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Study of some immunological parameters in hematological malignancies patients

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***Abstract*---**This study aimed to verify the number of differentiation aggregates that include HLA-DR, CD5 and CD7 In patients with hematological malignancies and comparing them with the healthy group, where the levels of these subgroups were detected in the peripheral blood of 40 males and females, Where samples were collected by 20 samples from patients with malignant hematomas, Plus 20 people from the healthy group. All blood samples and available data were collected from patients with hematological malignancies, from the Center for Oncology and Hematology, in partnership with the laboratories of medical centers and Al-Jawadeen National Laboratory, for the period from November 2021 to May 2022. The immunophenotypes of HLA-DR, CD5 and CD7 were studied in the samples using the flow cytometry technique, where the rate of differentiation aggregates in patients with leukemia was much higher than in healthy people, where the results showed significant differences with statistical significance at the level of P≤ 0.05 in the proportion of CD5, CD7 and HLA-DR in samples of patients with hematological malignant diseases, and a slight increase in the level of CD5 and HLA-DR was found in males than in females, and a slight decrease in the level of CD7 was observed in males than in females.

Keywords---immunological parameters, hematological malignancies, patients.

Introduction

Malignant hematomas are a disease resulting from the defective differentiation of hematopoietic stem cells. There are three main forms of malignant hematomas:

leukemia, lymphoma, and myeloma (Seita & Weissman, 2010). Where there is an abnormal and uncontrolled proliferation of cells, which is formed due to genetic deviation, which inhibits normal blood formation, leading to a change in physiological activities, and defects in cell differentiation may be either from myeloid or lymphoid (Arber et al., 2016). Flow cytometry identifies cell surface markers (differentiation aggregates) that are expressed at different stages of cell development by applying monoclonal antibodies against them, which helps in classifying leukemia (Olaniyi, 2011), The detection of deviations in blood cells is of diagnostic and prognostic importance, as abnormal expression of antigens is associated with adverse results, and this contributes to the approval of the treatment regimen for patients.

The HLA-DR is a class II cell surface receptor encoded by the human leukocyte antigen complex, and is important in the induction of T-cell responses through the use of antigen presenting cells to HLA-DR molecules to present protein fragments to T cells. In many autoimmune conditions, disease resistance and disease susceptibility (Keskinen et al., 1997), As for CD5, it is a glycoprotein and one of the differentiation aggregates found on the surfaces of T cells and is also found, but in a lower density, in B cells (Brown & Lacey, 2010), where it negatively regulates TCR signaling from the onset of T-cell activation, and plays a pivotal role in mediating T-cell activation. Results of cell survival or programmed cell death, in addition to preventing both autoimmunity and cancer (Benjamin Chun-Kit Tong, 2017), The research also includes the use of one of the differentiation groups that appear early in the emergence of T cells, CD7, which is a transmembrane glycoprotein and is expressed by most T cells in the periphery (Sempowski et al., 1999), which has an important role in initiating and amplifying T cell activation, and it has been proven that It is active in terms of cost and signal transmission (Stillwell & Bierer, 2001)

One of the useful features in studying the immunophenotypes represented by CD5, CD7, and HLA-DR is to determine the incidence of hematological malignancies using flow cytometry, which is a common technique used for clinical and research purposes, based on laser to describe cells based on complexity, shape and size as prepared The ability to take fluorescence measurements at multiple wavelengths has made the flow cytometer a practical resource in the use of fluorescent conjugated antibodies, DNA-binding dyes, fluorescent proteins, viable dyes and life indicator dyes, It is worth noting that with the advancement of technology, the number of parameters that the flow cytometer can measure has increased significantly, and through which cellular characteristics are measured, and different sub-cells can be collected and sorted for further analysis. It is capable of quantitative analysis and rapid multiparameter analysis from a group Heterogeneous cells on a cell-by-cell basis to provide a single-cell analysis (Schmit et al., 2021).

Materials and Methods

From each person included in the study, 1 ml of blood was drawn by venipuncture and by a 3 ml disposable medical syringe. 1 ml of blood was transferred to test tubes containing the anticoagulant EDTA TUBE for the purpose of testing CD7, CD5, HLA- DR The sample was used to perform

quantitative analysis by flow cytometry device, where (100 µl) of blood was placed in an Eppendrof tube, Then add (5 µl) either of (CD5 KIT and using fluorescent objects phycoerythrin (PE)) or (HLA-DR KIT and using fluorescein isothiocyanat (FITC), or (CD7 KIT and using fluorescent objects Percp)) peridinin-chlorophyll protein becton dickinson) to the tube containing blood, and the sample was placed in a vortex device for five seconds, and the samples were placed in the incubator at room temperature for 30-45 minutes, 100 µl of Erythrocyte lysing (reagent A) was added and the sample was placed in a vortex device for five seconds and leave the 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 a Micro centrifuge device for five minutes at a speed of 5000 rpm, after that we got a precipitate and a filtrate, The filtrate is WBC and the precipitate is RBC. The precipitate RBC was removed. (1 ml) of Buffer solution was added to the sample. The sample was placed in a micro centrifuge device for five minutes at a speed of 5000 rpm for getting rid of impurities and after getting rid of impurities (600 µl) was added. From the buffer solution to the filtrate, the sample was transferred to a plastic test tube or the so-called can tube. The sample was placed in a flow cytometry device, and the result was read in less than one minute.

Statistical analysis

All analyzes were carried out using the program (Statistical package for the social acience) (SPSS.V.28) and the analysis of variance was used Anova and graphs

Results and Discussion

CD5, CD7 and HLA-DR differentiation aggregates in patients with hematological malignancies and in healthy group

Differentiation aggregates: a protocol used to identify and investigate cell surface molecules present in leukocytes, providing targets for immunophenotyping of cells in terms of physiology (Zola et al., 2005). The gradual accumulation of genetic and epigenetic alterations leads to the emergence of human cancers. Hematological malignancies arise from cells that spread easily and travel through the body, and can arise very rapidly and untestable unlike solid tumors, and they often involve malignant hematological events. Chromosomal rearrangements that lead to the activation of the silent gene naturally, and human cancers arising from hematopoietic cells have the ability to proliferate in the absence of growth signals, escape from apoptosis and immune surveillance, in addition to inhibiting growth and differentiation (Garner & de Visser , 2020).

The current study showed an increase in the percentage of CD5, CD7 and HLA-DR in patients with hematological malignancies compared with the percentage of CD5, CD7 and HLA-DR in the healthy group, where this increase is diagnosed when there is an accumulation of malignant cells in the peripheral blood, bone marrow and nodes. The reason for this accumulation is due to the reduction of programmed cell death, aging and increased proliferation, in addition to defects in the negative regulation of BCR and TCR-induced signals and different signaling

pathways, which are contributors to gene changes on the external surface of the cell and the activation of internal mutations (Bardet et al., 2015) and (Jaseb et al., 2019) , (Campoli & Ferrone, 2008).

Stem cells throughout the life of the individual maintain the balance of hematopoiesis through self-renewal and defects in self-renewal and differentiation lead to deficiencies in hematopoiesis and the development of hematological malignancies (Ramdass et al., 2013). Inflammatory cells also have a strong influence on tumor development when the inflammatory response becomes chronic. These cells are potent inducers of tumors, facilitate genetic instability, promote angiogenesis, and produce an attractive environment for tumor growth. This can result in cell mutations and proliferation, which leads to the creation of an environment Also, chemokines, cytokines, adhesion molecules, and inflammatory enzymes produced by inflammatory cells affect the entire tumor, including tumor cells, lymphocytes, and endothelial cells, regulating the growth, migration, and differentiation of all types of cells in the tumor microenvironment. Immune cell functions by shifting inflammatory mechanisms in favor of the spread of malignancy such as the release of growth factors and survival, promoting angiogenesis and lymphatic vessels, stimulating DNA damage to facilitate invasion, and remodeling cancer cells to provide receptors for cell diffusion through lymphatic vessels and capillaries and by avoiding host defenses (Singh et al., 2019).Its microenvironment (Schmidt & Weber, 2006), and some results indicate that chronic antigen stimulation from allergic conditions may increase the risk of developing some hematological malignancies (Söderberg et al., 2004).

The results of the test in Table (1) showed that there were significant statistically significant differences at the level of P≤ 0.05 in the proportion of CD5, CD7 and HLA-DR in samples of patients with hematological malignancies, where the level of CD5, CD7 and HLA-DR increased in patient samples compared with The healthy group, and this rise is a significant increase.

Table No. (1) normative values of immunological criteria CD5,CD7 and HLA-DR

Sig.	F	Mean Square	df	Sum of Squares		
.000	35.240	9529.850	3	28589.551	Between Groups	HLA
		270.429	36	9735.457	Within Groups	
			39	38325.008	Total	
.000	17.256	2860.181	3	8580.542	Between Groups	CD5
		165.747	36	5966.886	Within Groups	
			39	14547.428	Total	
.000	11.632	1534.762	3	4604.286	Between Groups	CD7
		131.939	36	4749.813	Within Groups	

			39	9354.099	Total

The distribution of patients with hematological malignancies according to gender

The study samples were divided according to the effect of sex into two groups, the male group and the female group, where the results showed at the time of the study that the infection rate in males is higher in the CD5 and HLA-DR differentiation groups than the infection rate in females and a slight decrease in the infection rate in males than females in CD7, indicating a preponderance of infection in males, Where previous studies have shown that blood malignancies tend to occur more frequently in males than females, and in many cases the rate among males is more than double that of females for many subtypes of blood cancers, whether myeloid or lymphatic, and this difference is evident in both children and adults (Smith et al., 2011), (Cartwright et al., 2002). This may be due, as indicated by some current evidence, that alcohol consumption increases the risk of many cancers (Psaltopoulou et al., 2018), and some investigations conducted on heavy smokers showed positive associations with malignant tumors (Pasqualetti et al. , 1997), There is a role for family history and lifestyle of leukemia, and the increased risks depending on the occupational life that the individual leads, including exposure to agriculture and UV rays and exposure of hairdressers to a variety of chemicals including organic solvents, dyes and ammonia (Slager et al., 2014), Aging (aging), exposure to polluted environments (residence or occupation near traffic, stations, garages, dust, smoke and industrial areas) and global and local climate variability (dry and rainy seasons) increase the global burden of hematomas, as well as sex factors, infectious syndrome, sepsis, bacteremia, and virus Human immunodeficiency (Nkanga et al., 2017), Some studies showed the harmful effects of smoking on the fetus during pregnancy and in childhood, as it increases the risk of hematological malignancies (Antonopoulos et al., 2011), and there is evidence that the disease affects aspects of life differently in both sexes, where physical and emotional performance is observed in males. More than females and awareness of the disease is particularly relevant in male or female patients (Sztankay et al., 2011).

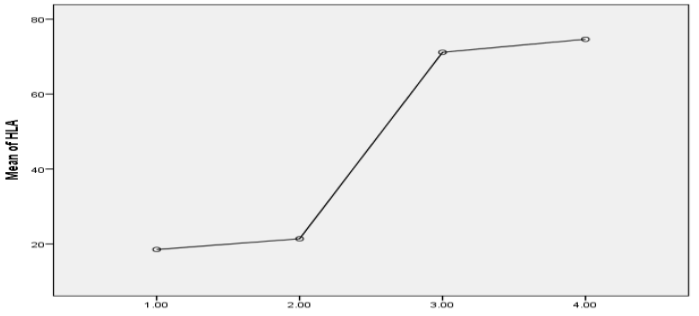

Figure No. (1) The average concentrations of HLA-DR for males and females in the study groups

Where the number 1 refers to the group of healthy females, the number 2 to the group of healthy males, the number 3 to the group of females with malignant

hematological diseases, and the number 4 to the group of males suffering from malignant hematological diseases

The results of Figure No. (1) Showed a slight increase in the percentage of HLA-DR in healthy males compared to healthy females and a slight increase in the percentage of HLA-DR in sick males compared to female patients, while we notice a sharp increase in the percentage of HLA-DR in the group of patients compared with the healthy group

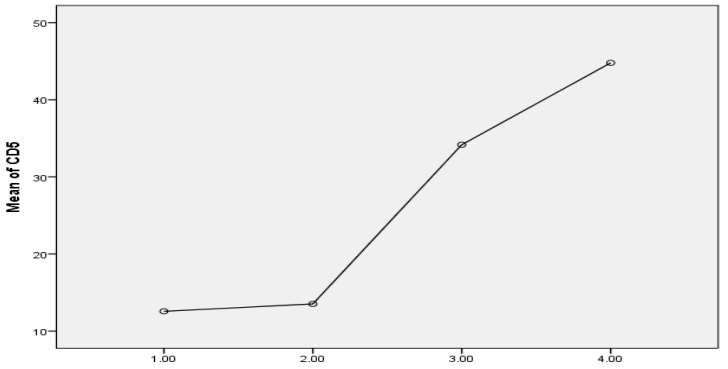

Figure No. (2) Average CD5 concentrations for males and females in the study groups

Where the number 1 refers to the group of healthy females, the number 2 to the group of healthy males, the number 3 to the group of females with malignant hematological diseases, and the number 4 to the group of males suffering from malignant hematological diseases

The results of Figure No. (2) Showed a slight increase in the percentage of CD5 in healthy males compared to healthy females and a significant increase in the percentage of CD5 in sick males compared to female patients, while we notice a sharp increase in the percentage of CD5 in the group of patients compared with the healthy group

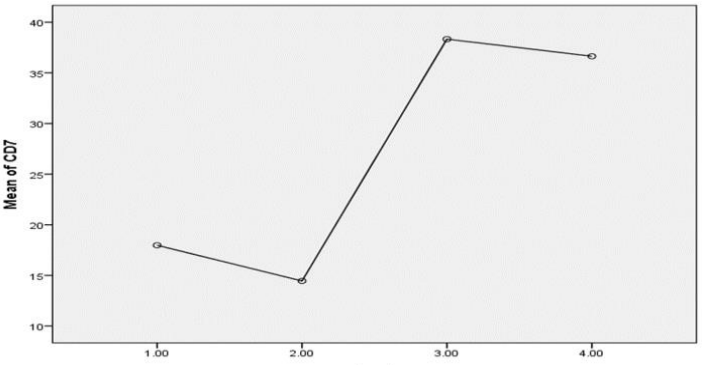

Figure (3) Average CD7 concentrations for males and females in the study groups

Where the number 1 refers to the group of healthy females, the number 2 to the group of healthy males, the number 3 to the group of females with malignant hematological diseases, and the number 4 to the group of males suffering from malignant hematological diseases

The results of Figure No. (3) Showed a sudden drop in CD7 percentage in healthy males compared to healthy females, and a slight decrease in CD7 percentage in sick males compared to female patients, while we notice a sharp increase in CD7 percentage in the group of patients compared with the healthy group

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