HERPES SIMPLEX VIRUSES 1 AND 2

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OBJECTIVES

• Examine the evolution and classification of Herpes Simplex Viruses 1 and 2 (HSV-1 and HSV-2)

• Explain the symptoms, transmission, management, and prevention of infection

• Describe the testing modalities for Herpes Simplex Viruses 1 and 2 along with their indications and limitations
**HERPESVIRIDAE FAMILY**

- Enveloped virus with linear, double stranded DNA (Straus, 1990)
- Includes Herpes Simplex viruses 1 and 2, Varicella-zoster virus, Epstein-Barr virus, Cytomegalovirus, and Human herpes virus 6, 7, Kaposi sarcoma-associated herpesvirus (HHV-8), Herpes B virus (Bennett, 2019)
- Divided further into subfamilies:
  - **Alpha herpes viruses:** Rapid growth in many tissues, destroy host tissues
  - **Beta** – slow growing in limited cell types
  - **Gamma** – slow growing in lymphoid cells
- Only primates infected by two herpes simplex viruses (Wertheim, 2014)
HERPES SIMPLEX VIRUS STRUCTURE

• DNA: Linear (Ahmad, 2020)

• Capsid: ~125 nm diameter icosahedral

• Envelope: Derived from host organelle with viral membrane proteins
  • Glycoproteins B and D help virus bind and enter host cells (Straus, 1990)

• Tegument: Complex multi-subunit protein layer between the capsid and envelope (Ahmad, 2020)
THE EVOLUTION OF THE HUMAN HERPES SIMPLEX VIRUSES (WERTHEIM, 2014)
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HERPES SIMPLEX VIRUS 1

• Primarily oral transmission (World Health Organization, 2022)
  • HSV-1 infections with genital lesions increasing in frequency, especially in young women and men who have sex with men (CDC, 2021)

• Mostly childhood infections (World Health Organization, 2022)

• Estimated 3.7 billion people <50 years old
  • Highest in low and middle income countries (Johnston, 2021)
  • Serologies >90% in sub-Saharan Africa and Latin America

• 50-70% of healthy adults in United States have positive serologies (Mandell, 2020)
HERPES SIMPLEX VIRUS 2

• Primarily sexually transmitted (World Health Organization, 2022)
• Estimated 491 million people ages 15-49
  • 11.9 % of people ages 14-49 have been infected in the United States (CDC, 2021)
• Almost 2 times more women than men (World Health Organization, 2022)
• More frequent recurrence and subclinical shedding (CDC, 2021)
• 2 to 3 fold increased risk in acquiring HIV
## COMPARISON OF HSV1 AND HSV2

<table>
<thead>
<tr>
<th></th>
<th>HSV-1</th>
<th>HSV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of lesions</td>
<td>Primarily oral, increasingly genital</td>
<td>Primarily genital</td>
</tr>
<tr>
<td>Prevalence (Worldwide)</td>
<td>3,700,000,000</td>
<td>491,000,000</td>
</tr>
<tr>
<td>Prevalence (United States)</td>
<td>50-70%</td>
<td>11.9%</td>
</tr>
<tr>
<td>Associated risks</td>
<td></td>
<td>HIV, women 2x more than men</td>
</tr>
<tr>
<td>Clinical considerations</td>
<td>If infected by HSV-2, 3x more likely to be subclinical</td>
<td>More subclinical shedding, more frequent recurrence</td>
</tr>
</tbody>
</table>

(World Health Organization, 2022; CDC 2021, Mandell, 2020)
SYMPTOMS

• Initial infection: Fever, body aches, swollen lymph nodes, sores (Johnston, 2021)

• Virus dormant in sensory nerve ganglions (Straus, 1990)

• Subsequent outbreaks: burning or tingling prior to sores appearing (Johnston, 2021)
  • “Cold sores”

• Encephalitis: Altered mental status, headache, seizures (Leonard, 2022)
TRANSMISSION

• Contact with the virus (World Health Organization, 2022)
  • Open sores, saliva, body fluid, mucus membranes
  • Greatest risk with active sores, but can also transmit while dormant
  • Rarely from mother to baby
  • 10 in 100,000 births worldwide
NEONATAL HERPES

• Transmission (Demmeer-Harrison (1), 2022):
  • Intrauterine: Rare, 1 in 250,000 deliveries
    • Ascending infections with prolonged rupture of membranes
  • Perinatal: 85%
  • Postnatal: 10%

• Risk (Demmeer-Harrison (2), 2022):
  • 2% if active lesions
  • 25 to 60% if first time infection
  • <37 weeks gestation, use of scalp electrodes, skin lacerations
Placenta Infarcts
- Necrotizing, calcifying funisitis
- Plasma cell deciduitis
- Lymphocytic villitis
- Hydrops fetalis
- Fetal/Neonatal demise
- Microcephaly, hydranencephaly
- Eye watering, pain, conjunctival erythema
- Skin sores and scars

Liver: Geographic necrosis

Viral cytopathic change

Hyperbolic pigmentation

Necrotizing Pneumonia

https://en.wikipedia.org/wiki/Microcephaly
https://en.wikipedia.org/wiki/Hydranencephaly
https://www.pratisandhi.com/the-aftermath-of-pregnancy/
https://expertpath.com
MANAGEMENT OF MATERNAL INFECTIONS

- Not recommended to screen pregnant women for HSV infections, but should collect a thorough history (CDC, 2021)
- If partner is infected by HSV and mother not previously infected, refrain from sex in the third trimester
- Cesarean delivery for active genital lesion (Preboth, 2000)
- No active lesion or prodromal symptoms may proceed with vaginal birth
- Consider antiviral therapy
TESTING, SYMPTOM MANAGEMENT, AND PREVENTION

• Testing: (CDC, 2021)
  • Symptomatic, high risk populations including ≥10 sexual partners, HIV+, positive for their sexually transmitted diseases

• Symptom Management:
  • Antiviral medications: Acyclovir, famciclovir, and valacyclovir
  • Reduces viral shedding and allows sores to heal quicker (Pethboth, 2000)

• Prevention (World Health Organization, 2022):
  • Avoid oral or sexual contact especially with those with open sores
    • Condoms offer some protection (CDC, 2021)
  • Don’t share food, beverages, or cutlery
AVAILABLE TESTING

• Current lesions (Leonard, 2022):
  • Viral culture – traditional gold-standard
    • Direct Fluorescent Antibody Stain
  • Nucleic Acid Amplification Test (NAAT)/Polymerase Chain Reaction (PCR) testing

• History of lesions in the past:
  • Serologic testing

• Immunohistochemical staining
VIRAL CULTURES

- Direct Fluorescent Antibody Stain
  - Rapid results
  - Lower sensitivity, must confirm with viral culture

[Diagram showing steps of virus culture process]
VIRAL CULTURES

1. Centrifuge
2. Incubate
3. Centrifuge
4. Cytopathic Effects or Immunofluorescence

PCR Components

- DNA Sample
- Primers + Probe
- Nucleotides
- Taq Polymerase
- Mix Buffer
- 96 well plate
- PCR Cycle

Thermal Cycler
**PCR Components**

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- Taq Polymerase
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**PCR Process (One Cycle)**

1. **Denaturing**
   - 95°C - Strands Separate

**PCR Cycle**

Thermal Cycler
**PCR Components**

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**PCR Process (One Cycle)**

1. **Denaturing**

   - 95°C - Strands Separate

**Thermal Cycler**

- PCR Cycle
PCR Components

DNA Sample
Primers + Probe
Nucleotides
Mix Buffer
96 well plate

Taq Polymerase

Thermal Cycler

PCR Process (One Cycle)

1. Denaturing
95°C - Strands Separate (5 sec.)

2. Annealing
56°C - Primers Bind Template (20 sec.)

3. Extension
76°C - Synthesise New Strand
PCR TESTING PROBES

Hybridization Probe

TaqMan Probe

https://en.wikipedia.org/wiki/TaqMan
PCR DATA
PCR TESTING - TYPING

- Melting Curve Stage
  - Denature
  - Anneal
  - Slowly heat until denatured
- Better matching of probe = higher melting point
SEROLOGIC TESTING

- Chemiluminescent Immunoassay
- Do not order in neonates (Leonard, 2022)
- Options: Combined vs Type specific
SEROLOGIC TEST INTERPRETATION

- Combined IgG develops in days to weeks (Leonard, 2022)
- IgG specific to HSV-1 or HSV-2 may take up to 6 months to form

- But why do we even need to know the type?
  - HSV-2 more subclinical shedding and recurrence
  - Epidemiologic information

- Repeat testing in one month
IMMUNOHISTOCHEMICAL STAINING

• Stain thin sections on glass microscope slides (ThermoFisher Scientific, 2022)
• Specific antigens targeted by antibodies
• Coupled to fluorophore or pick up stain to visualize
• Dark brown nuclear staining (Solomon, 2022)

<table>
<thead>
<tr>
<th>Test Modality</th>
<th>Indications</th>
<th>Benefits</th>
<th>Limitations</th>
<th>Cost (aruplab.com)</th>
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</thead>
</table>
| Herpes Virus Culture                             | Acute infection with active lesions, especially in neonates                  | Specific                                      | Time  
May not differentiate type  
False negatives late in disease                                                                     | $$                 |
| DFA                                              | Acute infections with active lesions, generally not used alone                | Quick                                         | Lower Sensitivity, must confirm negatives with cultures                                              | $$                 |
| PCR                                              | CSF, blood                                                                   | Quick, sensitive and specific, able to determine type | False-negatives early in the disease, little data in use in neonates                                   | $$$                |
| Herpes Simplex Virus Combined, IgG and IgM       | Blood                                                                        | No active lesions needed                      | IgM is not clinically relevant, cross reactive  
Does not differentiate type  
False negative early on                                               | $$                 |
| Herpes Simplex Virus type specific glycoproteins, IgG | Differentiates type to aid with treatment and counseling, blood          | No active lesions needed                      | False negative early on, some patients never develop type specific                                     | $                   |
| Immunohistochemistry                             | Paraffin imbedded tissue, body fluid                                        | Invasive sample collection, cannot distinguish HSV1/2 |                                                                                                       | $$$                |
SUMMARY

• Herpes Viridae family with an envelope and double stranded, linear DNA

• Symptoms: Flu-like symptoms, burning/tingling, sores involving mouth, nose, eyes, or genitals

• Transmission: Contact with infected person, more likely if symptomatic

• Symptom Management: Acyclovir

• Testing: DFA, viral culture, PCR, serology, immunohistochemical stain
REFERENCES


• Herpes Simplex Virus. World Health Organization, accessed September 14th 2022.

• Johnston, C., A. Wald. (2021). Epidemiology, Clinical Manifestations, and Diagnosis of Herpes Simplex Virus Type 1 Infection. Up to Date, accessed September 14th 2022.


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