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

Carol S. Hefley, Samantha G. Rice, *Alishbah A. Malik, Dr. Randy S. Wymore*

Tulsa Community College, Oklahoma State University – Center for Health Sciences

Department of Pharmacology and Physiology

Abstract

Morgellons disease is a complex dermopathy 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 burgdorfori,* and *Treponema denticola*.
- *Borrelia burgdorfori* 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 dermopathy is present.

Introduction

Morgellons is a multisystem infective disease that is characterized as a mysterious condition which is misunderstood in the medical community. This condition can be debilitating and disabling, as non-healing lesions with unique colored fiber-like filaments emerge from open wounds. Crawling sensations on and under the skin, with intense itching, severe fatigue, difficulty concentrating, and short-term memory loss are also associated within the sign and symptoms of Morgellons disease. Due to similarities in sign and symptoms to those of a mental illness involving false beliefs of infestation by parasites, this disease is often mistaken as delusional parasitosis.

Methods

Microbial Techniques

❑ **MacConkey Agar**

MacConkey agar is used to isolate and differentiate members of the Enterobacteriaceae.

- Ability to ferment lactose.
- Selective and differential culture media.
- Selectively isolate Gram-negative and enteric bacilli bacteria.

- Samples are plated using a lawn.
- Colonies are isolated.
- Molecular analysis is used to conducted further research.

Methods

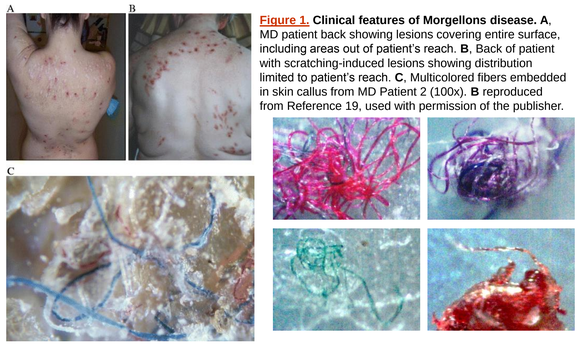


Figure 1. **Clinical features of Morgellons disease. A**, MD patient back showing lesions covering entire surface, including areas out of patient's reach. **B**, Back of patient with scratching-induced lesions showing distribution limited to patient's reach. **C**, Multicolored fibers embedded in skin callus from MD Patient 2 (100x). **B** reproduced from Reference 19, used with permission of the publisher.

Molecular Techniques

❑ **DNA Extraction**:

De-identified epithelial tissue samples are weighed out to 0.1g and added to a 1.5 µl microcentrifuge tube. Contents are spun at 12,000 x g for 1 minute and 250 µl of resuspension media are added. Vortex to mix and set tubes in a dry bath at 37°C for approximately 30 minutes. After incubation, add 250 µl TNE 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. Remove supernatant to clean microcentrifuge tube and add equivalent amount of chloroform: isolamyl. 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 tilt. Remove DNA clot from supernatant with loop and place in a clean microcentrifuge tube containing 100-200 µl of TE.

❑ **Polymerase Chain Reaction:**

PCR was used to amplify our gene of interest.

- *Borrelia* 16S ribosomal RNA small subunit or CTP synthase gene F: 5'-CCTGGCTTAGAACTAACG-3'; R: 5'-CCTACAAAGCTTATTCCTCA-3'
- *Treponema* specific 16S ribosomal RNA F: 5'-AARCATGCAAGTCGARCGGCAAG-3'; R: 5'-TCCATTGCGGAATATTCTTA-3'
- *Bartonella* 16S rRNA F: 5'-CCTCCTTCAGTTAGGCTGG-3'; R: 5'-GAGATGGCTTTTGGAGATTA-3'
- *Helicobacter pylori* Urease gene F: 5'-GCCAATGGTAAATTAGTT-3'; R: 5'-CTCCTTAATTGTTTTTAC-3'

❑ **Gel 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.

❑ **Nanodrop:**

1µl of DNA sample was used to measure the nucleic acid concentration.

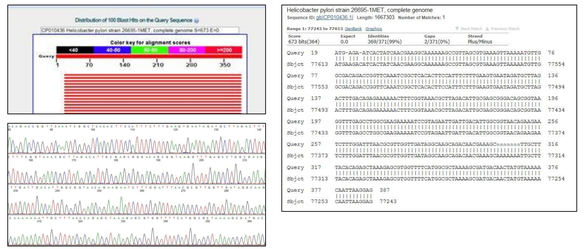
Results

Examples of our positive PCR results in 2% agarose gel:

***Helicobacter pylori* strain 26695-1MET, complete genome**

GCTCTCCGGGAACTTTTGCGCATGTAACACGCTCTTTAGCTCTGTGTAAAGCAATTTTTTTGCTTTCGTTGTCTGCTTGCC
TATCAACCAACGCGGTTAAATCCAAAGATTCTTCTGTTACCGCCAATGTCAATCAATTCTACGGATTTTTCTTCGCCAGGCTC
AAACCTTACCGCTGTCCCGCTCGCAATGTCTAAGCGTTTACCGAAAGTTTTTTTCTCTGTCAAAGTCTAAGCATCTATTCAC
TTCAAAGAAATGGAAGTGTGAGCCGATTTGAACCGGTCTGTCGCCAACATTTTTAACTTTCACGCTAACGGCTTTTTTGCC
TTCGTTGATAGTGATGTCTTCATTTTTTAAGAACAACTCACCAGGAACTAATTTACCATTGGCA

NCBI Blastn and Blastx results



Bartonella henselae	BIF
Borrelia burgdorfori	B1,B2,B6,BIF,BOR1, BIG, #1, 1A, 1B, 1C, 1F, 1G, 1J1, 1K, 1M, 2B
Helicobacter pylori	HIL, H17, H9, 1L, 2M, 2N
Treponema denticola	T2, TM1, T4,3S, TN6, 16W, P35, TP4, T1, #1, #2, 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1K, 1L, 1M, 1O, 1P, 1R, 1S, 1U, 1V, 1W, 1X, 1Y, 1Z, 2F, 2K, 2L, 2M, 2P, BM2

Environmental Contaminant Controls:
(All controls Were Negative)
BJ- Betty Jo (Epithelial Cells)
C- Carol (Epithelial Cells)
S- Sam (Epithelial Cells)
W- Wymore (Epithelial Cells)
A- Autoclave
B- Women's bathroom
ST- Stairwell (Main)
WA- Water Fountain

Conclusions

- Unusual microbial organisms have been identified in Morgellons epithelial tissue samples.
- Research suggests there may be an infectious etiology of the dermopathy is present.
- Future research needs to be conducted to continue exploring etiologies to support our findings.
- Future research includes culturing epithelial cells to study fiber growth.
- *Borrelia burgdorferi* is a bacterial species of the spirochete class of the genus *Borrelia*, the predominant cause is Lyme Disease.
- *Bartonella henselae* is a member of the class of the *Bartonella* genus, one of the most common types of bacteria in the world, including cat-scratch disease.
- *Treponema denticola* is a Gram-negative, obligate anaerobic, motile and highly proteolytic spirochete bacterium that is associated with human periodontal disease and related to syphilis human pathogen.
- *Helicobacter pylori* is a helix-shaped bacterium that is classified as a curved rod, not spirochete, but is a Gram-negative bacterium.

References

- Middelveen, Marianne J., Cherl Bandoski, Jennie Burke, Eva Sapi, Katherine Filush, Yean Wang, Agustin Franco, Peter J. Mayne, and Raphael B. Stricker. "Exploring the Association between Morgellons Disease and Lyme Disease: Identification of *Borrelia Burgdorferi* in Morgellons Disease Patients." *BioMedCentral* (2015): 1-14. *Biomedical Central*. Middelveen, 12 Feb. 2015. Web. 12 June 2015.
- Pearson, Michele L., Joseph V. Selby, Kenneth A. Katz, Virginia Cantrell, Christopher R. Braden, Monica E. Parise, Christopher D. Paddock, Michael R. Lewin-Smith, Victor F. Kalanisky, Felicia C. Goldstein, Allen W. Hightower, Arthur Papier, Brian Lewis, Sarita Motipara, and Mark L. Eberhard. "Clinical, Epidemiologic, Histopathologic and Molecular Features of an Unexplained Dermopathy." *Clinical, Epidemiologic, Histopathologic and Molecular Features of an Unexplained Dermopathy*. PLOS One, 25J Jan. 2012. Web. 12 June 2015.
- Middelveen, Marianne J., Peter J. Mayne, Douglas G. Kahn, and Raphael B. Stricker. "Characterization and Evolution of Dermal Filaments from Patients with Morgellons Disease." *Clinical, Cosmetic and Investigational Dermatology*. Dove Medical Press, n.d. Web. 26 June 2015.

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