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Study the effect of natural killer cells in pationts with blood cancer disease from other when the injury-infected corona emerging

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***Abstract*---**Lymphocytes and subgroups of natural killer cells (CD16 and CD56) play an important role in maintaining the function of the immune system. After infection with COVID-19, the total number of lymphocytes and subgroups changes, indicating a possible association between lymphocyte subgroup change and causative mechanisms. For viral diseases, the study showed a clear decrease in peripheral lymphocytes in patients with coronavirus-19. The reason may be attachment to the virus or indirectly due to immune injuries from inflammatory mediators. It is possible that the secretion of lymphocytes circulating in inflammatory lung tissues also leads to lymphocytopenia. And the viral infection can cause an imbalance in the levels of lymphocyte subgroups, as the cell surface molecules of CD16 and CD56 natural killer cells participate in the humoral and cytotoxic immunity against viral infection, where a decrease in the level of these cells was observed less in severe cases compared to mild cases. Patients with lymphocytic leukemia often suffer from weak immune status When they are exposed to risk factors for contracting COVID-19.

***Keywords*---**blood cancer, COVID-19, Lymphocytes.

Introduction

Corona virus-19 is an infectious disease caused by the SARS-Covid-2 virus. This virus is constantly evolving with changes in the genetic code (genetic mutations) during genome replication, and genetic strains of SARS-Covid-2 virus have appeared and spread throughout the world since the beginning of the pandemic Corona-19 (Zhou et al., 2021), and the virus can cause mild to severe respiratory infections in humans, and because it is highly contagious, it has overwhelmingly

surpassed SARS and Middle East respiratory syndrome in terms of the number of infected and the spatial extent of the epidemic areas (Hu et al. , 2021).

Natural killer cells are a type of cytotoxic lymphocyte because they have a role similar to that of (CD8+T cytotoxic T cells) in the adaptive immune response. Natural killer cells provide rapid responses to cells infected with virus and other pathogens, they form a bridge Between innate and adaptive immune responses, however, these cells are unique in that they have the ability to recognize and kill stressed cells in the absence of antibodies and the MHC, allowing for a much faster immune response. Natural killer cells are renowned for detecting and controlling early signs of cancer. (Eissens et al., 2012), and recent studies showed a significant decrease in the subgroups of natural killer cells in patients infected with COVID-19 compared to healthy people, where the change of the surrounding lymphocyte subset was associated with clinical characteristics of COVID-19 (Wan et al., 2012). ., 2020), which should be noted that the decrease in the number of surface molecules of natural killer cells in the disease The number of people infected with SARS-CoV-2 than the normal limit is less in severe cases than in moderate and mild cases, where the proportion of (NK) negatively correlated with inflammatory indicators through an increase in the levels of interleukin-6 IL-6, interleukin-10 IL-10 and C-reactive protein (CRP) C-Reaction Protein), a protein made by the liver that is sent to the bloodstream in response to inflammation when an infection occurs, in addition to an increase in the erythrocyte sedimentation rate (ESR) in patients infected with the virus, which reveals the presence of an active infection in the body and helps diagnose Inflammation or following the progress of the disease by knowing the rate of red blood cells (F. Wang et al., 2020).

Studies have indicated a clear decrease in surrounding lymphocytes and natural killer cells in COVID-19 patients with lymphomas. % to 4.2%, and there was an increase in the percentage of cells with cytotoxic potential, as SARS-CoV-2 caused the release of large amounts of cytokines, and lymphocytes decreased by less in patients with severe cases than in patients in moderate cases (Pasin et al., 2020)

Materials and Methods

The study included (90) people in three groups, the first group included 30 cases of patients infected with Corona-19 virus, who were confirmed to have contracted the virus by PCR smear, and their ages ranged between (16-60 years), and the second group included 30 cases of leukemia patients (where they were Relying on previous diagnostic reports in the records of patients in cancer centers) who were confirmed to be infected with the Corona virus-19 by PCR swab, their ages ranged between (16-60 years), and the third group was represented by the control group and it included 30 apparently healthy people. Their ages ranged from (16-60 years), as the study samples were collected from the Al-Zahraa Center for Expatriates and the centers of cancerous tumors and blood diseases in Karbala, Najaf and Babylon after they were diagnosed through clinical and laboratory tests, for the period from October 2021 to April 2022 after obtaining official approvals and with the help of the staff The medical supervisor supervising the disease in these institutions, and the control group (the healthy ones) were selected similarly in terms of age and gender, with a total of 30 samples

In the study of the immunophenotypic pattern of surface markers, the use of a six-color flowcytometry device was adopted, and different fluorochromes can be used to distinguish between separate sub-populations, where the fluorescent compound absorbs the light energy, and the electronic system then converts the optical signals into electronic signals that can be Computer processed, using fluorescent markers to detect expression of cellular molecules (Büscher, 2019)

Method for measuring the level of CD16 and CD56 in the blood using a flow cytometry device :
(100µl) of blood was placed in an Eppendrof tube, (5 µl) of (CD16 KIT and using fluorescein isothiocyanate) (FITC) or (CD56 KIT and FITC)(fluorescein isothiocyanate) is added to the tube The blood container, then the sample was placed in the vortex device for five seconds, then the samples were placed in the incubator at room temperature for 30-45 minutes, then (100 µl) of Erythrocyte lysing (reagent A) was added, the sample was placed in the vortex device for five seconds and left Sample for 10 minutes in a dark room To get rid of RBC in the sample, (1 ml) of Erythrocyte lysing (reagent B) was added to the sample and the sample was placed in a vortex device for five seconds and then left in a dark room at room temperature for 20 minutes, the sample was placed In the Micro centrifuge apparatus for five minutes at a speed of 5000 revolutions / min, after that we obtained a precipitate and a filtrate, the precipitate is WBC and the filtrate is RBC, the filtrate RBC was eliminated, (1 ml) of Buffer solution was added to the sample, and the sample was placed in the Micro centrifuge device for Five minutes at a speed of 5000 revolutions per minute for the purpose of getting rid of impurities, and after getting rid of impurities, (600 microliters) of buffer solution was added to the precipitate, the sample was transferred to a plastic test tube or the so-called can tube, and the sample was placed in a Flow cytometry device, Result read in less than 1 minute.

Statistical Analysis

All analyzes were performed using the program (Statistical package for the social acience) (SPSS.V.28) and the following tests were used: Ancova, Least significant defferences

Results and Discussion

A cluster of differentiation (CD) is a protocol used to identify and investigate cell surface molecules present in leukocytes, providing targets for immunophenotyping of cells. receptor-activated part) is important for the cell (Zola et al., 2005). The surface marker CD16 is a group of differentiation molecules found on the surface of natural killer cells, neutrophils, monocytes, and macrophages, functions as a transmembrane receptor and participates in signal transduction, and has normal cytotoxicity. low, and this marker also plays an important role in the early activation of natural killer cells (Cooper et al., 2001), and the surface marker CD56 is a homologous glycoprotein that is highly associated with natural killer cells as well as dendritic cells, and is also expressed on the surfaces of neurons and glia and skeletal muscle, and it is seen as a neuronal cell adhesion molecule because of its role in cell adhesion, neurotrophic factors, learning and memory (Van Acker et al., 2017). The current study shows

that natural killer cells show dysfunction in patients infected with COVID-19 if CD16, CD56 values decrease, and this decrease is correlated with the intensity of inflammation and may result from programmed cell death caused by COVID-2 virus (Bi, 2022).

The current study showed a marked imbalance in the immune system through a decrease in the numbers of natural killer cells (CD16, CD56) in patients with lymphatic malignant blood diseases and those infected with Coronavirus-19 and patients with Coronavirus-19 without blood diseases, as patients with Of the blood diseases and those infected with the virus, there is a decrease in the number of surface markers at a higher rate compared to patients infected with Corona-19 virus and not infected with blood diseases (Kalicińska et al., 2021). Lymphocytes or by immune apoptosis of lymphocytes (chu et al., 2016), the normal antiviral immune response requires activation of the inflammatory pathways of the immune system leading to excessive production of pro-inflammatory cytokines that lead to lymphocyte depletion and exacerbation of distress syndrome Acute respiratory and large-scale tissue damage leading to multiple organ failure (Ragab et al., 2020), possible causes of vinegar depletion may include Lymphocytes in the context of SARS-CoV-2 infection: suppression of bone marrow, migration of T cells into inflamed tissues, directly (via ACE-2 receptors) and indirectly (by stimulation of the proinflammatory cytokines IL-6 and TNFa or production of metabolic molecules such as lactic acid). , which may inhibit lymphocyte proliferation), lymphocyte destruction (Tan et al., 2020).

Table No. (1) the differences recorded between the values of the surface marker CD16 in the three experimental groups (the healthy group, the group of lymphocytic leukemia patients infected with Coronavirus, the group of patients infected with Coronavirus without leukemia patients)

N	S. D	Mean	Age categories	Gender	The sample
6	3.57	11.93	16-25	Male	control
2	0.00	13.50	26-35		
4	0.70	8.30	36-45		
2	0.71	7.80	46-55		
2	0.14	7.70	More then 55		
16	3.10	10.18	Total		
4	5.60	14.65	16-25	Female	
4	0.61	11.05	26-35		
4	0.81	10.20	36-45		
2	0.07	11.55	More then 55		
14	3.31	11.91	Total		
30	3.26	10.98	Total		
5	2.12	10.12	16-25	Male	Hematology with covid-19
1		7.70	26-35		
3	2.02	6.17	36-45		
3	2.80	8.47	46-55		
4	3.14	7.68	More then 55		
16	2.63	8.31	Total		
5	2.38	5.10	16-25	Female	
1		11.70	26-35		

3	3.17	7.40	36-45		
2	1.91	5.35	46-55		
3	3.84	7.60	More then 55		
14	3.05	6.64	Total		
30	2.91	7.53	Total		
5	3.17	8.66	16-25	Male	covid-19
3	3.02	9.10	26-35		
4	2.16	9.70	36-45		
1		6.00	46-55		
3	3.31	6.57	More then 55		
16	2.81	8.44	Total		
5	4.16	8.56	16-25	Female	
3	1.01	6.30	26-35		
2	1.56	10.40	36-45		
1		4.90	46-55		
3	2.85	9.93	More then 55		
14	3.17	8.37	Total		
30	2.93	8.41	Total		
30	4.34	9.81	16-25	Total	
14	2.79	9.77	26-35		
20	2.20	8.72	36-45		
9	2.19	6.96	46-55		
17	2.95	8.32	More then 55		
			1.44	the sample lsd	
			1.66	age categories lsd	

The results of Table (1) also showed that there were no significant differences according to the sample as well as according to the age at the level of P≤ 0.05 in the percentage of CD16 in lymphatic leukemia patients infected with Corona virus and patients infected with Corona virus without leukemia compared with the healthy group, where the level of CD16 decreased in Patient samples compared with the healthy group

Table No. (2) The differences recorded between the values of the surface marker CD56 in the three experimental groups (the healthy group, the group of lymphocytic leukemia patients infected with Coronavirus, the group of patients infected with Coronavirus without leukemia patients)

N	S. D	Mean	Age categories	Gender	The sample
6	0.98	15.40	16-25	Male	control
2	1.34	13.25	26-35		
4	0.78	13.30	36-45		
2	0.57	10.90	46-55		
2	0.78	10.75	More then 55		
16	2.00	13.46	Total		
4	1.40	14.45	16-25	Female	
4	0.95	13.03	26-35		
4	2.22	14.60	36-45		

2	0.21	11.05	More then 55		
14	1.85	13.60	Total		
30	1.89953	13.5267	Total		
5	2.93	8.90	16-25	Male	Hematology with covid-19
1		12.90	26-35		
3	2.75	10.97	36-45		
3	2.75	12.17	46-55		
4	3.48	7.23	More then 55		
16	3.29	9.73	Total		
5	2.49	8.50	16-25	Female	
1		13.60	26-35		
3	1.42	9.87	36-45		
2	2.47	6.05	46-55		
3	0.76	12.33	More then 55		
14	2.85	9.63	Total		
30	3.04	9.68	Total		
5	3.41	12.20	16-25	Male	covid-19
3	2.30	12.27	26-35		
4	2.09	12.75	36-45		
1		9.80	46-55		
3	4.11	8.37	More then 55		
16	3.13	11.48	Total		
5	2.70	9.14	16-25	Female	
3	2.03	10.50	26-35		
2	0.07	13.95	36-45		
1		3.70	46-55		
3	4.72	11.07	More then 55		
14	3.51	10.14	Total		
30	3.33	10.86	Total		
30	3.63	11.49	16-25	Total	
14	1.73	12.39	26-35		
20	2.31	12.65	36-45		
9	3.61	9.32	46-55		
17	3.34	9.87	More then 55		
			1.23	The sample lsd	
			1.37	Age categories lsd	

The results of the test in Table (2) showed that there were significant differences according to the sample as well as according to the age with a statistical significance at the level of P≤ 0.05 in the percentage of CD56 in lymphatic leukemia patients infected with Corona virus and patients infected with Corona

virus without leukemia compared with the healthy group, where The level of CD56 decreased in patient samples compared with the healthy group, and this decrease was significantly reduced

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