ASSESSMENT OF TELOMERE LENGTH BY Q-FISH AND THE EFFECT OF RESVERATROL ON TELOMERE STABILITY IN PREMATURE AGING SYNDROMES

Submitted By

Peter Safwat Fahmy Erian

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Master in Clinical & Chemical Pathology, Faculty of Medicine,

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A Thesis Submitted in Partial Fulfillment
Of
The Requirement for the Doctor of Philosophy Degree
In
Environmental Sciences

Department of Environmental Medical Sciences
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APPROVAL SHEET

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Peter Safwat Fahmy

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List of Abbreviations

ATM	Ataxia-Telangectasia mutated gene
BER	Base excision repair
BM	Bone marrow
BN	Binucleated cells
CA	Comet assay
CAT	Catalse enzyme
CBMNA	Cytokinesis-block micronucleus assay
DC	Dyskeratosis congenita
DEB	Diepoxybutane (1,3-Butadiene diepoxide)
DDR	DNA damage response
DDT	Dichloro-diphenyl-trichloroethane
DSB	Double-strand breaks
DSBR	Double strand break repair
FA	Fanconi anemia
FISH	Fluorescence in situ hybridization
GPx	Se-dependant glutathione peroxidase enzyme
GR	Glutathione reductase enzyme
HGPS	Hutchinson-Gilford progeria syndrome
hTERC	Human telomerase RNA protein
hTERT	Human telomerase reverse transcriptase protein
LTL	Leukocyte telomere length
MGE	Microgel electrophoresis
MN	Micronucleus assay
MNi	Micronuclei
NBUDs	Nuclear buds

NER	Nucleotide excision repair
NPBs	Nucleoplasmic bridges
PARP	Poly (ADP-ribose) polymerase protein
PAS	Premature aging syndromes
PCR	Polymerase chain reaction
PIBIDS	Photosensitivity, Ichthyosis, Brittle hair, Intellectual impairment, Decreased fertility and Short stature syndrome
PMNCs	Peripheral blood mononuclear cells
POT1	Protection of telomeres 1 protein
Q-FISH	Quantitative Fluorescence in situ hybridization
Rap1	Repressor activator protein 1
RNP	Reverse transcriptase ribonucleoprotein
ROS	Reactive oxygen species
SCGE	Single cell electrophoresis
SOD	Superoxide dismutase enzyme
T/C	Telomere/Centromere
TERT	Telomerase reverse transcriptase
TIN2	TRF1-interacting protein 2
TPP1	Tripeptidyl peptidase 1 protein
TRF	Telomerase restriction fragment analysis
TRF1	Telomere repeat binding factor 1 protein
TRF2	Telomere repeat binding factor 2 protein
TTD	Trichothiodystrophy
WES	Whole exome sequencing
WS	Werner syndrome
XP	Xeroderma pigmentosa

ABSTRACT

Background: Premature aging syndromes are associated with telomere shortening originated from defective telomerase function or mutations in the DNA repair system, which in turn can increase the susceptibility for malignant tumors.

Aim: This study aims to assess the effect of Resveratrol - which is a natural extract - on the stability of chromosomes in premature aging syndromes patients using in vitro micronucleus assay as a parameter of improvement, and to assess the telomere length by Q-FISH technique in premature aging syndromes.

Methods: The present study was conducted on 27 patients and 10 normal controls matching in age and sex. Induction of breakage by 1,3-Butadiene diepoxide, evaluation of the antioxidant effect of Resveratrol on genomic stability were done using in vitro micronucleus assay technique and assessment of telomere length by Q-FISH.

Results: The proper dose of Resveratrol was adjusted in vitro, and significant reduction in the level of micronuclei in cultures treated with Resveratrol was found. Q-FISH was a powerful diagnostic method for the measurement of telomere length, and it helped us to evaluate the relation between the severity of the patients' phenotype and the mean telomere length of each patient.

Conclusion: This study recommended the use of in vitro micronucleus assay and Q-FISH techniques as prognostic markers for the severity of the disease. Further clinical assessments and adjustment of the dose of Resveratrol is needed to ensure its safety if used as an adjuvant therapy.

Keywords: Resveratrol - Micronuclei - Fanconi anemia - Genomic stability - Premature aging syndromes - Q-FISH.

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INTRODUCTION

INTRODUCTION

Aging is a natural process characterized by a progressive functional decline of tissues, organs and organ systems, which leads to an increased susceptibility to age-related diseases and, ultimately, to death. A persistently DNA damage response (DDR), known as "cellular senescence", is one of the main contributing factors to age associated tissue dysfunction, reduced regenerative capacity and age-related diseases **(Boccardi et al., 2015)**.

Telomeres are the physical ends of eukaryotic linear chromosomes. Telomeres form special structures that cap chromosome ends to prevent degradation by nucleolytic attack and to protect chromosome termini from DNA double-strand breaks **(Fajkus et al., 2005)**.

Telomeres are important for replication of the chromosomes during cell division in reproducible cells as skin, blood, bone and many other kinds of cells. Telomeres also play an important protective role in our cells, as their presence prevents important genetic material from being lost during cell division. Telomeres serve as caps on the ends of chromosomes, protecting chromosome ends from breakage; as broken chromosomes trigger unwanted biological responses **(Diotti & Loayza, 2012)**.

In proliferative cells and unicellular organisms, telomeric DNA is replicated by the actions of telomerase, a specialized reverse transcriptase ribonucleoprotein (RNP) composed of two

main components, a telomerase reverse transcriptase (TERT) protein and a noncoding RNA component (TER, telomerase RNA), which form an integral and essential part of the enzyme **(Gilson & Geli, 2007; Prasad et al., 2017)**. In the absence of telomerase, some cells employ a recombination-based DNA replication pathway known as alternative lengthening of telomeres. However, mammalian somatic cells that naturally lack telomerase activity show telomere shortening with increasing age leading to cell cycle arrest and senescence. In another way, mutations or deletions of telomerase components can lead to inherited genetic disorders. Also, the depletion of telomeric proteins can elicit the action of distinct kinases-dependent DNA damage response, culminating in chromosomal abnormalities that are incompatible with life. In addition to the intricate network formed by the interrelationships among telomeric proteins, long noncoding RNAs that arise from subtelomeric regions, named telomeric repeat-containing RNA, are also implicated in telomerase regulation and telomere maintenance **(Cesare & Reddel, 2010)**.

Telomerase is extensively important in the maintenance of genome integrity. Therefore, telomerase dysfunction may result in defects in various highly proliferative cells or tissues, and, ultimately leading to aging-related degenerative diseases. (**Prasad et al., 2017)**

In humans, the average telomere length typically ranges from 10 to 15 kb. Telomeric DNA inevitably shortens upon each cell replication at a rate of 50-200 bp **(Zhao et al., 2009)**. When

telomeres become critically short, a cellular response is triggered, signaling cells to exit the cell cycle and to senesce. This process indicates that the cells have reached their maximum proliferation capacity, known as the Hayflick limit **(Hayflick, 1965)**. Few cells escape this natural crisis and loss critical cell cycle checkpoints and components of the DNA damage repair machinery. These cells that bypassed the crisis are now considered immortalized and emerge with multiple chromosomal abnormalities and mutations. Some of these cells bear mutations in genes responsible for inducing tumorigenesis, making them prone to promote cancer formation **(Kong et al., 2013)**.

Recent reports showed that telomere shortening is a potential contributor to the pathogenesis of many premature aging syndromes as dyskeratosis congenita, Werner syndrome, Bloom syndrome, ataxia telangiectasia, Nijmegen breakage syndrome, Fanconi anemia, Aplastic anemia and idiopathic pulmonary fibrosis. A common hallmark of these diseases is that patients have critically short telomeres compared with healthy individuals of the same sex and age **(Boccardi et al., 2015)**.

Different methods had been adopted to measure telomere length, among these methods is the Quantitative fluorescence in situ hybridization (Q-FISH) technique. Q-FISH is based on the use of the peptide nucleic acid (PNA) telomere oligonucleotides probes and appropriate digital image analysis system designed to quantify fluorescence signals. The advantages of Q-FISH include: measuring the individual length of all telomeres in a given genome,