Mutant P53 expression in chronic myeloid leukemia

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Summary:

Background: Chronic Myeloid Leukemia (CML) occurs due to malignant transformation of a pluripotent stem cell. Progression is insidious from chronic to aggressive accelerated or blastic phases. Studies revealed a significant role of the tumor suppressor gene P53 in disease progression.

Objectives: To evaluate the immunohistochemical expression of mutant P53 protein in CML at different clinical phases.

Subjects, materials and methods: This case - control study was conducted over 8 months period from January to August in 2010 on 60 preserved bone marrow trephine biopsies of patients with CML (30 in chronic phase, 15 in accelerated phase and 15 in blastic phase) collected from the Department of Histopathology, Teaching laboratories, Medical City directorate, Baghdad. Mutant P53 was evaluated by immunohistochemistry technique of Dakopatts Corporation and scored in the department of pathology / college of medicine / University of Baghdad.

Results: This study was done on 60 CML patients whose age mean was 41.6 ± 16.8 years and range from 14 to 81 years with male to female ratio of 1.06:1. Positive mutant P53 expression was detected in 10 out of 30 cases (33.3%) in chronic phase while it was positive in 8 out of 15 cases (53.3%) in the accelerated phase and positive in 13 out of 15 cases (86.7 %) in the blastic phase with p value of 0.003.

Conclusions: There was a significant difference in the immunohistochemical expression of mutant P53 in the bone marrow biopsies of CML patients among different phases and its expression was more in advance phases of the disease. Moreover the mutant P53 expression show a significant correlation with the blast percentage in the accelerated and blastic phases of CML.

Keywords: CML, P53, immunohistochemistry.

Introduction:

Chronic Myeloid Leukemia is a myeloproliferative disorder seen typically in older adults (4th - 6th decades) [1,2]. According to Iraqi Cancer Registry (2005), leukemia is regarded as the fourth malignancy in adults and CML is the third among leukemia [3]. CML in >90% is associated with Philadelphia chromosome which is a reciprocal translocation t(9; 22) with fused genes BCR and ABL resulting in expression of the constitutively active protein tyrosine kinase [4,5,6]. The identification of BCR-ABL fusion product by PCR or FISH established the diagnosis of CML [7], which is effectively treated with tyrosine kinase inhibitor (imatinib mesylate) [8]. Genomic instability with inhibition of the activity of the tumor suppressor protein P53 which contributes to the maintenance of genomic integrity by repairing DNA damage or by inducing apoptosis, play a role in progression of CML [9,10]. Many anticancer agents activate P53 to induce apoptosis in response to cellular injury [11], so mutation in P53 mostly is associated with resistance to chemotherapy [7]. Immunohistochemistry is the application of a labeled antibody to identify a specific antigen, which is visualized under light microscopy by means of a color signal [12]. Mutant

protein P53 has a prolonged half-life making it easily immunologically detectable[7]. The present study was done to evaluate the value of immunohistochemical expression of the mutant P53 protein in CML and to investigate it's expression throughout different clinical phases of CML.

Pateints and Methods:

In this case control study, we used an immunohistochemical technique to study the expression of mutant P53 protein in the histological sections of bone marrow trephine biopsies from (60) patients with CML whom were diagnosed by senior hematologists in the Departments of hematology, Teaching Laboratories, Medical City directorate / Baghdad. Diagnosis of CML was done by peripheral blood and bone marrow aspirate findings while bone marrow biopsy is done for prognostic purpose. The diagnosis of these CML cases was done in the period from the 1st of January 2008 to the 31st of December 2009. These biopsies were collected and processed for this study over 8 months from January to August in 2010. Thirty cases with CML in chronic phase have been chosen randomly in a systematic way when ordered according to the time of doing the biopsy (every two cases then the third was chosen), while all the cases that were

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diagnosed in that 2 years period as an accelerated or blastic phase have been chosen (15 cases for each). All the cases with repeated or inadequate bone marrow trephine biopsies or deficient in clinical data were excluded. The patients' clinical features, peripheral blood and bone marrow aspirates findings including blast percentages were documented from the patients' laboratory records. This study work was done in the laboratory of the department of pathology in the college of medicine / University of Baghdad. Paraffin blocks of bone marrow trephine biopsy specimens that have been fixed in Bouin's solution, were cut in serial sections of 5 μm in thickness by a microtome and mounted onto charged slides for staining with immunohistochemical staining. By following the procedure that has been developed by Delellis RA et al (1979) in ordered steps with noting that the slides should not be dried and using the products that provided by Dakopatts Corporation, Denmark: After deparaffinization in oven at 65°C for 30 minutes and in xylene jar for 20 minutes, rehydration of these sections in serial alcohols and water was done. Antigen retrieval was done with target retrieval solution at pH 9 (Dakopatts) by micro-wave for 15 minutes at a temperature of 95 °C. After washing for 5 minutes in Tris-buffered saline (TBS) (Dakopatts), we added peroxidase blocking reagent of the EnVision visualization system (Dakopatts) by dropping for 10 minutes followed by washing in TBS. The primary antibody [Monoclonal mouse anti-human P53 protein (clone DO-7, Dakopatts) was diluted in antibody diluent (Dakopatts) with a dilution ratio of 1:25] was dropped on slides and incubated at room temperature (25 °C) for 30 minutes then washed in TBS twice followed by adding labeled polymer-HRP of the EnVision visualization system for 30 minutes at room temperature and washing in TBS three times. We added 0.02% diaminobenzidine hydrochloride (DAB) HRP of the EnVision visualization system as chromogen for 10 minutes at room temperature in darkness then washed in TBS and distilled water and stained lightly with Mayer's hematoxylin counterstain (Dakopatts), rinsed with tap water and mounted with mounting medium (Dakopatts), covered to be examined under light microscope. With each batch, technical controls were used; an oral sequamous cell carcinoma biopsy as a positive control for P53 staining. Negative technical controls were achieved by omitting the primary antibody. Ten bone marrow trephine biopsies obtained from 10 subjects having anemia were used as normal controls to determine the cut-off value. Each slide was examined under light microscope in different magnification powers and 400 - 1000 cells were screened. Only cells exhibiting a clear brownish nuclear coloration without any cytoplasmic or background staining [13] were counted as positively stained cells. The cutoff value was 5% and obtained by calculation the average positive staining percentage of normal controls. The percentage of

positively stained cells was determined by screening the slides with high magnification power (X40) then counting these cells with oil immersion magnification power (X100): The extent of P53 positivity was scored as 0 in cases with either complete absence of staining or in cases with less than 5% have positive brown nuclear staining on (X100), while it was scored as 1 if more than 5% up to 10% of all nucleated cells showed positive staining on (X100). Score 2 was given to cases when more than 10% up to 25% of all nucleated cells showed positive staining on (X100), score 3 was given to cases when more than 25% up to 50% of all nucleated cells showed positive staining on (X100). Finally, a very high expression with score 4 was given to cases when more than 50% of all nucleated cells showed positive staining on (X100). The intensity of staining of the brownish coloration was given score as 0 if there is no staining at all even with magnification power of (X100), scored as 1 if only can be detected at high magnification powers of (X40), while it was scored as 2 if it was detected with difficulty at low magnification power of (X10) and scored as 3 if it was detected very clearly at magnification power of (X10) [14].

Total score (TS) = intensity score (IS) + percentage score (PS).

(TS range = 0-7) [15].

Total score of 3 or more is considered positive.

All results were tabulated and calculated using the Statistical Package for the Social Sciences (SPSS) and the Microsoft Office Excel software. Statistics were analyzed by means of Chi square test, median, mean, standard deviation, range, frequencies and percentages. P values <0.05 were considered statistically significant.

Results:

Among sixty patients with CML, 31 (51.7%) were males and 29 (48.3%) were females. Male to female ratio (M: F) was 1.06:1. Age at diagnosis ranged between (14 - 81) years; mean age was 41.6 ± 16.8 years (mean $\pm$ SD). Most CML patients encountered at the 4th - 6th decades of life where 48.3 % of the cases were observed at this range. The most frequent clinical manifestation was pallor observed in 17 cases (28.3 %) followed by weakness in 13 cases (21.7 %), while splenomegaly alone was found in 43 cases (71.7%). In the chronic phase, 10 out of 30 cases (33.3 %) were positive for mutant P53 staining. In the accelerated phase, 8 out of 15 cases (53.3 %) were positive for mutant P53 staining, while in the blastic phase 13 out of 15 cases (86.7 %) were positive for mutant P53 staining. Those results had a high statistical significance with p value of 0.003 (figure 1).
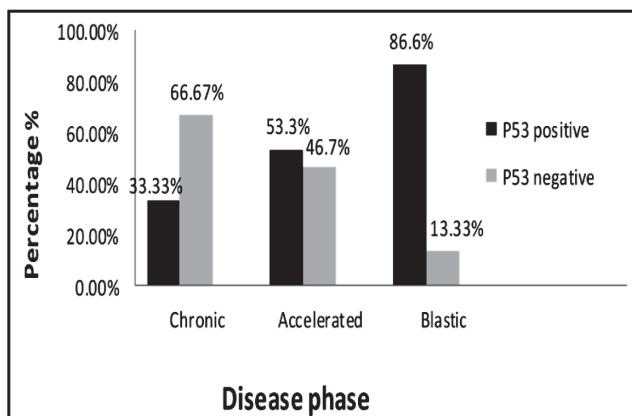
Figure (1): Relationship between disease phase and P53 staining results.

Positive P53 staining was noted in 13 out of 15 cases (86.7%) with bone marrow aspirate blasts $\geq$ 20%, while positive P53 staining was observed in 10 out of 30 cases (33.3%) with bone marrow aspirate blasts < 10% with a high statistical significance with p value of 0.003 (figure2).
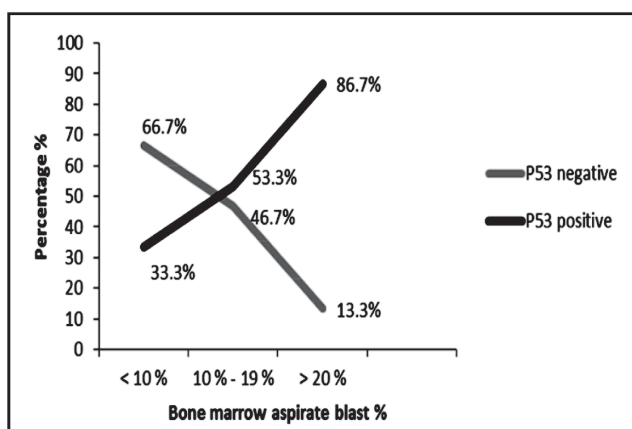

Figure (2): Relationship between bone marrow aspirate blast percentages with P53 staining results.
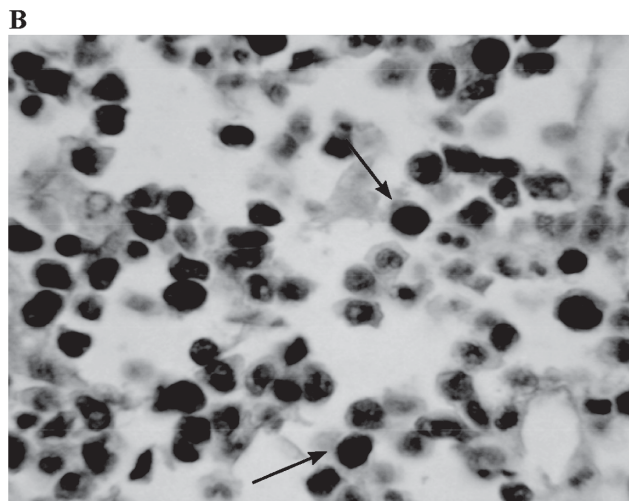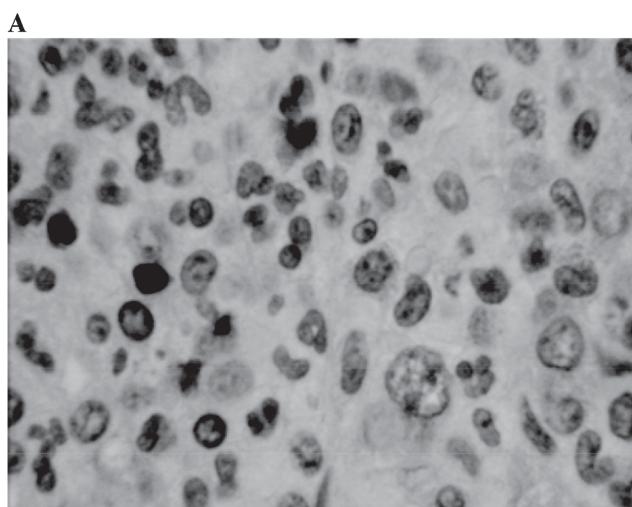
A

B

Figure (3): B.M. trephine biopsy of CML, P53 immunohistochemical staining (X100). (A) Chronic phase; negative nuclear staining. (B) Blastic phase; positive nuclear staining (arrows).

Discussion

This study included sixty patients, 51.7 % of them were males and 48.3 % were females. Male to female ratio was 1.06:1. Those findings were slightly different from what was reported in a previous Iraqi study done in 2003 [16], where out of 32 patients, 62.5% were males and 37.5% were females and male to female ratio was 1.6: 1. This difference could be attributed to the variation in sample sizes between both studies. While in Brazil, a study done in 1996 [17], showed out of 45 patients, 55.6% were males and 44.4% were females and those results were comparall to the current study. At the time of diagnosis, the age of our patients ranged from 14 to 81 years. Mean age of our patients was 41.6 years, which was slightly lower than that reported by Turkish study done in 1995 [18], where the mean age was 45.4 years; this is maybe due to ethnic variations between Iraqi and other countries populations.

Almost half of our patients (48.3%) were in the age group 41 - 60 years, which represents middle age, this was much similar to the previously mentioned Iraqi study [16], where 46.9% of the patients presented in the age group 35 - 60 years; this is expected since CML is a disease of middle age and early elderly. In this study, the most frequent clinical manifestation at diagnosis was pallor which was observed in 28.3 % of patients; weakness was the second most frequent clinical manifestation which was found in 21.7 % of cases and abdominal pain was observed in 16.7 % of cases. In comparison to a previous Iraqi study [16], abdominal pain was the prominent clinical feature which was found in 28.2 % of patients followed by weakness in (25 %). While in an Indian study done in 2001 [19], pallor was observed in

66.6% of 21 patients. In another study done in Germany, 1994 [20], the most common manifestations were weakness in (62.7%) of 513 patients followed by weight loss in 20.4 % of the patients. The possible reason for this variation is the difference in sample sizes among these studies.In the present study, splenomegaly was found in 71.7 % of all patients in the three phases, which is slightly lower than that reported in a similar Iraqi study [16] where it was found in 87.5 % of patients. The incidence of splenomegaly in the two studies were much higher than a study conducted in Portland, 2004 [21] where only (33%) of 141 patients had splenomegaly. The discrepancy of those results may be due to that in Iraq most of the patients were presented with advanced stages of diseases. In this study, we found that the expression of mutant P53 protein increases with the progression of CML to more aggressive stages (p = 0.003), similar to a Serbian study [7]. Thus we may propose that the expression of the mutant P53 protein increases with the progression of the disease. Moreover the mutant P53 protein expression increased significantly with high bone marrow blasts percentage. This is again goes with the suggestion that the expression of the mutant P53 protein increases with the evolution of the disease.

Conclusions:
There was a significant increase in the immunohistochemical expression of mutant P53 in the bone marrow biopsies of CML among chronic, accelerated and blastic phases and its expression is more in advance phases of the disease. Moreover the mutant P53 expression show a significant correlation with the blast percentage of the accelerated and blastic phases of CML.

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