RAD51 Homologous Recombination Repair Gene Polymorphism and Risk of Acute Myeloid Leukemia

Thesis

Submitted for partial fulfillment of master degree (M.Sc.) in Clinical and Chemical Pathology.

By

Marian Ramzy Saad

M.B.B.Ch, Cairo University

Supervised by

Prof. Dr. Sahar Kamal El-Din Hussein

Professor of Clinical & Chemical Pathology

Faculty of Medicine

Cairo University

Dr. Asmaa Ahmed Abd El-Aal

Lecturer of Clinical & Chemical Pathology

Faculty of Medicine

Cairo University

Dr. Reham Abd El-Alem Afifi

Lecturer of Clinical & Chemical Pathology

Faculty of Medicine

Cairo University

Faculty of Medicine

Cairo University

2009

Abstract

Key words:
Acute Myeloid Leukemia, Repair Genes, RAD51, Polymorphism.

Results:
Acute myeloid leukemia is a clonal haemopoietic disorder frequently associated with genetic instability. Polymorphisms in DNA repair enzymes are thought to increase the risk of development of AML. In our study we investigated 40 cases of AML and 37 controls for the polymorphism in DNA homologous recombination repair gene-RAD51 by PCR-RFLP. We found, among the cases there were 19 cases with positive expression of the polymorphic gene, while in controls we found only 7 candidates with positive expression of the same polymorphism. These results suggest a strong correlation between the presence of polymorphic RAD51-G135C allele and the incidence of AML.

Acknowledgement

First of all, I would like to thank God for his grace and mercy and for giving me the effort to complete this work.

I would like to express my deepest gratitude and greatest respect to Professor ***Dr. Sahar Kamal El-Din Hussein***, Professor of Clinical and Chemical Pathology, Cairo University under whose supervision I had the honor and pleasure to proceed with this work. Her constant guidance, encouragement and foresight made all the difference.

My deepest appreciation goes to **Dr. *Asmaa Ahmed Abd El-Aal,***
Lecturer of Clinical and Chemical Pathology, Cairo University for her supervision, advice, effort and for allowing me a free access to her precious time.

I would like to express my sincere thanks to **Dr. Reham Abd El-Alem Afifi,** lecturer of Clinical and Chemical Pathology, Cairo University for her continuous guidance encouragement, creativity and offering me years of technical experience.

Special thanks goes to all patients attending the oncology clinic of Kasr El-Aini Hospital, Cairo University, for their help and cooperation without which this work could not be possible.

And last-but, by no means least, I would like to thank ***my parents, my husband and my daughters*** for all their patience, love, support and sacrifice which made this work possible.

CONTENTS

Title	Page No.
• **List of Abbreviations**.....	**i**
• **List of Tables**…….	**iv**
• **List of Figures**……...	**v**
• **Introduction and Aim of the Study**…….	**1**
• **Review of Literature**……	**3**
❖ **Chapter 1: DNA damage and repair**……...	**3**
• DNA damage……..	**3**
• DNA repair…….	**9**
• DNA repair genes……...	**11**
• Mechanisms of DNA repair……...	**22**
• Genetic polymorphisms…….	**30**
❖ **Chapter2: RAD51 gene**……	**33**
• Cytogenetic location……...	**33**
• RAD51 structure…….	**34**
• Role of RAD51 in DNA repair……...	**35**
• RAD51 polymorphism……...	**37**
❖ **Chapter 3: Acute Myeloid Leukemia**…….	**40**
• Pathogenesis……..	**41**
• DNA repair genes in AML…….	**44**
• Hematopoietic growth factors and cytokines…….	**48**
• Classification of AML……	**51**
• Prognostic criteria……..	**71**
• **Subjects and Method**s……..	**73**
• **Results**……	**87**
• **Discussion**…….	**104**
• **Summary**……	**111**
• **Conclusion**……	**113**
• **Recommendations**……	**114**
• **References**…….	**115**
• **Arabic Summary**……...	**136**

LIST OF ABBREVIATIONS

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ANLL	Acute non lymphoblastic leukemia
AP	Accelerated phase
APL	Acute promyelocytic leukemia
ASCL	Autologous stem cell transplantaton
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia and RAD3-related
ATRA	All-trans-retinoic acid
AUL	Acute undifferentiated leukemia
BC	Blastic crisis
BCR	Breakpoint cluster region
BER	Base excision repair
BM	Bone marrow
CAE	Chloroacetate esterase
CALLA	Common acute lymphoblastic leukemia
CBC	Complete blood count
CD	Cluster of differentiation
CDNB	1-chloro2,4dinitrobenzene
CEL	Chronic eosinophilic leukemia
CFU	Colony forming unit
CFU-GM	Colony forming unit-granulocyte and monocyte
CHR	Complete haematological remission
CI	Confidence interval
CR	Complete remission
DIC	Disseminated intra-vascular coagulopathy
DLCL	Diffuse large B cell lymphoma
DNA	Deoxy-ribonucleic acid
DSB	Double strand break
ECM	Extracellular matrix
ELISA	Enzyme linked immunosorbant assay
EM	Electron microscope
ET	Essential thrombocytopenia
FAB	French-American-British classification
FISH	Fluorescence in situ hibridization
FLT3	Fms-like tyrosine kinase 3 receptor
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte-monocyte colony stimulating factor

GST	Glutathione-S-Transferase
Hct	Haematocret value
HES	Hyper-eosinophilic syndrome
HGFs	Haematopoietic growth factors
HLA	Human leucocyte antigen
HPLC	High performance liquid chromatography
HR	Homologous recombination
ICL	Interstrand cross links
Ig	Immunoglobulin
IL-1	Interleukin-1
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-7	Interleukin-7
INF-α	Interferon- α
IPT	Immunophenotyping
IR	Incomplete remission
LDH	Lactate dehydrogenase
LM	Light microscope
MDR	Multi drug resistance
MDS	Myelodysplastic syndrome
Mg CL	Magnesium chloride
MMM	Myelosclerosis with myeloid metaplasia
MPD	Myeloproliferative disorders
MPO	Myeloperoxidase
MRD	Minimal residual disease
mRNA	messenger Ribose Nucleic Acid
NAP	Neutrophil alkaline phosphatase
NEC	Non erythroid cell
NER	Nucleotide excision repair
NHEJ	Non-homologous endjoining
NK	Natural killer
NSCLC	Non small cell lung carcinoma
NSE	Non specific esterase
OR	Odd's ratio
PAH	Polcyclic aromatic hydrocarbons
PAS	Periodic acid Schiff
PB	Peripheral blood
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor

PV	Polycythemia vera
RAEB	Refractory anemia with excess blasts
RAD50	Homolog of S. cerevisiae RAD50
RAD51	Homolog of RecA of E.coli
RB	Retinoblastoma
RBC	Red blood cells
RFLP	Restriction fragment length polymorphism
ROS	Reactive oxygen species
RNA	Ribonucleic acid
RPA	Replication protein A
SBB	Sudan black B
SCF	Stem cell factor
SD	Standard deviation
Sm	Surface membrane
SNP	Single nucleotide polymorphism
SSB	Single strand break
t-AML	Therapy related Acute myeloid leukemia
TAE	Tris-Acetate EDTA
Taq	Thermus aquaticus
TCR	T-cell receptor
Tdt	Terminal deoxy nucleotidyl transferase
TLC	Total leucocytic count
UAL	Undifferentiated acute leukemia
UTR	Untranslated region
WBC	White blood cells
WHO	World Health Organisation
WT1	Wilm's tumor gene
XRCC3	X-ray cross-complementing 3

LIST OF TABLES

Number	Title	Page
1	Human DNA repair genes	11
2	Morphologic FAB classification of AML	52
3	Immunophenoytping markers in AML	54
4	The WHO classification of AML	55
5	Score for biphenptypic leukemia	66
6	Prognostic implications of chromosome findings in AML	71
7	Preparation of PCR components in a 25 µl reaction volume	80
8	Individual data of the AML patients	92
9	Individual data of the control group	94
10	Laboratory diagnostic data of AML cases and controls	97
11	Genotype expression in AML cases and controls	97
12	Statistical comparison between different age groups and gene polymorphism.	98
13	Statistical relation between sex and gene polymorphism.	100
14	Statistical relation between lab. data and gene polymorphism	101
15	Statistical relation between blast counts and gene polymorphism	101
16	Frequency distribution in FAB classes in AML cases	102
17	Statistical relation of gene polymorphism and outcome of the disease.	103

LIST OF FIGURES

Number	Title	Page
1	DNA repair by base excision	24
2	DNA repair by nucleotide excision	26
3	RAD51 cytogenetic location	33
4	Major pathways of DNA repair in eukaryocytes	37
5	RFLP-PCR analysis of RAD51 expression in AML patients	96
6	Genotype expression in AML cases and controls	98
7	Comparison between different age groups and gene polymorphism.	99
8	Relation between sex and gene polymorphism.	100
9	Relation of gene polymorphism and FAB classification.	102
10	Relation of gene polymorphism and outcome.	103

INTRODUCTION AND AIM OF WORK

Acute myeloid leukemia (AML) is a clonal hemopoietic disorder that is frequently associated with genetic instability characterized by a diversity of chromosomal molecular changes. Most cases of AML arise de novo; however, 10%-20% of all cases of AML arise after exposure to chemotherapy or radiotherapy after the treatment of a primary malignancy [therapy-related AML (t-AML)] ***(Pederson-Bjergaard et al., 2002).***

DNA is at constant risk for damage from both endogenous and exogenous sources. A large number of highly complex mechanisms have evolved to protect DNA from damage including DNA repair pathways and systems that protect against oxidative stress and other damaging agents ***(Knudsen et al., 2001).***These pathways play vital role in maintaining genetic integrity. The ability of an individual to prevent and repair damage is genetically determined and is the result of combinations of multiple genes that may display subtle differences in their activity ***(DeBoer, 2002).***

Genetic polymorphisms have now been identified in a number of DNA repair genes and damage-detoxification genes, Polymorphisms can affect gene function, promoter activity, mRNA stability, and splice variants and hence, can result in a change in the cellular ability to cope with DNA damage , which contributes to an altered disease susceptibility .The genotype distributions of a number of these polymorphic genes have been shown to be associated with AML and/or t-AML***(Seedhouse et al., 2004).***

Double-strand breaks (DSBs) in DNA are arguably the most important class of DNA damage because they may lead to either cell death or loss of genetic material resulting in chromosomal aberrations. The balance of DSB repair activity appears to be critical to the genetic stability of cells. Too little repair leads to acquisition and persistence of mutations, whereas elevated levels of repair can inhibit the apoptotic pathway and can enable a cell with badly damaged DNA to attempt repair, potentially mis-repair , and survive, DSBs are predominantly repaired by either homologous recombination(HR) repair or non homologous end-joining pathways in mammalian cells***(Rollinson et al.,2007).***

One of the central proteins in the HR pathway is RAD51 ***(Li and Heyer, 2008).***Cells lacking RAD51 are characterized by an accumulation of chromosomal breaks before cell death. Hence the role of RAD51 is vital in maintaining genetic stability within a cell ***(Seedhouse et al., 2004***). Potential associations between variants of RAD51 family genes and specific forms of cancer have been reported ***(Thacker, 2005).***

A G/C polymorphism at position-135 in the5'untanslated region of the RAD 51 gene has been identified ***(Wang et al., 2001).***This polymorphism has been the focus of a number of studies in breast and ovarian cancer ***(Dufloth et al., 2005).***

DNA DAMAGE AND REPAIR

The DNA contained in every mammalian cell is under constant attack by agents that can either directly damage one of its three billion bases or break the phoshodiester backbone on which the bases reside ***(Michael et al., 2004).*** Life on Earth has evolved to deal with metabolic and external sources of DNA damaging agents through the development of elegant mechanisms that repair damage to the DNA ***(Ford 2004).***

DNA Damage :

DNA in most cells is regularly damaged by endogenous and exogenous mutagens. Unrepaired damage can result in apoptosis or may lead to irregular cell growth and cancer. If DNA damage is recognized by cell machinery, several responses may occur to prevent replication in the presence of genetic errors. At the cellular levels, checkpoints can be activated to arrest the cell cycle; transcription can be up-regulated to compensate for the damage, or the cell can apoptose ***(Vispe et al., 2000).***

Alternatively, the damage can be repaired at the DNA level enabling the cell to replicate as planned. Complex pathways involving numerous molecules have evolved to perform such repair. Because of the importance of maintaining genomic integrity in the general and specialized functions of cells as well as in the prevention of carcinogenesis, genes coding for DNA repair molecules have been considered as candidate cancer-susceptibility genes ***(Shields and Harris, 1991)***

The pattern of DNA damage is often complex but has characteristics associated with the damaging agent. Such damage occurs at a frequency too high to be compatible with life. As a result cell death and tissue degeneration, aging and cancer are caused. To avoid this and in order for the genome to be reproduced, these damages must be corrected efficiently by DNA repair mechanisms. Eukaryotic cells have multiple mechanisms for the repair of damaged DNA. These repair systems in humans protect the genome by repairing modified bases, cross links and double-strand breaks ***(Tuteja and Tueja, 2001).***

Any human cancer susceptibility syndromes arise from mutations in genes involved in DNA damage responses. Most therapeutic agents that are currently used to treat malignancies, including radiation therapy and many chemotherapeutic agents, are responsible for most of the side effects such as bone marrow suppression, gastrointestinal toxicities, and hair loss. These are all attributable to DNA damage induced cell death of proliferating progenitor cells in these tissues ***(Froelich et al., 1995).***

❖ **Agents that damage DNA:**

DNA can be damaged in a variety of ways;

1. Certain wave length and irradiation:

As energy released by exposure to an external source of ionizing radiation (Gamma and X-rays) ***(Thompson et al., 2002)*** , Ultraviolet (UV 200-300nm) radiation from the sun, cancer chemotherapy and radiotherapy ***(Lunne et al., 1999).***Hydrolysis or thermal disruption at elevated temperature increases the rate of

depurination (loss of purine bases from the DNA backbone) and single strand breaks ***(Ohta et al., 2009).***

2. Highly reactive oxygen species:

Exposure of the cellular DNA to reactive oxygen species (ROS) generated either by the normal metabolism of the cell or by chemical and physical exogenous agents, is at the origin of lesions that can have genotoxic or mutagenic consequences ***(Marsin and Bignami., 2003).***

3. Chemicals used in therapies:

- Alkylating chemicals can modify purine bases and can cause intrastrand or interstrand cross links that require additional molecular interventions for them to be reserved.

- Inhibitors of DNA topoisomerases can lead to enhanced single or double strand break (DSBs) depending on which topoisomerase is inhibited and on the phase of the cell cycle ***(Michael et al., 2004).***

4. Other stresses:

Such as intermittent or prolonged exposure to abnormally low level of oxygen nutrients ***(Lu et al., 2001).***

5. Smoking:

Cigarette smoking may induce DNA damage ***(Ford, 2004),*** as it contains large quantities of carcinogens, including polycyclic aromatic hydrocarbon, such as benzo[α]pyrene, which damage DNA by covalent binding or oxidation, leading to base modification, strand breaks, and cross linkage between bases on

opposite strands, or between DNA and protein, and numerous other defects ***(Ito et al., 2004).***

6. **Viruses:** as Epstein-Barr virus (EBV) infection ***(Devita et al., 2001).***

❖ **Types of DNA damage:**

Genomic disorders are a clinically diverse group of conditions caused by gain, loss or re-orientation of a genomic region containing dosage-sensitive genes ***(Oriscoll, 2008).***

1. Base loss:

Single base alteration may occur due to depurination, deamination of cytosine to uracil, deamintion of adenine to hypoxanthine, alkylation of a base, insertion or deletion of nucleotide and base-analogue incorporation. Whereas, two base alteration may be due to UV-induced thymine-thymine dimer or bi-functional alkylating agent cross linkage. Within a typical mammalian cell, several hundreds pyrimidines are spontaneously lost per haploid genome per day. Loss of purine or pyrimidine base creates an apurinic apyrimidinic (AP) site which is also called abasic sites ***(Deutsch & Hegde, 2005).***

Abasic sites are frequent cellular DNA lesions that are formed by spontaneous base loss, by exposure to radiation or antitumor drugs, or as intermediates during base excision repair of oxidized, deaminated or alkylated bases ***(Georgakilas et al., 2004).***

Their persistence can yield block to RNA transcription and DNA synthesis and can be a source of mutations. Organisms have