Serum Phosphorylated Neurofilament Heavy Chain Level in Relapsing Remitting Multiple Sclerosis

Thesis

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List of Contents

List of Tables

List of Figures

List of Abbreviations

Abb.	Full term
AGE	Agarose gel electrophoresis
Alb	Albumin
AQP	Aquaporins
BBB	Blood–brain barrier
CD	Cluster of differentiation
CIS	Clinically isolated syndrome
CL	Cross linker elements
CMV	Cytomegalovirus
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CSF	Cerebrospinal fluid
CXCL	Chemokine ligand
CXCR5	Chemokine receptor 5
DIS	Disseminated in space
DIT	Dissemination in time
DMD	Disease-modifying drug
DSS	Disability Status Scale
EAE	Experimental autoimmune encephalomyelitis
EBNA	Epstein–Barr nuclear antigen 1
EBV	Epstein-Barr virus
ECL	Electrochemiluminescence
EDSS	Expanded Disability Status Scale
ELISA	Enzyme-linked immunosorbent assay
FLAIR	Fluid-attenuated inversion recovery
GFAP	Glial Fibrillary Acidic Protein
GWAS	Genom Wide Association Studies
HLA	Human Lekocytic Antigen
HMG-CoA	Hydroxy-3-methylglutaryl-coenzyme A
HRP	Horseradish Peroxidase

List of Abbreviations Cont...

Abb.	Full term
IEF	Isoelectric focusing
IFN	Interferon
IFNb	Interferon beta
Ifs	Intermediate filaments
Ig	Immunoglobulin
IgG	Immunoglobulin G
IL	Inerleukin
Int	Internexin
IQR	Interquartile range
JC viral titers	John Cunningham viral titer
JCV	John Cunningham virus
kDa	Kilo Dalton
KIR4.1	Inward rectifier (Kir) potassium channel subunit 4.1
Loc	Local
MBP	Myelin basic protein
MHC	Major Histocompatibility Complex
miRNA	Mitochondrial RNA
MMPs	Matrix metalloproteinases
MRI	Magnetic resonance image
mRNA	Messenger RNA
MRZR	Measles, Rubella, and Varicella Zoster
MS	Multiple sclerosis
MT	Microtubules
Nab	Neutralizing Antibody
NCAM	Neural cell adhesion molecule also called CD56
NEFH	Neurofilament Heavy gene
NEFL	Neurofilament Light gene
NEFM	Neurofilament Medium gene

List of Abbreviations Cont...

Abb.	Full term
NF-H	Neurofilament Heavy
NF-L	Neurofilament Light
NF-M	Neurofilament Meduim
NFs	Neurofilaments
NGF	Nerve growth factor
NMO	Neuromyelities optica
OCBs	Oligoclonal bands
OD	Optical density
ON	Optic neuritis
OPN	Osteopontin
PAGE	Polyacrylamide gel electrophoresis
PBMCs	Peripheral blood mononuclear cells
PML	Progressive multifocal leucoencephalopathy
pNF-H	Phosphorylated Neurofilament Heavy chain
PPMS	Primary progressive MS
PRMS	Progressive relapsing MS
Q	Quotient
RIS	Radiologically isolated syndrome
ROC	Receiver Operating Curve
RR-MS	Relapsing remitting Multiple Sclerosis
S	Serum
S100B	S100 Calcium Binding Protein B
sCD163	Soluble CD163
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SiMoA	Single Molecule Array
SNPs	Single nucleotide polymorphisms
SPMS	Secondary progressive MS
SPSS	Statistical package for Social Science

List of Abbreviations Cont...

Abb.	Full term
SR	Synthesis rate
T reg	Regulatory T cells
T2	Tesla 2
TH	T-helper
TNF-α	Tumor necrosis factor-α
US	United States
VCAM-1	Vascular cell adhesion molecule-1
VLA-4	Very late antigen-4
WML	White matter lesion

INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated inflammatory disease that attacks myelinated axons in the central nervous system (CNS), destroying the myelin and the axon in variable degrees. It affects approximately 2.5 million people world widely of all races. As true to most autoimmune disease, it affects females more than males ***(Ebers, 2008; Paul et al., 2018).***

Multiple sclerosis is a disease of clinical diagnosis. There is no single specific diagnostic test available. No single clinical feature or diagnostic test is sufficient to diagnose MS. Hence the necessity to research the presence of new diagnostic, prognostic markers and markers of activity of the disease ***(Cosh and Carslaw, 2014).***

The cytoskeleton of a nerve cell is critical for its shape and physiology. It is composed of three classes of protein polymers called microtubules, microfilaments and intermediate filaments, each formed by the self-association of protein subunits. Neurofilaments (NFs) are intermediate filaments with a diameter of 10 nm, similar to that of neurons. NFs from the CNS are heteropolymers that are composed of four subunits, namely neurofilament heavy, medium and light polypeptides (NF-H, NF-M and NF-L, respectively) as well as a-internexin (Int), whereas in the peripheral nervous system, neurofilaments are made up of NF-H, NF-M, NF-L and peripherin ***(Yan et al., 2007)***.

The three major neurofilament protein subunits form the backbone of the axonal cytoskeleton and, following axonal injury or death, can be detected in serum or cerebrospinal fluid (CSF) allowing their potential use as biomarkers of neurodegeneration ***(Gresle et al., 2011).***

In MS, it has been difficult to directly assess the efficacy of current and emerging therapies for reducing axonal injury and the likely pathological substrate of progressive neurological decline. Given the likely association between axonal degeneration and disability progression in MS, a serum biomarker of axonal injury could prove useful as a prognostic or monitoring tool ***(Dutta and Trapp, 2011).***

The axonal neurofilament heavy chain is strongly phosphorylated, resists proteolysis and highly immunogenic. These properties make phosphorylated neurofilament heavy chain (pNF-H) an ideal target for immunological detection. And since it is only found in axons, its presence in serum points towards axonal damage ***(Gresle et al., 2011).***

AIM OF THE STUDY

This study aims to associate between the level of serum phosphorylated neurofilament heavy chain (pNF-H) and the clinical activity of relapsing remitting Multiple Sclerosis (RRMS) and disability measured by **Kurtzke Expanded Disability Status Scale** (EDSS score).

Chapter 1

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is an immune-mediated inflammatory disease that attacks myelinated axons in the central nervous system (CNS), destroying the myelin and the axon in variable degrees. In most cases, the disease follows a relapsing-remitting pattern, with short-term episodes of neurologic deficits that resolve completely or almost completely. A minority of patients experience steadily progressive neurologic deterioration ***(Luzzio and Dangond, 2017).***

Epidemiology

Worldwide, approximately 2.5 million people are affected by MS. The disease is seen in all parts of the world and in all races, but rates vary widely ***(Jo¨rg et al., 2016).*** Prevalence estimates for MS in the United States (US) vary from 58 to 95 per 100, 000 population ***(Noonan et al., 2010)*** and some studies reported an increase to 149.2 per 100, 000 individuals in 2012 ***(Dilokthornsakul et al., 2016).*** The overall MS prevalence in Middle East and North Africa, was 51.52/100, 000 ***(Heydarpour et al., 2015).*** In Egypt, a community-based survey in Al Quseir, Egypt, has found that MS prevalence was 13.74/100, 000 ***(El-Tallawy et al., 2013).***

As is true of autoimmune diseases in general, MS is more common in women. The female-to-male ratio of MS

incidence has increased since the mid-20th century, from an estimated 1.4 in ***1955*** to 2.3 in ***2000 (Kampman and brustad, 2008)*** with some studies suggested an increase in ratio to reach 3.13 in the US in ***2012 (Dilokthornsakul et al., 2016).*** MS is usually diagnosed in persons aged 15-45 years; however, it can occur in persons of any age. The average age at diagnosis is 29 years in women and 31 years in men ***(Luzzio and Dangond, 2017).*** In Egypt, a cross sectional study held in Ain Shams University Hospitals revealed female-to-male ratio of 2.57:1 and mean age at disease onset of 26 years ***(Zakaria et al., 2016).***

Etiology of MS

The etiology of MS is unknown. Epidemiological data indicates that both environmental and genetic factors play key roles in the development of MS ***(Evans et al., 2013).***

I. Environmental Factors

i. Diet

A low vitamin D intake or low exposure to sunlight, its most important source, has been associated with a high risk of developing MS, as well as worsening of the disease and an increased risk of relapses. Indeed, the geographical distribution of the disease is usually associated with a reduced availability of vitamin D through low sun exposure. Serum concentrations of vitamin D were significantly lower in patients with MS than in healthy subjects ***(Bagur et al., 2017).***