



Reduced Natural Killer Cells Percentage in Patients with Lung Cancer

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Abstract:

Increased as well as decreased percentages of NK cells of lung cancer patients in comparison to healthy control subjects have been reported. Percentage of NK cells in the peripheral blood were analyzed in 30 patients with lung cancer and 16 subjects of healthy control by flow cytometer technique using CD56⁺CD3⁻ marker. Our result indicated that 13.3% of NK cells percentage of lung cancer patients was within normal range (with mean value 6.250 ± 0.30204 %) compared with 25% of healthy control subjects (with mean value 10.500 ± 0.84764) as compared with normal NK cells percentage (5-15%).

Keywords: Natural Killer cells, Flocytometer, lung cancer.

نقص النسبة المنوية لخلايا القاتلات الطبيعية في مرضى سرطان الرئة

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الخلاصة:

زيادة نسبة Natural Killer cells إضافة إلى نقصانها في مرضى سرطان الرئة بالمقارنة مع الأصحاء تم إثباته. تم تحليل مستويات القاتلات الطبيعية في الدم المحيطي في 30 من مرضى سرطان الرئة و 16 من الأصحاء من قبل تقنية التدفق الخلوي باستخدام معلمات الخلايا CD56⁺CD3⁻. أشارت دراستنا إلى إن 13.3% من مستوى القاتلات الطبيعية في مرضى سرطان الرئة كان ضمن المعدل الطبيعي مع القيمة المتوسطة (6.250 ± 0.30204 %) مقارنة مع 25% للأصحاء مع القيمة المتوسطة (10.500 ± 0.8476) بالمقارنة مع المستويات الطبيعية للقاتلات الطبيعية (5-15%).

Introduction:

Natural killer cells (NK) are a type of cytotoxic lymphocyte critical to the innate immune system; have gradually been recognized as a distinct lymphoid subpopulation ,initially by their large granular morphology and more recently by a CD-3, CD16+/- or CD56+ cell surface phenotype [1,2].

Typically immune cells detect MHC (Major histocompatibility complex) presented on infected cell surfaces, triggering cytokine release causing lysis or apoptosis. NK cells are unique, they have the ability to recognize stressed cells in the absence of antibodies and MHC, and they do not require prior sensitization for lytic activity [3]. They account for about 5 to 15% of circulating lymphocytes [1,4]. The ability to reliably measure NK activity in human body fluids or tissues and to enumerate cells which express NK cell-associated surface markers has contributed considerably toward a better definition of NK cell involvement in human diseases. NK cell participates either directly or indirectly

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in multiple developmental, regulatory, and communication networks of the immune system. They represent an efficient effector cell that is not only equipped for killing but is also capable of rapid response to exogenous or endogenous signals by producing a variety of cytokines and factors involved in interactions between immune and non immune cells by their production of interferon-gamma (IFN- γ). IFN- γ is a key cytokine that drives antitumor cellular immune response, it is critical for activation of macrophages and resistance to intracellular bacterial infections [5-7].

The NK cell phenotype is defined by their expression of neural cell adhesion molecule (NCAM) (CD56) and lack of expression of the T cell marker CD3 [2,8]. Peripheral blood NK cells is recognized in to two distinct populations (CD56^{bright} & CD56^{dim}) where initially description of them in healthy individuals was based on their cell surface density of CD56 [6,9,10], this considered as NK cell markers for clinical and basic research purposes, when most NK cells (~90%) express low levels of CD56 and are designated CD56^{dim} while a minority (5-10%) express high levels and are subsequently called CD56^{bright} NK cells [6]. This research was accomplished using the technique of flow cytometry and since there are no or little local studies estimated the natural killer cells percentages in diseased and healthy subject by this technique the goal of this research is to estimate of NK CD56 percentage in lung cancer patients and healthy Iraqi subjects using flow cytometry technique.

Materials and Methods:

Subjects and blood samples collection:

Subject involved: 30 lung cancer patients and 16 healthy Iraqi subjects. Blood samples were collected from 30 lung cancer patients admitted to Hospital of Radiotherapy and Nuclear Medicine .While 16 individuals; were healthy Iraqi subjects (lived in different places of Baghdad), blood samples collection started during September 2012 and lasted till April 2013 In Biotechnology research center/Al-Nahrin University. Three milliliters (3ml) of venous blood were taken by venipuncture from all patients and healthy subjects included in this study and put in vacuumed tube with anticoagulant (0.2% EDTA) and gently shaken. These samples should be worked within 24 hr.

Human peripheral blood mononuclear cells isolation:

The Ficoll-isopaque separation fluid is a sterile, ready to use for purification of lymphocytes in high yield and purity from small or large volumes of human peripheral blood, using a simple and rapid centrifugation procedure based on the method which developed by [11,12].

Viable counting of isolated lymphocytes:

The cell count and viability were determined by procedure of [13]. Viable cells were determined by trypan blue solution within (24) hrs of blood collection, to distinguished round and small lived cells from dead cells that appeared swollen, large, dark blue with loss of membrane integrity.

Detection of CD3⁻ & CD56⁺ NK cells subset by Flow cytometry:

Two color Fluorescein isothiocyanate (FITC)-anti human-CD56 monoclonal antibody, and Phycoerythrin (PE)-anti human-CD3 monoclonal antibody [14] provided by (USBiological /USA) were used to determine the immunophenotype of NK cells (defined by their expression of CD56 and lacked the expression of T cell marker CD3 [8]). Isolated lymphocytes were gated on a forward scattered versus- side scattered cytogram.

Two hundred μ l of peripheral blood mononuclear cells (PBMCs) for each sample was transferred into two separated sterile Eppendorf tubes, one tube with marker and the second without marker (the same treated but without marker), one Eppendorf tube was subjected to CD3 & CD56 estimation protocol (USBiological/USA) using 10 μ l of FITC (for CD56 marker), and EP (for CD3 marker) – conjugated monoclonal anti- human CD marker. Each tube was incubated in the dark at room temperature for 30 min, then treated with (500 μ l) of lysis buffer solution and incubated in the dark at room temperature for 20 min, washed with deionized water, and centrifuged at 300g for 5min, The pellet was suspended with PBS (500 μ l), and analyzed by flow cytometer (Apogee/USA) to read relatively amount of CD3⁻ CD56⁺ marker.

Results and discussion:

Estimation the percentage of NK cells by CD3⁻ CD56⁺:

Table 1 showed the percentage of NK cells in lung cancer patients and healthy Iraqi subjects. Percentage of NK cells was calculated out of total lymphocytes.

According to many researches, the normal content of NK cells comprise 5-15% of all lymphocytes [3,15]. From the results of the present study (Table-1), it was found that the mean value of NK cells percentage for total lung cancer patients was 2.4333 \pm 0.30204 and for total healthy Iraqi subjects was

5.8125±0.84764. When compared these results of NK cells percentage for subjects of lung cancer patients and healthy control blood samples with the normal content of NK cells 5-15%, we showed that 13.3% of lung cancer patients (with mean value 6.250±0.30204) represented by four patients (P4, P27, P29, P30), and 25% of healthy control subjects (with mean value 10.5000±0.84764) represented by four control subjects only (S1, S2, S5, S10) was appeared within the normal range of NK cells. This may indicated that the four patients have stabilized disease while the other 26 patients have progressive one.

Natural Killer cells represent the innate first line of defense for cellular immunity, by sensing transformed tumor cells in the blood stream and killing them thus it ultimately prevent the formation of tumor mass [15,16].

Chemotherapy treatments for cancer patients cause decrease of NK cells after two weeks. These number reached to the lowest level during third and fourth weeks and this fact varies according to the drug used and the stage of the disease [17,18]. Decreased activity or low numbers of circulating NK cells in peripheral blood have been reported to be associated with progression of cancer in humans [19].

Also NK cells suppressed during radiotherapy and returned to pre-radiotherapy baseline levels by the end of the 6-week post-radiotherapy observation period [20] but this depend on the dose of radiation (high dose and chronic low dose caused suppression to immune cells especially T, B, NKT, and NK cells). Other subjects of 26 lung cancer patients (86.6%) appeared with NK cells percentage below the baseline level ,where lung tumors induce aberrant expansion of CD4+ regulatory T cells, and inhibits cytotoxic T-cell and natural kill cell activity [21]. Results of present study may be explained according to Al Omar & his group who demonstrated that there were no significant differences in the median proportions of NK cells for patients compared with healthy control [22].

It was found in a study in the same field that patients with high absolute numbers and activity of NK cell noted at the end of therapy, as many as 70% to 80% of the peripheral blood lymphocytes (PBLs) were NK cells during the time of evaluation because of the depletion of T cells and B cells during chemotherapy, NK cells did not show significant declines in respond to chemotherapy according to the drug used ,as showed by many studies , also it was observed that NK cells play an important role in the host response against fungal infections and other opportunists in cancer patients and this fact may explain the simultaneous of percentage in patients of cancer [23]. As shown in figure 1.

Table 1-NK cells percentage in lung cancer patients and healthy Iraqi subjects.

	CD56 cells (%)in lung cancer patients			CD56 cells(%)in healthy control
P1	2		S1	13
P2	1		S2	12
P3	1		S3	4
P4	9		S4	4
P5	2		S5	9
P6	3		S6	2
P7	1		S7	4
P8	1		S8	4
P9	2		S9	1
P10	2		S10	8
P11	3		S11	4
P12	1		S12	4
P13	1		S13	2
P14	1		S14	4

P15	1		S15	2
P16	2		S16	1
P17	4			
P18	3			
P19	4			
P20	2			
P21	3			
P22	3			
P23	1			
P24	1			
P25	1			
P26	1			
P27	6			
P28	1			
P29	5			
P30	5			

P=Patient

S=Healthy control

The results of the present study indicated that 75% of healthy control subjects appeared with NK cells ranged below the baseline level 5-15%, while 25% were with normal range, this might be attributed to other diseases since it was found that decreased or absent NK cell numbers or activity is often associated with the development or progression of cancer, acute or chronic viral infections, autoimmune diseases, immunodeficiency syndromes, and psychiatric illness [15].

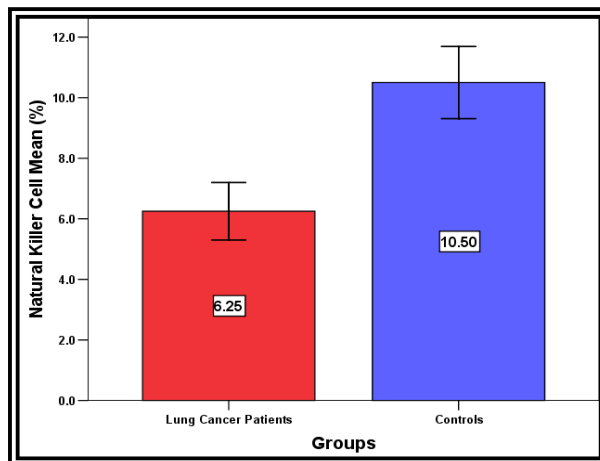


Figure 1-NK cells percentage in cancer patients and negative control.

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