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Assessment of Immune and Molecular Changes in T Lymphocytes, Natural Killer Cells and Vasoactive Neuropeptides in Chronic Fatigue Syndrome

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Abstract

Immune dysregulation due to infection, inflammation or compromises to immune and molecular mechanisms can have detrimental effects on normal physiological functions. Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is a disorder of unknown mechanism where diagnosis is often delayed and evidence-based effective treatments are lacking. CFS/ME is characterised by severe fatigue, flu-like symptoms, pain and cognitive disturbances. To date a mechanism explaining the relationship between these symptoms and the physiological irregularities presented by patients remains obscure. As such, CFS/ME patients spend a considerable amount of money on health service cost in pursuit of the most effective treatment to alleviate their symptoms. An important initiative towards better management of CFS/ME is the development of accurate diagnosis. The principal aim of this research is to identify feasible biomarkers that can be used in effective diagnosis of CFS/ME. This research examines the status of lymphocytes in CFS/ME with a specific focus on their cellular function including apoptosis, protein secretion, receptor expression and gene expression. It is hypothesized that irregularities in these cellular processes contribute to the mechanism of CFS/ME and thus may form a suite of diagnostic markers for assessing CFS/ME patients.

Participants for the study comprised 95 CFS/ME patients and 50 non-fatigued controls at baseline. Data for 50 CFS/ME and 27 non-fatigued controls was available for the follow up study at 6 and 12 months. Participants were aged between 25-65 years. The criteria for inclusion were based on the Centre for Disease Prevention and Control (CDC) 1994 clinical diagnostic criteria. Whole blood was collected from each participant at baseline, at 6 months and at 12 months. Flow cytometric protocols were

employed in examining measures of cytotoxic activity in $CD8^{+}$T and Natural Killer (NK) cells, levels of $CD56^{bright}CD16^{negative}$ NK cells $CD56^{dim}CD16^{positive}$ NK phenotypes, $CD4^{+}$T helper cytokine secretion and levels of Foxp3 and vasoactive neuropeptide receptor (VPACR2). The expression pattern of cytotoxic related genes including granzyme A (*GZMA*), granzyme K (*GZMK*), perforin (*PRF1*) and interferon-gamma (*IFN-G*) and the expression of microRNA (miRNA) molecules in NK and $CD8^{+}$T cells were investigated.

At baseline, compared to the non-fatigued controls, CFS/ME patients exhibited significant decreases in cytotoxic activity of NK and $CD8^{+}$T cells, levels of $CD56^{bright}CD16^{negative}$ NK cells and repression of *IFN-G, GZMA* and *GZMK* gene expression. At the same time, significant increases in cytokines, IL-10, IFN-γ and TNF-α, FOXP3, VPACR2 and *PRF1*expression were noticed in the CFS/ME patients compared to controls. At 6 months follow up, significant reductions in NK activity, $CD56^{bright}CD16^{negative}$ NK cells, IL-10 and IL-17A were observed in the CFS/ME patients compared to the non-fatigued controls. At 12 months, in contrast to the non-fatigued controls, CFS/ME patients continued to demonstrate significant decreases in NK activity and significant increases in only IL-2. Assessment of miRNA expression revealed a significant down-regulation of a number of miRNAs in CFS/ME patients in comparison to the non-fatigued controls, specifically, *miR-21, miR-146a, miR-223, miR-17-5p, miR-103, miR-106, miR-10a, miR-191* and *miR-152* were significantly down-regulated mainly in the CFS/ME patients in comparison to the controls.

In conclusion, the results from this study have elucidated the extent of decreases in cytotoxic activity in CFS/ME. In addition, this study has identified unique immune

related processes and molecules that are compromised in CFS/ME patients. These novel parameters may have important implications in the development of biomarkers for CFS/ME. Moreover the consistent decrease in cytotoxic activity and NK phenotypes over the 12 month period strongly supports their usefulness as biomarkers for diagnosing CFS/ME. These biomarkers if implemented in the clinical setting could potentially assist in improving diagnosis and demonstrating a clear pathomechanism for CFS/ME. Eventually, this may result in the development of better therapeutic and treatment strategies for managing CFS/ME.

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Abbreviations

AB	Antibody
APC	Antigen presenting cell
AIDS	Acquired Immune Deficiency Syndrome
AU	Australia
cAMP	Cyclic adenosine monophosphate
CCR	Chemokine receptor
CD	Cluster of differentiation
CDC	Centre for disease prevention and control
CFS/ME	Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis
CNS	Central Nervous System
CB4	Coxsackie's B4 virus
CD2BP2	CD2 (cytoplasmic tail) binding protein 2
CTSC	Cathepsin C
CXC	Chemokine
DEFB1	Defensin beta 1
EBV	Epstein Barr Virus
EIF2B4	Eukaryotic translation initiation factor 2B, subunit 4
EIF4G1	Eukaryotic translation initiation factor 4 gamma, 1
FasL	Fas ligand
EGR3	Early growth response 3
FCγR	Fc gamma receptor
FOXP3	Forkhead/winged helix transcription
GSN	Gelsolin (amyloidosis, Finnish type)

GZMA	Granzyme A
GZMB	Granzyme B
GZMK	Granzyme K
HHPV	Human Herpes Virus
HIF1A	Hypoxia inducible factor 1 alpha
HLA-DR	Human Leuckocyte Antigen
HTLV-1	Human T-lymphotropic Virus Type I
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IL-10RA	Interlukin 10 receptor alpha
IL-6R	Interleukin 6 receptor
IL-6ST	Interleukin 6 signalling transducer
JAK	Janus kinase
LPS	Lipopolysaccharide
LTα	Lymphotoxin alpha
LU	Lytic units
MAPK9	Mitogen-activated protein kinase 9
miRNA	Micro ribonucleic acid
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B cells
NFKBIZ	Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor zeta
NK	Natural Killer
PACAP	Pituitary adenylate cyclase activating polypeptide
Prf1	Perforin

RISC	RNA induced silencing complex
RT-qPCR	Reverse transcriptase quantitative polymerase chain reaction
SOCS	Suppressor of cytokine signalling
STAT	Signal transduction and activators of transcription
Th	T helper cell
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A
TRAF	TNF receptor associated factor family of proteins
TRAIL	TNF-related apoptosis inducing ligand
Treg	Regulatory T lymphocytes
US	United States of America
VN(s)	Vasoactive neuropeptide(s)
VIP	Vasoactive intestinal peptide
VPACR	VIP and PACAP receptor

Statement of Novelty

To the best of my knowledge I, Ekua Weba Brenu, declare that this work is an original documentation of the present research. All components of the written work were produced by the candidate unless otherwise specified where due acknowledgement has been given in the form of references.

Ekua Weba Brenu

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