

Research Article

The Role of IFN- γ Level In The Patients of Non-Small Cell Carcinoma And Small Cell Carcinoma of Lung Cancer At IV Stage Compared With Other Stages

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Abstract

IFN- γ used as a marker to know the suppression or development of disease of lung cancer, 200 samples were collected from lung cancer disease patients, where all cases of the disease were collected from the center of Middle Euphrates Cancer in AL-Najaf City (January 2023 to April 2023), with 130 and 70 samples for both males and females and 80 samples represented the control group and were divided into 50 and 30 samples for males and females respectively, the age, smoking and types of lung cancer factors such as sex, age, and subtypes of main types of lung cancer disease that were taken in studying research. All samples which 155 cases were comprised of non-small cell carcinoma cases of patients representing 78% divided into 75 (38%) cases of squamous cell carcinoma, 50 (25%) cases of adenocarcinoma (33.3%), and 30 (15%) cases of large cell carcinoma, while 45 (22%) represented cases of small cell carcinoma, the blood samples from all patients cases have separated the serum from plasma to detect levels of IFN- γ by (ELISA) apparatus to measure its levels of IFN- γ in the patients' serum of lung cancer, the results were (0.3995 ± 0.00282) pg/ml increased significantly at $(p \leq 0.05)$ compared with the control group (0.2009 ± 0.003593) pg/ml, the study also was showed significantly $(p \leq 0.05)$ at stage IV of the disease (0.1971 ± 0.04218) pg/ml in comparison with stage III (0.184 ± 0.05346) pg/ml and stage I-II (0.1708 ± 0.08292) pg/ml. Furthermore, the study observed IFN- γ levels in the patients' serum of a type of lung cancer for NSCLC (0.4632 ± 0.05331) pg/ml increased significantly $(p \leq 0.05)$ compared with type SCLC (0.2003 ± 0.06652) pg/ml in addition to high significant in subtypes adenocarcinoma (0.5984 ± 0.05379) pg/ml compared with squamous cell carcinoma (0.4973 ± 0.04301) pg/ml and large cell carcinoma (0.294 ± 0.06313) pg/ml.

1. Introduction

Lung tumors represent one of the most frequent and considered second among diagnosed cancer malignancy lead to cause tumorous death firstly, around the world [1]. There are two essential categories of lung cancer that included non-small cell lung carcinoma (NSCLC) which forms eighty-five percent of lung cancer cases while fifteen percent of cases represent the second type of lung cancer called small cell lung carcinoma (SCLC) which is known as hostile lung tumor who related to smoking and often the identification of patients with disease of metastasis on the other hand, more sub - classify of NSCLC into large cell carcinoma, squamous cell carcinoma and, adenocarcinoma that is considered more common cases frequently [2].

NSCLC refers to the most frequent kind of histological tissues which account for eighty- five percent of all cases of lung tumors, around thirty percent of recent cases from NSCLC are diagnosed as topical more developed or advanced stages that included a broad group of various clinical cases in addition to non-homogenous spectrum of medical therapeutic options [3], especially the higher non-homogenous group of lung cancer that represented by stage three of NSCLC where diagnosed by various sizes of tumors significantly, participation of lymph nodes as well as local infiltration, in stage three for NSCLC treatment, which rely on the crucial role of many modes of therapy encompassing surgical ways, broad range options of systemic therapies, and also radiotherapy [4].

The stages of lung cancer can be taken and its studying from a biopsy or patch via an involved piece removes from tissue, especially lung tissue or lymph nodes then microscopical examination, stages are divided into four cases I, II, III, and IV, where all of these appeared in the second type of lung cancer who called NSCLC while these stages are limited in a type SCLC who represents the first type of lung cancer, as well as the stages of NSCLC, can be subdivided into another subclasses as IIIA, IIIB or IIIC , SCLC and NSCLC of lung cancer detected by TNM system where the number T, N, and M referred to the site and size of the tumors (T), means the spread of tumor in the lymphatic node and also referred to increasing the tumor close to the basic structures in the thoracic such as big airways of the lung and heart, also in the major blood vessels or tissues exterior from the lung, L referred to the spread of cancer to the lymph nodes that are considered a part of the immune system then tumor cells expand into the other parts of the lymphatic system while M means a stage of metastases referring to the spread of tumors which take place in all body parts and also it grows in other organs or tissues [5]. Stage III of NSCLC required optimum management of the proper staging of pathological and clinical especially mediastinal and hilar lymph nodes thus, the patients be candidates correctly in order to reach the approach of surgery or connected the chemicals with radiotherapy [6]. The studies of imaging that consist of scans of computed tomography (CT), ultrasound, and scan of positron emission tomography (PET) are broadly utilized to monitor and organize or stage for numbers various cancers. On the other hand, the special setting for the staging of lung tumors, and staging of mediastinal through needle aspiration by using ultrasound to each endobronchial (EBUS) and transbronchial (TBUS) that has been advanced and at present substituted from mediastinoscopy approximately, therefore all of the techniques that remembered previously depict the primary thing for the approach proper to NSCLC who local advanced [7].

Cytokines represent the first organizer for each adaptive and innate immunity through an essential role for them in the organization of responses of immune against infectious agents, cancer, and disorders of autoimmune diseases as well as cytokines have many additional functions as providing of the protection and assist to develop illness of autoimmune and cancer especially in an extreme of activation or very deficiently [8].

IFN- γ considered a lone member of the interferon family of inhibition of viruses where first described by Frederick Wheelock as an inhibitor for viruses that cause phytohemagglutinin produced from activation of white cells of the blood, IFN- γ represents a protein that is coded by the specific gene for IFN- γ and it consists of two chains of polypeptide related by the anti-parallel manner, IFN- γ in the blood of human appears in three parts with molecular various mass, two parts represent a mature form of IFN- γ while another one part considered the free active form of IFN- γ [9]. IFN- γ in the cells of tumors is released principally after the activation of immune cells as T-helper cells (Th1), CD8, and natural killer cells [10]. The proofs also refer to other immune cells may be produced IFN- γ as macrophages, (DC), and B-cells from the pathway of innate immunity , the releasing of IFN- γ induced via other cytokines specifically interleukin 12(IL-12), colony-stimulating factor of macrophage (M-CSF), and interleukin 18(IL-18) also by signals of receptors that activate receptors of NK cells, CD16 in NK cells, and receptors of T-cells in [11].

On the other hand, IFN- γ introduces the actions of antitumor via two manners either directly way to inhibition of growth or by indirectly way via increased kill by cells of the immune system, IFN- γ has the influence on inhibition of growth for tumor cells by stimulating the death of cells with apoptosis, necrosis, and stopping of growth via aging as well as there are the presence of effects for immune induction for each of increased of major histocompatibility 1(MHC-1) , offerings of antigen, and characters of antigenicity, that have been broadly identification of models for tumor and infection [12].

2. Materials and Methods

Samples

The study was conducted at the Center of Al-amen to Advanced Research in the Imam Ali Holy Shrine, where the collected specimens from the center of Middle Euphrates Cancer in Al-Najaf the period from (November 2022 to the end of April 2023). 176 blood samples were then separated by centrifugation at 3000 rpm for 3 minutes to separate the serum from plasma, 120 cases of specimens referred to lung cancer diseases and included both males and females in addition to control cases with 56 samples, all cases reached less than 35 years to 85 years, as well as all cases divided into 96 cases with percent 80% comprise Non-small cell carcinoma type while about 24 cases comprise small cell carcinoma type that represents 20% from all cases.

Estimation concentration of IFN- γ (Elabscience kit, USA)

The samples to each well that contained a specific antibody specifically comprised the horseradish peroxidase enzyme with a particular antibody of IFN- γ that was added to each microelisa strip plate well and then placed in an incubator, washing after removing the free components where it gave a blue color when the attach of HRP but, the appearance of color after added of stop solution to each well, the rack of wells was read at 450 nm by spectrophotometer to know optical density (OD) which represents levels of IFN- γ in each sample and it was calculated via comparing the value of OD with a standard curve of each sample.

Procedures

The test was performed relying on the leaflet of the company that manufactured it where the concentrations were calculated by the equation remembered in the kit of assay.

Table 1: Samples distribution based on factors (sex, age, smoking).

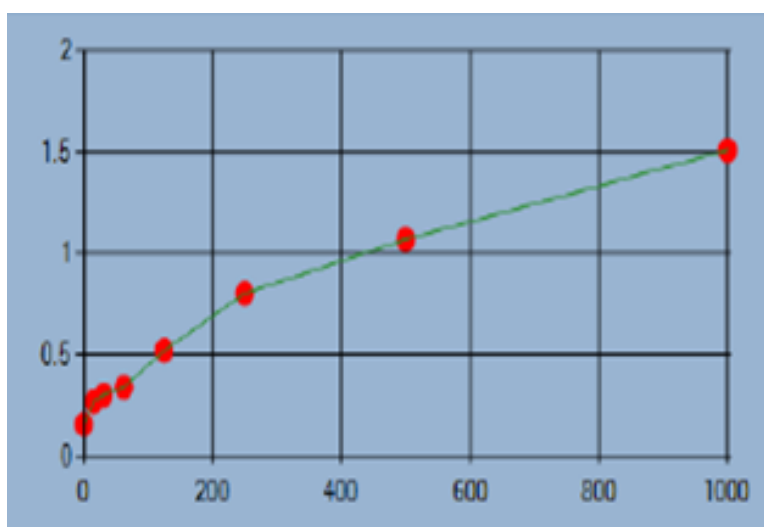
Factors	Groups	Lung Cancer	(%)	Control	(%)
Sex	Male	130	65	50	63
	Female	70	35	60	37
Age	≤ 35-45	30	15	14	18
	46-55	47	23	9	11
	56-65	58	29	38	48
	66-75	35	18	8	10
	76-85	30	15	11	13
Smoking	yes	110	55	60	75
	No	90	45	20	25

Table 2: Types of lung cancer.

Clinical Types of Lung Cancer			
NSCLC Squamous	NSCLC Adenocarcinoma	NSCLC Large Cell Carcinoma	SCLC Small Cell Carcinoma
75	50	30	45
38%	25%	15%	22 %

Results Calculation

The levels of IFN- γ for each standard solution through its related to an optical density (OD) via used spectrophotometer apparatus based on the log scale for both axes (X and Y) so the sample concentration of IFN- γ was calculated by the value of OD as well as the calculated IFN- γ concentration on the Y-axis via multiplying it by the dilution factor Figure 1.

**Figure 1:** Standard curve of IFN- γ concentration (Elabsience , USA)

Analysis Manner Statistic

Graph Pad Prism version 5 was used to analyze the data obtained in the current research. An unpaired t-test was illustrated via a one-way ANOVA table to determine the significance presence or not at $P \leq 0.05$ [13].

3. Results and Discussion

Samples Distribution

200 samples were collected from lung cancer disease patients and all were divided into 80 samples representing the control group where all specimens were collected from the center of Middle Euphrates Cancer in AL-Najaf city, with 130 and 70 specimens for both males and females who infect cases of disease respectively while control cases were divided into 50 and 30 samples for males and females respectively, the age, smoking and types of lung cancer factors that were taken in studying research as shown in Table 1,2.

Table 1,2 demonstrates the occurrence of lung cancer disease with 65 % and 35% incidence in men and females respectively where the current study is identical to the previous studies by [14] Who found that the lung cancer disease in men more than females with 67% and 33% in Al-Najaf province, the research referred the aged (56-65) years was more frequency with 29% that was identical with another study and it followed aged (46-55) years with 23% [15] but, researcher Siegel and his colleagues in 2023 showed more occurrence of lung cancer disease in those aged over 70 years may be because of alteration in the lifestyle that reflected the exposure to infected by disease as well as

the role of smoking may play pivotal in occurs of disease where the research consisted of 110 samples with 55% thus, the incidence of lung cancer disease especially in men than women and at aged 50 years, which may belong to an increased percentage of smoking in males in comparison with females, the results in the table above also agreed with the study introduced by [16]. Who showed that smoking represents a noteworthy factor to occur of lung cancer disease beside it can cause various types of cancers such as the larynx and sore throat that the cigarette consists of many carcinogens substances as aromatic compounds contain hydrocarbons, polycyclic and nitrosamine also nicotine substance which represents addictive for smokers. The research comprised the types of lung cancer with a higher percentage of NSCLC cases than SCLC cases where reaching 78% and 22% respectively these percent agreed with another result introduced by [17]. Who obtained the percentages of 84% and 13% for NSCLC and SCLC and another researcher showed a percentage 15% for SCLC.

Evaluation of IFN- γ levels in the patients' serum and control group

The research study showed a significant increase at ($p \leq 0.05$) in the concentrations of IFN- γ in persons serum infected by lung cancer (0.3995 ± 0.00282) pg/ml when compared with the control group (0.2009 ± 0.003593) pg/ml as in Figure 2.

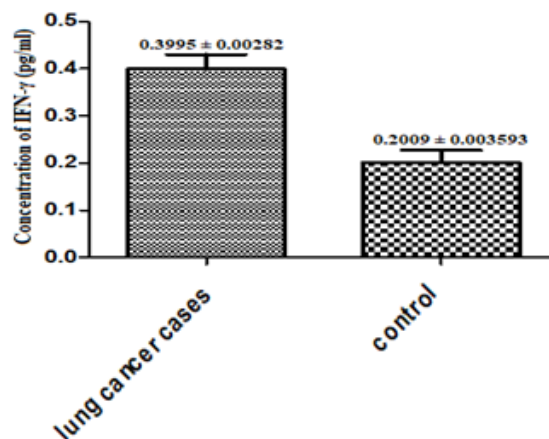


Figure 2: IFN- γ levels in patients serum and control group. * Significance at a probability level of $p \leq 0.05$

IFN- γ plays a role as an antitumor on immunological levels where it had a cytotoxic role effectively when it was found with other immune factors such as granzyme B and perforin which are utilized to induce apoptosis for tumor cells [9], where the results in the research agreed with another study introduced by Kanai and his colleagues in 2020 that illustrated higher levels of IFN- γ in the serum of lung cancer patients than in control cases while Boutsikou and his colleagues in 2018 obtained their results from the study on some cytokines included an IFN- γ in serum patients of lung cancer and that referred were increasing levels of its which agreed with our research.

IFN- γ and some cytokines had an importance in the metastasis and progression of lung cancer disease through induction of proliferation and angiogenesis via directed or undirected effect and defective apoptosis processes [18] also, based on the results of researcher Kanai and his colleagues and who showed that the levels of IFN- γ may be a response to mitogen that rely on cases of the immunological system for any patient and it observed the decreasing levels of IFN- γ after the treatment process which be seen noticed.

On the other hand, the results in the study showed IFN- γ levels in the patients serum of NSCLC (0.4632 ± 0.05331) pg/ml were higher significantly ($p \leq 0.05$) than SCLC (0.2003 ± 0.06652) pg/ml beside that illustrated high significant in adenocarcinoma (0.5984 ± 0.05379) pg/ml in comparison with squamous cell carcinoma (0.4973 ± 0.04301) pg/ml and large cell carcinoma (0.294 ± 0.06313) pg/ml as in Figure 3.

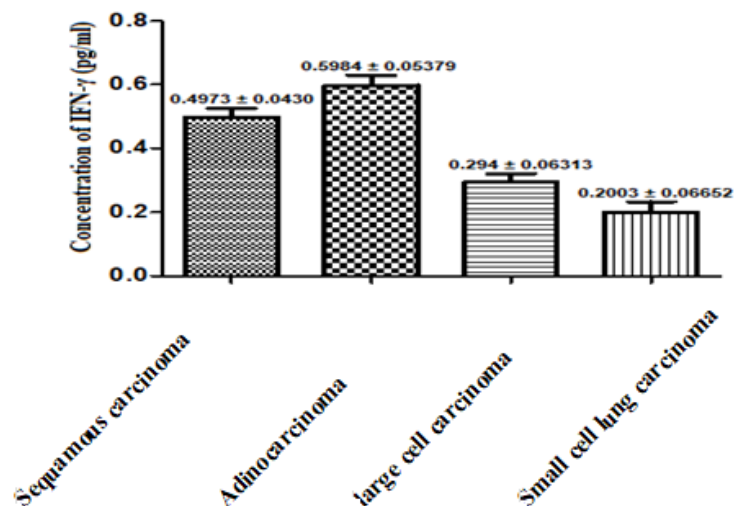


Figure 3: The level of IFN- γ in patients serum according to the of lung cancer types. * Significance at a probability level of $p \leq 0.05$

IFN- γ acts as an antitumor as well as the generation of tumors in various stages of immune responses thus, increased expression of IFN- γ led to induced death of CD4+ T cells, alteration in the ratio of CD4:CD8, and weakness in secondary response of the immune system to antitumor also IFN- γ can organize of protein in apoptosis called programmed cell death 1 ligand (PD-L1) that it related at a membrane of checkpoint molecule of immunity found on the surfaces of tumor cell by active CD8+ T-cell that connected to PD-L1 causing apoptosis of T-cell and give the sign for IFN- γ to help cancer cell to escape from the antitumor agent, affect cytotoxicity, and cancer cell survive of cancer cell but, it bind of the cells (CD47) to PD-L1 causing protumorigenic that make IFN- γ play a role in the regulation for expression of CD47 cell [19]. The results obtained in the research agreed with another study by [20] where they referred to increased levels of IFN- γ rely on the development of the disease after an indicator of immunotherapy used as immune checkpoint Inhibitors (ICIs) for days of treatment at T1 and compared that patients at day T3 related with different immune events where found a significantly less and IFN- γ can be used as a biomarker to patients at NSCLC.

Finally, the study showed higher significance ($p \leq 0.05$) in the levels of IFN- γ in patients' serum with developed stages of the disease detected last stage IV (0.1971 ± 0.04218) pg/ml compared with other stages, stage III (0.184 ± 0.05346) pg/ml and stage I-II (0.1708 ± 0.08292) pg/ml as in Figure 3. IFN- γ plays a role in the peripheral blood causing the induction of chemokines in cases disease of lung cancer but, it may be limited while, another chemokine called chemokine ligand 9 (CXCL9) has increased levels that are connected to danger for lung cancer also as it found IFN- γ levels which were induced of chemokines in plasma, especially in the patients' cases of NSCLC where higher levels in the IV stage at metastasis which compare with other stages of disease [21], the other study by [20] showed lower significant in INF- γ levels specifically in first and second stages than other last stages at advanced cases disease while the researcher Lee and his colleagues at 2021 found higher significantly INF- γ levels in stage IV and especially in the patients' serum with NSCLC type of lung cancer. So, specifically, the results of the research were identical also with the results in another study introduced by [18].

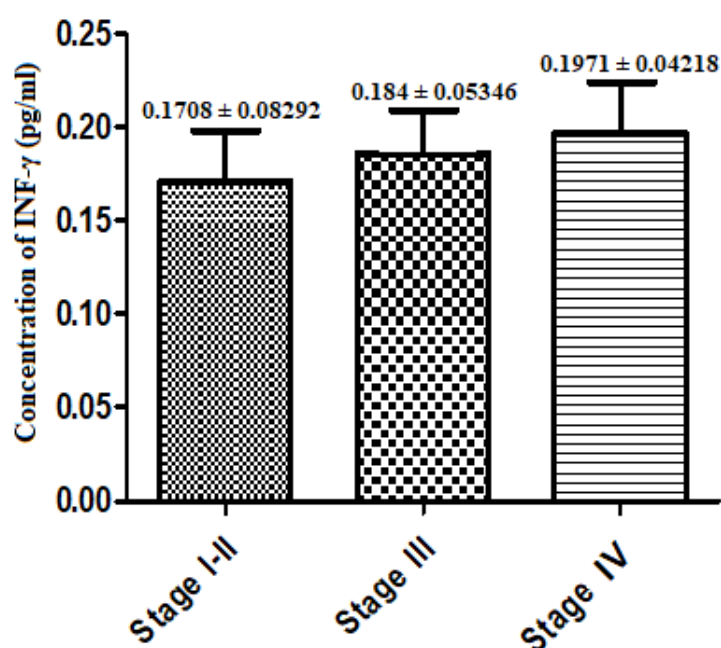


Figure 4: IFN- γ levels in patients' serum based on stages of disease. * Significance at a probability level of $p \leq 0.05$

4. Conclusion

The levels of IFN- γ in the patients' serum of lung cancer, specifically adenocarcinoma, were increased levels compared with the other two types, on the one hand, and also, the control group from other hand as well as in the IV stage compared to the other stages that may belong role of its in the study in the progression of lung cancer such as advanced disease tumorigenesis or carcinogenesis.

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