Kleine-Levin Syndrome: Antibodies in Patient Serum and Their Reactivity with Proteins From Neural Tissue

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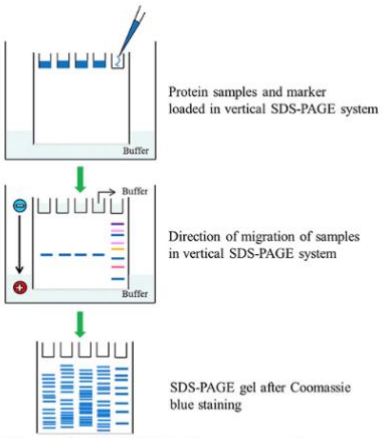
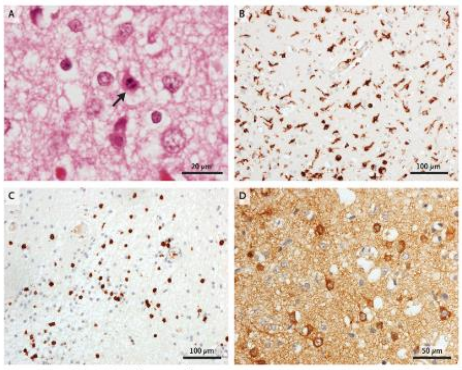
What is this project?

The basis of this research is testing for the presence of antibodies in patient sera that react with tissue proteins from the hypothalamus, thalamus, caudate nucleus, and portion of the parieto-temporal region. This will be found through the biochemical methods called SDS-PAGE and immunohistochemistry. SDS-PAGE uses electrophoresis in which an electrical current is applied to a gel, separating proteins by their mass (otherwise known as western blotting). Protein extraction from brain cells and tissues will be applied to the electrophoretic gel, followed by repeating this with patient sera at several concentrations. This leads to the ability to detect proteins in patients' sera (antibodies) that react with the proteins from the brain extract. Following this method, immunohistochemistry will be completed. Sections of normal brain tissue will be cut micrometers thin, and patient sera will be applied to these sections of brain tissue. Normal brain tissue is chosen because it is a proper aspect of control and serum would inevitable bind to abnormal tissue. Patients are predominately normal with regard to neurological function…until the onset of KLS. Both of these are readily used in biochemistry and immunology, and have been successful in studying many other diseases and disorders. Patient sera will be obtained from collaborator Dr. Arnulf, as well as a positive control of narcolepsy. Brain tissue will be obtained from one of the repositories through the Neurobiobank of the NIH.



Example SDS-PAGE process taken from Sigma-Aldrich.



Example IHC analysis – source: New England Journal of Medicine.

Why?

Most prior studies on KLS have focused on analyzing brain function through imaging, blood and spinal fluid analysis, and treatment trials for patients. There have been very few projects that explore the pathophysiology of this disorder, and none have been performed using both patient serum and brain tissue. This project has the potential to provide critical insights into the immunopathology and etiology mechanisms of KLS patients.

Anticipated results:

The anticipated result of SDS-PAGE is specific binding of serum antibody from patients' sera with one or more proteins separated from either neural cell line and/or tissue protein extracts. Conversely, the lack of binding of antibodies from control sera would indicate the specificity of binding and lack of a false positive. Binding of antibodies from patient sera to specific neural proteins may provide evidence that auto-antibodies present in patient sera are capable of reacting with separated proteins from the brain. As human sera contains hundreds of proteins, electrophoresis allows separation of proteins via molecular weight thus narrowing the search for presumptive identification of the auto-antigen. This preliminary result would provide rationale for immunohistochemistry to determine if antibodies in the patients sera can also physically bind to intact brain tissue.

The anticipated result is notable visual representation of antibodies from KLS patient serum binding to neural tissue, with lack of similar findings from control sera. Quantification of the antibody binding is completed using a computer software. This would be a groundbreaking study as the finding of antibodies binding to brain tissue concretely suggests that there is an autoimmune mechanism that infiltrates the central nervous system of patients with Kleine-Levin Syndrome, and will further the hypothesis of autoimmune etiology. In addition, such finding will open the door for replicated studies as well as force more pathophysiology research to be completed for this devastating disorder.

Budget:

SDS-PAGE

Item	Supplier	Cost
Tissues	France hospital, Sigma-Aldrich, Inc., Neurobiobank	$1,686.99
Procedure Materials	ThermoFischer Scientific, Sigma-Aldrich, Inc., Bio-Rad Laboratories, Boston BioProducts, Abcam	$2,431.49
Laboratory Supplies	ThermoFisher Scientific	$500.00
Subtotal A		$4,618.48

Immunohistochemistry

Item	Supplier	Cost
Procedure Materials	R&D Systems, BioCare Medical, ThermoFisher Scientific, Sigma-Aldrich, Inc.	$1,782.70
Laboratory Supplies	ThermoFisher Scientific	$783.45
Outgoing Shipping	FedEx	$200.00
Subtoal B		$2,766.15

Direct Costs	Subtotal A+B	$7,384.63
Indirect Costs	6%	$443.08
Overall Total		$7,827.71

Timeline:

May/June 2020 – Submit proposal to FAU Division of Research GrantsERA. Submit proposal to the Kleine-Levin Syndrome Foundation.

July 2020 – Submit IBC Application to FAU Division of Research.

August/September – Order and receive serum from Dr. Isabelle Arnulf, France.¬¬ Order and obtain and all necessary materials.

September/October– Begin experimental procedures.

March/April – Finalize results and submit finished paper for publication.