

## P53 Expression in Acute Lymphoblastic Leukemia in Sudanese Patients Using Flow Cytometer

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### Abstract:

**Background:** *Leukemia is abnormal condition in which abnormal cells divide without control. Cancer cells keep on growing and form new cancer cells. In most types of cancer and ALL is uncontrolled and exaggerated growth and accumulation of cells called*

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*“lymphoblasts” or “leukemic blasts,” which fail to function as normal blood cells. .*

*The p53 is a well-known tumor suppressor gene found in 50%of cancer because have ability to modulate various cellular processes including apoptosis, cell cycle arrest and DNA repair. This study was conducted to detect of p53 expression in acute lymphocytic leukemia in Sudanese patients using the FlowCytometry and data were collected from the patients interview using designed questionnaire attending into FlowCytometrylabrotary .The results showed that 39 of the patients with ALL in this study had negative p53,of which 56.4% were male and 43.6%were female. While 11of the patient with ALL had positive p53 of which 72.7% were male and 27.3 were female.*

**Method:** *This was prospective cross sectional study in which 50blood and/or bone marrow samples of newly diagnosed patients as acute lymphoid leukemia (ALL).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:***60% of the study populations were male and 40%were female. We found that p53 was expressed in 11(22%) patient’s samples this indicate that P53 mutation plays a role in leukomogenesis. In our study, we found that the flowcytometric parameters of p53 expression showed low significant importance in the differentiation between types of ALL. 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.*

**Key words:** P53 Expression, acute Lymphblastic Leukemia, Sudanese Patients, Flow Cytometer

## **Introduction**

Acute lymphoblastic leukemia, also known as acute lymphocytic leukemia or acute lymphoid leukemia (ALL), is an acute form of leukemia, or cancer of the white blood cells, characterized by the overproduction and accumulation of cancerous, immature white blood cells, known as lymphoblasts.[4] In persons with

ALL, lymphoblasts are overproduced in the bone marrow and continuously multiply, causing damage and death by inhibiting the production of normal cells (such as red and white blood cells and platelets) in the bone marrow and by spreading (infiltrating) to other organs. ALL is most common in childhood, with a peak incidence at 2–5 years of age and another peak in old age.[4]

ALL was one of the first cancers for which an effective chemotherapeutic treatment was developed. [5][6]

P53 is Tumor protein also known as p53, cellular tumor antigen p53, phosphoprotein p53, tumor suppressor p53, antigen NY-CO-13, or transformation-related protein 53 is any isoform of a protein encoded by homologous genes in various organisms, such as *TP53* (humans) and *Trp53* (mice). [7]

Activated p53 binds DNA and activates expression of several genes including microRNA miR-34a,[8] WAF1/CIP1 encoding for p21 and hundreds of other down-stream genes. p21 (WAF1) binds to the G1-S/CDK (CDK4/CDK6, CDK2, and CDK1) complexes (molecules important for the G1/S transition in the cell cycle) inhibiting their activity.

When p21(WAF1) is complexes with CDK2 the cell cannot continue to the next stage of cell division. A mutant p53 will no longer bind DNA in an effective way, and, as a consequence, the p21 protein will not be available to act as the "stop signal" for cell division.[9] Studies of human embryonic stem cells (hESCs) commonly describe the nonfunctional p53-p21 axis of the G1/S checkpoint pathway with subsequent relevance for cell cycle regulation and the DNA damage response (DDR). Importantly, p21 mRNA is clearly present and upregulated after the DDR in hESCs, but p21 protein is not detectable. In this cell type, p53 activates numerous microRNAs (like miR-302a, miR-302b, miR-302c, and miR-302d) that directly inhibit the p21 expression in hESCs.[10]

## **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  $\geq 30\%$  of the population expressed the marker. The parentheses were also recorded for most of the markers.

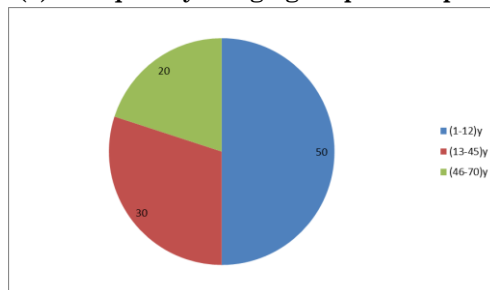
### **Results:**

In our study, 25(50%) of the patients were below 12 years old, 15(30%) were between (13-45) years and 10 (20%) were between (46-70) years old (Figure1). 30(60%) were male and 20(40%) were female (Figure2).17(34%) of samples were blood and 33(66%) were bone marrow (Figure3). The results showed that 11(22%) of patients showed positive p53 and 39(78%) were negative. (Figure4). p53 positive expression in sub types of ALL

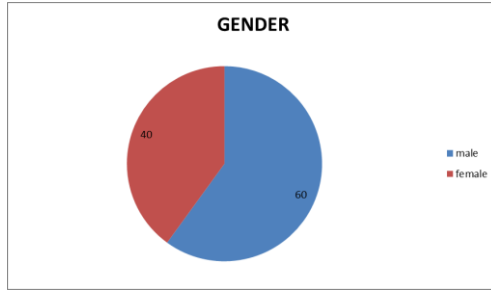
was as follows: 9(25.5%) was B-ALL2(15%) T-ALL while expression negative was follow 28(75.5%) was B-ALL and 11(85%) was T-ALL (Figure5). P53 percentage level of ALL sub types was: B-ALL was (75.6%) negative, (5.4%) low, (18.9%) high. T-ALL was (84.6%) negative, (7.6%) low, (7.6%) high Figure (3) (Figure6). P53 remark of ALL sub types was (24.5%) of B-ALL positive and (75.5%) negative. (15%) of T-ALL positive and (85%) negative. P53 mean intensity level in ALL sub types was: B-ALL (24.3%) dim and (75.7%) bright. T-ALL : (7.6%) dim and (92.4%) bright (Figure7).

The expression of p53 among age: we found that in the group of (1-12) years: (46.1%) was negative and (63.6%) positive. The group of (13-45) years: (30.7%) was negative and (27.7%) was positive. The group of (46-70)years: (23%) was negative and (9%) was positive. The expression of p53 among gender: we found that (56.4%) of male was negative and (72.7%) was positive, for female (43.5%) was negative and (72.2%) was positive. The expression of p53 among sample type: we found that (41%) of peripheral samples were negative and (9%) were positive. For bone marrow samples (58.9%) were negative and (90.9%) were positive. (Figure 8)

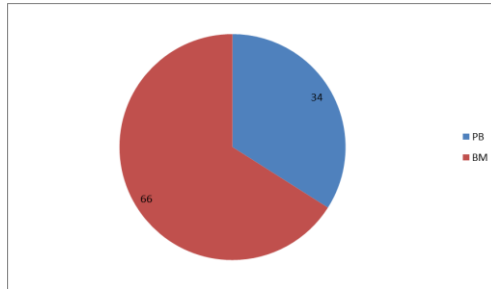
**Figure (1): Frequency of age groups with percentage**



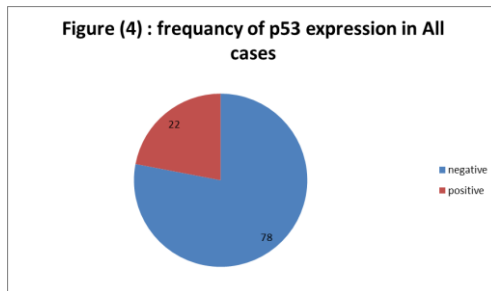
**Figure (2) : Frequency of gender with percentage**



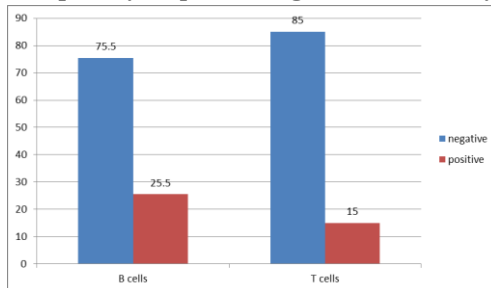
**Figure (3): Frequency of sample type with percentage**



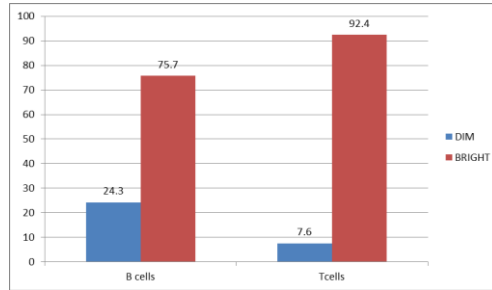
**Figure (4) : frequency of p53 expression in All cases**



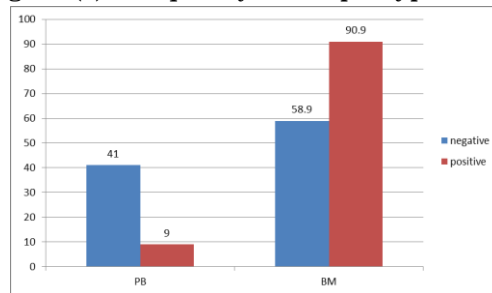
**Figure 5. Frequency of percentage level of sub type of ALL**



**Figure (7): Frequency of min level in type of ALL**



**Figure (8): Frequency of sample type of ALL**



## Discussion

Our study showed that there were no significant differences in the p53 between the sub types of ALL among age, gender and sample types (p. value = 0.24, 0.33, & 0.050, respectively). When we studied the p53 percentage level of ALL cases we found that both of B-ALL and T-ALL showed low p53 percentage also we found both of them with increase of negative percentage. Regarding mean intensity of p53, we found that both of B-ALL and T-ALL showed dim p53 mean intensity with lowest degree but decrease in T-ALL more than B-ALL while B-LL showed dim more than T-ALL. The brightest expressions appear with both of B-ALL and T-ALL with high degree but in T-ALL more than T-ALL.

Regarding peak wide (Pw) of P35 we found that T-ALL showed heterogeneous with lowest degree but increase of homogenous while B-ALL have more heterogeneous than T-ALL and low homogenous than B-ALL.

Also the study showed that increase the positive of p35 in male than female also increase in children in age of (1-12)y and increase positive p53 in bone marrow more than blood.

By the general view, we found that relations of p53 flowcytometric parameters (Percentage. pwttype and mean intensity) were insignificant in the differentiation between btypes of ALL (p.value= 0.41, 0,14 and 0.20, respectively) while remark we

Significant (p.value=51). ) our findings agree with study done by Wijdan N. Ibrahim MBChB MSc PhD, that investigate the status of p53 protein and their relation to the occurrence of TEL-AML-1 translocation in children with acute lymphoblastic leukemia (ALL) attending the oncology unit at Basrah maternity and children hospital during the period from May 2009 to April 2010. [11]

## **Conclusion:**

In our study, we found that the flowcytometric parameters of p53 expression showed low significant importance in the differentiation between sub types of ALL. 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.

## **ACKNOWLEDGMENT:**

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