The Investigation of the Microbial and Molecular Analysis of Morgellons Epithelial Tissue Samples

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Abstract

Morgellons disease is a complex dermatopathy that is controversial in the medical community. Unfortunately, there is not enough evidence to the etiology or transmission of this disease. Due to the lack of information, the debate surrounding Morgellons is considerable. There are currently no markers for diagnosis, which leads patients to a common differential diagnosis of delusional parasitosis or delusional infestation. However, with further investigation, potential etiologies can be explored. In this study:

- Lesions from patients are collected and de-identified so they are anonymous to the researchers.
- Lesions are studied for unusual microbial organisms; specifically, Bartonella henselae, Helicobacter pylori, Borrelia burgdorferi, and Treponema denticola have been detected in dermatological specimens, providing a base line for spirochetal cause
- We hypothesize microbial organisms could be the infectious cause of Morgellons disease.
- Identifying these related strains will help to determine if an infectious etiology of the dermatopathy is present.

Methods

Molecular Techniques

- **DNA Extraction:** De-identified epithelial tissue samples are weighed out to 0.1g and added to 1.5 ml microcentrifuge tube. Contents are spun at 12,000 x g for 1 minute and 250 μl of resuspension media are added. Vortex and spin at 10,000 x g for 3 minutes. Remove supernatant to clean microcentrifuge tube and add equivalent amount of chloroform: isoamyl. Vortex and spin again at 10,000 x g for 3 minutes. Remove supernatant to blood culture tube. Add 2 volumes of 95% ethanol and 3 volumes of TE and place in a -20°C freezer for 30 minutes. Remove supernatant and spin again at 12,000 x g for 1 minute and 250 μl of TE solution and vortex to mix. Set in a dry bath for one hour. Add 500 μl Phenol: Chloroform: isoamyl Solution. Vortex and spin at 10,000 x g for 3 minutes.

- **Polymerase Chain Reaction (PCR):** PCR was used to amplify our gene of interest. 
  
  - Borrelia 16S ribosomal RNA small subunit or CTP synthase gene F: 5'-CCGGCTTGAACGACTACGCGGAG-3'; R: 5'-CCTACAAAGTCTTCTCTAAT-3' 
  - Treponema specific 16S ribosomal RNA F: 5'-AACATGAAGCAGTCGCGGACG-3'; R: 5'-CTCAATGCGGAATATTCTTA-3' 
  - Bartonella 16S-RNA F: 5'-GGCTTCTCGATTTGAGGCTGG-3'; R: 5' GAGATGTTCGGTTAAGATT-3' 
  - Helicobacter pylori Urease gene F: 5'-GCGAATGAAATTTAGTT-3'; R: 5'-GGCCAATGGGAATTATTTCTA-3' 

- **DNA Electrophoresis:** Ten microliters of each PCR product was run on a 2% agarose gel at 75 Volts for 2 hours. The gel was stained using ethidium bromide solution for 5 minutes and viewed with a Bio-Doc IT UV transilluminator.

- **Gel Purification:** Gel purification was conducted with a kit provided by Invitrogen. After purification 12 μl is removed and stored in a clean 0.5 μl tube. DNA is sent for sequencing with subsequent bioinformatics analysis.

- **Cloning:** 1μl of DNA sample was used to measure the nucleic acid concentration.

Environmental Contaminant Controls: (All controls Were Negative)

- BJ: Betty Jo (Epithelial Cells)
- C: Carol (Epithelial Cells)
- S: Sam (Epithelial Cells)
- W: Wymore (Epithelial Cells)

- Autoclave
- Women's bathroom
- ST- Stairwell (Main)
- WA- Water Fountain

References


Acknowledgements

We would like to extend our gratitude to OSU Center for Health Sciences for their strong support, in particular, the Pharmacology and Physiology Department. Thanks to the Charles E. Holman Foundation for continued funding. Thank you to Dr. Allen in the Forensics Department for allowing us to use the Bio-Doc IT UV transilluminator. We would also like to thank TABELC for their amazing commitment to bioscience education. Without their support and funding the summer research program would not be possible. Thank you, especially to Dr. Marino for your leadership, Dr. Diana Spencer for your time and training in Lab Sprits with the assistance of Dusti Sloan and Donita Gray. A special thank you to Betty Jo Westefeld for your support and assistance throughout the summer. DNA sequencing was performed by the DNA/Protein Resource Facility at OSU Stillwater.