance equality between groups was checked by Levene test. Results were shown as mean ± standard deviation (median, min-max) for continuous variables and frequency for categorical variables. One-way analysis of variance was utilised to compare more than two groups when assumptions of normality and variance homogeneity were met. When these assumptions were not satisfied, Kruskal-Wallis test was applied for comparison of more than two groups. Dunn's nonparametric comparison was utilised as post hoc testing after Kruskal-Wallis test. Pearson's chi-square test was applied to compare categorical variables. The relation between two continuous variables was investigated via Pearson's correlation. Multiple linear regression analysis was applied to estimate the relation between the independent variables and the outcome by adjusting confounding variables, such as age.

Results

Numbers of female to male (%) patients in COPD, asthma and ACOS groups were 14/30 (31.8/68.2%), 32/19 (62.7/37.3%) and 23/17 (57.5/42.5%), respectively. 19 (43.1%) of COPD patients, 8 (15.6%) of asthma patients and 11 (27.5%) of ACOS patients were smokers. Demographic features and laboratory findings of the patients are summarized in Table I. There was a statistically significant difference among patients with COPD, asthma and ACOS respect to the age; COPD patients were about ten years older than other patients ($p < 0.001$, Table I). Also, the number of cigarettes smoked in units of packages per years (ppy) was significantly higher in COPD group as compared to other groups ($p < 0.001$, Table I).

Mean white blood count (WBC) and C-reactive protein (CRP) levels in three patient groups were statistically similar. All of the patients had bronchitis or upper airway infections, but none of them had pneumonia or other severe infections

Table I. The demographic features and laboratory findings of the patients.

	COPD (n = 44)	Asthma (n = 51)	ACOS (n =40)	*p*-value
Age (years)	59.5 ± 10.9 (60, 31-82)	47.8 ± 15.1 (46, 15-89)	50.1 ± 15.8 (54, 22-82)	< 0.001[a]
Number of cigarettes (ppy)	34.3 ± 24.8 (30, 0-100)	7.5 ± 16.2 (0, 0-80)	12.6 ± 14.0 (7.5, 0-41)	< 0.001[a]
WBC (K/μL)	8209 ± 2252 (8095, 100-13880)	7537 ± 2038 (7200, 3800-13500)	8198 ± 1952 (7835, 4500-13100)	0.116a
CRP (mg/L)	19.8 ± 51.6 (5.2, 3-331)	11.8 ± 17.3 (4.1, 3-88.5)	16.1 ± 55.8 (4.1, 0.6-348)	0.414[a]
FEV_1 (%)	62.6 ± 8.88 (63.5, 35-75)	70.4 ± 7.94 (73, 51-80)	72.1 ± 9.14 (75, 48-80)	< 0.001[a]
FVC (%)	71.0 ± 11.9 (70, 37-91)	75.6 ± 10.7 (75, 59-101)	83.0 ± 15.6 (81, 49-128)	< 0.001[a]
FEV_1/FVC	66.5 ± 6.5 (67.5, 53-83)	78.5 ± 11.1 (79, 38-100)	76.9 ± 9.1 (78.5, 54-96)	< 0.001[a]
FEF 25-75 (L/s)	37.9 ± 11.9 (37, 18-73)	52.1 ± 15.1 (51, 26-84)	56.0 ± 24.1 (54.5, 5-107)	< 0.001[a]
Native thiol (μmol/L)	441.5 ± 68.6 (442, 301-565)	448.9 ± 61.0 (449, 301-564)	451.0 ± 57.3 (457, 317-559)	0.761[b]
Disulphide (μmol/L)	21.1 ± 6.5 (21.7, 7-30.8)	18.0 ± 7.4 (17.6, 0.7-35.1)	19.6 ± 7.0 (20.6, 0.4-30.3)	0.097[a]
Total thiol (μmol/L)	483.9 ± 70.8 (485, 350-617)	484.9 ± 59.0 (479.6, 336-588)	490.24 ± 59.2 (494, 361-591)	0.818[a]
Disulphide/native thiol (%)	4.9 ± 1.7 (4.65, 1.75-8.77)	4.15 ± 1.8 (4.54, 0.13-9.66)	4.4 ± 1.65 (4.56, 0.07-7.35)	0.115[b]
Disulphide/total thiol (%)	4.4 ± 1.4 (4.2, 1.69-7.4)	3.7 ± 1.6 (4.1, 0.13-8.09)	4.0 ± 1.4 (4.1, 0.07-6.4)	0.112[b]
Native thiol/total thiol (%)	91.1 ± 2.8 (91.4, 85-9)	92.4 ± 3.2 (91.6, 83.8-99.7)	91.9 ± 2.8 (91.6, 87.1-99.8)	0.112[b]

Note: Results are demonstrated as mean ± standard deviation (median, min-max) for continuous variables. [a,b]: *p*-values are obtained via Kruskal Wallis test, and ANOVA, respectively.

that needed hospitalization. Mean FEV_1%, FEV_1/FVC, FEF25-75 (L/s) were statistically lower in the COPD group than in the other groups. Since all of the patients were selected to have a moderate obstruction in PFTs and homogenous for this aspect, the numerical differences were not considered clinically significant. When thiol/disulphide homeostasis parameters such as levels of native thiols, disulphide, total thiols and the ratios of these parameters were analysed, the findings were similar among three patient groups ($p > 0.05$, Table I).

We also evaluated the correlations of demographic features or laboratory findings of patients with thiol/disulphide homeostasis parameters (Table II). All of the parameters other than age and CRP levels did not correlate significantly with thiol/disulphide parameters. Correlation analysis showed a significant negative correlation of age with native thiol and total thiol measurements (Table II, $p < 0.001$). Also, native thiol and total thiol levels were significantly and negatively correlated with CRP levels (Table II, $p < 0.001$). A number of cigarettes (ppy), WBC, FEV_1, FEV_1/FVC, FVC measurements did not show any significant correlation with any of thiol/disulphide homeostasis parameters ($p > 0.05$).

Since disulphide level is an important marker of oxidation in tissues, we analysed this parameter further in patients with different smoking status. As presented in Figure 1, disulphide levels were statistically similar in patients with different smoking status, i.e. smokers, non-smokers, ex-smokers ($p = 0.537$). When the association between disulphide levels and CRP, which is an important marker for inflammation was analysed, the correlation between these 2 parameters did not yield a significant association ($r^2 = 0.0001$, $p = 0.909$, Figure 2).

When measurements were adjusted for age and number of cigarettes by using regression analysis, similarity for thiol/disulphide homeostasis parameters among three patient groups persisted (Table III and IV).

Table II. The Pearson's correlation coefficient between demographic features/laboratory findings of the patients and thiol/disulphide homeostasis parameters.

	Native thiol	Total disulphide	Disulphide/ thiol	Disulphide/ native thiol	Native thiol/ total thiol	Total thiol
Age (years)	-0.389	-0.005	-0.387	0.153	0.145	-0.095
	(0.001)*	(0.952)	(0.001)*		(0.094)	(0.094)
Number of cigarettes (ppy)	-0.095)	0.101	-0.072	0.111	0.112	0.112
	(0.271	(0.244)	(0.409)	(0.198)	(0.194)	(0.194)
WBC (K/μL)	-0.128	-0.028	-0.134	0.013	0.014	-0.014
	(0.138)	(0.749)	(0.122)	(0.883)	(0.874)	(0.874)
CRP (mg/L)	-0.195	0.010	-0.191	0.065	0.065	-0.065
	(0.024)*	(0.909)	(0.027)*	(0.457)	(0.457)	(0.457)
FEV_1 (%)	0.156	-0.081	0.137	-0.143	-0.140	0.140
	(0.070)	(0.349)	(0.114)	(0.099)	(0.105)	(0.105)
FVC (%)	0.141	0.024	0.145	-0.056	-0.046	0.046
	(0.102)	(0.784)	(0.092)	(0.516)	(0.597)	(0.597)
FEV1/FVC	0.168	-0.084	0.147	-0.142	-0.142	0.142
	(0.052)	(0.332)	(0.089)	(0.101)	(0.100)	(0.100)
FEF 25-75 (L/s)	0.178	-0.052	0.165	-0.012	-0.116	0.116
	(0.038) *	(0.548)	(0.056)	(0.166)	(0.179)	(0.179)

Note: The numbers in the parenthesis are *p*-values. * denotes for statistical significance.

Discussion

In this study, we demonstrated for the first time in the literature that thiol/disulphide homeostasis parameters were similar among patient groups with three pulmonary obstructive disorders; namely COPD, asthma and ACOS. In recent years, Dutch hypothesis has proposed that COPD and asthma have common origin, share similar physiopathology and clinical expressions are determined by endogenous factors, such as heredity, age and gender or exogenous factors, such as allergens, smoking, viruses, and air pollution[11,13-17]. Our results may support this view of Dutch hypothesis in that COPD, asthma and ACOS patients showed similar biochemical features regarding levels of thiol/disulphide homeostasis parameters. Another view, as called British hypothesis, proposes that these disorders are totally different clinical entities with distinct biochemical and clinical features. To the best of

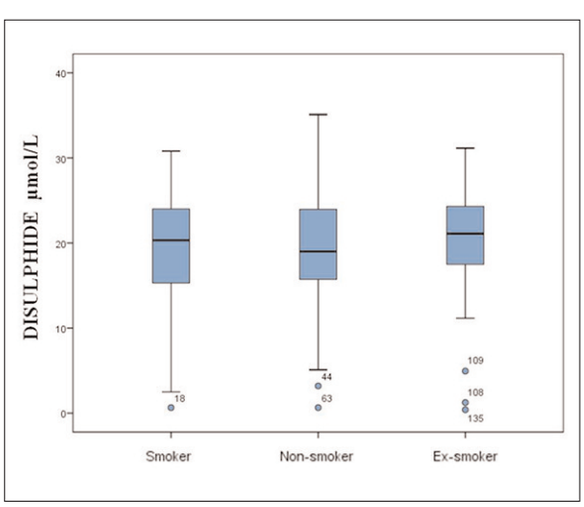

Figure 1. The distribution of disulphide levels (μmol/L) in patients with their smoking status ($p = 0.537$).

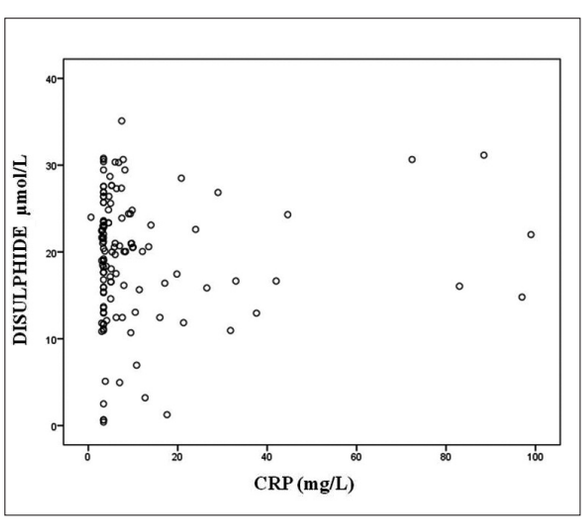

Figure 2. The relationship between CRP (mg/L) and disulphide (μmol/L) levels ($r^2 = 0.0001$, $p = 0.909$). Two outliers with CRP > 300 mg/L are not shown here for a clearer presentation.

Table III. Multiple linear regression results of native thiol (μmol/L) with independent predictors.

		95% CI		
	β ± SE	Lower	Upper	*p*
Constant	532.542 ± 18.919	495.113	569.971	0.000
Age (years)	-1.730 ± 0.359	-2.441	-1.019	0.000
Number of cigarettes (ppy)	-0.132 ± 0.268	-0.662	0.398	0.624
COPD *vs*. asthma	16.067 ± 14.326	-12.276	44.410	0.264
ACOS *vs*. asthma	6.735 ± 12.334	-17.666	31.136	0.586

n = 135 R^2 = 0.160 F = 6.178.

our knowledge, this is the first study to compare thiol/disulphide homeostasis parameters in COPD, asthma and ACOS patient groups.

Determination of dynamic thiol/disulphide status in diseases where oxidative stress plays a major role in pathogenesis would be important[12]. Thiol is reducible and interventions that may increase thiol levels may prevent pathological processes resulting from distortions in thiol/disulphide homeostasis. In this study, comprehensive measurement of dynamic thiol/disulphide parameters was performed by using a fully automated method recently developed by Erel and Neselioglu[12]. Before this new measurement technique, it was possible to measure the concentrations of thiol and disulphide only in low molecular weight compounds such as cysteine, reduced glutathione (GSH) and oxidized glutathione (GSSG). However, a small portion of the thiol pool of the body is composed of low molecular weight thiols (cysteine, cysteinylglycine, GSH, homocysteine and g-glutamylcysteine); thiols of albumin and other proteins generally make up the larger portion[18]. Therefore, thiol and disulphide concentrations measured by older methods may not reflect the exact thiol/disulphide status of the body.

Oxidative stress plays an important role in the pathogenesis of COPD[19]. COPD was associated with lower total thiol levels in comparison with healthy individuals[20]. Oxidative stress in COPD patients plays an important role in the progression of disease severity[21]. Oxidative stress is also an important factor in asthma. Plasma total thiol levels were found reduced in asthmatic patients. It was shown that, treatment with montelukast was able to limit oxidative stress and restored plasma total thiol levels[22]. There has been no report about thiol levels in ACOS patients, so far. When we evaluated thiol/disulphide homeostasis parameters among three patient groups, all parameters were found similar among the groups (Table I). This result may have been resulted from selection of patients with moderate levels of obstruction ($50 < FEV_1\% < 80$) according to their PFTs. We selected only patients with moderate obstruction to be able to compare a homogenous group. In Table I, the differences among three patient groups for mean age and an average number of cigarettes were statistically significant. Also, age had a significant correlation with thiol/disulphide homeostasis parameters. When the biochemical measurements were adjusted for age and number of cigarettes by us-

Table IV. Multiple linear regression results of native thiol (μmol/L) with independent predictors.

		95% CI		
	β ± SE	Lower	Upper	*p*
Constant	3.544 ± 0.581	2.396	4.693	0.000
Age (years)	0.013 ± 0.011	-0.009	0.034	0.258
Number of cigarettes (ppy)	0.001 ± 0.008	-0.015	0.017	0.894
COPD *vs*. Asthma	0.586 ± 0.440	-0.284	1.455	0.185
ACOS *vs*. Asthma	0.225 ± 0.379	-0.524	0.974	0.553

n = 135 R^2 = 0.160 F = 6.178.

ing regression analysis, similarity for thiol/disulphide homeostasis parameters among three patient groups persisted (Tables III-IV).

Smokers or patients with infections were included in the study. When we investigated the relationship between smoking status and disulphide levels, we found that there were no significant differences among patient groups of smokers, non-smokers or ex-smokers (Figure 1). Since CRP is a good marker of inflammation and infection, we sought an association with CRP levels and disulphide levels[23]. The correlation between CRP and disulphide levels was non-significant (Figure 2). Similarly, there was no statistically significant difference in WBC and CRP levels among patient groups with COPD, asthma or ACOS (Table I).

Conclusions

We report that biochemical parameters related with thiol/disulphide homeostasis were found similar in COPD, asthma and ACOS patient groups with moderate levels of obstruction. Further studies that would include milder or more severe levels of obstruction may provide a clearer picture for thiol/disulphide homeostasis in obstructive pulmonary disorders.

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Ethics Approval

The study was approved by the Local Ethics Committee of Yildirim Beyazid University (14.1.2015/21).

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Slats A, Taube C. Asthma and chronic obstructive pulmonary disease overlap: asthmatic chronic obstructive pulmonary disease or chronic obstructive asthma? Ther Adv Respir Dis 2016; 10: 57-71.
2) Bateman ED, Reddel HK, van Zyl-Smit RN, Agusti A. The asthma-COPD overlap syndrome: towards a revised taxonomy of chronic airways diseases? Lancet Respir Med 2015; 3: 719-728.
3) Miller MR, Levy ML. Chronic obstructive pulmonary disease: missed diagnosis versus misdiagnosis. Brit Med J 2015; 351: h3021.
4) Ding B, Enstone A. Asthma and chronic obstructive pulmonary disease overlap syndrome (ACOS): structured literature review and physician insights. Expert Rev Respir Med 2016; 10: 363-371.
5) Rahman I. Oxidative stress in pathogenesis of chronic obstructive pulmonary disease: cellular and molecular mechanisms. Cell Biochem Biophys 2005; 43: 167-188.
6) Rahman I, MacNee W. Role of transcription factors in inflammatory lung diseases. Thorax 1998; 53: 601-612.
7) Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. Am J Respir Crit Care Med 1996; 154: 1055-1060.
8) Kips J. Asthma: Epidemiology, Anti-Inflammatory Therapy And Future Trends. Acta Clin Belg 2001; 56: 70-71.
9) Cremers CM, Jakob U. Oxidant sensing by reversible disuphide bond formation. J Biol Chem 2013; 288: 26489-26496.
10) Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol 2006; 71: 551-564.
11) Diagnosis of Diseases of Chronic Airflow Limitation: Asthma, COPD and Asthma-COPD Overlap Syndrome (ACOS), 2015. Avaliable at: http://www.goldcopd.org/asthma-copd-overlap.html
12) Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014; 47: 326-332.
13) Postma DS, Weiss ST, van den Berge M, Kerstjens HA, Koppelman GH. Revisiting the Dutch hypothesis. J Allergy Clin Immunol 2015; 136: 521-529.
14) Smolonska J, Koppelman GH, Wijmenga C, Vonk JM, Zanen P, Bruinenberg M, Curjuric I, Imboden M, Thun GA, Franke L, Probst-Hensch NM, Nurnberg P, Riemersma RA, van Schayck CP, Loth DW, Brusselle GG, Stricker BH, Hofman A, Uitterlinden AG, Lahousse L, London SJ, Loehr LR, Manichaikul A, Barr RG, Donohue KM, Rich SS, Pare P, Bosse Y, Hao K, van den Berge M, Groen HJ, Lammers JW, Mali W, Boezen HM, Postma DS. Common genes underlying asthma and COPD? Genome-wide analysis on the Dutch hypothesis. Eur Respir J 2014; 44: 860-872.
15) Soler X, Ramsdell JW. Are Asthma and COPD a Continuum of the Same Disease? J Allergy Clin Immunol Pract 2015; 3: 489-495.
16) Weiss ST. The Dutch hypothesis meets genomics. Am J Respir Crit Care Med 2015; 191: 722-723.
17) Papaiwannou A, Zarogoulidis P, Porpodis K, Spyratos D, Kioumis I, Pitsiou G, Pataka A, Tsakiridis K, Arikas S, Mpakas A, Tsiouda T, Katsikogiannis N, Kou-

GIOUMTZI I, MACHAIRIOTIS N, SIMINELAKIS S, KOLETTAS A, KESSIS G, BELEVESLIS T, ZAROGOULIDIS K. Asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS): current literature review. J Thorac Dis 2014; 6 Suppl 1: 146-151.

18) TURELL L, RADI R, ALVAREZ B. The thiol pool in human plasma: the central contribution of albumin to redox processes. Free Radic Biol Med 2013; 65: 244-253.

19) ANTUS B, HARNASI G, DROZDOVSZKY O AND BARTA I. Monitoring oxidative stress during chronic obstructive pulmonary disease exacerbations using malondialdehyde. Respirology 2014; 19: 74-79.

20) ERDEN ES, MOTOR S, USTUN I, DEMIRKOSE M, YUKSEL R, OKUR R, OKTAR S, YAKAR Y, SUNGUR S, GOKCE C. Investigation of Bisphenol A as an endocrine disruptor, total thiol, malondialdehyde, and C-reactive protein levels in chronic obstructive pulmonary disease. Eur Rev Med Pharmacol Sci 2014; 18: 3477-3483.

21) AHMAD A, SHAMEEM M, HUSAIN Q. Altered oxidant-antioxidant levels in the disease prognosis of chronic obstructive pulmonary disease. Int J Tuberc Lung Dis 2013; 17: 1104-1109.

22) DILEK F, OZKAYA E, KOCYIGIT A, YAZICI M, GULER EM, DUNDAROZ MR. Plasma total thiol pool in children with asthma: Modulation during montelukast monotherapy. Int J Immunopathol Pharmacol 2016; 29: 84-89.

23) ASSASI N, BLACKHOUSE G, CAMPBELL K, HOPKINS RB, LEVINE M, RICHTER T, BUDDEN A. CADTH Health Technology Assessments Comparative Value of Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) Testing in Combination Versus Individually for the Diagnosis of Undifferentiated Patients With Suspected Inflammatory Disease or Serious Infection: A Systematic Review and Economic Analysis Ottawa (ON): Canadian Agency for Drugs and Technologies in Health Copyright (c) CADTH 2015.