CHAPTER I

1.1 INTRODUCTION

Despite decades of dramatic progress in treatment and prevention, infectious diseases remain a major cause of death and debility and are responsible for worsening the living conditions of many millions of people around the world. Meningitis is one of the quite serious, with fairly high death rate disease in developing countries like Nepal.

During the first six months of 1983, an epidemic of serogroup A meningococcal meningitis occurred in the Kathmandu valley of Nepal, resulting in 875 cases and 95 deaths. The annual attack rate was 103 cases per 100,000 populations, with a peak attack rate occurring in April (Cochi *et al.,* 1987).

In the present context, meningitis as a disease is still one of the serious manifestation in developing countries. The first recorded major outbreak occurred in Geneva in 1805. The highest burden of meningococcal disease occurs in sub-Saharan Africa, which is known as the "Meningitis Belt". During the first 11 weeks of 2009 (January 1st - March 15th), a total of 24,868 suspected cases of meningitis, including 1,513 deaths, occurred particularly in Nigeria and Niger with predominance of *Neisseria meningitidis* serogroup A ("Epidemiological Record", 2009). Outbreaks of meningococcal disease were reported in Mongolia 1973-1974, Viet Nam 1977, China 1979 and 1980, Nepal 1983, and 1994-1995, Saudi Arabia 1987, and Yemen 1988 (WHO, 1997). The Confenderation of Meningitis Organization (CoMO) has marked April 25th, 2009 and April 24th, 2010 as Meningitis Day to create awareness among people.

Meningitis is the inflammation of the coverings of the brain and/or spinal cord (meninges), which consist of the pia mater, arachnoid mater and dura mater from inner to outside. When inflammation occurs in the dura mater, the disease is termed as pachymeningitis and when the arachnoid and pia mater are involved, it is called as leptomeningits, or meningitis proper (Wilson, 1995). Encephalitis is the inflammation

of brain parenchyma. Concomitant meningitis that occurs with encephalitis is khown as meningoencephalitis (Forbes *et al*., 2007). The blood-brain barrier (BBB) is a diffusion barrier, which impedes influx of most compounds from blood to brain. Increase in BBB permeability is seen in cases with inflammatory condition (Ballabh *et al*., 2004).

Meningitis may be generally classified as acute, subacute or chronic (Kasper *et al*., 2005). Two or more bacterial species are isolated on culture of patient's initial CSF specimen, in case of mixed bacterial meningitis (Marcandin *et al*., 2005). Recurrent bacterial meningitis is defined as two or more episodes of meningitis caused by different bacterial organism or, alternatively , a second or further episodes caused by the same organisms with a greater than 3 weeks interval after the completion of therapy for the initial episode (Tebruegge & Curtis, 2008).

Wide range of microorganisms can cause meningitis. The three causative agents of most cases of bacterial meningitis are *Neisseria meningitidis, Haemophilus influenzae* and *Streptococcus pneumonia*e. They are transmitted from person to person through the exchange of respiratory secretions. Other includes *Staphylococcus aureus*, Group B *Streptococcus*, *Listeria monocytogenes*, *Escherichia coli, Treponema pallidum* (CDC, 1998).

Central nervous system tuberculosis accounts for about 5% of all extra pulmonary tuberculosis and tuberculous meningitis (TBM) is most serious complication. Mortality rates vary from 7%-45%. HIV contributes to increased disease burden of TBM (Bhigjiee *et al.*, 2007).

Chronic meningitis is the common presentation, although fungal CNS infection may present acutely, mimicking bacterial meningitis. The fungal meningitis is associated with different predisposing factors, which include *Histoplasma capsulatum* (infants), Zygomycetes (metabolic disturbances), *Cryptococcus* spp. (Intravenous drug abuse), *Cryptococcus neoformans* (immunocompromised people), and *Candida albicans* (nosocomial superinfection) (Mahon & Manuselis, 2000).

The main parasitic cause of eosinophilic meningitis besides *Angiostrongylus cantoenesis* includes cysticercosis, paragonimiasis, gnathostomiasis and schistosomiasis (Pien & Pien, 1999).

A rare form of self limiting benign recurrent aseptic meningitis with CSF pleocytosis consisting of large friable epitheloid cells termed Mollaret's cells, are found in case of Mollaret's meningitis (Chandrika *et al*., 2009).

Recurrent bacterial meningitis is much less common phenomenon and it has been estimated that annual incidence of recurrent meningitis to be around 0.12 cases per 100,000 adults (Tebruegge & Curtis, 2008).

A case of adult polymicrobial anaerobic meningitis caused by MRSA, *Bacteroides fragilis,* and *Morganella morgagnii*, after colorectal surgery has been reported by Lechuz *et al*. (2000).

Antiobiotic therapy has changed meningitis from an uniformly fatal disease to frequently curable one. Meningitis associated brain injury and neuronal death is not mediated simply by the presence of viable bacteria but occurs as a consequence of the host reaction to bacterial components (Scheld *et al.*, 2002).

Examination of the Cerebrospinal fluid (CSF) is the key to the definitive diagnosis of acute bacterial meningitis. The CSF should be examined in every patient after their clinical suspicion of the possibility of meningitis. The CSF is generally examined for pressure, clarity, presence of Leukocyte, presence of RBCs, concentration of glucose, proteins, Gamma Globulin, Lactic acid and inorganic ions, presence of endotoxin and bacterial antigen. Normal CSF is crystal clear containing less than 5 WBCs/mm^3 in an adult.

Bacterial meningitis is characterized by marked pleocytosis of CSF, consisting predominantly of Polymorphonuclear Leukoctes or Neutrophils (PMN), while in case of aseptic meningitis moderate pleocytosis consisting mainly lymphocytes.

CSF total protein is more likely to be elevated in bacterial meningitis. Glucose levels are usually normal in viral infections and reduced markedly in pyogenic meningitis (Collee *et al*., 1996).

CSF can be tested for the diagnosis of variety of neurological diseases. Lumbar puncture is performed in an attempt to count the cells in the fluid and to detect the levels of protein and glucose. These parameters alone may be extremely beneficial in the diagnosis of central nervous system infections (such as meningitis). Culture examination yields microorganisms that have caused the infection.

Therefore, the present study was done to determine routine parameters and perform microbiological study of CSF from the meningitis suspected patients (3DOL-75years) attending TUTH. The study also aimed to find association and correlation in between different parameters so obtained.

CHAPTER II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To diagnose bacterial meningitis from meningitis suspected patients attending Tribhuvan University Teaching Hospital (TUTH).

2.2 SPECIFIC OBJECTIVES

- To determine bacteriological profile of CSF from suspected meningitis patients.
- To study macroscopic and microscopic observation of CSF.
- To correlate direct microscopy, leukocyte count, protein and glucose level with bacteriological findings.
- To describe antibiotics susceptibility test (AST) of isolates.

CHAPTER III

3. LITERATURE REVIEW

3.1 MENINGITIS

Meningitis is a disease involving inflammation (swelling), or irritation, of the meninges (membrane surrounding brain and spinal cord). It generally refers to inflammatory process of leptomeninges and Cerebrospinal fluid (CSF) (Kumar *et al*., 2005). This inflammation causes changes in the Cerebrospinal fluid (CSF) that surrounds the brain and spinal cord. The brain and spinal cord are relatively well protected within bony compartments from outside and blood brain barrier (BBB) from the blood circulation internally (Chaudhary, 1999). The interior of the cranium is lined with dura mater; the surface of the brain is covered with the pia mater. Between the two in contact with the dura mater lies the arachnoid mater, which is connected to the pia by many fine filamentous processes (arachnoid meninges). These three layers constitute the meninges (Sinnatamby, 2004). When the inflammation occurs in dura mater, the disease is termed as pachymeningitis and when arachnoid and pia mater are involved, it is called as leptomeningitis or meningitis proper (Brain & Walton, 1969).

3.2 ANATOMY & PHYSIOLOGY OF CENTRAL NERVOUS SYSTEM (CNS)

3.2.1 Anatomy of meninges

The cranial meninges are covering of brain that lies immediately internal to cranium. Meninges are composed of three membranous connective tissue layers: dura mater, arachnoid mater, and pia mater.

The dura mater (Latin: dura, “hard”; mater, “mother”), the outermost layer, is composed of tough, nonelastic, dense connective tissue and adheres to the skull and vertebral column. The dura is covered on its innermost surface by squamous epithelial cells. The

dura mater, a bilaminar membarane; is also called pachymeninx (Greek: pachy,"thick"; meninx,"membrane").

The arachnoid (Greek: arachnoeides, "like a cob web"), the middle layer, is composed of fibroblast and dense collagenous and elastic connective tissue, adheres to inner surface of dura by pressure of CSF.

The pia mater (Latin: pia, "tender"; mater, "mother"), the innermost layer. It intimately clothes the surface of brain dipping into the sulci.

The arachnoid mater and pia mater (arachnoid-pia) develop from a single layer of mesenchyme surrounding the embryonic brain: becoming parietal part (arachnoid mater) and visceral part (pia mater) of lepto meninx. CSF filled spaces form within this layer and coalesce to form the subarachnoid or lepto meningeal space (Moore & Dalley, 2006; Gray & Fedorko, 1992).

The extensions of subarachnoid space are called as subrachnoid cisterns. The cerebello medullary cistern lies between the inferior surface of the cerebellum and the posterior surface of medulla. The cistern points continuous with this lies anteriorly to the pons and continued forward into the cistern lying in front of the optic chiasma-the cistern chiamatis (Brain & Walton, 1969).

3.2.2 Blood Brain Barrier (BBB)

For the control of passage of substances in and out of the nervous system two sites of exchange have been postulated. The blood-CSF barrier is concerned with transfer of organic and inorganic materials in between plasma and CSF. The other termed Blood Brain Barrier is the locus of interchange between blood stream and neural parenchyma. Both are profoundly altered by inflammation of meninges. These changes in permeability may contribute to aberration of spinal fluid in meningitis and may affect the course of disease (Harter & Petersdorf, 1960).

The blood-brain barrier (BBB) is a diffusion barrier, which impedes influx of most compounds from blood to brain. Three cellular elements of the brain microvasculature compose the BBB, viz. endothelial cells, astrocyte end-feet, and pericytes (PCs) (Ballabh *et al*., 2004). The features that distinguish cerebral capillaries from other capillaries throughout the body are: adjacent endothelial cells fused together by pentalaminar tight junctions (Zonulae occludens) that prevent intercellular transport, rare or absent pinocytic vesicles, and abundant mitochondria. Furthermore the capillary is surrounded by a basement membrane which in turn is partially surrounded by foot processes (cytoplasmic extensions) of the astrocyte, one of glial cells of brain. The blood brain barrier prevents many toxic substances that might be circulating in the blood from reaching the cells of brain. It also makes it very difficult to deliver certain antibiotics and other types of medication to the cells of brain (Creager, 2004).

Through the blood-brain barrier, only lipid soluble molecules can pass because the plasma membrane of the endothelial cells is composed of primarily of lipid molecules. Essential water soluble molecules like glucose, amino acids etc. are recognized by the carrier proteins and transported across the barrier (Carola *et al*., 1992).

Large protein molecule and most of the antibiotics cannot enter at all. Among the antibiotics penicillin cannot cross where sulfonamides, tetracycline, chloramphenicol and many other lipid soluble drugs can. Inflammations can damage the tight junctions which results in the loss of the barrier function; in such cases penicillin can cross the barrier (Chaudhary, 1999).

3.2.3 Ventricles of brain

There are four ventricles numbered from top of the brain to downwards. They are left and right ventricles of the cerebral hemispheres, the third ventricle of diencephalon, and the fourth ventricle of the pons and medulla oblongata. Each lateral ventricle is connected to the third ventricle of the diencephalons through the small intra-ventricular foramen known as foramen of Monro. The third ventricle is continuous with the fourth

ventricle through a narrow channel called as the cerebral aqueduct (Sylvius of mid brain) (Carola *et al.*, 1992).

Highly vascularized villi of pia mater projects into four ventricles (cavities) within the brain and are covered with ependymal epithelial cells. These projections are known as the choroid plexuses and are the sites at which the fluid component of blood is modified (by secretion and absorption of certain solutes) and secreted into the ventricles. This modified and the secreted fluid is CSF (Gray & Fedorko, 1992).

3.2.4 Cerebrospinal fluid (CSF)

Water of the body together with its dissolved solute is called body fluid which may be extracellular (ECF) or intracellular (ICF). CSF the portion of ECF separated from the other ECF by epithelial membrane is called transcellular fluid (Karim *et al.*, 1996). The cerebrospinal fluid (CSF) is the watery substance that surrounds the brain and spinal cord within subarachnoid space and within the ventricles and the central canal of the spinal cord (Wilson, 1995). It is the clear, colorless liquid that is essentially an ultrafiltrate of blood (Carola *et al.*, 1992).

Formation of CSF: The sites of CSF production are the choroid plexuses (Koroyd: membrane like) network of capillaries (microscopic blood vessels) in the wall of ventricles. The capillaries are covered by ependymal cells that form CSF from blood plasma by filtration (minor) and secretions (major). CSF is formed at the rate of approximately 500 ml/day, which is about 3 times as much as the total volume of the fluid in the entire CSF system. Probably two thirds or more of these fluids originates as secretion from the choroid plexuses in the 4 ventricles mainly in the two lateral ventricles. Additional amount of the fluid are secreted by all the ependymal surfaces of the ventricles and the arachnoid membrane, and small amount comes from the brain itself through the perivascular spaces that surrounds the blood vessels entering the brain (Standring, 2005). First the Sodium ions are actively secreted into the ventricles. These ions being positively charged draw negatively charged ions particularly chloride ions into the ventricles. The presence of all these ions in ventricles fluid increases its osmotic

pressure (OP) within the ventricles and causes water to move by osmosis from the blood into the ventricles. The OP of CSF remains about 160 mm H_2O i.e. about 5 times that of blood (Creager, 2004). Less important transport processes move small amount of glucose into CSF and both potassium and bicarbonate ions out of CSF into the capillaries (Guyton, 1991).

Penetration of substances into brain: The BBB permits certain substances to enter the CSF but excludes others protecting the brain and spinal cord from potentially harmful blood borne substances. CO_2 and O_2 penetrate the brain with ease. Glucose is the major ultimate source for nerve cells. Glucose uptake into the cells is a process of facilitated diffusion mediated by glucose transporter GLUT-1. The brain contains two forms of GLUT-1: GLUT-1 & GLUT-2. Another transporter in cerebral capillaries is an unique Na^+- K^+-Cl^- cotransporter that is stimulated by ET1 & ET3 and apparently induced by humoral factors from astrocyte. It may help keep the brain K^+ low (Standring, 2005).

Circulation of CSF: The fluid flows slowly from the two lateral ventricles of the brain where much of the fluid is formed, through the paired Interventricular foramina to the 3^{rd} ventricle. The fluid then passes along the aqueduct of Sylvius into the 4^{th} ventricle, where a small amount of additional fluid is added. It then passes out of 4^{th} ventricle through 3 small openings, two Lateral foramina of luschka and midline foramen of magendie entering magna a large fluid filled space that lies behind the medulla and beneath the cerebellum.

Some CSF slowly makes its way down the spinal cord to the lumbar cistern. Most of the fluid however circulates slowly toward the top of the brain through the subarachnoid space, toward the cerebrum.

From the cerebral subarachnoid spaces the fluid flows into multiple arachnoid villi that project into large saggital venous sinus and other venous sinuses. Finally the fluid empties into the venous blood through the surface of villi (Standring, 2005).

Absorption of CSF: The CSF is absorbed through arachnoid villi into veins primarily the cerebral venous sinuses. The vill consists of projections of fused arachnoid membrane endothelium (Ganong, 2005). The endothelium of arachnoid villi shows pores. When the pressure of the CSF within the arachnoid villi is high, the pores open up and the fluid escapes into the venous blood, but the venous blood cannot enter the villi (Chaudhary, 1999).

The CSF is secreted and reabsorbed at the same rate, so the volume remains constant. Thus the pressure within the ventricles and other cavities containing CSF remains constant. However conditions such as tumor, infections and hemorrhages in the brain can disrupt the normal flow of CSF causing pressure to change (Creager, 2004). At 112mm CSF formation and absorption equals and at 68mm CSF absorption is zero (Ganong, 2005). In man the rate of formation of CSF is 20ml/hrs or 500ml/day (Burtis *et al*., 2006). The rate of secretion is 0.35-0.40 ml/day. The content of CSF in man is 150 ml of which about 30ml is in ventricular system and remainder is in the subarachnoid space (Guyton, 1991).

Function of CSF: The major function of CSF is to cushion the brain with its solid vault since the brain and CSF have approx same specific gravity so that the brain simply floats in the fluid. It also acts as fluid buffer, regulates the volume of cranial content, maintains balance of extracellular fluid, acts as a medium through which nutritive as well as waste products are interchanged in brain and forms the supporting framework for arteries, veins and venous sinuses (Guyton, 1991; Standring, 2005).

Composition and appearances of CSF: The fluid will appear turbid with more than 200×10^6 WBC/L or 400×10^6 RBC/L. The presence of bacteria or aspirated epidural fat also causes turbidity of specimen. Clot formation may occur when protein concentration is significantly elevated. CSF may be colored yellow by bilirubin or rarely by carotenoid; red (or more usually pink/orange) by oxyhaemoglobin; or brown by methaemoglobin. Pigmentation due to bilirubin is called xanthochromia. This term has also been used to describe discoloration due to oxyhaemoglobin (Marshall & Bangert,

1995). Xanthochromia is present in more than 90% of patients within 12 hours of subarachnoid hemorrhage onset and in patients with serum bilirubin levels between 10 to 15 mg/dL (171-256.5 µmol /L) (Seehusen *et al*., 2003).

CSF vol: Child:- 60-100ml
Adult:- 100-160 ml

CSF (Water:- 99.13%, Solid:- 0.87%, pH:-7.3)

Physical characteristics of CSF in normal condition:-	
Appearance:	Clear, colourless
Specific gravity:	1.003-1.008
Osmolality(mosm/kg H_2O)	289.0
Pressure: newborn	30-80mm water
children	50-100mm water
adult	70-200mm water (average 125)
(Kelle & Neil, 1972)	

Diagnostic value of CSF sample: CSF is invaluable as the diagnostic aid in evaluation of an inflammatory conditions, infectious or non infectious, involving brain, spinal cord and meninges. Combining a set of CSF variables referred to as routine parameters (i.e. determination of protein, albumin, immunoglobulin, glucose, lactate and cellular changes, as well as specific antigen and antibody testing for and infectious agents) will increase diagnostic sensitivity and specificity (Deisenhammer *et al*., 2006). Examination of CSF is essential when acute or chronic infection of brain or meninges is suspected (Munro & Edward, 1995). CSF analysis also allows immunologic confirmation of certain infections (e.g. Lyme disease) and fractionation of CSF proteins (e.g. myelin basic protein) (Waldman, 2005).

Except in unusual circumstances, a lumbar puncture (spinal tap) is one of the first steps in the workup of the patient with suspected CNS infection, in particular meningitis (Munro & Edwards, 1995). If intracranial pressure is raised lumbar puncture may occasionally be followed by herniation of the brain stem through the foramen magnum

(coning) potentially a fatal event (Collier *et al.*, 1998). CSF is collected aseptically inserting a needle into subarachnoid space, usually at level of lumbar spine (Forbes *et al.*, 2007). Lumbar puncture for CSF examination is urgently warrentd in individual in whom meningitis is clinically suspected (Razonable & Keating, 2010)

Only 3-5ml of fluid should be collected for the removal of larger volume may lead to headache, and the rate of collection should be slow; about 4-5 drops a second (Collee *et al.*, 2006). The puncture can be performed either between the third and fourth lumbar spine (L3-L4) or fourth and fifth spine (L4-L5) (Brain & Walton, 1969). CSF also can be obtained from the cisterna magna by a tap below the external occipital protuberance (Waldman, 2005).

After making the patient ready for lumbar puncture, the skin is cleaned with alcohol and ether and painted with iodine: a local anesthetic may be applied so as to comfort patient (Brain & Walton, 1969). The needle is introduced midway between the spinous processes in the sagittal plane. A 20-gauge needle for adults, or a 22-gauge needle for children, is used typically (Waldman, 2005). After the pressure has been measured, the fluid is collected in two sterile screw capped containers consecutively, about 3 ml each. The needle is withdrawn (Brain & Walton, 1969). Only a few milliliters are needed for basic studies (e.g. protein, glucose). Specialized tests that require concentration of the CSF (e.g. cell count, specific antibody studies) require more CSF (Waldman, 2005).

3.3 ETIOLOGICAL AGENTS OF MENINGITIS

3.3.1 Bacterial meningitis

The etiological agents of bacterial meningitis depend on the season of year, the age, ethnic background, and geographic location of the patients. *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumonia* are the leading causes of bacterial meningitis. Other frequent etiologic agents of bacterial meningitis include *Staphylococcus aureus*, *Escherichia coli*, *Borrelia burgdorfi sensu lato*, *Treponema pallidum*, *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Streptococcus* spp.

Other than group B, *S. epidermidis, Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Acinetobacter* spp. (Gray & Fedorko, 1992; Domelier *et al*., 2006), *Pseudomonas aerugonisa* (Stevens *et al*., 1984).

The rare causative agents of bacterial meningitis are also known. Bacterial meningitis caused by *Bacteroids fragilis, Achromobacter xylosoxidans, Gordona aurantiaca (Rhodococcus aurantiacus), Lactobacillus* spp., *Corynebacterium aquaticum, Streptococcus mitis, Pasteurella multocida, Haemophilus influenzae* type f, *Psychrobacter immobilis* have been reported by Gray & Fedorko (1992). Bacterial meningitis due to *Brucella* spp. *Campylobacter fetus, Leptospira interrogans, Mycoplasma pneumoniae*, Coagulase negative Staphylococci, *Tropheryma whipplei, Rickettsia* spp., *Coxiella burnetti, Salmonella* spp. and *Flavobacterium meningosepticum* have been reported by Deisenhammer *et al*. (2006). Neonatal meningitis due to *Leuconostoc mesenteroides* which was vancomycin resistant was reported by Friedland *et al*. (1990). *Serratia marcescens* meningitis following ear surgery was reported by Theccanat *et al*. (1991). *Helicobacter cinaedie,* previously known as *Campyloacter* like organism Type I and *C. cinaedi* was known to cause septicaemia and meningitis in newborn (Orlicek *et al*., 1993). Meningitis due to *B. anthracis* has been reported by Dixon *et al*. (1999); & Tabatabaiae & Syadati (1993). A report of 1st cause of *Bacteroids thetaiotaomicron* meningitis was published by Feuillet *et al.* (2005). *Gemella haemolysans* rare cause of bacterial meningitis in 17 months old boy was reported by Ozkalay *et al*. (2007). First reported case of *Ureaplasma urealyticum* meningitis in an adult was reported by Geibdorfer *et al.* (2007). *Streptococcus pyogenes* is an agent rarely associated to meningitis and corresponds to 0.2-1% of cases (Arnoni *et al*., 2007). *Streptococcus* is found to cause meningitis and septic shock (Vamer *et al*., 2008). *Stomatococcus mucilaginosus* meningitis has been reported in 2 months old child (Rizvi *et al*., 2008). *Stenotrophomonas maltophila* meningitis associated with neurosurgical procedures has been reported by Yemisen *et al.* (2008). An infrequent case of gonococcal meningitis in pregnant adolescent has been reported by Martin *et al*. (2008). Member of CC17, a notorious emerging nosocomial

clone of *E. faecium* was knowm to cause enterococcal meninigitis (Jaspan *et al*., 2010). *Streptococcus salivarus* meningitis report was published on MMWR, 2010.

3.3.2 Viral meningitis

Viral cause of meningitis includes Herpes simplex virus (HSV) 1 & 2, varicella-zoster Virus (VZV), enteroviruses, echoviruses, coxsackie virus (A & B), Human immunodeficiency virus (HIV) type 1 & 2, Epstein-Barr virus (EBV), cytomegalovirus, adenovirus, Human T-cell Leukaemia Virus type I (HTLV-I), Influenza and Parainfluenza viruses, Lymphocytic chorio-meningtis (LCM) virus, mumps virus Polio viruses, Rabies virus, Rota virus, rubella virus, Sandfly virus (Cheesbrough, 2000; Deisenhammaer *et al*., 2006).

3.3.3 Fungal meningitis

The fungi causing meningitis includes: *Aspergilllus fumigatus*, *Candida* spp., *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Blastomyces dermatitis* (Cheesbrough, 2000)

3.3.4 Parasitic meningitis

Wide range of parasites is known to cause meningitis. They are *Acanthaemoeba* spp. *Echinococcus granulosus*, Cysticercus (*Taenia* spp.), Malarial parasites, *Nagleria fowleri*, *Pargonimus westermani*, *Schistosoma* spp., *Strongyloides stercolis*, *Toxoplasma gondii*, and *Trichinella spiralis* (Deisenhammer *et al*., 2006).

3.4 PATHOGENESIS AND PATHOPHYSIOLOGY

The pathogenesis and pathophysiology of bacterial meningitis follows as: nasopharyngeal colonization, local invasion, bacteraemia, endothelial cell injury, meningeal invasion, subarachnoid space inflammation, increased CSF outflow resistance, hydrocephalus, interstitial edema, increased intracranial pressure, decreased cerebral blood flow. Endothelial cell injury and subarachnoid space inflammation causes increased BBB permeability, vasogenic edema and increased edema.

Subarachnoid space inflammation causes bacteraemia, cytoxicity edema, cerebral vasculitis, cerebral infarction, leading to decreased cerebral blood flow (Tunkel & Scheld, 1993).

Infants younger than 32 weeks gestation receive little of maternal Immunoglobulin received by full term infants. The meninges also show increased permeability. Inefficiency in the neonate alternate complement pathway comprises their defense against encapsulated bacteria. T cell deficient migration and B cell activity also are compromise. Finally deficient migration and phagocytosis by Neutrophil contributes to neonatal vulnerability to pathogen of even low virulence (Dredge & Krishnamoorty, 2010; Yu & Grauaug, 1963).

3.4.1 Routes of an infection

Inflammation of the meninges occurs either as a primary or secondary to disease in some other parts of the body (Smith & Easmon, 1990). Infection may originate in the meninges (meningitis) or in the brain (encephalitis) and then spread from one site to another (Wilson, 1995). There are four principal routes by which infectious microbe enter the nervous system (Graham & Lantos, 2002).

Direct spread from an infected site: It may occur as a result of fracture in the skull, either in case of penetrating wounds of cranial vault or fractures of the base when organisms may spread to the meninges from the nasopharynx (Brain & Walton, 1969). The extension of an infection close to or contiguous with the CNS can occasionally occur; examples of such infections include otitis media (infection of middle ear), sinusitis and mastoiditis (Baron *et al*., 1994). Organisms may also be introduced by surgical procedures such as lumbar puncture (Brain & Walton, 1969) or congenital malformations (Graham & Lantos, 2002)

Haematogenous spread: In this case, meningitis occurs by entry of organisms into the subarachnoid space through the choroids plexus or through other blood vessels of the brain. This is the most common way that CNS gets infected (Baron *et al.*, 1994). In such

cases, meningitis follows bacteraemia. It may be the only or the principal manifestation of this, as in so called primary pneumococcal meningitis, meningococcal meningitis, or the infection of the meninges may be secondary to focal infection elsewhere in the body, for example, pneumonia, empyema, osteomyelitis, erysipelas, typhoid fever, etc. in which case bacterium may or may not be associated with endocarditis due to infecting organism. Tuberculous meningitis may thus be the part of a general miliary dissemination of tuberculosis (Brain & Walton, 1969). Retrograde venous spread can occur through anastomotic connection between the veins of face and cerebral circulation (Graham & Lantos, 2002).

Anatomic defects in CNS structures: Anatomic defects as a result of surgery, trauma, or congenital abnormalities can allow microorganism easy and ready access to the CNS. Local extension occur secondary to an established infection in an air sinus, most often the mastoid or frontal; an infected tooth; or a surgical site in the cranium or spine causing osteomyelitis, bone erosion and propagation of an infection into CNS (Graham & Lantos, 2002).

Travel along the nerves leading to the brain (direct intraneural): Some viruses spread to CNS by invading and travelling through cranial nerves (Herpesviruses) or peripheral nerves (Rabies viruses) (Mahon & Manuselis, 2000).

Intracellular microorganisms use wide variety of mechanisms to enter their human hosts: through contaminated food (e.g. *L. monocytogenes*, *Salmonella*, and *Brucella* spp.), through the bite of infected arthropods (*R. rickettsii*, *R. prowazekii*, and *E. chaffeensis*), and through inhalation (*M. tuberculosis* and *C. burnetii*). This is contrast to extracellular neuroinvasive bacteria such as *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*, which infect humans via. respiratory tract (Drevets *et al.*, 2004). Neonates are colonized by *S. aureus* soon after birth; major niches include umbilical stump, perineal area, skin, and gastrointestinal tract. Later in life, major niches include anterior nares (Zumo *et al.*, 2000).

3.4.2 Bacterial virulence factors

Several bacterial virulence factors are responsible for initiation of bacterial meningitis.

Fimbriae: The fimbriae of *N. meningitidis* and *H. influenzae* mediate adherence of an organisms to nasopharyngeal epithelial cells (Stephens & McGee, 1981).

Polysaccharide capsule: Among the six encapsulated types of *H. influenzae* (a through f) type b strains constitute less than 5% of nasopharyngeal isolates, although more than 95% of meningeal and systemic infections are caused by type b strains (Smith *et al*., 1987). *S. pneumoniae* adheres to nasopharyngeal cells and was most found on desquamated cells. Nasopharyngeal mucus may provide a protected nidus from which pneumococci may spread (Freter, 1980). About 84% of cases of neonatal meningitis due to *E. coli* are caused by strains bearing the K1 antigen, which is antigenically related to capsular material of serogroup B meningococci and type III group B streptococci (Robbins *et al.,* 1974). Antiphagocytic properties of the polysaccharide capsule are the key to the organism's virulence (Forbes *et al*., 2007).

Other bacterial components: Hemocin, the bacteriocin produced by *H. influenzae*, has been shown to be strongly associated with type b encapsulated strains and may play a role in host nasopharyngeal colonization and/or systemic invasion by this organism. Studies with pneumococcus have identified two toxins responsible for permanent loss of neurons in the hippocampus. Pneumolysin, a pore forming toxin, and hydrogen peroxide produced by the bacterium induce apoptosis by release and translocation of mitochondrial apoptosis-inducing factor. For group B streptococci, an important pathogen in neonatal meningitis, hemolysin has recently been identified as the toxin that triggers host cell apoptosis (Flier *et al*., 2003).

Bacterial hemolysin and cytokines have direct lethal effects on host tissues (Mertsola *et al.*, 1991). Many pathogenic *Neisseria*, *Haemophilus*, and *Streptococcus* species produce IgA1 protease that cleave IgA in the hinge region of immunoglobulin

molecule; these enzymes have pathogenic role by facilitating adherence of bacterial strains to mucosal through local destruction of IgA (Mulks *et al.*, 1982).

Neisseriae species have additional mechanisms of an antigenic variation such that a single clone of bacterial gene gives rise to multiple antigenic types allowing newly arisen antigenic variants to escape immune response (Serkin & Seifert, 1998).

Pneumococci of all serotype are known to secrete a highly specific endopeptidase which cleaves a Pro-Thr peptide bond in the hinge region of human IgA1 (Colier *et al*., 1998).

3.4.3 Clinical outcomes

Cerebral blood flow: Cerebral blood flow initially increases as a result of vasoactive neuropeptide release and then steadily decreases due to vasoconstriction and pressure of surrounding cerebral edema (Pfister *et al*., 1990). Cerebral blood flow was increased when systemic blood pressure was raised and decreased when blood pressure was lowered, indicating that the flow was pressure passive. These blood flow alteration may lead to regional hypoxia, increased concentration of Lactate in the brain secondary to utilization of glucose by anaerobic glycolysis, and CSF acidosis (Tureen *et al*., 1992). Nitric oxide freely diffuses into the cytosol and stimulates guanylate cyclase, which transforms guanylate triphosphate into cyclic guanylate monophosphate, raising the intracellular cyclic GMP levels and causing relaxation of smooth muscles (Leib *et al*., 1998). Decrease in cerebral flow is accompanied by steady increase in intracranial pressure in CSF and CSF Lactate concentration, which is a sign of deleterious metabolic changes (Tureen *et al*., 1992).

Bacterial meningitis exerts profound effects on blood vessels coursing through the subarachnoid space. Phlebitis of the major cortical draining vessels or dural sinuses or both may result in thrombosis with secondary brain infarction, focal neurologic deficits and prominent seizures activity (Tunkel & Scheld, 1993).

Neuronal cell death: In bacterial meningitis, hypoxia, neurotoxic bacterial products, and host mediators combine to cause neuronal injury. Whether the damage is bacterial or leukocyte derived, the final toxic element is often the free radical. These oxidants including reactive Oxygen intermediates and reactive Nitrogen intermediates have toxic effects on neurons (Quagliarello & Scheld, 1992).

Inflammatory mediators in the CSF trigger the activation of caspases. Activation of caspases results in neuronal apoptosis. This neuronal loss may explain some of the learning and memory difficulties seen in survivors of meningitis (Nau *et al*., 1999).

Oxidants such as reactive oxygen species and reactive nitrogen intermediates are terminal mediators of brain damage in bacterial meningitis (Koedel *et al*., 2002).

Neuronal cell death pathways may be divided into necrotic pathways, caspase-independent apoptotic pathway, and caspase-dependent apoptotic pathway (Flier *et al*., 2003).

Alternation of blood brain barrier (BBB): Bacterial meningitis, like many other disease state increases the permeability of BBB. The major sites of BBB are arachnoid membrane, choroid plexus epithelium, and cerebral microvascular endothelium. The increased BBB permeability seen in this disorder must occur at the level of choroid plexuses epithelium, the cerebral microvascular endothelium or both. The increased BBB permeability that occurs during bacterial meningitis at the level of cerebral capillary endothelial cell may result from the separation of intercellular tight junctions, from increased pinocytosis or from both alternations (Quagliarello *et al*., 1986). Matrix metalloproteinase released by the leukocyte digest intercellular tight junction and the basal membrane during diapedesis (Syrogiannopoulos *et al.*, 1987). These morphologic changes correlated with the functional penetration of albumin across the BBB, with the highest values of albumin entry occurring within 18hrs (Lesse *et al*., 1988).

Bacterial toxins in blood or Cerebrospinal fluid (CSF), such as Gram negative Lipopolysacharide (LPS), Gram positive peptidoglycan, and cytotoxins, engage Toll

like receptors of endothelial cells and activate their downstreaming signalling cascades. The endothelial cells then release mediators, such as Tumor Necrosis Factor (TNF-α), Nitric Oxide, and Metalloproteinase-2 (MMP-2), which increases endothelial permeability (Quagliarello & Scheld, 1992).

Vascular endothelial growth factor (VEGF) is intrathecally released from invading Neutrophils in CSF in bacterial meningitis. VEGF induces the formation of transcellular canals called vesiculo-vacuolo organelles and causes loss of intercellular tight junctions (Feng *et al*., 1998).

Subarachnoid space inflammation: Pneumococcal cell wall lytic products, released during antibiotic induced autolysis during treatment of bacterial meningitis, LPS and Teichioc acid contribute to host inflammatory response in subarachnoid space (Tunkel & Scheld, 1993). Elevated concentration of PGE2, prostacyclin, IL-1β, and TNF in CSF were found in majority of an infants and children with bacterial meningitis (Mustafa *et al.*, 1989). Intercellular Calcium is an important mediator of an inflammatory cellular response (Flier *et al*., 2003). The invasion of the bacteria into the subarachnoid spaces induces an inflammatory response in bacterial meningitis. The activation of this coagulation cascade in brain blood vessels leads to thrombosis (vasculitis, endarteritis) and cortical infarctions. The resulting hypoperfusion and hypoxia aggravate the inflammation, contributing the blood-CSF barrier breakdown, brain edema, neuronal death and brain injury (Kowalik *et al*., 2007). Inflammation activates the coagulation cascade, since the advanced bacterial meningitis is associated with thrombosis and ischemia. Several plasma enzyme systems are activated during inflammation. These include the complement system, vasoactive nonapeptide bradykinin, and vasoactive amine histamine (Lorenzl *et al.*, 1996).

Increased intracranial pressure: The major element that contributes to an increase in intracranial pressure during bacterial meningitis is the development of cerebral edema, which may be vasogenic, cytotoxic, and/or interstitial in origin and may result in life threatening cerebral herniation and other complication (Nugent *et al*., 1979). Vasogenic

cerebral edema is principally a consequent of an increased BBB permeability. Interstitial edema reflects obstruction of flow in normal CSF pathways. An increase in outflow resistance to CSF movement may cause interstitial brain edema and/or the resultant hydrocephalus during bacterial meningitis (Scheld *et al.*, 1980). Release of vasoconstrictive agents, such as the endothelins, and vasodilator agents, such as nitric oxide, causes loss of autoregulation of cerebral perfusion pressure (Flier *et al.*, 2003).

Meningism: The occurrence of meningeal signs in absence of meningitis, which has been variously described as serious meningitis or meningism, is due to disturbance in the osmotic relationship between the blood and the CSF. At the onset of acute infectious diseases (pneumonia, typhoid fever, or the acute exanthemata and so forth), there is retention of fluid in the body and dilution of the blood. The dilution of the blood makes it hypotonic to the CSF. The state of abnormal relationship between the osmotic pressure of the blood and the CSF is transient one and clears up with diuresis or when sufficient time has elapsed for equilibrium between the rate of formation and absorption of the CSF to be established. When this occurs the symptoms disappear (Merritt, 1967).

3.4.4 Host defense mechanisms

Cytokines: Cytokines are key regulators of an immune response, released by macrophages, microglia, astrocytes, ependyma, and endothelia. Four major avenues exist to intervene in the inflammatory response at the cytokine level: inhibition of the cytokine gene, blockade of cytokine itself or by cleavage to activate the procytokine, and antagonism of cytokine receptor and its down streaming signal. Anti inflammatory cytokines such as IL-10 and transforming growth factor beta are known to downregulate the host inflammatory response (Koedel *et al.*, 1996).

Leukocytes: One of the hallmarks of bacterial meningitis is the development of neutrophilic pleocytosis within the CSF. The complement component C5a has been suggested as one chemotactic substances in CSF (Tonnesen *et al.*, 1984). The anaphylotoxins C3a and especially C5a are potent chemotaxins. The inflammatory

functions of C3a and C5a are mediated through the specific C3a receptor and C5a receptor (CD88) (Zwijnenburg *et al.*, 2006).). Chemokines contribute to leukocyte recruitment by activating integrins and by promoting the migration of adherent leukocyte across the endothelium and through the extracellular matrix (Ehrlich *et al.*, 1998). CXCL8, CXCL1, CCL2, CCL3, CCL4, AND CCL5 (RANTES) were measured in the CSF of patient with bacterial meningitis, whereas in control CSF samples CXCL1, CCL3 and CCL4 levels were undetectable and CXCL8 and CCL2 were present in very low concentrations (Spanaus *et al.*, 1997). Besides chemokines other cytokines also exert chemoattractant properties. Elevated levels of IL-6, MMP-9 (Matrix Metalloproteinase- 9), UparR (urokinase type Plasminogen Activator Receptor) system is found in bacterial meningitis and correlated with pleocytosis (Zwijnenburg *et al.*, 2006).

The absence of humoral immune components in CSF prevents opsonization of bacteria and subsequent effective phagocytosis and killing by Neutrophils. In clinical case series, in which bacterial meningitis patient with an initial low CSF leukocyte count, before initiation of an antiobiotic therapy, generally have a worse outcome than patient with initially higher CSF leukocyte counts (Spananus *et al.*, 1997).

Complement: Complement components in CSF are usually absent or present in only minimal concentrations. Meningeal inflammation leads to increased but low, concentration of complement in CSF. The important of this relative complement deficiency in normal and infected CSF may be critical, since a specific antibody and/or complement are essential for opsonization of encapsulated meningeal pathogens, and efficient phagocytosis (Simberkoff *et al.*, 1980).

The capsular polysaccharide of *S. pneumoniae* activates the alternative complement pathway, resulting in cleavage of C3 and subsequent attachment of C3b to bacterial surface, thereby facilitating opsonization, phagocytosis and intravascular clearance of an organism (Broome *et al.*, 1980). The complement cascade is also activated by *H. influenzae* type b. This cascade is essential host defense mechanism in protection

against invasive disease by *N. meningitidis*. Patients with deficiencies in the terminal complement component (C5, C6, C7, C8 and perhaps C9), the so called membrane attack complex, are particulary prone to infection with neisserial species, including *N. meningitidis* (Ross & Densen, 1984).

3.5 TYPES OF MENINGITIS

Infectious meningitis is broadly classified into acute pyogenic (usually bacterial meningitis), subacute, chronic (usually Tuberculous, Spirochetal or Cryptococcal), aseptic (usually acute viral meningitis) (Baron *et al*., 1994; Kumar *et al*., 2005) and Recurrent (Arnoni *et al*., 2007).

3.5.1 Acute bacterial meningitis

Bacterial meningitis is an acute purulent infection within the subarachnoid space. It is associated with a CNS inflammatory reaction that may result in decreased consciousness, seizures, raised intracranial pressure (ICP), and stroke (Kasper *et al*., 2005). Effective antimicrobial agents markedly reduce mortality associated with meningitis. In an immunosuppersed patient, purulent meningitis may be caused by other agents such as *Klebsiella* or an anaerobic organism, which may have an atypical course and uncharacteristic CSF findings (Quagliarello & Scheld, 1997).

The microorganisms that cause acute pyogenic meningitis vary with the age of patient (Durand, 1993). Which the etiological agents involved as the cause of any occurrence of bacterial meningitis depends upon several underlying host factors, but age of the patient is most important (Baron *et al*., 1994). On the basis of this, they may be “Neonatal meningitis”, “Meningitis in children”, and “Meningitis in adults”.

Neonatal meningitis: Neonatal meningitis is inflammation of the meninges due to bacterial invasion in the first 90 days of life (Dredge & Krishnammorty, 2010). Neonatal meningitis is known to occur in about 1/ 6 th of all invasive pneumococcal

infection. The highest meningitis attack rate occurs in 1st week of life when the invading organisms are probably acquired and infection common in winter (Collier *et al*., 1998).

Neonatal meningitis is known to happen between birth and first 28 days of life. The mortality varies based on the treatment with survival rates of 17% to 29% and with complication rate of 15% to 68%. Among the predictive factors for the diagnosis of this condition are premature birth, newborn weight, type of bacteria, predisposition of or microbial germ, and length of treatment and complication (Silva *et al.,* 2007).

Among US neonates, group B Streptococci are the most commonly identified organisms, implicated in roughly 50% of all cases of bacterial meningtis and *E .coli* accounts for another 20%. *Listeria monocytogenes* is the third most common pathogen with 5-10% of cases. Studies from an underdeveloped country suggest that Gram negative bacilli, especially *Klebsiella* organisms and *E. coli* may be more common than group B streptococci. In their study from Africa to South Asia, Tiskumara *et al.* noted that 75% of cases of late onset meningitis were due to Gram negative bacilli (Dredge & Krishnamoorthy, 2010). In the review studies from Asia, Africa and Latin America Zaidi *et al.* (2009) reported that most common organisms were *Klebsiella* spp., *E. coli* and *S. aureus* (Dredge & Krishnamoorthy, 2010).

Meningitis in children: Organisms identified in cases of bacterial meningitis in 889 French children aged 29 days to 18 years, *Neisseria meningitidis* 395 (44.4%), Serogroup B 232 (26.1%), Serogroup C 130 (14.6%), Serogroup A, W 135, or Y 11 (1.2%), Unknown group 22 (2.5%), *Streptococcus pneumoniae* 374 (42.1%), *Streptococcus agalactiae* 50 (5.6%), Other Streptococci 9 (1%), *Haemophilus influenzae* type b 22 (2.5%), Other bacteria 39 (4.4%), *Escherichia coli* (19), *Haemophilus influenzae* non-b type (13), *Listeria monocytogenes* (4), *Pasteurella multocida* (2), and *Fusobacterium necrophorum* (1) (Dubos *et al*., 2008).

Of 482 children included in analysis, 210 (44%) children had meningitis caused by *H. influenzae* type b, 106 (22%) by *S. pneumoniae,* 60 (12%) by *N. meningitidis,* and 14 (3%) by another organisms; 5 *Salmonella enteritidis,* 2 *E. coli,* 2 Group B

Streptococcus, 2 *Haemophilus* type a, 1 *Pseudomonas aerugonisa*, 1 *Staphylococcal aureus* and 1 *Acinetobacter* spp. Malnutrition may be an important cofactor explaining poor outcome of childhood bacterail meningitis (BM) in developing countries. A study conducted in Latin America children (2months-5 yrs) showed that underweight child is prone to have a severe form of bacterial meningitis with more sequel and deaths than his/her normal weight counterparts. Overall severe underweight increased risk to die of bacterial meningitis 5.85 times, moderate underweight 2.55 times, mild underweight 1.98 times (Roine *et al.*, 2010).

Meningitis in an adult: Respiratory tract infection is the primary route of entry of many etiological agents of meningitis in adults. Alcoholism, splenectomy, diabetes mellitus, prosthetic devices, immunosupression contribute to increased risk. Important agents are meningococci, pneumococci, *Listeria monocytogenes*, and less commonly *Staphylococcus aureus* and Gram negative Bacilli. The later organisms reach to meninges via. hematogenous seeding from various sources, including urinary tract infections (Baron *et al*., 1994). Two groups of adults are at higher risk for group B *Streptococcus* infection: puerperal women and patient with serious underlying disease (Durand *et al*., 1993). Meningitis in 16 year old caused by *Leuconostoc* spp. was reported from South Africa by Coovadia *et al*., 1987. In 1998, 28 adults patients with Bacterial meningitis aged 15 years or above, *S. agalactiae* was isolated in 11 cases, *S. pneumoniae* in 3 cases, *K. pneumoniae* in two cases and *Pseudomonas pseudomallei* in 1 case (Smith *et al*., 2000).

Neisserial infection: The first report of bacterial infection underlying meningitis was done by the Austrian bacteriologist Anton Weichselbaum, who in 1887 described the *meningococcus* ("Wikipedia", 2009). The existence of multiple serotypes of Neisseriae results in meningitis in some people on exposure to new strain (Serkin & Seifert, 1998).

Twelve subtypes or serogroup of *N. meningitidis* have been identified ad four (A, B, C, and W135) are recognized to cause epidemics. A, B, C, X, Y, Z, Z' (29E) and W135 having pathological importance has been determined. Further serogroup H, I, K, L have

also been described but their pathological significance is not yet clear. A group D was described by Branham (1958) but no capsular polysaccharide specific for this group has yet been demonstrated (Collee *et al*., 2006).

N. meningitidis causes both endemic and epidemic disease principally meningitis and meningococcemia (CDC, 1997).

The risk of epidemic meningococcal disease differs in between serogroups. Serogroup A, B and C can cause outbreaks. Other serogroup (group D, E29, W135 and Z) have so far been associated with outbreaks. Serogroup A meningococcus has historically been the main cause of epidemic meningococcal disease and still dominates in Africa during both endemic and epidemic periods. The major and most exclusive epidemics of meningococcal meningitis have also been almost exclusively associated with serogroup A, as in Brazil (1974), North America and Europe prior to the mid 1950s, Finland (1974), Nepal (1983-1985), Rwanda (1978), Saudi Arabia (1987), Sudan Ethiopia (1988-1989), Kenya, Uganda and Burundi (1982-1992), United republic of Tanzania or in West Africa especially in Burkina Faso and Mali (1995-1997). Serogroup B generally associated with sporadic disease mey cause some upsurges or outbreaks as in Norway (mid 1970), Cuba (1982-1984), Chile (1986, 1993), Brazil (1989) and Oregon, USA (1994). Serogroup C like serogroup A, has been reported for large outbreaks in Brazil (1972-1974), Vietnam (1977-1978), Northern Nigeria (1975), Burkania Faso and Mali (1979) (WHO, 1997).

Meningococcal meningitis commonly designated as Cerebrospinal Meningitis, is the only form of bacterial meningitis which causes epidemics (WHO, 1998).

A characteristics feature of menigococcal meningitis is presence of petechiae or purpura in skin (Collier *et al.*, 1998).

Apart from epidemics, meningococcal meningitis occurs sporadically thoroughout the world with seasonal variation and accounts for variable proportion of endemic bacterial meningitis (WHO, 1998).

Serogroup A-C of *N. meningitidis* accounts for most cases of meningococcal meningitis thoughout the world. Although serogroup A dominates across Africa serogroup B and C are responsible for most cases in an industrialized country. Serogroup C meningococcal meningitis has been recorded in Africa, the Middle East and the Indian sub continent. It has been responsible for 25-65% of all meningococcal cases in Europe, America and Australia (Van *et al.*, 2000).

The most prevalent serogroups are serogroup B (62%) and more virulent serogroup C (22%). Meningococcal meningitis is also more common among the following groups; persons of black race, smoke, person exposed to overcrowding and among binge drinkers (Boos *et al.*, 2004).

The distribution of the Hb receptor gene hmbR was investigated among disease and carriage *N. meningitidis* isolates, revealing gene was detected at significantly higher frequency among disease isolate than among carriage isolates (Harrison *et al.*, 2009).

Currently available meningococcal vaccines that consists of pure capsular polysaccharide (Serogroup A, C, Y and W135) are generally safe and efficacies in an adults and children under age >2yrs (Shao *et al*., 2009).

Serogroup B and W-135 were 2 predominant serogroup to cause pediatric meningococcus meningitis in children younger than 1 year. The identified serogroups were B (43.75%), W135 (31.25%), A (6.25%), Y (6.25%) and undetermined (12.5%) (Tuan *et al*., 2009).

Pneumococcal infection: *S. pneumoniae* is an important pathogen which to high degree is ascribed to its polysaccharide capsule (Colier *et al.*, 1998).

Meningitis in individuals at the extreme of age infants, young children and the elderly is commonly caused by *S. pneumoniae* (CDC, 1998).

In sub Saharan meningitis belt pneumococci were the leading causative agents if nonepidemic meningitis and other bacteriaemic disease followed by Hib. Mortality was

high and deaths were predominantly due to pneumococcal meningitis; the overall hospital case fatality rate in 17 studies was 45% (549 of 1211 patients) (Peltola *et al*., 2001).

The most common etiologic agent of bacterial meningitis in the latest US surveillance study was *S. pneumoniae* (61) (Dery & Hasbun, 2007).

A recent review reports the serotypes of 11,556 pneumococcal isolates from patients under the age of 18 years detected in 16 European countries. Serotype 14 was the commonest (19.47%), followed by serotypes 6 (16.26%), 9 (15.14%), and 23 (10.29%) (Tzanakakai & Mastrantonio, 2007).

Streptococcus pneumoniae is the commonest cause of community acquired bacterial meningitis in youg children and the elderly. Recently, a novel penicillin, cephalosporin, and macrolides resistant and Multi-Drug Resistant (MDR) strains of DRSP was isolated in Taiwan (Karunanayake *et al.*, 2007).

Of total 401 CSF specimen cultured, culture positive observed with 55 (13.7%) cases in which *S. pneumoniae* was identified from 20 (36.4%) culture positive cases observed by Alam *et al*. (2007).

Prior to the development and the use of 7-valent protein polysaccharide pneumococcal conjugate vaccine (PCV 7), Invasive pneumococcal disease (IPD) was considered the predominant cause of morbidity and mortality throughout the industrialized and non industrialized countries (Dery & Hasbun, 2007).

Pneumococcal meningitis can result in substantial morbidity and mortality, not only in developing countries, but also in developed countries. It has high case fatality rate and up to 30% of those who survive infection may develop long term sequel, such as neurological conditions or impaired hearing (Prieto *et al*., 2009).

***Haemophilus influenzae* meningitis:** Most childrens are colonized with a species of *H. influenzae* only 2-55% harbours Hib. The organism is able to penetrate the respiratory

mucosa and enters the blood stream. This is the result of combination of factors and subsequently the organisms gain access to CSF, where infection is established and inflammation occurs. Meningitis is the most common severe form of Hib disease, in most countries. However more cases and deaths are due to pneumonia than meningitis (CDC, 1998).

Haemophilus meningitis is rare in adults. In a study conducted by Tang *et al.* (1998) in Taiwan, meningitis accounting for 1.8% of 326 bacteriologically proven adult cases of meningitis diagnosed between Jan 1984-May 1996.

In Gambia, mortality attributable to Hib meningitis in children less than 5 years was 23 per 100,000 per year (Peltola, 2001).

Significant proportion (6.7%) of patients in Salvador, Brazil developed Hib meningitis due to strains resistant to ampicillin and chloramphenicol, antibiotics used routinely for the empirical treatment of bacterial meningitis in Brazil (Reis *et al.*, 2002).

The incidence rate of 20.1 Hib meningitis cases per 100, 000 children <5 years of age in 2004 in the district of Colombo, Sri Lanka has been reported by Batuwanthadawe *et al.*, (2005).

The introduction of vaccines to prevent invasive *H. influenzae* type b disease in children under 5 years of age is effective in reducing the incidence of bacterial meningitis. It has been estimated 78% of annual cases of Hib meningitis are currently prevented (Dery & Hasbun, 2007).

A total of 218 cases of acute bacterial meningitis were identified. Incidence of Hib meningitis from 1998, declined significantly after the introduction of combined Hib (DPT-Hb/hib) pentavalent vaccines (Paredes *et al.*, 2007).

Listeriosis: *Listeria monocytogenes* is a Gram positive, motile facultative intracellular bacterium that causes severe food borne infection. Pregnant women, their neonates, the elderly and immunosuppressed persons (e.g. transplant or AIDS patients) are

particularly susceptible to severe *L. monocytogenes* infection. In neonates L. *monocytogenes* may cause disseminated disease (granulomatosis infantiseptica) and exudative meningitis, both of which are seen in immunosuppressed adults (Kumar *et al.*, 2005).

3.5.2 Sub acute meningitis

Meningitis is considered sub acute when signs and symptoms have been present for 1-7 days. Patients with sub acute meningitis typically have an unrelenting headache, stiff neck, low-grade fever, and lethargy for days to several weeks before they present for evaluation. Cranial nerve abnormalities and night sweats may be present. This syndrome overlaps that of chronic meningitis. Common causative organisms include *M. tuberculosis*, *C. neoformans*, *H. capsulatum*, *C. immitis*, and *T. pallidum*. Initial infection with *M. tuberculosis* is acquired by inhalation of aerosolized droplet nuclei. Fungal infections are typically acquired by the inhalation of airborne fungal spores. The most common pathogen causing fungal meningitis is *C. neoformans*. This fungus is found worldwide in soil and bird excreta. *H. capsulatum* is endemic to the Ohio and Mississippi River valleys of the Central United States and to parts of Central and South America. *C. immitis* is endemic to the desert areas of the southwest United States, northern Mexico, and Argentina. Syphilis is a sexually transmitted disease that is manifested by the appearance of a painless chancre at the site of inoculation. *T. pallidum* invades the CNS early in the course of syphilis. Cranial nerves VII and VIII are most frequently involved (Kasper *et al*., 2005).

3.5.3 Chronic meningitis

Chronic inflammation of the meninges (pia, arachnoid, and dura) can produce profound neurologic disability and may be fatal if not successfully treated. The condition is most commonly diagnosed when a characteristic neurologic syndrome exists for ≥4 weeks and is associated with a persistent inflammatory response in the Cerebrospinal fluid (CSF).

Inflammatory deposits seeded via. the CSF circulation are often prominent around the brainstem and cranial nerves and along the undersurface of the frontal and temporal lobes. Such cases, termed basal meningitis, often present as multiple cranial neuropathies, with visual loss (CN II), facial weakness (CN VII), hearing loss (CN VIII), diplopia (CNs III, IV, and VI), sensory or motor abnormalities of the oropharynx (CNs IX, X, and XII), decreased olfaction (CN I), or facial sensory loss (CN V). Patients with slowly progressive involvement of multiple cranial nerves and/or spinal nerve roots are likely to have chronic meningitis (Kasper *et al.*, 2005).

Tuberculous meningitis: Patient with Tuberculous meningitis usually has symptoms of headache, malaise, mental confusion and vomiting. Moderate pleocytosis made up of mononuclear or mixture of polymorphonuclear and mononuclear cells. HIV positive patients are also at risk for infection by *M. avium*, *M. intercellulare* usually in setting of disseminated infection (Kumar *et al.*, 2005). Tuberculous meningitis normally develops more slowly and there may well be accompanying focal neurological signs including optochaismayic tuberculoma (Akhadar *et al.*, 2001) as well as a history suggestive of tuberculosis (Smith & Easmon, 1990). Infection of the meninges by TB bacilli is usually caused by rupture of the subependymal tubercle into the subarachnoid space rather than by haematogenous seeding of the meninges. It can also be a complication of miliary TB (Al-Abbasi *et al*., 2002).

Tuberculous meningitis (TBM) is one of the common forms of central nervous system (CNS) infection especially in many developing countries when tuberculous remains highly endemic (Youssef *et al*., 2006). WHO has noted that the global incidence of TB is increasing by 0.4% per annum. CNS TB accounts for about 5% of all extra pulmonary TB and TBM is most serious complication. A more recently developed technique is the use of Mycobacteriophage amplication which detects viable organisms in specimen (Bhigjee *et al*., 2007). An estimated 47,315 cases of TB in Nepal with 21,245 new smears positive TB has been recorded. The mean CSF ADA activity in TBM (13.62 ± 8.45 IU/L) was found to be significantly higher as compared to NTBM (6.51 ± 2.41 IU/L) and NTBNM (2.35 ± 1.16 IU/L) (Gautam *et al*., 2007).

Neurosyphilis: *Treponema pallidum* is one of the etiological agents responsible for chronic meningitis (Baron *et al*., 1994). Neurosyphilis is the tertiary stage of syphilis and occurs in only about 10% patient with untreated infection (Kumar *et al*., 2005).

Fungal meningitis: The causative agents are *Cryptococcus neoformans*, *Candida* spp., *Histoplasma capsulatum*, *Coccidioides immitis* and *Blastomyces dermatitidis*. The later three are more common in the US. Cryptococcus neoformans, capsulated yeast, spreads from a focus in the lungs into the blood stream and hence in the meninges (Smith & Easmon, 1990). A case of fungal meningitis in an immunocompetent woman caused by *Aspergillus fumigatus* has been described by Verweij *et al*. (1999). Fungal disease of CNS is encountered primarily in immunocompromised patient. The mucoid encapsulated yeasts can be visualized in CSF by India ink preparation and in tissue section by PAS and Mucicarmine as well as Silver stain (Kumar *et al*., 2005).

3.5.4 Recurrent bacterial meningitis

Epidemics of meningococcal disease are recurrent in that part of Sub Saharan Africa known as "menigntis belt" which extends from Senegal in the west to Ethopia in the east (WHO, 1998). Recurrent meningitis in children may be due to immune deficiency, presence of indewelling devices in the ventricular system or brekage of mucocutaneous barrier between skin and CSF has been reported by Sumanasena & Lamabadusuriya (2003). Recurrent bacterial meningitis is much less common phenomenon but generally possesses a considerable diagnosis challenge caused by different bacterial organisms or alternatively a second or further after completion of therapy for the initial episodes (Tebruegge & Curtis, 2008). The distribution of causative pathogens for episodes of recurrent meningitis differed from that for the episodes of non recurrent meningitis has been reported by Driel *et al*. (2008).

3.5.5 Mixed meningitis

Unusual case of culture proven pneumococcal and meningococcal mixed meningtis was reported by Marchandin *et al*. (2005). Patient with heterozygous sickle cell diseae HbsD

with mixed meningitis due to *S. pneumoniae* and *M. tuberculosis* presenting as primary spontaneous ventriculitis with acute hydrocephalus was reported by Nagarathna *et al*. (2007).

3.5.6 Acute aseptic meningitis

Aseptic meningitis is characterized by an increase in Lymphocyte and other mononuclear cells (pleocytosis) in the CSF (in contrast to purulence, the PMN response characteristics of bacterial meningitis) and negative bacterial and fungal cultures. Patients may have fever, headache, stiff neck and nausea and vomiting. It is commonly associated with viral infections and is usually self limiting infection (Forbes *et al*., 2007). The disease is generally of viral, and rarely of bacterial or other etiology. Though viral meningitis is main cause of increased lymphocytes in CSF; there are other etiologies of infectious nature (Almeida *et al*., 2007).

Viral Meningitis: Viral meningitis occurs world-wide in sporadic and epidemic forms. The incidence during non-epidemic conditions is rarely known. Seasonal variations can be observed and depend on the causative agent. Enteroviruses are the most common cause of epidemics of viral meningitis and they occur in general in late summer or early winter periods, affecting mainly infants and young children. Mumps virus is another important agent of viral meningitis in nonimmunized populations. Outbreaks of aseptic meningitis in late winter may be due mainly to mumps. The most affected in these outbreaks are children in the age group 5-9 years. Arboviral meningitis outbreaks, e.g. West Nile virus may occur under special favouring conditions (Romania 1996) such as periods of increased vector activity. Outbreaks of enteroviral meningitis occurred in some countries in the Eastern Mediterranean Region which caused great concern among the public as they coincided with the vast wave of meningococcal outbreaks in Africa and the increased public awareness of the disease (WHO, 1997). The viral aseptic meningitis is usually shelf limiting and treated symptomatically. In an appropriate 70% of cases a pathogen can be indentified most commonly an enterovirus, echovirus, coseckie virus and nonparalytic poliomyelitis are responsible for up to 80% of these

cases. HIV aseptic meningitis occurs within 1-2 weeks of seroconversion in about 10% of patient; antibodies to HIV can be demonstrated and virus can be isolated from CSF (Almeida *et al.*, 2007).

Drug induced aseptic meningitis (DIAM): A true non infectious process has now been associated with some classes of medications including NSAIDs (non steroid anti inflammatory drugs) and antibiotics with sulfa, intravenous immunoglobulins, isoniazid, and Muromonab-CD3. This entity has been termed drug induced aseptic meningitis (Jolles *et al.*, 2000; Sekul *et al.*, 1994).

Two possible ways in which DIAM arises are, direct irritation of the meninges by intrathecal administration of the drug, and immunological hypersensitivity to the drug (Jolles *et al.*, 2000). Aseptic meningitis like picture may also develop subsequent to rupture of an epidermoid cyst into subarachnoid space or the introduction of chemical irritant (chemical meningitis). Some intrathecal medicine including metrotexate, anaesthics, aracytin, baclofen, corticoids, or contrast chemicals, can cause chemical meningitis (Almeida *et al.*, 2007).

Carcinomatous meningitis: This dessimaination occurs more frequently in acute haematological diseases, such as leukemia and lymphomas. Among solid tumors, the dissemination is more frequent with melanomas and breast or lung cancer (De Luca *et al.*, 1995). Elevated levels of interleukin-6 (IL-6), an inflammatory cytokine produced by B and T Lymphocytes have been found in infectious and noninfectious non malignant inflammatory disorders. Elevated IL-10 with an IL-10 to IL-6 ratio greater than 1.0 is a strong predictor of the presence of lymphoma cells in the CSF. Alternatively, an IL-10 to IL-6 ratio of less than 1.0 is characteristics of an infectious or noninfectious nonmalignant inflammatory disorder (Faller *et al.*, 2001).

3.5.7 Mollaret's meningitis

Mollaret's in 1944 described a rare form of self limiting aseptic recurrent benign meningitis which has been termed as Mollaret's meningitis. Recurrent episodes of

severe headache, meningism and fever; attacks separated by symptoms free interval of weeks to months; CSF pleocytosis with large "endothelial" cells, neutrophils, and lymphocytes; spontaneous remission of symptoms and signs; no causative etiologic agent detected are the clinical diagnosis criteria for Mollaret's meningitis. Mollaret's meningitis is an extremely rare condition, till 2002 approximately 50 cases of recurrent HHSV meningitis have been described in USA and Europe (Hoque *et al*., 2004).

3.6 MORPHOLOGICAL FEATUERS

The types of Meningitis and their morphology have been described by Victor *et al*. (2000); Kumar *et al*. (2005); & Quagliarrello & Scheld (1997).

3.6.1 Acute pyogenic meningitis

The normally clear CSF is cloudly and someties frankly purulent. In acute meningitis an exudates is evident within the leptomeninges over the surface of the brain. The meningeal vessels are engorged and stand out prominently. The location of exudates varies; in *H. influenzae* meningitis it is usually basal, whereas in pneumococcal meningitis it is often densest over the cerebral convexities near the saggital sinus. When meningitis is fulminant the inflammation may extend to the ventricles producing ventriculitis. Leptomeningeal fibrosis and consequent hydrocephalus may follow pyogenic meningitis, although if it is treated early, there may be little remaining evidences of an infection. In some infections, particulary in pneumococcus meningitis large quantities of capsular polysaccharide of an organism produce particularly gelatinous exudates that encourage arachnoid fibrosis, and chronic adhesive arachnoiditis.

3.6.2 Listeriosis

In an acute human infection, *L. monocytogenes* evokes an exudative pattern of an inflammation with numerous Neutrophils. The meningitis it causes is microscopically and macroscopically indistinguishable from that caused by other pyogenic bacteria. The finding of Gram positive mostly intracellular bacilli in CSF is virtually diagnostic. More

varied lesion may be encountered in neonates and immunosuppressed adults. Focal abscesses alternate with grayish or yellow nodule representing necrotic amorphous basophilic tissue debris.

3.6.3 Acute aseptic meningitis

There is no distinctive macroscopic characteristic except for the brain swelling, seen in some instances. On a microscopic examination there is either no abnormality or mild to moderate infiltration of the leptomeninges with Lymphocytes.

3.6.4 Tuberculous meningitis

On macroscopic observation, the subarachnoid space contains gelatinous or fibrinous exudates, most often at the base of brain obliterating the cisterns and encasing cranial nerves. There may be discrete, white granules scattered over the leptomeninges. On microscopic examination there are mixture of lymphocyte, plasma cells, and macrophages. Organisms can often be seen with Acid Fast stain. The infectious process may spread to the choroid plexuses and ependymal surface, travelling through the CSF of long standing duration, a dense fibrous adhesive arachnoiditis may develop, most conspicuous around the base of brain. Another manifestation of disease is development of single or often multiple well circumscribed intraparenchymal mass (Tuberculoma) which may be associated with meningitis. A tuberculoma may be upto several centimeters in diameter causing significant mass effect. On microscopic examination there is usually a central core of caseous necrosis surrounded by atypical tuberculous granulomatous reaction, calcification may occur in an inactive lesion.

3.6.5 Neurosyphilis

Meningovascular neurosyphilis is a chronic meningitis involving the base of brain and variably also the cerebral convexities and spinal leptomeninges. In addition there may be an associated obliterative endertities (Heubuer arterities) accompanied by distinctive perivascular inflammatory reaction in plasma cells and lymphocytes. Cerebral gummas

(plasma cell-rich lesion) may also occur in relation to meninges and extending into cererbral hemisphere, diencephalon or spinal cord.

3.6.6 Fungal meningitis

With cryptococal infection, the brain shows chronic meningitis affecting the basal leptomeninges, which are opaque and thickened by reactive connective tissue and may obstruct the outflow of CSF from the foramina of lushka and magendie, giving rise to hydrocephalus. Sections of brain disclose a gelatinous material within the subarachnoid space cyst within a parenchyma ("soap bubbles") which are especially prominent in the basal ganglia. Parenchymal lesion consist of aggregates of an organisms within expanded perivascular (Virchow-Robins) spaces associated with minimal or an absent inflammation or gliosis. The meningeal infiltrates consists of chronic inflammatory cells and fibroblasts admixed with cryptococci. Well formed granulomas are not seen ordinarily, in some cases however, there is marked chronic inflammatory and granulomatous reaction to that seen with *M. tuberculosis.*

3.7 EPIDEMIOLOGY OF BACTERIAL MENINGITIS

3.7.1 Global status of Bacterial meningitis

Patients with *H. influenzae* or enteric Gram negative bacilli in their CSF had meningitis significantly more often than did other kinds of bacteria ($P < 0.001$ & < 0.01 respectively). The number of *Sreptococcus pneumoniae, N. meningtidis,* and *Listeria monocytogenes* were too small to permit statistical evaluation (Olson & Hoeprich, 1984).

The etiological agents and mortality rates (0-54%) of bacterial meningitis depends upon season of the year and age, sex, ethnic background, and geographic location of the patient. *H. influenzae* was the most frequent cause of bacterial meningitis (2.9 cases per 100, 000) and paradoxically was associated with the lowest fatality rate 3% of the most frequent bacterial agents. *Listeria monocytogenes* was reported relatively infrequently

(0.2 cases per 100,000 population) but had highest fatality rate (22%). (Gray & Fedorko, 1992).

Acute bacterial meningitis in an adult is usually caused by *S. pneumoniae, N. meningitidis*, and Gram negative bacilli (Domingo *et al*., 1997).

Acute bacterial meningitis (ABM) is a major cause of death and disability in developing countries. The case fatality rate of ABM remains around 10-30%. An additional 5-40% of cases have only partial recovery with late sequel (Van *et al*., 2002).

The epidemiology of bacterial meningitis has changed significantly in recent years, reflecting a dramatic decline in the incidence of meningitis due to *Haemophilus influenzae*, and a smaller decline in that due to *Neisseria meningitidis*, following the introduction and increasingly widespread use of vaccines for both these organisms. Currently, the organisms most commonly responsible for community-acquired bacterial meningitis are *Streptococcus pneumoniae* (50%), *N. meningitidis* (25%), group B streptococci (15%), and *Listeria monocytogenes* (10%). *H. influenzae* now accounts for 10% of cases of bacterial meningitis in most series (Kasper *et al*., 2005).

Acute bacterial meningitis constitutes significant global public health problems. World wide it has been estimated that 1-2 million cases of bacterial meningitis occur annually. The problem is more significant in resource poor countries including those in some regions of Sub Saharan Africa, South East Asia and Latin America (Van *et al*., 2006).

Bacterial meningitis was attributable to *S. pneumoniae* (39.5%), to *N. meningitidis* (28.9%); serogroup B-3, serogroup C-2, ACYW-135 group-1, unidentified-5 cases, to *Listeria monncytogenes* in 7.9%, to *E. coli* in 7.95% and due to other Streptococci in 15.8% among them 3 were attributable to *S. suis*, and single cases of *S. agalactiae, S. salivarus* and *S. bovis* (Kowalik *et al*., 2007).

The analyzed microbiological cause of meningtis by study period (1993-1998) Vs (1999 -2003), in inner city referral hospital in Mexico, it has been identified that prior to the introduction of Hib vaccine (1993-1998) the most frequent pathogen was Hib in 64% of

cases; *S. pneumoniae* occured in 22% and *N. meningitidis* in 0.5% cases, other pathogens were found in 5.55% of cases and no bacterial pathogen was found in 8%. The no. of Hib meningitis cases during 1999-2003 study period was 8 (14%), *S. pneumoniae* was found in 54%, *N. meningitidis* in 5%, other pathogens in 8% and no pathogen in 19% of cases. Therefore the incidence of Hib decreased from 64% to 14% ($p < 0.001$) following the introduction of Hib vaccine (Paredes *et al.*, 2007).

3.7.2 National Status of Bacterial meningitis

A study done to find out epidemiological aspects of Meningococcal Meningitis it was found that there was a high death rate (19.2%) of all admitted cases of meningitis in the different hospitals in Kathmandu valley (Pradhan, 1983).

A study done in the period of April 1974 to 1978, out of the 4.45% of the central nervous system disorder admitted in Kanti Children's Hospital 41.1% was meningitis out of which 33% comprised septic meningitis and 50% occurred in infants below one year of age, while 85% occurred below 5 years of age .The death rate was 24% (Shrestha, 1983).

A retrospective study conducted at Kanti Children's Hospital pointed out that 2.8% of the total children admitted at Kanti Children's Hospital were patients with meningitis out of which 84.2% were with pyogenic meningitis, 15.7% with tubercular meningitis; 31.2% were below one year of age and 66.6% were males. The commonest modes of presentation were fever (91.6%) and vomiting (72.9%) (Sharma, 1983).

In other 2 year study at Kanti Children's Hospital, it was found that in the two year period, meningitis constituted 3.2% of the total admission at Kanti Children's Hospital out of which 80.6% were due to pyogenic meningitis and 19.3% were tuberculous in origin. 23.5% were below the age of one year and 61.3% were males. The overall mortality rate was 7.9% (Sharma and Dixit, 1984).

A study done in the Pediatrics Ward of Bheri Zonal Hospital concluded that meningitis was the second most frequent disease of the central nervous system admitted in Bheri Zonal Hospital (Subedi, 1985).

In an epidemiological study, it was found that during the first six months of 1983, an epidemic of serogroup A meningococcal meningitis occurred in the Kathmandu valley of Nepal, resulting in 875 cases and 95 deaths. The annual attack rate was 103 cases per 100,000 populations, with a peak attack rate occurring in April. Epidemic meningococcal disease had not been recognized previously in Nepal. Early in 1984, a review of hospital-based data on pyogenic meningitis in Kathmandu showed three times as many cases per month compared with the same period the previous year, suggesting that a recurrent epidemic was unfolding (Cochi *et al.*, 1987).

In a study done by Tiwari *et al*. (2002) at TUTH, highest incidence of meningitis was found in neonates (0-1month). The rate of incidence of meningitis among children up to 14 yrs was found to be 8.70%.

First case of Cryptococcal meningitis in BP Koirala Institute of Health and Service Hospital, Nepal, that occurred in patient with no obvious predisposing factors was reported by Khanal *et al.* (2002).

A prospective study of 42 children (with range of ages from 1 month to 44 months) admitted to Kanti Children's Hospital, Katmandu from the June 1993 to February1994 suspected to be suffering from meningitis and whose clinical features and microscopic and biochemical studies of the Cerebrospinal fluid suggested bacterial meningitis showed Latex agglutination test (LAT) positive in 33 (80.5 %) out of the 41 cases. 18 (54.5%) of the LAT positive results revealed *Haemophilus influenzae*, 10 (30.3%) revealed *Neisseria meningitidis*, 4 (12.1%) revealed *Streptococcus pneumoniae*, and 1 (3.0%) revealed group B *Streptococcus*. LAT and positive result of Gram stain corresponded in 71.87% cases (Tiwari, 2003).

Study was conducted among the children suspected of meningitis attending Kanti Childern's Hospital, June, 2006 to September, 2006, where 431 CSF samples were processed and out of 431 samples only 21(4.87%) samples showed culture positive. Among 21 bacterial isolates, 11 (52.38%) were Gram negative organisms where as 10 (47.62%) were Gram positive organisms (Tuladhar, 2006).

Children aged 2 months to 5 years who were admitted to KCH from November 2004 through March 2007 with fever and/or a possible clinical diagnosis of pneumonia, meningitis, or septicemia were considered for enrollment in the study. A total of 2,528 children with suspected invasive bacterial disease were recruited, of whom 9.6% had meningitis. A total of 300 CSF samples were collected and cultured; of which 11 (3.7%) were positive for *S. pneumoniae,* and 5 (1.7%) were positive for *H. influenzae.* A total of 244 CSF specimens were tested by latex agglutination, of which 19 (7.8%) had positive results and 229 CSF specimens been tested for pneumococcal antigen, of which 23 (10.0%) had positive results. Of the 1,485 children in the age group 0-11 months, 25 (1.7%), of the 547 children in the age group 12–23 months, 4 (0.7%), and of the 496 children in the age group 24–59 months, 4 (0.8%) had *S. pneumoniae* meningitis (Shah *et al.*, 2009).

All children aged 2-59 months (inclusive) who were admitted to the pediatric ward at Patan Hospital from April 2005 through December 2006 with fever and/or suspected pneumonia, meningitis, or bacteremia were recruited. CSF samples were obtained from 199 children. 9 (4.5%) yielded a pathogenic organism, 7 (78%) of which were positive for *S. pneumoniae* (Williams *et al.*, 2009).

Meningitis is a communicable disease which is kept under the class "other communicable disease" in Nepal. Nepal planned to introduce pentavalent (DPT-HepB-Hib) vaccine from April 2009. The estimated target population for the fiscal year (2008/ 2009) was 27,383,773. Of the total population 45.28% visited OPD and 0.32% was found to visit OPD for other communicable disease in 91 hospitals. The death rate among In patients with communicable disease (0.20%) is significantly lower than that

of non communicable disease (1.34%) among the hospitalized cases in the country. Among the five leading causes of morbidity among In patient as reported by central hospitals, meningitis is the 4th cause found in Kanti Children's Hospital. Five leading causes of mortality among In patients by central hospitals, meningitis is 3rd cause recorded in Kanti Children's hospital. A total of 5,082 confirmed cases of meningitis have been recorded. It has been found that 72,674 laboratory facility were available in Nepal for culture and routine examination of CSF, among total 31,21,197 available Microbiology Facility Laboratory ("Annual Report", 2007/2008).

3.8 LABORATORY INVESTIGATIONS OF MENINGITIS

The most important consideration in the management of a patient with acute bacterial meningitis are determining the most likely etiological agent and initiating immediate empirical antimicrobial therapy within 30 min of presentation (Gray & Fedorko, 1992). It is difficult to distinguish various forms of meningitis (Smith & Easmon, 1990). The principal specimen is CSF (Collee *et al*., 1996). CSF and blood specimen should be obtained from patient with clinical signs and symptoms of meningitis and transported to laboratory without delay (Washington, 1991). The collection of clinical specimen is important for the isolation and identification of bacterial agents that causes meningitis. It is recommended that the clinical specimen be obtained before antimicrobial therapy is begun to avoid loss of viability of an etiological agents (CDC, 1998). Examinations of CSF macroscopically, microscopically, chemically and immunologically and culture methods is necessary to distinguish meningitis from non specific syndromes and to distinguish among bacterial, fungal and aseptic meningitis (Smith & Easmon, 1990).

CSF should be collected by an experienced medical officer. The collection procedure should be aseptic (Baron *et al*., 1994). The physician thoroughly cleans the skin of the lumbar region below the termination of spinal cord where the cauda equine goes through the spinal cord. He/ she makes small belb in the skin over the space between 3rd and 4th or 4th and 5th Lumbar vertebrae with 2% Procaine and introduces spinal needle (22 gauge, 3.5" (9cm) long) through the belb into spinal canal , pressure maintained

within 50-150 mm measured by manometer 3-4 ml of fluid is allowed to drip into plain tubes (sterile).

Only 3-5 ml of fluid should be collected for the removal of larger volume my lead to headache, and rate of collection should be slow about 4-5 drops a second. When there is increased intracranial pressure it may be unsafe to undertake lumbar pucture as removal of fluid may draw down the cerebellum to the foramen magnum and compress the medulla (Collee *et al*., 2006). Volumes of 1-2 ml are usually significant to detect bacteria but the isolation of fungi and mycobaceria requires a minimum of 3ml (preferably 10-15ml) of CSF for each culture (Dalton & Nottebart, 1986).

Label the specimen, hand carry it (whenever feasible) to the laboratory as soon as possible (CDC, 1998). CSF is hypotonic therefore Neutrophils may lyse and counts may decrease by 32% after 1hr and 50% by 2 hr in CSF specimen held at room temperature (Steele *et al*., 1988). *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* are fastidious organisms that may not survive long transit time or variation in temperature. Refrigeration may prevent recovery of these organisms; thus CSF specimen should be stored at room temperature or in an incubator (37℃) if they cannot be processed immediately (Kasten, 1990). Do not expose CSF to sunlight or extreme heat or cold. If *N. meningitidis* is suspected to be the cause of illness and delay of several hours in processing specimen is anticipated incubating the CSF (with screw caps loosen) at 35℃ in 5% CO_2 atm or candle jar may improve bacterial survival. If same day transport to lab is not possible CSF should be inoculated into Trans-isolate (TI) biphasic medium at 35℃ (CDC, 1998). In suspected cases of viral infection, a portion of CSF is sent to virology laboratory on ice or a cold pack in an insulated container and for tissue culture (e.g. for enteroviruses). Blood sera may provide a baseline for serodiagnosis (e.g. for mumps, HSV) or other agents *(Mycoplasma pneumoniae*) (Collee *et al*., 1996). Now-a-days PCR techniques have been practiced widely which has high sensitivity and specificity. Nasopharyngeal specimen may be value in meningococcal or *Hemophilus* infections both for established aetiology and for screening close contact (Smith & Easmon, 1990). To increase the chances for the isolation of viruses in suspected viral

meningitis, generally, faeces, a rectal swab, an anal swab and throat swab is practiced in addition to CSF collection (Cheesbrough, 1984).

3.8.1 Macroscopic observations of CSF

The CSF should be examined with naked eyes for the presence of turbidity and any sign of contamination with blood from the puncture wound (Collee *et al.*, 1996). The color of CSF supernatant generally reflects the conditions or causes. The yellow colour of CSF supernatant is due to blood breakdown products, hyperbilirubinemia or CSF protein (≥ 150mg/dL), orange colour is due to blood breakdown products or hgh carotenoid ingestion, pink colour is due to blood breakdown products, green due to purulent CSF and brown due to Meningeal melanomatosis (Seehusen *et al.*, 2003).

3.8.2 Microscopic observation of CSF

When <0.5 ml of CSF is received into microbiology laboratory the entire unconcentrated specimen is used for microscopical examination and culture. When >0.5 ml of CSF is available for microscopic examination and culture, the CSF should be concentrated by centrifugation 3000 rpm/10min (Murray & Hampon, 1980). Mix the sediment with vortex mixture. The sediment is used to inoculate culture media and prepare smear for staining (Dalton & Nottebart, 1986). As cells and bacteria may be scanty, it is generally helpful to make a thick film within an area of about 10mm diameter. When CSF is highly turbid and proteinaceous, part of film should be thin, for sometimes a wholly thick film, although dried and fixed by heat, become washed of the slide in course of staining. A very careful search for bacteria should be made particularly in areas of film where there are plenty of leukocytes and search should be continued at least 10min before accepting the result as negative. The findings of bacterial forms resembling meningococci, pneumococci, haemophiIi, coliform bacilli, *Streptococcus,* and *Listeriae* should once be reported to physician. If a variety of bacterial form is seen in the film the probability that specimen being contaminated with live or death bacteria should be suspected (Collee *et al*., 2006). Based on the

demographic and clinical data of patient and Gram staining morphology the etiology of the majority of cases of bacterial meningitis can be presumptively determined within 1st 30min after receiving specimen (Baron *et al.*, 1994).

AFB is difficult to detect in CSF. The chances can be increased by centrifugation for 30min with higher angular velocity (3600 g/min) and utilizing several drops. Usually the AFB is few and therefore a prolonged search is required. Examination of an auramine stained smear by fluorescence microscopy is a more sensitive method of detecting AFB in CSF (Cheesbrough, 1984). If insufficient quantity of CSF is received the specimen should be used directly for smear and culture (Baron *et al.*, 1994).

An alternative method of concentrating bacteria in CSF specimen to be cultured is membrane filtration technique. CSF (usually >2ml) is filtered through 0.45 μm pore sized sterile disposable filter. The "upstream" side of the filter is especially placed face down onto the chocolate agar (Gray & Fedorko, 1992).

3.8.3 Chemical examinations of CSF

Combining a set of CSF variables referred to as routine parameters (i.e. detection of protein, albumin, immunoglobulin, glucose, Lactate and cellular changes as well as specific antigen and antibody testing for an infectious agents) will increase the diagnostic sensitivity and specificity (Deisenhammer *et al.*, 2006). The supernatant of the centrifuged CSF sample or uncentrifuged CSF smple if clear is taken for chemical analysis (Cheesbrough, 2000). Lactate and C-reactive protein (CRP) levels are useful indications to distinguish bbacterial from aseptic meningitis. Detection of lipopolyaccharide (LPS) in the CSF reveals the meningitis caused by Gram negative bacteria (Smith & Easmon, 1990).

Glucose estimation: If the glucose is not preserved with fluoride, the glucose must be estimated within 20 minutes of the fluid being withdrawn otherwise a false low result will be obtained due to glycolysis. The enzymatic, colorimetric techniques or simplier semiquantitave technique using Benedict's reagent can be used. The normal

concentration of glucose level in CSF is about half to 2/3rd to that of blood, i.e. 2.2-4.0 mmol/L (45.72 mg %). Reduce CSF levels are found in most forms of meningitis especially in pyogeinic meningitis markedly reduced and may even be undetectable. In viral meningitis the level is usually normal. A raised CSF glucose is found in hyperglycemia and sometimes with encephalitis (Cheesbrough, 2000).

The glucose concentration in CSF should be related to blood concentration. CSF glucose/serum ratio is preferable. Pathological changes in this ratio are supportive of bacterial or fungal meningitis. Normal value of glucose ratio is >0.4-0.5. In the 1st 6 months of life the ratio is 1 (Deisenhammer *et al*., 2006). Whatever the cause, lowered ratio indicates diffuse generalized meningeal disease; acute purulent meningitis, tuberculous meningitis, fungal menignits, carcinomatous meningitis, acute syphilitic meningitis, rheumatoid meningitis etc. (Marshall & Bangert, 1995).

Lactate: CSF Lactate is normally less than 2.5 mmol/L and independent of arterial blood values indicating that it is a product of metabolism within CSF (Deisenhammer *et al.*, 2006).

Measurement of total protein and globulin: Some 80% CSF protein is derived from plasma proteins which have passively diffused across the various blood-CSF barriers. Two specific proteins present in CSF are of special note. Tau protein is desialyted Transferrin which may arise during the process of receptor mediated Transferrin transfer. In the systemic circulation desialyted Transferrin is removed by asialoreceptors of reticuloendothelial system, which are not present in CNS; Tau protein can thus serve as a specific protein marker for CNS. Prealbumin (Transthyretin) is synthesized by choroidal epithelium (Marshall & Bangert, 1995).

CSF protein concentrations may increase when there is increased permeability of blood-CSF most commonly due to inflammatory conditions. Total protein, albumin, α2 macroglobulin and electrophoresis have all been advocated as an investigation appropriate to the assessment of barrier permeability. Permeability of blood CSF barrier alters with age (Marshall & Bangert, 1995; Brackenridge, 1962).

Albumin is particulary suitable indicator protein because it is neither synthesized nor metabolized intrathecally. In CSF that is free of contaminating blood albumin must necessarily come from plasma through blood-CSF barrier (Burtis *et al.*, 2006).

Total protein concentration upto 60mg/dl are normal in healthy adults. The low levels of protein in CSF limit the methods that are used to measure total protein in it. Turbidimetric methods and several of CBB dye binding metods are commonly used for this purpose. The most serious defect of turbidimetric method is the requirement for 0.2-0.5 mL of sample. Coomassie Brilliant Blue (CBB) methods are sensitive enough for use with sample as little as 25µL; but they underestimate globulins. Because albumin is the highest concentration protein in CSF this underestimation may not be serious enough to preclude the use of CBB method. Nephelometry, Immunoturbiditmetry, Electroimmunodiffusion and RID are most often used assays for the measurement of albumin and IgG in CSF. Precipitation of proteins for turbidimetric or nephelometric assays is achieved with Sulfosalicyclic acid alone or with Sulfosalicyclic acid in combinationwith sodium sulphate or trichloroacetic acid (TCA) or with TCA alone. Direct photometric methods are used for CSF after removal of interfering molecules by gel filtration requires the use of high quality spectrophometer (Burtis *et al.*, 2006).

Total protein can be measured in CSF using colorimetric technique or visual comparative technique. Pandy's test is screening test which detects rise in CSF globulin (Cheesbrough, 2000).

CRP is a protein synthesized by hepatocytes. Normally they are less than 50µg/L. Concentration greater then 600µg/L is observed in bacterial meningitis (Marshall & Bangert, 1995).

3.8.4 Microbiological examinations of CSF

Culture: Bacterial culture is enexpensive and still accepted as "gold standard" for the diagnosis of bacterial meningitis (Gray & Fedorko, 1992). Although culture is not rapid,

it is important to obtain viable organisms for antimicrobial sensitivity and for epidemiological investigation as well for detail identification (Smith & Easmon, 1990). The sediments should be used for inoculating in culture media. The media routinely used for bacterial culture of CSF are 5% Sheep blood agar, enriched chocolate agar, and a enrichment broth (e. g. Thioglycolate, Columbia, Brucella, supplemented peptone). The culture plates should be incubated for at least 72hrs at 37°C in an atmosphere containing 5-10% CO_2. A candle jar may also be used if CO_2 indicator is not available. The enrichment broth with the caps loosen should be incubated at 37°C in air for at least 5 days. If the Gram stain demonstrates the presence of Gram negative rods resembling members of the family Enterobacteriaceae, a Mac Conkey (MAC) agar plate can also be inoculated. If the Gram stain reveals organisms that morphologically resemble anaerobic bacteria or if the patient is known to have an underlying conditions predisposing the patient to an anaerobic infection (such as chronic otitis media, a pilonidal sinus, dermal sinus or brain abscesses), an anaerobic blood agar plate should can be added to the routine culture media, and the plate should be incubated at 37°C in an anaerobic atmosphere.

If fungal meningitis is suspected, two Sabouroued Dextrose sugar (SDA) tubes are inoculated on the slant and incubated for at least 7 days, one at 30°C and another at 35°C. If tuberculous meningitis is suspected the CSF sediment is centrifuged at 3600 × g for 30 min to concentrate the bacteria. The supernatant is decanted and the sediment is vortexed thoroughly and inoculated on the Lowenstein Jension or modified Ogawa slant. The tube should be inoculated at 37°C for at least 8 weeks. If amoebic meningoencephalits is suspected free living amoebae can be co cultivated on an artificial media if they are supplied with a living nutrient such as *Klebsiella pneumoniae* or *E. coli.*

CSF may be inoculated directly to tissue culture for the detection of a viral agents, diagnosis of viral encephalitis is often accomplished by isolation of the virus from a throat culture, faeces or blood so these specimens should be submitted in aditon to CSF for identification of an etiological agent. 0.25ml of CSF per tube is inoculated into tubes

of primary Monkey Kidney Hep-2 continuous cell line, and human fetal diploid foreskin fibroblast cell cultures. These culture systems are incubated at 25℃ in air for varying amounts of time depending on the cell culture system (Baron *et al*., 1994; Gray & Fedorko, 1992).

Antibiotics Susceptibility Test (AST): Chemical efficacy is also dependent on achieving staisfactory drug concentration at the site of the infection; this is influenced by the standard pharmacological factors of absorption, distribution, metabolism and excretion (Stephen *et al.,* 2005). Chloramphenicol is regularly detected in the CSF when blood levels greater than 10μg/ml is reached and the concentration in the spinal fluid is approximately 25% of that in the blood. When large amounts of penicillin are given parenterally, there seems to be a slight increament in the amount of the drug found in CSF, but this is not linear relationship. There is general agreement that the transfer of penicillin from blood to spinal fluid is increased in meningitis (Harter & Petersdorf, 1960).

The blood brain barrier prevents many toxic substances that might be circulating in the blood from reaching the cells of brain. It also makes it very difficult to deliver certain antibiotics and other types of medication to the cells of the brain (Creager, 2004). Trauma, certain toxins and an inflammation can cause breakdown of BBB (Tortora & Derrickson, 2006).

β Lactam antibiotics inhibits peptidoglycan cross linking through interaction of the common β Lactam ring with the transpeptidase enzyme e.g. penicillin, ampicillin, cloxacillin, cephalosporin, cefotaxime, ceftriazone, ceftazidime. Amikacin is an Aminoglycoside-aminocyclitol antibiotic (AGACs). One effect of AGACs is to interfere with initiation and the assembly of bacterial ribosomes. Aminoglycoside binds to A side of 16S rRNA and interfers with accurate recognition of cognate tRNA by rRNA during translation and may also perturb translocation of tRNA from A side to peptidyl-tRNA side (P site). Macrolide antibiotics group selectively inhibits protein synthesis in broad range of bacteria by binding to 50S subunit and blocks translocation,

e.g. Erythromycin. The fluoroquinolones selectively inhibits topoisomerases II and IV which are not found in mammalian cells. These are capable of catalyzing a variety of change in DNA topology, e.g. Ciprofloxacin, Norfloxacin, and Ofloxacin. Cotrimoxazole is the combination of Sulphamethoxazole with Trimethoprim, where DHFR inhibitor is used in combination with Sulphonamides, to achieve a double interference in Folate metabolism (Denyer *et al*., 2005).

3.8.5 Serology of CSF

When the antigen detection tests are anticipated the specimen should be stored at ≤4°C because bacterial polysaccharide antigen often tends to break down faster at room temperature and 37 °C than at ≤ 4°C (Gray & Fedorko, 1992).

The antigen and antibody complex is detected as line of precipitation in agarose gel in Counter current electrophoresis (CIE). The antigen detection limit is 30-100ng/ml. Latex aggulation (LA) and coagglutination (COAG) test requires no specific equipments and are more sensitive than CIE. *N. meningitidis* group B and *E. coli* serotype K1, however, presented particular problems because of the poor immunogenicity as the antigen concerned and the consequently lack good antisera. Enzyme immunoassay (EIA) for the detection of bacterial antigen in CSF uses specific (primary) antibodies bound to a solid support such as plastic microwell tray or tube or polystyrene beads. EIA are better suites for testing specimens in a batch mode than for testing of individual CSF specimens.The Quellung capsular reaction is rarely used; however it can be used to confirm the presence of an organism with morphology typical of *S. pneumoniae*, *N. meningitidis*, or *H. influenzae* type b. The VDRL test is the useful test for detecting antibody against *Trepoema pallidum* in CSF. ELISA is a rapid test and is used to detect antigen of pathogen (such as Hib, meningococci, pneumococci, mycobacteria) in CSF by utilizing enzyme labelled specific antibodies. LAL assay can be used to give an early and presumptive diagnosis of Gram negative meningitis with a reported sensitivity of 93% and specificity of 99% compared with CSF culture. LAL

assay detects only Gram negative bacteria. Assay doesn't differenciate types of Gram negative organisms (Mahon & Manselis, 2000).

3.8.6 Molecular technique

PCR is potentially sensitive enough to detect a single microbe in any patient sample and being rapid, a large number of samples can be processed in a day. This method is of particular importance to detect slow growing organisms such as *Mycobacterium tuberculosis* (at least 8 weeks) and *Cryptococcus neoformans* (>2 days) and is equally important for other organisms. Compared with culture the sensitivity of the universal PCR for detection and identification of a bacterium directly from CSF was 92.3% (Lu *et al.*, 2000).

CHAPTER IV

4. MATERIALS AND METHODS

This study was conducted at TUTH emergency laboratory and Microbiology laboratory of Alka hospital from 16th September, 2009 to 16th February, 2010. During this period CSF samples from 183 patients were obtained from TUTH, of which 162 samples from the patient suspected of meningitis were processed in the laboratory. The samples were processed according to the standard protocol. Briefly, the appearance of the samples was noted, cell count was done, Gram staining was done, protein and glucose levels were estimated from CSF, the serum glucose level was also estimated from 162 samples, samples were cultured in different types of the media. After isolation antibiotic susceptibility pattern of different isolates were also observed.

4.1 MATERIALS

All the materials required for present work have been listed in the Appendix-I**.**

4.2 METHODS

4.2.1 Collection of samples

The CSF samples were collected in the Hospital by medical officer by puncturing the lumbar region at L3-L4 level. For every patient, duplicate sample was collected; one for the cell count and other for culture and an additional blood specimen were collected. The sample was immediately transported to the laboratory at the room temperature. As soon as the samples were received in the laboratory, the samples were processed.

4.2.2 Sample processing

Each CSF samples were processed macroscopically, microscopically and microbiologically. The routine parameters were also determined. From the blood sample glucose test was performed.

4.2.3 Macroscopic observations

Each CSF sample was observed for its physical appearance with naked eye. The color and the turbidity of the samples were noted. The samples were categorized as clear when holding test tube of CSF against white printed page; CSf should be as clear as similar test tube filled with water (CSF, 2008), slightly turbid when turbidity visible but news print read easily through the tube, cloudy when newsprint not read easily and definitely purulent when newsprint cannot be seen in terms of its turbidity (CSF, 2010) and bloody (mixed with blood) in terms of its color.

4.2.4 Cytological examinations

A drop of CSF sample was taken from the fresh CSF sample with the help of sterile micropipette and transferred into a clean dried small test tube (5ml). Then a drop of Turk's Solution was added to the tube to obtain 1:2 dilutions if the CSF sample was clear. For the turbid CSF sample, further dilutions were made as follows:

Four drops and nine drops of Turk's Solution were added to obtain 1:5 and 1:10 dilutions. To obtain 1:100 dilutions, a drop of 1:10 diluted CSF sample was taken in another clean test tube and then nine drops of Turk's Solution was added. The diluted samples were gently shaken.

A Neubauer's Counting Chamber with 0.1mm depth of counting surface and 9 squares with $1mm^2$ areas each on each counting side was used. The slide was placed safely on the horizontal bench plane and a cover slip was placed on the slide. A well washed micropipette was used to charge the counting chamber. The charged counting chamber was allowed to stand for about a minute and observed in the microscope under 10X objective. The leukocytes were counted on each corner square. Total number of leukocytes per mm^3 was obtained by using following calculation:

$$\text{Total Leukocyte Count/mm}^3 = \frac{\text{Total cell X Dilution Factor X Depth Factor}}{\text{Area Counted}}$$

Where,

Depth Factor = 10

Area counted = 4

4.2.5 Estimation of sugar and protein

The GOD-POD method was used for detection of serum and CSF glucose concentration. The glucose is determined after enzymatic oxidation in presence of Glucose oxidase. The formed hrdrogen peroxide reacts under catalysis of peroxidase with Phenol and 4 aminophenazone to red-violet quinoneimine dye as an indicator.

Reaction principle:

$$\text{Glucose} + O_2 + H_2O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4\text{ aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinonemine} + 4H_20$$

Photometric test using Pyragallol red was the method used for detection of protein concentration in CSF. Together with pyragallol red/molybdate, proteins form a red compex. The intensity of colour is directly proportional to protein concentration in specimen.

The procedure for estimating CSF sugar and protein and Blood sugar is given in Appendix IV.

4.2.6 Microscopic examinations

The CSF samples were centrifuged at 3000x g revolutions per minute for 10 minutes. From the deposit of the centrifuged samples Gram staining was done according to the standard protocol. Gram staining procedure is given in Appendix III (A). Gram stained smear was observed for the presence of microorganisms for its cell morphology and number.

4.2.7 Culture

The sample was inoculated onto the chocolate agar plate, blood agar plate and MacConkey agar plates in order. Blood agar and chocolate agar plates were incubated in candle jar (5-10% CO_2) at 37^oC for overnight and Mac Conkey agar plates were incubated at 37^oC in incubator for overnight.

4.2.8 Identification of the isolate

The culture plates were examined after overnight incubation and the organism showing the growth on the streaked line were identified with the use of standard microbiological techniques as described in the Bergey's manual which include observation of colony morphologies, staining reactions, and biochemical properties. Standard protocol provided by Cheesbrough (2000) and Collee *et al.* (1996) and Gray & Fedorko (1992) was followed for identification of bacteria isolated from CSF specimens.

Biochemical tests: Appropriate biochemical tests were performed for the confident identification of the bacterial isolates. For that, the growth of the bacteria from the primary culture plates was subcultured onto the different agar plates as required to obtain the pure culture which were inoculated onto different biochemical media. Optochin disc and Bacitracin discs were also placed on the subcultured plate in order to differentiate pneumococcus from other gram positive cocci.

Gram-positive organisms were identified primarily on the basis of their response to Gram's staining, catalase, oxidase and coagulase tests.

For the identification of *Streptococcus pneumoniae,* bile solubility test was done. The procedure is given in Appendix-XIII.

The biochemical tests used for the identification of Gram-negative bacterial isolates include Catalase test, Oxidase test, Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Triple Sugar Iron (TSI) test, Urease test, Motility test and Gas production tests.

The composition and preparation of biochemical media and reagents used in the biochemical test are mentioned in the Appendix-II. The procedure for performing biochemical tests are mentioned in Appendix-V.

4.2.9 Antibiotic susceptibility pattern of the isolate

Standard Kirby-Bauer disc diffusion method was followed for the antibiotic susceptibility test. The pure culture of the isolate was inoculated into Nutrient broth and incubated for about 4 hours at 37^0C and then using a sterile swab the organism was swabbed on Mueller Hinton agar surface plate. For the isolates like *S. pneumoniae* Mueller Hinton agar containing 10% of sheep blood was used for testing its antibiotic susceptibility testing. Commercially available antibiotic discs manufactured by Hi media were used. The antibiotics discs were taken out from refrigerator and after bringing to room temperature, appropriate discs were placed over the agar plates containing the carpet culture of the isolates with the help of sterile forceps. The plates were then incubated at 37^0C for overnight. Similarly, MHA containing 10% blood and chocolate agar plates were incubated anaerobically in candle jar incubator for overnight.

Then after incubation the size of zone of inhibition were measured and the results were interpreted on the basis of the standard format.

4.2.10 Quality control for tests

All tests were performed with regular quality control.

During the study, the sterility of each batch of the test medium was confirmed by incubating uninoculated plates and tubes overnight at 37^oC. The incubated plates and tubes of the batch of the medium were not used if those plates and tubes showed the evidence of bacterial growth and other visual reactions after incubation.

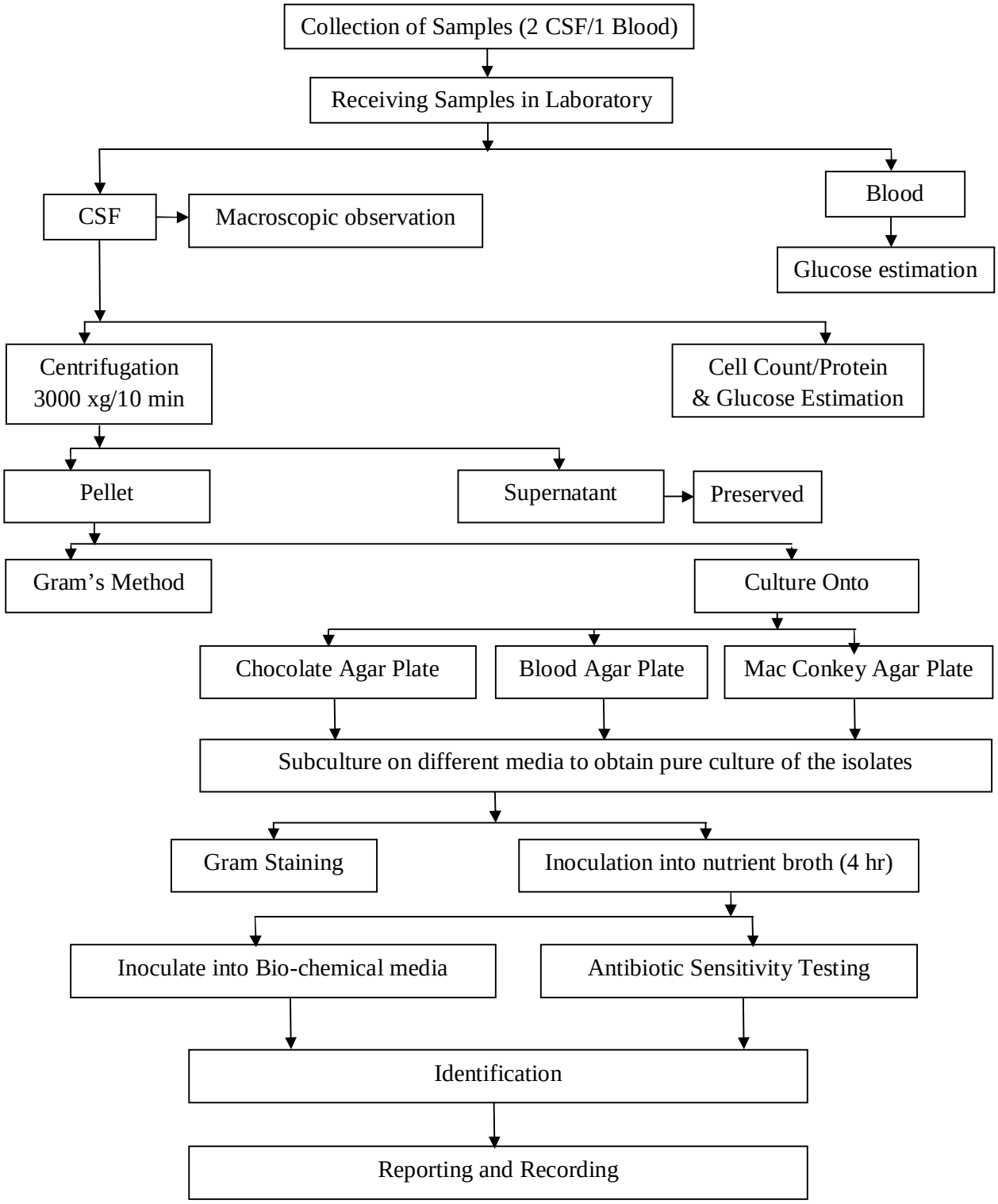
Control test were also made to confirm that test medium has been made correctly or not. For this, from each batch, one test medium was inoculated with a standard culture of bacterium known to give a positive reaction. Control strains of *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used to check the quality of the

medium from each batch. During identification of the organism, for each test ATCC control positives and control negatives was taken simultaneously. Quality of sensitivity tests was maintained by maintaining the thickness of MHA and MBHA at 4 mm and the pH at 7.2-7.4. Similarly antibiotics discs containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all the procedures.

4.2.11 Data Analysis

Chi square test was done to find association in between cases of meningitis and gender, and association in between higher count of Leukocyte with cases of meningitis. Karl Pearson test was done to find Correlation in between isolation of organism in Gram stain of fresh CSF sample and growth of an organism in the culture medium. The Calculation of Specificity and Positive Predictive value were made.

FLOW CHART SHOWING THE PROCESSING OF CSF SAMPLES



CHAPTER V

5. RESULTS

The results so obtained from the study are presented in tables and photomicrographs.

Table 5.1 Sexwise age distribution pattern and total positive cases from meningitis suspected patients attending TUTH.

S.N.	Age	Male		Female		Total Samples	Total +ve cases (%)
		No. of samples	BM +ve (%)	No. of samples	BM +ve (%)		
1	0–45 days	24	3 (12.5)	24	1 (4.1)	48	4 (8.3)
2	45 days-1 yr	14	0	20	0	34	0
3	1 - 14 yrs	12	1(8.3)	5	0	17	1 (5.8)
4	14 – 60 yrs	32	2 (6.2)	18	3 (16.6)	50	5 (10)
5	>60 yrs	7	1 (14.2)	6	0	13	1 (7.6)
Total		89	7 (7.8)	73	4 (5.4)	162	11 (6.7)

Of total 162 CSF samples so received, 11 (6.8%) isolates were recovered. Neonates 4 (8.3%), children 1 (5.8%), adults 5 (10%) and elderly 1 (6.7%) were known to have laboratory confirmed cases of meningitis. The highest percentages of isolates 5 (45.5%) were recovered from the adult patient (14years-60years). It was followed by neonates from whom 4 (36.4%) organisms were isolated (Table: 5.1). Male and female were found not to differ significantly with cases of meningitis ($P \leq 0.05$, M:F=1.75) as calculated by χ^2 test (Appendix XI-A). (**BM**: Bacterial meningitis)

Table 5.2 Bacterial isolates recovered from different age and gender of patients.

Age group	sex	*E.coli*	Gram negative diplococci	*K.pneumoniae*	*P.aerugonisa*	*S.aureus*	*S.pneumoniae*	Total
0-45 days	M	1				1	1	3
	F	1						1
45- 365 days	M							0
	F							0
1-14 Years	M					1		1
	F							0
14-30 Years	M			1	1			2
	F		1	1				2
30-60 Years	M						1	1
	F							0
>60 Years	M						1	1
	F							
Total		2	1	2	1	2	3	11

Among 11 isolates *S. aureus* (1), *S. pneumoniae* (1), and *E. coli* (2) from neonates; *S. aureus* (1) from children age group 1 -14 years were isolated and identified. 1 gram negative diplococcus was isolated from age group 14-30years. *K. pneumoniae* (2), *P. aerugonisa* (1) from age group 14-30years , *S. pneumoniae* (1) from age group 30-60 years and *S. pneumoniae* from age group >60 years were isolated and identified.
(*S. aureus: Staphylococcus aureus, S. pneumoniae: Streptococcus pneumoniae*)

Table 5.3 Percentage distribution of organisms isolated

S.N	Organisms	no. of organisms	Percentages %
1	*Eccherichia coli*	2	18.2
2	Gram negative diplococi	1	9
3	*Klebsiella pneumoniae*	2	18.2
4	*Staphylococcus aureus*	2	18.2
5	*Streptococcus pneumoniae*	3	27.4
6	*Pseudomonas aerugonisa*	1	9
	Total	11	100

3 (27.4%) *Streptococcus pneumoniae*; 2 (18.2%) each *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* and 1 (9%) *Pseudomonas aerugonisa* and gram negative diplococcus were recovered from the CSF (Table: 5.3).

Table 5.4 Total isolates obtained from Gram stain of fresh CSF and culture

Organism observed on Gram's stain		**Organism isolated on culture**	
Yes	**No**	**Yes**	**No**
E. coli (2), *K. pneumoniae* (2), gram negative diplococcic(1) *Streptococcus pneumonia*e (3), *P. aerugonisa (1)*	*S. aureus* (1)	*E. coli* (2), *K. pneumoniae* (2) *S. aureus* (2) *Streptococcus pneumonia*e (3), *P. aerugonisa* (1)	Gram negative diplococci

Among 11 bacterial isolates, Gram negative organisms were more predominant than Gram positive organisms. Of these isolates 1 *S. aureus* couldn't be isolated from Gram's stain and 1 Gram negative diplococcus couldn't be grown on culture medium. High degree of positive correlation (r=0.79) in between the organisms isolated in Gram stain of fresh CSF sample and growth in culture medium was found (Appendix XI-C). (*S. aureus: Staphylococcus aureus, S. pneumoniae: Streptococcus pneumoniae*)

Table 5.5 Macroscopic appearances of CSF, no. of isolates and range of leukocytes.

S.N	**Appearances**	**No. of CSF samples**	**Cases of BM**	**Range of leukocyte**
1	Clear	127	0	0-100
2	Slightly turbid	9	1	100-500
3	Cloudy	7	7	500-11,000
4	Definitely purulent	2	2	>11,000
5	Bloody	17	1	100-500

Of total 127 clear sample having leukocyte within range 0-100 no isolates were obtained. From 9 slightly turbid samples, leukocyte within range 100-500 only 1 isolate was obtained. From 7 cloudy samples, leukocyte range 500-11,000, 7 isolates were

obtained. From 17 bloody samples only 1 isolate was obtained (Table 5.5). The association of higher count of leukocyte in CSF and cases of meningitis was found to be statistically significant (P=0.05) (Appendix XI-B).

Table 5.6 Types of an organism isolated from turbid CSF samples.

S.N	Appearances	observed on Gram's stain	Organisms isolated on culture medium
1	Slightly turbid	+	*E. coli* (1)
2	Cloudy	+	*S. pneumoniae* (3), *K. pneumoniae* (1), *E. coli* (1), *S. aureus* (1), Gram negative diplococcus (1)
3	Definitely purulent	+	*P. aerugonisa*(1), *K. pneumoniae* (1)
4	Bloody	-	*S. aureus* (1)

Organisms were isolated only from turbid samples in Gram's stain. One isolate *S. aureus* could only be isolated on culture and was not seen on Gram's staining, which was recoverd from bloody sample. (*S. aureus: Staphylococcus aureus, S. pneumoniae: Streptococcus pneumoniae*)

5.7 Value of CSF glucose in suspected cases of meningitis

	Bacterial meningitis	
CSF glucose	**present**	**absent**
Below normal	11	18
normal	0	133

Of total 29 CSF samples showing low CSF glucose value than the normal value, only 11 cases of bacterial meningitis were determined (normal values given in Appendix VI-A). The Specificity was 88.07% and PPV was37.93% (Appendix X-A)

5.8 Value of CSF protein in suspected cases of meningitis

CSF protein	Bacterial meningitis	
	present	**absent**
Above normal	11	24
normal	0	127

Of total 35 CSF samples showing high protein values than the normal value; only 11 cases of bacterial meningitis were determined (normal values given in Appendix VI-A). The Specificity was 84.10% and PPV was calculated to be 31.42% (Appendix X-B)

5.9 Value of CSF /serum glucose ratio in suspected cases of meningitis

CSF/serum glucose ratio	Bacterial meningitis	
	present	**absent**
Below normal	11	5
normal	0	146

Of total 16 CSF samples having CSF/serum glucose values below normal range of 0.4-0.5 (Deisenhammer *et al*., 2006) only 11 cases of bacterial meningitis were determined. The Specificity of the test was 96.68% and PPV 69.43% (Appendix X-C).

Table 5.10 Antibiotic Susceptibility Testing (AST) of bacterial isolates

S.N	Organisms/antibiotics	Sensitive (S)	Intermediate Resistant (IR)	Resistant (R)
A.	*Streptococcus pneumoniae* (N=3)			
1	Cefotaxime (CE)	2	1	0
2	Chloramphenicol (C)	3	0	0
3	Ciprofloxacin (CF)	3	0	0
4	Co-Trimoxazole (CO)	0	1	2
5	Erythromycin (E)	3	0	0
6	Penicillin (P)	3	0	0
B.	*Staphylococcus aureus* (*N*=2)			
1	Amikacin (AK)	2	0	0
2	Cefotaxime (CE)	1	0	1
3	Ceftazidime (CA)	2	0	0
4	Chloramphenicol (C)	2	0	0
5	Cloxacillin (OXA)	2	0	0
6	Ofloxacin (OF)	0	0	2

Streptococcus pneumoniae (3) showed Resistant against CO (2) and Intermediate Resistant against CE (1) and CO (1). *Staphylococus aureus* (2) showed Resistant against CE (1) and OF (2). Both Gram positive organisms were Sensitive towards other antibiotics so tested (Table 5.10; A & B).

S.N	**Organisms/antibiotics**	**Sensitive**	**Intermediate Resistant**	**Resistant**
C.	*Escherichia coli* (*N*=2)			
1	Amikacin (AK)	1	0	1
2	Cefotaxime (CE)	1	1	0
3	Ceftriaxone (CI)	2	0	0
4	Chloramphenicol (C)	2	0	0
5	Co-Trimoxazole (CO)	1	0	1
6	Ofloxacin (OF)	0	1	1
D.	*Klebsiella pneumoniae* (*N*=2)			
1	Amikacin (AK)	1	0	1
2	Cefotaxime (CE)	1	1	0
3	Ceftriaxone (CI)	0	0	2
4	Chloramphenicol (C)	1	1	0
5	Co-Trimoxazole (CO)	2	0	0
6	Ofloxacin (OF)	2	0	0
E.	*Pseudomonas aerugonisa* (*N*=1)			
1	Amikacin (AK)	1	0	0
2	Cefotaxime (CE)	1	0	0
3	Ceftazidime (CA)	1	0	0
4	Chloramphenicol (C)	0	0	1
5	Ciprofloxacin (CF)	0	0	1
6	Ofloxacin (OF)	1	0	0

E. coli (2) showed Resistant against Amikacin (1), Co-Trimoxazole (1) and Ofloxacin (1); Intermediate Resistant against Cefotaxime (1) and Ofloxacin (1). *K. pneumoniae* (2) showed Resistant against AK (1) and CI (2), IR against CE (1) and C (1). *P. aerugonisa* (1) showed Resistant against C and CF. All gram negative organisms were sensitive towards other antibiotics so tested.

PHOTOMICROGRAPHS

1. Gram stain result of CSF sample showing Gram negative diplococci with pus cells.

2. Investigator performing lab work

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

Acute bacterial meningitis caused by variety of microorganisms is a serious disease. Immediate diagnosis and proper treatment is required to reduce the rate of mortality, morbidity and development of neurological sequeale

The CSF was collected in two sterile vials numbered 1 and 2. Vial no.1 was used for culture and Gram stain and no. 2 was used for all other investigations which include cell count, protein and glucose estimation. Only 3-5 ml of fluid should be collected (Collee *et al.*, 2006). 1 ml of fluid in sterile container no.1 and 2-3ml in other container no.2 is collected. In the container 1, first sample collected is used for culture and in other container for other investigations (Cheesbrough, 2005). The CSF was centrifuged in a sterile tube at 3000 rpm/10 min; the pellet was used for staining and culture. Tiwari *et al.* (2002), also suggested the centrifugation of CSF for microbiological study at 3000 rpm/10 min.

Altogether 162 samples collected from patients suspected of meningitis were analyzed and studied. 11 (6.7%) cases of patients with meningitis are recorded. Retrospective data of meningitis calculated by Tiwari *et al*. (2002), showed 7.12% of isolated cases of meningitis. No significant differences were found in both of these studies ($P \leq 0.05$). Shaha *et al*. (2005) gave report of 22 (44%) of patients with pyogenic meningitis. Chandramuki (2007) gave report of 284 (73.8%) of culture positive cases of meningitis.

The low percentage of cases isolated in TUTH may be due to outreach of hospital services within patients from only Kathmandu and nearby valley areas, where effective

vaccination against Hib and Pneumococcus have been practiced and also the hygienic living condition of the people in this area.

Although 7 males and 4 females were identified as having bacterial meningitis (BM), there was no significant differences in between male and female ($P \leq 0.05$; M:F::1.75:1). No significant differences was observed from the study conducted by Tiwari *et al*. (2002), ($P \leq 0.005$; M:F::1.28:1). The highest percentage of isolates i.e. 10% (5), were from adults (14-60 yrs), followed by neonates 8.3% (4) and elderly people 7.6% (1). 4 (80%) cases were from adults below 29 years of age.

Anyone can contract meningitis, but those most at risk are children under five, young adults (the 14-25 age group), and older people (over 55) (Innes, 2001). Bacterial meningitis strikes schools particularly colleges annually, since the college students are in close contact with other students (in classrooms and dormitories). The University of Central Florida and Marian College having two cases each of bacterial meningitis have been reported by Gerics (2007). High risk of people for meningitis is in age in between 15 and 24 as reported by Kerek (2009). The median age group at which BM is diagnosed has shifted from 15 months to 25 years ("Mayoclinic", 2010). The age distribution of acute bacterial meningitis (ABM) has shifted from children to adults. At the same time, older population is increasing (Laguna-Del-Estal *et al*., 2010).

Escherichia coli (2), *Staphylococcus aureus* (1), *Streptococcus pneumoniae* (1), were found in neonates. Gram negative intracellular diplococci (1), *Klebsiella pneumoniae* (2), *Pseudomonas aerugonisa* (1), and *Streptococcus pneumoniae* (1) were found in adults. 1 *Staphylococus aureus* and 1 *Streptococcus pneumoniae* was isolated in children and elderly respectively.

Meningococcal and pneumococcal meningitis can occur at any age. Staphylococcal meningitis is most common in first few months of life. *H. influenzae* meningitis occurs between the ages of three months and five years (Smith & Easmon, 1990). The incidence of an organism differs greatly with age (Baron *et al*., 1994).

In a Canadian review of 101 cases of neonatal meningitis done by Newman *et al*. (2001) determined meningitis was caused by *E.coli*. In babies older than 1 week old *Streptococcus pneumoniae* was found to cause bacterial meningitis (Vergnano *et al*., 2005). *Escherichia coli*, and *Staphylococcus aureus* causing newborn BM has been reported by Silva *et al*. (2007). Studies from underdeveloped countries suggest that gram negative bacilli such as *Escherichia coli* may be more common than group B streptococci (Dredge & Krishnamoorty, 2010). In a review of studies from Asia, Africa and Latin America, Zaidi, *et al*. (2009) reported that the most common organisms were *Escherichia coli* and *Staphylococcus aureus. Streptococcus pneumoniae* is the cause of pediatric meningitis resulting in substantial morbidity and mortality in developing countries (Prieto *et al*., 2009).

Community-acquired *Staphylococcus aureus* meningitis (CASAM) in children hospitalized from 1983 to 1998 at Nossa Senhora da Glória Children's Hospital (HINSG) has been reported by Rodrigues *et al*. (2000). The third striking clinical observation is the preponderance of *K. pneumoniae* as a cause of community-acquired bacterial meningitis in adults in Taiwan, even in the absence of liver abscess or other sites of infection. The proportion of cases of culture-proven bacterial meningitis due to *K. pneumoniae* in one Taiwanese hospital increased from 8% during 1981 and 1986 to 18% during 1987 to 1995. In a recent large review cases of community acquired bacterial meningitis from the Massachusetts General Hospital were reported due to *K. pneumoniae* (Ko *et al*., 2002). 60 (49.1%) of adult *Klebsiella* meningitis patients have been identified at Kaohsiung Chang Gung Memorial Hospital by Chang *et al*. (2002). 25 cases of CSF culture proven *Pseudomonas aerugonisa* Adult Bacterial Meningtis (ABM) reported in 17 men and 8 women by Huang *et al*. (2007).

Bacterial meningitis remains a highly lethal disease in older adults. The spectrum of etiologic bacterial organisms is broader than that for a younger population. The responsible bacterial organisms include *S. pneumoniae;* and, less commonly, *N. meningitidis* or *H. influenzae* (Choi *et al*., 2001). With the 'UN International Day for

Older People' on October 1 fast approaching, the Meningitis Trust issued a warning about the dangers of meningitis to the over 55s as cases in this age group are on the increase. Approximately 10% of bacterial meningitis cases occur in the over 55s, so they are considered an 'at risk' group (“Meningitis Trust”, 2008).

In a recent Taiwan study, conducted by Tang *et al.* (1999), *N. meningitidis* was no longer a common organism responsible for infants and children meningitis but *Klebsiella pneumoniae* was the most important in both community acquired and nosocomial meningitis in an adults. Though the common pathogens associated with CAABM are *Streptococcus pneumoniae, Haemophilus influenzae,* and *Neisseria meningitidis* the etiological agents and their relative frequency may vary in different geographical areas. As compared to Western studies the relative incidence of meningitis cause by *H. influenzae, N. meningitids,* and *L. monocytogenes* is less in South-East Asia on contrary gram negative bacilli such as *Klebsiella pneumoniae* and *Pseudomonas aerugonisa* are increasingly been recognized as important pattern of community acquired as well as nosocomial meningitis (Chandramuki *et al.*, 2007).

Among 11 bacterial isolates, 6 (54.54%) were Gram negative organisms where as 5 (45.45%) were Gram positive organisms. Thus Gram negative organisms were found to be predominant in this study group also which correlates with the study done by Gorman *et al.* (1962) and Tankhiwale *et al.* (2001). The predominance of Gram-negative organisms reported as etiological agents of bacterial meningitis was also seen in the study done by Rao *et al.* (1998) where Gram-negative bacteria were isolated from 63.3%, while Gram-positive bacteria were found only in 36.7% of the cases.

The highest percentage of isolates were *Streptococcus pneumoniae* (27.3%), followed by *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae*. 1 (9.09%) were Gram negative diplococci, and *Pseudomonas aerugonisa.* Retrospective study of Community Acquired Acute Bacterial Meningitis (CAABM) done by Chandramuki, *et al.* (2007), reported *Streptococcus pneumoniae* as the predominant cause of meningitis.

In this study, Gram stain detected organism in 10 (90.9%) and culture positive was seen in 10 (90.9%). Gram negative intracellular parasite so detected by Gram stain couldn't be grown on culture media and a organism so not detected on Gram stain from bloody sample was isolated on culture. High degree of positive correlation (r=+0.79) in between the organisms isolated in Gram stain of fresh CSF sample and growth in culture medium was found.

Tiwari *et al*. (2002) also gave high degree of positive correlation (r= +0.8) of direct Gram staining and growth of organism. 115 (45.45%) culture negative cases were reported by Chandramuki *et al*. (2007). In 60 (23.71%) culture negative cases the etiological pathogen was recognized only by Gram stain. The low yield of bacteria on culture are prior antibiotics therapy, delay in transport of specimen to microbiological laboratory, no availability of special media for specific pathogen, presence of autolysis enzyme in CSF and lack of 24hr facility for processing CSF specimen. A simple Gram stain smear can offer the immediate clues to aid a diagnosis of pyogenic meningitis. Studies have reported a CSF Gram stain sensitivity of 60-90% and high specificity, stressing its importance in rapid and accurate diagnosis of the causative bacteria. The Gram stain positive in 89% of cases was reported by Dubos *et al.* (2008), its performance depends on the density of organisms (and possibly duration of illness at diagnosis), as well as the skill of the examiner. The Gram stain sensitivity varies by study from 55% to 90%. Viable counts below or equal to 10^3 CFU/ml for all organisms were associated with poor microscopic results (La Scolea *et al*., 1984).

9 (100%) isolates were recovered from cell count >4,000 and 75% isolates from 500-4,000 Polymorphonuclear neutrophils (PMN). 9 (100%) isolates were seen from definitely purulent and cloudy specimen and 1 (11.11%) isolate from slightly turbid specimen. 1 (5.88%) isolate was obtained from bloody CSF. The cell count was done with the help of Neubauer's chamber. No isolates were recorded from clear CSF. Slight increase in value of protein from bloody CSF was recorded. Although CSF samples had cell count more than normal value, in most of the cases organisms were not isolated.

This may be due to aseptic cases (viral meningitis) or patients with meningism which can be correlated only with serological, chemical and clinical findings of the patient. Prior administration of antibiotics to the patients decreases the rate of isolation of the organisms.

One of the hallmarks of bacterial meningitis is the development of Neutrophilic pleocytosis within the CSF (Tunkel & Scheld, 1993). Cytological evaluation should be performed within 2hr after puncture, preferably within 30min because of lysis of both RBC and WBC. Lymphocytes and Monocytes at the resting phase and occasionally ependymal cells are found in normal CSF. An increase in number of neutrophilic granulocytes can be found in bacterial and acute viral CNS infection (Deisenhammer *et al.*, 2006). A white cell count with an indication whether the cells are pus cells or lymphocytes, is required when the CSF appears slightly cloudy. Improved Neubauer ruled chamber is used and cells are counted in 4 large squares and reported in cells/L of CSF (Cheesbrough, 2005). Large number of Leukocytes was seen on microscopy with higher bacterial concentration and low bacterial numbers were associated with rare Leukocytes (Scolea & Dryja, 1984). A correction for the presence of blood due to traumatic tap by subtracting 10mg/L for every 10^9 erythrocytes/L is recommended by Marshall & Bangert (1995). Peripheral blood in the CSF after a "traumatic tap" will result in an artificial increase in WBCs by one WBC for every 500 to 1,000 RBCs in the CSF. This correction factor is accurate as long as the peripheral WBC count is not extremely high or low. A traumatic tap occurs in approximately 20 percent of lumbar punctures (Seehusen *et al*., 2003).

The CSF protein was increased and glucose concentration was decreased in the cases with meningitis. The ratio of CSF/serum was shown to decrease below the normal range in case of meningitis.

The CSF glucose concentration is reduced in most forms of meningitis, except viral meningitis. In pyogenic bacterial meningitis it is markedly reduced and may even be undetectable (Cheesbrough, 2005). Striking elevation in CSF total protein is seen in

bacterial meningitis. Bacterial meningitis causes inflammation of the meninges which increases permeability of blood-CSF barrier to plasma protein (Burtis *et al.*, 2006).

Glucose is actively transported across the blood brain barrier. The CSF glucose levels are directly proportional to the plasma and therefore simultaneous measurement in CSF and blood is required. A CSF/serum ratio less than 0.4-0.5 is considered to be pathological. Normal CSF glucose concentration is 50-60% of the serum value. Decreased CSF/ serum glucose ratio indicates bacterial infection (Deisenhammer *et al.*, 2006). In health ratio of CSF to plasma glucose of 0.6-0.8 is maintained through glucose utilization by cells close to CSF. A contemporaneous blood specimen for glucose measurement should always accompany a CSF for glucose analysis, both specimens being suitably preserved. The CSF/serum glucose ratio falls below normal range in number of conditions and most notable reductions are observed in bacterial, tuberculous and some fungal meningitis (Marshall & Bangert, 1995).

Markedly decreased CSF glucose with markedly increased total protein, high WBC count with 89% Neutrophils, and the presence of a large number of Polymorphonuclear Leukocytes and bacteria in the Gram-stained smear of the CSF sediment are the most striking laboratory results in bacterial meningitis (Harrington & Plenzler, 2004).

18 CSF samples were known to have below normal range of glucose concentration. In vitro glycolysis is seen in presence of leukocytes which decreases the glucose level with delay in processing of CSf sample (Burtis *et al.*, 2006). CSF from 24 patients were shown to have high protein value but with no recognized cases of bacterial meningitis. Aseptic meningitis is characterized CSF pleocytosis and negative bacterial and fungal culture. Aseptic meningitis is usually associated with viral infection and is also a component of Syphilis and some other spirochaetal disease (e.g. Leptospirosis and Lyme borreliosis) (Forbes *et al.*, 2007). Aseptic meningitis was applied to an illness of acute onset with clinical features of meningitis, leukocytes but no bacteria in the CSF, and short and benign course. Aseptic meningitis is almost commonly caused by viral agent, especially an enterovirus but may also be due to other infective agents. It may

also result from Tumor, Cysts, chemicals, sarcoidosis or other noninfectious cause (Chakraborty, 2005).

Bacterial infections of CNS, especially acute infections such as BM requires immediate, invariably empiric antibiotic therapy.

Among 3 isolates of *S. pneumoniae* all were sensitive (S) towards Chloramphenicol (C), Penicillin (P), Ciprofloxacin (CF), Cefotaxime (CE) and Erythromycin (E).1 isolate showed Intermediate resistant (IR) against Co-Trimoxazole (CO), and Cefotaxime (CE). 2 isolates showed Resistant (R) against Co-Trimoxazole (CO). *S. pneumoniae* showed Resistant to Co-Trimoxazole, where 16% showed IR, and resistant to Cefotaxime (CE). All isolates were susceptible to Chloramphenicol (C), Erythromycin (E), and Penicillin (P) (Shah *et al*., 2009; Williams *et al*., 2009). 3.5% of *S. pneumoniae* isolates showed resistance to Ceftriaxone (CE) (Jones *et al*., 2003).

Among 2 isolates of *Staphylococcus aureus* all showed their sensitivity towards Amikacin (AK), Cloxacillin (CX), Ofloxacin (OF) and Ceftazidime (CA). Only 1 isolate was sensitive to Cefotaxime (CE) while other was resistant. 1 isolate was sensitive towards Chloramphenicol (C) and 1 showed IR. Only 0.2% of *S. aureus* showed resistant against Ceftazidime (Rogues *et al*., 2003). 57% of *S. aureus* were shown to be highly resistant against Cefotaxime (CE) (Shakibaie *et al*., 2000).

Among 1 isolates of *E. coli* was sensitive towards Amikacin (AK), Ceftriaxone (CI), and Chloramphenicol (C). 1 isolate was sensitive to Co-Trimoxazole (CO), and Cefotaxime (CE). 1 isolate showed IR against Ofloxacin (OF), and Cefotaxime (CE). 1 isolate showed resistant against Co- Trimoxazole (CO), and Ofloxacin (OF) and Amikacin. Isolates of *E. coli* being Cefotaxime and Fluoroquinolones resistant were reported by Lee *et al*., 2004. *E.coli* showing resistant against Ceftriaxone (CI) was reported by Jones *et al*. (2003). Multiple drug resistance is a condition enabling a disease causing organism to resist distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating the organism (Wikipedia, 2010). Cases of MDR *E. coli* has been reported by Sonavane *et al*. (2008) and Garcia (2005)

Among 2 isolates of *K. pneumoniae* both were sensitive towards Co-Trimoxazole (CO) and Oflxacin (OF). 1 isolate was sensitive towards Amikacin (AK), Cefotaxime (CE), and Chloramphenicol (C). 1 isolate showed IR towards Cefotaxime (CE), and Chloramphenicol (C). Both isolates were resistant against Ceftriaxone (CI) and 1 isolate was resistant against Amikacin (AK). Ceftriaxone (CI) resistant in *K. pneumoniae* was reported by Jones *et al*. (2003).

Emergence and spread of antibiotic resistant gene among bacterial pathogen is becoming a serious problem worldwide. The Amikacin (AK) is very useful in treatment of multidrug resistant infection because only limited no. of modifying enzymes, such as AAC (6′)-I type acetyltransferases are able to inactivate it. Multi resistant strain harbouring AAC (6′)-I type enzyme especially AAC (6′)-Ib, has seriously limited the successful use of Aminoglycoside including Amikacin (AK) (Bistue *et al*., 2009).

Pseudomonas aerugonisa was sensitive towards Amikacin (AK), Cefotaxime (CE), Ofloxacin (OF), Ceftazidime (CA). It was resistant against Ciprofloxacin (CF) and Chloramphenicol (CO). 43% of isolates of *P. aerugonisa* showing Ciprofloxacin (CF) resistant have been reported by Rogues *et al*. (2003).

Although even the most potent and recently developed antimicrobial drugs are available throughout the world, in developing countries their use is confined to those who are wealthy enough to afford them. In tertiary referral hospitals such as the Kenya National Hospital in Nairobi, Kenya, the first line antimicrobial drugs used are Ampicillin, Chloramphenicol, Co-amoxiclav, Co-trimoxazole, Erythromycin, Gentamicin, Penicillin, and Tetracycline. Amikacin, Cefuroxime, and Ciprofloxacin, are used as second line agents. In district hospitals only the first line agents are available, but sometimes not even these are available (Hart & Kariuki, 1998).

High resistance to Cotrimoxazole (CO) could be the result of frequent prescribing, easy availability of the drug, and the practice of prescribing it to treat suspected bacterial pneumonia cases. In our study, isolates showed no resistance to Chloramphenicol (C), which may reflect the rare use of this drug in Nepalese hospitals and in the community.

In a study done by Dutta and Bhatnagar (2001) on a rational antibiotics therapy in bacterial meningitis, they stated that the neonatal meningitis is best treated with a combination of ampicillin and a third generation cephalosporin for the wide range of Gram positive and Gram negative bacilli and third generation cephalosporin with or without ampicillin for pneumococci, haemophilli and meningococci. They further supported that the therapy should be modified, if necessary, on availability of culture susceptibility pattern. Combination of Ceftriaxone or Cefotaxime with Vancomycin for empiric therapy of pneumococcal CNS infection has been suggested by Jones *et al*. (2003).

6.2 CONCLUSION

Hence the comparative evaluation of the macroscopical observation, microbiological test and determined routine parameters from the CSF sample collected from patients attending TUTH, suspected of meningitis was done to diagnose meningits. The significant association in between the cell count and bacterial isolates was determined. The suspected cases of meningitis were supported with the protein, glucose and ratio of CSF/serum glucose value for diagnosis. No recurrent cases of meningitis were found.

CHAPTER VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. Of total 183 samples of CSF so obtained in the Laboratory, 162 samples suspected of Meningitis were processed during period of 5 months (16^{th} Sept 2009, to 16^{th} Feb 2010). Among them 89 (55%) were Male and 73 (45%) were Female.

2. The highest percentage of patients 30.8% (50) suspected of Meningitis was from age group 14-60 years.

3. Of total patients of Meningitis, 7 (63.6%) were Male and 4 (36.3%) were Female, but Male and Female patients were found not to differ significantly with cases of Meningitis ($P \leq 0.05$, M:F=1.75).

4. A total 11 (6.7%) isolates were recovered. The highest percentage of isolate was obtained from age group within 14-60 years i.e. 5 (10%).

5. *Streptococcus pneumoniae* 3 (27.4%), 2 (18.2%) each *E. coli*, *K. pneumoniae*, *S. aureus*, and 1 (9%) each *Pseudomonas aerugonisa* and a Gram negative diplococci were isolated.

6. From definitely purulent and cloudy samples, 9 isolate were obtained, 1 from slightly turbid sample, and 1 from bloody sample.

7. From the CSF sample having leukocyte count >4000, 6 isolates were recovered; 3 were isolated from leukocyte within range 500-4000, 1 was isolated from leukocyte range of 200-500, 1 of isolate obtained from leukocyte range of 100-200.

8. Most of the isolates were sensitive towards Chloramphenicol (C).

9. The specificity and PPV of CSF glucose analysis in Bacterial Meningitis was 88.07% and 37.93%, of CSF protein analysis was 84.10% and 31.42%, and analysis of the ratio of CSF/ serum glucose was 96.68% and 69.43%.

7.2 RECOMMENDATIONS:

1. Notmal CSF protein concentration should be related to patient's age (higher in neonates and age > 60yrs) and site of collection.

2. Not only measured value of CSF glucose concentration but the ratio of CSF/serum glucose should be taken into consideration. Pathological changes in this ratio or in Lactate concentration are supportive for bacterial and fungal meningitis.

3. Where different disciplines examine CSF, co-operation in between different department will be necessary for correct diagnosis.

REFERENCES

Akhadar A, El-Hassani MYR, Chakir N and Jiddane M (2001) Optochaismatic tuberculoma as complication of tuberculous meningitis: A new case and literature review. J Neurol 28: 137-42.

Al-Abbasi AM (2002) Tuberculous meningoencephalitis in Baghdad, 1993–99: a clinical study of 224 cases. East Med Health 2: 231-40.

Alam MR, Saha SK, Nasreen T, Latif F, Rahman SR and Gomes DJ (2007) Detection, Antimicrobial susceptibility and serotyping of *Streptococcus pneumoniae* from Cerebrospinal fluid from suspected meningitis patients. Bangladesh J Microbiol 24: 24-29.

Almeida SM, Nogueira MB, Raboni SM and Vidal LR (2007) Laboratorial Diagnosis of Lymphocytic Meningits. Brazalian J Infect Dis 11: 489-95.

Annual Report 2064/2065 (2007/2008), Government of Nepal, Ministry of Health and Population. Department of Health Services, Kathmandu, Nepal.

Arnoni MV, Berezin EN, Safadi MA, Almedia FJ and Lopes CR (2007) *Streptococcus pyogenes* meningitis in children: Report of two cases and Literature review. The Brazilian J Infect Dis 11: 375-77.

Ballabh P, Braun A and Nedergaard M (2004) The blood brain barrier: an overview structure, regulation and clinical implication. Neurobiol Dis 16: 1-13.

Baron EJ, Petterson LR and Finegold SM (1994) Meningitis and other infections of central nervous system. In: Bailey and Scott's Diagnostic Microbiology, 9th ed., Mosby-Year Book, Inc, pp. 907-16.

Batuwanthudawe R, Rajapakse L, Somaratne P, Dassanayake M and Abeysinghe N (2009) Incidence of childhood *Haemophilus influenzae* type b meningitis in Sri Lanka. Int J Infect Dis 30: e1-5.

Bhigjie AL, Padayachee R, Paruk H, Pillay KDH, Marais S and Connoly C (2007) Diagnosis of tuberculous Meningitis: Clinical and Laboratory parameters. Int J Infect Dis 11: 384-85.

Bistue AJCS, Bistuae S, Martin FA, Vozza N, Ha H, Joaquin JC, Zorreguieta A and Tolmaskey MC (2009) Inhibition of aac (6′)-Ib- mediated amikacin resistance by nuclease resistant external guide sequence in bacteria. PNAS 106: 13230-35.

Boos C, Daneshvar C, Hinton A and Dawes M (2004) An unusual case of chronic Meningitis. BMC Family Practice 5:21.

Brackenridge CJ (1962) Cerebrospinal Fluid protein fraction in health and disease. J Clin Path 15: 206-10.

Brain L and Walton JN (1969) Brain's disease of nervous system, 7th ed. Oxford University press, London, pp. 355-86.

Broome CV, Facklam RR, Allen JR, Fraser DW and Austrian R (1980) Epidemiology of pneumococcal serotypes in the United States. J Infect Dis 141: 191-23.

Burtis CA, Ashwood ER and Brunus DE (2006) TIETZ textbook of Clinical Chemistry and Molecular Diagnosis, 4th ed. Elesevier, India, pp. 529-48.

Carola R, Harley PJ and Noback RC (1992) The Spinal Cord and Spinal Nerves. In: Human Anatomy and Physiology, 2nd ed. pp. 361-200.

CDC and WHO (2003) Manual for the laboratory identification and Antimicrobial Susceptibility Testing of bacterial pathogens of public importance in the developing world. pp. 5-62 (WHO/CDS/CSR/RMD/2003.6)

Center for Disease Control and Prevention (CDC). Morbidity and Mortality Weekly Report (MMWR), 1997; 46: 1-14.

Centers for Disease Control and Prevention (CDC) (1998) Laboratory methods for the diagnosis of Meningitis caused by *Neisseria meningitidis, Streptococcus pneumoniae,* and *Haemophilus influenzae* pp. 1-63.

Chakraborty P (2005) A textbook of Microbiology. New Central Book Agency, India, pp. 679-84.

Chandramuki A, Wasiulla R, Mani R, Pradhan S and Nagarathna S (2007) Bacteriological profile of community acquired acute bacterial meningitis. A ten year retrospective study in tertiary neurocare center in South India. Indian J Med Microbiol 25: 108-14.

Chandrika DAJ, Pathirana KD and Abeywickrema LAA (2009) A patient with recurrent Aseptic Meningitis (Mollaret's meningitis) due to Herpes Simplex type I infection. Galle Med J 14: 64-65.

Chang LU, Nerg CW and Win CH (2002) *Klebsiella pneumoniae* Meningitis in Audlts: Clinical features, prognostic factors and therapeutic outcomes. J Clin Neuroscience 9: 533-38.

Chaudhary S (1999) Medical Physiology, 1st ed. Central Publication, India.

Cheesbrough M (1984) Collection, Transport, and Examination of Specimens. In: Medical Laboratory Manual for Tropical Countries, 1st ESBL ed. Cambridge University Press, UK 2: 160-74.

Cheesbrough M (2005) District laboratory Practice in Tropical Countries, Part 2. Gopsons Paper Limited, Noida, pp. 116-24.

Cheesbrough Monica (2000) Microbiological tests: Examination of Cerebrospinal Fluid. In: District Laboratory Practice in Tropical Countries, Low price edition; Cambridge University Press, UK 2: 116-24.

Choi C (2001) Bacterial Meningitis in aging adults. CID 33: 1380-85.

Cochi SL, Markowitz LE, Joshi D, Owens RC, Stenhouse DH, Regmi WR, Shrestha RPB, Acharya IL, Manandhar M, Gurubacharya VL, Owens D and Reingold AL (1987) Control of epidemic Group A meningococcal Meningitis in Nepal. Int J Epidemio 6: 91-97.

Collee JG, Marmion BP, Fraser AG and Simmons A (2006) Mackie and Mc Cartney practical Medical Microbiology, 14th ed. Rajkamal electric press, India, pp.77-80.

Collee JG, Mormion BP, Fraser AG and Simmons A (1996) Mackie and Mc Cartney Practical Medical Microbiology, 14th ed. Churchill Livingstone.

Collier L, Balows A and Sussman M (1998) Topley & Wilson's Microbiology and Microbial infections, 9th ed., Vol II & III. Oxford University Press, USA.

Coovadia YM, Solwa Z and Ende JVD (1987) Meningitis caused by vancomycin resistant *Leuconostoc* sp. J Clin Microbiol 25: 1784-85.

Creager JG (2004) Human Anatomy and Physiology. Wadsworth publishing company, California, pp. 291-94.

Dalton HP and Nottebart A (1986) Intrepretative Medical Microbiology. Churchill Livingstone, New York, pp. 189-93.

De Luca A, Antinori A, Cingolani A, *et al*. (1995) Evaluation of Cerebrospinal Fluid EBV-DNA and IL-10 as markers for in vitro diagnosis of AIDS- related primary central nervous system lymphoma. British J Haemato 90: 844-49.

Deisenhammer F, Bartos A, Egg R, Gilhus NE, Giovannoni G, Rauer S and Sellebjerg F (2006) Routine Cerebrospinal Fluid (CSF) Analysis. Eur J Neurol 13: 913-22.

Denyer SP, Hodges NA and Gorman S (Eds.) (2005) Hugo and Russell's Pharamaceutical Microbiology, 7th ed. Replika press, India, pp. 233-35.

Dery MA and Hasbun R (2007) Changing epidemiology of Bacterial meningitis. Cur Infect Dis Reports 9: 301-07.

Dixon TC, Meselson M, Guilleman J and Hanna PC (1999) Anthrax. N Eng J Med 30: 462-65.

Domingo P, Barquet N, Alvarez M, Coll P, Nava J and Gram J (1997) Group B Streptococcal Meningitis in an adults: report of twelve cases and review. Clin Infect Dis 25: 1180-87.

Domelier AS, Marquet NM, Grandet A, Meneghetti L, Rosinau A and Quentin R (2006) Loss of catabolic function in *Streptococcus agalactiae* strains and its association with neonatal meningitis. J Clin Microbiol 44: 3245-50.

Dredge and Krishnamoorty (2010) Neonatal Meningitis. http://emedicine.medscape.com/article/117690_overview

Drevets DA, Leenen PJM and Greenfield RA (2004) Invasion of central nervous system by intracellular bacteria. Clin Microbiol Rev 17: 323-47.

Driel JJV, Bekker V, Spanjaard L, Eride AV and Kuijpers TW (2008) Epidemiologic and Microbiologic characterstics of recurrent bacterial and fungal meningitis in the Netherland, 1988-2005. CID 47: e42-51.

Dubos F, Rocque FDL, Levy C, Bingen E, Aujard Y, Cohen R and Beart G (2008) Sensitivity of bacterial Meningitis score in 889 children with Bacterial Meningitis. J Pediatr 152: 378-82.

Durand ML, Calderwood SB, Weber DJ, *et al*. (1993) Acute bacterial meningitis in adults: a review of 493 episodes. N Eng J Med 328: 21-8.

Dutta S and Bhatnagar R (2001) Rational antibiotics therapy in bacterial meningitis. Indian J Pediatr 68: 32-39.

Ehrlich LC, Hu S, Sheng WS, Sutton RL, Rockswold GL, Peterson PK and Chao CC (1998) Cytokine regulation of human microglial cell IL-8 production. J Immunol 160: 1944-48.

Epidemiological Record (2009); 84: 117-92 (http://www.who.int/wer).

Faller DV, Mentzer SJ and Perine SP (2001) Induction of the Epstein Barr virus tymidine kinase gene with concominant nucleotide antivirals as a therapeutic strategy for Epstein Barr virus associated malignancies. Current Opin Oncol 13: 360-67.

Feng D, Nagy JA, Pyne K, Dvorak HF and Dvorak AM (1998) Neutrophils emigrate from venules by a transendothelial cell pathways in response to FMLP. J Exp Med 187: 903-15.

Feuillet L, Carvajal J, Sudre I, Pelletier J, Thomassin JM, Drancourt M and Cherif AA (2005) First isolation of *Bacteroides thetaiotaomicron* from a patient with a Cholesteatoma and Experiencing Meningits. J Clin Microbiol 43: 1467-69.

Flier M, Geelen SPM, Kimpen JLL, Hoepelman IM and Tuomanen EI (2003) Reprogramming the host response in Bacterial Meningitis: How best to improve outcome. Clin Microbiol Rev 16: 415-29.

Forbes BA, Sahm DF and Weissfiwld AS (2007) Bailey and Scott's Diagnostic Microbiology, 12th ed. Mobsby Elsevier, USA, pp. 822-31.

Freter R (1980) Prospects for preventing the association of harmful bacteria with host mucosal surfaces. In: Beachy EH (Ed.) Bacterial adherence. Champman and Hall, New York, 6: 441-59.

Frieland IR, Snipelisky M and Khousal M (1990) Meningitis in a neonate caused by *Leuconostoc* sp. J Clin Microbiol 28: 2125-26.

Ganong JG (2005) Review of Medical Physiology. Lange medical publication, Singapore, p.163.

Garcia R (2005) Multidrug resistant E. coli outbreak in neonatal intensive care unit (NICU). American J Infect Control 33: e122-23

Gautam N, Aryal M, Bhatta N, Bhattacharya SK, Baral N and Lamsal M (2007) Comparative study of Cerebrospinal Fluid Adenosine deaminase activity in patients with Meningitis. NMCJ 9: 104-06.

Geibdorfer W, Sander G, John S, Gessner A, Schoerner C and Schroppel K (2003) *Ureaplasma urealyticum* Meningits in an adult patient. J Clin Microbiol 46: 1141-43.

Gerics J, Nov 10 (2007). http://neurologicalillness.suite101.com/article.cfm/bacterial_meningitis-scare

Gorman CA (1962) Bacterial meningitis II. Infections caused by certain Gram negative enteric organisms. Proceedings of the Staff Meetings of the Mayo Clinic 37: 703-05.

Graham D and Lantos P (2002) Greenfields Neuropathology, Arnold London.

Gray LD and Fedorko DP (1992) Laboratory diagnosis of Bacterial Meningitis. Clin Microbiol Rev 5: 130-45.

Griffis JM and Bertram MA (1977) Immunoepidemiology of meningococcal disease in Military recruits. II. Blocking of serum bactericidal activity by circulating Ig A early in the course of invasive disease. J Infect Dis 136: 733-39.

Guyton AC (1991) Textbook of Medical Physiology, 8th ed. WB Saunder's company, India, pp. 679-84.

Harrington BJ and Plenzler M (2004) Case study: Misleading Gram stain findings on smear from CSF specimen. Lab Med 35: 475-78.

Harrison OB, Nicholas JE, Blair JM, Grimes HS, Tinsley CR, Nassif X, *et al.* (2009) Epidemiological evidence for the role of Hb receptor in meningococcal virulence. J Infect Dis 200: 94-98.

Hart CA and Kariuki S (1998) Antimicrobial resistance in developing countries. BMJ 317: 647-50.

Harter DH and Petersdorf RG (1960) A consideration of the pathogenesis of the Bacterial Meningitis: Review of experimental and clinical status. Yale J Bio Med 32: 280-09.

Hoque MA, Chowdhury N, Hoque Z, Siddiqui NA, Islam R and Ahmad Q (2004) Mollaret's Meningitis: A case report and review of literature. TAJ 17: 41-46.

http://en.wikipedia.org/wiki/Cerebrospinal_fluid (2009) (Wikipedia, 2009)

http://en.wikipedia.org/wiki/Multiple_drug_resistance (Wikipedia, 2010)

Huang CR, Lu CH, Chuang YC, Tsai NW, *et al*. (2007) Adult *Pseudomonas aerugonisa* meningitis: High incidence of underlying medical and/or postneurological condition and high mortality ratio. Jpn J Infect Dis 60: 397-99.

Innes AR (CDR weekly 2001)
http://www.health24.com/medical/condition-centers/777-792-824-1855,13738.asp

Jander S, Schroeter M, and Stoll G (2000) Role of NMDA receptor signalling in the regulation of inflammatory gene expression after focal brain ischemia. J Neuroimmunol 109: 181-87.

Jaspan HB, Brothers AW, Campbell AJP, Mc Guire JK, Browd SR, Manley TJ, Pak D and Weissman SJ (2010) Multidrug resistant *Enterococcus faecium* Meningitis in a toddler. Pediatr Infect Dis J. (www. pidj.com)

Jolles S, Sewell WA and Leighton C (2000) Drug-induced aseptic meningitis: diagnosis and management. Drug Saf 22: 215-26.

Jones ME, James AK, Draghi DC and Sahm DF (2003) Clinical isolation of *Streptococcus pneumoniae* with different susceptibility to ceftriaxone and cefotaxime. Antimicrob Agent Chemother 47: 3155-60.

Karim R, Rahaman M and Rahaman A (1996) Reflex A Guide to Physiology and Bio-Chemistry, 6th ed. Shadow printing and packaging, Dhaka, pp. 11.2-11.3.

Karunanayake L, Bandara D and Dunusinghe P (2007) Fatal Meningitis in child due to multi drug resistant *Streptococcus pneumonia*. Ceylon Med J 52: 32-33.

Kasper DL, Fauci AS, Longo DL, Braunwald E, Hanser SL and Jameson JL (Eds.) (2005) Harrison's Principles of Internal Medicine, 16th ed. Mc Graw Hill Medical Publishing Division, New York, pp. 2471-84.

Kasten BL (1990) Microbiology. In: DS Jacobs, BL kasten, WR Demott, and WL Wolfson (Eds.), Laboratory Test hand book, 2nd ed. The Williams and Wilkins Co., Baltimore, pp. 688-89.

Kellee CA and Neil E (1972) Samson Wright's Applied physiology, 12th ed. Oxford University Press, London, p. 347.

Kerek O (2009) (www.bbc.co.UK)

Khanal B, Sharma SK and Deb M (2002) Cryptococcal Meningitis in non AIDS patient. JNMA 41: 323-25.

Ko WC, Paterson DI, Sagnimeni AJ, *et al*. (2002) Community Acquired *Klebsiella pneumoniae* Bacteremia: global differences in clinical patterns. Emerg Infect Dis 8: 160-66.

Koedel U, Bernatowicz A, Frei K, Fontana A and Pfister HW (1996) Systemically (but not Intrathecally) administered IL-10 attenuates pathophysiology alternation in experimental pneumococcal meningitis. J Immunol 157: 5185-91.

Koedel U, Winkler F, Angele B, Fontana A and Pfister HW (2002) Meningitis associated central nervous system complications are mediated by the activation of Poly (ADP- ribose) polymerase. J Cereb Blood Flow Metab 22: 39-49.

Kowalik MM, Smiatacz T, Hlebowicz M, Pajuro R and Trocha H (2007) Coagulation, coma and outcome in Bacterial Meningitis: An observational study of 38 adult cases. J Infect 55: 141-48.

Kumar V, Abbas AK and Fausto N (2005) Robbins and Cotran pathologic basis of disease, 7th ed. Elsevier, India.

La Scolea JR, J Leonard and Dryja D (1984) Quantitation of bacteria in Cerebrospinal Fluid and Blood of children with Meningitis and its diagnostic significance. J Clin Microbiol 19: 187-90.

Laguna-Del-Estal P, Garicia-Madero R, Gill-Navarro M, Garcia-Zubiric C and Agud FM (2010) Acute Bacterial Meningitis in older people. Rev Clin Esp 210: 57-64.

Lechuz JMG, Hernangomcz S, Juan RS and Bouza E (2000) Feculent meningitis: polymicrobial meningitis in coclorectal surgery. Diagn Microbiol Infect Dis 38: 169-70.

Lee K, Kim YA, Park YJ, *et al.* (2004) Increasing prevalence of vancomycin-Resistant Enterococci and Cefoxitin, Imipenem and Fluoroquinolone resistant Gram negative bacilli: A KONSAR study in 2002. Yonsei Med J 45: 598-08.

Leib SL, Kim YS, Black SM, Tureen JH and Tauber MG (1998) Inducible Nitric Oxide Synthase and the effect of aminoguanidine in experimental neonatal meningitis. J Infect Dis 177: 692-700.

Lesse AJ, Moxon ER, Zwahlen A and Scheld WM (1988) Role of Cerebrospinal fluid pleocytosis and *Haemophilus influenzae* type b capsule on blood brain barrier permeability during experimental meningitis in rat. J Clin Invest 82: 102-09.

Lorenzl S, Koedel K, Frel K and Pfister HW (1996) Effect of the bradykinin B2 receptor antagonist Hoel 140 in experimental pneumococcal Meningitis in Rat. Eur J Pharmacol 308: 335-41.

Lu JJ, Perng CL, Lee SY and Wan CC (2000) Use of PCR with universal primers and restriction endonuclease digestion for detection and identification of common Bacterial Pathogen in Cerebrospinal Fluid. J Clin Microbiol 38: 2076-80.

Mahon CR and Manuselis G (2000) Diagnostic Microbiology, 2nd ed. Elesevier, Philadelphia.

Marchandin H, Ventura V, Alfonso JM and Perre PV (2005) Mixed Bacterial Meningitis due to *Streptococcus pneumoniae* and *Neisseria meningitidis* in an 18 month old child. J Clin Microbiol 43: 1477-79.

Marshall W, and Bangert SK (1995) Clinical Biochemistry Metabolic and Clinical Aspects. Person professional Limited, USA, pp. 562-68.

Martin MC, Perez F, Moreno A, Moral A, Alvarez MA, Mendez FJ and Vazquez F (2008) *Neisseria gonorrhoeae* meningitis in pregnant adolescent. Emerg Infect Dis 14: 1672-73.

Mayoclinic 2010 (www.mayoclinic.com.2010)

Meningitis Trust (2008) (http://www.maturetimes.co.uk/node/6546)

Merritt HH (1967) A text book of neurology, 4th ed. Kothari book depot, India.

Mertsola J, Kennedy WA, Waagner D, Llorens XS, Olsen K, Hansen EJ and McCracken GH (1991) Endotoxin concentration in Cerebrospinal Fluid

correlates with clinical severity and neurologic outcome of *Haemophilus influenzae* type b Meningitis. Am J Dis Child 145: 1099-03.

Mittal R, Wang Y, Hunter CJ, Gomez IG and Prasadaro NV (2009) Brain damage in new born Rat model of Meningitis by *Enterobacter sakazakii*. Lab Invest 89: 263-77.

Mittal S, Saxena A and Garg P (2009) Unusual presentation of *Salmonella* Typhi infection in children. Trop Doct 39: 27-28.

Moore KL and Dalley AF (2006) Clinically Oriented Anatomy, 5th ed. Lipponcott Williams & Wilkins A Wolters Kulwer Company, London.

Mulks MH, Kornfld SJ, Frangione B and Plant AG (1982) Relationship between the specificity of IgA proteases and serotypes in *H. influenzae*. J Infect Dis 146: 266-74.

Munro J and Edwards C (Eds.) (1995) Macleod's Clinical Examination, 9th ed. Churchill Livingstone, pp. 251-52.

Murray PR and Hampon A (1980) Recovery of pathogenic bacteria from CSF. J Clin Microbiol 12: 554-57.

Mustafa MM, Ramilo O, Syrogiannopoulos GA, Olsen KD, Mc Cracken GH and Hansen EJ (1989) Induction of meningeal inflammation by outer membrane vesicles of *Haemophilus influenzae* type b. J Infect Dis 159: 917-22.

Nagarathna S, Grag N, Satish S, Rafi W, Chandramuki A, Seshagiri S and Pal P (2007) Co existence of pneumococcal and tubercular mixed meningitis in heterozygous sickle cell disease: a case report. Intern J Infect Dis 12: 560-62.

Nau R, Soto A and Bruck W (1999) Apoptosis of neurons in dentate gyrus in human suffering from Bacterial Meningitis. J Neuropathol Exp neurol 58: 265-74.

Newman N, Doctor BA and Minich NM (2001) Clinical outcomes of neonatal meningitis in very-low birth-weight infants. Clin Pediatr (Phila) 40: 473-80.

Nugent SK, Bausher JA, Moxon ER and Rogers MC (1979) Raised intracranial pressure: its management in *Neisseria meningitidis* meningoencephalitis. Am J Dis Child 133: 260-62.

Olson DA and Hoeprich PD (1984) Analysis of bacterial isolates from Cerebrospinal Fluid. J Clin Microbiol 19: 144-46.

Orlicek SL, Welch DF and Kuhls TL (1993) Septicaemia and Meningitis caused by *Helicobacter cinaedi* in a Neonate. J Clin Microbiol 31: 569-71.

Ozkalay N, Anil M, Heluaci M, Agus N, Guler O, Dikerler A and Kanar B (2007) Meningitis due to *Gemella haemolysans* in pediatric case. J Clin Microbiol 45: 2337-39.

Paredes CF, Lammoglia L, Herandez I and Preciado JIS (2007) Epidemiology and outcomes of Bacterial Meningitis in Mexican children: 10 year experience (1993-2003). Int J Infect Dis 12: 380-86.

Peltola H (2001) Burden of meningitis and other severe bacterial infections of children in Africa: Implication for prevention. Clin Infect Dis 41: 64-75.

Pfister HW, Koedel U, Habert RI, *et al.* (1990) Microvascular changes during the early phase of experimental Bacterial Meningitis. J Cerebral Blood Flow Metab 10: 914-22.

Pien FD and Pien BC (1999) *Angiostrongylus castonensis* Eosinophilic meningitis. Int J Infect Dis 3: 161-63.

Pradhan S (1983) Epidemiological Aspects of Meningococcal Meningitis. Nepas Journal 2: 113-19.

Prieto RG, Montero JSR, Alejandre CG, Meca LAA, Rivero A and Miguel AG (2009) Epidemiology of pneumococcal Meningitis hospitalization in pediatrics population in Spain (1998-2006). Vaccine 27: 2669-73.

Quagliaerello V and Scheld WM (1997) Treatment of Bacterial meningitis. N Eng J Med 336: 08.

Quagliarello V and Scheld WM (1992) Bacterial Meningitis: pathogenesis, pathophysiology and progress. N Eng J Med 327: 864-72.

Quagliarello VJ, Long WJ and Scheld WM (1986) Morphologic alteration in BBB with experimental Meningitis in rat. J Clin Invest 77: 657-72.

Rao BN, Kashbur IM, Shembesh NM and El-Bargathy SM (1998) Etiology and occurrence of acute bacterial meningitis in children in Benghazi, Libyan Arab Jamahiriya. Eas Med Health 4: 50-57.

Razonable RR and Keating MR (2010) Meningitis: treatment and medication http:/emedicine.medscape.com/article/232915-treatment.

Reis JN, Lima JB, Ribeiro GS, Corderio SM, Salgado K, Reis MG and Ko AI (2002) Antimicrobial resistant in *Haemophilus influenzae* isolated during population based surveillance for Meningitis in Salvador, Brazil. Antimicrob Agents Chemother 46: 3641-43.

Rizvi M, Fatima N, Shukalal I and Atzal K (2008) *Stomatococcus mucilaginous* Meningitis in a healthy 2 month old child. J Med Microbiol 57: 382-83.

Robbins JB, Mc Cracken GH, Gotschich EL, *et al*. (1974) *E. coli* K1 capsular polysaccharide associated with neonatal meningitis. N Eng J Med 290: 518-24.

Rodrigues MM, Patrocinio SJ and Rodrigues MG (2000) *Staphylococcus aureus* Meningitis in children. A review of 30 community acquired cases. Arq Neuropsiquiatr 58: 843-51.

Rogues A, Draghi DC, karlowsky JA, Sahm DF and Bradley JS (2003) Prevalence of antimicrobial resistant in bacteria isolated from central nervous system specimen as reported by US hospital Laboratories from 2000-2002. http://www.annclinmicrob.com/content/3/1/3

Roine I, Weisstaub G and Peltola H (2010) influence of Malnutrition on course of childhood bacterial Meningitis. Pediatr Infect Dis J 29: e1-4.

Ross SC and Densen P (1984) Complement deficiency states and infection: Epidemiology, pathogenesis and consequences of Neisserial and other infections in an immune deficiency. Medicine 63: 243-73.

Scheld MW, Koedel U, Nathan B and Pfister (2002) Pathophysiology of Bacterial Meningitis: Mechanism(s) of neuronal injury. J Infect Dis 186: S225-33.

Scheld WM, Daecy RG, Winn HR, Welsh JE, Jane JA and Sande MA (1980) Cerebrospinal Fluid outfole resistance in rabbits with experimental Meningitis. Alteration with penicillin and Methyl prednisolone. J Clin Invest 62: 243-53.

Scheld WM, Koedel U, Nathan B and Pfister HW (2002) Pathophysiology of bacterial meningitis: Mechanism(s) of neuronal injury. J Infect Dis 186: 225-33.

Seehusen DA, Reeves MM and Fomin DA (2003) Cerebrospinal fluid analysis. Am Fam Physician 68: 1103-08.

Sekul EA, Cupler EJ and Dalakas MC (1994) Aseptic Meningitis associated with high dose intravenous immunoglobulin therapy: frequency and risk factors. Ann Intern Med 121: 259-62.

Serkin CD and Seifert HS (1998) Frequency of pilin antigenic variation in *Neisseria gonorrhoeae*. J Bacteriol 180: 95.

Shah AS, Knoll MD, Sharma PR, and Moisi JC, Kulkarni P, Lalitha MK, Steinhoff M and Thomas K (2009) Invasive pneumococcal disease in Kanti Children Hospital, Nepal as observed by the South Asian pneumococcal alliance network. CID 48: S123-28.

Shaha AC, Nag MN, Deb A and Nath NC (2005) Correlation of laboratory findings with etiology of patients presenting with fever followed by altered consciousness in Teaching Hospital. TAJ 18: 25-29.

Shakibaie MR (2000) Plasmid pattern of Antibiotic resistance in β lactamase producing *Staphylococcus aureus* strain isolated from hospitals in Kerman, Iran. http://www.ams.ac.ir//AIM/9922/Shakibaei9922.html

Sharma PR (1983) Childhood Meningitis: A Five Months Survey. Nepas Journal 2: 132-38.

Sharma PR and Dixit H (1984) Bacterial Meningitis-2 Years Experience at Kanti childrens Hospital. Journal IOM 6: 23-30.

Shrestha YB (1983) A Five Year Review of Central Nervous System Disorders. Nepas Journal 2: 207-25.

Silva LPA, Cavalheiro LG, Queiros F, Nova CV and Lucena R (2007) Prevalence of newborn Bacterial Meningitis and sepsis during the pregnancy period for public health care system participants in Slvador, Brazil. Brazilian J of Infect Dis 11: 272-76.

Simberkoff MS, Moldover HN and Rahal JJ (1980) Absence of detectable bactericidal and opsonic activity in normal and infected human Cerebrospinal Fluid. A regional host defense deficiency. J Lab Clin Med 95: 362-72.

Sinnatamby CS (2004) Last's Anatomy, 10th ed. Churchill Livingstone, pp. 477-78

Smith AL (1987) Pathogenesis of *Haemophilus influenzae* Meningitis, Pediatr Infect Dis 6: 783-86.

Smith GR and Easmon CSF (1990) Topley and Wilson's Principles of Bacteriology , Virology, and Immunology, 8th ed., Vol 1-4. Edward Arnold Publicaiton UK.

Sonavane AE, Baradkar VP and Mathur M (2008) Pattern and antibiotic susceptibility of bacteria isolated in clinically suspected cases of meningitis in children. J Pediatr Neurosci 3:131-33.

Spanaus KS, Nadal D, Pfister HW, Seebach J, Winder U, Frei K, Gloor S and Fontana A (1997) C-X-C and C-C chemokines are expressed in the CSF in Bacterial Meningits and mediate chemotactic activity on peripheral blood-derived PMN and Mononuclear cells in Vitro. J Immunol 158: 1956-64.

Standring S (2005) Gray's Anatomy and Physiology. Wadsworth publishing company, California, pp. 291-94.

Steele RW, Marmer DJ, O' Brien MD, Tyson ST and Steele CR (1986) Leukocyte survival in Cerebrospinal Fluid. J Clin Microbiol 23: 965-66.

Stephens DS and McGee ZA (1981) Attachment of *Neisseria meningitidis* to human mucosal surface: influence of pili and type of receptor cell. J Infect Dis 143: 525-32.

Stevens D, Leiberman M, Mc Nitt T and Price J (1984) Demonstration of Uronic acid capsular material in the Cerebrospinal Fluid of patient with meningitis caused by mucoid *Pseudomonas aerugonisa*. J Clin Microbiol 19: 942-43.

Subedi BK (1985) The Top Nine Diseases in the Paediatric Ward of Bheri Zonal Hospital. JNMA 24: 47-49.

Sumanasena SP and Lamabadsuriya SP (2005) Child with CSF rhinorrhoea complicated by Recurrent Meningitis. Sri Lanka J Child Health 34: 128-9.

Syrogiannopoulos GA, Hansen EJ, Erwin Al, *et al*. (1987) Dexamethasone in the treatment of experimental *H. influenzae* type B Meningitis. J Infect Dis 155: 213-19.

Tabatabiae P and Syadati A (1993) *Bacillus anthracis* as a cause of Bacterial Meningitis. Pediatr Infect Dis 12: 1035-37.

Tang LM, Chen ST and Wu YR (1998) *Haemophilus influenzae* Meningits in an adults. Diag Microbiol Infect Dis 32: 27-32.

Tang LM, Chen ST, Hsu WC and Lyu RK (1999) Acute Bacterial meningitis in adults: a hospital based epidemiological study. Q J Med 92: 719-25.

Tankhiwale SS, Jagtap PM, Khadse RK and Jalgaonkar SV (2001) Bacteriological study of pyogenic meningitis with special reference to C-reactive protein. Indian J Med Microbiol 19: 159-60.

Tebruegge M and Curtis N (2008) Etiology, pathogenesis, and diagnosis of recurrent Bacterial Meningitis. Clin Microbiol Rev 21: 519-27.

Theccanat G, Hirschfield L and Isenberg H (1991) *Serratia marcescens* Meningitis. J Clin Microbiol 29: 822-23.

Tiwari KB (2002) Study of Meningitis in patients visiting Tribhuvan University Teaching Hospital. A dissertation submitted to Central Department of Microbiology.

Tiwari PN (2003) Meningitis in children- A prospective study with latex agglutination tests of cerebrospinal fluid.
http://www.nepas.org.np/jnepas/jnepas 21/meningitis.pdf

Tonnensen MG, Smedley LA and Henson PM (1984) Neutrophil endothelial cell interaction. Modulating of neutrophil adhesiveness induced by complement fragments C5a and C5a des arg and formyl methonyl leucyl phenylalanine in vitro. J Clin Invest 74: 1581-92.

Tortora GJ and Derrickson B (2006) Principles of Anatomy and Physiology, 11th ed. John Wiley & Sons, Inc, Hoboken.

Tuan PL, Li WC, Huang YC, Chiu CH and Lin TY (2009) Invasive pediatric *Neisseria meningitidis* infections. J Microbiol Immunol Infect 42: 427-32.

Tuladhar R (2007) Comparative evaluation of microscopic and cultural examination in bacterial meningitis among the patients attending Kanti children hospital. A dissertation submitted to central department of microbiology, Tribhuvan University.

Tunkel AR and Scheld WM (1993) Pathogenesis and pathophysiology of Bacterial Meningitis. Clin Microbiol Rev 6: 118-36.

Tureen JH, Tauber MG and Sande M (1992) Effect of hydration status on cerebral blood flow and Cerebrospinal Fluid lactic acidosis in rabbits with experimental meningitis. J Clin Investig 89: 947-53.

Tzanakakai G and Mastrantonio P (2007) Aetiology of Bacterial Meningitis and resistant to antibiotics of causative pathogens in Europe and in the Mediterranean region. Int J Antimicrob Agents 29: 621-29.

Vamer GTS, Dominguez MC, Esgleas M, Osaki M, Takamatsu D, Segura M and Gottschalk M (2008) Disruption of Srt A gene in *Str. suis* results in decreased interaction with an endothelial cells and extracellular matrix protein. Vet Microbiol 127: 417-28.

Van BD, Schmand B, De GJ, *et al*. (2002) Cognitive impairment in adults with good recovery after Bacterial Meningitis. J Infect Dis 186: 1047-52.

Van D, Brandtzarg P and Van MJWM (2000) Update on Meningococcal disease with emphasis on pathogenesis and clinical management. Clin Microbiol Rev 13: 144-46

Van de BD, de Gans J, Tunkel AR and Wijdicks FM (2006) Community acquired Bacterial Meningitis in Adults. N Engl J Med 354: 1429-32.

Vergnano S, Sharland M, Kazembe P and Mwansambo C (2005) Neonatal sepsis: an international prespective. Arch Dis Child Fetal Neonatal Ed 90: f220-24.

Verweij PE, Brinkman K, Kremer HPH, Kullberg BJ and Meis JFGM (1999) *Aspergillus* Meningitis: Diagnosis by non culture based microbiological methods and management. J Clin Microbiol 37: 1186-89.

Victor M, Ropper AH, and Adanis RD (2000) Adamas and Victors principle of neurology. Mc Graw Hill, New York.

Waldman AL (2005) Lumbar Puncture (Examination of CSF). http://www.emedicine.com/neuro.

Washington JA (1991) Medical Bacteriology. In: Laboratory procedures in Clinical Microbiology, 2nd ed. The WB Saunders Co., Philadelphia, pp.1025-73.

WHO (1997) Control of epidemic meningococcal disease, WHO practical guidelines, 2nd ed. http://www.who.int/emc (WHO/EMC/BAC/98.3)

Williams EJ, Thorson S, Maskey M, Mahat S, Hamaluba M, Dongol S, *et al*. (2009) Hospital based surveillance of invasive pneumococcal disease among young children in urban Nepal. CID 48: S114-22.

Wilson KJW (1995) The nervous system. In: Ross and Wilson Anatomy and Physiology in Health and Illness, 7th ed. ELBS Churchill-Livingstone, pp. 245-48

www.comoonline.org/pdf/CoMO=APPA_Conference_ release_China_240909.pdf.

www.brooksidepress.org (CSF, 2008)

www.medialabinc.net/turbidity-keyword.aspx (CSF, 2010)

Yemisen M, Mete B, Tunali Y, Yentur E and Ozturk R (2008) A meningitis case due to *Stenotrophomonas maltophila* and review of literature . Int J Infect Dis 12: e125-27.

Youssef FG, Afifi SA, Azab AM, Wasty MM, Aziz KMA, Parker TM, Oun SA, *et al*. (2006) Differentiation of tuberculous Meningitis from acute Bacterial Meningitis using simple clinical and laboratory parameter. Diagn Microbiol Infect Dis 55: 275-78.

Yu JS and Grauaug A (1963) Purulent meningitis in the neonatal Period. Arch Dis Child 38: 391-96.

Zaidi AK, Thaver D, Ali SA and Khan TA (2009) Pathogens associated with sepsis in newborns and young infants in developing countries. Pediatr Infect Dis J 28: S10-8.

Zumo LA, Gondim FAA and Greenberg A (2000) Staphylococcal Meningitis. Q J Med 93: 45-53.

Zwijnenburg PJG, Poll T, Roord JJ and Furth AM (2006) Minireview: Chemotatic factors in Cerebrospinal Fluid during Bacterial meningitis. Infect Immun 74: 717-27.