J Surg Med. 2020;4(12):1104-1107.
DOI: 10.28982/josam.820913

Research article
Araştırma makalesi

Journal of Surgery and Medicine
e-ISSN=2602-2079

Can ischemia modified albumin (IMA) and total sulfhydryl level (TSH) be used as a biomarker in the diagnosis of bladder tumor? A prospective case-control study

İskemi modifiye albümin (İMA) ve toplam sülfhidril seviyesi (TSH) mesane tümörü tanısında biyobelirteç olarak kullanılabilir mi? İleriye dönük vaka kontrol çalışması

Recep Eryılmaz [1], Canan Demir [2], Rahmi Aslan [1], Halit Demir [3], Kerem Taken [1]

[1] Department of Urology, Yuzuncu Yil University, School of Medicine, Van, Turkey
[2] Van Yuzuncu Yıl University, Vocational School of Health Services, Tuşba-Van, Turkey
[3] Van Yuzuncu Yıl University, Department of Biochemistry, Tuşba-Van, Turkey

ORCID ID of the author(s)
RE: 0000-0002-4506-8784
CD: 0000-0002-4204-9756
RA: 0000-0002-4563-0386
HD: 0000-0002-2746-7941
KT: 0000-0002-4370-4222

Corresponding author / Sorumlu yazar:
Recep Eryılmaz
Address / Adres: Yüzüncü Yıl Üniversitesi, Tıp Fakültesi, Üroloji Kliniği, Van, Türkiye
E-mail: recepuro@hotmail.com

Ethics Committee Approval: The approval for the research was received from the interventional ethics committee of Van Yuzuncu Yıl University on 3/4/2020 with the decision number 2020/09. All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.
Etik Kurul Onayı: Van Yüzüncü Yıl Üniversitesi müdahale etik kurulundan 04.03.2020 tarih ve 2020/09 sayılı kararla araştırma onayı alındı. İnsan katılımcıların katıldığı çalışmalardaki tüm prosedürler, 1964 Helsinki Deklarasyonu ve daha sonra yapılan değişiklikler uyarınca gerçekleştirilmiştir.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Published: 12/21/2020
Yayın Tarihi: 21.12.2020

Abstract

Aim: Bladder tumor is one of the most common cancers. Cystoscopy, which is an invasive procedure, is used in its diagnosis. We conducted a study to determine whether a more non-invasive method can be used for this purpose. In this study, the uses of Ischemia Modified Albumin (IMA) and total sulfhydryl level (TSH), which are both antioxidant markers, were investigated for the diagnosis of bladder tumor.

Methods: Ischemia Modified Albumin (IMA) and total sulfhydryl level (TSH) were identified by the spectrophotometric method. Patients with primary bladder tumors who did not receive any prior treatments or undergo any interventions were included in this prospective case control study. Those with severe cardiac and neurological diseases, other malignancies, acute and chronic infectious diseases, active organ failure, chronic obstructive pulmonary diseases, and other ischemic immunosuppressive diseases, along with individuals with severely low or high serum albumin levels (<20 or >55 g/L) were excluded from the study.

Results: Forty-two primary bladder tumors and 45 healthy volunteers were included in the study. Serum IMA and TSH levels of the patient and control groups were compared. Patients with bladder tumors had high serum IMA (P=0.045) levels and low TSH levels (P=0.033).

Conclusion: Both IMA and total TSH can be considered non-invasive biomarkers in the diagnosis of bladder tumor. Since there are few studies on this subject in the literature, further, larger studies are needed.

Keywords: Bladder tumor, IMA, Total TSH

Öz

Amaç: Mesane tümörü en yaygın görülen kanserlerden biridir.Tanısında sistoskopi gibi invazif bir yöntem kullanılmaktadır.Bu çalışmada, mesane tümörü tanısında kulanılmak üzere daha non invaziv bir yöntem belirlemek için bir oksidan belirteç olan İskemi Modifiye Albümin (İMA) ve yeni bir antioksidan belirteç olan total sülfhidril (TSH) düzeyinin mesane tümörlü hastalarda bir biyobelirteç olup olamayacağını araştırmayı amaçladık.

Yöntemler: İskemik Modifiye Albümin (İMA) ve total sülfhidril seviyesi (TSH) spektrofotometrik yöntemle belirlendi. Çalışmaya, primer mesane tümörü olan ve daha önce herhangi bir tedavi almayan aynı zamanda herhangi bir cerrahi müdahalesi olmayan hastalar dahil edilirken, ağır kalp ve nörolojik hastalıkları, diğer maligniteleri, akut ve kronik enfeksiyon hastalıkları, aktif organ yetmezliği, kronik obstrüktif akciğer hastalığı olan ve diğer iskemik immünsüpresif hastalıkları olan hastalar çalışma dışı bırakıldı. Ciddi derecede düşük veya yüksek serum albumin seviyeleri (<20 veya >55 g/L) olan kişiler de çalışmadan çıkarıldı.

Bulgular: 42 primer mesane tümörü ve 45 sağlıklı gönüllü çalışmaya dahil edildi. Hasta ve gönüllü gruplarının serum İMA ve total TSH düzeyleri karşılaştırıldı. Kontrol grubuna göre mesane tümörü olan hastaların serum İMA (P=0,045) düzeyleri yüksek ve total TSH düzeyleri düşük (P=0,033) izlendi.

Sonuç: Hem İMA hemde Total TSH düzeyi, mesane tümörü tanısında daha noninvaziv bir biyobelirteç olarak düşünülebilir. Literatürde bu konuda çok fazla çalışma bulunmadığından daha geniş çaplı çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Mesane tümörü, İMA, Total TSH

How to cite/Atıf için: Eryılmaz R, Demir C, Aslan R, Demir H, Taken K. Can ischemia modified albumin (IMA) and total sulfhydryl level (TSH) be used as a biomarker in the diagnosis of bladder tumor? A prospective case-control study. J Surg Med. 2020;4(12):1104-1107.

Introduction

Bladder tumor is the most common type of cancer among individuals above the age of 65 years; and it constitutes 7% of all cancers. Its etiology includes tobacco dependence, schistosomiasis, eating and drinking habits and lifestyle changes. Lifestyle, eating and drinking habits are particularly effective on the oxidant-antioxidant balance [1]. Recently, research on the role of free radicals in cancer development and the protective effects of antioxidants has increased. Oxidative stress (OS) occurs during cell proliferation due to both hydrogen peroxide and superoxide [2].

Ischemia Modified Albumin (IMA), a systemic marker of oxidative stress, and total sulfhydryl, an antioxidant marker, are among the new biochemical markers [3,4].

Albumin is a protein consisting of 585 aminoacids. The last amino acid terminal in the albumin structure is capable of binding heavy transition metals (nickel, cobalt) [5]. It reversibly binds the drugs in blood, bilirubin, hormone, fatty acids, cations (Ca^{+2}, Na^{+2} and K^{+}) and other ligands [6]. Amino end (N terminal) of albumin molecule is the primary binding site of transitive metal ions, especially the aspartyl-alanyl-histidyl-lysine amino acid sequence, which binds cobalt (Co^{+2}), nickel (Ni^{+2}), copper (Cu^{+2}) [7]. Free radical damage and disruption of cell membrane integrity cause the formation of damaged albumin by reducing the binding of these heavy metals to the N-terminal of albumin. This modified form of albumin is called Ischemia Modified Albumin (IMA) and measured spectrophotometrically by albumin cobalt binding test [8]. It was examined in different diseases such as pulmonary embolism, cancer, paralysis, and particularly, ischemic heart diseases, all of which yielded IMA levels above the normal range [9,10]. Plasma proteins are highly sensitive to oxidation due to the free sulfhydryl groups in serum albumin structure. Therefore, it is stated that measuring sulfhydryl groups bound to proteins is a significant indicator to identify oxidative stress [11,12].

We herein investigated the use of IMA level, which is an oxidant marker, and total sulfhydryl level, which is a new antioxidant, as biomarkers in diagnosing bladder tumors in patients.

Materials and methods

This prospective study includes 42 patients with primary bladder cancer, who were diagnosed in our urology clinic in a tertiary university hospital between 04/03/2020-10/10/20 and 45 healthy individuals. No sample selection was made from the universe. All patients enrolled in the study had primary bladder tumors which were diagnosed during cystoscopy and none had received any previous treatments or interventions. Patients with severe cardiac and neurological diseases, other malignancies, acute and chronic infectious diseases, active organ failure, chronic obstructive pulmonary diseases, other ischemic immunosuppressive diseases, and severely low or high serum albumin levels (<20 or >55 g/L) were excluded from the study. Since the formation of ischemia modified albumin is related to disruption of the cell membrane and normal albumin in serum, too high or low albumin levels would affect its levels.

The control group comprised completely healthy volunteers who did not have any disease or history of drug use and had not undergone any surgeries.

Demographic, clinical, and biochemical data of both groups were recorded and analyzed comparatively. Written consents were obtained from patients and volunteers.

The approval for this research was received from the interventional ethics committee of Van Yuzuncu Yıl University on 04/03/2020 with the decision no 2020/09.

Sampling and analysis

Blood samples were drawn from the antecubital vein into serum biochemistry tubes and centrifuged at 5000 rpm for 10 minutes. The obtained serum samples were stored at -80 ° C until biochemical analysis.

IMA measurement

Serum IMA level was measured by colorimetric analysis developed by Bar-Or. To measure IMA, 50 ml of cobalt chloride was added to 200 ul of serum, shaken slightly, and the mixture solution was incubated for 10 minutes to ensure proper binding of cobalt to albumin. Then, coloring substance 50 uL of 1.5 mg/ ml dithiothreithol (DTT) (Sigma-Germany) was added and binding reaction was stopped by adding 1.0 ml% 0.9 NaCl after 2 minutes. A colorimetric control was prepared for each sample. 50 uL distilled water was used instead of 50 uL 1.5 mg/ml DTT for control samples. The obtained color complex was measured by spectrophotometric method at 470 nm. The results were reported as absorbance unit (ABSU).

Total sulfhydryl groups (SH) were measured by the methods described initially by Elmman (Ellman, 1959) and total sulfhydryl level was identified with the method developed by Hu (Hu, 1984). Here, thiols react with 5, 5'-dithiobis- (2-nitrobenzoic acid) (DTNB). It then gives the maximum peak at 412nm. 25uL of serum was added to 1 mL of Tris-EDTA buffer (0.25 mmol / L Tris base, 20 mmol / L EDTA, pH 8.2) and absorbance was read at 412 nm (A1). After that, a 25L aliquot of DTNB stock solution (10mmol/L in absolute methanol) was added to the primary solution. After 15 minutes at ambient temperature, the absorbance was read again (A2) together with a DTNB blank (B). The concentration of sulfhydryl groups was calculated by using reduced glutathione as sulfhydryl group standard and the result was expressed in mmol/L.

Statistical analysis

The descriptive statistics for the features mentioned are Mean and Standard Deviation. Kolmogorov-Smirnov test was used to verify that continuous variables were normally distributed. Normally and non-normally distributed binary groups were compared with the T-test and Mann Whitney U test, respectively. ROC curve analysis was performed to evaluate the performance of the biomarkers in distinguishing the patient group from the control group. The statistical significance level was 5%. SPSS statistical packaged software was used for all calculations.

Results

The mean age of the patients and the control group were 63.2 (3.22) years and 60.66 (4.15) years, respectively. There were 26 males (62%) in the patient group and 30 males (67%) in the control group. The mean tumor size was 3.27 (1, 35) cm.

None of our patients had muscle invasion and all were papillary urothelial tumors. The size of the tumor was larger than 3 cm in 24 patients while it was smaller in 18. Eighteen patients had low-grade and 24 patients had high-grade bladder tumors. The demographic characteristics of the patient and control groups were similar (Table 1). The diagnostic features of the patients are presented in Table 2.

Table 1: Demographic characteristics of the patient and control groups

Variable	Bladder cancer(n=42)	Control group(n=45)
Mean Age/year	63.2 (3.22)	60.66 (4.15)
Male	26	30
Female	16	15
Body Mass Index (BMI) kg/m^2	25.32 (6.45)	26.55 (4.63)
Smoker	30	33

Table 2: The diagnostic properties of patients

Variable	Bladder cancer(n=42)
Tumor size	
<3cm	18
>3cm	24
Grade	
Low grade	18
High grade	24
Pathology	
Papillary urothelial tumor	34
Carcinoma in situ	8
Other pathologies	0
Detrusor invasion -	42
Detrusor invasion +	0
TNM Stage	
Ta	24
Tis	8
T1	10

The descriptive statistics and comparison results of both IMA and TSH levels are shown in Table 3. The differences between the means of patient and control groups in terms of both IMA and TSH levels were statistically significant ($p<0.05$). Serum IMA levels were higher and serum TSH levels were lower in patients with tumors larger than 3 cm compared to those with smaller tumors. Likewise, patients with high-grade tumors had higher serum IMA levels and lower serum TSH levels compared to those with low-grade tumors ($p<0.05$ for both). While the mean IMA level of the patient group was significantly higher than that of the control group (Figure 1), the mean TSH of the patient group was significantly lower (Figure 2).

Table 3: Descriptive statistics and comparison results

	Group	n	Mean (SD)	P-value
IMA (Absorbance unit)	Control	45	0.962 (0.090)	<0.05
	Patient	42	3.1976 (0.977)	
TSH (nmol/mg protein)	Control	45	0.203 (0.065)	<0.05
	Patient	42	0.031 (0.022)	
IMA	>3cm	24	4.0187 (0.06033)	<0.05
	<3cm	18	2.1483 (0.40493)	
IMA	High Grade	24	4.0058 (0.08632)	<0.05
	Low Grade	18	2.0565 (0.11324)	
TSH	>3cm	24	0.0142 (0.00504)	<0.05
	<3cm	18	0.0535 (0.01367)	
TSH	High Grade	24	0.0142 (0.00504)	<0.05
	Low Grade	18	0.0535 (0.01367)	

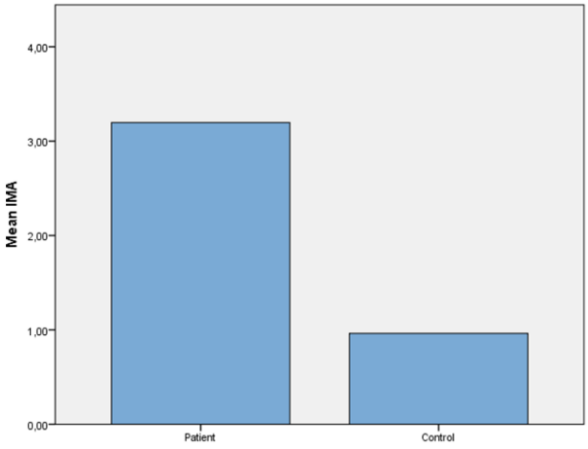

Figure 1: Serum IMA levels in patients and controls

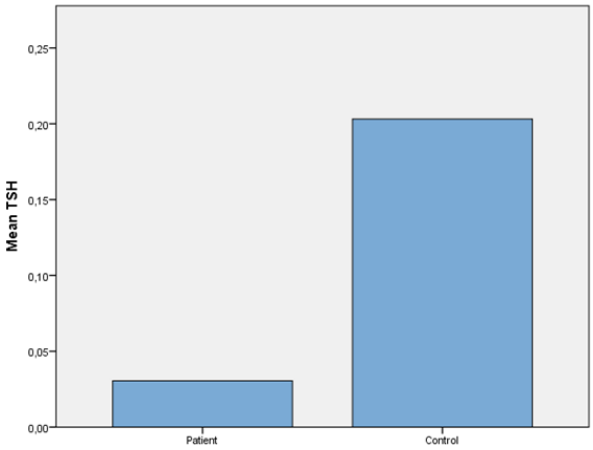

Figure 2: Serum TSH levels in patients and controls

Discussion

Oxidative stress may play a role in the development of many cancers. Discussions continue about whether oxidative stress is a factor in tumor progression or cancer pathophysiology. Some results show that ROS is a mutagenic factor causing DNA damage, suppressing apoptosis, and triggering proliferation, invasion, and metastasis [13].

Oxidative stress causes cancer development by stimulating tissue protein denaturation, DNA damage, lipid peroxidation and altering normal metabolic activity [9]. Reactive oxygen species (ROS) forming oxidative stress cause cancer by stimulating DNA damage and genetic mutation. Excessive ROS production leads to formation of ischemia modified albumin (IMA) by modifying serum albumin with oxidation [10].

In a study regarding the correlation between antioxidants and bladder cancer performed by Islam et al. [14], there was a decrease in the activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione and paraoxonase) in the tissues of patients with bladder cancer. As a result, imbalance occurs between oxidizers and antioxidants. It shows that the imbalance has a potential role in etiology and progress of bladder cancer.

How oxidant-antioxidant balance plays a role in cancer pathogenesis has been mentioned in detail in the studies above. Therefore, we think that IMA and serum total sulfhydryl levels, both of which are antioxidant markers, can be used as biomarkers to diagnose bladder tumors. In this study, there is a statistical significance in the parameters of both patient and control groups when serum IMA and total sulfhydryl level were compared. No previous study has been conducted regarding serum total sulfhydryl level in patients with bladder tumor. In this sense, our study is a first.

There are many studies revealing the correlation between serum IMA level and cancer. In a study by Qing-Xing Huangai et al. [15], serum IMA levels strongly correlated with serum albumin levels in patients with advanced gastric cancer. Serum IMA level in patients with pre-operative advanced gastric cancer was indicated as an independent prognostic factor for operation.

Erkut et al. [16] identified that IMA could be used as a hypoxic indicator in acute leukemias.

In another study, Da Silveria RA et al. [17] found that both inflammatory and oxidative processes increased in prostate

cancer, and accordingly, serum IMA level was high while antioxidant defense decreased.

In the study of Ellidag et al. [18], total antioxidant status (TAS) level was lower compared to the control group, and total antioxidant status (TOS) and IMA levels were higher. Also, serum albumin levels were significantly low in these patients.

Currently, there is no strong non-invasive test to diagnose bladder tumors early. Early diagnosis is significant for the patients at risk of bladder tumor. When bladder tumor is diagnosed with the current diagnostic techniques, more than 70% of the cases are non-muscle invasive and 30% have already invaded the muscle or are metastatic. The 5-year survival rate of early bladder tumor is 94%, while it is lower than 50% in muscle-invasive stage and lower than 20% if metastatic [19].

Studies have reported that serum IMA levels are high in diverse types of cancer and hypoxic diseases. These studies state that serum IMA level is directly related to cancer. It can be also used as a biomarker in some types of cancer and as a prognostic factor in others. In the studies of Wong et al. [19], the importance of early diagnosis of bladder tumor was indicated in detail on the survey.

In our study, serum IMA levels were significantly higher in the bladder tumor patient group compared to the controls, and especially higher among patients with a tumor size greater than 3 cm and high-grade tumors. According to these results, serum IMA level can be used as a biomarker in patients with bladder tumors. Although the specificity of serum IMA level is lower than cystoscopy in bladder tumor, it is a more non-invasive method.

Total sulfhydryl groups are a significant part of the antioxidant defense against free radicals [20]. Another target of free radical attacks is sulfhydryl groups bound to proteins. Compounds with sulfhydryl have a significant role in protecting cells against particularly reduced glutathione free radical damage [21]. Total sulfhydryl would be a protective biomarker against cancer as an antioxidant, which is why serum total sulfhydryl level was measured in patients with bladder tumors in this study. It was significantly lower in the overall patient group, in those with tumor sizes greater than 3 cm and high-grade tumors.

Limitations

The limitations of our study are the small number of our patients and the fact that IMA and total TSH levels were not checked in the follow-up of patients after primary surgery.

There is a need for large-scale studies which investigate the use of IMA and total TSH levels in patients with bladder tumors.

Conclusions

We determined that serum IMA level is high, and serum total sulfhydryl level is low in bladder tumors. Both parameters are non-invasive biomarkers in the diagnosis of bladder tumor. The results were more significant in high grade tumors and those larger than 3 cm in size. In addition, IMA and TSH may play key roles in the etiopathogenesis of bladder tumors. Therefore, the measurement of total sulfhydryl level, especially in bladder tumors, is a first in the literature. There is a need for further, comprehensive studies on the subject.

References

1. Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: a global overview and recent trends. European Urology. 2017;71(1):96–108.
2. Sawicka E, Kratz EM, Szymańska B, Guzik A, Wesołowski A, Kowal P, et al. Preliminary Study on Selected Markers of Oxidative Stress, Inflammation and Angiogenesis in Patients with Bladder Cancer. Pathol Oncol Res. 2019 Mar 4. doi: 10.1007/s12253-019-00620-5.
3. Awadallah SM, Atoum MF, Nimer NA. Ischemia modified albumin: an oxidative stress marker in beta-thalassemia major. Clin Chim Acta 2012; 413(9-10):907-10.
4. Uzar E, Tamam Y, Evliyaoglu O, Tuzcu A, Beyaz C, Acar A, et al. Serum prolidase activity and oxidative status in patients with diabetic neuropathy. Neurol Sci. 2012;33(4):875-80.
5. Chawla R, Goyal N, Calton R, Goyal S. Ischemia modified albumin: A novel marker for acute coronary syndrome. Indian J Clin Biochem. 2006;21:77-82.
6. Lee E, Eom JE, Jeon KH, Kim TH, Kim E, Jhon GJ, et al. Evaluation of albumin structural modifications through cobalt-albumin binding (CAB) assay. J Pharm Biomed Anal. 2014;91:17-23.
7. Sbarouni E, Georgiadou P, Voudris V. Ischemia modified albumin changes review and clinical implications. Clin Chem Lab Med 2011;49:177-84.
8. Zurawska-Płaksej E, Grzebyk E, Marciniak D, Szymańska-Chabowska A, Piwowar A. Oxidatively modified forms of albumin in patients with risk factors of metabolic syndrome. J Endocrinol Invest. 2014;37:819-27.
9. Sbarouni E, Georgiadou P, Voudris V. Ischemia modified albumin changes - review and clinical implications. Clin Chem Lab Med. 2011; 49(2):177-84.
10. Cakir M, Karahan SC, Mentese A, Sag E, Cobanoglu U, Polat TB, et al. Ischemia modified albumin levels in children with chronic liver disease. Gut Liver 2012;6(1):92-7.
11. Hu ML. Measurement of protein thiol groups and glutathione in plasma. Meîhod Enzymol. 233:380(S994).
12. Stockcr R, Frei B. Endogenous antioxidant defences İn human blood plasma,"Oxidative Stress: Oxidants and Antioxidants, editör: Sies H, Acedemic Press, London (1991):213.
13. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006;160:1–40.
14. Islam MO, Bacchetti T, Ferretti G. Alterations of Antioxidant Enzymes and Biomarkers of Nitro-oxidative Stress in Tissues of Bladder Cancer. Oxid Med Cell Longev. 2019 May 5;2019:2730896. doi: 10.1155/2019/2730896. eCollection 2019
15. Huang QX, Ma J, Wang YS. Significance of preoperative ischemia-modified albumin in operable and advanced gastric cancer. Cancer Biomark. 2018;22(3):477-85.
16. Erkut N, Mentese A, Ozbas HM, Sumer A, Orem A, Topbas M, et al. The indicator of hypoxia in acute leukemia: Ischemia-modified albumin. Cancer Biomark. 2015;15(5):559-65.
17. Da Silveira RA, Hermes CL, Almeida TC, Bochi GV, De Bona KS, Moretto MB. et al. Ischemia-modified albumin and inflammatory biomarkers in patients with prostate cancer Clin Lab. 2014;60(10):1703-8.
18. Ellidag HY, Eren E, Aydin O, Akgol E, Yalcinkaya S, Sezer C, et al. Ischemia modified albumin levels and oxidative stress in patients with bladder cancer Asian Pac J Cancer Prev. 2013;14(5):2759-63.
19. Wong R, Rosser CJ. UroSEEK gene panel for bladder cancer surveillance Transl Androl Urol. 2019 Dec;8(Suppl 5):S546-9.
20. Ryrfeldt A, Bannenberg G, Moldeus P. Free radicals and lung disease. British Med Bull. 1993;49(3):588-603.
21. Cotgreave IA, Johansson U, Moldeus P, Brattsand R. The effect of acute cigarette smoke inhalation on pulmonary and systemic cysteine and glutathione redox states in the rat. Toxicology. 1987;45:203-12.

This paper has been checked for language accuracy by JOSAM editors.
The National Library of Medicine (NLM) citation style guide has been used in this paper.