SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Acute myelogenous leukemia (AML) is a clonal, malignant disease of hematopoietic tissues that is characterized by accumulation of abnormal blast cells, principally in the marrow, and impaired production of normal blood cells. There is considerable heterogeneity between cases of AML as regards morphology, immunological phenotype, associated cytogenetic and molecular abnormalities, and patterns of gene expression. Pretreatment conventional cytogenetic studies identify an acquired clonal abnormality in approximately 50% - 60% of patients with de novo AML, of which 10% - 12% are complex karyotypes that have very poor outcome. The rest 40% - 50% of cases, have no karyotypic abnormality, yielding an AML with normal karyotype. All such cases of cytogenetically normal AML are currently categorized in the intermediate-risk group, yet this group is quite heterogeneous.

The backbone of remission induction consists of an anthracycline (daunorubicin) and cytosine arabinoside (Ara-C), a regimen that has not changed in over 30 years. Daunorubicin is given at a dose of 45 mg/m^2/day for 3 days, in combination with Ara-C, which is administered as a continuous infusion at 100 mg/m^2/day for 7 days (frequently referred to as 3+7 chemotherapy). It is unusual for induction chemotherapy not to clear most of the leukemic blasts; however, this is at a cost of 3 - 4 weeks of severe pancytopenia. Supportive care throughout the period of marrow suppression is crucial to treatment outcome.

The human genome is constantly attacked by endogenous reactive metabolites, therapeutic drugs and a plethora of environmental mutagens that impact its integrity. Thus it is obvious that the stability of the genome must be under continuous surveillance. Nucleotide excision repair (NER) is a major DNA repair pathway in eukaryotic cells. It is responsible for the repair of Bulky DNA adducts such as UV-light-induced photo-lesions, intra-strand cross-links and large chemical adducts generated from exposure to genotoxic agents. XPD enzyme is a member of TFIIH complex which is essential in the NER pathway. The anticancer activity of most chemotherapy drugs relies on the induction of DNA damage in rapidly cycling tumor cells. Consequently, the regulation of DNA repair pathways in tumor cells is a critical factor for their response to chemotherapy drugs and may influence drug toxicity and remission.

Aim of our study:

Evaluation of two *XPD* polymorphisms Asp312Asn (G→A) and Lys751Gln (A→C) in relation to the response to induction chemotherapy as well as the chemotherapy-induced toxicities in adult cytogenetically normal AML patients.

This study included:

51 adult Egyptian patients (over 18 years old) with newly diagnosed de novo cytogenetically normal AML of both sexes presented to the Department of Hematology, Medical Research Institute, Alexandria University. Those over 60 years old and those with comorbid conditions and/or poor performance status were excluded. Patients with acute promyelocytic leukemia (AML M3) were not enrolled in this study either, because of the different chemotherapy regimen adopted for them.

Patients were subjected to a number of investigations:

Clinical examinations and laboratory tests were performed at presentation and a three milliliter sample of venous blood was collected from each patient. Patients were treated by the standard remission induction protocol (3+7). During chemotherapy, patients were monitored for possible toxic effects induced by chemotherapy. Response to chemotherapy was evaluated after regeneration from chemotherapy-induced bone marrow aplasia, patients who failed to achieve complete remission were subjected to a second similar course of chemotherapy. DNA was extracted from the collected blood samples and the two *XPD* polymorphisms (*XPD* Asp312Asn and *XPD* Lys751Gln) were determined using PCR-RFLP. And statistical analysis of the obtained data was performed.

Results of the study:

XPD Asp312Asn polymorphism was not associated with the response to chemotherapy or with any of the studied chemotherapy-induced toxicities. On the other hand *XPD* Lys751Gln polymorphism, was associated with the response to chemotherapy and with cardiotoxicity but not with hepatotoxicity, nephrotoxicity or metabolic toxicity. Patients with the common *XPD* 751 genotype (AA) were more likely to achieve complete remission. While Patients with *XPD*751 CC variant (Gln/Gln) were more likely to suffer chemotherapy-induced cardiotoxicity.

Conclusions:

In the present work, we identified *XPD* codon 751 polymorphism as a prognostic marker in de novo cytogenetically normal AML patients treated with chemotherapy. Although it is still early to establish a firm conclusion based on our work, yet this data might represent a small step towards better understanding of the role played by the DNA repair genes polymorphisms in the outcome of acute myeloid leukemia therapy.

Recommendations:

Up to our best knowledge, no reports studied these polymorphisms in relation to response to chemotherapy as well as chemotherapy-induced toxicities in newly diagnosed cytogenetically normal AML patients. Nonetheless our study has limitations due to the relatively small sample size. Clearly, these analyses need to be conducted in larger populations. Furthermore, future large studies should include multiple SNPs of genes involved in the different DNA repair pathways as well as in xenobiotic and apoptotic pathways. This is necessary to validate the association between variants in DNA repair genes and outcome and to clarify the underlying mechanisms involved in AML progression. Further work to identify how those factors interact may allow for the development of individualized treatment regimens for AML patients.

REFERENCES

References

1. Weber GF. Molecular Mechanisms of Cancer. 1st ed. Springer, Dordrecht. 2007, pp. 525-50 (Hematologic Malignancies).

2. Baer MR, Greer JP. Acute Myeloid Leukemia in Adults. In : Wintrobe's Clinical Haematology. Greer JP, Foerster J, Rodgers GM, Paraskevas F, Glader B, Arber DA, et al (eds). 12th ed. Lippincott Williams & Wilkins, Philadelphia. 2009, pp. 1843-89.

3. Liesveld JL, Lichtman MA. Acute Myelogenous Leukemia. In : Williams Hematology. Kaushansky K, Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Prchal JT (eds). 8th ed. McGraw-Hill, New York. 2010, pp. 1277-330.

4. Appelbaum FR. Acute myeloid leukemia in adults. In: Abeloff's Clinical Oncology. Abeloff MD, Armitage JO, Niederhuber JE. Kastan MB, McKenna WG (eds). 4th ed. Elsevier, Philadelphia. 2008, pp. 2215-34.

5. Deschler B, Lubbert M. Acute myeloid leukemia: epidemiology and etiology. Cancer 2006; 107(9): 2099–107.

6. Ruddon RW. Cancer Biology. 4th ed. Oxford, New York. 2007, pp. 62-116 (The Epidemiology of Cancer).

7. Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. CA Cancer J Clin. 2002; 52(1): 23–47.

8. Boyd SD, Arber DA. Acute myeloid leukemias. In : Blood and Bone Marrow Pathology. Porwit A, McCullough J, Erber WN (eds). 2nd ed. Elsevier, Philadelphia. 2011, pp. 273-88.

9. Burnett AK, Venditti A. Acute myeloid leukaemia. In : Postgraduate Haematology. Hoffbrand AV, Catovsky D, Tuddenham EG, Green AR (eds). 6th ed. Blackwell, Oxford. 2010, pp. 415-32.

10. Wetzler M, Byrd JC, Bloomfield CD. Acute and Chronic Myeloid Leukemia. In : Harrison's Hematology and Oncology. Longo DL (ed). 1st ed. McGraw-Hill, New York. 2010, pp. 166-81.

11. Howlader N, Noone AM, Krapcho M, Garshell J, Neyman N, Altekruse SF, et al. SEER Cancer Statistics Review, 1975-2010, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2010/.

12. Elattar I. Cancer Registry of National Cancer Institute of Egypt. 2002-2003. 2003.

13. Pulsoni A, Pagano L, Latagliata R, Casini M, Cerri R, Crugnola M, et al. Survival of elderly patients with acute myeloid leukemia. Haematologica 2004; 89(3): 296–302.

14. Estey EH. How I treat older patients with AML. Blood 2000; 96: 1670–3.

15. Sekeres MA, Stone RM, Zahrieh D, Neuberg D, Morrison V, De Angelo DJ, et al. Decision-making and quality of life in older adults with acute myeloid leukemia or advanced myelodysplastic syndrome. Leukemia 2004; 18: 809–16.

16. American Cancer Society. Cancer Facts and Figures 2005. Atlanta: American Cancer Society; 2005.

17. Rowe JM, Tallman MS. Therapy for Acute Myeloid Leukemia. In : Hematology: Basic Principles and Practice. Hoffman R, Benz Jr. EJ, Shattil SJ, Furie B, Silberstein LE, McGlave P, et al (eds). 5th ed. Churchill Livingstone, Philadelphia. 2008.

18. Catenacci DV, Schiller GJ. Myelodysplasic syndromes: a comprehensive review. Blood reviews 2005; 19(6): 301-19.

19. Sharathkumar A, Kirby M, Freedman M, Abdelhaleem M, Chitayat D, Teshima IE, et al. Malignant hematological disorders in children with Wolf-Hirschhorn syndrome. Am J Med Genet 2003; 119A: 194-9.

20. Turhan AG, Lemoine FM, Debert C, Bonnet ML, Baillou C, Picard F, et al. Highly purified primitive hematopoietic stem cells are PML-RARA negative and generate nonclonal progenitors in acute promyelocytic leukemia. Blood 1995. 85(8): 2154-61

21. van Lom K, Hagemeijer A, Vandekerckhove F, de Greef GE, Sacchi N, Löwenberg B. Clonality analysis of hematopoietic cell lineages in acute myeloid leukemia and translocation (8;21): Only myeloid cells are part of malignant clone. Leukemia 1997; 11: 202-5.

22. Look AT. Oncogene transcription factors in human acute leukemias. Science 1997; 278: 1059-64.

23. Kelly LM, Gilliland DG. Genetics of myeloid leukemias. Annu Rev Genomics Hum Genet 2002; 3: 179-98.

24. Pabst T, Mueller BU. Transcriptional dysregulation during myeloid transformation in AML. Oncogene 2007; 26: 6829-37.

25. Bacher U, Schnittger S, Haferlach T. Molecular genetics in acute myeloid leukemia. Curr Opin Oncol 2010; 22: 646–55.

26. Marcucci G, Haferlach T, Dohner H. Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. J Clin Oncol 2011; 29: 475–86.

27. Gilliland DG, Jordan CT, Felix CA. The molecular basis of leukemia. Hematology Am Soc, Hematol Educ Program 2004: 80–97.

28. Ferrara F, Schiffer CA. Acute myeloid leukaemia in adults. Lancet 2013; 381(9865): 484-95.

29. Frohling S, Scholl C, Gilliland DG, Levine RL. Genetics of myeloid malignancies—pathogenetic and clinical implications. J Clin Oncol 2005; 23: 6285–95.

30. Estey E, Dohner H. Acute myeloid leukaemia. Lancet 2006; 368(9550): 1894-907.

31. Shen Y, Zhu YM, Fan X, Shi JY, Wang QR, Yan XJ, et al. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood 2011; 118: 5593–603.

32. Mauritzson N, Albin M, Rylander L, Billström R, Ahlgren T, Mikoczy Z, et al: Pooled analysis of clinical and cytogenetic features in treatment-related and de novo adult acute myeloid leukemia and myelodysplastic syndromes based on consecutive series of 761 patients analyzed 1976–1993 and on 5098 unselected cases reported in the literature 1974–2001. Leukemia 2002; 16(12): 2366-78.

33. Taylor AM, Metcalfe JA, Thick J, Mark YF. Leukemia and lymphoma in ataxia telangiectasia. Blood 1996; 87(2): 423-38.

34. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. Blood 1994; 84(5): 1650-5.

35. Minelli A, Maserati E, Rossi G, Bernardo ME, De Stefano P, Cecchini MP, et al. Familial platelet disorder with propensity to acute myelogenous leukemia: genetic heterogeneity and progression to leukemia via acquisition of clonal chromosome anomalies. Genes Chromosomes Cancer 2004; 40(3): 165-71.

36. Smith ML, Cavenagh JD, Lister TA, Fitzgibbon J. Mutation of CEBPA in familial acute myeloid leukemia. N Engl J Med 2004; 351(23): 2403-7.

37. Preston DL, Kusumi S, Tomonaga M, Izumi S, Ron E, Kuramoto A, et al. Cancer incidence in atomic bomb survivors. Part III: Leukemia, lymphoma and multiple myeloma, 1950-1987. Radiat Res 1994; 137(2s): S68-97.

38. Hayes RB, Yin SN, Dosemeci M, Li GL, Wacholder S, Travis LB, et al. Benzene and the dose-related incidence of hematologic neoplasms in China. Chinese Academy of Preventive Medicine–National Cancer Institute Benzene Study Group. J Natl Cancer Inst 1997; 89: 1065–71.

39. Doll R. Cancers weakly related to smoking. Br Med Bull 1996; 52: 35–49.

40. Pogoda JM, Preston-Martin S, Nichols PW, Ross RK. Smoking and risk of acute myeloid leukemia: results from a Los Angeles County case-control study. Am J Epidemiol. 2002; 155: 546–53.

41. Smith MT. Benzene, NQO1, and genetic susceptibility to cancer. Proc Natl Acad Sci U S A 1999; 96(14): 7624-6.

42. Larson RA, Wang Y, Banerjee M, Wiemels J, Hartford C, Le Beau MM, et al. Prevalence of the inactivating 609C→T polymorphism in the NAD(P)H:Quinone Oxidoreductase (NQO1) gene in patients with primary and therapy-related myeloid leukemia. Blood 1999; 94: 803-7.

43. Smith MT, Wang Y, Kane E, Rollinson S, Wiemels JL, Roman E, et al. Low NAD(P)H:quinine oxidoreductase 1 activity is associated with increased risk of acute leukemia in adults. Blood 2001; 97: 1422-6.

44. Bowen DT, Frew ME, Rollinson S, Roddam PL, Dring A, Smith MT, et al. CYP1A1*2B (Val) allele is overrepresented in a subgroup of acute myeloid leukemia patients with poor-risk karyotype associated with NRAS mutation, but not associated with FLT3 internal tandem duplication. Blood 2003; 101: 2370-74.

45. Curtis RE, Boice JD Jr, Stovall M, Bernstein L, Holowaty E, Karjalainen S, et al. Relationship of leukemia risk to radiation dose following cancer of the uterine corpus. J Natl Cancer Inst 1994; 86: 1315-24.

46. Curtis RE, Boice JD Jr, Stovall M, Bernstein L, Greenberg RS, Flannery JT, et al. Risk of leukemia after chemotherapy and radiation treatment for breast cancer. N Engl J Med 1992; 326: 1745-51.

47. Youn B-S, Mantel C, Broxmeyer HE. Chemokines, chemokine receptors and hematopoiesis. Immunol Rev 2000; 17: 150–74.

48. Anderlini P1, Luna M, Kantarjian HM, O'Brien S, Pierce S, Keating MJ, et al. Causes of initial remission induction failure in patients with acute myeloid leukemia and myelodysplastic syndromes. Leukemia 1996; 10: 600–8.

49. Seymour JF, Pierce SA, Kantarjian HM, Keating MJ, Estey EH. Investigation of karyotypic, morphologic and clinical features in patients with acute myeloid leukemia blast cells expressing the neural cell adhesion molecule (CD56). Leukemia 1994; 8: 823–6.

50. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 1976; 33(4): 451–8.

51. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002; 100(7), 2292-302.

52. Swerdlow SH, Campo E, Harris NL. WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. 4th ed. IARC, Lyon. 2008.

53. Heerema-McKenney AE, Arber DA. Acute Myeloid Leukemia. In : Hematopathology. His ED (ed). 2nd ed. Elsevier, Philadelphia. 2012.

54. Lowenberg B, Downing J R, Burnett A. Acute myeloid leukemia. N Engl J Med 1999; 341(14), 1051-62.

55. Baer MR, Barcos M, Farrell H, Raza A, Preisler HD. Acute myelogenous leukemia with leukemia cutis. Eighteen cases seen between 1969 and 1986. Cancer 1989; 63: 2192-200.

56. Cohen PR, Talpaz M, Kurzrock R. Malignancy-associated Sweet's syndrome: review of the world literature. J Clin Oncol 1988; 6: 1887–97.

57. Cooper TM, Hasle H, Smith FO. Acute Myeloid Leukemia, Myeloproliferative and Myelodysplastic Disorders. In: Principles and practice of pediatric oncology. Pizzo PA, Poplack DG, (eds). 6th ed. Lippincott Williams & Wilkins, Philadelphia. 2010:567-611.

58. Castagnola C, Nozza A, Corso A, et al. The value of combination therapy in adult acute myeloid leukemia with central nervous system involvement. Haematologica 1997; 82: 577-80.

59. Holmes R, Keating MJ, Cork A, Broach Y, Trujillo J, Dalton WT Jr, et al. A unique pattern of central nervous system leukemia in acute myelomonocytic leukemia associated with inv(16) (p13q22). Blood 1985; 65: 1071-8.

60. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 2010, 115(3), 453-74.

61. Lichtman MA, Rowe JM. Hyperleukocytic leukemias: rheological, clinical, and therapeutic considerations. Blood 1982; 60(2): 279-83.

62. Bunin NJ, Pui CH. Differing complications of hyperleukocytosis in children with acute lymphoblastic or acute nonlymphoblastic leukemia. J Clin Oncol 1985; 3(12), 1590-5.

63. McPhee SJ, Hammer GD. Pathophysiology of Disease: An Introduction to Clinical Medicine. 6th ed. McGraw-Hill, New York. 2010. (Neoplasia)

64. Khalidi HS, Medeiros LJ, Chang KL, Brynes RK, Slovak ML, Arber DA. The immunophenotype of adult acute myeloid leukemia: High frequency of lymphoid antigen expression and comparison of immunophenotype, French-American-British classification, and karyotypic abnormalities. American Journal of Clinical Pathology 1998; 109: 211–20.

65. Grimwade D. The clinical significance of cytogenetic abnormalities in acute myeloid leukaemia. Best Pract Res Clin Haematol 2001; 14(3): 497-529.

66. Mrozek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004; 18(2): 115-36.

67. Fröhling S, Skelin S, Liebisch C, Scholl C, Schlenk RF, Döhner H, et al. Comparison of cytogenetic and molecular cytogenetic detection of chromosome abnormalities in 240 consecutive adult patients with acute myeloid leukemia. J Clin Oncol. 2002; 20(10): 2480-5.

68. Lugthart S, van Drunen E, van Norden Y, van Hoven A, Erpelinck CA, Valk PJ, et al. High EVI1 levels predict adverse outcome in acute myeloid leukemia: prevalence of EVI1 overexpression and chromosome 3q26 abnormalities underestimated. Blood 2008; 111(8): 4329-37.

69. Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 1999; 13(12): 1901-28.

70. Mrózek K1, Prior TW, Edwards C, Marcucci G, Carroll AJ, Snyder PJ, et al. Comparison of cytogenetic and molecular genetic detection of t(8;21) and inv(16) in a prospective series of adults with de novo acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol 2001; 19(9): 2482-92.

71. Grimwade D. Screening for core binding factor gene rearrangements in acute myeloid leukemia. Leukemia 2002; 16(5): 964-9.

72. Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Kodera Y, Miyawaki S, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood 2001; 97(8): 2434-9.

73. Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/ enhancer binding protein-α (C/EBPα), in acute myeloid leukemia. Nat Genet 2001; 27(3): 263-70.

74. Caligiuri MA, Schichman SA, Strout MP, Mrózek K, Baer MR, Frankel SR, et al. Molecular rearrangement of the ALL-1 gene in acute myeloid leukemia without cytogenetic evidence of 11q23 chromosomal translocations. Cancer Res 1994; 54(2): 370-3.

75. Bos JL, Toksoz D, Marshall CJ, Verlaan-de Vries M, Veeneman GH, van der Eb AJ, et al. Amino-acid substitutions at codon 13 of the N-ras oncogene in human acute myeloid leukaemia. Nature. 1985; 315(6022): 726-30.

76. King-Underwood L, Renshaw J, Pritchard-Jones K. Mutations in the Wilms' tumor gene WT1 in leukemias. Blood 1996; 87(6): 2171-9.

77. Gari M, Goodeve A, Wilson G, Winship P, Langabeer S, Linch D, et al. c-kit protooncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia. Br J Haematol. 1999; 105(4): 894-900.

78. Osato M, Asou N, Abdalla E, Hoshino K, Yamasaki H, Okubo T, et al. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2αB gene associated with myeloblastic leukemias. Blood 1999; 93(6): 1817-24.

79. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Massé A, et al. Mutation in TET2 in myeloid cancers. N Engl J Med 2009; 360(22): 2289-301.

80. Langemeijer SM, Kuiper RP, Berends M, Knops R, Aslanyan MG, Massop M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet 2009; 41(7): 838-42.

81. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med 2009; 361(11): 1058-66.

82. Dohner K, Dohner H. Molecular characterization of acute myeloid leukemia. Haematologica. 2008; 93(7): 976-82.

83. de Labarthe A, Pautas C, Thomas X, de Botton S, Bordessoule D, Tilly H, et al. Allogeneic stem cell transplantation in second rather than first complete remission in selected patients with good-risk acute myeloid leukemia. Bone Marrow Transplant 2005; 35(8): 767–73.

84. Schlenk RF, Döhner K, Krauter J, Fröhling S, Corbacioglu A, Bullinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med 2008; 358(18): 1909–18.

85. O'Donnell MR, Abboud CN, Altman J, Appelbaum FR, Arber DA, Attar E, Borate U, et al. Acute myeloid leukemia. J Natl Compr Canc Netw 2012, 10(8), 984-1021.

86. Giles FJ, Keating A, Goldstone AH, Avivi I, Willman CL, Kantarjian HM. Acute myeloid leukemia. Hematology Am Soc Hematol Educ Program. 2002: 73-110.

87. Grimwade D, Walker H, Harrison G, Oliver F, Chatters S, Harrison CJ, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood 2001; 98(5): 1312:20.

88. Löwenberg B, Suciu S, Archimbaud E, Haak H, Stryckmans P, de Cataldo R, et al. Mitoxantrone versus daunorubicin in induction-consolidation chemotherapy—The value of low-dose cytarabine for maintenance of remission, and an assessment of prognostic factors in acute myeloid leukemia in the elderly: Final report. European Organization for the Research and Treatment of Cancer and the Dutch-Belgian Hemato-Oncology Cooperative Hovon Group. J Clin Oncol 1998; 16(3): 872-81.

89. ECOG / Zubrod Performance Status. Available from http://www.ecog.org/general/perf_stat.html

90. Sorror ML, Maris MB, Storer B, Sandmaier BM, Diaconescu R, Flowers C, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: infl uence of pretransplantation comorbidities. Blood 2004; 104(4): 961–8.

91. Raza A, Preisler HD, Li YQ, Larson RA, Goldberg J, Browman G, et al. Biological characteristics of newly diagnosed poor prognosis acute myelogenous leukemia. Am J Hematol 1993; 42(2): 359-66.

92. Stone RM, O'Donnell MR, Sekeres MA. Acute myeloid leukemia. Hematology Am Soc Hematol Educ Program. 2004: 98-117.

93. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and post-remission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group study. Blood 2000; 96(13): 4075-83.

94. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood 2010; 116(3), 354-65.

95. Mrózek K. Acute Myeloid Leukemia with a Complex Karyotype. Semin Oncol 2008; 35(4): 365–77.

96. Foucar K, Reichard K, Czuchlewski D. Bone Marrow Pathology. 3rd ed. ASCP Press, Chicago. 2010, pp. 377-431 (Acute Myeloid Leukemia).

97. Dang H, Jiang A, Kamel-Reid S, Brandwein J, Chang H. Prognostic value of immunophenotyping and gene mutations in elderly patients with acute myeloid leukemia with normal karyotype. Human pathology 2013; 44(1): 55-61.

98. Mrózek K, Döhner H, Bloomfield CD. Influence of new molecular prognostic markers in patients with karyotypically normal acute myeloid leukemia: recent advances. Curr Opin Hematol. 2007; 14(2): 106-14.

99. Fröhling S, Schlenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K,et al. Acute myeloid leukemia. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood 2002; 100(13): 4372-80.

100. Gale RE, Green C, Allen C, Mead AJ, Burnett AK, Hills RK, et al. The impact of FLT3 internal tandem duplication mutant level, number, size and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood. 2008; 111(5): 2776-84.

101. Verhaak RG, Goudswaard CS, van Putten W, Bijl MA, Sanders MA, Hugens W, et al. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other genetic abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood. 2005; 106(12): 3747-54.

102. Thiede C1, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood 2006; 107(10): 4011-20.

103. Preudhomme C, Sagot C, Boissel N, Cayuela JM, Tigaud I, de Botton S, et al. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). Blood 2002; 100(8): 2717-23.

104. Marcucci G1, Maharry K, Radmacher MD, Mrózek K, Vukosavljevic T, Paschka P, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features:a Cancer and Leukemia Group B study. J Clin Oncol 2008; 26(31): 5078-87.

105. Pabst T, Eyholzer M, Fos J, Mueller BU. Heterogeneity within AML with CEBPA mutations; only CEBPA double mutations, but not single CEBPA mutations are associated with favourable prognosis. Br J Cancer 2009; 100(8): 1343-6.

106. Boissel N, Leroy H, Brethon B, Philippe N, de Botton S, Auvrignon A, et al. Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia 2006; 20(6): 965-70.

107. Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K, et al. KIT-D816 mutations in AML1-ETO–positive AML are associated with impaired event-free and overall survival. Blood 2006; 107(5): 1791-9.

108. Miller KB, Pihan G. Clinical Manifestations of Acute Myeloid Leukemia. In : Hematology: Basic Principles and Practice. Hoffman R, Benz Jr. EJ, Shattil SJ, Furie B, Silberstein LE, McGlave P, et al (eds). 5th ed. Churchill Livingstone, Philadelphia. 2008.

109. Lowenberg B, Griffin JD, Tallman MS. Acute myeloid leukemia and acute promyelocytic leukemia. Hematology Am Soc Hematol Educ Program 2003: 82-101.

110. Sekeres MA, Elson P, Kalaycio ME, Advani AS, Copelan EA, Faderl S, et al. Time from diagnosis to treatment initiation predicts survival in younger, but not older, acute myeloid leukemia patients. Blood 2009; 113(1): 28-36.

111. Atkins CD. Single nucleotide polymorphisms and anthracycline cardiotoxicity in children: potential implications for adult oncology. J Clin Oncol 2012; 30(28): 3563.

112. Büchner T, Hiddemann W, Berdel WE, Wörmann B, Schoch C, Fonatsch C, et al. 6-thioguanine, cytarabine, and daunorubicin (TAD) and high-dose cytarabine and mitoxantrone (HAM) for induction, TAD for consolidation, and either prolonged maintenance by reduced monthly TAD or TAD-HAM-TAD and one course of intensive consolidation by sequential HAM in adult patients at all ages with de novo acute myeloid leukemia (AML): a randomized trial of the German AML Cooperative Group. J Clin Oncol 2003; 21(24): 4496-504.

113. Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, Tantravahi R, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res 1998; 58(18): 4173-9.

114. Moore JO, George SL, Dodge RK, Amrein PC, Powell BL, Kolitz JE, et al. Sequential multiagent chemotherapy is not superior to high-dose cytarabine alone as post-remission intensification therapy for acute myeloid leukemia in adults under 60 years of age: Cancer and Leukemia Group B Study 9222. Blood 2005; 105(9): 3420-7.

115. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990; 75(3): 555-62.

116. Koreth J, Schlenk R, Kopecky KJ, Honda S, Sierra J, Djulbegovic BJ, et al. Risk adapted post-remission therapy in acute myeloid leukemia: results of the German Multicenter AML HD93 treatment trial. Leukemia. 2003; 17(8): 1521-8.

117. Cornelissen JJ, van Putten WL, Verdonck LF, Theobald M, Jacky E, Daenen SM, et al. Results of a HOVON/SAKK donor versus nodonor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middleaged adults: benefits for whom? Blood 2007; 109(9): 3658-66.

118. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and post-remission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood 2000; 96(13): 4075-83.

119. Breems DA, Lowenberg B. Acute myeloid leukemia and the position of autologous stem cell transplantation. Semin Hematol 2007; 44(4): 259-66.

120. Whitman SP, Ruppert AS, Marcucci G, Mrózek K, Paschka P, Langer C, et al. Long-term disease-free survivors with cytogenetically normal acute myeloid leukemia and

MLL partial tandem duplication: a Cancer and Leukemia Group B study. Blood 2007; 109(12): 5164-7.

121. Estey E, Kornblau S, Pierce S, Kantarjian H, Beran M, Keating M. A stratification system for selecting and evaluating therapies in patients with relapsed or primary refractory acute myeloid leukemia. Blood 1996; 88(2): 756.

122. Estey EH. Treatment of relapsed and refractory acute myeloid leukemia. Leukemia 2000; 14(3): 476–9.

123. Christmann M, Tomicic MT, Roos WP, Kaina B. Mechanisms of human DNA repair: an update. Toxicology 2003; 193(1): 3-34.

124. López-Camarillo C, Lopez-Casamichana M, Weber C, Guillen N, Orozco E, Marchat LA. DNA repair mechanisms in eukaryotes: Special focus in Entamoeba histolytica and related protozoan parasites. Infect Genet Evol. 2009; 9: 1051–6.

125. Aylon Y, Kupiec M. DSB repair: the yeast paradigm. DNA repair 2004; 3(8), 797-815.

126. Daley JM, Palmbos PL, Wu D, Wilson TE. Nonhomologous end joining in yeast. Annu Rev Genet 2005; 39: 431-51.

127. Sassanfar M, Dosanjh MK, Essigmann JM, Samson L. Relative efficiencies of the bacterial, yeast, and human DNA methyltransferases for the repair of O6-methylguanine and O4-methylthymine. Suggestive evidence for O4-methylthymine repair by eukaryotic methyltransferases. J Biol Chem 1991; 266(5): 2767-71.

128. Marti TM, Kunz C, Fleck O. DNA mismatch repair and mutation avoidance pathways. J Cell Physiol 2002; 191(1): 28-41.

129. Krokan HE, Standal R, Slupphaug G. DNA glycosylases in the base excision repair of DNA. Biochem J 1997; 325(Pt 1), 1-16.

130. Boiteux S, Guillet M. Abasic sites in DNA: repair and biological consequences in Saccharomyces cerevisiae. DNA repair 2004; 3(1): 1-12.

131. Le May N, Egly JM, Coin F. True lies: the double life of the nucleotide excision repair factors in transcription and DNA repair. J Nucleic Acids 2010; 19: 1-11.

132. Mullenders LH, Berneburg M. Photoimmunology and nucleotide excision repair: impact of transcription coupled and global genome excision repair. J Photochem Photobiol B 2001; 65(2-3): 97-100.

133. Friedberg EC. How nucleotide excision repair protects against cancer. Nat Rev Cancer 2001; 1(1): 22-33.

134. Vermeulen W, de Boer J, Citterio E, van Gool AJ, van der Horst GT, Jaspers NG, et al. Mammalian nucleotide excision repair and syndromes. Biochem Soc Trans 1997; 25(1): 309-15.

135. Balajee AS, Bohr VA. Genomic heterogeneity of nucleotide excision repair. Gene 2000; 250(1-2): 15-30.

136. Hanawalt PC. Controlling the efficiency of excision repair. Mutat Res 2001; 485(1): 3-13.

137. Ogi T, Limsirichaikul S, Overmeer RM, Volker M, Takenaka K, Cloney R, et al. Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells. Mol Cell 2010; 37(5) 714-27.

138. Fousteri M, Mullenders LH. Transcription-coupled nucleotide excision repair in mammaliancells: molecular mechanisms and biological effects. Cell Res 2008; 18(1): 73-84.

139. Compe E, Egly JM. TFIIH: when transcription met DNA repair. Nat Rev Mol Cell Biol. 2010; 13(6): 343-54.

140. Schultz P, Fribourg S, Poterszman A, Mallouh V, Moras D, Egly JM. Molecular structure of human TFIIH. Cell 2000; 102(5): 599–607.

141. Sandrock B, Egly JM. A yeast four-hybrid system identifies Cdk-activating kinase as a regulator of the XPD helicase, a subunit of transcription factor IIH. J Biol Chem 2001; 276(38): 35328–33.

142. Chang WH, Kornberg RD. Electron crystal structure of the transcription factor and DNA repair complex, core TFIIH. Cell 2000; 102(5): 609–13.

143. Egly JM, Coin F. A history of TFIIH: two decades of molecular biology on a pivotal transcription/repair factor. DNA repair 2011; 10(7): 714-21.

144. Iben S, Tschochner H, Bier M, Hoogstraten D, Hozák P, Egly JM, et al. TFIIH plays an essential role in RNA polymerase I transcription. Cell 2002; 109(3): 297–306.

145. Barski A, Chepelev I, Liko D, Cuddapah S, Fleming AB, Birch J, et al. Pol II and its associated epigenetic marks are present at Pol III-transcribed noncoding RNA genes. Nat Struct Mol Biol 2010; 17(5), 629–34.

146. Lehmann AR. The xeroderma pigmentosum group D (XPD) gene: one gene, two functions, three diseases. Genes Dev 2001; 15(1): 15-23.

147. Liu H, Rudolf J, Johnson KA, McMahon SA, Oke M, Carter L, et al. Structure of the DNA repair helicase XPD. Cell 2008; 133(5): 801–12.

148. Chen J, Suter B. Xpd, a structural bridge and a functional link. Cell Cycle 2003; 2(6): 503-6.

149. Winkler GS, Araújo SJ, Fiedler U, Vermeulen W, Coin F, Egly JM, et al. TFIIH with inactive XPD helicase functions in transcription initiation but is defective in DNA repair. J Biol Chem 2000; 275(6): 4258-66.

150. Andressoo JO, Mitchell JR, de Wit J, Hoogstraten D, Volker M, Toussaint W, et al. An Xpd mouse model for the combined xeroderma pigmentosum/Cockayne syndrome exhibiting both cancer predisposition and segmental progeria. Cancer Cell 2006; 10(2): 121-32.

151. Rudolf J, Makrantoni V, Ingledew WJ, Stark MJ, White MF. The DNA repair helicases XPD and FancJ have essential iron-sulfur domains. Mol Cell 2006; 23(6): 801-8.

152. Fuss JO, Tainer JA. XPB and XPD helicases in TFIIH orchestrate DNA duplex opening and damage verification to coordinate repair with transcription and cell cycle via CAK kinase. DNA repair 2011; 10(7): 697-713.

153. Fan L, Fuss JO, Cheng QJ, Arvai AS, Hammel M, Roberts VA, et al. XPD helicase structures and activities: insights into the cancer and aging phenotypes from XPD mutations. Cell 2008; 133(5): 789-800.

154. Singleton MR, Dillingham MS, Wigley DB. Structure and mechanism of helicases and nucleic acid translocases, Annu Rev Biochem 2007; 76: 23-50.

155. Lehmann AR. XPD structure reveals its secrets. DNA repair 2008; 7(11): 1912-5.

156. Boon EM, Livingston AL, Chmiel NH, David SS, Barton JK. DNA-mediated charge transport for DNA repair. Proc Natl Acad Sci U S A 2003; 100(22): 12543-7.

157. Wolski SC, Kuper J, Hänzelmann P, Truglio JJ, Croteau DL, Van Houten B, Kisker C. Crystal structure of the FeS cluster-containing nucleotide excision repair helicase XPD. PLoS Biol 2008; 6(6): 1332-42.

158. Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol. 2008; 9: 297–308.

159. Monzo M, Brunet S, Urbano-Ispizua A, Navarro A, Perea G, Esteve J, et al. Genomic polymorphisms provide prognostic information in intermediate-risk acute myeloblastic leukemia. Blood 2006; 107(12): 4871-9.

160. Kuptsova N, Kopecky KJ, Godwin J, Anderson J, Hoque A, Willman CL, et al. Polymorphisms in DNA repair genes and therapeutic outcomes of AML patients from SWOG clinical trials. Blood 2007; 109: 3936-44.

161. Chu E, McGowan M, El-fiky A, Kang SP, Harrold LJ, Tiedmann D et al. Chemotherapeutic and Biologic Drugs. In : Physicians' Cancer Chemotherapy Drug Manual 2008. Chu E, DeVita VT (eds). Jones and Bartlett Publishers. 2008, pp. 11-372.

162. Langerak AD, Dreisbach LP. Chemotherapy Regimens and Cancer Care. Landes Bioscience, Georgetown. 2001. (Chemotherapeutic Drug Toxicities and Mechanisms of Action).

163. Bain BJ, Briggs C. Basic haematological techniques. In : Dacie and Lewis Practical Haematology. Bain BJ, Bates I, Laffan MA, Lewis SM (eds). 11th ed. Churchill Livingstone, Philadelphia. 2011, pp. 23-56.

164. Bates I, Burthem J. Bone marrow biopsy. In : Dacie and Lewis Practical Haematology. Bain BJ, Bates I, Laffan MA, Lewis SM (eds). 11th ed. Churchill Livingstone, Philadelphia. 2011, pp. 123-37.

165. Higgins T, Beutler E, Doumas BT. Hemoglobin, Iron, and Bilirubin. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 509-26.

166. Panteghini M, Bais R. Enzymes. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 317-36.

167. Lamb EJ, Price CP. Creatinine, Urea, and Uric Acid. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 363-72.

168. Sacks DB. Carbohydrates. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 373-401.

169. Scott MG, LeGrys VA, Klutts JS. Electrolytes and Blood Gases. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 432-49.

170. National Cancer Institute: Common terminology criteria for adverse events, v. 4.0. Available from: http://ctep.cancer.gov/reporting/ctc.html

171. Johnson AM. Amino Acids and Proteins. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 286-316.

172. Batar B, Güven M, Baris S, Celkan T, Yildiz I. DNA repair gene XPD and XRCC1 polymorphisms and the risk of childhood acute lymphoblastic leukemia. Leuk Res 2009; 33: 759-63.

173. Database of single nucleotide polymorphisms (SNPs) and multiple small-scale variations that include insertions/deletions, microsatellites, and non-polymorphic variants. Available from http://www.ncbi.nlm.nih.gov/snp.

174. Yagi T, Morimoto A, Eguchi M, Hibi S, Sako M, Ishii E, Mizutani S, Imashuku S, Ohki M, Ichikawa H. Identification of a gene expression signature associated with pediatric AML prognosis. Blood 2003; 102(5): 1849-56.

175. Byrd JC, Mrózek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 2002; 100(13): 4325-36.

176. American Cancer Society, Cancer in Africa. Available from http://www.cancer.org/research/cancerfactsfigures/globalcancerfactsfigures/global-facts-figures-2nd-ed

177. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999; 286(5439): 487-91.

178. Evans WE, Johnson JA. Pharmacogenomics: the inherited basis for interindividual differences in drug response. Annu Rev Genomics Hum Genet 2001; 2: 9-39.

179. Bullinger L, Döhner K, Bair E, Fröhling S, Schlenk RF, Tibshirani R, et al. Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. N Engl J Med 2004; 350(16): 1605-16.

180. Efferth T, Volm M. (2005). Pharmacogenetics for individualized cancer chemotherapy. Pharmacol Ther 2005; 107(2): 155-76.

181. Ishikawa K, Ishii H, Saito T. DNA damage-dependent cell cycle checkpoints and genomic stability. DNA Cell Biol 2006; 25: 406–411.

182. Kennedy RD, D'Andrea AD. DNA repair pathways in clinical practice: lessons from pediatric cancer susceptibility syndromes. J Clin Oncol 2006; 24: 3799–808.

183. Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002; 11(12): 1513-30.

184. Bhatla D, Gerbing RB, Alonzo TA, Mehta PA, Deal K, Elliott J, et al. DNA repair polymorphisms and outcome of chemotherapy for acute myelogenous leukemia: a report from the Children's Oncology Group. Leukemia 2008; 22(2): 265-72.

185. Cai J, Damaraju VL, Groulx N, Mowles D, Peng Y, Robins MJ, et al. Two distinct molecular mechanisms underlying cytarabine resistance in human leukemic cells. Cancer Res 2008; 68(7): 2349-57.

186. Bouwman P, Jonkers J. The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. Nat Rev Cancer 2012; 12(9): 587-98.

187. Seker H1, Butkiewicz D, Bowman ED, Rusin M, Hedayati M, Grossman L, Harris CC. Functional Significance of XPD Polymorphic Variants Attenuated Apoptosis in Human Lymphoblastoid Cells with the XPD 312 Asp/Asp Genotype. Cancer Res 2001; 61(20): 7430-4.

188. Spitz MR, Wu X, Wang Y, Wang LE, Shete S, Amos CI, et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res 2001; 61(4): 1354-7.

189. Ziemba A, Derosier LC, Methvin R, Song CY, Clary E, Kahn W, et al. Repair of triplex-directed DNA alkylation by nucleotide excision repair. Nucleic Acids Res 2001; 29(21): 4257-63.

190. Zheng H, Wang X, Warren AJ, Legerski RJ, Nairn RS, Hamilton JW, et al. Nucleotide excision repair-and polymerase η-mediated error-prone removal of mitomycin C interstrand cross-links. Mol Cell Biol 2003; 23(2): 754-61.

191. Tirode F1, Busso D, Coin F, Egly JM. Reconstitution of the transcription factor TFIIH: assignment of functions for the three enzymatic subunits, XPB, XPD, and cdk7. Mol Cell 1999; 3(1): 87-95.

192. Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, et al. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 2000; 21(4): 551-5.

193. Shen J, Desai M, Agrawal M, Kennedy DO, Senie RT, Santella RM, et al. Polymorphisms in nucleotide excision repair genes and DNA repair capacity phenotype

in sisters discordant for breast cancer. Cancer Epidemiol Biomarkers Prev. 2006; 15(9): 1614-9.

194. Shuck SC, Short EA, Turchi JJ. Eukaryotic nucleotide excision repair: from understanding mechanisms to influencing biology. Cell Res 2008; 18: 64-72.

195. Lamerdin JE, Stilwagen SA, Ramirez MH, Stubbs L, Carrano AV. Sequence Analysis of theERCC2Gene Regions in Human, Mouse, and Hamster Reveals Three Linked Genes. Genomics 1996; 34(3): 399-409.

196. Della Coletta L, Rolig RL, Fossey S, Morizot DC, Nairn RS, Walter RB. Characterization of the Xiphophorus fish (Teleostei: Poeciliidae) ERCC2/XPD locus. Genomics. 1995; 26(1): 70-6.

197. Shen MR, Jones IM, Mohrenweiser H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res. 1998; 58(4): 604-8.

198. Lauria F, Raspadori D, Ventura MA, Rondelli D, Testoni N, Tosi P, et al. The presence of lymphoid-associated antigens in adult acute myeloid leukemia is devoid of prognostic relevance. Stem Cells 1995; 13(4): 428-34.

199. Sperling C, Büchner T, Creutzig U, Ritter J, Harbott J, Fonatsch C, Sauerland C, Mielcarek M, Maschmeyer G, Löffler H, et al.. Clinical, morphologic, cytogenetic and prognostic implications of CD34 expression in childhood and adult de novo AML. Leuk Lymphoma 1995; 17(5-6): 417-26.

200. Chang H, Brandwein J, Yi QL, Chun K, Patterson B, Brien B. Extramedullary infiltrates of AML are associated with CD56 expression, 11q23 abnormalities and inferior clinical outcome. Leuk Res 2004; 28(10): 1007-11.

201. Kobayashi R, Tawa A, Hanada R, Horibe K, Tsuchida M, Tsukimoto I. Extramedullary infiltration at diagnosis and prognosis in children with acute myelogenous leukemia. Pediatr Blood Cancer 2007; 48(4): 393-8.

202. Allan JM, Smith AG, Wheatley K, Hills RK, Travis LB, Hill DA, Swirsky DM, Morgan GJ, Wild CP. Genetic variation in XPD predicts treatment outcome and risk of acute myeloid leukemia following chemotherapy. Blood 2004; 104(13): 3872-7.

203. Strom SS, Estey E, Outschoorn UM, Garcia-Manero G. Acute myeloid leukemia outcome: role of nucleotide excision repair polymorphisms in intermediate risk patients. Leuk Lymphoma 2010; 51(4): 598-605.

204. Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei DD, Groshen S, Lenz HJ. A Xeroderma pigmentosum group D gene polymorphism predicts clinical outcome to platinum-based chemotherapy in patients with advanced colorectal cancer. Cancer Res 2001; 61(24): 8654-8.

205. Gurubhagavatula S, Liu G, Park S, Zhou W, Su L, Wain JC, et al. XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy. J Clin Oncol 2004; 22(13): 2594-601.

206. Hu JJ, Hall MC, Grossman L, Hedayati M, McCullough DL, Lohman K, Case LD. Deficient nucleotide excision repair capacity enhances human prostate cancer risk. Cancer Res 2004; 64(3): 1197-201.

207. Cheng L, Spitz MR, Hong WK, Wei Q. Reduced expression levels of nucleotide excision repair genes in lung cancer: a case-control analysis. Carcinogenesis 2000; 21(8): 1527-30.

208. Wei Q1, Cheng L, Amos CI, Wang LE, Guo Z, Hong WK, Spitz MR. Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. J Natl Cancer Inst 2000; 92(21): 1764-72.

209. Matullo G, Palli D, Peluso M, Guarrera S, Carturan S, Celentano E, et al. XRCC1, XRCC3, XPD gene polymorphisms, smoking and 32P-DNA adducts in a sample of healthy subjects. Carcinogenesis 2001; 22(9): 1437-45.

210. Hou SM, Fält S, Angelini S, Yang K, Nyberg F, Lambert B, et al. The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk. Carcinogenesis 2002; 23(4): 599-603.

211. Au WW, Salama SA, Sierra-Torres CH. Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. Environ Health Perspect. 2003; 111(15): 1843-50.

212. Hu JJ, Hall C, Torti FM, McCullough DL, Lohman K, Grossman L. Deficient DNA repair and human prostate cancer risk. Proc Am Assoc Cancer Res 2000; 41: 360.

213. Hemminki K, Xu G, Angelini S, Snellman E, Jansen CT, Lambert B, Hou SM. XPD exon 10 and 23 polymorphisms and DNA repair in human skin in situ. Carcinogenesis 2001; 22(8): 1185-8.

214. Mehta PA1, Alonzo TA, Gerbing RB, Elliott JS, Wilke TA, Kennedy RJ, et al. XPD Lys751Gln polymorphism in the etiology and outcome of childhood acute myeloid leukemia: a Children's Oncology Group report. Blood 2006; 107(1): 39-45.

215. Dastugue N, Payen C, Lafage-Pochitaloff M, Bernard P, Leroux D, Huguet-Rigal F, et al. Prognostic significance of karyotype in de novo adult acute myeloid leukemia. The BGMT group. Leukemia 1995; 9(9): 1491-8.

216. Leith CP, Kopecky KJ, Godwin J, McConnell T, Slovak ML, Chen IM, et al. Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. Blood 1997; 89(9): 3323-9.

217. Hiddemann W, Kern W, Schoch C, Fonatsch C, Heinecke A, Wörmann B, et al. Management of acute myeloid leukemia in elderly patients. J Clin Oncol 1999; 17(11): 3569-76.

218. Stone RM. The difficult problem of acute myeloid leukemia in the older adult. CA Cancer J Clin 2002; 52(6): 363-71.

219. Kuptsova-Clarkson N, Ambrosone CB, Weiss J, Baer MR, Sucheston LE, Zirpoli G, et al. XPD DNA nucleotide excision repair gene polymorphisms associated with DNA repair deficiency predict better treatment outcomes in secondary acute myeloid leukemia. Int J Mol Epidemiol Genet. 2010; 1: 278–94.

220. Bosken CH, Wei Q, Amos CI, Spitz MR. An analysis of DNA repair as a determinant of survival in patients with non-small-cell lung cancer. J Natl Cancer Inst 2002; 94(14): 1091-9.

221. Levati L, Marra G, Lettieri T, D'Atri S, Vernole P, Tentori L, et al. Mutation of the mismatch repair gene hMSH2 and hMSH6 in a human T-cell leukemia line tolerant to methylating agents Genes Chromosomes Cancer 1998; 23(2): 159-66.

222. Rajewsky MF1, Engelbergs J, Thomale J, Schweer T. DNA repair: counteragent in mutagenesis and carcinogenesis—accomplice in cancer therapy resistance. Mutat Res 2000; 462(2-3): 101-5.

223. Krajinovic M, Labuda D, Mathonnet G, Labuda M, Moghrabi A, Champagne J, et al. Polymorphisms in genes encoding drugs and xenobiotic metabolizing enzymes, DNA repair enzymes, and response to treatment of childhood acute lymphoblastic leukemia. Clin Cancer Res 2002; 8(3): 802-10.

224. Robles AI, Wang XW, Harris CC. Drug-induced apoptosis is delayed and reduced in XPD lymphoblastoid cell lines: possible role of TFIIH in p53-mediated apoptotic cell death. Oncogene 1999; 18(33): 4681-8.

225. Chang-Claude J, Popanda O, Tan XL, Kropp S, Helmbold I, von Fournier D, et al. Association between polymorphisms in the DNA repair genes, XRCC1, APE1, and XPD and acute side effects of radiotherapy in breast cancer patients. Clin Cancer Res 2005; 11(13): 4802-9.

226. Coin F, Marinoni JC, Rodolfo C, Fribourg S, Pedrini AM, Egly JM. Mutations in the XPD helicase gene result in XP and TTD phenotypes, preventing interaction between XPD and the p44 subunit of TFIIH. Nat Genet 1998; 20(2): 184-8.

227. Shen M, Berndt SI, Rothman N, Demarini DM, Mumford JL, He X, et al. Polymorphisms in the DNA nucleotide excision repair genes and lung cancer risk in Xuan Wei, China. Int J Cancer 2005; 116(5): 768–73.

228. Le Morvan V, Bellott R, Moisan F, Mathoulin-Pélissier S, Bonnet J, Robert J. Relationships between genetic polymorphisms and anticancer drug cytotoxicity vis-à-vis the NCI-60 panel. Pharmacogenomics 2006; 7(6): 843–52.

229. Guven M, Guven GS, Oz E, Ozaydin A, Batar B, Ulutin T, et al. DNA repair gene XRCC1 and XPD polymorphisms and their association with coronary artery disease risks and micronucleus frequency. Heart Vessels 2007; 22(6): 355–60.

230. Botto N,Masetti S, Petrozzi L, Vassalle C, Manfredi S, Biagini A, et al. Elevated levels of oxidative DNA damage in patients with coronary artery disease. Coron Artery Dis. 2002; 13: 269–74.

231. Frosina G. Overexpression of enzymes that repair endogenous damage to DNA. Eur J Biochem. 2000; 267: 2135–49.

PROTOCOL

تأثير تعدد الأشكال فى جين *XPD* على الإستجابة للعلاج الكيماوى الحثى فى مرضى سرطان الدم النقوى الحاد

Effect of *xeroderma pigmentosum complementation group D (XPD)* gene polymorphisms on the response to induction chemotherapy in acute myeloid leukemia patients

خطة بحث مقدمة إلى
معهد البحوث الطبية
جامعة الإسكندرية
إيفاء جزئيا لشروط
الحصول على درجة

Protocol of a thesis submitted to the
Medical Research Institute
University of Alexandria
in partial fulfillment of the
requirements of the degree of

الماجستير فى الكيمياء الطبية التطبيقية

Master of Applied Medical Chemistry

من

By

محمد رمضان محمود بركات

Mohammad Ramadan Mahmoud Barakat

بكالوريوس علوم (كيمياء/كيمياء حيوية)
كلية العلوم
جامعة الإسكندرية
٢٠٠٥

Bachelor of Science (Chemistry/Biochemistry)
Faculty of Science
University of Alexandria
2005

دبلوم تكنولوجيا حيوية
معهد الدراسات العليا و البحوث
جامعة الإسكندرية
٢٠٠٦

Diploma in Biotechnology
Institute of Graduate Studies and Research
University of Alexandria
2006

قسم الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية
٢٠١٠

Department of Applied Medical Chemistry
Medical Research Institute
University of Alexandria
2010

Supervisors المشرفون

Dr. Mervat Abd El-Fattah El-Toukhy الدكتورة/ ميرفت عبدالفتاح الطوخى
Professor, Department of Applied Medical Chemistry أستاذ بقسم الكيمياء الطبية التطبيقية
Medical Research Institute معهد البحوث الطبية
University of Alexandria جامعة الإسكندرية

Dr. Neveen Abd El-Moneim Hussein الدكتورة/ نيفين عبد المنعم حسين
Lecturer, Department of Applied Medical Chemistry مدرس بقسم الكيمياء الطبية التطبيقية
Medical Research Institute معهد البحوث الطبية
University of Alexandria جامعة الإسكندرية

Dr. Ahmed Mohamed Lotfy Bedewy الدكتور/ أحمد محمد لطفى بديوى
Lecturer, Department of Hematology مدرس بقسم أمراض الدم
Medical Research Institute معهد البحوث الطبية
University of Alexandria جامعة الإسكندرية

Background

Acute myelogenous leukemia (AML) is a clonal, malignant disease of hematopoietic tissues that is characterized by accumulation of abnormal (leukemic) blast cells, principally in the marrow, and impaired production of normal blood cells. Thus, the leukemic cell infiltration in marrow is accompanied, nearly invariably, by anemia and thrombocytopenia.[(1)] The overall annual incidence of AML is 3.7 per 100,000 persons, and age-dependent mortality is 2.7 to nearly 18 per 100,000 persons.[(2)] In adults, AML accounts for 80 to 90% of cases of acute leukemia.[(3)]

The etiology of most cases of AML is unclear,[(2)] where the majority of patients have not been exposed to an antecedent causative factor.[(1)] There is considerable heterogeneity between cases of AML with respect to morphology, immunological phenotype, associated cytogenetic and molecular abnormalities and patterns of gene expression.[(4)] A number of clinical and biologic features predict prognosis in AML including clinical state, morphology, surface/enzyme markers, cytogenetics & molecular markers.[(3)]

The backbone of AML treatment for 30 years has been the combination of daunorubicin and cytarabine for remission induction chemotherapy protocol.[(4)] These chemotherapeutic drugs bring about their effect by causing DNA damage.[(5)] This damage causes activation of DNA-repair mechanisms in the different phases of the cell cycle. Cells resume cell-cycle progression once damage has been repaired, whereas cells which fail to repair the DNA damage undergo permanent cell-cycle arrest or apoptosis.[(6)]

There are six pathways of DNA repair: homologous recombination repair (HRR), non-homologous end-joining (NHEJ), base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), and methyltransferase repair.[7] Nucleotide excision repair is a major DNA repair pathway in eukaryotic cells. It involves more than 30 proteins including Xeroderma pigmentosum (XP) groups A to G.[8]

Xeroderma pigmentosum complementation group D (*XPD)* gene encodes a helicase which is a component of transcription factor IIH (TFIIH) and functions in transcription initiation and nucleotide excision repair in eukaryotes.[9] Functional DNA repair capacity is thought to differ significantly between the different polymorphic variants of *XPD*.[10,11]

Among the polymorphic variants described for *XPD* gene are Lys751Gln (A>C) and Asp312Asn (G>A).[12] The association between these variants and the response to chemotherapy in AML has been subjected to recent research in order to predict the prognosis of AML patients.[5,13]

Aim of the work

This study aims to investigate the association of *XPD* polymorphic variants and response to remission induction chemotherapy in patients with acute myeloid leukemia. In addition, it aims to investigate the association between *XPD* polymorphic variants and chemotherapy-induced toxicities during remission induction in patients with acute myeloid leukemia.

Subjects and Methods

This study will include 30 patients with newly diagnosed acute myeloid leukemia presented to the Department of Hematology, Medical Research Institute, Alexandria University.

Inclusion criteria:

- Adult patients with de novo AML of both sexes.

Exclusion criteria:

- Patients over 60 years old.
- Patients with co morbid conditions (liver, kidney or heart disease).
- Patients with poor performance status.

All patients will be subjected to the following at presentation:

- Full medical history taking
- Thorough clinical examination
- Complete blood picture [14]
- Bone marrow aspiration [15]
- Liver function tests (Bilirubin, ALT, AST, Alkaline phosphatase) [16,17]
- Kidney function tests (Urea, Creatinine, Uric Acid) [18]
- Blood glucose level, serum calcium, potassium, sodium and magnesium [19,20]
- X-ray chest
- Ultrasound Abdomen
- Sample collection and Genotyping:

- 3ml of venous blood will be collected from each patient before chemotherapy; immediately after collection, whole blood will be stored in aliquots at -20^{o}C until assayed.
- 2 *XPD* polymorphisms: Lys751Gln (A>C) and Asp312Asn (G>A) will be analyzed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.[21]

Patients will be treated by the standard remission induction protocol (3+7) comprising:[4]

- Cytosine Arabinoside 100 mg/m^2 on days 1-7 by continuous intravenous infusion.
- Daunorubicin 45 mg/m^2 on days 1-3 by intravenous push.

During remission induction, patients will be monitored for possible toxic effects induced by chemotherapy:

- Genitourinary toxicity (creatinine increase, proteinuria) [18,22]
- Gastrointestinal toxicity (nausea, diarrhea, mucositis)
- Liver Toxicity (increase of bilirubin, alkaline phosphatase, transaminases) [16,17]
- Lung Toxicity (dyspnea, cough, pulmonary edema, pneumonitis/infiltrates)
- Metabolic toxicity (hypoglycemia, hyperglycemia, hypocalcemia, hypokalemia, hyponatremia, hypomagnesemia) [19,20]

Response to chemotherapy will be evaluated after regeneration from chemotherapy-induced bone marrow aplasia as regards clinical picture, complete blood picture and bone marrow aspiration.[14,15]

Analysis of the Results

The obtained data will be tabulated and statistically analyzed using SPSS software.

References

1- Liesveld JL, Lichtman MA. Acute Myelogenous Leukemia. In : Williams Hematology. Kaushansky K, Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Prchal JT (eds). 8th ed. McGraw-Hill, New York. 2010, pp. 1277-330.

2- Deschler B, Lubbert M. Acute myeloid leukemia: epidemiology and etiology. Cancer 2006;107:2099-107.

3- Baer MR, Greer JP. Acute Myeloid Leukemia in Adults. In : Wintrobe's Clinical Haematology. Greer JP, Foerster J, Rodgers GM, Paraskevas F, Glader B, Arber DA, Means RT (eds). 12th ed. Lippincott Williams & Wilkins, Philadelphia. 2009, pp. 1843-89.

4- Burnett AK, Venditti A. Acute myeloid leukaemia. In : Postgraduate Haematology. Hoffbrand AV, Catovsky D, Tuddenham EG, Green AR (eds). 6th ed. Blackwell Publishing, Oxford. 2010, pp. 415-32.

5- Kuptsova N, Kopecky KJ, Godwin J, Anderson J, Hoque A, Willman CL, et al. Polymorphisms in DNA repair genes and therapeutic outcomes of AML patients from SWOG clinical trials. Blood 2007; 109:3936-44.

6- Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol 2008; 9:297–308.

7- López-Camarillo C, Lopez-Casamichana M, Weber C, Guillen N, Orozco E, Marchat LA. DNA repair mechanisms in eukaryotes: Special focus in Entamoeba histolytica and related protozoan parasites. Infect Genet Evol 2009; 9:1051-6.

8- Le May N, Egly JM, Coin F. True lies: the double life of the nucleotide excision repair factors in transcription and DNA repair. J Nucleic Acids 2010; 19: 1-11.

9- Liu H, Rudolf J, Johnson KA, McMahon SA, Oke M, Carter L, et al. Structure of the DNA repair helicase XPD. Cell 2008; 133:801–12.

10- Shuck SC, Short EA, Turchi JJ. Eukaryotic nucleotide excision repair: from understanding mechanisms to influencing biology. Cell Res 2008; 18: 64–72.

11- Osawa K. Gene Polymorphisms and Chemotherapy in Non-small Cell Lung Cancer. Chin J Lung Cancer 2009;12:837-40.

12- Wang F, Chang D, Hu FL, Sui H, Han B, Li DD, Zhao YS. DNA repair gene XPD polymorphisms and cancer risk: a meta-analysis based on 56 case-control studies. Cancer Epidemiol Biomarkers Prev 2008; 17: 507-17.

13- Kuptsova N, Ambrosone CP, Weiss J, Baer MR, Sucheston LE, Zirpoli G, et al. *XPD* DNA nucleotide excision repair gene polymorphisms associated with DNA repair deficiency predict better treatment outcomes in secondary acute myeloid leukemia. Int J Mol Epidemiol Genet 2010; 1: 278-94.

14- Bain BJ, Lewis SM, Bates I. Basic haematological techniques. In : Dacie and Lewis Practical Haematology. Lewis SM, Bain BJ, Bates I (eds). 10th ed. Churchill Livingstone, New York. 2006, pp. 26-59.

15- Bates I. Bone marrow biopsy. In : Dacie and Lewis Practical Haematology. Lewis SM, Bain BJ, Bates I (eds). 10th ed. Churchill Livingstone, New York. 2006, pp. 116-31.

16- Higgins T, Beutler E, Doumas BT. Hemoglobin, Iron, and Bilirubin. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 509-26.

17- Panteghini M, Bais R. Enzymes. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 317-36.

18- Lamb EJ, Price CP. Creatinine, Urea, and Uric Acid. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 363-72.

19- Sacks DB. Carbohydrates. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 373-401.

20- Scott MG, LeGrys VA, Klutts JS. Electrolytes and Blood Gases. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 432-49.

21- Batar B, Güven M, Baris S, Celkan T, Yildiz I. DNA repair gene *XPD* and *XRCC1* polymorphisms and the risk of childhood acute lymphoblastic leukemia. Leuk Res 2009; 33:759-63.

22- Johnson AM. Amino Acids and Proteins. In : Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, Bruns DE, Sawyer BG (eds). 6th ed. Saunders Elsevier, Philadelphia. 2008, pp. 286-316.

PUBLISHED PAPER

Original research paper

XPD gene polymorphisms and the effects of induction chemotherapy in cytogenetically normal *de novo* acute myeloid leukemia patients

Mervat A. El-Tokhy[1], Neveen A. Hussein[1], Ahmed M. L. Bedewy[2], Mohammad R. Barakat[1]

[1]Applied Medical Chemistry Department, Medical Research Institute, Alexandria University, Alexandria, Egypt, [2]Hematology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt

Background: Cytogenetically normal acute myeloid leukemia (AML) represent nearly half of newly diagnosed *de novo* AML cases. XPD is one of the DNA repair proteins, whose genetic polymorphisms are thought to affect their function as regards response to chemotherapeutic drugs and chemotherapy-induced toxicities.
Subjects and methods: We investigated the *XPD* Asp312Asn and Lys751Gln polymorphisms by polymerase chain reaction-restriction fragment length polymorphism in 51 newly diagnosed cytogenetically normal *de novo* AML patients. The response to the standard induction chemotherapy protocol and chemotherapy-induced toxicities were monitored.
Results: The *XPD* Asp312Asn GG genotype was the most frequent (57%) followed by the GA variant (37%), and the AA variant was the least frequent (6%). As regards the *XPD* Lys751Gln polymorphism, the AA genotype was the most frequent (49%), followed by the AC (39%) and CC (12%) variants. These variants were not associated with age, sex, FAB subtype, CNS infiltration, chemotherapy-induced hepatotoxicity, nephrotoxicity, or metabolic toxicity. The XPD Lys751Gln CC polymorphic variant was associated with chemotherapy-induced cardiotoxicity and lower chance to achieve response to induction chemotherapy.
Conclusion: *XPD* Lys751Gln and not Asp312Asn polymorphism was associated with chemotherapy-induced cardiotoxicity and response to induction chemotherapy in newly diagnosed cytogenetically normal AML patients. Pretreatment assay of *XPD* Lys751Gln may help to anticipate cardiotoxicity in those at risk. Moreover, it may be considered a prognostic marker in AML cases. However, further large scale research is needed to verify its usefulness.

Keywords: AML, XPD, Chemotherapy

Introduction

Acute myeloid leukemia (AML) is a clonal, malignant disease of hematopoietic tissues that is characterized by accumulation of abnormal (leukemic) blast cells, principally in the marrow, and impaired production of normal blood cells. Thus, the leukemic cell infiltration in marrow is accompanied, nearly invariably, by anemia and thrombocytopenia.[1] The overall annual incidence of AML is 3.7 per 100 000 persons, and age-dependent mortality is 2.7 to nearly 18 per 100 000 persons.[2] In adults, AML accounts for 80–90% of cases of acute leukemia.[3]

There is considerable heterogeneity between cases of AML as regards morphology, immunological phenotype, associated cytogenetic and molecular abnormalities, and patterns of gene expression.[4] A number of clinical and biologic features predict prognosis in AML including clinical state, morphology, surface/enzyme markers, cytogenetics, and molecular markers.[3]

The backbone of AML treatment for 30 years has been the combination of daunorubicin and cytarabine for remission induction chemotherapy protocol.[4] These chemotherapeutic drugs bring about their effect by causing DNA damage.[5] This damage causes activation of DNA-repair mechanisms in the

Correspondence to: Ahmed M. L. Bedewy, Hematology Department, Medical Research Institute, Alexandria University, 8 Mahmoud Younes street, Smouha, 21615 Alexandria, Egypt.
Email: dr_ahmed_bedewy@yahoo.com

DOI 10.1179/1607845413Y.0000000144

different phases of the cell cycle. Cells resume cell-cycle progression once damage has been repaired, whereas cells which fail to repair the DNA damage undergo permanent cell-cycle arrest or apoptosis.[6]

There are six pathways of DNA repair: homologous recombination repair, non-homologous end-joining, base excision repair, mismatch repair, nucleotide excision repair (NER), and methyltransferase repair.[7] NER is a major DNA repair pathway in eukaryotic cells. It involves more than 30 proteins including Xeroderma pigmentosum (XP) groups A to G.[8]

Xeroderma pigmentosum complementation group D (*XPD*) gene encodes a helicase which is a component of transcription factor IIH (TFIIH) and functions in transcription initiation and NER in eukaryotes.[9] Functional DNA repair capacity is thought to differ significantly between the different polymorphic variants of *XPD*.[10,11]

Among the polymorphic variants described for *XPD* gene are Lys751Gln (A > C) and Asp312Asn (G > A).[12] The association between these variants and the response to chemotherapy in AML has been subjected to recent research in order to predict the prognosis of AML patients.[5,13]

In this work, we aimed to investigate the association of *XPD* polymorphism with the response to remission induction chemotherapy. In addition, we explored the association of *XPD* polymorphism with chemotherapy-induced toxicities during remission induction in patients with de novo cytogenetically normal AML.

Subjects and methods

This study included 51 patients with newly diagnosed *de novo* cytogenetically normal AML presented to the Department of Hematology, Medical Research Institute, Alexandria University. Patients over 60 years old and those with co-morbid conditions (liver, kidney, or heart disease) were excluded from the study. All patients were subjected at presentation to full medical history taking, thorough clinical examination, routine laboratory, and imaging workups.

Patients received the standard remission induction protocol (3 + 7) comprising: Cytosine Arabinoside 100 mg/m^2 on days 1–7 by continuous intravenous infusion and daunorubicin 45 mg/m^2 on days 1–3 by intravenous push.[4,14] Patients were monitored for toxic effects induced by chemotherapy, namely gastrointestinal toxicity (nausea, diarrhea or mucositis), hepatotoxicity (increased serum bilirubin, alkaline phosphatase, or transaminases levels), nephrotoxicity (increased serum creatinine or presence of proteinuria), metabolic toxicity (hypoglycemia, hyperglycemia, hypocalcemia, hypokalemia, hyponatremia, or hypomagnesemia) and cardiac toxicity (conduction abnormalities, palpitations, prolonged QTc interval, arrhythmias, ischemia/infarction, hypertension, hypotension, left or right ventricular dysfunction, pericarditis, pulmonary hypertension, or cardiomyopathy). Monitoring included clinical examination, laboratory evaluation (liver and kidney functions, blood electrolytes, and cardiac enzymes assays), electrocardiography, and imaging studies including echocardiography. The grade of toxicity was recorded according to the National Cancer Institute common terminology criteria (version 4.0).[15]

Response to chemotherapy was evaluated after regeneration from chemotherapy-induced bone marrow aplasia.[4] Patients who failed to achieve complete remission were subjected to a second similar course of chemotherapy.

Molecular studies for XPD polymorphisms

Sample collection and DNA extraction

Three milliliters of venous blood were collected before receiving chemotherapy after obtaining signed consent. Immediately after collection, whole blood was stored in aliquots at −20 °C until assayed. Genomic DNA was extracted from leukocytes using Gene JET DNA purification kit (Feremntas, Germany) according to the manufacturer's instructions.

Polymerase chain reaction-restriction fragment length polymorphism assay for XPD Lys751Gln (A > C)

The *XPD* Lys751Gln (A > C) genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of DNA samples collected previously.[16] The PCR primers were: forward, 5′-GCCCGCTCTGGATTATACG-3′; and reverse, 5′-CTATCATCTCCTGGCCCCC-3′. PCR was performed in 50-μl containing 2 mM MgCl2, 0.04 mM deoxynucleotide triphosphates, 2.5 units of Taq polymerase, and the manufacturer's buffer [20 mM Tris-HCI (pH 8.4) and 50 mM KCl]. After an initial denaturation at 94°C for 3 minutes, there were 38 cycles of 45 seconds at 94°C, 45 seconds at 60°C, and 60 seconds at 72°C, and then a final extension step of 7 minutes at 72°C. After overnight digestion of the PCR product with PstI, 15 μl of the digested products were resolved on a 3% agarose gel containing ethidium bromide. The homozygous wild-type allele (Lys 751) produced two DNA bands (290 and 146 bp), whereas the mutant allele (Gln 751) produced three DNA bands (227, 146, and 63 bp). Heterozygotes displayed all four bands (290, 227, 146, and 63 bp).

PCR-RFLP assay for XPD Asp312Asn (G > A)

XPD Asp312Asn (G > A) polymorphism was analyzed by PCR-RFLP method.[16] The oligonucleotide

primers used were: 5′-CTGTTGGTGGGTGCCCG TATCTGTT-GGTCT-3′ and 5′-TAATATCGGGG CTCACCCTGCAGCACTTCCT-3′. PCR was performed in 25 µl reaction mixtures containing 1.5 mM MgCl2, 0.2 mM deoxynucleotide triphosphates, 3% DMSO, 0.2 µM primers, 1 µg of template DNA, and 1.5 units of Taq polymerase in PCR buffer (10 mM Tris-HCl (pH 9.0 at 25°C), 50 mM KCl, and 0.1% Triton X-100 (Promega)). After an initial denaturation at 94°C for 4 minutes, the DNA was amplified by 30 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 60 seconds at 72°C, and then by a final extension step of 5 minutes at 72°C. Fifteen microliters of the PCR product was digested with *Sty*I for 8 hours at 37°C. The digestion products were then resolved on a 3% agarose gel (5 V/cm) containing ethidium bromide. The homozygous wild type *(Asp/Asp)* was identified by two DNA bands (507 and 244 bp), the homozygous mutant type *(Asn/Asn)* produced three bands (474, 244, and 33 bp); and heterozygotes *(Asp/Asn)* displayed all four bands (507, 474, 244, and 33 bp).

Statistical analysis of the data

Data were fed to the computer using IBM SPSS software package, version 20.0. Comparison between different groups regarding categorical variables was tested using χ^2 test. When more than 20% of the cells have expected count <5, correction for χ^2 was conducted using Monte Carlo correction. Parametric tests were used for normally distributed data, while non-parametric tests were used for abnormally distributed data. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

Results

Fifty one newly diagnosed *de novo* cytogenetically normal AML patients were enrolled in this study (28 males and 23 females). Age of patients ranged from 19 to 57 years with a median of 33 years. AML FAB subtype M2 was the most frequent while FAB subtype M7 was the least frequent among the studied patients. Six patients had CNS infiltration

Table 1 XPD Asp312Asn and Lys751Gln polymorphisms in relation to the studied clinical and laboratory parameters

		XPD Asp312Asn polymorphism				XPD Lys751Gln polymorphism			
		GG (Asp) (*n* = 29)	GA (Asp/Asn) (*n* = 19)	AA (Asn) (*n* = 3)	*P*	AA (Lys) (*n* = 25)	AC (Lys/Gln) (*n* = 20)	CC (Gln) (*n* = 6)	*P*
Age (years) (19–57)	Median (min–max)	31 (19–56)	36 (21–57)	47 (25–50)	0.302*	35 (19–57)	31 (21–53)	39.5 (21–50)	0.623*
Sex	Male (*n* = 28)	12	13	3	0.051**	11	12	5	0.186**
	Female (*n* = 23)	17	6	0		14	8	1	
FAB subtype	M0 (*n* = 3)	1	2	0	0.292**	2	1	0	0.118**
	M1 (*n* = 11)	9	2	0		5	4	2	
	M2 (*n* = 12)	7	5	0		3	9	0	
	M4 (*n* = 11)	7	2	2		7	2	2	
	M5 (*n* = 8)	3	4	1		6	1	1	
	M6 (*n* = 4)	2	2	0		1	3	0	
	M7 (*n* = 2)	0	2	0		1	0	1	
CNS infiltration	No (*n* = 45)	26	17	2	0.489**	22	18	5	0.905**
	Yes (*n* = 6)	3	2	1		3	2	1	
Hepatic toxicity	No (*n* = 37)	22	13	2	0.829**	18	16	3	0.351**
	Yes (*n* = 14)	7	6	1		7	4	3	
Renal toxicity	No (*n* = 44)	24	17	3	0.624**	21	17	6	0.579**
	Yes (*n* = 7)	5	2	0		4	3	0	
Metabolic toxicity	No (*n* = 39)	24	14	1	0.148**	19	16	4	0.794**
	Yes (*n* = 12)	5	5	2		6	4	2	
Cardiac toxicity	No (*n* = 44)	26	15	3	0.445**	24	18	2	<0.001**,***
	Yes (*n* = 7)	3	4	0		1	2	4	
Response to induction chemotherapy	Complete response (*n* = 30)	19	11	0	0.089**	19	8	3	0.046**,***
	Resistance (*n* = 21)	10	8	3		6	12	3	
Number of induction courses to complete response	One course (*n* = 19)	16	2	1	0.239**	12	5	1	0.531**
	Two courses (*n* = 11)	9	1	1		5	5	1	

* Kruskal Wallis test *P* value.
**Monte Carlo test *P* value.
***Significant.

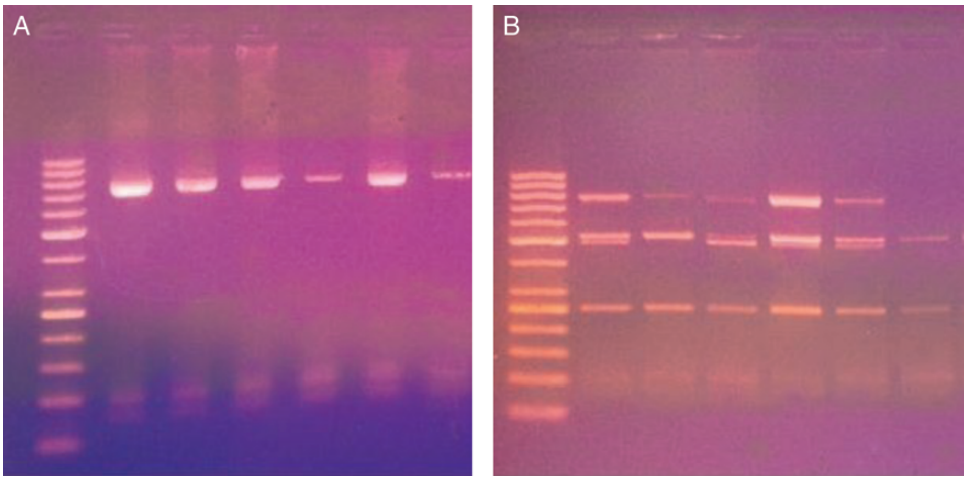

Figure 1 PCR products (A) and digestion products (B) for Asp312Asn polymorphism of 6 cases. The first lane to the left is DNA ladder (50–1000 base). Lanes 2, 4, 5, and 6 represent GA genotype. Lanes 3 and 7 represent GG genotype.

(11.8%). Among the chemotherapy-induced toxicities, hepatotoxicity was the most frequent (27%) followed by metabolic toxicity (24%), while nephrotoxicity and cardiac toxicity were the least frequent (13%) (Table 1).

Thirty patients (59%) achieved complete response while 21 patients (41%) failed to respond to induction chemotherapy. Among the responders, 19 patients achieved the complete response after one course of induction chemotherapy while 11 patients achieved it after 2 courses (Table 1).

The *XPD* Asp312Asn GG genotype was the most frequent (57%) followed by the GA variant (37%), and the AA variant was the least frequent (6%) (Fig. 1). No significant association was found between these variants and any of the studied parameters, namely age, sex, FAB subtypes, CNS infiltration, and different types of chemotherapy-induced toxicities (Table 1).

As regards the *XPD* Lys751Gln polymorphism, the AA genotype was the most frequent (49%), followed by the AC (39%) and CC (12%) variants (Table 1) (Fig. 2). These variants were not associated with age, sex, FAB subtype, CNS infiltration, chemotherapy-induced hepatotoxicity, nephrotoxicity, or metabolic toxicity. However, while they were significantly associated with chemotherapy-induced cardiotoxicity, which was more frequent among patients having the CC polymorphic variant. In addition, the response to induction chemotherapy was significantly associated with these polymorphic variants with more patients having the AA genotype achieved complete remission (Table 1).

The response to induction chemotherapy among the studied patients was not associated with the age, sex, FAB subtype, or *XPD* Asp312Asn polymorphism, while it was significantly associated with CNS infiltration and *XPD* Lys751Gln polymorphism. No patient with CNS infiltration achieved complete response and patients with the C allele of *XPD* Lys751Gln polymorphism had lower chance to achieve complete response (Table 2).

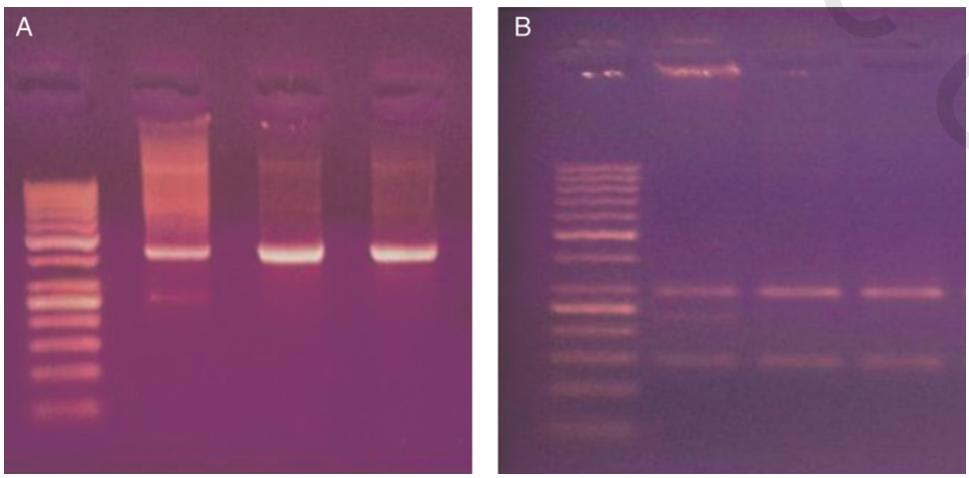

Figure 2 PCR products (A) and digestion products (B) for XPD Lys751Gln polymorphism of 3 cases. The first lane to the left is DNA ladder (50–1000 base). Lane 2 represents AC genotype. Lanes 3 and 4 represent AA genotype.

Table 2 The response of acute myeloid leukemia patients to induction chemotherapy in relation to the studied parameters

		Response to induction chemotherapy		
		Complete response ($n = 30$)	Resistance ($n = 21$)	*P*
Age (years)	Median (min–max)	31 (19–57)	33 (21–54)	0.985*
Sex	Male	16	12	0.788**
	Female	14	9	
FAB subtype	M0	2	1	0.196***
	M1	9	2	
	M2	4	8	
	M4	7	4	
	M5	6	2	
	M6	1	3	
	M7	1	1	
CNS infiltration	No	30	15	0.002***,†
	Yes	0	6	
XPD Asp312Asn polymorphism	GG (Asp)	19	10	0.089***
	GA (Asp/Asn)	11	8	
	AA (Asn)	0	3	
XPD Asp312Asn polymorphism	GG	19	10	0.265**
	GA/AA	11	11	
XPD Lys751Gln polymorphism	AA (Lys)	19	6	0.046***,†
	AC (Lys/Gln)	8	12	
	CC (Gln)	3	3	
XPD Lys751Gln polymorphism	AA	19	6	0.015**,†
	AC/CC	11	15	

* Kruskal Wallis test *P* value.
**Chi square test *P* value.
***Monte Carlo *test P* value.
†Significant.

Discussion

AML is a highly malignant and cytogenetically heterogeneous type of cancer. Cytogenetically normal AML accounts for approximately 40–50% of adult patients with AML and represents a heterogeneous group with an intermediate prognosis.[17]

Human DNA repair mechanisms protect the genome from DNA damage caused by endogenous and exogenous agents including chemotherapy.[18] *XPD* gene product is a helicase that is a component of the TFIIH complex. XPD plays a role in transcription and NER through reversal of DNA crosslinks and oxidative damage induced by chemotherapy. *XPD* polymorphic variants are supposed to possess different DNA repair capacities and consequently, result in variable effects of the chemotherapeutic agents.[18,19]

In this study, the *XPD* Asp312Asn polymorphism was not associated with chemotherapy-induced toxicities or with the response to induction chemotherapy. As regards the *XPD* Lys751Gln polymorphism, it was not associated with age, sex, FAB subtype, CNS infiltration, chemotherapy-induced hepatotoxicity, nephrotoxicity, or metabolic toxicity. However, patients with CC genotype (Gln/Gln) had a higher chance to suffer chemotherapy-induced cardiotoxicity.

According to Shen and his colleagues, *XPD* Lys751Gln is the main polymorphism that induces amino acid changes in the protein and consequently, modifies its DNA repair ability.[20] As described by Spitz *et al.*[21], Rzeszowska-Wolny *et al.*,[22] and Shen *et al.*,[23] variant allele of *XPD* gene codon 751 (C allele) is associated with impaired DNA repair activity. The impaired DNA repair activity was reported to enhance drug toxicities.[13,24] On the contrary, a report by Lunn *et al.*[25] relates suboptimal repair of X-ray-induced DNA damage to the common allele (A). However, this can be attributed to the diferenct genotoxic element evaluated and to the *in vitro* model of their study.

It has been generally agreed with that toxicity from chemotherapeutic drugs depends on the pharmacological effects and drug dosages of regimen, patient's performance status, organ functions, and previous treatments. However, the toxicity may be quite different even though the patients with the similar body status received the same drug dosage. It is now hypothesized that the toxicity could vary for patients with different genotypes; a milestone towards the individualized medicine.

The reason for which, cardiotoxicity was significantly associated with the XPD Lys751Gln polymorphism is not clear. However, recent attention was focused on the association between chemotherapy-induced cardiotoxicity and DNA polymorphisms in order to help selecting the chemotherapeutic regimen most likely to benefit each individual patient.[26]

A report by Guven *et al.*[27] studied the XPD Lys751Gln polymorphism in patients with coronary artery disease. They found an association between the

variant allele C and the evidence of genotoxicity in their patients. It has been suggested that oxidative stress and the generation of reactive oxygen species may play an important role in the induction of DNA damage.[28] NER together with base excision repair is responsible for repair of the oxidative DNA damage induced by chemotherapy.[29] Accordingly, genetic polymorphisms in the *XPD* DNA repair gene may influence individual variation in DNA repair capacity in response to chemotherapy, leading to increased risk of developing cardiotoxicity in those having the inefficient C allele.

In this work, an association between the response to chemotherapy and the *XPD* Lys751Lys common genotype (AA) was found. This issue was raised by different authors. However, none of the them reported similar findings. Kuptsova-Clarkson *et al.*[13], Spitz *et al.*,[21] and Shen *et al.*[23] found the variant allele of *XPD* gene codon 751 (C allele) to be associated with impaired DNA repair activity. They concluded that impaired DNA repair activity due to this variant, led to lower resistance to chemotherapy and higher anticipation to achieve CR and longer survival. On the other side, Qiu *et al.*[30] and Rumiato *et al.*[31] denied any association between this polymorphism and response to chemotherapy.

Up to our best knowledge, no reports studied this polymorphism in newly diagnosed cytogenetically normal AML patients. A possible explanation for our finding may be the intact DNA repair machinery granted by the common (A) allele limits the genome mutations, which would cause drug resistance, within the leukemic clone. Consequently, a better response to chemotherapy is achieved. Obviously, further research is required before establishing a firm conclusion in this regard.

Conclusion

XPD Lys751Gln and not Asp312Asn polymorphism was associated with chemotherapy-induced cardiotoxicity and response to induction chemotherapy in newly diagnosed cytogenetically-normal AML patients. Pretreatment assay of *XPD* Lys751Gln might help to anticipate cardiotoxicity in those at risk. Moreover, it may be considered a prognostic marker in AML cases. However, further large scale research in needed to verify its usefulness.

Acknowledgment

We here appreciate the support of the Hematology Department, Medical Research Institute, Alexandria University.

References

1 Liesveld JL, Lichtman MA. Acute myelogenous leukemia. In: Kaushansky K, Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Prchal JT, (eds.) Williams Hematology. 8th ed. New York: McGraw-Hill; 2010. pp. 1277–330.
2 Deschler B, Lubbert M. Acute myeloid leukemia: epidemiology and etiology. Cancer 2006;107:2099–107.
3 Baer MR, Greer JP. Acute myeloid leukemia in adults. In: Greer JP, Foerster J, Rodgers GM, Paraskevas F, Glader B, Arber DA, Means RT, (eds.) Wintrobe's clinical haematology. 12th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. pp. 1843–89.
4 Burnett AK, Venditti A. Acute myeloid leukaemia. In: Hoffbrand AV, Catovsky D, Tuddenham EG, Green AR, (eds.) Postgraduate haematology. 6th ed. Oxford: Blackwell Publishing; 2010. pp. 415–32.
5 Kuptsova N, Kopecky KJ, Godwin J, Anderson J, Hoque A, Willman CL, *et al.* Polymorphisms in DNA repair genes and therapeutic outcomes of AML patients from SWOG clinical trials. Blood 2007;109:3936–44.
6 Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol. 2008;9:297–308.
7 López-Camarillo C, Lopez-Casamichana M, Weber C, Guillen N, Orozco E, Marchat LA. DNA repair mechanisms in eukaryotes: Special focus in Entamoeba histolytica and related protozoan parasites. Infect Genet Evol. 2009;9:1051–6.
8 Le May N, Egly JM, Coin F. True lies: the double life of the nucleotide excision repair factors in transcription and DNA repair. J Nucleic Acids 2010;19:1–11.
9 Liu H, Rudolf J, Johnson KA, McMahon SA, Oke M, Carter L, *et al.* Structure of the DNA repair helicase XPD. Cell 2008;133: 801–12.
10 Shuck SC, Short EA, Turchi JJ. Eukaryotic nucleotide excision repair: from understanding mechanisms to influencing biology. Cell Res 2008;18:64–72.
11 Osawa K. Gene polymorphisms and chemotherapy in non-small cell lung cancer. Chin J Lung Cancer 2009;12:837–40.
12 Wang F, Chang D, Hu FL, Sui H, Han B, Li DD, *et al.* DNA repair gene XPD polymorphisms and cancer risk: a meta-analysis based on 56 case-control studies. Cancer Epidemiol Biomarkers Prev. 2008;17:507–17.
13 Kuptsova N, Ambrosone CP, Weiss J, Baer MR, Sucheston LE, Zirpoli G, *et al. XPD* DNA nucleotide excision repair gene polymorphisms associated with DNA repair deficiency predict better treatment outcomes in secondary acute myeloid leukemia. Int J Mol Epidemiol Genet. 2010;1:278–94.
14 Estey EH. Acute myeloid leukemia: 2013 update on risk-stratification and management. Am J Hematol. 2013;88(4):318–27. doi: 10.1002/ajh.23404.
15 National Cancer Institute: Common terminology criteria for adverse events, v. 4.0. Available from: http://ctep.cancer.gov/reporting/ctc.html.
16 Batar B, Güven M, Bariş S, Celkan T, Yildiz I. DNA repair gene XPD and XRCC1 polymorphisms and the risk of childhood acute lymphoblastic leukemia. Leuk Res. 2009;33(6):759–63. doi: 10.1016/j.leukres.2008.11.005. Epub 2008 Dec 19.
17 Heo SG, Hong EP, Park JW. Genetic risk prediction for normal-karyotype acute myeloid leukemia using whole-exome sequencing. Genom Inform. 2013;11(1):46–51. doi: 10.5808/GI.2013.11.1.46. Epub 2013 Mar 31.
18 Manuguerra M, Saletta F, Karagas MR, Berwick M, Veglia F, Vineis P, *et al.* XRCC3 and XPD/ERCC2 single nucleotide polymorphisms and the risk of cancer: a HuGE review. Am J Epidemiol. 2006;164(4):297–302. Epub 2006 May 17.
19 Crespan E, Garbelli A, Amoroso A, Maga G. Exploiting the nucleotide substrate specificity of repair DNA polymerases to develop novel anticancer agents. Molecules 2011;16(9): 7994–8019. doi: 10.3390/molecules16097994.
20 Shen MR, Jones IM, Mohrenweiser H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res. 1998;58(4): 604–8.
21 Spitz MR, Wu X, Wang Y, Wang LE, Shete S, Amos CI, *et al.* Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res. 2001;61(4): 1354–7.
22 Rzeszowska-Wolny J, Polanska J, Pietrowska M, Palyvoda O, Jaworska J, Butkiewicz D, *et al.* Influence of polymorphisms in DNA repair genes XPD, XRCC1 and MGMT on DNA damage induced by gamma radiation and its repair in lymphocytes in vitro. Radiat Res. 2005;164(2):132–40.
23 Shen M, Berndt SI, Rothman N, Demarini DM, Mumford JL, He X, *et al.* Polymorphisms in the DNA nucleotide excision

repair genes and lung cancer risk in Xuan Wei, China. Int J Cancer 2005;116(5):768–73.
24 Le Morvan V, Bellott R, Moisan F, Mathoulin-Pélissier S, Bonnet J, Robert J. Relationships between genetic polymorphisms and anticancer drug cytotoxicity vis-à-vis the NCI-60 panel. Pharmacogenomics 2006;7(6):843–52.
25 Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, *et al.* XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 2000;21(4):551–5.
26 Atkins CD. Single nucleotide polymorphisms and anthracycline cardiotoxicity in children: potential implications for adult oncology. J Clin Oncol. 2012;30(28):3563; author reply 3563–4. Epub 2012 Aug 27.
27 Guven M, Guven GS, Oz E, Ozaydin A, Batar B, Ulutin T, *et al.* DNA repair gene XRCC1 and XPD polymorphisms and their association with coronary artery disease risks and micronucleus frequency. Heart Vessels 2007;22(6):355–60. Epub 2007 Nov 26.
28 Botto N, Masetti S, Petrozzi L, Vassalle C, Manfredi S, Biagini A, *et al.* Elevated levels of oxidative DNA damage in patients with coronary artery disease. Coron Artery Dis. 2002;13:269–274.
29 Frosina G. Overexpression of enzymes that repair endogenous damage to DNA. Eur J Biochem. 2000;267:2135–2149.
30 Qiu M, Yang X, Hu J, Ding X, Jiang F, Yin R, *et al.* Predictive value of XPD polymorphisms on platinum-based chemotherapy in non-small cell lung cancer: a systematic review and meta-analysis. PLoS One 2013;8(8):e72251. doi: 10.1371/journal.pone.0072251.
31 Rumiato E, Cavallin F, Boldrin E, Cagol M, Alfieri R, Basso D, *et al.* ERCC1 C8092A (rs3212986) polymorphism as a predictive marker in esophageal cancer patients treated with cisplatin/5-FU-based neoadjuvant therapy. Pharmacogenet Genom. 2013; 23(11):597–604.

ARABIC SUMMARY

جامعة الإسكندرية
معهد البحوث الطبية
قسم الكيمياء الطبية التطبيقية

تأثير تعدد الأشكال فى جين *XPD* على الإستجابة للعلاج الكيماوى الحثى فى مرضى سرطان الدم النقوى الحاد

رسالة مقدمة
بقسم الكيمياء الطبية التطبيقية، معهد البحوث الطبية،
جامعة الإسكندرية

ضمن متطلبات درجة
الماجستير
فى
الكيمياء الطبية التطبيقية

من
محمد رمضان محمود بركات
بكالوريوس علوم فى الكيمياء/الكيمياء الحيوية (٢٠٠٥)، كلية العلوم، جامعة الإسكندرية
دبلوم تكنولوجيا حيوية (٢٠٠٦)، معهد الدراسات العليا و البحوث، جامعة الإسكندرية

أغسطس ــ ٢٠١٤

جامعة الإسكندرية
معهد البحوث الطبية
قسم الكيمياء الطبية التطبيقية

تأثير تعدد الأشكال فى جين *XPD* على الإستجابة للعلاج الكيماوى الحثى فى مرضى سرطان الدم النقوى الحاد

رسالة مقدمة من

محمد رمضان محمود بركات

للحصول على درجة

الماجستير

فى

الكيمياء الطبية التطبيقية

لجنة الحكم و المناقشة: **التوقيع:**

أ.د./ ميرفت عبدالفتاح الطوخى .....
قسم الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية

أ.د./ أحمد محمد لطفى بديوى .....
قسم أمراض الدم
معهد البحوث الطبية
جامعة الإسكندرية

أ.د./ محمد أحمد عبد المحسن .....
قسم الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية

أ.د./ نادية السيد زكى .....
قسم أمراض الباطنة، وحدة أمراض الدم
كلية الطب
جامعة الإسكندرية

التاريخ / /

لجنة الإشراف **الموافقين**

أ.د./ ميرفت عبدالفتاح الطوخى .....

قسم الكيمياء الطبية التطبيقية

معهد البحوث الطبية

جامعة الإسكندرية

أ.د./ أحمد محمد لطفى بديوى .....

قسم أمراض الدم

معهد البحوث الطبية

جامعة الإسكندرية

د./ نيفين عبد المنعم حسين .....

قسم الكيمياء الطبية التطبيقية

معهد البحوث الطبية

جامعة الإسكندرية

الملخص العربى

الملخص العربى

تأثير تعدد الأشكال فى جين *XPD* على الإستجابة للعلاج الكيماوى الحثى فى مرضى سرطان الدم النقوى الحاد

سرطان الدم النقوى الحاد هو مرض نسيلي خبيث من الأنسجة المكونة للدم، و يتميز بتراكم الخلايا الأرومية الغير طبيعية بصورة أساسية فى النخاع مما يؤدى إلى ضعف إنتاج خلايا الدم الطبيعية. هناك تباين كبير بين حالات سرطان الدم النقوى الحاد من حيث المورفولوجيا، النمط الظاهري المناعي، التشوهات الوراثية الخلوية و الجزيئية، وأنماط التعبير الجيني. تحليل الوراثيات الخلوية التقليدى قبل العلاج يتعرف على خلل نسيلي مكتسب في ما يقرب من ٥٠٪ - ٦٠٪ من مرضى سرطان الدم النقوى الحاد الحديث، بما فيهم من ١٠% - ١٢% وراثيات خلوية مركبة لديهم نتائج سيئة للغاية. أما ٤٠% - ٥٠% من الحالات ليس لديهم تشوهات وراثية خلوية. هذه الحالات من سرطان الدم الحاد بوراثيات خلوية طبيعية يتم تصنيفها فى المجموعة متوسطة المخاطر، لكن هذه المجموعة شديدة التباين.

يتكون علاج الحث الكيماوى لسرطان الدم النقوى الحاد بصورة أساسية من انثراسيكلين (داونوروبيسين) و ارابينوزيد السيتوزين، و هو نظام علاجى لم يتغير منذ ٣٠ سنة. يعطى الداونوروبيسين بجرعة ٤٥ مج\م٢ لكل يوم لمدة ثلاثة أيام بالتوازى مع السيتوزين أرابينوزيد بجرعة ١٠٠ مج\م٢ لكل يوم لمدة ٧ أيام عن طريق الوريد (لذلك يسمى هذا النظام العلاجى ٣+٧). إنه من غير المألوف لعلاج الحث الكيماوى ألا يقوم بمسح معظم الخلايا الأرومية الإبيضاضية، و لكن يكون هذا على حساب ٣ إلى ٤ أسابيع من قلة شديدة و شاملة فى كريات الدم. الرعاية الداعمة خلال فترة قمع النخاع أمر حاسم لنتائج العلاج.

الجينوم البشري يهاجم باستمرار من قبل نواتج أيض داخلية متفاعلة، و كذلك عقاقير علاجية ومطفرات بيئية مما يؤثر على سلامته. و لذلك فمن الواضح أن استقرار الجينوم يجب أن يكون تحت المراقبة المستمرة. إستئصال النيوكليوتيدات هو مسلك رئيسى لإصلاح الحمض النووى في الخلايا حقيقية النواة. وهو مسؤول عن إصلاح نواتج الإضافة للحمض النووي ذات الحجم الكبير مثل الأضرار الناتجة عن الأشعة فوق البنفسجية، و الوصلات المتكونة بين الضفائر و كذلك النواتج الكيماوية الناجمة عن التعرض للمواد السامة. يعمل إنزيم XPD كجزء من مركب TFIIH، و هذا المركب ضرورى لمسار إصلاح الحمض النووى باستئصال النيوكليوتيدات. النشاط المضاد للسرطان الخاص بمعظم أدوية العلاج الكيماوى يعتمد على استحثاث التلف في الحمض النووي داخل الخلايا السرطانية سريعة الإنقسام. وبالتالي، فإن تنظيم مسارات إصلاح الحمض النووي في الخلايا السرطانية يشكل عاملا حاسما لاستجابتها لعقاقير العلاج الكيماوى ويمكن أن تؤثر على سمية الدواء و سكون المرض.

الهدف من البحث:

تقييم اثنين من تعدد الأشكال فى جين *XPD* (Asp312Asn (G→A) و Lys751Gln (A→C)) فيما يتعلق بالإستجابة للعلاج الكيماوى الحثى فضلا عن السميات التي يسببها العلاج الكيماوى في مرضى سرطان الدم النقوى الحاد الحديث فى البالغين ذوى الوراثيات الخلوية الطبيعية.

شملت هذه الدراسة:

٥١ مريضاً مصرى بالغ تم تشخيصهم حديثا بسرطان الدم النقوى الحاد الحديث ذو الوراثيات الخلوية الطبيعية من كلا الجنسين (قسم أمراض الدم، معهد البحوث الطبية، جامعة الإسكندرية). تم إقصاء المرضى فوق الستين و من يعانون من حالات مرضية متزامنة أو حالات ضعف أداء. المرضى الذين يعانون من سرطان الدم النقوى الحاد من نوع M3 لم يتم تسجيلهم في هذه الدراسة بسبب إختلاف نظام العلاج الكيماوى الخاص بهم.

تم إجراء عدد من الفحوصات على المرضى:

تم إجراء فحوص سريرية وتحاليل مخبرية في بداية دخول المستشفى و جمع عينة بحجم ٣ ملليلتر من الدم الوريدي لكل مريض. تم علاج المرضى بالنظام الكيماوى الحثى (٣+٧). خلال العلاج الكيماوى، تابعنا المرضى لكشف الآثار السامة المحتملة الناجمة عن العلاج. ثم تم تقييم الاستجابة للعلاج الكيماوى بعد التعافى من تثبيط نماء نخاع العظم الناجم عن العلاج. بينما المرضى الذين لم يستجيبوا للعلاج المبدئى تم تعريضهم لدورة ثانية مماثلة من العلاج الكيميائى. قمنا بعد ذلك باستخلاص الحمض النووى (DNA) من عينات الدم و تعيين تعدد الشكال فى جين *XPD* عن طريق PCR-RFLP. ثم قمنا بإجراء التحاليل الإحصائية على البيانات التى تم الحصول عليها.

نتائج الدراسة:

التعدد الشكلى *XPD* Asp312Asn لم يترافق مع الإستجابة للعلاج الكيماوى أو مع أي من السميات التي يسببها العلاج الكيماوى. و من ناحية أخرى، التعدد الشكلى *XPD* Lys751Gln كان مرتبطا مع الإستجابة للعلاج الكيماوى ومع السميات فى القلب ولكن ليس مع السميات فى الكبد، أو الكلى أو السميات الأيضية. المرضى الذين يملكون الشكل الوراثى *XPD*751 (AA) كانوا أكثر قابلية لتحقيق هدوء كامل للمرض. في حين أن المرضى الذين يملكون الشكل الوراثى *XPD*751 (CC) كانوا أكثر عرضة للمعاناة من السميات فى القلب التي يسببها العلاج الكيماوى.

الإستنتاجات:

لقد حددنا فى الدراسة الحالية التعدد الشكلى فى *XPD*751 كعلامة تنبؤية لاستجابة مرضى سرطان الدم النقوى الحاد الحديث ذو الوراثيات الخلوية الطبيعية للعلاج الكيميائى. على الرغم من أنه ما زال من المبكر تشكيل إستنتاج مؤكد من هذا البحث، إلا أن هذه النتائج قد تمثل خطوة صغيرة فى فهم الدور الذى يلعبه تغير الأشكال فى جينات إصلاح الحمض النووى على نتيجة علاج مرضى سرطان الدم النقوى الحاد.

التوصيات:

على قدر علمنا هذا أول بحث يقوم بدراسة تعدد الأشكال فى جين *XPD* فيما يتعلق بالإستجابة للعلاج الكيماوى الحثى فضلا عن السميات التي يسببها العلاج الكيماوى في مرضى سرطان الدم النقوى الحاد الحديث فى البالغين ذوى الوراثيات الخلوية الطبيعية. مع ذلك دراستنا محدودة نظرا لصغر حجم العينة نسبيا. لذلك تحتاج هذه التحليلات إلى إجرائها على عدد أكبر من المرضى. علاوة على ذلك، ينبغي أن تشتمل الدراسات المستقبلية الكبيرة على عدد أكبر من الأشكال المتعددة فى الجينات الخاصة بالمسارات المختلفة لإصلاح الحمض النووى، كذلك الجينات الخاصة بالتخلص من السميات و تلك الخاصة بموت الخلية المبرمج. هذا ضرورى للتحقق من صحة الارتباط بين المتغيرات في جينات إصلاح الحمض النووي ونتائج العلاج وتوضيح الآليات الكامنة وراء تطور مرض سرطان الدم النقوى الحاد. المزيد من الأبحاث لتحديد كيفية تفاعل هذه العوامل قد تسمح بتطوير نظم علاج فردية لمرضى سرطان الدم النقوى الحاد.