Evaluation Levels of IL-17 and IL-10 in Serum Patients of Lung Cancer in Both Types Non-Small Cell Carcinoma and Small Cell Carcinoma in Al Najaf province/Iraq

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Abstract. The current study utilized cytokines as biomarkers where play a pivotal role in the suppressive or progression of lung cancer and are used to regulate of immune response of patients with lung cancer, samples were collected from cases of patients who suffer from clinical symptoms of disease and from both sexes specifically from the center of Middle Euphrates Cancer in the government of Al-Najaf at the period from (December, 2022 to the end of March, 2023) where comprised 176 samples as 120 cases represent lung cancer and 56 cases as the apparently healthy control group. 120 cases were comprised as non-small cell carcinoma cases of patients representing 96 (80%) divided in to into 46(47.9%) cases of squamous cell carcinoma,32 cases of adenocarcinoma (33.3%), and 18 (18.7%) cases of large cell carcinoma, while 24 (20%) represented cases of small cell carcinoma, taking of blood samples from all patients cases then separated to obtain of serum for detection levels of IL-17 and IL-10 via the using of Enzyme-Linked Immunosorbent Assay (ELISA) apparatus that measured the serum levels of IL-17 in lung cancer patients and were showed (0.2309 ± 0.06659) pg/ml significantly at (p≤ 0.05) compared with the apparently healthy control group (0.1377 ± 0.0117) pg/ml, as well as it observed higher significant (p ≤ 0.05) for levels of IL-10 in patients serum (0.392 ± 0.002333) pg/ml than the apparently healthy control group(0.2089 ± 0.003074) pg/ml.

1 Introduction

Lung cancer classifies considered the most cancer distributed in the world and leads to cause death in both women and men one lung cancer type that for 20% as small cell carcinoma lung cancer (SCLC), and another type that form percentage more than 85% of lung cancers belong to a type called non-small cell lung cancer (NSCLC) that includes essentially of squamous cell carcinoma (SCC) and adenocarcinoma (AC) thus, the obvious vision for progression or development that related to NSCLC incompletely that back to the vital role of the microbiome and their genes in diseases and healthy effect [1]. The deviations in micro biota be attributed to tumors by the understanding of various
mechanisms as, altering the stability of the host genome due to the production of toxins by bacteria, the release of metabolites from microbial cells that induce cancer, and also harm in the barrier of the immune system locally. The growth and metastatic spread of tumor cells may be affected directly by microbial intratumor, William Busch in the early year 1868 registered a decline spontaneously in the tumor in persons who suffer from infections caused by streptococci and from that period, the proof referred to the causative agent as diplococci bacteria which was related to tumors in humans specifically lung cancer [2, 3].

On the other hand, even though the suggestion of numerous bacteria were responsible for causing tumors in humans, there was no player essential in the tumorigenicity of the lung therefore, a recent study at present to know the occurrence of tumorigenesis particularly in NSCLC of lung cancer via the role of diplococci bacteria in addition to finding a proof for the function of oncogenic to microbiota in a progressing case of lung cancer [4]. However, infections caused by microbes and anticancer represent major diseases that threaten life in the world because of a high rate of mortality, and more infectious pathogens have been high resist to some types of medicines [5].

A biomarker refers to an indicator that measuring any biological process and can be noticed classes of biomarkers used for prognostic, detection and predict for the response of treatment, Thus, the selection of cytokines such as Interleukin 10 (IL-10,) and Interleukin 17(IL-17) as indicators or factors to verify in the tumor microenvironment (TME) of lung cancer [6,7].

2 Materials and methods

2.1 Specimens Collection

The current study was performed at Al-amen Center to Advanced Research and Biotechnology / Imam Ali Holy Shrine, samples were collected from the center of Middle Euphrates Cancer in the government of AL-Najaf at the period from (December, 2022 to the end of March , 2023). The study comprises collected 176 blood samples which separated from plasma and take of serum, also that comprises 120 cases of lung cancer diseases from patients and that included both male and female with 85 and 35 samples respectively in addition to healthy cases that considered a control group with 56 samples (36 male and 20 female), and aged of all cases reached a round less than 35 years to 85 years, all cases divided in to ninety six case with percent 80% comprises Non-small cell carcinoma type while about twenty four case comprises small cell carcinoma tyype that represents 20% from all cases.

2.2 Estimation level of IL-17 and IL-10 (Sun long kit, China and Elabscience kit, USA)

The samples or standards were added to the wells of microelisa strip plate that bind to the specific antibody. The horseradish peroxidase enzyme with specific antibody of interleukin 17 or 10 was added to each well of microelisa strip plate and placed in an incubator, the free components was washed to remove. Then A substrate solution was added to each well and only wells that contain of interleukin 17 or 10, HRP bind with antibody interleukin 17 or 10 give a blue color while, yellow color appear after addition of stop solution for each well, the well plate placed in spectrophotometer at wave length 450 nm to measure of optical density (OD), OD value represents the level of interleukin 17 or 10 in the sample, then it was calculated of interleukin 17 or 10 concentration in each samples by comparing the value of OD for each samples with the standard curve.
2.3 Procedures

The test was achieved according to the company of manufacture and leaflet then calculated of concentrations based on the equation referred to in the kit.

2.4 Results Calculation

The concentration of interleukin for each standard solution and its related to optical density(OD) reading relied on the log scale for both X-axis and Y-axis, so the sample concentration of IL-17 and IL-10 were measured by the value of OD, and the concentration on the Y-axis was measured via multiplying it by the factor of the dilution fig (1) and fig (2).

![Standard curve of IL-10 (Elabscience, USA).](image1)

![Standard curve of IL-17 (Sun long, China).](image2)

2.3 Statistical Analyze

The data obtained in the study were analyzed and performed by using the program Graph Pad Prism version 5 with an unpaired t-test, it was demonstrated via one-way ANOVA table at P ≤ 0.05 to know the variance significantly [8].
3 Results and discussion

3.1 Distribution of specimens for lung cancer

One hundred twenty specimens were taken from patients who suffered from lung cancer disease as well as fifty-six samples that were considered as the apparently healthy control group where all specimens were collected from the center of Middle Euphrates Cancer in AL-Najaf city, 85 and 35 specimens represented males and females for infection cases respectively while 36 specimens of males and 20 specimens of females represented apparently healthy control group, the difference between males and females according to age, smoking and types of lung cancer shown in the table (1).

<table>
<thead>
<tr>
<th>Factors</th>
<th>Groups</th>
<th>Lung Cancer (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>85 70.8</td>
<td>36 64.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>35 29.2</td>
<td>20 35.8</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;35-45</td>
<td>20 17</td>
<td>9 16</td>
</tr>
<tr>
<td></td>
<td>46-55</td>
<td>32 27</td>
<td>7 13</td>
</tr>
<tr>
<td></td>
<td>56-65</td>
<td>38 31.6</td>
<td>26 46.4</td>
</tr>
<tr>
<td></td>
<td>66-75</td>
<td>20 17</td>
<td>6 11</td>
</tr>
<tr>
<td></td>
<td>76-85</td>
<td>10 8</td>
<td>8 14.2</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>78 65</td>
<td>20 35.8</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>42 35</td>
<td>36 64.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NSCLC Squamous</th>
<th>NSCLC Adenocarcinoma</th>
<th>NSCLC Large Cell Carcinoma</th>
<th>SCLC Small Cell Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>32</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>38%</td>
<td>27%</td>
<td>15%</td>
<td>20%</td>
</tr>
</tbody>
</table>

The research referred to the occurrence of lung cancer in men 70.8 % compared to 29% incidence in females, So many previous studies identical with this study [9] they found the occurrence of lung cancer in men more than females with 67% and 33% respectively in the province of Al-Najaf, the frequency of aged (56-65) years in the current study with 31.6% that was nearly in another study and followed 27% for aged (46-55) years [10], while [11] they showed more incidence of lung cancer in those aged over 70 years, because of changes in the style of life that may be reflected the exposure to lung cancer infection. Furthermore, a number of smoking in the current study seventy-eight samples form 65% thus, leading to more incidence of lung cancer in men than women and in groups more than 50 years, which may belong to an increased percentage of smoking in males in comparison with females as well as other risk factors that was more exposure in males than females which one of these as smoking of cigarette.

The study was agreed with another study was presented by [12] they referred to smoking of cigarette considered a noteworthy factor for the incidence of lung cancer, smoking can cause different types of cancers as lung, throat, larynx, and others because of cigarettes contain of materials carcinogens as aromatic hydrocarbons that consist of polycyclic compounds as well as nitrosamine in addition to nicotine material considered addictive and it often consumption by smokers via smoking, as well as in the fig (3) showed the percentage of NSCLC cases was higher than the percentage of SCLC cases in the current study which reached 80% and 20% respectively. Similar results were also reported.
by [13], where they observed that 84% was for NSCLC type while 13% was for SCLC type. Also, [14] they showed 15% for SCLC.

![Pie chart showing percentage distribution of lung cancer types and subtypes]

**Fig. 3.** Percentage distribution of lung cancer types and subtypes.

### 3.2 Estimation levels of IL-17 in the serum of lung cancer patients and apparently healthy control group.

The results showed that the serum levels of IL-17 among patient cases with lung cancer (0.2309 ± 0.06659) pg/ml were the presence of significant (p≤ 0.05) that compared with apparently healthy control group (0.1377 ± 0.0117) pg/ml as in fig (4). IL-17 represents one of the pro-inflammatory cytokines that take part in the stimulation and progression of tumors as well as the induction for growth of non-small carcinoma type and activation of angiogenesis [15]. IL-17 was produced by immune cells as T helper cells (Th 17) that specialize for IL-17, CD8 cells, mast cells, granulocyte cells, and natural killer cells (NKs) where IL-17 plays a principal role in the regulation process of autoimmune disease and inflammation as well as organized -regulation of Th 17 responsible for the release of IL-17 that lead to various cases of human carcinoma [16]. The previous studies identical with this study, [15] who observed to increase level of IL-17 in the serum of lung cancer patients compared with control group because of the contributions of IL-17 in the progression of tumors and the formation of the microenvironment that provides it growth in addition to the pivotal role of IL-17 in the disease fibrosis in organs as lung [17]. Also, [18] they demonstrated a higher significantly in level of IL-17 of tumor tissues than level of IL-17 in tumor tissues near and normal distant tissues of lung.
3.3 Levels appreciation of IL-17 according to the types of lung cancer

The results found the level of IL-17 was higher significantly (p≤ 0.05) of NSCLC (0.3617 ± 0.01731) pg/ml than it level of SCLC (0.1851 ± 0.01396) pg/ml as well as it showed highly signficant (p≤ 0.05) among types of NSCLC where reached (0.4692 ± 0.01868,0.3651 ±0.02014, and 0.2508±0.01312) pg/ml for squamous cell carcinoma, adenocarcinoma, and large small carcinoma respectively as in fig (5), another study by [19] they referred to stimulation of the progression of tumors through the stimulation of angiogenesis for fibroblasts and endothelial cells via administered of injection of NSCLC and cancer cells which increasing expression of IL-17, which led to stimulating tumorigenicity thus it gave a high level of IL-17A in the group of NSCLC than others groups in the study, while [20] who showed that results agreed with these results where observed high expression of IL-17 in the serum of patients specifically in stages III and IV of lung cancer during an increasing activity of immune cells as Th 17 and γδT17 that responsible for IL-17 secretion led to high levels of IL-17 in the serum of patients.

The current study was identical to other study ,[21] they illustrated an increasing level of IL-17 in the serum of the patients in comparison with healthy control because of the pivotal role of IL-17 in the progression of NSCLC for patients where observed higher significantly (p≤ 0.05) specifically in squamous subtype than other subtypes as adenocarcinoma and large small carcinoma. Also, another study [22] showed an increasing level of IL-17 in patients with NSCLC of lung cancer compared with a health control group where its levels were related to the size of the tumor and also observed relation of that increasing levels of IL-17 in the serum of patients to the malignancies that spread within the lung.

The study was demonstrated increasing the immune cells that secreted of IL-17, especially in the tissues of tumors that binding to increasing of angiogenesis of lymph specifically in NSCLC as well as weak survival for life, Also, this study was agreed with[16] they showed the observation to increasing of IL-17 levels in the serum of NSCLC patients of lung cancer.
Fig. 5. Level of IL-17 in patients serum according to the types of lung cancer.

3.4 Estimation levels of IL-10 in the serum of lung cancer patients and apparently healthy control group

The results showed increased significantly \((p \leq 0.05)\) of IL-10 levels in the serum of patients with lung cancer \((0.392 \pm 0.002333) \text{ pg/ml}\) compared with the apparently healthy control group \((0.2089 \pm 0.003074) \text{ pg/ml}\) as in fig (6). IL-10 has a role in the regulation of angiogenic and immune responses as well as acts to stimulate the survival of cancer cells and metastasis also proliferation via control of immunological antitumor, IL-10 was produced by tumor cells in addition to several cells of the immune system as lineages of lymphoid and myeloid \((\text{Ouyang and [23,24]})\). IL-10 play a role as a multifaceted in the pathogenesis of tumor as well as the stimulation of tumor and angiogenesis while it acts as a support to inhibit the growth of tumor and metastasis thus, it represents a master key to immunity of antitumor and at the same time promotion inflammation of tumor so that the levels of IL-10 were dysregulation lead to a cause of carcinogenesis and development of tumor \([25]\).

Other studies were introduced by \([26]\) who found the high levels of IL-10 were related with environmental activation of inflammation in tumor and also suggested that the elevated levels of IL-10 in the serum patients of NSCLC for lung cancer have higher danger to immunological events that it related adversely and one of these most events as pneumonitis where the levels of IL-10 play trustworthy role in the prediction of occurrence. Also , \([27]\) they reported that lung cancer patients have increasing levels of IL-10 in their serum compared with the health control group, \([28]\) who referred to the serum levels of IL-10 in lung cancer patients as higher significantly obviously, than the healthy persons and the patients at high levels of expression had lower rates of survival, also it was concluded that IL-10 levels that released from tumor cells and cancers related macrophages cells have the pivotal role in the progression of lung cancer.
3.5 Estimation levels of IL-10 according to the types of lung cancer

The current study showed that the levels of IL-10 in patients with NSCLC (0.3251 ± 0.04750) pg/ml were significantly (p≤ 0.05) than SCLC (0.0979± 0.005753) pg/ml also showed highly significance (p≤ 0.05) in large small carcinoma of NSCLC (0.4911 ± 0.05803) pg/ml compared with adenocarcinoma (0.2914 ± 0.004608) pg/ml and squamous (0.1927 ± 0.03839) pg/ml that showed in fig (7). The level of IL-10 can be used as a predictor of the marker of NSCLC in the next stage, another study by [29] who referred to low expression of IL-10 in the early stage of the disease where it considered a projection of shabby as well as the signal of IL-10 was represented as influencing aim for treated of NSCLC and it can be used of these signals in the constraining of her molecules thus, the high expression of it that connected to predicting of disease. Also, [30] they showed higher expression of IL-10 in the normal region around the tumor region of the lung and related to the diameter of the tumor in the patients of NSCLC specifically large cell carcinoma comparison with squamous, adenocarcinoma, and SCLC.
4 Conclusion

Estimated levels of IL-17 and IL-10 in the serum of patients with lung cancer, such as non-small cell lung cancer, were clearly elevated compared to the two types, on the one hand, and to the control group, on the other hand, which appeared healthy. Clear, which indicated the pivotal role of the interleukins selected in our study in the development of lung cancer such as advanced tumorigenesis or carcinogenesis.

References