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بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات:
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Detection of MicroRNA levels in amyotrophic lateral sclerosis patients

Thesis

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In Neurology

By

Radwa Mohammed Ahmed Soliman
(M.B.,B.Ch. M. Sc.)

Supervised by

Prof. Dr. Nagia Aly Fahmy
Professor of Neurology
Faculty of Medicine - Ain Shams University

Prof. Dr. Ahmed Osman Mostafa
Professor of Biochemistry
Faculty of science - Ain Shams University

Prof. Dr. Ramez Reda Moustafa
Professor of Neurology
Faculty of Medicine - Ain Shams University

Prof. Dr. Nabila Hamdi
Professor of Molecular Pathology
Faculty of science - German University *in Cairo*

Dr. Hebatallah R. Rashed
Associate Professor of Neurology
Faculty of Medicine - Ain shams University

Faculty of Medicine
Ain Shams University
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Radwa Soliman

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List of Abbreviations

AHCs .....Anterior Horn Cells

ALS.....Amyotrophic Lateral Sclerosis

ALSFRS-R.....ALS Functional Rating Scale

C9ORF.....chromosome 9 Open Reading Frame

CNS .....Central Nervous System

CSF.....Cerebrospinal Fluid

ECAS .....Edinburgh Cognitive and Behavioural *ALS* Screen

fALS.....Familial ALS

FTD ..... Frontotemporal Degeneration

FUS.....Fused in Sarcoma

Has .....Homo Sapiens (Humans)

HC .....Healthy Controls

miRNA.....MicroRNA

MNs .....Motor Neurons

MOCA .....Montreal Cognitive Assessment

qPCR .....Quantitative Polymerase Chain Reaction

sALS .....Sporadic ALS

SOD1 .....Super Oxide Dismutase

TARDBP .....TAR DNA-Binding Protein

TDB43 .....TAR DNA-Binding Protein 43

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal, adult-onset neurodegenerative disease. It is one of the motor neuron diseases in which motor neurons (MNs) are selectively lost. This progressive loss of MNs results in muscle denervation and atrophy, leading usually to death within 3-5 years of symptom onset ***(Pasinelli and Brown, 2006; Rothstein, 2009).***

It is a common neurodegenerative disorder with an incidence of 1-2 / 100,000 and a prevalence of approximately 6/100,000 ***(Shaw and Wood, 2010)***.

There is no definitive diagnostic test for ALS; diagnosis is based on clinical findings, electromyography results and exclusion of mimics.

All suspected ALS patients should undergo electrophysiological testing which allows identification of Lower Motor Neuron features of ALS in both clinically affected and as yet clinically silent regions. In this way, the disease may be shown to be more widespread than is evident clinically and an earlier working diagnosis can be made. Typical electromyographic features of ALS include evidence of active denervation (positive sharp waves, fibrillation potentials, fasciculation potentials) and chronic denervation evidenced by large motor unit potentials ***(Daube, 2000).***

MicroRNAs (miRNAs) are small, regulatory RNAs that regulate the expression of protein-coding RNAs. miRNAs direct mRNA degradation or translational repression by partial binding to the 3'-UTR of mRNAs after first being incorporated into an RNA-induced silencing complex containing Argonaute-2 (Ago2) ***(Gregory et al., 2005; Chekulaeva and Filipowicz, 2009; Ha and Kim, 2014).***

Emerging data demonstrate that miRNAs are powerful regulators of physiological and pathological cellular processes ***(Naeini, 2010; O'Connell et al., 2010 and Sayed and Abdellatif, 2011).***

Therefore, miRNA expression is often dysregulated in disease and thus can be used as both diagnostic and therapeutic targets ***(Chen et al., 2008; Koval et al., 2013).***

Because only partial complementarity is required for miRNA-mRNA interactions, a single miRNA can potentially regulate hundreds of mRNAs ***(Selbach et al., 2008).***

MicroRNAs are 20 to 25 nucleotide-long noncoding transcripts that regulate biological processes via mRNA cleavage or translational repression of mRNA. There are over 1000 miRNAs in humans and they are typically measured using RNA sequencing. Overall, similar to the

results in tissues from patients with ALS, most miRNAs levels are decreased in the CSF and serum of patients with ALS when compared with healthy and diseased controls. miRNA181a-5p, miRNA-143-5p, miRNA-574-5p, and miRNA-338-3p have been found to be increased in CSF from patients with ALS while miRNA 206, 106b and 181a-5p are thought to be increased in serum of patients with ALS ***(Eitan and Hornstein, 2016; Benigni et al., 2016).***

A recent study showed reduced levels of miR-126-5p in pre-symptomatic ALS mice models, and a subsequent myocytes expression of diverse ALS-causing mutations promote axon degeneration and neuromuscular junction (NMJ) dysfunction, thus considering a novel mechanism underlying ALS pathology, in which alterations in miR126-5p facilitate a non-cell-autonomous mechanism of motor neuron degeneration in ALS and claimed that, overexpressing miR126-5p is sufficient to transiently rescue axon degeneration and NMJ disruption both in vitro and in vivo ***(Maimon et al., 2018).***

Aim of the Work

The aim of this study is to measure certain microRNAs in the plasma of amyotrophic lateral sclerosis patients versus normal controls, attempting to verify their potential role as possible biomarkers of the disease.

Chapter (1): Amyotrophic Lateral Sclerosis

Epidemiology of ALS:

The incidence of ALS is approximately 2.6 cases per 1000 00 persons annually, whereas the prevalence is approximately 6 cases per 100 000. Men have a higher incidence of disease than women (3/ 100000 compare to 2.4 /100000 person). In familial cases the incidence is the same. The average survival from onset to death is 3–4 years ***(Logroscino et al., 2009)***.

There is no population based study about ALS in Egypt, thus, there are no reports about the incidence or prevalence of ALS. This is due to the lack of specialized ALS centers across the country, which resulted in the absence of ALS registry and database. The delay in diagnosis results in delayed treatment with FDA approved medications and other symptomatic therapies ***(Rashed & Tork, 2020)***. In a recent Egyptian study, 30 patients were recruited from ALS clinic where they showed that the mean time from symptom onset to diagnosis (diagnostic delay) was 18.5±16.3 months and the mean age at onset of symptoms was 52.6±10.9 years ***(Rashed & Tork, 2020).***

Environmental factors

Over the years a multitude of environmental exposure and lifestyle risk factors have been proposed as potential causes of ALS. The possible environmental factors evaluated include the intense physical activity, football, cigarette smoking, manual work, armed services and deployment, exposure to lead/solvents, pesticides and chemicals, heavy metal, electric shock, geographical clustering and cyanotoxins ***(Al-Chalabi and Hardiman, 2013).***

Unfortunately, no conclusive data are available and further studies are needed to define exogenous risk factors for ALS ***(Sutedja et al., 2009)***. Interestingly, environmental exposures may result in heritable changes to genes, without altering the DNA sequence. This phenomenon is defined epigenetic and represents the most important point of convergence between genetic predisposition and environmental exposures ***(Paez-Colasante et al., 2015).***

Pupillo and coworkers (2018) found evidence that certain foods and nutrients (red and processed meat, animal protein, and sodium, zinc and glutamic acid) were associated with a higher risk of ALS, whereas others (coffee, tea, whole bread, raw vegetables, and citrus fruits) might be associated with a lower risk of ALS but no