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The difference in the immunological parameters of T-cells and B-cells for patients with cancerous blood diseases compared to others when infected with the emerging corona virus

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***Abstract*---**Lymphocytes and T-cell subsets (CD4+T and CD3+T) and CD19 B cells play an important role in maintaining the function of the immune system. The study showed a clear decrease in peripheral lymphocytes in patients with COVID-19, and the reason may be attachment to the virus or indirectly due to immune injuries from inflammatory mediators, and it may lead to the secretion of lymphocytes circulating in the lung tissue Inflammatory conditions also lead to lymphocytopenia, and viral infection can cause an imbalance in the levels of lymphocyte subgroups. The cell surface molecules of CD3 and CD4 T cells and CD19 B cells participate in the humoral immunity against viral infection. 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 infection with the Corona virus-19

***Keywords*---**immunological, T-cells, B-cells, cancerous blood, corona virus.

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).

Repentant cells are part of the immune system. They coordinate multiple aspects of the adaptive immune response, including responses to pathogens, allergens, and tumors. They can be responsible for inflammatory or autoimmune diseases. T cells are distinguished from other cells by the presence of T cell receptors. T Cell Recepter on the surface of the cell, and it is not activated until it finds its specific antigen, and T cells can turn into memory cell cells that help in eliminating the antigen (B. V. Kumar et al., 2018). The number of T-lymphocytes and the severity of COVID-19 has been observed. As the disease progresses, CD4+ T cells and CD3+ T cells decrease significantly in critically ill patients and to a lesser extent in moderately ill patients. Excessive activation of T cells after infection is associated with an overexpression of TH17. This explains the observations of immune damage in patients. Th1 activation of pathological cells leads to an increase in interleukin-6 (IL-6), which causes monocytes to function. Inhibition of GCSF or IL-6 is expected to prevent immune damage and is a potential mechanism involved in disease deterioration Corona virus-19 (Yang et al., 2021).

B cells are a type of lymphocyte responsible for the humoral immune component of the adaptive immune system. These cells produce antibodies, which play a key role in immunity, as they work to prevent foreign substances from harming the body, and they contain B cell receptors on their surface, which they use to bind Once the B cells bind to the protein called the antigen, they release antibodies that stick to the antigen and prevent it from harming the body and present it as prey to T cells, which swallow and digest it and eliminate this intruder. Then they secrete cytokines to attract other immune cells, in addition to its prominent function in the field of hematology (Lebien & Tedder (2008), CD19 frequency in B cells is increased in severe COVID-19 cases compared to mild cases, and these lymphocytes can serve as potential biomarkers and even active participants in adaptive antiviral responses against SARS-CoV-2. The humoral immune response, which is based primarily on the production of neutralizing antibodies by plasma cells, performs a protective function. This can be done by controlling infection in later stages of the disease and possibly preventing future re-infection (Sosa-Hernández et al., 2020).

Multiple studies also showed a marked defect in the immune system through a decrease in the numbers of T cells (CD4, CD3) in patients with blood diseases and those infected with Coronavirus-19, and patients with malignant blood diseases affected by Coronavirus-19 are characterized by a lack of cells Lymphatic, the lack of T-lymphocytes during infection with COVID-19 is associated with impaired inflammatory response, which is manifested by an increase in proinflammatory cytokines (IL-10, IL-2-IL-4, TNFa, TNFg), which in turn can cause severe lung injury during infection. SARS infection Possible causes of lymphocyte depletion in the context of SARS-Covid-2 infection may include bone marrow suppression and T-cell migration into inflamed tissues directly by ACE-2 receptors and indirectly by stimulation of proinflammatory cytokines (IL-6, TNFa) or production

of T-cells. Metabolism such as lactic acid, lymphocyte destruction (Kalicińska et al., 2021)

Materials and Working 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 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. Reliance in the study of the immunophenotypic pattern of surface markers using a six-color flowcytometry device, 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 light signals into electronic signals that can be processed By computer, fluorescent markers are used to detect expression of cellular molecules (Büscher, 2019).

Method for measuring the level of CD3, CD4 and CD19 in the blood using a flow cytometry device

(100µl) of blood was placed in an Eppendrof tube, then (5 µl) of (CD3 KIT and using fluorescent antibodies phycoerythrin (PE)) or from (CD4 KIT and using fluorescent antibodies phycoerythrin (PE)) or from (CD19 KIT and using fluorescent antibodies (Percp)) to the tube containing blood, the sample was placed in a vortex device for five seconds, the samples were placed in the incubator at room temperature for 30-45 minutes, (100 µl) of Erythrocyte lysing (reagent A) was added. The sample was placed in a vortex device for five seconds and the sample was left 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 a micro centrifuge device for five minutes at a speed of 5000 rpm, after that we obtained a precipitate and a filtrate, the precipitate is WBC and the filtrate is RBC, the filtrate RBC was discarded, and (1 ml) of the Buffer solution was added. To the sample, the sample was placed in a M. device micro centrifuge for five minutes at a speed of 5000 revolutions per minute for the purpose of getting rid of impurities, and after getting rid of the impurities, (600 µl) 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 device Flow cytometry, the result was read in less than one 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. The part that activates the receptor) is important for the cell (Zola et al., 2005), and the CD3 surface marker is a protein complex and a co-receptor involved in the activation of both CD8 cytotoxic T cells and CD4 helper T cells, and this antigen remains present in all T-cell lymphomas. and leukemia and therefore can be used to distinguish them from phenotypically similar B-cell tumors and myeloid tumors (Chetty & Gatter, 1994), and the CD4 surface marker is a type of lymphocyte that helps coordinate the immune response by stimulating other immune cells such as macrophages, B lymphocytes, and B cells CD8 toxic T lymphocytes and that depletion of these markers during viral infection leads to a weakening of the immune system (Luckheeram et al., 2012), where lymphocytosis is one of the main laboratory findings in patients with COVID-19 with diagnostic and prognostic potential, as dysregulated immune responses are involved in the pathogenesis of COVID-19 disease. CD4 increased with increasing disease severity, where a slight decrease was observed in patients with mild to moderate symptoms and a severe decrease in persons with severe disease compared to healthy people (Bobcakova et al., 2021), and as the surface marker CD19 transmembrane protein present On the surfaces of B cells, it plays two major roles in human cells, as it acts as a transducer protein to recruit cytoplasmic signaling proteins to the membrane and on the other hand acts to reduce B-cell receptor signaling. Immunohistochemistry for leukemia (K. Wang et al., 2012), and in vitro studies indicate that these lymphocytes can serve as potential biomarkers and even active participants in the anti-coronavirus response, as the frequency of CD19 is increased in mild to moderate cases of COVID-19 infection compared to cases severe as it decreases with the severity of the disease (Sosa-Hernández et al., 2020).

The current study showed a marked imbalance in the immune system through a decrease in the numbers of T-cells (CD3,CD4) and B-cells (CD19) in patients with lymphatic malignant blood diseases and those infected with Coronavirus-19 and patients infected with Coronavirus-19 without blood diseases, as Patients with blood diseases and infected with the virus are characterized by a significantly lower number of surface markers compared with patients infected with COVID-19 and without blood diseases (Kalicińska et al., 2021). COVID-19 affects lymphocytes or by immune apoptosis of lymphocytes (chu et al., 2016), and the normal antiviral immune response requires activation of the inflammatory pathways of the immune system, which leads to the excessive production of pro-inflammatory cytokines that leads to cell depletion. lymphatic, exacerbation of acute respiratory distress syndrome and widespread tissue damage leading to multiple organ failure (Ragab et al., 2020). Possible causes may include: The

effects of lymphocyte depletion in the context of SARS-Covid-2 infection: suppression of bone marrow, migration of T cells to inflamed tissues, directly (via ACE-2 receptors) and indirect (by stimulation of the proinflammatory cytokines IL-6 and TNFa or production of metabolic molecules such as Lactic acid, which may inhibit lymphocyte proliferation), destroy lymphocytes (Tan et al., 2020)

Table 1
The differences recorded between the values of the surface marker CD3 in the three experimental groups (the healthy group, the group of lymphocytic leukemia patients infected with the Corona virus, the group of patients infected with Corona virus without leukemia patients)

N	S. D	Mean	Age categories	Gender	The sample
6	5.06	54.24	16-25	Male	control
2	0.00	66.70	26-35		
4	1.79	49.45	36-45		
2	0.00	48.70	46-55		
2	0.00	51.40	More then 55		
16	6.38	53.55	Total		
4	13.78	64.07	16-25	Female	
4	1.33	67.15	26-35		
4	16.63	69.20	36-45		
2	0.00	42.90	More then 55		
14	13.69	63.39	Total		
30	11.40	58.14	Total		
5	5.39	50.56	16-25	Male	Hematology with covid-19
1		53.30	26-35		
3	13.01	44.43	36-45		
3	8.65	48.90	46-55		
4	11.07	35.13	More then 55		
16	10.42	45.41	Total		
5	7.64	41.96	16-25	Female	
1		45.10	26-35		
3	11.75	44.27	36-45		
2	7.71	29.15	46-55		
3	9.51	50.80	More then 55		
14	10.11	42.74	Total		
30	10.19	44.17	Total		
5	12.52	49.74	16-25	Male	covid-19
3	9.41	48.37	26-35		
4	4.12	47.50	36-45		
1		39.00	46-55		
3	11.43	43.40	More then 55		

16	9.18	47.06	Total		
5	11.80	42.76	16-25		
3	4.94	40.90	26-35		
2	2.62	52.15	36-45		
1		34.20	46-55	Female	
3	8.87	43.60	More then 55		
14	8.89	43.27	Total		
30	9.10	45.29	Total		
30	11.32	50.23	16-25		
14	12.17	54.87	26-35		
20	12.88	51.75	36-45	Total	
9	10.25	41.73	46-55		
17	9.79	43.68	More then 55		
			4.65	The sample lsd	
			7.69	Age categories lsd	

The results of the test in Table (1) 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 CD3 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 CD3 decreased in the samples of patients compared with the healthy group, and this decrease was significantly reduced

Table 2
The differences recorded between the values of the surface marker CD4 in the three experimental groups (the healthy group, the group of lymphocytic leukemia patients infected with the Corona virus, the group of patients infected with Corona virus without leukemia patients)

N	S. D	Mean	Age categories	Gender	The sample
6	9.85	35.63	16-25		
2	0.00	37.80	26-35		
4	3.75	33.95	36-45	Male	
2	0.00	29.30	46-55		
2	0.00	29.80	More then 55		
16	6.59	33.96	Total		
4	10.45	36.75	16-25		control
4	12.12	44.40	26-35		
4	9.30	47.65	36-45	Female	
2	0.00	27.10	More then 55		
14	11.46	40.67	Total		
30	9.64	37.09	Total		
5	4.93	23.32	16-25		
1		27.60	26-35		Hematology with covid-19
3	7.58	21.17	36-45	Male	
3	9.30	20.40	46-55		

4	4.89	16.90	More then 55		
16	6.30	21.03	Total		
5	8.37	20.90	16-25		
1		21.00	26-35		
3	9.14	20.00	36-45	Female	
2	4.03	9.45	46-55		
3	10.60	20.73	More then 55		
14	8.34	19.04	Total		
30	7.26	20.10	Total		
5	7.92	25.44	16-25		
3	8.51	23.70	26-35		
4	5.26	22.55	36-45	Male	
1		18.70	46-55		
3	11.01	22.50	More then 55		
16	7.16	23.42	Total		
5	8.89	19.76	16-25		covid-19
3	5.95	21.27	26-35		
2	2.83	25.20	36-45	Female	
1		13.90	46-55		
3	3.46	20.93	More then 55		
14	6.27	20.69	Total		
30	6.79	22.15	Total		
30	10.36	26.93	16-25		
14	12.47	31.19	26-35		
20	12.10	29.53	36-45	Total	
9	8.77	19.03	46-55		
17	7.28	21.99	More then 55		
90	10.98	26.45	Total		
			4.02	The sample lsd	
			4.03	Age categories lsd	

The current study shown in Table (2) found that there were significant differences according to the sample with a statistical significance at the level of P≤ 0.05 in the percentage of CD4 in lymphoid leukemia patients infected with Corona virus and patients infected with Corona virus without leukemia compared with the healthy group, where the expression level of CD4 surface marker was decreased in patient samples compared with the healthy group and this decrease was significant

Table 3
The differences recorded between the values of the surface marker CD19 in the three experimental groups (the healthy group, the group of lymphocytic leukemia patients infected with the Coronavirus, the group of patients infected with Coronavirus without leukemia patients)

N	S. D	Mean	Age categories	Gender	The sample
6	13.20	21.50	16-25	Male	control
2	0.00	18.00	26-35		

4	2.25	5.75	36-45		
2	0.00	11.00	46-55		
2	0.00	3.70	More then 55		
16	10.82	13.59	Total		
4	4.10	14.05	16-25		
4	9.01	15.60	26-35		
4	1.39	21.20	36-45	Female	
2	0.00	8.50	More then 55		
14	6.42	15.74	Total		
30	8.95	14.59	Total		
5	2.16	10.52	16-25		
1		9.40	26-35		
3	2.76	10.37	36-45		
3	1.66	11.27	46-55	Male	
4	1.90	8.98	More then 55		
16	2.02	10.18	Total		Hematology with covid-19
5	2.45	8.12	16-25		
1		11.70	26-35		
3	0.38	9.17	36-45		
2	0.64	1.95	46-55	Male	
3	2.50	11.00	More then 55		
14	3.44	8.34	Total		
30	2.88	9.32	Total		
5	1.65	9.62	16-25		
3	0.80	8.67	26-35		
4	0.90	8.45	36-45		
1		7.90	46-55	Female	
3	3.01	6.80	More then 55		
16	1.79	8.51	Total		
5	2.38	8.70	16-25		covid-19
3	2.01	9.20	26-35		
2	0.21	9.85	36-45		
1		5.60	46-55	Female	
3	1.77	8.67	More then 55		
14	1.96	8.74	Total		
30	1.84	8.62	Total		
30	7.70	12.33	16-25		
14	5.81	12.36	26-35		
20	5.64	11.00	36-45	Total	
9	4.10	8.13	46-55		
17	2.75	8.22	More then 55		
			2.37	The sample lsd	
			2.98	Age categories lsd	

As for the results shown in Table (3), it was found 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 CD19 in lymphoid leukemia patients infected with Corona virus and patients infected with

Corona virus without leukemia compared with the healthy group , where the level of CD19 decreased in patient samples compared with the healthy group, and this decrease was significant

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