ATM GENE DELETION AND MUTANT p53 EXPRESSION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: CORRELATION WITH RISK FACTORS AND DISEASE OUTCOME

Thesis

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فقدان الجين ATM وظهور p53 ذو الطفرة الجينية فى مرض سرطان الدم الليمفاوى المزمن B: علاقتهم بعوامل الخطورة ومآل المرض

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DISCUSSION

Chronic lymphocytic leukemia is one of the most common hematological malignancies that results in significant morbidity and mortality. It is caused by the clonal expansion of B cells with a distinctive morphological appearance and surface immunophenotype. One of the most striking features of CLL is the extent of its clinical variability. Recent studies have been developed to understand this variability in terms of biological heterogeneity ***(Pettitt, 2007).***

The p53 protein is a transcription factor that is activated by DNA damage and coordinates the cellular response to such damage by triggering apoptosis or cell-cycle arrest. It also contributes directly to the repair of some forms of DNA damage. Inactivation of P53 by mutation or deletion occurs in approximately 50% of human cancers and is associated with genomic instability and resistance to chemotherapy ***(Carter et al., 2004).*** Mutant p53 is present at increased levels owing to its prolonged half-life although it lacks wild-type function ***(Pettitt, 2007).***

The ATM protein, a member of PIKK family, is the main integrator of cellular response after DNA double strand breaks and is responsible for the phosphorylation and stabilization of p53 ***(Giene et al., 2008).*** Critically, ATM is responsible for activating the p53 tumor suppressor protein, leading to the upregulation of p53-responsive genes that promote cell cycle

arrest and apoptosis. Outcomes of p53 activation, however, depend on the cellular context and the severity of the induced DNA damage, implying that in a given cell other cellular factors influence whether the outcome of the response will be survival or death ***(Stankovic et al., 2004).***

To highlight the role of ATM and p53 in B-CLL, this thesis was conducted to study the incidence of ATM gene deletion and detect mutant p53 expression in B-CLL patients in order to assess their prognostic impact and to correlate them with the clinical status, standard risk factors as well as patients' outcome.

Thirty newly diagnosed B-CLL patients. They were studied for ATM gene deletion by CCA and FISH technique and for mutant p53 expression by flow cytometry technique.

Conventional cytogenetic analysis was done and yielded successful metaphases in 16/30 (53.3%) cases. It revealed normal karyotype in 50%, deletion 13q14 in 25%, trisomy 12 in 12.5%, 14q rearrangement in 6.25% and deletion 11q in 6.25% of cases with successful metaphases. Follow up of the patients was done over a period of 24 months and prognosis was assessed according to the response to treatment. Patients with deletion 13q14 and 14q rearrangement had good prognosis while those with trisomy 12 and deletion 11q had bad prognosis. 62.5% of patients with normal karyotype had good prognosis and 37.5% had bad prognosis.

Our results are in accordance with ***Stilgenbauer et al. (2002)*** who found normal karyotype in 51% of cases, trisomy 12 in 19% of cases, deletion 13q14 in 10%, 14q rearrangement in 8%, deletion 11q in 8% and deletion 17p in 4% of cases but their CCA yielded successful metaphases in 91% of cases. They also found that trisomy 12, deletion 11q, deletion 17p and 14 q rearrangement were associated with bad prognosis and deletion 13q was associated with good prognosis.

In disagreement with our results, ***Oscier et al. (2002)*** used CCA and yielded successful metaphases in 71.1% of cases and found normal karyotype in 38%, deletion 13q14 in 17%, trisomy 12 in 29%, deletion 11q in 10% and deletion 17p in 6% of cases.

Deletion of the long arm of chromosome 11 is found in CLL and is associated with poor outcome. The deletion includes the ATM gene located at 11q22.3 to 11q23.1. The ATM protein is a central component of the signal transduction pathway that is activated in response to DNA double-strand breaks and results in DNA repair. The cellular consequences of ATM dysfunction include chromosomal radiosensitivity, radioresistant DNA synthesis and loss of cell cycle checkpoints ***(Austen et al., 2007).***

In the current work, metaphase/interphase FISH analysis was applied to all patients. It revealed positive ATM deletion in 6/30 (20%) cases versus CCA which showed deletion 11q in 1/16 (6.25%). Out of the other 5 patients with ATM deletion, 3

showed failed metaphases thus CCA could not be applied and the other 2 showed normal karyotype and trisomy 12.

Our results were confirmed by ***Cotter and Auer (2007)*** who reported that not all patients with deletions of 11q have evidence of an ATM mutation and vice versa. Thus, FISH technique showed a higher ability to detect ATM gene deletion than CCA.

In accordance with our results, ***Glassman and hays (2005)*** detected ATM gene deletion in 11% of patients by CCA and in 23% by FISH analysis. Also ***Ripolles et al. (2006)*** detected different chromosomal aberrations in 36.7% of CLL patients using CCA and in 68.4% of patients using FISH technique.

These findings were explained by the fact that FISH is considered a more sensitive and specific method than CCA in detecting chromosomal aberrations as it allows the evolutions of a large number of either dividing (metaphase) or nondividing (interphase) cells***(Galteland and colleagues, 1999)***.

The aforementioned findings were further assured by ***Bentley et al. (2005).*** They attributed the superiority of FISH over CCA in the demonstration of cytogenetic abnormalities to the fact that; although CCA is considered a golden method for cytogenetic analysis as it allows simultaneous visualization of all human chromosomes and screens for chromosomal aberrations without a prior knowledge of any abnormality, yet

its use is hampered by a high failure rate especially in LPDs due to the very low mitotic activity of cells and poor response to mitogens which in turn renders CCA not always informative as a routine analysis in LPDs.

The results of ATM deletion using FISH technique go in agreement with ***Oscier et al. (2002)*** who detected ATM deletion in 19% of B-CLL patients and both ***Stilgenbauer et al. (2002)*** and ***Shanafelt et al. (2003)*** who found it in 18% of patients. ***Schroers et al. (2005)*** also detected ATM deletion using FISH technique in 17% of patients and ***Galssman and Hayes (2005)*** found it in 23% of patients. Similarly, ***Dickinson et al. (2006)*** and ***Ripolles et el. (2006)*** found ATM gene deletion in 17.5% of patients. ***Berkova et al. (2008)*** detected ATM gene deletion less frequently being in 15% of B-CLL patients.

In disagreement with our results, ***Xu et al. (2008)*** used interphase FISH and found ATM gene deletion in 9.5% of Chinese patients with B-CLL. This difference in results could be attributed to different ethnic origin.

Studies using other techniques showed some variability. ***Stakovic et al. (2002)*** used RT-PCR to detect ATM mutation in B-CLL patients. They detected ATM mutation in 32% of patients; 4% of patients had loss of one allele and mutation in the other, 10% had 2 ATM mutations, 10% had a single point mutation and 8% had a single small insertion or deletion predicted to lead to protein truncation.

According to the results of FISH analysis in the present study, B-CLL patients were divided into 2 groups; a group with ATM gene deletion and another group without ATM deletion and they were both compared to the different studied parameters.

As regards the clinical data, patients with ATM deletion showed higher incidence of splenomegaly and older age with undetectable significance as regards sex, hepatomegaly and lymphadenopathy when compared to patients without ATM deletion.

Regarding laboratory findings, patients with ATM deletion revealed higher TLC, absolute lymphocyte count, percentage of BM lymphocytes, LDH concentration and CD38 expression and lower Hb level and platelet count than patients without ATM deletion.

The CLL is a complex disease with a very heterogeneous outcome. The development of newer prognostic factors has allowed for further discrimination of patients into risk categories. Thus, progressive and smoldering forms of the disease can now be separated more accurately than was previously possible using Rai's and Binet's staging systems. Genomic aberrations are important independent predictors of disease progression and survival in multivariate analysis and permit outcome prediction irrespective of the clinical stage allowing a risk assessment for individual patients early in the course of their disease ***(Cotter and Auer, 2007).***

The prognostic impact of ATM gene deletion was evaluated by a correlation study with the standard prognostic factors, response to therapy and analysis of variables. A highly significant association between ATM deletion and splenomegaly, higher total leucocytic count, higher absolute lymphocyte count, higher percentage of BM lymphocytes, higher CD38 positivity, lower Hb level and platelet count was obtained, however, no significant association with age, sex, hepatomegaly or LDH concentration

In agreement with these results, ***Starostic et al. (1998)*** stated that patients with ATM deletion showed lower platelet count and Hb level compared to patients without ATM deletion. Also, ***Cuneo et al. (2002)*** found ATM deletion to be associated with increased TLC, serum LDH concentration and B2-microglobulin. Moreover, ***Dickinson et al. (2006)*** found higher CD38 expression in ATM deletion group but on the contrary they found no association with the absolute lymphocyte count.

On the contrary, ***Xu et al. (2008)*** found no significant association between ATM gene deletion and age, sex, lymphocyte count, serum LDH concentration, β2-microglobulin or ZAP-70. However, frequency of ATM gene deletion was obviously higher in CD38-positive group than CD38-negative group.

Our results revealed insignificant statistical association between lymph node enlargement and ATM gene deletion, although all our patients with ATM gene deletion had

lymphadenopathy. ***Stilgenbauer et al. (2002)*** found that patients with deletion 11q exhibited extensive lymphadenopathy. Also, ***Dickinson et al. (2006)*** found that ATM deletion group had bulky abdominal and mediastinal lymphadenopathy. In 2007, ***Joshi and his colleagues*** used real time PCR and found that ATM gene was consistently underexpressed in B-CLL patients with bulky lymphadenopathy.

As for the relation between ATM gene deletion and its relation to disease outcome, ATM gene deletion was found to be significantly associated with poor response to treatment and poor prognosis as all patients (100%) with ATM deletion had unfavorable outcome and poor prognosis compared to patients without ATM deletion where only 41.5% of them had unfavorable outcome.

Our results go in agreement with ***Stilgenbauer et al. (2002)*** who found higher association of ATM gene deletion with inferior molecular remission rate after high dose therapy, shorter treatment free interval and reduced overall survival. Similarly, ***Oscier et al. (2002)***, ***Glassman and Hayes (2005) and Joshi et al. (2007)*** found that ATM gene deletion is associated with poor prognosis. Moreover, ***Ripolles et al. (2006)*** stated that the most frequent abnormality in patients with progressive B-CLL disease was ATM gene deletion. ***Giene et al. (2008)*** also found that inactivation of the ATM gene by deletion of 11q22.3-23.1 or by mutation occurs in a

proportion of B-CLL patients with poor prognosis and is an alternative cause of p53 dysfunction. Recently, ***Hauswirth and Jager (2008)*** reported that 11q deletions, harboring ATM gene, are associated with bulky disease and are associated with short survival times. This is even more pronounced than 17p deletions which predict for very poor outcome.

In discordance with the previous results, ***Xu et al. (2008)*** found no evidence of important association between disease outcome and incidence of ATM gene deletion in Chinese patients with B-CLL.

The p53 pathway plays a central role in cancer biology by limiting clonal expansion, maintaining genomic integrity and contributing to the action of chemotherapy ***(Pettitt et al., 2007).***

In this current study, MFI values were taken as numerical measurement of mutant p53 levels. To make analysis more objective we used the receiver operator curve (ROC) method to identify the appropriate cut off value of 12.5. Above 12.5, cases were assigned as highly expressing mutant p53. Our results revealed high expression in 10/30 (33.3%) cases with compatible results reported by ***Carter et al. (2004)*** who observed high mutant p53 expression in 5/21(23.8%) using the same technique with cut off value 15 and also confirmed the results by western blotting analysis.

Studies using FISH technique, showed some variability; ***Dohner et al. (1997)*** and ***Dickinson et al. (2006)*** detected P53 deletion in 10% of cases. Also, ***Galssman and Hayes (2005)*** detected the deletion in 12% of cases and ***Ripolles et al. (2006)*** detected it in 8.7% of cases. Similarly, ***Xu et al. (2008)*** found P53 gene deletion in 16.8% of patients whereas ***Berkova et al. (2008)*** found it in 17% of cases using FISH technique as well. ***Stankovic et al. (2002)*** detected P53 mutation in 6/50 (12%) of B-CLL cases using RT-PCR. Moreover, ***Pettitt et al. (2001)*** and ***Lin et al. (2002)*** measured the level of mutant p53 by western blotting and found it in 6/42 (14.3%) and 8/71 (11%) of B-CLL cases respectively. This observed discrepancy between our results and other studies could be attributed to different methodology.

According to the results of flow cytometric analysis of mutant p53, our patients were divided into 2 groups; a group with high expression of p53 and another group with low expression and they were both compared to the different studied parameters.

As regards clinical data, there was a significantly higher incidence of splenomegaly in patients with high mutant p53 expression. No significant difference was detected as regards age, sex, hepatomegaly or lymphadenopathy.

As for the laboratory data, patients with high mutant p53 expression had significantly higher TLC and CD38 expression than those with low mutant p53 expression. No significant

difference was detected as regards Hb level, platelet count, absolute lymphocyte count, percentage of bone marrow lymphocytes or serum LDH concentration.

In the present study, the prognostic relevance of mutant p53 was assessed by a correlation study with the studied prognostic factors. A statistically significant association between high mutant p53 expression and splenomegaly, higher TLC, higher absolute lymphocyte count and higher CD38 expression was obtained.

Association studies with predicted disease outcome revealed that cases with high mutant p53 expression were significantly associated with poor response to treatment and poor prognosis as 80% of our patients with high mutant p53 expression showed unfavorable outcome whereas only 40% of cases with low expression had unfavorable outcome.

These findings were further explained by ***Pettitt et al. (2007)*** who reported that such mutated p53 proteins do badly due to rapid clonal expansion, clonal instability and resistance to chemotherapy

In accordance with our results, ***Pettitt et al. (2001), Slilgenbauer et al. (2002), Lin et al. (2002)*** and later ***Xu et al. (2008)*** reported that P53 mutations are associated with large cell transformation, shortened survival and poor response to chemotherapy.

Moreover, ***Malcikova et al. (2007)*** showed that 12% of patients manifested a P53 gene alteration using RT-PCR and that most of affected patients (58%) exhibited a complete gene inactivation (deletion/mutation). However single allele missense mutations or deletions also occurred in (26% or 16%) of p53 affected patients respectively. They also confirmed that patients with deletion/mutation manifested the worst prognosis which differs significantly from both other groups with no P53 abnormality and with single allele aberration.

In 2004, ***Thornton and his associates*** stated that the incidence of P53 gene abnormalities is significantly different between patients with milder indolent course of the disease (7%) and pretreated/refractory group (50%). They confirmed that abnormal P53 gene was predicted for shorter survival regardless of the method used and that testing for P53 deletion by FISH and protein overexpression by flow cytometry is an effective and simple way of screening patients who are likely to have aggressive disease. DNA sequencing adds little to these methods in identifying population at risk.

Following the occurrence of double stranded breaks, ATM phosphorylates p53 leading to the upregulation of p53 levels in the cell nucleus where it plays a critical role in determining the final cell fate after DNA damage. It appears to be a crucial factor for the switch between the induction of cell cycle arrest, thus allowing DNA repair, and the induction of

apoptosis which may occur in the presence of extensive or persistent DNA damage ***(Bree et al., 2004).***

We found no significant association between ATM deletion and mutant p53 expression. In 2007, ***Cotter and Auer*** reported that P53 mutations are only present in tumors without an ATM deletion or loss of ATM protein. This finding was formerly explained by ***Carter et al. (2004)*** who detected 2 types of defects causing p53 dysfunction; type A defect which is due to P53 mutation and type B defect which is due to ATM gene deletion. In type A defect the baseline levels of p53 are increased reflecting the prolonged half life of mutant p53 as compared with the wild type protein. In type B defect the baseline levels of p53 are not increased and there is impaired accumulation of p53 in response to irradiation.