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Evaluation of the Expression of CD-4 and CD-45 Count among Patients Having Non-Small Cell Lung Cancer

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ABSTRACT:

Lung cancer is one of the most lethal types of cancer in case of both men and women. It has a higher mortality rate than the three most widespread malignancies (colon, breast, and pancreas combined).The demographic makeup of lung cancer has evolved during the last few years. The majority of reports, however, have limitations due to their small sample sizes, brief follow-up periods, and uneven patterns. There hasn't been a thorough analysis of long-term tendencies that change throughout time. The clinical importance of CD-4 and CD-45 expression in patients with non-small cell lung cancer was investigated using tissue microarrays created from the biopsies of 50 patients with non-small cell lung cancer.Immunohistochemistry was performed to identify the expression of CD-4 and CD-45 in a tissue microarray (IHC).Patients' overall survival (OS) was tracked through telephone contact. For statistical analysis, BIM SPSS statistics 22 and GraphPad primes 8 were both employed.The expression of CD-4 and CD-45 in the same patients was significantly positively correlated by immunohistochemical staining (Person correlation=0.4, P<0.0001). According to Kaplan-Meier survival analysis, CD-4high patients had better survival than CD-4low patients;Compared to CD-4lowCD-45low patients, CD-4highCD-45high individuals had a greater OS.The expression of CD-4 and CD-45 in tumor tissue was positively connected with OS and showed a favorable association with CD-4 and CD-45 in non-small cell lung cancer.

Keywords: Non-small cell lung cancer (NSCLC); Immunohistochemistry (IHC); CD-4+; CD-45+; Lung cancer; Tissue microarray.

Introduction:

Non-small cell lung cancer (NSCLC) is one of the most lethal malignant tumors in people and the leading cause of cancer deaths globally.Small-cell lung carcinoma and non-small-cell lung carcinoma (NSCLC) are the two primary subtypes of lung cancer, comprising nearly 15% and 85% of all cases respectively [1].The three subtypes of NSCLC include squamous-cell carcinoma, adenocarcinoma, and large-cell carcinoma [2]. Non-small cell lung cancer immunotherapy aims to increase the patient's cytotoxic T lymphocyte activity, assist in activating lymphoid organ-specific cytotoxic T cells, and create efficient and long-lasting anti-tumor immunity [3].CD-4+ and CD-8+ are two basic type of T cells. By controlling the tumor microenvironment or by removing tumor cells through cytolytic processes, CD-4+ T cells can target and kill cancerous cells. Here, we focus primarily on CD-4 and CD-45's clinical importance in non-small cell lung cancer. Both the B cell response and the activity of cytotoxic T lymphocytes can be improved by CD-4+ T cells. The majority of lymphocytes infiltrating lung cancer are CD-4+ T cells, which are also known as immune system helpers and are involved in a variety of immunological responses [4]. They are crucial for antitumor immunity as they secrete granzyme B and perforin, which kill target cells. Leukocyte Common Antigen (LCA), also known as CD-45, is generally prevalent on the surface of all white blood cells and is also highly expressed on the surface of T cells [5]. The development and maturation of lymphocytes, function control, and signal transmission all depend on CD-45, a crucial molecule for signal transduction on the cell membrane. The expression of CD-4 and CD-45 in non-small cell lung cancer tissues, as well as their connection to predictive survival, were examined for this study to better understand the part the tumor microenvironment plays in the management of lung cancer [6].

Materials and Methods:

Samples:

The biopsies specimen was taken from 50 non-small cell lung cancer patients with medical records from a public sector hospital located at West Bengal, India.The name of the hospital was not disclosed in this study due to the confidentiality clause. The hospital information system was used to acquire the clinicopathological data of patients with tissue samples. Patients who have previously received care elsewhere were not included. For the first two years, patients were followed up with every two months. Then, a progressive increase in the follow-up interval was made. At the conclusion of

the trial, overall survival (OS) was calculated from the date of diagnosis to the date of death or the date of last follow-up. Each patient's clinical and pathological information was also gathered from hospital records. Before being accessible, all patient data were completely anonymized with prior consent.

Tissue Microarray and Immunohistochemistry (IHC):

50biopsy samples of lung cancer from the 50 patients were included in a confirmed, formalin-fixed, paraffin-embedded array of human non-small cell lung cancer tumor tissues.Immunohistochemistry (IHC) was used as a standard procedure for lung cancer specimens. ADC tissue samples were put through the necessary driver mutation tests. The tissue chip was used for performing IHC. The tissue chip slice was 0.04μm thick and it was heated for a duration of 1 hour at 60°Cas per the standard literature reports.Tissues were first deparaffinized and pre-treated at 98°C for 20 min with the Epitope Retrieval Solution (pH = 8.9-9.1). Peroxidase blocking was done for 10 minutes following washing with wash buffer [7].The tissues were once again cleansed before being exposed to the main antibody for 30 minutes. We chose immunological markers for innate and adaptive immunity. The tissue chip was dewaxed, blocked with hydrogen peroxide, antigen-retrieved with citric acid (BL604A, Biosharp), and blocked with goat serum (SL038, Solarbio) for 30 minutes at 37°C. Therefore, incubated overnight at 4 °C with the CD-4 rabbit monoclonal antibody (ab183685, Abcam) and the CD-45 rabbit polyclonal antibody (ab10558, Abcam). The tissue chip was first stained with DAB Chromogenic Kit for 5 minutes at room temperature, followed by counterstaining with Mayer's Hematoxylin solution [8,12]. The tissue chip was then incubated with the HRP-coupled-goat-anti-rabbit secondary antibody (1:500) (abab6721) at room temperature for 2 hours (G1080, Solarbio). Slides were scanned using a Microscope slide scanner.

Digital Image Analysis and IHC:

The DensitoQuant software module was used to evaluate the images taken by the Panoramic Viewer software and to compute the tissue's immune-positive rate.In order to measure immune cell numbers, the proportion of cells with the minimal intensity that were deemed positive was used. A stain-intensity measurement tool called DenstioQuant in Quant Center allows for user-controlled whole-slide analysis on a pixel basis. In order to obtain trustworthy monochromatic intensity values, the IHC signal was first manually adjusted and controlled. Following that, the positive reaction's pixel intensity levels were scaled and shown in three levels: faint (yellow), moderate (orange), and strong (red).Blue-stained nuclei and white, unstained pixels served as a representation of the negative cells' backdrop [9,10].Utilizing the overall pixel area and individual pixel intensity levels, the module automatically determined the immuno-positive rate. Background pixels and ratios for weak, moderate, and strong positives were also created. For further analysis, the immuno-positive rate indicating particular immunolabeling on each plate was used.

Statistics:

For statistical analysis, immuno-positive rates below 35% and over 35% were defined as high and low expression, respectively.The expression of CD-4 and CD-45 in non-small cell lung cancer tissues was examined using IBM SPSS Statistics 22 software's chi-square test, and the association between CD-4 and CD-45 expression and patients' overall survival was examined using GraphPad Primes 8's Kaplan-Meier method [11].

Results:

lung cancer tissue chip expression of CD4 and CD45:

The expression of CD-4 and CD-45 in non-small cell lung cancer tissue was examined using an immunohistochemical study of lung cancer tissues on a microarray chip. The total of 47 samples were evaluated in this experiment after three of the 50 samples were eliminated due to serious tissue damage.According to Figure 1(A), a positive rate for CD-4 or CD-45 that was higher than 35% was regarded to have a high expression (CD-4^{high}, CD-45^{high}), whereas a positive rate that was lower than 35% was considered to have a low expression (CD-4^{low}, CD-45^{low}).Out of the 47 tissue samples, there were 18 samples with CD-4^{high} expression, 29 samples with CD-4^{low} expression; 19 samples with CD-45^{high} expression, and 28 samples with CD-45^{low} expression. Analyzed by SPSS software, 12 out of 47 samples (25.53%) are CD-4^{high} CD-45^{high}, and 22 out of 47 samples (46.80%) are CD-4^{low} CD-45^{low} expression [Figure 1(B)].Person correlation was determined using SPSS, and it was 0.4 ($P<0.0001$). The findings demonstrated a substantial link between the two expressions, and this association was statistically significant [Figure 1(B)]. We performed a T-test to evaluate the connection between CD-4 and CD-45. The study of the CD-4 and CD-45 expression in 50 cancer tissues. According to the findings, $r = 0.9$, $P < 0.0001$. In non-small cell lung cancer tissue, CD-4 and CD-45 are linked.

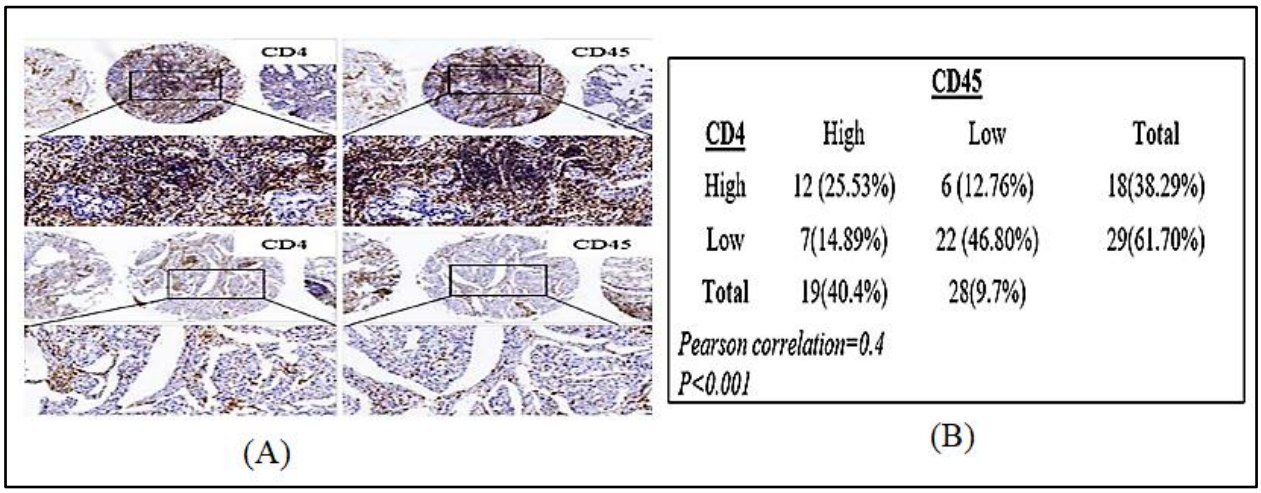

Figure 1.(A) Immunohistochemical analysis of CD-4 and CD-45 expression ;(B) Pearson correlation analysis based on the expression of CD-4 and CD-45 in immunohistochemistry (IHC).

Association between prognosis and CD-4 CD-45 expression:

We used tissue microarray to acquire the positive rate of gene expression in 50 non-small cell lung cancer patients in order to examine the association between the expression of CD-4/CD-45 and the predictive survival of patients (with 3tissue-damaged samples removed).Telephone follow-up was used to determine the survival time of the samples that were included; according to 60-month data, the survival time is larger than 60 months. The statistical analysis and artwork were done in GraphPad Primes 8.The findings demonstrated that patients with CD-4^{high} had substantially longer prognosis survival times than patients with CD-4^{low}. Between CD-45^{high} and CD-45^{low} patients, there was no discernible difference in overall survival(OS).We separated 47 samples into four groups: CD-4+CD-45+, CD-4-CD-45+, CD-4+CD-45-, in order to determine the effect of CD-4 and CD-45 co-expression on the prognosis of patients with non-small cell lung cancer.The Kaplan-Meier survival analysis was performed on the CD-4+CD-45+ and CD-4-CD-45- groups. The results showed that the survival period was much longer for CD-4+CD-45+ than for CD-4-CD-45-. Consequently, it can be concluded that increasing the expression of CD-4 and CD-45 can enhance non-small cell lung cancer patients' prognosis for survival.

Discussion and Concluding Remarks:

The largest cause of cancer-related death worldwide is lung cancer, and this is likely to be the case for the foreseeable future. The GLOBACON report for 2018 states that lung cancer killed 1.8 million people, accounting for 18.4% of all cancer-related fatalities, and affected an estimated 2.1 million people (11.6% of all cancers). According to the aforementioned data, 48,698 (8.5%) of the 67,795 new cases of lung cancer that were diagnosed in India in 2018 were in men. This figure represents 5.9% of all cancer cases [13,18]. In addition, lung cancer contributed to 63,475 fatalities or 8.1% of all cancer-related deaths [14].

The majority of cases of lung cancer are non-small cell lung cancer (NSCLC). There are known risk factors for developing NSCLC, with smoking being a significant risk factor along with other environmental and genetic risk factors. Patients may be eligible for several therapies, including surgery, radiation therapy, chemotherapy, and targeted therapy, depending on the stage of their lung cancer [15]. Specific mutations have been uncovered to better target treatment for particular patients thanks to genetics and biomarkers testing advances. This review discusses the most recent therapies, such as surgery, chemotherapy, radiotherapy, and immunotherapy, as well as how biomarker testing has increased patients with NSCLC's survival rates. According to preliminary research conducted in our lab, there was a general drop and a lower absolute CD-4 count in lung cancer patients than the reference value. T lymphocytes have the surface molecules CD-4 and CD-45 [16]. They will cause the active expression and secretion of several cytokines, such as IL-4, IL-5, IL-6, IL-10, and IL-13, when activated by antigen stimulation, which will hasten immune regulation and enhance immune resistance function [17]. This study discovered a statistically significant link between the expression of CD-4 and CD-45 in non-small cell lung cancer tissues. This related action may be mediated by CD-45 when combined with the cell signaling effect of CD-45.

Conflict of Interest:

No potential conflict of interest was reported among the authors in this reported study.

Author's Contribution Statement:

All the authors have equal contributions to this reported study.

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