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P53 Expression in Acute Myeloid Leukemia in Sudanese Patients Using Flow Cytometry

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Abstract:

Background*: Acute myeloid leukemia (AML) is a malignant disease of the bone marrow in which immature myeloid cells are predominant cells involved (myeloid blasts)* [(1)]*. p53 is a cell cycle check point control protein that assesses DNA damage and acts as a transcription factor regulating genes, which control cell growth, DNA repair, and apoptosis. P53 mutations have been found in a wide*

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variety of different cancers including hematological cancer [2]. We used the Flowcytometer to assess the pattern of p53 protein expression in different cases of acute myeloid leukemia.

***Method**: This is prospective cross sectional study in which 50 blood and/or bone marrow samples of newly diagnosed patients as acute myeloid leukemia (AML). All samples were treated with fix and permeabilation reagents and incubated with p53 anti human antibody for 10 minutes in dark place and then run by the flowcytometer.*

***Result**: 54% of the study populations were male and 46% were female. We found that p53 was expressed in all types of acute myeloid leukemia and observed in 31 of 50(62%) patient's samples this indicate that P53 mutation plays a role in leukomogensis. In our study, we found that the flowcytometric parameters of p53 expression showed low significant importance in the differentiation between sub types of AML. We recommended that increasing of sample size may help in the understanding of these relations especially if correlate with prognosis outcome of the treatment.*

Key words: p53 protein; flowcytometry; leukemia.

Introduction

Acute myeloid leukemia (AML), also known as acute myelogenous leukemia or acute non lymphocytic leukemia (ANLL) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age accounting for approximately 1.2% of cancer deaths in the United States [3]. The symptoms of AML include fatigue, shortness of breath, easy bruising and bleeding, and increased risk of infection. Several risk factors and chromosomal abnormalities have been identified, but the specific cause is not

clear. As an acute leukemia, AML progresses rapidly and is typically fatal within weeks or months if left untreated [4].

Tumor protein p53 also known as p53 cellular tumor antigen, p53 phosphoprotein, p53 or tumor suppressor p53 is a protein that in humans is encoded by the p53 gene. The p53 is crucial in multicellular marker where it regulates the cell cycle. The p53 has been described as the guardian of the genome. Its role in conserving stability by preventing genome mutation [5]. P53 is 53-kilodalton (KDa) protein based on calculations from amino acid residues [6]. In humans, the p53 gene is located on the short arm of chromosome 17 (17p13.1) [7] and play a role in apoptosis, genomic stability, inhibition of angiogenesis, and reproduces the cell cycle when there is DNA damage. If there is a mutation in p53, the cell cycle damaged DNA, leading to uncontrolled cell proliferation and cancer tumors. The aim of this study was to determine the P53 expression in acute myeloid leukemia among Sudanese patients, and define the role of p53% and mean florescent intensity (MFI) to differentiate between different sub types of (AML) and the relationship between p53 and myeloid specific markers during Mar 2015 to Jun 2015.

Materials and Method

This is a prospective, descriptive cross-sectional study conducted in Khartoum state, patients attended in the Flowcytometry laboratory in the period from Mar-2015 till Jun-2015. Data was collected using combined interview questionnaires and observation check list including the demographic data, age, sex, gender and residence with the clinical data. We collected 50 fresh samples taken in EDTA container. The analyzed samples were either of peripheral blood (PB) or bone marrow aspirate (BMA) according to availability and presence of blast cells. Samples running were performed on

the flowcytometer (COULTER EPICS XL-MCL™ Flowcytometer - Miami, Florida - USA). Data was analyzed using SPSS. Ethical consents were obtained from all patients included in the study.

Quality Control:

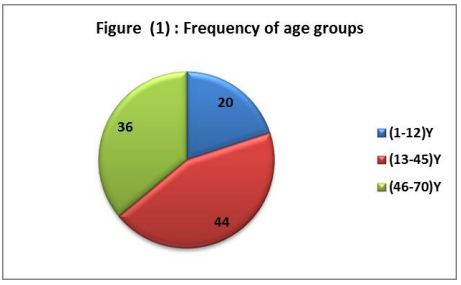
Depending upon pilot study in the quality control results (that saved in the Q.C system II software file) of EPICS XL flowcytometer, which adjusted the cut off points between negative and positive scale for every marker, Positivity was considered when ≥30% of the population expressed the marker. The parentheses were also recorded for most of the markers.

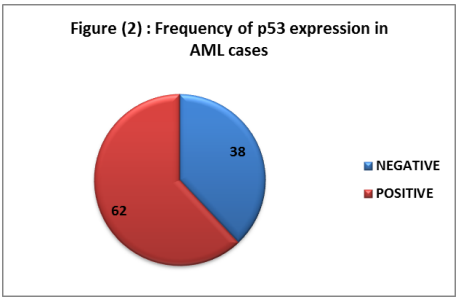
Results:

In our study, 10 (20%) of the patients were below 10 years old, 22 (44%) were between (13-45) years and 18 (36%) were between (46-70) years old (Figure 1). 27 (54%) were male and 23(46%) were female. 23(46%) of samples were blood and 27(54%) were bone marrow. The results showed that 31(62%) of patients showed positive p53 and 19(38%) were negative (Figure 2). p53 positive expression in sub types of AML was as follow: 10 (20%) was M0, 1(2%) was M1, 19 (38%) was M2, 5(10%) was M3, 6 (12%) was M4, 5 (10%) was M5, 4 (8%) was M7. (Figure 3). P53 percentage level of AML sub types was: M0 was (30%) negative, (60%) low, (10%) high. M1 was (100%) low. M2 was (26.3%) negative, (15.7%) low, (57.8%) high. M3 was (40%) negative, (60%) high. M4 was (16.6%) negative, (16.6%) low, (66.6%) high. M5 was (40%) low, (60%) high and M7 was (25%) negative, (25%) low, (50%) high, (Figure 4).P53 remark of AML sub types was: (60%) of M0 negative and (40%) positive. (100%) of M1 was positive. (31%) of M2 was negative and (69%) was positive. (40%) of M3 was negative and (60%) was positive.

(33%) of M4 was negative and (67%) was positive. (20%) of M5 was negative and (80%) was positive. (25%) of M7 was negative and (75%) was positive (Figure 5). P53 mean intensity level in AML sub types was: M0: (80%) dim and (20%) bright. M1: (100%) dim. M2: (52.6%) dim and (47.4%) bright. M3: (80%) dim and (20%) bright. M4: (50%) dim and (50%) bright. M5: (60%) dim and (40%) bright. M7: (100%) was dim (Figure 6).

The expression of p53 among age: we found that in the group of (1-12) years: (40%) was negative and (60%) positive. The group of (13-45) years: (36%) was negative and (64%) was positive. the group of (46-70) years: (39%) was negative and (61%) was positive. The expression of p53 among gender: we found that (44%) of male was negative and (56%) was positive, for female (30%) was negative and (70%) was positive. The expression of p53 among sample type: we found that (35%) of peripheral samples were negative and (65%) were positive. For bone marrow samples (41%) were negative and (59%) were positive.





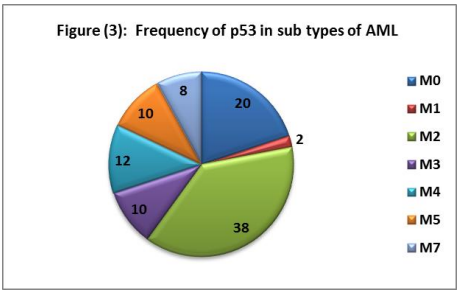


Figure (4): p53 percentage level in sub types of AML

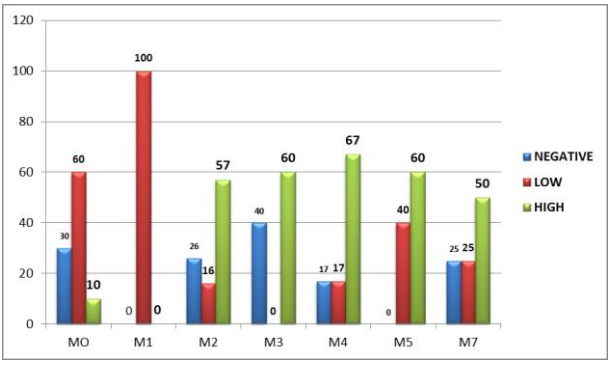


Figure (5): Remark expression of p53 according to sub types of AML

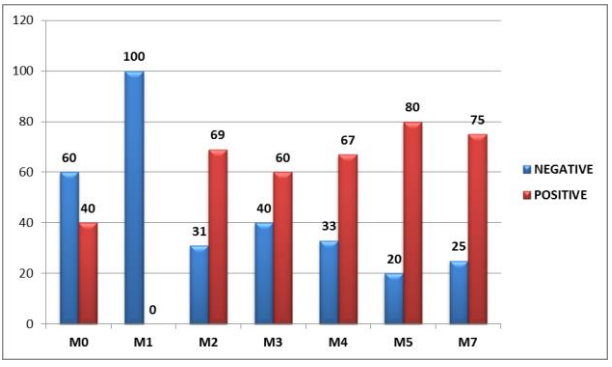
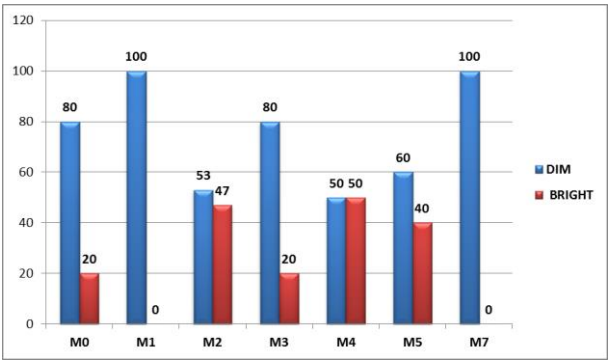


Figure (6): P53 mean intensity level of sub types of AML



Discussion

Our study showed that there were no significant differences in the p53 between the sub types of AML among age, gender and sample types (p.value = 0.98, 0.31, & 0.67, respectively). When we studied the p53 percentage level of AML cases, we found that all cases of M1 showed low p53 percentage with no high level. While the other types of AML (M2, M3, M4, M5, M6) showed considerable high percentage of p53. AML M5 showed no any negative result of p53 while M3 showed no low percentage of p53. The highest positive cases of p53 appear with M5 and the lowest with M1 and the most negative cases for p53 observed with M1. Regarding mean intensity of p53, we found that all sub types of AML showed dim expression. So all M1 and M7 were showed dim p53 mean intensity while M2 and M4 showed the lowest degree. The brightest expressions appear with M4 and M2 cases while the lowest bright expression observed with M3 cases.

These findings of p53 pattern can help to some extent in the differentiation between sub types of AML as follow: A-between M1 and other types due to free of high percentage. B-between (M1, M7) and other types due to free of bright mean

intensity. C- between M3 and other types due to free of low percentage. D- between (M1, M5) and other types due to free of negative results. E- between M4 and other types due to the highest percentage of p53 and the brightest expression which observed with M4 cases. By the general view, we found that all the relations of p53 flowcytometric parameters (Percentage, remark and mean intensity) were insignificant in the differentiation between subtypes of AML (p.value = 0.60, 0.57, and 0.43, respectively).our findings agree with study done by Wattel and Preudhomme (8) and therefore detection of P53 mutation is not used routinely in diagnosis of AML.

Conclusion:

In our study, we found that the flowcytometric parameters of p53 expression showed low significant importance in the differentiation between sub types of AML. We recommended that increasing of sample size may help in the understanding of these relations especially if correlate with prognosis and outcome of the treatment.

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