http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 17 (7): 891-897, 2014
ISSN 1028-8880 / DOI: 10.3923/pjbs.2014.891.897

Detection P53 Gene Deletion in Hematological Malignancies Usimg Fluorescence *In situ* Hybridization: A Pilot Study

Aaedah F. Annooz, Alice K. Melconian, Ban A. Abdul-Majeed and Ali M. Jawad
College of Medicine, Kufa University, P.O. Box 18, Najaf, Iraq

Abstract: P53 as a tumor suppressor gene plays a major role in cancer development, it is essential for cell growth regulation and apoptosis. The deletion of p53 is known to be associated with aggressive diseases in several hematological malignancies. The evidence indicated that p53 deletions can be acquired as a result of chemotherapy. Therefore, a follow-up study for p53 gene deletion by fluorescence *in situ* hybridization technique (FISH) was carried out for the patients group who affected with different hematological malignancies before and after chemotherapy. The main goals from screening of p53 deletion were to assess the correlation between p53 deletion and chemotherapy resistance, overall median survival and chromosomal abnormalities. It is concluded from the present study that p53 deletion has a cardinal effect on the clinical outcome (chemotherapy resistance, overall median survival) and outcome of chromosomal abnormalities (quality and quantity of chromosomal abnormalities) of the patients who were affected with hematological malignancies before and after chemotherapy.

Key words: P53 gene, fluorescence *in situ* hybridization, hematological malignancies

INTRODUCTION

The p53 gene is a tumor suppressor gene, located on chromosome 17 p13.1 and encodes a 53-kD nucler phosphoprotein with specific DNA binding properties (Hollstein *et al*., 1991), it plays a critical role in cell cycle regulation, cellular response to DNA damage and induction of apoptosis (Shimamura and Fisher, 1996), Often inactivated by deletion and or point mutation in most types of solid tumors (Hahn *et al*., 1999; Peller and Rotter, 2003). In hematologic malignancies, P53 gene mutations are found in 25-30% of Burkitt's lymphoma and Chronic Myeloid Leukemia (CML) in blast crisis, 15% of Chronic Lymphocytic Leukemia (CLL) and 5-10% of Acute Myeloid Leukemia (AML), Myeloysplastic Syndrome (MDS) and large cell non-Hodgkin's Lymphoma (NHL), but are very rare in Acute Lymphocytic Leukemia (ALL, except in Burkitt's ALL or in relapse) in multiple myeloma and in follicular NHL (except after histologic progression) (Wattel *et al*., 1994).

The most hematological malignancies respond well to the conventional chemotherapy. Most of chemotherapeutic drugs work by impairing mitosis and effectively targeting fast dividing cells so-called (blast cells). Multiple stress conditions activate p53 including DNA damage, hypoxia, redox stress, ribonucleotide imbalance, cell adhesion and oncogenes (Ferbeyre *et al*., 2000, 2002; Gaumont-Leclerc *et al*., 2004; Giaccia and Kastan, 1998). The correlation between p53 mutations and resistance to chemotherapy in CLL were observed (Bonnefoi *et al*., 2002). However, no other attempt at correlating p53 mutations to response to chemotherapy and survival in hematologic malignancies has been made, to our knowledge. More surprisingly, it was found that other extra p53 deletions may be acquired as a result of chemotherapy (Venkat, 2003).

In the present study, we looked for p53 deletions in group of hematologic malignancies using fluorescence *in situ* Hybridization (FISH). In this report, we tried to correlate the presence of those deletions to results of chemotherapy treatment.

MATERIALS AND METHODS

Patient characteristics: Heparinized peripheral blood and/or bone morrow samples were obtained from eight selected patients with different types of Hematological Malignancies at hematology clinic of Baghdad Teaching Hospital from June 2006 to August 2007 and 10 of blood samples control. Their diagnosis was established in the Hematology Section of Medical City Teaching Laboratories, based on blood film, bone marrow aspiration and biopsy findings. The patients were; Two patients with AML, one patient with ALL, one patient with multiple myeloma, one patient with non-Hodgkin lymphoma and three patients with CML.

Corresponding Author: Aaedah F. Annooz, College of Medicine, Kufa University, P.O. Box 18, Najaf, Iraq Tel: 009647902306594

Cytogenetics: Chromosome analysis obtained through short term was done according to standard procedures (Czepulkowski *et al*., 1992). A total of 20 metaphases from unsynchronized peripheral blood and bone marrow culture were analyzed. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (Shaffer *et al*., 2009).

Fluorescence *in situ* hybridization (FISH): Fluorescence *in situ* Hybridization (FISH) using LSI P53 (17p13.1) spectrum orange probe (Abbott molecular/Vysis, USA) was applied according to manufacturer's instructions (Al-Achkar *et al*., 2007). A total of 50 metaphase spreads were analyzed, each using a fluorescence microscope (Axioplan, Zeiss) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain DAPI (Diaminophenylindol). Image capturing and processing were carried out using an ISIS imaging system (MetaSystems, Altlussheim, Germany).

Statistical analysis: Both of the Chi-Square test (symbolized as X2-test) and correlation test or so-called simple linear regression (symbolized as r-test) were used in the statistical analysis of our results. The Chi-Square test was applied according to (Daniel, 1985a). While correlation test was applied according to (Daniel, 1985b). P53 FISH probe was tested on the ten different normal control samples (peripheral blood from individuals with no evidence of a hematologic malignancy). Patients were considered evaluable if at least 50 metaphase could be scored from each slide (Chang *et al*., 2005).

RESULTS

P53 gene using FISH in eight patients was evaluated. The clinical information for the patients was illustrated in (Table 1). No cells with p53 deletion were detected in our control group. Before chemotherapy, the minimum cells with p53 deletion were estimated 16% in the patient (No.6). The number of cells with p53 deletion after chemotherapy of five patients was shown in (Table 1) as follow as: In patients No. 1, No. 2, No. 3 and No. 5, the number of cells with p53 deletion was decrease after chemotherapy, but without significant difference for patient No. 1. While, with significant difference for the patients No. 2 and No. 3 were showed. In patient No. 4, the number of cells with p53 deletion was increased after chemotherapy, but without significant difference. No results obtained for the patients No. 6, No. 7 and No. 8 after chemotherapy, as a consequence of cell culture failure.

Comparison the number of cells with p53 deletion between patients No. 6 and No. 7 and between patients No. 7 and No. 8 was carried out (Table 1). The X^2-test is

Table 1: Clinical characteristics, treatment, percentage of cells with p53 deletion, response and outcome in NHL, ALL, AML, MM and CML with P53 deletion

					Percentage of cells with p53 deletion			No. of cells without p53 deletion		
PN	Sex/age	Hematological malignancy type	Kind of samples	Treatment	Before chemotherapy	After chemotherapy/ week No.	X^2 and p values	(Percentage of the cells)	Response	(r) value
1	F/62	NHL-Low-grade NHL	PB	Chlorambucil and Cyclophosphamide	34%	12 (24%) 13 (24%) 17 (34%)	1.212, p≥0.05 1.212, p≥0.05 1.212, p≥0.05	33 (66%)	Resistance	- 0.84
2	M/17	ALL- T cell leukemia	PB	Doxorubicin, 6-MP, ARA-C, Cyclophosphomide, Vincristine, Methotrexate and Daunorubicin	56%	10 (32%) 32 (24%)	5.82, p≥0.05 10.6, p≥0.05	22 (44%)	Dead after 30 months	- 0.95
3	F/25	AML- M3	PB	ATRA, Doxorubicin, 6-MP and Methotrexate	66 %	12 (10%) 28 (24%) 33 (28%)	33.2, p≥0.05 17.8, p≥0.05 14.48, p≥0.05	17 (34%)	Dead after 20 months	- 0.62
4	M/70	Multiple myeloma-Stage I	PB	Cyclophosphamide	24%	17 (26%)	0.052, p≥0.05	38 (76 %)	Dead after 10 months	- 0.44
5	M/18	AML- M3	PB	Daunorubicin, ATRA, 6-MP and Methotrexate	44%	13 (30%) 15 (54%) 17 (32%)	- 6, p≥0.05 0.052, p≥0.05	28 (56 %)	Dead after 30 months	- 0.20
6	M/27	CML- chronic phase	PB	Imatinib	8 (16%)	-	-	42 (84%)		-
7	M/68	CML-blastic phase	PB	Imatinib	20 (40%)	-	-	30 (60%)*	Dead after 1 month	-
8	M/65	CML- chronic phase	PB	Imatinib	9 (18%)	-	-	41 (82%)**	Dead after 20 months	-

PB: Peripheral blood sample, *X2: 7.142, Df: 1, p≤0.05 (significant), **X2: 5.87662, Df: 1, p≤0.05 (significant)

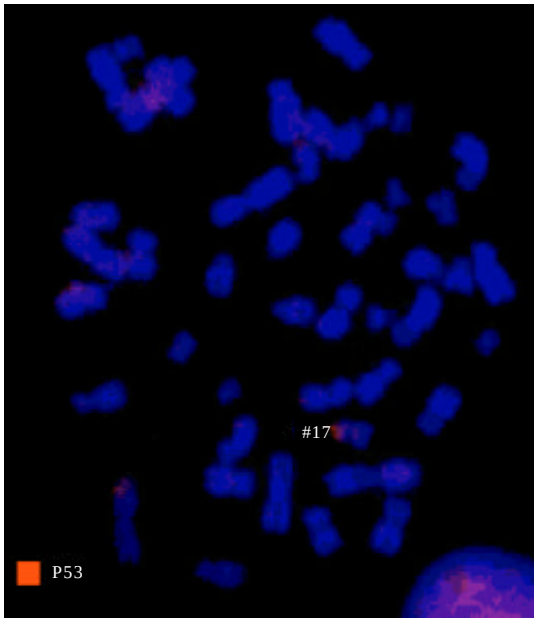

Fig. 1: Fluorescence *in situ* Hybridization (FISH) using probe for P53 (red) showed one copy of P53. Abbreviations: # = chromosome

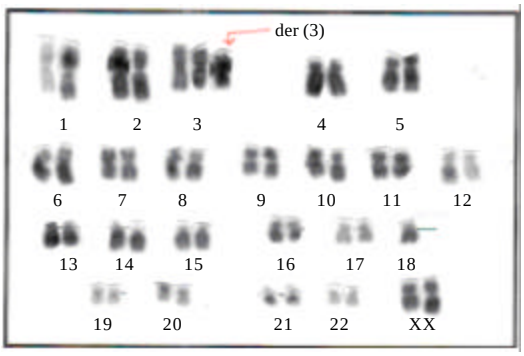

Fig. 2: GTG-banding revealed a 46, XX,+der(3),-18. Derivative chromosome was indicated by the arrowhead

used in the assessment, a significant difference was obtained.

The correlation between p53 deletion and survival of our patients who affected with different hematological malignancies and submitted to study of p53 deletion by FISH was studied. The correlation test or (r) is used in the assessment A negative correlation between the number of cells with p53 deletion before chemotherapy (at first diagnosis) and survival of the patients was obtained (r = -0.52).

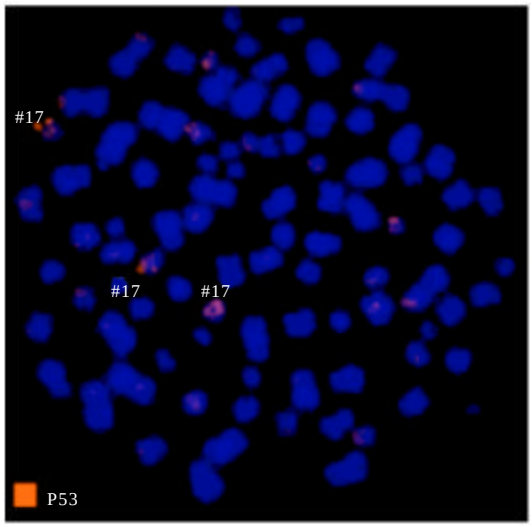

Fig. 3: Fluorescence *in situ* Hybridization (FISH) using probe for P53 (red) showed three copy of P53. Abbreviations: # = chromosome

Conventional cytogenetic analysis was performed. Many chromosomal abnormalities in patient group were detected. In patient No. 1, aneuploidy was seen before and after chemotherapy (12 weeks), the metaphase showed 46, XX,+der(3), -18 and one signal of p53 (Fig. 1, 2). In patient No. 2 polyploidy and aneuploidy appeared before and after chemotherapy (Fig. 3). In the patient No. 3, aneuploidy was detected before and after chemotherapy. After 33 weeks of chemotherapy in patient No. 3 a unique complex karyotype t (14; 21),+12, +14, -19 and -22, appeared in the blood (Fig. 4, 5). In patient No. 4, aneuploidy was detected before and after chemotherapy. In patient No. 5 aneuploidy was detected before chemotherapy and persisted in the blood sample of the patient after (13 weeks of chemotherapy) and until the last dose of chemotherapy (17 weeks of chemotherapy). In contrast, aneuploidy was not detected in the bone marrow sample at the same period of chemotherapy (13 weeks of chemotherapy). Polyploidy was detected after (13 weeks of chemotherapy) in both blood and bone marrow samples and was disappeared after (15 weeks of chemotherapy).

The correlation between number of cells with p53 deletion and total number of chromosomal abnormalities were assessed before chemotherapy in the blood and bone marrow samples of the patients. The correlation was evaluated in the eight patients of our preliminary group, a negative correlation was obtained (r = -0.60). When this correlation was applied on each patient who submitted to follow-up of p53 before and after chemotherapy for the patients (No. 1 to No. 5), a negative correlations were

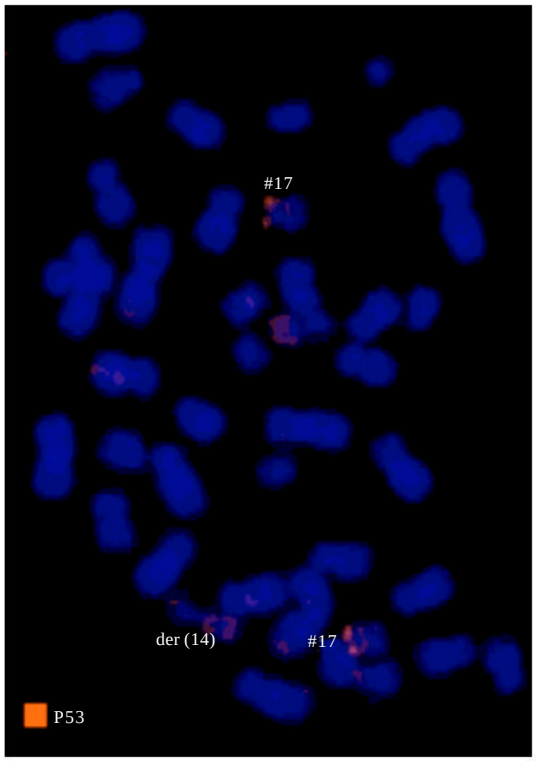

Fig. 4: Fluorescence *in situ* Hybridization (FISH) using probe for P53 (red) showed two copy of P53 and der (14) t (14;21). Abbreviations: # = chromosome, der = derivative chromosome

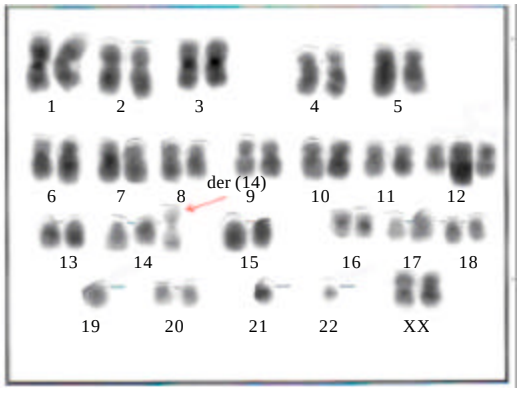

Fig. 5: GTG-banding revealed a 46, XX, t (14; 21), +12, +14, -19, -22. Derivative chromosome was indicated by the arrowhead

obtained between number of cells with p53 deletion and total number of chromosomal abnormalities before and after chemotherapy (Table 1).

DISCUSSION

The P53, is a tumor suppressor gene, is implicated in the regulation of cell cycle proliferation, differentiation and apoptosis. The loss of p53 function eliminates growth arrest in response to certain DNA damaging agents and enhances the frequency of gene amplification, suggesting a role for p53 in the control of cell cycle check point and in the maintenance of integrity of the genome (Hollstein *et al*., 1991). The fact that p53 mutations are mainly seen in advanced or relapsing hematologic malignancies suggests that they are associated with poor response to chemotherapy.

Each patient's results taken as a separate entity to compare p53 deletion before and after each dose of chemotherapy in relation to the effect of the drug. Whatever the function of the drugs is, the reduction in p53 deletion matches good response to chemotherapy. On the opposite, the appearance of a relapse state is associated with resistance to the chemotherapy (Venkat, 2003).

In patient No. 1 (Low-grade NHL), p53 deletion is present in (34%) of cells before chemotherapy. This ratio occurs within the range of p53 alterations limits that was reported previously in NHL (10-60%) (Kremer *et al*., 2003). The patient was treated on three stages (12, 13 and 17 weeks of chemotherapy), the p53 deletion reduced to (24, 24 and 34%, respectively). By using X^2-test after 12 and 13 weeks of chemotherapy, this reduction did not differ significantly from its value before chemotherapy. While after 17 weeks of chemotherapy the p53 deletion return to (34%), the same value before chemotherapy was detected. The patient was treated with Chlorambucil and Cyclophosphamide, they lead to disturb once of replication in the cells and if the DNA was not replicated, the cell will not divide or proliferate (Rubin and Hait, 2003). Therefore the number of malignant cells with p53 deletion was reduced on the 12 and 13 weeks of chemotherapy, the re-increasing of p53 deletion on the 17 week caused occurrence of chemotherapy resistance.

In the patient No. 2 (ALL), the ratio of cells with p53 deletion was (56%) before chemotherapy, that is slightly higher than ratio of p53 alterations (50%) (Olivier, 2008). To our knowledge, no previous registry was recorded the ratio of p53 deletion in ALL. The patient was treated on two stages (10 and 32 weeks of chemotherapy), the p53 deletion reduced to (32 and 24%, respectively). This value differs significantly before chemotherapy ($P = 0.05$). The patient was treated with Doxorubicin, 6-MP, ARA-C, Cyclophosphomide, Vincristine, Methotrexate and Daunorubicin, they chemotherapeutic agents succeeded in attacking or reducing the malignant cells that contain p53 deletion. The patient died after thirty months of chemotherapy.

In patient No. 3 (AML-M3), the ratio of cells with p53 deletion was (66%) before chemotherapy that is higher than ratio of p53 alterations (5-10%) (Christiansen *et al*., 2001). The patient was treated on

three stages (12, 28 and 33 weeks of chemotherapy), the p53 deletion reduced to (10, 24 and 28%, respectively). These values differ significantly before chemotherapy ($p \leq 0.05$). The patient was treated with ATRA, Doxorubicin, 6-MP and Methotrexate. They chemotherapeutic agents succeeded in attacking or reducing the malignant cells that contain p53 deletion. Although, the different percentage of p53 deletion among the stages of chemotherapy related to modulation of the pro-apoptotic and antiapoptotic molecules (Keshelava *et al*., 2001). The patient died after 20 months of chemotherapy.

In patient No. 4 (Multiple Myeloma), the ratio of cells with p53 deletion was (24%) before chemotherapy, this ratio occur within the range of p53 alterations limits that was reported previously in multiple myeloma (20-40%) (Chang *et al*., 2005). The patient was treated with Cyclophosphamide for 17 weeks, the slight increase in p53 deletion could predicts occurrence of chemotherapy resistance. The patient died after 10 months of chemotherapy.

In patient No. 5 (AML-M3), the ratio of cells with p53 deletion was (44%) before chemotherapy, that is higher than ratio of p53 alterations (5-10%) (Christiansen *et al*., 2001). The patient was treated on three stages (13, 15 and 17 weeks of chemotherapy), the p53 deletion reduced to (30, 54 and 32%, respectively). The patient was treated with Daunorubicin, ATRA, 6-MP and Methotrexate, the different percentage of p53 deletion among the stages of chemotherapy related to shortage in the treatment or unregulated of drug doses. The patient died after 20 months of chemotherapy.

In the patients No. 6, 7 and 8, the ratio of cells with p53 deletion was (16-40%) before chemotherapy, that is slightly more than ratio of p53 alterations (20-30%) (Olivier, 2008). The progression of CML from Chronic Phase (CP) to Blast Crisis (BC) is frequently associated with deletion of the short arm of chromosome 17 (Feinstein *et al*., 1991). The value of P53 deletion in the patient No.7 (BC) was higher than in the patients No. 6 and 8 (CP). The significant difference ($p \leq 0.05$ and $p \leq 0.05$) confirmed the fact that p53 deletion was associated with progression of CML patients (Di Bacco *et al*., 2000). These patients were treated with Imatinib (STI-571), the patients No. 7 and 8 died after 1 and 20 months of chemotherapy, respectively. The high levels of cells with p53 deletion in those patients have a more chance for chemotherapy resistance toward Imatinib. This is quite coincides with an evidence that acquired loss of p53 contributes to the blastic transformation (bcr/abl)-expressing hematopoietic cells and provides insights into the molecular mechanism for blast crisis of human CML (Honda *et al*., 2000).

A negative correlation was obtained ($r = -0.52$) between the number of cells with p53 deletion that was detected before chemotherapy and the survival of our preliminary patients group (Chang *et al*., 2005; Gandhi, 2007). Therefore, p53 deletion is considered a predictive factor for short survival of patients treated with chemotherapy (Drach *et al*., 1998; Lozanski *et al*., 2004).

Various chromosomal abnormalities were observed in our patients whom were affected with different types of hematological malignancies. The main causes for detection of those chromosomal abnormalities, firstly that in the presence of p53 deletion (Tweddle *et al*., 2001). Secondly, the determination of the cytogenetic abnormality is of prognostic value (Christiansen *et al*., 2001). Thirdly, chromosomal abnormalities related primary (before chemotherapy), or secondary (after chemotherapy). Since, extra chromosomal abnormalities may be obtained after chemotherapy (Connor and Ferguson-Smith, 2004).

In the patients No. 1, 3 and 4, the unique chromosomal abnormality (aneuploidy) which was detected before and after chemotherapy, the patients No. 2, the chromosomal abnormality (polyploidy and aneuploidy) was detected before and after chemotherapy, the patients No. 5, the chromosomal abnormality (aneuploidy) was before chemotherapy and polyploidy was after chemotherapy, the patient No. 7, the chromosomal abnormality (aneuploidy) was before chemotherapy. The presence of aneuploidy in hematological malignancies like ALL, Multiple myeloma, AML and CML represents a poor prognostic sign (Moorman *et al*., 2007; Yang *et al*., 2000; Grimwade *et al*., 1998; Stefanova *et al*., 2000; Fassas *et al*., 2002; Johansson *et al*., 2002).

Two alternative explanations could account for the apparent differences in the level of genetic instability observed in p53-deficient cells. One possibility is that these differences reflect true tissue-specific differences in p53 tumor suppression mechanisms (Bertrand *et al*., 1997). A second possibility is that p53 deficiency is necessary but not sufficient to induce chromosomal instability (Paulson *et al*., 1998).

After chemotherapy, it was shown that the negative correlation between the number of cells with p53 deletion and total number of chromosomal abnormalities still existed in patients (No. 1 to No. 5). These chemotherapeutic agents induce apoptosis in malignant cells harboring p53 deletion despite the presence of the chromosomal abnormalities and irrespective of them. This must direct researchers to find drugs which restore p53 function and repair system function and not only to kill malignant cells (Kojima *et al*., 2005).

In summary, Inactivation of p53 in turn facilitates tumor progression by a mechanism that, surprisingly, does not include the induction of chromosomal instability. Other studies are needed to underscore the complexity of p53 tumor suppression and the need to understand the mechanisms in the context of natural selective pressures occurring during caner evolution *in vivo* (Rubin and Hait, 2003).

ACKNOWLEDGMENTS

Many thanks to Baghdad Teaching Hospital, Teaching Laboratories. Also, many thanks to the Section of Medical Genetics especially Dr. Ahmed, Dr. Abedlkader, Kifaya, Yusra, Ragad and Israa. Furthermore, I'm greatly indebted to Baghdad Teaching Hospital, Department of Hematology especially, Prof. Ali Mohammed Jawad, Dr. Mohammed, Dr. Basam, Dr. Alaadin S., Dr. Waseem, Dr. Mazin, Dr. Ahmed Abed and Dr. Alaadin F. Also, I'm greatly indebted to the Laboratory of Cellular and Molecular Biology, Department of Genetics and Microbiology, University of Pavia, Italy, for their scientific support especially Prof. Elena Giulotto, Dr. Francesca, Dr. Marco, Dr. Alberto and Dr. Nino. Also, many thanks to Atomic Energy Commission of Syria especially Prof. Waleed Al-achkar and his assistant Abedsamad Wafa for their high support in the writing of this study.

REFERENCES

Al-Achkar, W., A. Wafa and M.S. Nweder, 2007. A complex translocation t(5;9;22) in Philadelphia cells involving the short arm of chromosome 5 in a case of chronic myelogenous leukemia. J. Exp. Clin. Cancer Res., 26: 411-415.

Bertrand, P., D. Rouillard, A. Boulet, C. Levalois, T. Soussi and B.S. Lopez, 1997. Increase of spontaneous intrachromosomal homologous recombination in mammalian cells expressing a mutant p53 protein. Oncogene, 14: 1117-1122.

Bonnefoi, H., A. Ducraux, S. Movarekhi, M.F. Pelte, S. Bongard, E. Lurati and R. Iggo, 2002. p53 as a potential predictive factor of response to chemotherapy: Feasibility of p53 assessment using a functional test in yeast from trucut biopsies in breast cancer patients. Br. J. Cancer, 86: 750-755.

Chang, H., C. Qi, Q.L. Yi, D. Reece and A.K. Stewart, 2005. p53 gene deletion detected by fluorescence *in situ* hybridization is an adverse prognostic factor for patients with multiple myeloma following autologous stem cell transplantation. Blood, 105: 358-360.

Christiansen, D.H., M.K. Andersen and J.P. Biergaard, 2001. Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype and a poor prognosis. J. Clin. Oncol., 19: 1405-1413.

Connor, M. and M. Ferguson-Smith, 2004. Cancer Genetics. In: Essential Medical Genetics, Connor, M. and M. Ferguson-Smith (Eds.). Blackwell Science, New York, pp: 162-178.

Czepulkowski, B.H., B. Bllatt and D.E. Rooney, 1992. Basic Techniques for the Preparation and Analysis of Chromosomes from Bone Marrow and Leukemia Blood. In: Human Cytogenetics: A Practical Approach, Rooney, D.E. and B.H. Czepulkowski (Eds.). Vol. 2, Oxford University Press, USA., pp: 1-24.

Daniel, W.W., 1985a. Biostatistics: A Foundation for Analysis in the Health Sciences. John Wiley and Sons, New York, pp: 275-321.

Daniel, W.W., 1985b. Biostatistics: A Foundation for Analysis in the Health Sciences. John Wiley and Sons, New York, pp: 363-394.

Di Bacco, A., K. Keeshan, S.L. McKenna and T.G. Cotter, 2000. Molecular abnormalities in chronic myeloid leukemia: Deregulation of cell growth and apoptosis. Oncologist, 5: 405-415.

Drach, J., J. Ackermann, E. Fritz, E. Kromer and R. Schuster *et al*., 1998. Presence of a p53 gene deletion in patients with multiple myeloma predicts for short survival after conventional-dose chemotherapy. Blood, 92: 802-809.

Fassas, A.B.T., T. Spencer, J. Sawyer, M. Zangari and C.K. Lee *et al*., 2002. Both hypodiploidy and deletion of chromosome 13 independently confer poor prognosis in multiple myeloma. Br. J. Haematol., 118: 1041-1047.

Feinstein, E., G. Cimino, R.P. Gale, G. Alimena and R. Berthier *et al*., 1991. P53 in chronic myelogenous leukemia in acute phase. Natl. Acad. Sci. USA., 88: 6293-6297.

Ferbeyre, G., E. de Stanchina, E. Querido, N. Baptiste, C. Prives and S.W. Lowe, 2000. PML is induced by oncogenic ras and promotes premature senescence. Genes Dev., 14: 2015-2027.

Ferbeyre, G., E. de Stanchina, A.W. Lin, E. Querido, M.E. McCurrach, G.J. Hannon and S.W. Lowe, 2002. Oncogenic ras and p53 cooperate to induce cellular senescence. Mol. Cell., 22: 3497-3508.

Gandhi, V., 2007. p53 as a predictor for chemotherapy response in CLL cells. Leukemia Lymphoma, 48: 219-220.

Gaumont-Leclerc, M.F., U.K. Mukhopadhyay, S. Goumard and G. Ferbeyre, 2004. PEA-15 is inhibited by adenovirus E1A and plays a role in ERK nuclear export and Ras-induced senescence. J. Biol. Chem., 279: 46802-46809.

Giaccia, A.J. and M.B. Kastan, 1998. The complexity of p53 modulation: Emerging patterns from divergent signals. Genes Dev., 12: 2973-2983.

Grimwade, D., H. Walker, F. Oliver, K. Wheatley and C. Harrison *et al*., 1998. The importance of diagnostic cytogenetics on outcome in AML: Analysis of 1,612 patients entered into the MRC AML 10 trial. Blood, 92: 2322-2333.

Hahn, W.C., C.M. Counter, A.S. Lundberg, R.L. Beijersbergen, M.W. Brooks and R.A. Weinberg, 1999. Creation of human tumour cells with defined genetic elements. Nature, 400: 464-468.

Hollstein, M., D. Sidransky, B. Vogelstein and C.C. Harris, 1991. P53 mutations in human cancers. Science, 253: 49-53.

Honda, H., T. Ushijima, K. Wakazono, H. Oda and Y. Tanaka *et al*., 2000. Acquired loss of p53 induces blastic transformation in p210 (bcr/abl)-expressing hematopoietic cells: A transgenic study for blast crisis of human CML. Blood, 95: 1144-1150.

Johansson, B., T. Fioretos and F. Mitelman, 2002. Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. Acta Haematol., 107: 76-94.

Keshelava, N., J.J. Zuo, P. Chen, S.N. Waidyaratne and M.C. Luna et al., 2001. Loss of p53 function confers high-level multidrug resistance in neuroblastoma cell lines. Cancer Res., 61: 6185-6193.

Kojima, K., M. Konopleva, I.J. Samudio, M. Shikami and M. Cabreira-Hansen *et al*., 2005. MDM2 antagonists induce p53-dependent apoptosis in AML: Implications for leukemia therapy. Blood, 106: 3150-3159.

Kremer, M., M. Spitzer, S. Mandl-Weber, K. Stecker and B. Schmidt *et al*., 2003. Discordant bone marrow involvement in diffuse large B-cell lymphoma: Comparative molecular analysis reveals a heterogeneous group of disorders. Lab. Invest., 83: 107-114.

Lozanski, G., N.A. Heerema, I.W. Flinn, L. Smith and J. Harbison *et al*., 2004. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood, 103: 3278-3281.

Moorman, A.V., C.J. Harrison, G.A.N. Buck, S.M. Richards and L.M. Secker-Walker et al., 2007. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): Analysis of cytogenetic data from patients treated on the Medical Research Council (MRC). Blood, 109: 3189-3197.

Olivier, M., 2008. P53 (protein 53 Kda): Tp53 (tumor protein, Li-Fraumeni syndrome). Atlas of Genetics and Cytogenetics in Oncology and Haematology. http://documents.irevues.inist.fr/handle/2042/15937?show=full

Paulson, T.G., A. Almasan, L.L. Brody and G.M. Wahl, 1998. Gene amplification in a p53-deficient cell line requires cell cycle progression under conditions that generate DNA breakage. Mol. Cell. Biol., 18: 3089-3100.

Peller, S. and V. Rotter, 2003. TP53 in hematological cancer: Low incidence of mutations with significant clinical relevance. Hum. Mutat., 21: 277-284.

Rubin, E.H. and W.N. Hait, 2003. Principles of Cancer Treatment. In: Holland-Frei Cancer Medicine, Kufe, D.W., R.E. Pollock, R.R. Weichselbaum, R.C. Bast and T.S. Gansler (Eds.). 6th Edn., BC Decker Inc., Hamilton, Canada, ISBN-10: 1-55009-213-8, pp: 2282-2298.

Shaffer, L., M. Slovak and L. Cambell, 2009. ISCN 2009: An International System for Human Cytogenetic Nomenclature. S. Karger, Basel.

Shimamura, A. and D.E. Fisher, 1996. p53 in life and death. Clin. Cancer Res., 2: 435-440.

Stefanova, M., J. Dierlamm, L. Michaux, P. Leberecht, D. Seeger, K. Hinz and D.K. Hossfelsd, 2000. Polysomy 13 with concomitant deletion of 13q13-14 involving the retinoblastoma gene and the D13S25 locus in a case of acute myeloid leukemia. Cancer Genet. Cytogenet., 119: 158-161.

Tweddle, D.A., A.J. Malcolm, N. Bown, A.D. Pearson and J. Lunec, 2001. Evidence for the development of p53 mutations after cytotoxic therapy in a neuroblastoma cell line. Cancer Res., 61: 8-13.

Venkat, P.C., 2003. Meet the tumor suppressor protein. Cell Biology and Genetics, The p53 Gene, August 3, 2003. http://www.clltopics.org/CellBio/p53Gene.htm.

Wattel, E., C. Preudhomme, B. Hecquet, M. Vanrumbeke and B. Quesnel *et al*., 1994. p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood, 84: 3148-3157.

Yang, C.P., J.H. Wu, I.J. Hung and T.H. Jaing, 2000. Cytogenetic pattern of childhood leukemia in Taiwan. J. Formosan Med. Assoc., 99: 281-289.