Clinical Phenotypes of Amyotrophic Lateral Sclerosis in Egyptian Patients and their Relation to Autoantibodies for Alpha Synuclein serum level.

Thesis

Submitted for partial fulfillment of master degree in Neuropsychiatry.

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2018

Acknowledgment

No words can express the great grace that **ALLAH** has endured upon me. He granted me the will and patience to fulfill this demanding piece of work.

I would like to express my deepest gratitude to my supervisors, **Dr. Nagia Aly Fahmy,** Professor of Neurology Faculty of Medicine, Ain Shams University for giving me the honor of working under her super vision and providing me with an example of what a doctor, leader and teacher should be like. This work would not have been possible without her guidance, encouragement and continuous support.

I would like to thank **Dr. Mohammed Salama,** assistant Professor of clinical toxicology Faculty of Medicine, Al-Mansoura University, for his kindness, valuable assistance, and guidance during this work.

My sincere thanks also go to **Dr. Hosam Shokry Mohammed,** lecturer of neuropsychiatry Faculty of Medicine, Ain Shams University for his kindness, useful remarks, encouragement and unlimited support.

My deepest thanks and appreciation are extended to **Dr. Hassan El-Fawal,** Professor and Dean School of Sciences and Engineering, American University in Cairo AUC who made this work possible by his great help during clinical work.

I am also indebted to **Dr. Ali Soliman shalash** Professor of Neurology, Faculty of Medicine, Ain Shams University for his continuous help and encouragement throughout this work

My sincere thanks to all staff members of neuropsychiatry department, Faculty of Medicine, Ain Shams University for their support and kindness.

Many thanks and gratitude to my patients and their families for their cooperation and commitment.

Dedication

To the soul of my mother who taught me the meaning of life, you will always be with me in every step of my life.

To my father the origin of my success. I am very grateful than ever to get the full support, advice and care you gave me.

To my brothers and sisters, for the endless support, may God bless you.

CONTENTS

LIST OF ABBREVIATIONS

AAbs	Autoantibodies
ADL	Activities of Daily Living
ALS	Amyotrophic Lateral Sclerosis
ALS2	Alsin
ALSFRS-R	Als Functional Rating Scale-Revised
ALS-MITOS	ALS Milano-Torino Staging
ALS/PDC	Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex
CHCHD10	Coiled-Coil-Helix-Coiled-Coil-Helix
CSF	Cerebrospinal Fluid
C9orf72	chromosome 9 open reading frame72
DLB	Dementia With Lewy Bodies
DM	Diabetes Miletus
DNA	Deoxyribonucleic acid
DPRs	Dipeptide Repeat Proteins
EAATs	Excitatory Amino Acid Transporters
fALS	Familial ALS
FDA	Food and Drug Administration
FLAIR	Fluid-Attenuated Inversion Recovery
FTLD	Frontotemporal Lobar Degeneration
FVC	Forced vital capacity
HHV	Human Herpesvirus
HTLV	T-Cell Lymphotrophic Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LMNs	Lower Motor Neuron
MiTos	Milano-Torino scale
MiRNAs	Micrornas
MMT	Manual muscle testing
MND	Motor Neuron Disease
MOCA	Montreal cognitive assessment
mRNA	Messenger ribonucleic acid
MSA	Multiple System Atrophy
MUNE	Motor Unit Number Estimation
MVIC	Maximum Voluntary Isometric Contraction
NFTs	Neurofibrillary Tangles
PD	Parkinson's Disease

LIST OF ABBREVIATIONS

PLS	Primary Lateral Sclerosis
PMA	Progressive Muscular Atrophy
REM	Rapid Eye Movement
RHIs	Round Hyaline Inclusions
RNA	ribonucleic acid
sALS	Sporadic amyotrophic lateral sclerosis
SLIs	Skein-Like Inclusions
SOD1	Superoxide Dismutase
UBQLN2	Ubiquilin-2
UMNs	Upper Motor Neurons
UPS	Ubiquitin-Proteasome System
VAPB	Vehicle-Associated Membrane Protein B
VCP	Valosin-Containing Protein

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ABSTRACT

Background: Amyotrophic lateral sclerosis (ALS) is a common neurodegenerative disorder with different phenotypes have been recognized. Till now no available definite marker developed for ALS.

Aim / Objectives: Assess the ALS Egyptian patients who attend neuromuscular clinic -Ain Shams University hospitals to recognize their clinical phenotypes and investigate the serum level of autoantibodies for alpha synuclein as a possible biomarker for the disease.

Subjects and Methods: The study included 30 ALS patients and 30 age and sex matching control.For patient's group, only 26 patients completed the study. They exposed to detailed history and clinical examination, screeningfor cognitive impairment,staging of diseases severity by specific staging scales.Full laboratory, neurophysiological and imaging studies that needed for diagnosis and exclusion of the mimicswere done. Finally blood samples takenfrom the patients and control groups for serum alpha synuclein autoantibodies assessment.

Results: This study showed that men were more affected by ALS than women (65.4%, 34.6% respectively), Themean age of onset of the disease was 46.15 year, 53% of patients were young (<_ 45 years). Familial cases represent 15% of total cases. History of identified risk factor for ALS was found in (73.8%) of patients. Classical ALS was the most common phenotype identified in (38.46%) of patients. Moreover (34.62%) of patients had cognitive impairment. The median time till diagnosis of the disease was 19 months. Diagnosis of ALS by Awaji criteria showed that (76.90%) of patients had definite ALS. Functional staging scales for the patients showed (19.20%) of patients were on stage of disease onset, whereas (11.50%) were on stage of complete dependency. Serum alpha synuclein autoantibodies(IgM and IgG) were significantly higher among patients than control with higher sensitivity and specificity (mainly for IgG). The titers of these autoantibodies not related to the age, gender, duration of the disease, clinical phenotypes or cognitive impairment There was a reversible relation between the titer of autoantibodies and ALS Functional Rating Score (ALSFRS), although statistically was not significant.

Conclusion: ALS is a heterogeneous disorder. It has different clinical phenotypes presentations which carry distinctive clinical and prognostic characteristics, strongly related to a complex interplay between gender and age. Alpha synuclein antibodies may have a role as a disease biomarker.

.**Keywords:** amyotrophic lateral sclerosis, clinical phenotypes, alpha synuclein, autoantibodies.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is one of the motor neuron diseases. In the USA it is commonly known as Lou Gehrig's disease, after the baseball player diagnosed with this disease in 1939. 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 are two broad categories of ALS identified familial and sporadic types. For familial ALS (FALS) more than fourteen gene mutations have been described as causing FALS, most of these have autosomal dominant inheritance, but recessive and x- linked inheritance were also prescribed. There is phenotypic variability across different mutations in the same FALS gene. Sporadic ALS (SALS) by definition is for patients who have no family history of ALS**(Anderson,2006)**.

Amyotrophic lateral sclerosis (ALS) has a heterogeneous clinical presentation, clinical phenotypes with consequent variability in disease progression and survival **(Kiernan et al., 2011)**.

The Classic amyotrophic lateral sclerosis is the most common type.Other types are known with lesser frequencies**(Al-Chalabi et al., 2016).**

ALS pathophysiology is complex. Although different animal models used to study how mutations cause motor neuron diseases, these models still have limitations. Mutations in several genes that have been implicated in the pathophysiology of amyotrophic lateral sclerosis (ALS) can exert motor

neuronal injury through more than one pathophysiological mechanism, although these mechanisms are often interlinked **(Hardimanet al., 2017).**

Mutation in Cu / Zn Superoxide dismutase 1(SOD1) gene is the longest-studied gene implicated in ALS and has been linked to the greatest number of pathophysiological mechanisms**(Deng et al., 1993).**

Mutations in (SOD1) gene account for 20% of all FALS cases **(Ince et al., 2011**).Moreover, misfolded SOD1 aggregates have been reported in sporadic ALS as well as mutant SOD1 familial ALS **(Bosco et al., 2010).**Thus suggesting SOD1 protein misfolding may indeed play a role in sporadic disease, although such findings remain controversial (**Liu et al., 2009).**

Aberrant RNA metabolism and impaired protein homeostasis are predominant factors linking multiple ALS causative genes to neuronal injury**(Pasqualiet al., 2014).** Mitochondrial dysfunction can arise from a mutation in CHCHD10 (coiled-coil-helix-coiled-coil-helix domain-containing 10) and from secondary respiratory chain deficiencies that arise from protein aggregates generated in the presence of other ALS-associated mutations. Both cases lead to an increase in oxidative stress, which puts further stress on an already impaired protein homeostasis system **(Geninet al., 2016).**

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

All suspected ALS patients should undergo electrophysiological testing which allows identification of LMN 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 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).**

Not all suspected amyotrophic lateral sclerosis patients require imaging but it is important in some presentations. Magnetic resonance imaging (MRI) of the spinal cord should be done in all limb-onset ALS patients without bulbar involvement as mixed cord and root compression is responsible for some 30% of ALS misdiagnoses. When symptoms and signs remain isolated to the bulbar region, MRI can be useful in identifying infiltrative lesions of tongue and pharynx **(Shaw and Wood, 2010)**

MRI studies have reported corticospinal tract degeneration, with extensive involvement of the frontal and temporal regions and basal ganglia in patients with ALS**(Turner et al., 2012)**.

Alpha-synuclein (ASN) which is a small protein that, along with β and ɣ synuclein comprises the synuclein family of proteins **(Surguchoeva, 2008)**. Although the function of alpha-synucleinis not well understood, studies suggest that it plays a role in maintaining a supply of synaptic vesicles in presynaptic terminals by clustering synaptic vesicles.

In recent years, investigations into the pathophysiology of amyotrophic lateral sclerosis (ALS) have led to suggestions that ALS lies on a pathological continuum with other neurodegenerative diseases calledα-Synucleinopathies.They include,Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA).All these disorders characterized by the abnormal accumulation of α-synuclein aggregates in neurons, nerve fibers or glial cells. This initiated the investigation of α-synucleinas a biomarker in the cerebrospinal fluid (CSF) and in peripheral fluids such as serum, plasma and saliva in PD patients (**Simonsen et al., 2016)**.

Small amounts of these α-synuclein pathologies can occur in some neurologically normal individuals who do not have associated neurodegeneration, the absence of neurodegeneration in such individuals precludes them from having a degenerative α-synucleinopathy, and it has yet to be established whether such individuals have a form of preclinical disease **(McCann et al., 2014)**.

The health service needs of ALS patients and their careers require integrated services across the allied health disciplines along with neurology, respiratory and palliative medicine specialists**(Corr et al., 1998 &Nget al., 2011)**. Clinical classification of phenotypes can improve communication with patients and health-care professionals, and provide an insight into prognosis and treatment needs. Therefore, a key requirement for an ALS classification system is phenotypic description.