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Chemotherapy Effect on Neutrophils Function in Leukemic Patients

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Abstract. Neutrophils are chief phagocytic leukocytes, which form an essential part of the innate immune system that plays a key role in defense against living pathogens. Neutropenia has been considered to be the most important risk factor for severe infection during chemotherapy. In this study, the activity of neutrophils' function in adult leukemic patients before and after chemotherapy induction has been investigated. Whole blood samples were obtained from 32 leukemic patients with different cases, 8 patients from each: acute lymphatic leukemia, acute myeloid leukemia, chronic lymphatic leukemia and chronic myeloid leukemia. Immunophenotyping test was used to count T-lymphocytes, B-lymphocytes and Natural-killer lymphocytes. The mean results of the immunophenotyping test were in normal range so the count of these cells was not affected by chemotherapy. An oxidative burst test was used to quantify the neutrophils functions. There was significant differences between leukemia groups after chemotherapy ($F = 3.923$, $p = 0.01$). Acute lymphatic leukemia was the lower significant ($p = 0.003$) compared to the controls. There was no significant difference between the other cases and the controls. Acute lymphatic leukemia case was the most affected by chemotherapy.

Keywords: Chemotherapy, Neutrophils, Leukemia, Oxidative burst test.

Introduction

Leukemia is a heterogeneous group of neoplastic disorders characterized by lethal overgrowth of poorly differentiated white blood cells (WBCs).

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It is divided into acute and chronic leukemia. Furthermore, leukemia can also be divided into myelogenous or lymphatic. The lymphocytic or lymphoblastic indicates that the cancerous change takes places in marrow cells that form lymphocytes. The myelogenous or myeloid indicates that the cell change takes place in marrow cells that normally differentiate to form red blood cells (RBCs), and in some type of WBCs, such as granulocytes or monocytes.

Neutrophils are the leucocytes that respond most rapidly to the invasion by pathogens. It is the chief phagocytic leukocytes that form an essential part of the innate immune system. It plays a key role in the front line of defense against invading pathogens. They normally make up 75% of the total WBCs. Low count of neutrophils (neutropenia) is considered to be the most important risk factor for severe infection during chemotherapy.

Chemotherapy is a drug treatment, which is used to kill cancer cells or to stop them from spreading. It acts by killing the cells that divide rapidly which are the main properties of cancer cells. It also harms cells that divide rapidly under normal circumstances such as in bone marrow, the digestive tract and hair follicles. All chemotherapeutic regimens can cause depression of the immune system by paralyzing the bone marrow and leading to the decrease of the WBCs, RBCs and platelets.

However, some patients may have infection undergoing chemotherapy as a result of impaired neutrophils' function. A study has shown the presence of impaired neutrophils function in children with acute lymphatic leukemia (ALL)[1]. In this study, the activity of neutrophils' function in adult leukemic patients before and after the chemotherapy induction by using Immunophenotyping and Phagoburst test has been investigated.

Materials and Methods

This study was performed in King Abdulaziz Hospital and Oncology Center for two years. All chosen leukemic patients were treated in the Oncology center and they consented to use their samples in this study.

Patients

This study used thirty-two leukemic patients aged 20-60 years with acute lymphatic leukemia (ALL); acute myeloid leukemia (AML),

chronic lymphatic leukemia (CLL) and chronic myeloid leukemia (CML). Eight patients for each type of leukemia were involved in this study. Two blood samples were collected in Heparinized tubes: one before, and one after the chemotherapy induction.

Each type of leukemia was treated with different protocols. ALL patients were treated by Hyper cyclophosphamide, vincristine, and doxorubicin regimen, while the AML patients were treated by cytarabine. Furthermore, CLL patients were treated by a regimen of Fludarabine, Cyclophosphamide, and Rituximab, while the CML were treated by Imatinib mesylate. The first sample was collected after diagnosis, while the second one was collected when the patients recovered. Thirty-two blood bank donors were used as control to compare the function of neutrophils between patients and healthy individuals.

Immunophenotyping (TBNK) Test

Principle

When the whole blood is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leucocytes surface antigens. The stained samples were treated with BD Multitest™ IMK kit lysing solution to lyse erythrocytes. During acquisition, the cells travel past the laser beam and scatter the laser light; the stained cells fluoresce. These scatter and fluorescence signals were detected by the instrument, which provides information about the cell's size, internal complexity, and relative fluorescence intensity.

BD Multitest™ reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population[2-4] to reduce contamination of unlysed or nucleated red blood cells in the gate. When BD Trucount tubes are used, a known volume of sample is stained directly in a BD Trucount tube. The lyophilized pellet in the tube dissolves by releasing a known number of fluorescent beads. During analysis, the absolute number (cells/μL) of positive cells in the sample can be determined by comparing cellular events to bead events. If appropriate software, such as BD FACSCanto clinical software or BD Multitest™ software is used, absolute counts are determined by the software.

Method

In this study, the BD Multitest™ - IMK Kit (Becton, Dickinson and Company; BD Biosciences, San Jose, CA USA) was used. For each patient sample, two BD Trucount tubes were used, and labeled tube 1 and tube 2. First, used was a pipette 20 μL of BD Multitest™ CD3/CD8/CD45/CD4 reagent into tube 1. Then, a 20μL of BD Multitest™ CD3/CD16 + CD56/CD45/CD19 reagent pipette into tube 2 was used. Afterward, a 50 μL of well- mixed, heparinized whole blood was pipette into each tube and incubated for 15 min in the dark at a room temperature (20^{o} - 25^{o}). After that, 450 μL of diluted 1:10 BD Multitest™ IMK kit lysing solution was added to each tube and incubated for 20 min in the dark at room temperature and then, analyzed by the flow cytometer.

Burst Test (PHAGOBURST)

Test kit was used for the quantification of the oxidative burst activity of Monocytes and Granulocytes in Heparinized whole blood, ORPEGEN Pharma, Germany.

Phagocytosis by polymorphonuclear neutrophils constitutes an essential arm of host defense against bacterial infection [5-6]. The Burst-test allows the quantitative determination of leukocyte oxidative burst. The kit contains, unlabelled opsonized E-coli bacteria as particulate stimulus, the protein kinase-C ligand phorbol 12-myristate 13-acetate (PMA) as high stimulus, the chemotactic peptide N-formyl – MetLeuPhe (FMLP) as low physiological stimulus, and dihydrohodamine (DHR) 123 as fluorogenic substrate[7] as well as other necessary reagents. Heparinized whole blood is incubated with the various stimuli at 37^{o}C; a sample without stimulus serves as negative background control. Upon stimulation, granulocytes produce reactive oxygen metabolites (superoxide anion, hydrogen peroxide, hypochlorous acid), which destroys bacteria inside the phagosome. Formation of reactive oxidation during the oxidative burst can be monitored by the addition and oxidation of DHR 123. The reaction was stopped by addition of LYSING SOLUTION, which removed the erythrocytes results in the partial fixation of leukocytes. After one washing step with WASHING SOLUTION, DNA STAINING SOLUTION is added to exclude aggregation artifacts of bacteria or cells. The percentage of cells having

produced reactive oxygen radicals are then analyzed as well as their mean fluorescence intensity (enzymatic activity).

In summary, it was compared the activity of neutrophils in each tube to find out the quantification of the oxidative burst activity of them.

Statistical Analysis

Results were analyzed using mean, one-way ANOVA, and Dunnett. The significance level was determined as $p < 0.01$.

Results

Immunophenotyping (TBNK) Test

The Immunophenotyping test was performed to clarify the count of T lymphocytes, B lymphocytes and Natural killer lymphocytes, as well as the ratio between T helper lymphocytes: T suppresser lymphocytes in leukemic cases (ALL, AML, CLL, and CML) before and after chemotherapy induction. As shown in Table 1, the mean results of immunophenotyping test for all cases were within the normal reference range before and after chemotherapy induction.

Table 1. Immunophenotyping test result for leukemia patients before and after chemotherapy. Values represent the mean %.

Cases	Total T (CD3+) Lymphocytes Reference range % 59 - 85		T Helper : T suppressor Ratio CD4 : CD8 Ratio Reference range % 1 – 3.6		Total B (CD19) Lymphocytes Reference range % 6 - 23		Total NK (CD16+ / CD56+) Lymphocytes Reference range % 6 – 31	
	Before	After	Before	After	Before	After	Before	After
ALL	76	62.4	1	1.2	17.3	10	6	22
AML	78.3	75	1	1.1	7.3	10	7.2	11
CLL	70	74	1	2	17	13	13	18
CML	72	76	1.3	1	11.5	6	10	9

Burst (Phagoburst) Test

Burst test was performed to measure the activity of neutrophils function in leukemia cases (ALL, AML, CLL, and CML) before and after chemotherapy induction. There were significant differences between Leukemia groups and within the leukemia groups after

chemotherapy (F = 3.923, p = 0.01). There were significant differences in the mean percentage of the activity of neutrophils function between normal controls and ALL patients (p = 0.003). However, the differences in the mean percentage of the activity of neutrophils function between controls and AML, CLL, and CML patients were not significant (p = 0.142, p = 0.428, and p = 0.023, respectively).

Discussion

The aim of this study was to clarify the activity of neutrophils' function in acute and chronic leukemia before and after chemotherapy induction. There is a previous study, which shows impaired neutrophils' function in the initial period of chemotherapy of ALL pediatric patients which were analyzed by Flow cytometry using the oxidative burst test. Most reports investigate the neutrophils' function either on one leukemic case or on acute leukemia by other methods than the flow cytometer and oxidative burst test[8-9]. The current study investigated and compared the activity of neutrophils' function of acute and chronic leukemia before and after chemotherapy induction.

The Immunophenotyping test was used to determine the count of T-lymphocytes, B-lymphocytes, and Natural killer lymphocytes before and after chemotherapy induction and compared it with healthy individuals. The test results for all cases (ALL, AML, CLL, and CML) followed the normal reference range and did not show any difference compared to the control results. Therefore, apparently, the chemotherapy induction does not affect the count of these cells.

The Oxidative burst test was used to determine the activity of neutrophils' function before and after chemotherapy induction in leukemic patients and compared it with controls. The test results showed that there was significant difference between the cases ALL, AML, CLL, and CML after chemotherapy induction (F = 3.923, p = 0.01).

Furthermore, there was a significant difference between the control and ALL patients (p = 0.003) after chemotherapy induction. However, there is no significant difference between the controls and AML, CLL, and CML patients.

As shown in Fig. 1, the activity of neutrophils' function before chemotherapy in ALL patients were about 87.5%, AML 37.5%, CLL and

CML both have been 62.5% of the activity. However, Fig. 2 shows the activity of neutrophils' function after chemotherapy induction was for ALL 12.5%, AML 37.5%, CLL 62.5% and CML 25%. Therefore, the case that was most affected by chemotherapy induction was ALL patients. In a previous study, physicians found in ALL pediatric patients the presence of neutrophils with normal oxidative burst after PMA stimulation was significantly lower ($p = 0.002$) compared with controls after chemotherapy induction[1].

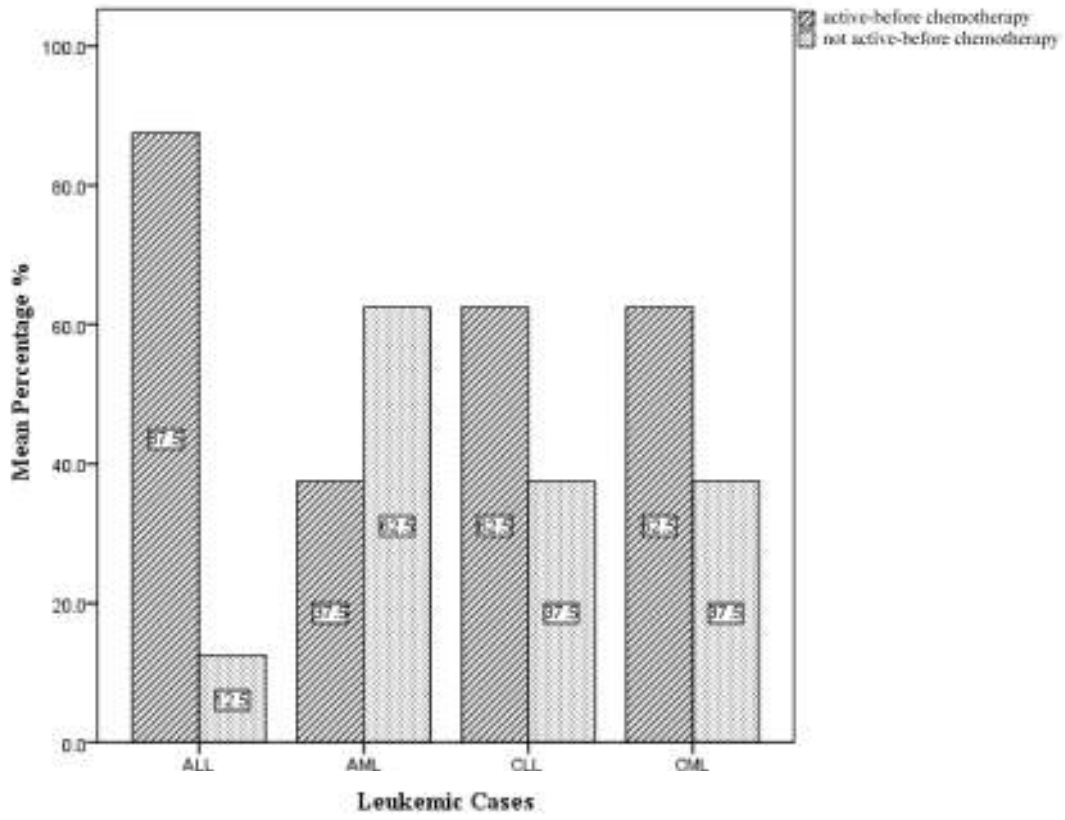

Fig. 1. Mean percentage (%) of the activity of neutrophils function in 32 leukemic cases; 8 patients from each case before chemotherapy induction.

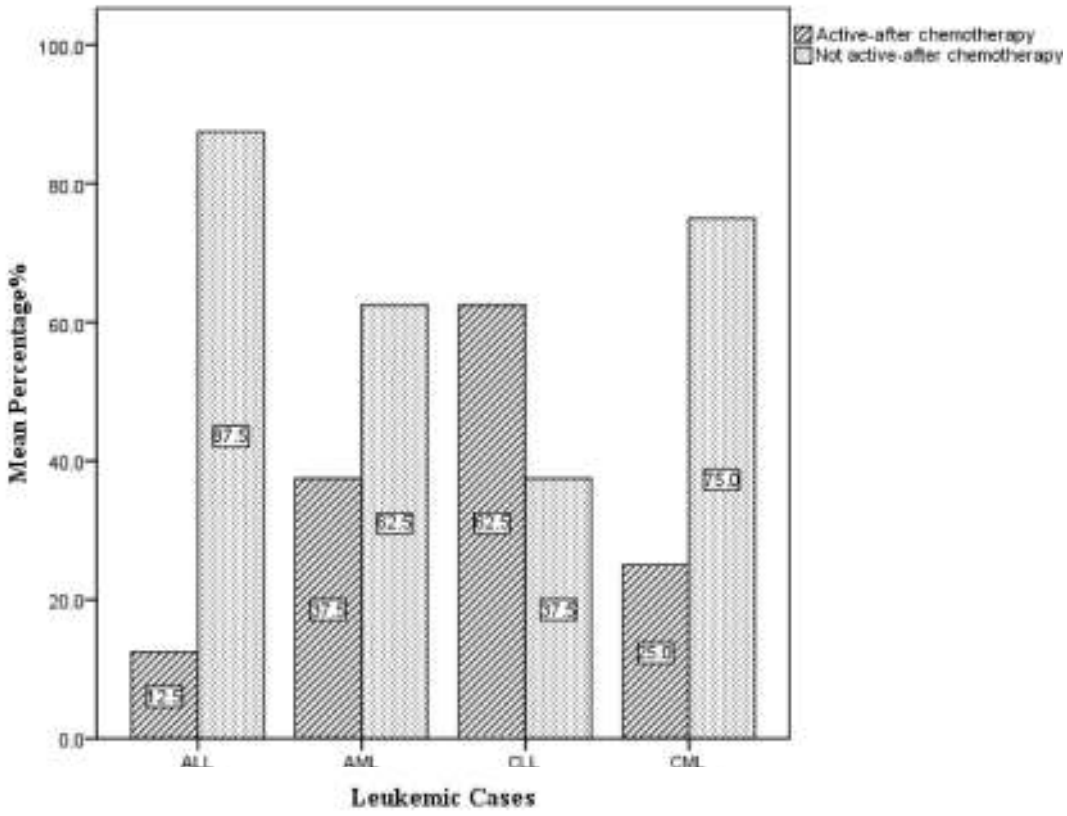

Fig. 2. Mean percentage (%) of the activity of neutrophils function in 32 leukemic cases; 8 patients from each case after chemotherapy induction.

However, in this study of adult ALL patients, the mean percentage of neutrophils' function with normal oxidative burst was significantly lower ($p = 0.003$) after chemotherapy induction than with the control.

Therefore, it is recommended to do a fast immune test to check T-lymphocyte and B lymphocytes functions.

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تأثير العلاج الكيميائي على وظيفة الخلايا المتعادلة لمرضى سرطان الدم

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المستخلص. الخلايا المتعادلة هي الخلايا البيضاء الرئيسة الآكلة التي تشكل جزءا أساسيا من جهاز المناعة الطبيعي، حيث تلعب دورًا رئيسيًا في الدفاع ضد العوامل الممرضة الحية. إن الانخفاض الحاد في عدد الخلايا المتعادلة يعتبر من أهم الآثار الجانبية الناتجة عن استخدام العلاج الكيميائي. في هذه الدراسة تم التحقق من نشاط وظيفة الخلايا المتعادلة في المرضى البالغين المصابين بسرطان الدم، قبل وبعد استخدام العلاج الكيميائي. تم الحصول على عينات دم كاملة من ٣٢ مريض مصابين بسرطان الدم، حيث تم أخذ ٨ عينات من مرضى: سرطان الدم اللمفاوي الحاد، سرطان الدم النخاعي الحاد، سرطان الدم اللمفاوي المزمن وسرطان الدم النخاعي المزمن، بعد استقراء العلاج الكيميائي. تم استخدام اختبار Immunophenotyping لعد الخلايا اللمفاوية (T, B) والخلايا القاتلة الطبيعية، حيث كان المتوسط الحسابي لنتائج هذا الاختبار يتبع المدى الطبيعي. إن اختبار Oxidative burst استخدم لقياس وظيفة الخلايا المتعادلة، وقد أظهرت النتائج وجود اختلاف ملحوظ بين أنواع سرطان الدم الحاد والمزمن بعد التعرض للعلاج الكيميائي، بمقدار (F=3.923, P=0.01)، وكان ابيضاض الدم اللمفاوي الحاد الأقل قيمة ملحوظة (P=0.003) مقارنةً بعينات أفراد طبيعيين ولم تظهر أي

اختلافات ملحوظة بين بقية الحالات الأخرى والأفراد الطبيعيين. ونستنتج من ذلك أن حالة ابيضاض الدم الحاد هي الأكثر تأثرًا بالعلاج الكيميائي.