

LABORATORY DIAGNOSIS OF MENINGITIS



What is meninges/leptomeninges?

- **Brain & spinal cord have two protective coverings**
 - **outer covering consisting of bones**
 - **inner covering of membranes (meninges).**

- **The meninges is a collective term for three distinct layers surrounding the brain & spinal column:**

Outer most memb.- Dura mater

Middle memb.- Arachnoid

Inner most memb.- Pia mater

- **The pia mater & the arachnoid membrane are collectively called leptomeninges**

Cerebrospinal fluid (CSF)

- **Envelops the brain & spinal cord**
- **Found in the subarachnoid space & within the cavities & canals of the brain & spinal cord**
- **Specialized secretory cells called choroid plexus produce it.**

Functions of C.S.F

- ❑ **Reduces weight of the brain**
- ❑ **Carries essential metabolites into the neural tissues**
- ❑ **Cleanse the tissues of waste**
- ❑ **Provides a mean by which the brain monitors changes in the internal environment**

What is Meningitis / Encephalitis /Meningo-encephalitis ?

- ❑ **Infection of the membranes surrounding the brain & spinal cord.**
- ❑ **Inflammation of parenchyma of the brain and is usually result of viral infections.**
- ❑ **Inflammation of the brain & meninges.**

Routes of Infection

- Hematogenous spread – most common
- Direct spread from infected site (e.g. otitis media, sinusitis, mastoiditis)
- Anatomic defects in CNS structure – as a result of surgery, trauma or congenital abnormalities can allow microorganisms easy & ready access
- Travel along the nerves (direct intraneural) – rabies virus, herpes simplex virus
- Through carelessly performed LP, an accidental wound or an infected neurosurgical wound

MENINGITIS

- Based on the host's immune response to the invading microorganism, meningitis is divided into two major categories:
 - **1. Purulent meningitis**
 - Marked, acute inflammatory exudate with large numbers of polymorphonuclear cells (PMNs)
 - Bacteria usually cause these infections

2. Aseptic meningitis

- Increase of lymphocytes & other mononuclear cells in the CSF
- Negative bacterial & fungal cultures
- commonly associated with viral infections & usually self limiting infection
- It is also a component of syphilis & other spirochetal diseases

Pathogenesis

▣ Host defense mechanisms

Blood brain barrier

- choroid plexus
- Arachnoid membrane
- cerebral micro vascular endothelium
(continuous intercellular junctions)

Factors

1. **Age of the host**

Neonates have highest prevalence because of

- immature immune system
- organisms present in the colonized female genital tract
- increased permeability of blood brain barrier
- lack of humoral antibody

CONTD.

2. Other factors

Alcoholism, splenectomy, diabetes mellitus, prosthetic devices, particularly, patients having CNS shunts, immunosuppression

3. Bacterial factors

1. production of IgA proteases
2. capsule
3. pilli
4. lipoteichoic acid

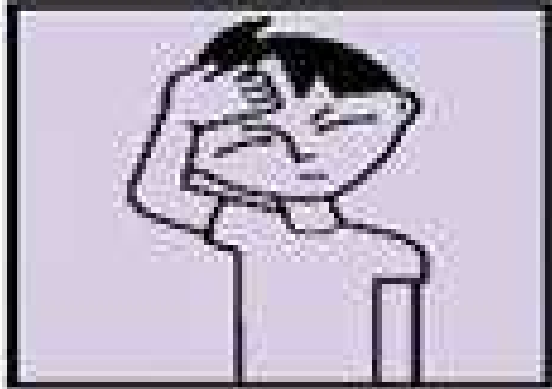
Clinical manifestations

Meningitis can be either acute or chronic in onset & progression of disease.

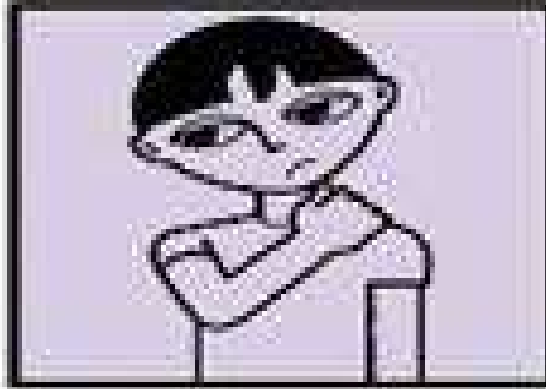
Acute meningitis

Chronic meningitis

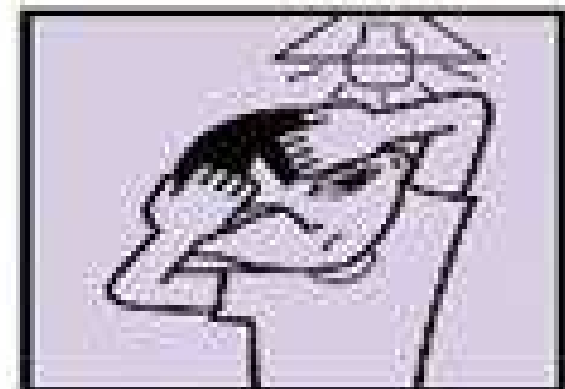
Signs & symptoms of meningitis



Severe headache



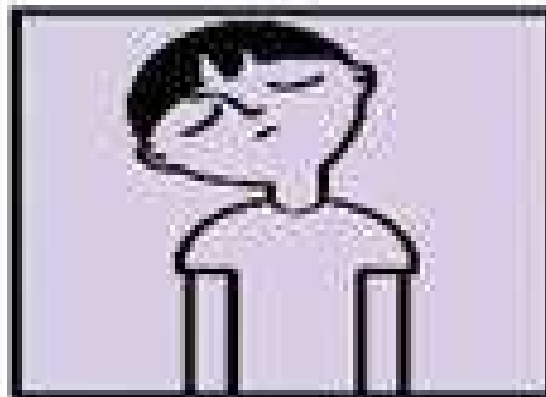
Stiff neck



Dislike of
bright lights



Fever/vomiting



Drowsy and less
responsive/
vacant



Rash (develops
anywhere on
body)

□ Etiologic agents – Acute meningitis

- **Neisseria meningitidis**
- **Haemophilus influenzae**
- **Streptococcus pneumoniae**
- **Staphylococcus aureus**
- **Escherichia coli**
- **Klebsiella sp.**
- **Streptococcus pyogenes**
- **Streptococcus group D**
- **Staphylococcus epidermidis**
- **Pseudomonas aeruginosa**
- **Listeria monocytogens**
- **Citrobacter sp.**
- **Enterobacter sp.**

□ **Chronic meningitis**

Etiologic agents

- **Mycobacterium tuberculosis (tuberculous meningitis)**
- **Cryptococcus neoformans**
- **Coccidioides immitis**
- **Histoplasma capsulatum**
- **Blastomyces dermatitidis**
- **Candida sp.**
- **Nocardia**
- **Actinomyces**
- **Treponema pallidum**
- **Brucella**
- **Salmonella**
- **Rare parasites – Toxoplasma gondii, cysticercus, Paragonimus westermanii, Trichinella spiralis**

Aseptic meningitis

□ Etiologic agents

- Enteroviruses – ECHO
 - Coxsackie
 - Polio
- Paramyxoviruses – Mumps
 - Measles
- Herpes viruses – Herpes simplex
 - Varicella-zoster
- Adenoviruses
- Arboviruses – Flavivirus – JEV
 - Buniyavirus
 - Arenavirus

Encephalitis / Meningo-encephalitis

□ Etiologic agents

1. VIRAL

- Enteroviruses – ECHO
 - Coxsackie
 - Polio
- Paramyxoviruses – Mumps
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2. PARASITIC

Direct invasion from nasal mucosa

- *Nagleria fowleri***
- *Acanthamoeba* spp.**

Through Hematogenous spread

- *Entamoeba histolytica***
- *Strongyloides stercoralis***
- *Taenia solium***
- *Toxoplasma gondii***

Laboratory diagnosis

Role of laboratory- Whether meningitis present/not
- to determine causative organism

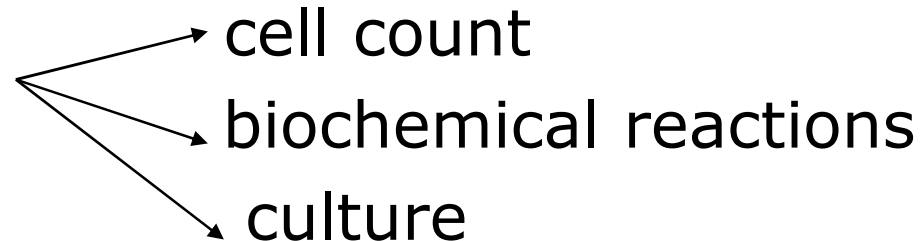
- **Clinical signs of meningeal irritation always suggest inf. of meninges, but it may occur**
 - in certain acute inf. not involving the meninges
 - with certain noninfectious cond. such as SAH

- **Infants may have meningitis without the usual localizing signs**

Collection of specimen

1. CSF –

collected in fresh sterile screw capped container by lumbar puncture under strict aseptic precautions. Preferably it should be collected in three different tubes



Should be hand delivered immediately to the laboratory

SHOULD NEVER BE REFRIGERATED.

Should be processed immediately, if delay incubate at 35° C.

2. Blood

Naked eye examination of CSF

□ **Appearance of CSF**

- * clear/cloudy/hazy/turbid/pus like
- * contains blood or not
- * presence of clot
 - indicates increased fibrinogen
 - found in pyogenic meningitis & spinal constriction
 - spider web clot on the surface of CSF is found in Tuberculous meningitis

Microscopic examination of CSF

1. CELL COUNT

Number of cells/cu. m.m.

**Type of the cell–polymorph/lymphocyte
predominant**

Presence of RBCs

2. GRAM'S STAIN

**prepare a smear from centrifuged deposit,
stain & examined microscopically**

GRAM'S STAIN

GN diplococci

GP diplococci

GPC

GNB

GPB

GP Yeast cells

3. Acid fast stain

- M. tuberculosis**

4. Wet preparation examination

- E. histolytica**
- Trypanosomes**
- Nagleria fowleri**

5. India ink preparation

- Cryptococcus neoformans**

6. D.G.I

- T. pallidum**
- Leptospira**

Chemical analysis

Glucose

Decrease or absent – Pyogenic meningitis

Normal – Viral meningitis

Protein

Increased

CRP detection

Direct Detection of antigen in CSF

Detection of bacterial antigen

- **Latex agglutination test**
- **Coagglutination test**

Detection of capsular antigen of C. neoformans

CULTURE

Bacterial culture

- ❖ **Chocolate agar**
 - ❖ 5% sheep blood agar
 - ❖ Enrichment broth – thioglycolate broth
- Incubation – 5-10% CO₂ env. in CO₂ incubator or candle jar
- 37⁰ C
 - for 72 hours
- ❖ MacConkey's agar – (if pt. is neonates)
 - ❖ Lowenstein Jensen medium-Tb. meningitis

- ❖ **Blood culture – positive in 40% cases of bacterial meningitis**

Fungal culture

- ❖ **If direct M/E shows fungal elements, then culture on**
 - **Sabouraud's dextrose agar**
 - **Brain heart infusion agar**
- ❖ **Incubation - 30⁰ C & 35⁰ C for 4 weeks**

Viral culture

- **c.s.f, feces, throat swab**
- **Tissue culture**

Differentiating features in CSF in different meningitis

Clinical setting	Glucose mg./dl	Protein mg./dl	Cell count /cu.m.m	Cell type	Gram/ AFB stain
Normal	45-100	15-50	0-5	Mononuclear	-
Viral infection	N	Slightly increased	2-2000	Mononuclear	-
Purulent menin.	Low/absent	markedly increased	5-20000	PMN	GNC/GPC/ GNB/GPB
Tb.Menin	N/low	increased	5-2000	Lympho	AFB



THANK YOU