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**Relation of O₂-free radicals to some biochemical markers
in patients with lung disease**

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ABSTRACT

O₂-free radicals play a major role in the pathogenesis of many inflammatory conditions and malignancy. Also, sialic acid (Total & Lipid-Bound) were recorded to be disturbed in inflammation and malignancy. So, the aim of this work is to study the relationship between O₂-free radicals, lipid-bound sialic acid (LSA), total sialic acid (TSA) and carcino-embryonic anitgen (CEA) in patients with chronic bronchitis & bronchogenic carcinoma. Also, to evaluate LSA & TSA as Tumor markers in patients with bronchogenic carcinoma and assessed the individual and combined values of LSA, TSA, & CEA determination in these patients,

To clarify this issue, two groups of patients were studied. The first group included 20 patients with chronic bronchitis. Their age ranged from (44-55) years. The second group included another 20 patients with bronchogenic carcinoma. Their age ranged from (45-62). These groups of patients were compared with 10 healthy, age and sex matched as controls.

The results of this study showed that; patients with chronic bronchitis and bronchogenic carcinoma have a significant increase of serum lipid peroxides (LP), LSA and TSA (P<0.001) while serum CEA is significantly increased only in patients with bronchogenic carcinoma compared with the control group (P<0.001). Also, there was a significant positive correlation between serum LP and LSA (P<0.01), TSA (P<0.001) in patients with chronic bronchitis while, there was non-significant positive correlation with serum CEA. Also, there was a significant positive correlation between serum LP and LSA (P<0.001), TSA (P<0.001) and CEA (P<0.05) in patients with bronchogenic carcinoma. Serum LSA is more sensitive biochemical marker (80%) than TSA (75%) and serum CEA (60%) while serum CEA is more specific marker (73.3%) in

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diagnosis of patients with bronchogenic carcinoma than serum LSA

(46.7%) and TSA (33.3%).

We could conclude that, the increase of O₂- free radicals may be the trigger for the increase of serum level of cell surface related sialoglycoprotein, sialoglycolipids, and tumor antigen (CEA). Neither one of the three biomarkers success to be absolutely diagnostic nor pathognomonic for bronchogenic carcinoma but, the combined measurement of LSA and CEA in serum may be helpful for better detection potential of bronchogenic carcinoma than either of the two markers alone. So, we recommend to interpretate the results carefully in these group of patients to assure that inflammation or other benign conditions are not causing changes in the test values.

INTRODUCTION AND AIM OF WORK

Oxidant stress seems to be involved in the pathogenesis of a variety of important disorders such as inflammatory diseases, cancer, atherosclerotic coronary heart disease, asthma, aging, and neurodegenerative disorders⁽¹⁾.

In most circumstances, a question can still be asked, whether the oxidant stress precedes and therefore is involved in the pathogenesis of tissue injury or is a result of injury and not of clinical importance. The data favor the former situation in several inflammatory conditions⁽²⁾.

Chronic infection contribute to the carcinogenic process⁽³⁾ Also, chronic inflammation resulting from non-infectious sources contributes to various pathological conditions leading to cancer e.g. asbestos exposure is a risk factor for cancer lung⁽⁴⁾.

Sialic acid, a family of acylated derivatives of neuraminic acid, is widely distributed in mammals and usually occurs as a terminal component of the non-reducing end of carbohydrate chains of glycoproteins and glycolipids⁽⁵⁾.

Selected glycoproteins and glycolipids may prove to be tumor markers⁽⁶⁾, since cell surfaces and membrane components play a prominent role in neoplastic behaviour⁽⁷⁾.

Carci no-embryonic antigen (CEA) is a large family of related cell-surface glycoproteins. The CEA family consists of about 10 genes located on chromosome 19. Up to 36 different glycoproteins have

having benign conditions such as cirrhosis, pulmonary emphysema, rectal polyp, benign breast disease and ulcerative colitis.

Also, CEA level is elevated in a variety of cancers such as colorectal, lung, gastric, breast, pancreatic, ovarian, and uterine⁽⁸⁾.

Most clinical laboratories have found that CEA testing lacks the specificity and sensitivity necessary for diagnostic screening⁽⁹⁾.

So, the aim of this work is to study the relationship between O₂-free radical and LSA, TSA & CEA in patients with chronic bronchitis and bronchogenic carcinoma. Also, to evaluate serum LSA & TSA as tumor markers in patients with bronchogenic carcinoma and assessed the individual and combined value of serum LSA & TSA with CEA determination in these patients.

SUBJECTS AND METHODS

Two groups of patients were selected from chest departments, Benha University Hospital.

First group: This group included 20 patients with chronic bronchitis. Their age ranged from (44-58 years). They were 12 males who were moderately smokers and 8 females.

Second group: This group included 20 patients with bronchogenic carcinoma. Their age ranged from (45-62 years). They were 14 males who were moderately smokers and 6 females. They have cancer lung with regional lymph node metastasis.

These two groups of patients were compared with 10 healthy normal subjects, their age ranged from (40-60 years) and formed of 7 males and 3 females as controls

Exclusion criteria:

-Diabetic, hypertensive and obese patients were excluded from this study.

-Also, patients with other inflammatory condition or organ metastasis were excluded.

All subjects included in this study were subjected to the followings:

-History and clinical examination.

-Plain X-ray of chest PA and lateral views.

-Pulmonary function tests.

-Bronchoscopic examination.

-Biopsies from lung with those suspected to have lung cancer.

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-CT scan of chest and abdomen.

- Electromagnetic resonance for some selected cases.
- Electrocardiography (E.C.G.).
- Echocardiography.
- Sampling: venous blood samples were taken from all subjects. One part was left unclotted for determination of ESR and complete blood picture. The other part left to be clotted. The sera were separated for routine investigations (SGOT⁽¹⁰⁾, SGPT⁽¹⁰⁾, S. urea⁽¹¹⁾, S. creatinine⁽¹²⁾, random serum glucose⁽¹³⁾). The remaining part of the sera were kept frozen at -80°C for determination of:
 - 1- Serum malondialdehyde⁽¹⁴⁾
 - 2- Serum lipid-bound sialic acid⁽¹⁵⁾
 - 3- Serum total sialic acid⁽¹⁶⁾
 - 4- Serum carcino-embryonic antigen by ELISA method⁽¹⁷⁾

Determination of serum lipid-bound sialic acid acid⁽¹⁵⁾

The improved procedure is as follows:

- 1-To a screw cap culture tube, 13 x 100 mm, 150 μ l distilled water was added to 44.7 μ l of serum. The contents were vortexed for 5 seconds. The tube was transferred to crushed ice.
- 2-Three ml cold (4-5°C) 2:1 (v/v) chloroform: methanol were added to the tube and the mixture was vortexed for 30 seconds.
- 3-To this mixture was added 0.5 ml cold distilled water. The tube was capped and the contents were mixed by repeatedly inverting the tube for 30 sec.
- 4-After centrifugating the tube 5 min. at room temperature at 2500 rpm, 1 ml of the upper layer was transferred into a 13 x 100mm culture tube.
- 5-Fifty μ l phosphotungstic acid solution (1 g/ml) were added and after mixing, the tube stood at room temperature for 5 min.
- 6-The tube was centrifuged for 5 min at 2500 rpm, and the supernatant was removed by suction.
- 7-one ml water was added, and the tube was vortexed until the precipitate was in suspension without grossly visible particles (about 1 min.).
- 8-One ml of resorcinol reagent was added, and the tube was mixed and placed in boiling water for exactly 15 min. (N.B. Resorcinol reagent was prepared according to Svennerholm⁽¹⁸⁾).

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Into a 100 ml volumetric flask were added 10 ml of the resorcinol stock solution, *0.25 ml of 0.1 M CuSO₄, 9.75 ml H₂O and

concentrated HCl to 100 ml. The resorcinol stock solution was 2% resorcinol in water. Both the stock solution and resorcinol reagent were kept refrigerated in light protected container).

9-Immediately, after the 15 min., the tube was transferred to an ice and water bath and left for 10 min.

10-To the ice-cold tube, 2 ml 85:15 (v/v) butyl acetate: n-butyl alcohol were added at room temperature and the tube was vortexed and centrifuged for 5 min. at 2500rpm.

11-The extracted blue color was determined by the use of a standard curve developed from a standard sample of n-acetyl neuraminic acid (Sigma chemical Co.) using the formula.

$$\text{LSA(mg/100ml serum)} = \frac{(X)(100,000 \text{ ul})}{(y) (44.7/\text{ul})(1000)}$$

Where: X= mg n-acetyl neuraminic acid read from standard curve of the sample.

Y=1 ml of supernatant divided by volume of entire supernatant, (this has been 1.00/1.30)

RESULTS

Table (1) showed a significant increase of S. lipid peroxides (LP), LSA and TSA in patients with chronic bronchitis and bronchogenic carcinoma when compared with the control group (P<0.001).

Meanwhile, there was no statistically significant difference of S. CEA in patients with chronic bronchitis and control group. But, there was a significant increase of CEA in patients with bronchogenic carcinoma (P<0.001) when compared with the control group.

Table (2) showed a correlation coefficient (r) between serum LP with S.LSA, TSA and CEA in patients with chronic bronchitis and bronchogenic carcinoma. There was a significant positive correlation between S. LP and LSA (P<0.01) in patients with chronic bronchitis and in patients with bronchogenic carcinoma (P<0.001) and TSA with (P<0.001) in both groups. Also, serum LP is positively correlated with CEA in both group which is significant in patients

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with bronchogenic carcinoma (P<0.05) and non-significant in patients with chronic bronchitis.

Table (3) showed that serum LSA is more sensitive

biochemical marker for patients with bronchogenic carcinoma (80%) than serum TSA (75%) and serum CEA (60%). On the other hand, serum CEA is more specific marker in patients with bronchogenic carcinoma (73.3%) than serum LSA (46.7%) and serum TSA (33.3%).

Table (1): The mean + SD and P values of serum lipid peroxides (LP), lipid-bound sialic acid (LSA), total sialic acid (TSA) and carcino-embryonic antigen (CEA) in patients with chronic bronchitis and bronchogenic carcinoma compared with the control group.

Biochemical Markers Groups	LP nmol/ml/h	LSA (mg/dl)	TSA (mg/dl)	CEA (ng/ml)
Control group (n=10)	4.54 ± 0.90	16.47 + 1.66	36.82 + 4.30	3.28 + 0.85
Chronic bronchitis (n=20)	5.62 ± 0.96 P<0.01	26.31 ± 4.74 P<0.001	47.90 + 3.04 P<0.01	3.85 + 1.23 N.S.
Bronchogenic carcinoma (n=20)	13.05 ± 1.29 P<0.001	27.05 ± 4.09 P<0.001	59.37 ± 8.91 P<0.001	5.67 + 1.62 P<0.001

Table (2): Correlation coefficient (r) between serum LP and LSA, TSA, and CEA in patients with chronic bronchitis and bronchogenic carcinoma.

Variables	Chronic bronchitis			Bronchogenic carcinoma		
	S. LSA	S. TSA	S. CEA	S. LSA	S. TSA	S. CEA
S. LP						
r	0.633	0.737	0.094	0.886	0.832	0.493
P	0.01	<0.001	N.S.	0.001	0.001	0.05

N.S. : Non-significant (P>0.05).

Table (3): The sensitivity and specificity of serum LP and LSA, TSA, and CEA in patients with bronchogenic carcinoma.

Groups	Non-bronchogenic carcinoma (n= 30)			Bronchogenic carcinoma (n= 20)		
	LSA	TSA	CEA	LSA	TSA	CEA
Studied parameter						
* Positive	16	20	8	16	15	12
Negative	14	10	22	4	3	8
Sensitivity (%)				80%	75%	60%
Specificity (%)				46.7%	33.3%	73.3%

* Cases considered positive if it is more than the mean values plus double the standard deviation

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DISCUSSION

The results of this study showed a significant increase of S. lipid peroxides in patients with chronic bronchitis (PO.01) and bronchogenic carcinoma (P<0.001) when compared with the control group (Table 1).

These results were compatible with the results of Buhl et al⁽¹⁹⁾, who found that free radicals injury were strongly implicated in the genesis and maintenance of several human pulmonary diseases such as chronic bronchitis, pulmonary disease, asthma, emphysema, bronchiectasis, adult respiratory distress syndrome, drug induced lung lesions, interstitial fibrosis and chronic interstitial pneumonitis. Also, Takase⁽²⁰⁾, recorded that lipid peroxide concentration was significantly higher in patients with lung cancer.

An increase in alveolar oxidant burden, potentially depleting alveolar and lung glutathione, low glutathione has been linked to abnormalities in the lung surfactant system and the interaction between glutathione and antiproteases in the epithelial lining fluid of patients. In inflammatory disorders, oxidants and proteases complement each other in their potential to destroy lung parenchyma. The increase of intracellular reactive oxygen species is believed to correlate with the elaboration of several cytokines⁽²¹⁾.

Our findings showed a significant increase of S.LSA & TSA in patients with chronic bronchitis and bronchogenic carcinoma (P<0.001) compared with the control group (Table 1).

Serum total sialic acid was recorded to be increased in non-neoplastic disease or inflammatory disorders⁽²²⁾. Also, in extensive study of Dwevidi et al.⁽²⁰⁾, total sialic acid was found to be increased in many types of cancers, breast cancer, colon cancer, ovarian, cervix, pancreas, prostate, thyroid, uterine, oesophageal and endometrial carcinoma. Moreover, serum LSA was found to be significantly increased in patients with chronic non-malignant diseases and malignancies such as lung cancer, gastro-intestinal cancer, head and neck squameous carcinoma and breast carcinoma*⁴⁾.

From the previous data serum LSA and TSA were significantly increased in chronic inflammatory disease and many types of cancers which give the documentation of alteration in the metabolism of cell

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surface glycoproteins and sialoglycolipids.

Our results showed a significant increase of serum CEA in patients with bronchogenic carcinoma ($P < 0.001$) while, there was non-significant increase in patients with chronic bronchitis when compared with the control group (Table 1).

Iwahashi^(2,3), found that serum CEA was elevated in the patients of lung cancer. However, Erbil et al.⁽⁵⁾ found a significant increase of CEA concentration in colorectal cancer. Also, Salvagno et al.⁽²⁶⁾, analyzed 74 patients with a variety of solid tumors and hematological malignancies and found that 21 patients has elevated CEA.

CEA is one of the oncofetal antigen that produced during fetal life. It is present in high concentration in the sera of fetuses and decreases to low levels or disappears after birth. In cancer patients, CEA reappears. The production of CEA demonstrates that certain genes are reactivated as the result of the malignant transformations of the cells⁽⁸⁾.

Our results showed a significant positive correlation between O_2 - free radicals and LSA (PO.01) and TSA (PO.001) in patients with chronic bronchitis. Also, a significant positive correlation between O_2 -free radical and LSA, TSA ($P < 0.001$) and CEA ($P < 0.05$) was found in patients with bronchogenic carcinoma (Table 2).

Recent evidence suggests that O_2 - free radicals induce cell damage that activates molecular responses, which may act as a signal transduct messenger. This produces. Two transcription factors, nuclear factor (NF) kappa B and activator protein (AP-1). Transcription factor results into gene expression that induces induction of anti-oxidant enzymes, differentiation and proliferation, adaptation, cell death or DNA repair⁽²⁷⁾. Oxidants stimulate cell divisions⁽²⁸⁾

A critical factor in mutagenesis is cell division when the cell rise to a mutation and therefore cancer transformation. So, chronic infection, high levels of particular hormones⁽²⁹⁾ and chemicals at doses that cause cell death⁽³⁰⁾ result in increased cell division and therefore an increased risk for cancer. Neoplastic transformation are accompanied by alterations in the composition of cell glycoproteins which are the major structural components of cell surfaces⁽²³⁾.

Comparative study of our findings (Table 3) showed that serum

LSA is more sensitive biochemical* markers for patients with bronchogenic carcinoma (80%) than serum TSA (75%) and CEA (60%). On the other hand, serum CEA is more specific marker in these patients (73.3%) than serum LSA (46.7%) and TSA (33.3%) (Table 3).

These results were in agreement with the findings of Salvagno et al.⁽²⁶⁾, who reported that LSA is more sensitive (92%) than CEA (28.4%) in patients with lung cancer.

However, the cause of discrepancy between sensitivity and specificity of CEA from one side and sialic acid (total and lipid-bound) may be due to difference in origin of the three biomarkers. As CEA is an immunologic marker and sialic acid (total and lipid-bound) reflects serum sialoglycoproteins derived from many sources in addition to tumor cell surface⁽⁵⁾.

We could conclude that, the increase of O₂-free radicals may be the trigger for the increase of serum level of cell surface related sialoglycoprotein, sialoglycolipids, and tumor antigen (CEA). Neither one of the three biomarkers success to be absolutely diagnostic nor pathognomonic for bronchogenic carcinoma but, the combined measurement of LSA and CEA" in serum may be helpful for better detection potential of bronchogenic carcinoma than either of the two markers alone. So, we recommend to interpretate the results carefully in these group of patients to assure that inflammation or other benign conditions are not causing changes in the test values.

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