An immunohistochemical study of tumour necrosis factor related apoptosis inducing ligand (TRAIL) in lung cancer patients

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Received 17 January 2013; accepted 22 June 2013
Available online 13 July 2013

KEYWORDS
Lung cancer; Adenocarcinoma; Squamous cell carcinoma; TRAIL

Abstract Lung cancer is a global problem and its incidence is dramatically increasing and expected to become the leading cause of cancer deaths. Several molecular genetic abnormalities have been described in lung cancer, one of the most recent such markers is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL).

Objective: To throw an outlook on tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression in tissue specimens obtained from lung cancer patients.

Patients and methods: Fifty Patients were included in this work selected on basis of being presenting with clinical and/or radiological picture suggestive of lung cancer. They were classified histopathologically into two groups, Group I comprised (37) non small lung cancer patients (NSCLC), and Group II comprised (13) small lung cancer patients (SCLC). All patients were subjected to thorough medical history, clinical examination and radiological examination. Fiberoptic bronchoscopic examination was done with different endoscopic sampling and the obtained specimens were subjected to special staining procedure for TRAIL expression by immunohistochemistry.

Results: Bronchial biopsy carried the highest diagnostic yield for lung cancer in both groups (60%) and (24%) in groups I and II, respectively. Histopathologically, the majority of cases
(86.48%) of group I were adenocarcinoma, (13.5%) were squamous, while all cases of group II (100%) were SCLC. TRAIL expression was positive in 67.6% of group I and in 23% of group II cases. 62.5% of adenocarcinoma patients were TRAIL positive, as well as 40% of squamous cell carcinoma patients while 23.1% only of SCLC were TRAIL positive. There was no statistical significant difference in the degree of cigarette smoking between TRAIL positive and negative subjects. The sensitivity of TRAIL immunostaining in lung cancer was 67.6%, while its specificity was 76.9% with accuracy of 70% in the studied groups.

Conclusion: TRAIL is overexpressed in the majority of NSCLC mainly adenocarcinoma which indicate that it may become a new adjuvant line for treatment of such cases.

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Introduction

Lung cancer is the leading cause of cancer-related mortality in the world. NSCLCs represent 75–80% of all types of lung cancer, and include squamous cell carcinomas, adenocarcinomas, and large-cell carcinomas [1].

Non-small cell lung cancer now accounts for about three-quarters of all cases of lung cancer and most patients continue to die of progressive metastatic disease despite new therapeutic strategies and advances in surgical oncology. This failure of conventional therapy is due to metastases as well as resistance to chemotherapy and radiation. Attempts to overcome resistance through increased dosage results in unacceptable toxicity [2].

Current interest is focused on the so-called “death ligands,” including TNF, FasL, and TRAIL [3]. Death ligands can trigger apoptosis in tumor cell lines via their cognate cell-surface death receptors, TNFR1, Fas, and DR4/DR5, respectively [4,5].

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo-2L) is a typical member of the tumor necrosis factor family, inducing apoptosis by activating death receptors [6]. A number of studies have shown that both the membrane-bound and the soluble extracellular domain of TRAIL can induce apoptosis in a wide variety of tumor cell lines without affecting most normal cells [7,8].

TRAIL receptor targeting agents represent an anti-cancer strategy in which activity is mainly dependent on the ability to induce apoptosis in tumor cells. Different from other members of the TNF super family (TNF and FasL), recombinant human (rh) TRAIL induces apoptosis in various tumor cells but not in normal cells. Furthermore, it has been demonstrated that systemic administration of rhTRAIL in mice or non-human primates results in anti-tumoricidal activity without any toxic effects on normal tissue [8].

Aim of the work

The aim of this work is to throw an outlook on tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression in tissue specimens obtained from lung cancer patients.

Patients and methods

This study had been carried out at the Chest and Pathology Departments, Faculty of Medicine, Zagazig University during the period between October 2008 and July 2011. Fifty patients were included in this work, (38) males and (12) females. Their age range was (35–80 years) with a mean (55 ± 10 years). Patients were selected on basis of being presenting with clinical and/or radiological picture suggestive of lung cancer according to Spiro [9]. Patients with prior history of malignancies, severe liver disease, renal and cardiac disease were excluded from the study; as such critical medical illness may not withstand invasive procedures like bronchoscopy and needle biopsies. Also, patients with liver disease suffer from bleeding tendency during the procedures. Patients with unstable angina and life-threatening arrhythmias are not suitable for invasive techniques. Lastly the result of TRAIL immunostaining may vary in the presence of such comorbid conditions. After histopathological examination, they were classified into two groups, Group I comprised (37) NSCLC patients, (28 males) and (9 females) with mean age (57 ± 11.4 years) and Group II comprised (13) SCLC patients, (10males) and (3 females) with mean age (56 ± 9.2 years). All patients were subjected to the following:

(1) Thorough medical history including:
(a) Smoking history.
(b) History of occupational exposure to carcinogenic elements such as, nickel, asbestos, etc. [10].
(c) Chest and other symptoms suggestive of lung cancer [9]. Cough, expectoration, dyspnea, chest pain, chest wheezes, hemoptysis, dysphagia, hoarseness of voice, features of Horner’s syndrome, superior vena cava obstruction symptoms (facial fullness, swelling of the upper extremities), constitutional symptoms (fever, chills, anorexia, weakness, significant weight loss), extrathoracic symptoms due to metastases (headache, nausea, vomiting, seizures, back pain, abdominal pain, bone pain) and non metastatic paramalignant manifestations (flushing, diarrhea, tingling and numbness of hands or feet).

(2) Clinical examination including general and local chest examination.

(3) Routine laboratory investigations including: complete blood picture, coagulation profile, erythrocyte sedimentation rate, liver functions tests, blood urea, serum creatinine and fasting blood sugar testing.

(4) Radiological examination including:
(a) Plain chest radiography (PA and lateral views)
(b) Computed Chest Tomography (CT). Percutanous trans-thoracic fine needle aspiration biopsy (TTNA) was done under CT guidance for 13 patients in this study from suspected peripheral pulmonary lesions by using spinal needle gauge 22 for cell block according to Lima [11].

(5) Histopathological examination.

(a) Tissue processing:

(1) Tissue was fixed in formalin for 24 hours and embedded in paraffin blocks.
(2) Sections were cut in 4μm thickness and stained by Hematoxylin Eosin (H&E) for histopathological examination.
(3) Immunohistochemical staining of TRAIL receptor was done usingavidin-biotin-peroxidase complex method.

(b) Immunohistochemistry:

(1) Tumor tissue sections were placed on charged slides. The slides were dewaxed with xylene and were washed with alcohol.
(2) To perform immunohistochemical staining, the slides were microheated in citrate buffer for 30 minutes to perform antigen retrieval assay.
(3) After antigen retrieval, the slides were washed with water and were blocked with 3% hydrogen peroxide for 30 minutes.
(4) After blocking, the slides were washed with water and were incubated for 30 minutes with anti-human TRAIL antibody at 4°C.
(5) After incubation, the slides were washed with water and were incubated for 30 minutes with biotinylated anti-rabbit secondary antibody.
(6) After incubation, the slides were washed with water and were incubated for 30 minutes with streptavidin–horseradish peroxidase complex.
(7) After incubation, the slides were washed with water and were developed with 3, 3-diaminobenzidine tetrahydrochloride as chromogen.
(8) The slides were counterstained with hematoxylin.

(c) Scoring:

(1) Interpretation of TRAIL expression was done by two independent investigators trained in pathologic interpretation.
(2) TRAIL expression was scored as follows:

- Positive: more than 25% of the tumor cells showed staining.
- Negative: 25% or less of the tumor cells showed staining.

(d) Statistical analysis:

(1) Adobe Photoshop and Cut Out software were used for data analysis.
(2) Data were statistically analyzed by using student t test.
(3) A p value of less than 0.05 was considered statistically significant.

Conclusion: TRAIL is overexpressed in the majority of NSCLC mainly adenocarcinoma which indicate that it may become a new adjuvant line for treatment of such cases.
An immunohistochemical study of tumour necrosis

(c) Assessment of metastatic workup through CT scan of the upper abdomen, to exclude the liver and adrenals metastasis (done for 8 cases). CT scan of the brain required if neurological symptoms or signs are present (done for 5 cases). Magnetic resonance image (MRI) is useful when evaluating a patient with spinal cord compression (done for 3 cases). Bone scintigraphy was required if patients report bone pain or if their serum calcium and/or alkaline phosphatase levels are elevated (done for 15 cases). Staging of lung cancer is the most accurate means to estimate prognosis and guide treatment decisions. The characteristics of the primary tumor (T), regional lymph nodes (N), and metastatic involvement (M) are used to stage NSCLC according to the TNM system. Staging of Small-Cell Lung Cancer was done using the old Veterans Administration staging system of “limited” and “extensive” disease categories. Limited disease is defined as disease confined to one hemithorax and the ipsilateral supraclavicular nodes. Extensive stage disease is defined as disease that has spread beyond these confines. Contralateral hilar lymph nodes, cervical lymph nodes, or distant organ metastasis is considered extensive stage disease [11].

(5) Sputum cytology for detection of malignant cells. It was done for (47) cases, all of them were presenting with productive cough.

(6) Fiberoptic bronchoscopic [12] examination using Olympus BF type 10 was one for all cases and well informed consent were obtained from all patients. Different endoscopic sampling was done including: endobronchial biopsy, bronchial washing, bronchoalveolar lavage (BAL) and bronchial brushing.

(7) Post bronchoscopic sputum cytology, for detection of malignant cells [13]. It was done for 50 cases.

(8) Cytological and histopathological examination of all types of specimens and biopsies and routine paraffin blocks were done from each biopsy sample and two sections were obtained from each paraffin block 4µ thick and were subjected to:

(a) Routine hematoxyline and eosin stain to verify histopathologic type [14]

(b) Special staining procedure (Human TRAIL/TNF-FSF10 Antibody) using monoclonal antibodies Daco [15] for detection of TRAIL expression: tumors showing ≤10% positively stained cells were considered negative. Samples showing >10% positively stained tumor cells were considered positive. All of the stained tumor biopsies were evaluated semi quantitatively for intensity of staining: no staining (0), weakly positive staining (1), positive staining (2), or strong positive staining (3).

Statistical analysis

Data were analyzed using statistical package for social science (SPSS) version (Chicago, IL, USA) [16].

Results

This table shows that there was no statistical significant difference in the mean age of the 2 studied groups. There was male predominance in the studied groups, where males represent 75.7% and 76.9% in groups I and II, respectively, with no significant statistical difference. 10.8% of group I patients were exposed to the risk of carcinogens in their work place while no such risk was present in group II patients with no statistical significant. 86.5% of group I patients were smokers (10.8% of them were Ex and the other 75.68% were current smokers) and 76.9% of group II patients were smokers (23% were Ex and the other 53.84% were current smokers). Smoking distribution between the 2 groups was statistically significant (P < 0.05).

Fig. 1 shows that the major presenting symptoms of lung cancer were cough (78.4% in group I and 100% in group II patients), dyspnea (75.7% in group I and 100% in group II patients) and expectoration (70.3% in group I and 92% in group II patients), while hoarseness of voice (16.2% in group I and 30.8% in group II patients) and dysphagia (5.4% in group I only) were the least presenting symptoms.

Fig. 2 shows that the presence of hilar shadow (51.4% in group I and 62% in group II) and mass lesion (43.2% in group I and 38% in group II) were the predominant radiological pictures in both groups.

Fig. 3 shows that pulmonary involvement of lung cancer in CT scanning were present in (67.6 of group I and 69.2% of group II), mass lesions were present in (70.3% in group I and 92% of group II), and both hilar and medistinal lymph nodes involvement were found in (51.4% of group I and 61.5% of group II).

Fig. 4 shows that the major bronchoscopic findings in both groups were broad spur (54.6%,61.5% in group I and group II, respectively) and Mass lesion (48.6%,61.5% in group I and group II, respectively), followed by bronchial narrowing (40.5%,46% in group I and group II, respectively). Ballow Table 2 shows that bronchial biopsy carried the highest diagnostic yield for lung cancer (60% and 24% in groups I and II, respectively), followed by bronchoalveolar lavage

![Figure 1](image-url) Distribution of presenting symptoms among both groups.
(50% and 16% in groups I and II, respectively) and post bronchoscopic sputum (34% in group I and 16% in group II).

This table shows that all cases (37 cases) of group I were of non small cell lung cancer type (32 cases were of adenocarcinoma type, 5 were of squamous cell type) while all cases (13) of group II were of small cell cancer.

Table 4 shows that TRAIL expression was positive in 67.6% of group I cases and 23% of group II cases. This difference in TRAIL expression among the 2 groups was statistically highly significant ($P < 0.01$).

Table 5 shows that (62.5%) of adenocarcinoma cases were TRAIL positive, while 40% of squamous and 23.07% of small cell cases were TRAIL positive. There was a high significant statistical difference in TRAIL immunostaining among different histopathological types of lung cancer ($P < 0.001$).

Table 6 shows that there was no statistical significant difference between male and female distribution as regard TRAIL immunostaining in both groups.

Table 7 shows that, there was no statistical significant difference in the degree of cigarette smoking between TRAIL positive and negative subjects ($P > 0.05$).

Table 8 shows that the sensitivity of TRAIL in lung cancer was 67.6%, while its specificity was 76.9% with accuracy of 70%. (Photos 1 and 2).
Discussion

Lung cancer is the most common cause of cancer mortality worldwide for both men and women, causing approximately 1.2 million deaths per year. In the United States, annually there are 200,000 new cases of lung cancer and 160,000 deaths, and recently, it surpassed heart disease as the leading cause of smoking-related mortality [17].

The incidence of lung cancer in Egypt is dramatically increasing and expected to become the leading cause of cancer deaths [18].

Cancer is a global problem, it comes next to cardiovascular disease as the cause of mortality in Egypt, lung cancer constitutes 2.8% of total malignancy in national registry published by NCI 2007 [19].

Unfortunately, in spite of the marked progress in the treatment of the disease many patients diagnosed with lung cancer still die because of their disease, but recent progress in the molecular biology with the increased understanding of molecular abnormalities in lung cancer, recent research efforts have focused heavily on identifying molecular targets and using this knowledge to develop molecular-targeted therapies [7]. The mean age at time of diagnosis was 57.08 ± 11.4 and 56.15 ± 9.2 in groups I and group II, respectively. 96.8% and 100% of cases in groups I and group II, respectively, were (40) years or above (Table 1). This finding is concomitant with that recorded by Ashour [20], (96%); Hasan [21], (96%); Mohamed et al. [22], (96%); El-Gamal et al. [23], El-Hefni et al. [19]. The present work elucidated that there was male predominance in the studied groups, where males represent 75.7% and 76.9% in groups I and II, respectively, with male to female ratio (3:1) and this ratio is typical with that recorded by El-Aassar [24], and El-Hefni et al. [19]. As per 2007 statistics, NCI registry, lung cancer was the fourth most frequently diagnosed cancer among males in Egypt, accounting for 1.54% of all cases. In females, lung cancer ranked the 9th, accounting for 2.8% of all cancer cases in females diagnosed in that year and the difference in the previous ratio may be attributed to the different number and locality of the studied cases and the high male to female ratio can explained by the fact that males more exposed to smoking, air pollution and occupational factors. It is now believed that males and females may differ in their susceptibility to carcinogenic effects of tobacco smoke and this difference may be due to differences in DNA repair mechanisms, although still considered controversial, in addition, differences in response to certain biologic therapies (e.g., epidermal growth factor inhibitors) and antiangiogenic agents have been observed between sexes [25].

In the current series, 10.8% of group I were exposed to an occupational risk in their work place while no such risk was present in Group II and this result was statistically non significant. This result questioned the minimal role of such factors in carcinogenesis and it runs parallel with the literature data in this concern where Wynder et al. [26], stated that beside tobacco smoking, a number of industrial process and byproducts increase the risk of lung cancer including arsenic, bischloromethyl ether, chromium, nickel, polycyclicaromatic hydrocarbons, chloromethyl and methyl ether. Molina et al. [27] stated that Asbestos exposure has been shown to be strongly associated with the causation of lung cancer, malignant pleural mesothelioma, and pulmonary fibrosis. It increases the risk of developing lung cancer by as much as 5 times. Tobacco smoke and asbestos exposure act synergistically, and the risk of developing lung cancer for persons who currently smoke tobacco and have a history of asbestos exposure approaches 80–90 times that of control populations. The role of smoking as the most important cause of lung cancer is undeniable [28]. In this study, it was found that 86.5% of group I patients were smokers (10.8% of them were ex smoker and the other 75.68% were current smokers) and 76.9% of group II patients were smokers (23% were Ex and the other 53.84% were current smokers) (Table 1). This result is consistent with that of Ashour [20]; Capwell et al. [29]; El-Shahat et al. [30]; Mobasher et al. [31]; Fawzy et al. [32]; El-Gamal et al. [23]; Doll et al. [26]; Alberg et al. [33] and El-Hefni et al. [19] who stated that three quarters of lung cancer cases can be attributed to smoking and the primary risk factor for the development of lung cancer is cigarette smoking, which is estimated to account for 90% of all lung cancers. The major clinical presentations of lung cancer in this work were cough (78.4% in group I and 100% in group II patients), dyspnea (75.7% in group I and 100% in group II patients) followed by expectoration (70.3% in group I and 92% in group II patients), chest pain (51.4% in group I and 69.2% in group II patients), chest wheezes (45.9% in group I and 53.8% in group II patients), while, hemoptysis (18.9% in group I and 38% in group II patients), hoarseness of voice (16.2% in group I and 30.8% in group II patients), and dysphagia (5.4% in group I patients only) were the least presenting symptoms in both groups (Fig. 1). This result is consistent with El-Gamal et al. [23] who reported that cough was the major clinical manifestation of lung cancer (100%), followed by dyspnea (86%), expectoration (48%) while horenes of voice (20%), dysphagia (4%) were the least presenting symptoms. In a study by Mohamed et al. [22], cough was the major clinical manifestation of lung cancer (98%), followed by expectoration (96%), dyspnea (86%), hemoptysis (48%), chest pain (42%), while chest wheezes (26%) horenes of voice (14%), dysphagia (10%) were the least presenting symptoms.

Fergusson [34], stated that cough is by far the most common symptom of lung cancer at presentation and any new cough that persists longer than 2 weeks should be regarded with suspicion, especially in patients over the age of 40 years.

Table 1  Demographic characteristics of the studied patients.

<table>
<thead>
<tr>
<th>Character</th>
<th>Group I (NSCLC)</th>
<th>Group II (SCLC)</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>35–80</td>
<td>43–72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>57.08 ± 11.4</td>
<td>56.15 ± 9.2</td>
<td>0.264</td>
<td>0.79</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>3</td>
<td>0.008</td>
<td>0.92</td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
<td>75.7</td>
<td>10.8</td>
<td>0.53</td>
</tr>
<tr>
<td>Occupation history</td>
<td>4</td>
<td>0</td>
<td>1.53</td>
<td>0.21</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Cig smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non smoker</td>
<td>5</td>
<td>13.5</td>
<td>3</td>
<td>23.7</td>
</tr>
<tr>
<td>Current</td>
<td>28</td>
<td>75.68</td>
<td>7</td>
<td>53.84</td>
</tr>
<tr>
<td>Exsmoker</td>
<td>4</td>
<td>10.8</td>
<td>3</td>
<td>23.7</td>
</tr>
<tr>
<td>*Goza smoking</td>
<td>24</td>
<td>64.9</td>
<td>7</td>
<td>532</td>
</tr>
</tbody>
</table>

who are tobacco smokers. Similar reports were recorded by Margolis [35] who stated that cough was the common initial symptom of lung cancer noted in (75%) case, followed by dyspnea in (60%) of cases, chest pain (45%) of cases, hemoptysis alone in (10%) and in combination with other symptoms in up to (50%) of cases while hoarseness of voice (18%), chest wheeze (10%), dysphagia (2%) were the least presenting symptoms. Spiro et al. [9] who stated that cough is reported to be the most common presenting symptoms of lung cancer (75%), followed by dyspnea in (58%) of cases, hemoptysis in (57%), chest pain in (49%) of cases, while hoarseness of voice (18%), chest wheeze and dysphagia (2%) were the least presenting symptoms. Regarding the radiological presentation of lung cancer in this study, the commonest radiological findings were mass lesion (43.2% in group and 61.9% in group II) and hilar shadow (54.1% in group I and 38.5% in group II), followed by heterogenous opacity (43.3% in group I and 30.7% group II), broad superior mediastinum (29.7% in group I and 15.4% in group II), while pleural effusion (24.3% in group I and 23.7% in group II), elevated hemidiaphragm (18.9%) in group I and 15.3% in group II), complete opacification of hemithorax (16.2% in group I and 7.6% group II), bilateral multiple nodules (13.5% in group I only), and cavitary lesion (5.4% in group I only) were the least presenting features (Fig. 2). This result is in agreement with Mohamed et al. [22] who found that both hilar shadow (54%) and mass lesion (54%), were the commonest presenting features, followed by heterogenic opacity (30%), broad superior mediastinum (30%), pleural effusion (24%), complete opacification of hemithorax (18%), bilateral multiple nodules (10%) and cavitary lesion (4%). Different results were reported by Fawzy et al. [32] who found that mass lesion was the commonest radiological finding (34%), followed by hilar shadow (27.2%), heterogenous opacity (18.1%), pleural effusion (10.9%), elevated hemidiaphragm (10.9%), complete opacification of hemithorax (3.6%), broad superior mediastinum (1.8%). Seaton et al. [10] reported that the chest radiograph is nearly always abnormal in patients with lung cancer at presentation and prominence of a hilar shadows is a common early finding in the case of a central tumor while mass shadow is in the case of a more peripheral situated tumor. Regarding the bronchoscopic finding, this study showed the major bronchoscopic findings in both groups were broad spur (54%, 61.5% in group I and group II, respectively) and Mass lesion (48.6%, 61.5% in group I and group II, respectively), followed by bronchial narrowing (40.5%, 46% in group I and group II, respectively) while carinal broadening (18.9% of group I and 38.5% of group II), vocal cord paralysis (18.9% of group I and 30.7% of group II), bronchial obstruction (8% of group I and 30.7% of group II), and tracheal involvement (13.5% group I, 7.6% of group II), were the least presenting features (Fig. 3). Mohamed et al. [22] found that bronchial involvement was detected in (84%) of cases followed by carinal broadening in (83%), vocal cord paralysis in (14%) and tracheal involvement in (4%) of cases. On review of the diagnostic techniques utilized in this study, it was clear that bronchial biopsy carried the higher diagnostic yield (60% and 24% in groups I and II, respectively) for the diagnosis of lung cancer (Table 2) and this result is consistent with Hanson and Collins [36] (45%); El-Shahat et al. [30] (56%) and Mobasher et al. [31]; (53%). also, higher results were recorded by Zavaleta [37] (85%); Shure and Astarita [38] (88%); Moustafa et al. [39] (85%); Fawzy et al. [31] (90%); Mohamed et al. [22] (84%) and El-Gamal et al. [23] (82%). This difference can be attributed to the variation in number and adequacy of tissue biopsy specimens obtained in one hand, and the different locations of the tumor lesions on the other hand. Histopathologically, all cases (37 cases) of group I were of non small cell lung cancer type (32 cases were of adenocarcinoma type, 5 were of squamous cell type) while all cases (13) of group II were of small cell cancer (Table 3). In a study by El-Shahat et al. [30], it was founded that, (20%) were squamous cell carcinoma, (24%) adenocarcinoma, (20%) small cell carcinoma, (16%) large cell carcinoma, (8%) metastatic carcinoma and (12%) were unclassified carcinoma. In a study by Hasan [21], it was found that (42.4%) squamous cell carcinoma, (24%) adenocarcinoma, (20%) small cell carcinoma, (16%) large cell carcinoma and (3%) were carcinoi tumour. Mobasher et al. [31]; recorded that (32.1%) small cell

### Table 2: Yield of different diagnostic techniques in the diagnosis of lung cancer.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I (NSCLC) No = 37</th>
<th>Group II (SCLC) No = 13</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Technique result</strong></td>
<td>Positive No</td>
<td>%</td>
</tr>
<tr>
<td><strong>Diagnostic technique</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre Bronchoscope sputum No = 47</td>
<td>6</td>
<td>12.8</td>
</tr>
<tr>
<td>Post Bronchoscope sputum No = 50</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Bronchial biopsy No = 50</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Broncho alveolar lavage No = 50</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Bronchial aspirate No = 50</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Brush biopsy No = 48</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>CT guided biopsy No = 13</td>
<td>3</td>
<td>23</td>
</tr>
</tbody>
</table>
carcinoma, (3.8%) large cell carcinoma, (6.4%) adenocarcinoma, (19.2%) squamous cell carcinoma and (38.5%) were undifferentiated cell carcinoma. Fawzy et al. [32] found that (74%) of lung cancer patients were adenocarcinoma, (18%) were squamous cell carcinoma and (8%) were small cell carcinoma. Korraa et al. [40] reported that (44%) small cell carcinoma, (20%) squamous cell carcinoma, (20%) large cell carcinoma and (16%) adenocarcinoma.

Mohamed et al. [22] reported that (82%) were adenocarcinoma, (4%) bronchoalveolar carcinoma, (4%) squamous cell carcinoma, (4%) lymphoma and (2%) anaplastic carcinoma. It is well known that patterns of histological types of lung cancer have changed over time. In the United States of America and many other countries, adenocarcinoma has become the most commonly diagnosed type of lung cancer [41]. In Europe, despite squamous cell carcinoma remaining the most predominant cell type, the rates of adenocarcinoma have increased steadily over time [42–44]. Further analysis of histological types by nationality revealed that adenocarcinoma predominated in Egyptian population and this contradictory may be attributed to some hypotheses, such as the changes in the characteristics of cigarettes and the consequent changes in the doses of carcinogens inhaled, the advances in diagnostic techniques for lung cancer and the possible role of occupational exposure and pollution, have been postulated to explain the recent shift in histopathology [45–46]. Nevertheless, the predominance of adenocarcinoma among lung cancer types in Egypt and squamous cell carcinoma in other Arab counties like Gulf area is a matter of interest that may be addressed in future studies.

Tumor necrosis factor-related apoptosis-inducing ligand or Apo 2 ligand (TRAIL/Apo2L) is a member of the tumor necrosis factor (TNF) family of ligands capable of initiating apoptosis through engagement of its death receptors. TRAIL induces apoptosis through interacting with its receptors. So far, four homologous human receptors for TRAIL have been identified, including DR4, KILLER/DR5, TRID/DecR1/TRAIL-R3 and TRAIL-R4/DecR2, as well as a fifth soluble receptor called osteoprotegerin (OPG), which was identified initially as a receptor for RANKL/OPGL and was shown later to bind TRAIL. Both the death receptors DR4 and DR5 contain a conserved death domain (DD) motif and signal apoptosis. The other three receptors appear to act as ‘decoys’ for their ability to inhibit TRAIL-induced apoptosis when overexpressed. Decoy receptor 1 (DecR1) and DecR2 have close homology to the extracellular domains of DR4 and DR5. DecR2 has a truncated, nonfunctional cytoplasmic DD, while DecR1 lacks a cytosolic region and is anchored to the plasma membrane through glycosylphosphatidylinositol moiety. The physiological relevance of OPG as a receptor for TRAIL is unclear, however, because the affinity for this ligand at physiological temperatures is very low [8].

Two main signaling pathways have been delineated to initiate the apoptotic suicide program in mammalian cells, the intrinsic and extrinsic pathways. The cell extrinsic pathway is initiated by members of the TNF superfamily. TRAIL has been shown to induce apoptosis through binding its respective receptors, DR4 and DR5. Ligation of TRAIL to its receptor results in trimerization of the receptor and clustering of the receptor’s intracellular DD, leading to the formation of the death-inducing signaling complex (DISC). Trimerization of the DDs leads to the recruitment of an adaptor molecule, FADD, and subsequent binding and activation of caspase-8 and -10. Activated caspase-8 and -10 then cleave caspase-3, which in turn leads to cleavage of the death substrates. The cell intrinsic pathway triggers apoptosis in response to DNA damage, cell cycle checkpoint defects, mitotic catastrophe hypoxia, loss of survival factors or other types of severe cell stresses. The pathway involves the activation of the proapoptotic arm of the Bcl-2 gene superfamily, which in turn engages the mitochondria to cause the release of apoptogenic factors such as cytochrome c and SMAC/DIABLO into the cytosol. In the cytosol, cytochrome c binds the adaptor APAF-1, forming an ‘apoptosome’ that activates the apoptosis-initiating protease caspase-9. In turn, caspase-9 activates ‘executioner’ protease caspase-3, -6, and -7. SMAC/DIABLO promotes apoptosis by binding to inhibitor of apoptosis (IAP) proteins and preventing these factors from attenuating caspase activation. The intrinsic and extrinsic apoptosis signaling pathways communicate with each other. Caspase-8 has been shown to cleave the proapoptotic Bcl-2 family member Bid. The cleavage of Bid by caspase-8 and the translocation of truncated Bid to the mitochondria to promote cytochrome c release through interaction with Bax and Bak provide a plausible mechanistic link between the extrinsic and intrinsic pathways. This apparently amplifies the apoptotic signal following death receptor activation, and different cell types may be more reliant on this amplification pathway than others. kidney, central nervous system, and thyroid. Mice bearing solid tumors, when injected with soluble TRAIL, showed increased tumor cell apoptosis, suppressed tumor progression, and improved survival without any normal-tissue toxicity. Administration of TRAIL to mice transplanted with human tumor xenografts derived from colon carcinoma, breast cancer, mammary adenocarcinoma, multiple myeloma, or malignant glioma, exerted marked antitumor activity without systemic toxicity. As an alternate to administration of soluble TRAIL, adenoviral gene therapy vehicles for TRAIL have been utilized with promising results both in vitro and in small experimental animals. Prolonged survival of nude mice bearing ovarian cancer xenografts have been reported upon administration of adenoviral vectors harboring the TRAIL gene. Therapeutic efficacy of TRAIL gene therapy has also been demonstrated with colon cancer, prostate tumors, glioblastoma, breast cancer, and hepatocellular carcinoma. Taken together, these results indicate substantial promise for TRAIL in the treatment of human cancers [6].

The result of the present study showed that (67.6%) of groups I and (23%) of group II were positive for TRAIL and this difference in TRAIL expression among the 2 groups was statistically highly significant (Table 4). Similar reports were recorded by Jin et al. [47]. Different figures were recorded by other investigators. Wiezorek et al. [48] showed that the death receptors for TRAIL in NSCLCs was expressed in 82% and absence of this receptors in SCLC. Voortman et al. [7] showed that All the NSCLC cell lines are positive for TRAIL receptor, lack of TRAIL-R2 expression was found in eight of 20 (40%) SCLC cell lines and in eleven of 20 (55%) NSCLC cell lines. Interestingly, in primary NSCLC, TRAIL-R2 was over expressed in seven (23%) of the 30 tumors tested, and all primary tumors expressed TRAIL-R2. Spierings et al. [15] showed that TRAIL receptors were expressed in 82% of NSCLC this expression may be translated into a successful rhTRAIL therapy.

Hopkins-Donaldson et al. [49] have demonstrated the resistance of small cell lung cancer cell lines to TRAIL-induced
apoptosis, which could be explained by an absence of TRAIL-R1 mRNA expression and a deficiency of surface TRAIL-R2 protein. Reduced levels of TRAIL-R1 in SCLC tumors compared to NSCLC tumors justify the resistance of SCLC cells to apoptosis mediated by death receptors. In the present study, it was found that (62.5%) of adenocarcinoma cases were TRAIL positive, while 40% of squamous and 23.07% of small cell cases were TRAIL positive and this result was statistically significant (Table 5). This result is consistent with that reported by Tsuchiya et al. [50] who reported that TRAIL is over expressed in 75% of adenocarcinoma, 30% of squamous and 15% of small cell, Srivastava [51] reported that TRAIL is over expressed in 90% of adenocarcinoma, 40% of squamous cell and lack of TRAIL expression in small cell line. This difference in TRAIL expression between adenocarcinoma and squamous cell is explained by Tsuchiya et al. [50] who reported a more frequent loss of chromosomes 3p and 17p in squamous cell carcinoma compared with adenocarcinoma. These differences between the two histopathological types might be caused by exposure to different carcinogenic agents and inactivation of chromosome 13q might not be as important in the genesis of adenocarcinoma, since allelic loss was relatively infrequent (19%). Loss of heterozygosity (LOH) on chromosome 2q was observed only in adenocarcinoma and implicates the possible existence of a tumor suppressor gene on this chromosomal arm associated with adenocarcinoma but not with squamous cell carcinoma [52].

The proapoptotic Bel-2 family member Bax is a frequent target for mutation in a subset of mismatch Repair (MMR)-deficient human tumors. It has been found that Bax is required for TRAIL-induced apoptosis of certain cancer cell lines, possibly by allowing the release of second mitochondria-derived activator of caspases (Smac)/direct IAP-binding family protein with low pH (DIABLO) and antagonizing the IAP protein family. Reintroduction of Bax into Bax-deficient cells restored TRAIL sensitivity. It was found that the tumor suppressor p53 was required for sensitizing the Bax-deficient tumors to TRAIL by chemotherapy, and the p53 downstream transcriptional target gene DR5 contributes significantly to TRAIL sensitization. Apoptosis induction in response to most DNA-damaging drugs usually requires the function of the tumor suppressor p53, which engages primarily the intrinsic apoptotic-signaling pathway. TRAIL induces apoptosis in a variety of cancer cell lines regardless of p53 status, and therefore it might be a useful therapeutic strategy, particularly in cells in which the p53-response pathway has been inactivated, thus helping to circumvent resistance to chemo- and radiotherapy. Although one of the attractive features of TRAIL is its ability to kill cancers with mutations in the p53 gene, the combination of TRAIL with chemotherapeutic agents has been found to be particularly effective in killing cancers with wild-type p53, presumably through induction of DR5 expression. Downstream of the TRAIL DISC, tumour resistance can be mediated by overexpression or mutations of Bcl-2 or IAP (inhibitor of apoptosis proteins) family members [49].

While the main function of TRAIL is the induction of cell death, accumulating evidences also suggest that this ligand is able to signal non-apoptotic pathways, such as Akt, NF-κB and MAPK, involved in cell survival and proliferation. Akt kinase was proposed to play a protective role against TRAIL toxicity in many tumour cells. Yet some Akt inhibitors such as

<table>
<thead>
<tr>
<th>Group</th>
<th>(NSCLC) N = 37</th>
<th>(SCLC) N = 13</th>
<th>$X^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAIL immunostaining</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
<td>67.6</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>32.4</td>
<td>10</td>
<td>76.9</td>
</tr>
</tbody>
</table>

### Table 5

The relationship between TRAIL immunostaining and histopathological types of lung cancer in the studied cases (Total = 50).

<table>
<thead>
<tr>
<th>TRAIL immunostaining</th>
<th>Squamous cells (No = 5)</th>
<th>Adenocarcinoma (No = 32)</th>
<th>Small cell (No = 13)</th>
<th>$X^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>40</td>
<td>20</td>
<td>62.5</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>60</td>
<td>12</td>
<td>37.5</td>
<td>10</td>
</tr>
</tbody>
</table>

### Table 6

Sex distribution of groups as regard TRAIL immunostaining result.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Group I (NSCLC) No = 37</th>
<th>Group II (SCLC) No = 13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male no = 28</td>
<td>Female no = 9</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>TRAIL immunostaining</td>
<td>Positive</td>
<td>21</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>$P$</td>
<td>0.11</td>
<td>1.0</td>
</tr>
</tbody>
</table>

A.H. Ghoneim et al.
amiloride, complestatin, quercetin, sulforaphane, arsenic or EGFR inhibitor enhance TRAIL-mediated apoptosis. On the other hand, however, more specific inhibitors of Akt (e.g., LY294002, Wortmannin, PI3K siRNA) were shown to be potent TRAIL inhibitors. TRAIL is thought to induce NF-κB activation through the serine/threonine kinase RIP1. Likewise, the NF-κB pathway is thought to inhibit TRAIL-induced cell death, but the issue remains controversial. NF-κB activation has either been shown to inhibit, or to promote TRAIL-induced apoptosis. Among the MAPK superfamily, p38, ERK and JNK, would favour TRAIL-induced apoptosis, whereas ERK would display either anti-apoptotic or pro-apoptotic functions. These apparent controversies could be due to our poor understanding of the TRAIL signalling pathway, and the molecular mechanisms involved in its regulation could be more complex than anticipated [51].

The present series elucidated that 75% and 20% of males in groups I and group II, respectively, and 44.4% and 33% of females in groups I and group II, respectively, were positive for TRAIL expression (Table 6). This result was of no statistical significant difference.

This finding is in agreement with Shi et al. [53] Thorburn [54]; McGrath [55] and Kris et al. [52] who stated that no significant relationship between TRAIL status and patient sex. In this study, there was no statistical significant difference in the degree of cigarette smoking between TRAIL positive and negative subjects in both groups (Table 7). This result is in agreement with results shown by Srivastava [51]; Zhang et al. [56] and Shi et al. [53] who found that no significant relationship between the degree of cigarette smoking and TRAIL status.

This is in disagreement with Kwon et al. [57]; Perez et al. [58] and Morissette et al. [59], who reported that there was a significant correlation between TRAIL apoptosis and the amount of life time cigarette consumption.

This finding goes parallel with the fact that H2O2 and cigarette smoke sensitized cells to TRAIL-mediated apoptosis in vitro. In fact, the cellular response to injury seems to be extremely important in allowing the TRAIL signaling pathway to induce apoptosis in various cell types. Thus, cellular response of the lung to injury induced by oxidative stress can lead to an increased alveolar sensitivity to TRAIL-mediated apoptosis [52]. This difference may be explained by the variations in the ability to metabolize tobacco carcinogens between different population. The Present work showed that the sensitivity of TRAIL in lung cancer was 67.6%, while its specificity was 76.9% with accuracy of 70% in the studied groups (Table 8), these observations are consistent with that reported by McGrath [55] who reported the specificity of TRAIL immunostaining in lung cancer was 80% while its specificity was 78% with accuracy of 80%.

False negative results of immunohistochemistry are due to technical (e.g., fixation) artefacts, beside that, in the present study, TRAIL expression was determined immunohistochemically in paraffin-embedded sections where receptors were detectable, but no membranous staining pattern was observed. However, this does not preclude the presence of DR4 or DR5 on the cell surface, because immunohistochemistry is not an optimal technique to detect membranous staining. Moreover, Zhang et al. [56] demonstrated that, although present on the cell surface, both DR4 and DR5 are predominantly located in the trans-Golgi network. Furthermore, they suggested that the localization in the trans-Golgi network might be a common feature of apoptosis-inducing TNF family receptors, because Fas and TNF were also reported to be located in the trans-Golgi network.

### Conclusion

(1) Apoptosis is frequently dysregulated in human cancers, and recent advancements in our understanding of the regulation of programmed cell death pathways has led to the development of novel agents to reactivate apoptosis in malignant cells. The activation of cell surface death...
receptors by tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) and death receptor agonists represent an attractive therapeutic strategy to promote apoptosis of tumor cells.

(2) TRAIL is overexpressed in the majority of NSCLC mainly adenocarcinoma. This indicate that rh TRAIL therapy may become a new adjuvant line for treatment of such cases.

References

An immunohistochemical study of tumour necrosis


