

SUMMARY AND CONCLUSIONS

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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/m²/day for 3 days, in combination with Ara-C, which is administered as a continuous infusion at 100 mg/m²/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.

Summary and Conclusions

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.

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PROTOCOL

تأثير تعدد الأشكال في 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**

Protocol of a thesis submitted to the
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Master of Applied Medical Chemistry

الماجستير فى الكيمياء الطبية التطبيقية

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الدكتورة/ ميرفت عبدالفتاح الطوخى
أستاذ بقسم الكيمياء الطبية التطبيقية
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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°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/m² on days 1-7 by continuous intravenous infusion.
- Daunorubicin 45 mg/m² 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

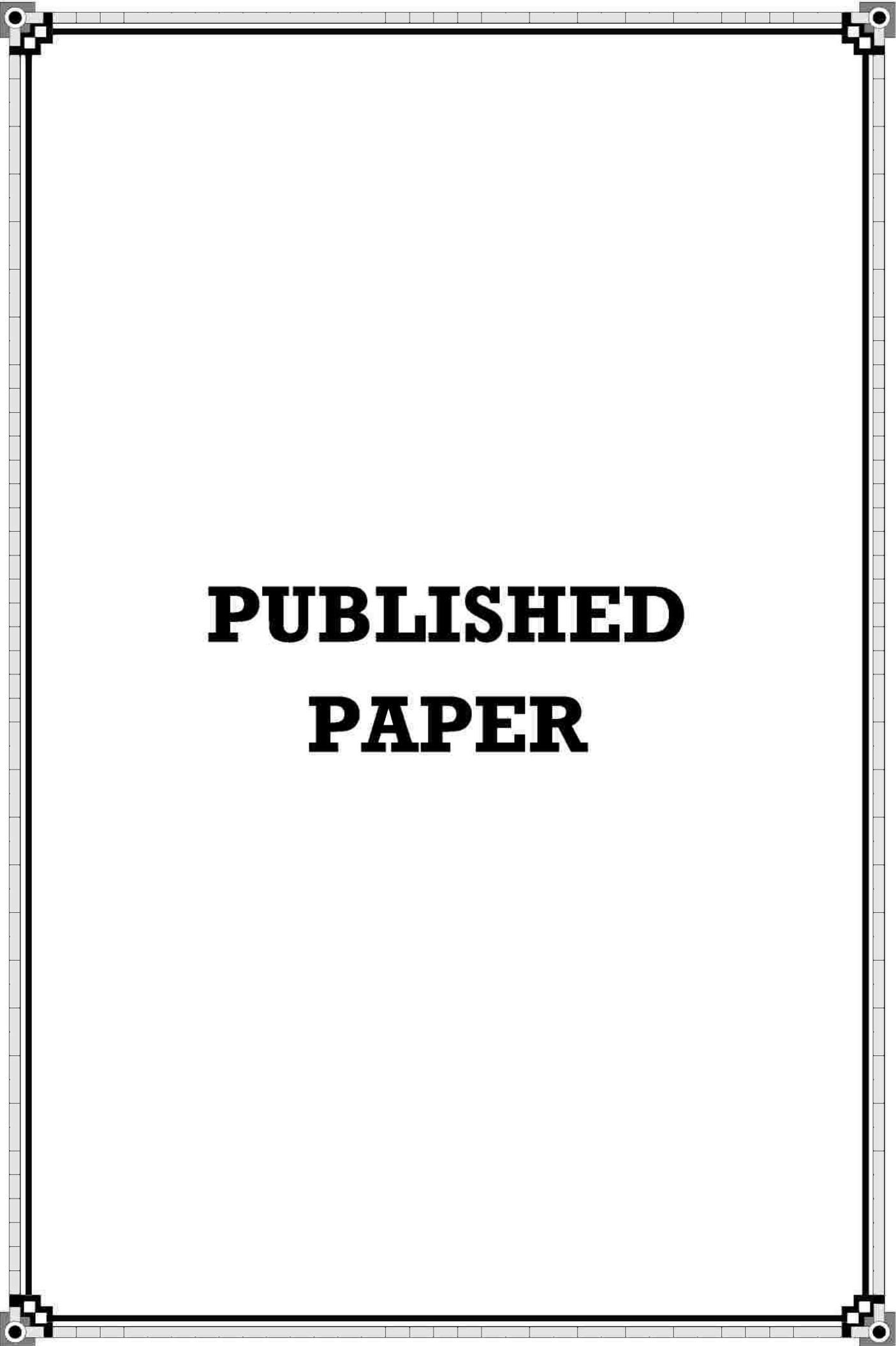
The obtained data will be tabulated and statistically analyzed using SPSS software.

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Original research paper

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

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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

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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 IIIH (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/m² on days 1–7 by continuous intravenous infusion and daunorubicin 45 mg/m² 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 (Fermentas, 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- μ l containing 2 mM MgCl₂, 0.04 mM deoxynucleotide triphosphates, 2.5 units of Taq polymerase, and the manufacturer's buffer [20 mM Tris-HCl (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 μ l 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'-CTGTTGGTGGGTGCCCCG TATCTGTT-GGTCT-3' and 5'-TAATATCGGGG CTCACCCTGCAGCACTTCT-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 *StyI* 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	
CNS infiltration	M7 (n = 2)	0	2	0	1	0	1	0.905**	
	No (n = 45)	26	17	2	22	18	5		
Hepatic toxicity	Yes (n = 6)	3	2	1	3	2	1	0.351**	
	No (n = 37)	22	13	2	18	16	3		
Renal toxicity	Yes (n = 14)	7	6	1	7	4	3	0.579**	
	No (n = 44)	24	17	3	21	17	6		
Metabolic toxicity	Yes (n = 7)	5	2	0	4	3	0	0.794**	
	No (n = 39)	24	14	1	19	16	4		
Cardiac toxicity	Yes (n = 12)	5	5	2	6	4	2	<0.001***	
	No (n = 44)	26	15	3	24	18	2		
Response to induction chemotherapy	Yes (n = 7)	3	4	0	1	2	4	0.046***	
	Complete response (n = 30)	19	11	0	19	8	3		
Number of induction courses to complete response	Resistance (n = 21)	10	8	3	6	12	3	0.531**	
	One course (n = 19)	16	2	1	12	5	1		
	Two courses (n = 11)	9	1	1	5	5	1		

* Kruskal Wallis test P value.

**Monte Carlo test P value.

***Significant.

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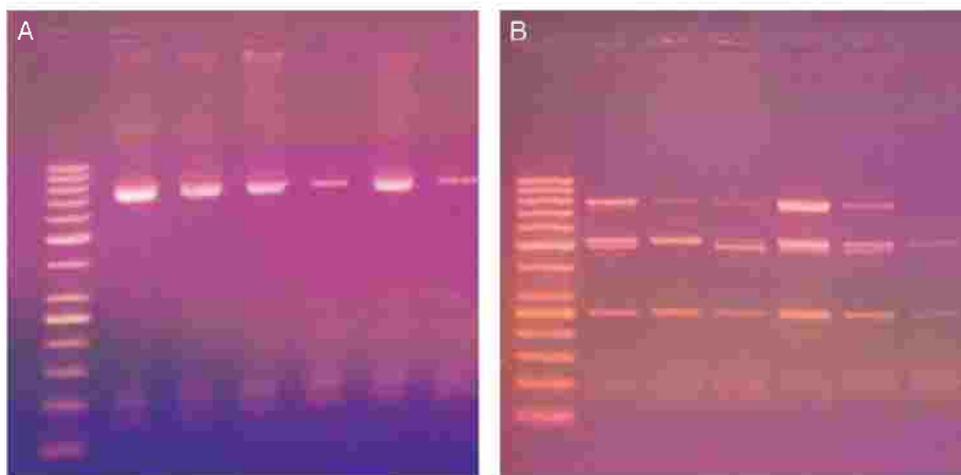


Figure 1 PCR products (A) and digestion products (B) for Asp312Asn polymorphism of 6 cases. The first lane to the left is DNA ladder (50–1000 base). Lanes 2, 4, 5, and 6 represent GA genotype. Lanes 3 and 7 represent GG genotype.

(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).

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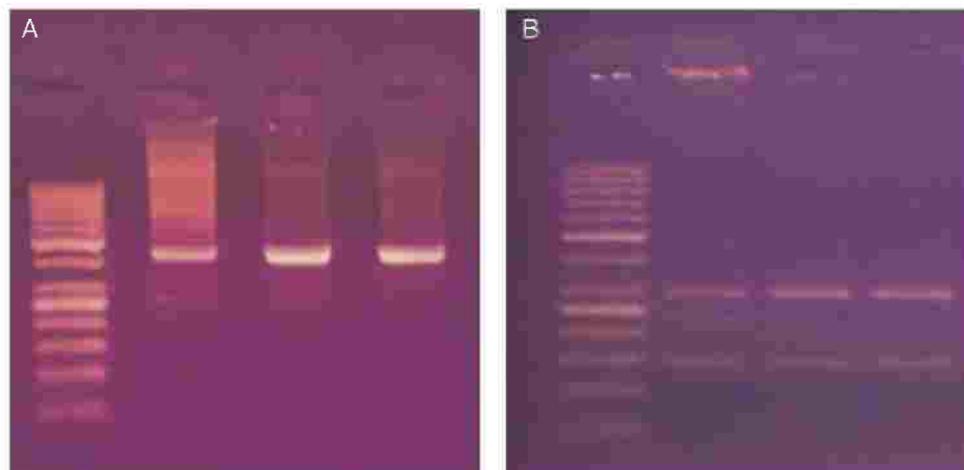


Figure 2 PCR products (A) and digestion products (B) for *XPD* Lys751Gln polymorphism of 3 cases. The first lane to the left is DNA ladder (50–1000 base). Lane 2 represents AC genotype. Lanes 3 and 4 represent AA genotype.

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

	Median (min-max)	Response to induction chemotherapy		P
		Complete response (n = 30)	Resistance (n = 21)	
Age (years)		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 different 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 is needed to verify its usefulness.

Acknowledgment

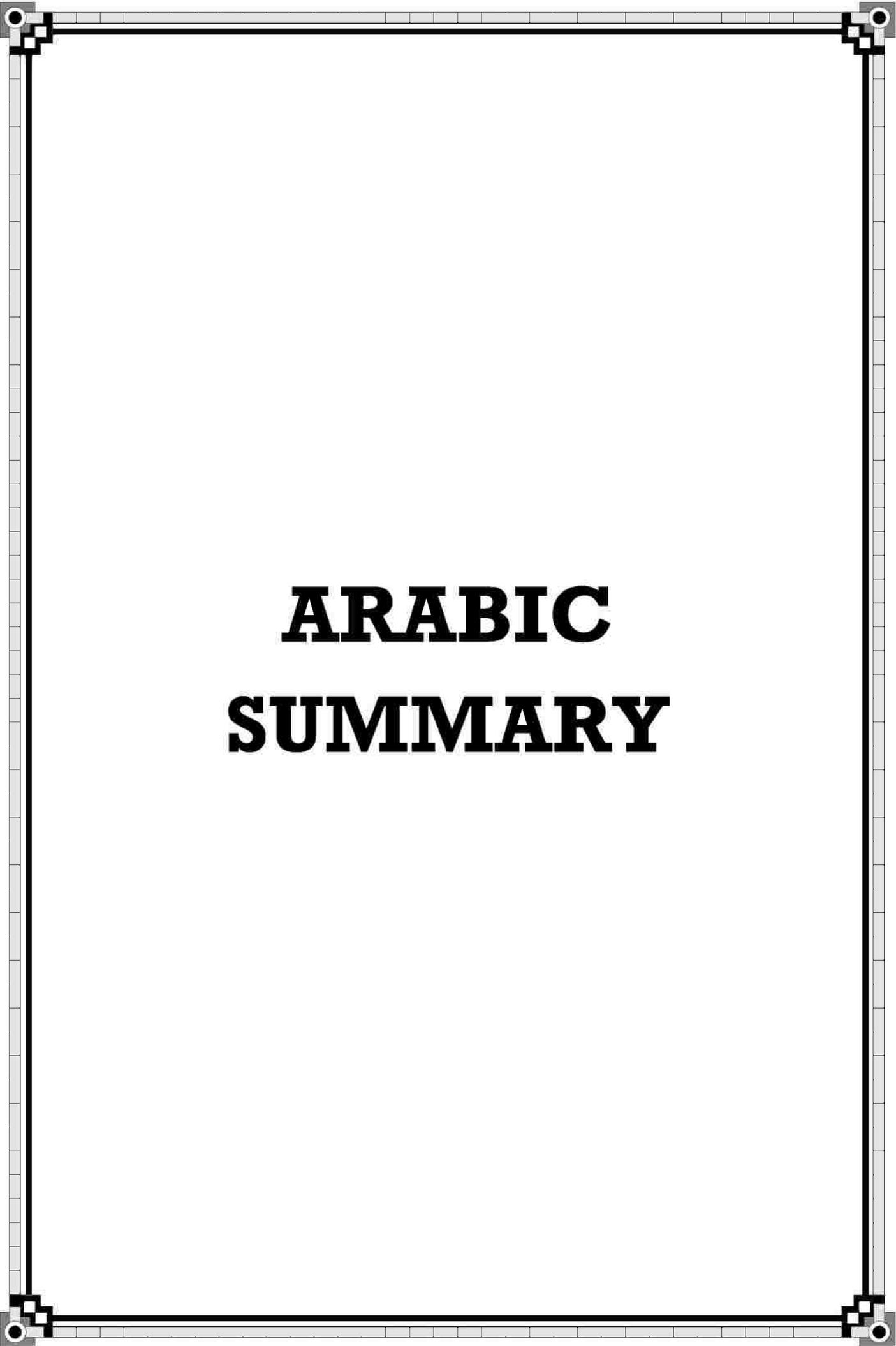
We here appreciate the support of the Hematology Department, Medical Research Institute, Alexandria University.

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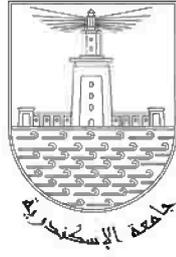
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ARABIC SUMMARY



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قسم الكيمياء الطبية التطبيقية

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

رسالة مقدمة
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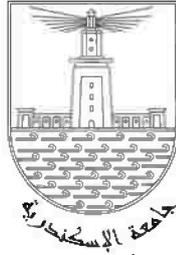
ضمن متطلبات درجة
الماجستير
فى
الكيمياء الطبية التطبيقية

من

محمد رمضان محمود بركات

بكالوريوس علوم فى الكيمياء/الكيمياء الحيوية (٢٠٠٥)، كلية العلوم، جامعة الإسكندرية
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أغسطس – ٢٠١٤



جامعة الإسكندرية
معهد البحوث الطبية
قسم الكيمياء الطبية التطبيقية

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رسالة مقدمة من
محمد رمضان محمود بركات

للحصول على درجة
الماجستير
فى
الكيمياء الطبية التطبيقية

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الملخص العربي

الملخص العربي

تأثير تعدد الأشكال في جين *XPD* على الإستجابة للعلاج الكيماوي 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 نخاع مما يؤدي 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 الوراثة الخلوية التقليدي 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 الكيماوي لسرطان الدم النقوى الحاد بصورة أساسية من انثراسيكلين (داونوروبيسين) و ارابينوزيد السيتوزين، و هو نظام علاجي لم يتغير منذ ٣٠ سنة. يعطى الداونوروبيسين بجرعة ٤٥ مج/م^٢ لكل يوم لمدة ثلاثة أيام بالتوازي مع السيتوزين أرابينوزيد بجرعة ١٠٠ مج/م^٢ لكل يوم لمدة ٧ أيام عن طريق الوريد (لذلك يسمى هذا النظام العلاجي ٧+٣). إنه من غير المؤلف لعلاج الحث الكيماوي 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 نخاع 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 رئيسي لإصلاح الحمض النووي فى 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 ضروري لمسار إصلاح الحمض النووي باستئصال النيوكليوتيدات. النشاط المضاد لسرطان الخاص بمعظم أدوية العلاج الكيماوي يعتمد على استحثاث التلف فى الحمض النووي داخل الخلايا السرطانية سريعة الإنقسام. وبالتالي، فإن تنظيم مسارات إصلاح الحمض النووي فى الخلايا السرطانية يشكل عاملا حاسما لاستجابتها لعقاقير العلاج الكيماوي 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 الكيماوي الحثى فضلا عن السميات التي يسببها العلاج الكيماوي فى مرضى سرطان الدم النقوى الحاد الحديث فى البالغين ذوى الوراثة الخلوية الطبيعية.

شملت هذه الدراسة:

٥١ مريضاً مصرياً بالغ تم تشخيصهم حديثاً بسرطان الدم النقوى الحاد الحديث ذو الوراثة 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 اختلاف 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 مليلتر من الدم الوريدي لكل مريض. تم علاج المرضى بالنظام الكيماوى الحثى (٧+٣). 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 الكيماوى. 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 التى يسببها العلاج الكيماوى. و من ناحية أخرى، التعدد الشكلى *XPD Lys751Gln* كان مرتبطاً مع الإستجابة للعلاج الكيماوى ومع السميات فى القلب ولكن ليس مع السميات فى الكبد، أو الكلى أو السميات الأيضية. المرضى الذين يملكون الشكل الوراثى (*AA*) *XPD751* كانوا أكثر قابلية لتحقيق هدوء كامل للمرض. فى حين أن المرضى الذين يملكون الشكل الوراثى (*CC*) *XPD751* كانوا أكثر عرضة للمعاناة من السميات فى القلب التى يسببها العلاج الكيماوى.

الإستنتاجات:

لقد حددنا فى الدراسة الحالية التعدد الشكلى فى *XPD751* كعلامة تنبؤية لاستجابة مرضى سرطان الدم النقوى الحاد الحديث ذو الوراثة الخلوية الطبيعية للعلاج الكيماوى. 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 في 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 الورااثيات 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 تشمل 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 فى جينات إصلاح الحمض النووى 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.