Examination of Cerebrospinal fluid (CSF)

Cerebrospinal fluid (CSF) is a clear, colorless fluid formed in the ventricles of the brain mainly by choroid plexus (meshwork of tiny small blood vessels in lateral third and fourth ventricles). It is mainly an ultrafiltrate of plasma. CSF is contained within the cerebral ventricles, the spinal canal and the subarachnoid space (space between arachnoid externally and pia mater internally) surrounding the brain and spinal cord (Fig. 6.1). CSF is reabsorbed into the blood through the arachnoid villi of dural venous sinuses.

COMPOSITION OF NORMAL CEREBROSPINAL FLUID IN ADULTS

- Total volume: 100-150 ml (10-60 ml in the newborn)
- Opening pressure: 60-180 mm of water (10-100 mm in infants and young children)
- Appearance: Clear and colorless with no clots (viscosity similar to water)
- Cells:
- Adults: 0-5 cells/cmm
- Infants: 0-30 cells/cmm
- 1-4 years: 0-20 cells/cmm
- 5-18 years: 0-10 cells/cmm
- Glucose: 45-80 mg/dl. (Normally CSF glucose is 60% or 2/3rds of blood glucose)
- Proteins: 15-45 mg/dl. (Normally CSF proteins are 1% of plasma proteins).
- Oligoclonal bands: Negative
- Chloride: 120-130 mEq/L (20 mEq/L more than serum level)
- Bilirubin: Absent.

FUNCTIONS OF CEREBROSPINAL FLUID

1. Protection of brain and spinal cord from injury by acting as a shock absorber.
2. To serve as a medium between blood and brain for supply of nutrients to and removal of waste products from brain.

COLLECTION OF CEREBROSPINAL FLUID

Some diseases produce characteristic alterations in composition of CSF, thus providing the basis for examination of CSF. Lumbar puncture or LP. Spinal or LP needle is passed between 3rd and 4th or between 4th and 5th lumbar vertebra (L3-L4 or L4-L5) and CSF is obtained from the subarachnoid space. LP is carried out at these levels to prevent injury to the spinal cord (spinal cord ends at about T12, below which are nerve roots or cauda equina).

Examination of CSF for diagnosis in suspected cases of:

- Central nervous system infections, especially meningitis (inflammation of leptomeninges) and encephalitis
- Meningeal involvement by leukemia or malignancy
- Subarachnoid hemorrhage (if CT scan is not available)
- Inflammatory diseases, e.g. multiple sclerosis (for diagnostic gammaglobulin findings), Guillain- Barré syndrome
- Neoplasms of central nervous system (CNS).

LABORATORY EXAMINATION OF CEREBROSPINAL FLUID

After collection, specimen of CSF should be transported immediately to the laboratory and examined without delay. This is because

(i) cells disintegrate rapidly.

(ii) reduction of glucose level occurs due to glycolysis. At the latest, CSF should be examined within 1 hour of collection, and CSF cell counts are always done within 30-60 minutes of collection. Glass tubes should not be used for collection since cell adherence to glass reduces the cell count. Specimen for bacterial culture should not be refrigerated as fastidious organisms (*Hemophilus influenzae, Neisseria meningitidis*) do not survive in the cold temperature.

CSF chemical examination results should always be compared with those in plasma since any change in plasma is reflected in CSF.

Examination of CSF includes:

1. Opening pressure
2. **Appearance**
3. **Total and differential cell counts**
4. **Chemical examination**
5. **Microbiological examination**
6. **Special investigations**

1. Gross Appearance of Cerebrospinal Fluid

Normal CSF is clear and colorless like distilled water, and does not clot. Abnormal CSF may appear turbid, blood-mixed, xanthochromic, or viscous (Fig. 6.5). Clot formation in CSF is abnormal and indicates increased proteins.

Turbid CSF may be due to the presence of:

- Leukocytes >200 cells/cmm
- Microorganisms like bacteria, fungi, or amebae
- Raised proteins
- Red cells: number of >400 cells/cmm the lood-mixed CSF Blood-stained CSF may result from traumatic tap (due to injury to venous plexus in spinal wall) or subarachnoid hemorrhage. Distinction of traumatic tap from subarachnoid hemorrhage is vitally important.

2. Cell Counts in Cerebrospinal Fluid

(1) **Total leukocyte count:** Cell count on CSF is done manually on undiluted sample in a counting chamber.

Total leukocyte counts increases in various disorders and along with differential count provides important diagnostic information. **An increase in cell count in CSF is called as pleocytosis**.

It is essential to do microscopic examination of all CSF samples since white blood cell (WBC) count upto 200/cmm and red cell count upto 400/cmm are associated with clear appearance of CSF.

Causes of increased cell count in CSF:

- Meningitis and other infections of CNS
- Intracranial of skull hemorrhage
- Meningeal infiltration by malignancy
- Repeated lumbar punctures

- Injection of foreign substances (e.g. radiographic contrast media, drugs) in subarachnoid space.
- Multiple sclerosis

Presence of blood in CSF due to traumatic tap or subarachnoid hemorrhage artefactually raises the leucocyte count by 1 WBC per 1000 red cells.

3. **Chemical Examination of Cerebrospinal Fluid**

Routine chemical examination of CSF consists of estimation of proteins and glucose. CSF from tube 1 is used for chemical examination.

1. **Estimation** of proteins in CSF: Normal CSF protein level in adults is 15-45 mg/dl. An increase in CSF protein is a sensitive but non-specific indicator of **CNS disease**. CSF proteins may be **normal during early stages of meningitis**. Significant elevation (>150 mg/dl) occurs in bacterial meningitis. There are various methods for estimation of CSF proteins. **Turbidimetric method using trichloroacetic acid** for precipitation of proteins is commonly used

CSF proteins are elevated in following conditions:

- **Increased capillary permeability of blood-brain barrier:** Meningitis
- **Mechanical obstruction to circulation of CSF (causing increased fluid reabsorption due to stasis):** Spinal cord tumor
- **Increased local (intrathecal) immunoglobulin (IgG) production:** Multiple sclerosis, neurosyphilis, sub- acute sclerosing panencephalitis
- **Both increased capillary permeability and increased local immunoglobulin (IgG) production:** Guillain- Barré syndrome
- **Hemorrhage in CSF:** Traumatic tap, subarachnoid hemorrhage.
- **Marked elevation** (>500 mg/dl) is noted in complete spinal block by a tumor, bacterial meningitis, and bloody CSF.

2. **Estimation of glucose in CSF:** Normal CSF glucose is 2/3rds of blood glucose (CSF to blood glucose ratio is 0.6). A sample for blood glucose should be drawn 1 hour before LP for comparison with CSF glucose. After collection, CSF sample should be immediately processed for glucose estimation because falsely low result due to glycolysis may occur.

CSF glucose is measured by glucose oxidase method. Normal range is 45-80 mg/dl. CSF glucose <40 mg/dl is abnormal.

CSF glucose is decreased due to utilization by bacteria (pyogenic or tuberculous), leucocytes, or cancer cells in CSF.

Decreased CSF glucose occurs in following conditions:

- Acute bacterial meningitis
- Tuberculous meningitis
- Fungal meningitis
- Meningeal involvement by malignant tumor (meningeal carcinomatosis)

Hypoglycemia

- CSF glucose is normal in viral meningitis

3. **Microbiological Examination**

Microbiological tests which can be carried out on CSF sample are:

- **Direct wet mount of CSF:** in suspected cases of cryptococcosis, amebic meningoencephalitis, Candida infection, and trypanosomiasis
- **Gram's smear:** should be done if CSF is turbid and neutrophils are increased.
- **Ziehl-Neelsen smear:** if tuberculous meningitis is suspected.
- **Latex agglutination tests:** for detection of bacterial and cryptococcal antigens.
- **Serologic tests for syphilis**
- **Culture** for bacteria and *Mycobacterium tuberculosis*
- **Polymerase chain reaction** for *Mycobacterium tuberculosis* and viruses.

Direct wet mount of CSF: One drop of CSF deposit (obtained after centrifugation) is placed on a glass slide, covered with a cover glass, and examined under the microscope with reduced illumination.

Observe for **motile trypanosomes** or **sluggishly moving amebae** ***(Naegleria fowleri)***. *N. fowleri* is a free-living ameba in water which enters through the nose and reaches central nervous system. It causes a fatal type of hemorrhagic meningoencephalitis.

Candida albicans may be seen in unstained wet mount; it appears as **oval budding forms and as pseudohyphae**.

When cryptococcal meningitis is clinically suspected, the wet mount is examined by dark-field microscopy. *Cryptococcus neoformans* appears as **spherical, budding yeast** forms, 2-10 µ in diameter and surrounded by a large unstained capsule. Cryptococci can be demonstrated by India ink preparation in 50% cases of cryptococcal meningitis.

(a) **Gram's smear:** This must be done if CSF is purulent and neutrophils are increased. Gram's smear is positive in 80% untreated cases and 60% partially treated cases of bacterial meningitis. Therefore, absence of bacteria on Gram's smear does not rule out bacterial infection.

Gram's method, and examined under the oil-immersion lens for bacteria (Fig. 6.9). Bacteria which commonly because meningitis are:

- **Meningococci:** Gram-negative diplococci located inside neutrophils.
- **Pneumococci:** Gram-positive diplococci surrounded by an unstained capsule.
- **Hemophilus *influenzae:*** Gram-negative coccobacilli.
- **Escherichia *coli:*** Gram-negative rods.

In all the above types of meninigitis, the accompanying cells are polymorphonuclear neutrophils. Detection of typical bacteria on Gram-staining suggests the etiologic agent of meningitis. However, definitive diagnosis requires culture of CSF.

There is an association between age of the patient and the causative organism of meningitis (Box 6.2).

(b) **Ziehl-Neelsen staining for *Mycobacterium tuber- culosis:***

(c) In tuberculous meningitis, number of tubercle bacilli is usually low in CSF. Ziehl-Neelsen or AFB staining is not a very sensitive method for detection of *M. tuberculosis* in CSF. AFB smears are negative in about 70% of cases of tuberculous meningitis. Fluorescent auramine stains have better sensitivity than Ziehl- Neelsen stain.

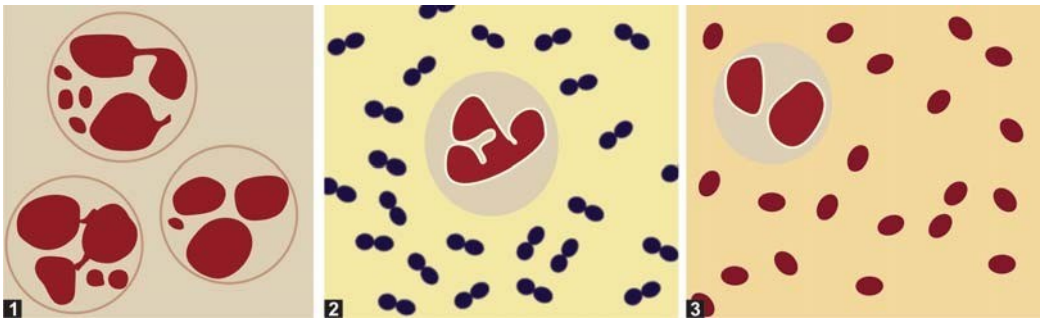

Fig. 6.9: Gram stained smears of CSF showing (1) *Neisseria meningitidis,* (2) *Streptococcus pneumoniae*, and (3) *Haemophilus influenzae*

Box 6.2: Association between age and organisms causing meningitis

- **0 to 6 months:** Group B streptococci, *Escherichia coli*, *Listeria monocytogenes*
- **6 months to 6 years:** *Streptococcus pneumoniae, Neisseria meningitidis*, *Hemophilus influenzae* type B, Enteroviruses
- **6 to 60 years:** *Neisseria meningitides*, *Streptococcus pneumoniae,* Enteroviruses, herpes simplex virus
- **>60 years:** *Streptococcus pneumoniae*, gram-negative bacilli, *Listeria monocytogenes*

(d) **Latex agglutination tests:** Latex agglutination tests for bacterial antigens are available commercially and are sensitive, rapid, and simple to perform. Currently available tests can detect *N. meningitidis* (groups A, B, C, Y, and W135), *H. influenzae* (capsular type B), *S. pneumoniae* and *S. agalactiae*. **Latex agglutination tests for cryptococcal antigens** are also available and have sensitivity of 90%.

(e) **Limulus lysate assay for endotoxin produced by gram-negative bacteria: Limulus amebocyte lysate** assay is a rapid, sensitive, and specific test for the presence of endotoxin. Endotoxin is produced by gram-negative bacteria like *N. meningitides, H. influenzae* type b, *E. coli*, and *Pseudomonas*. This test is particularly useful as a rapid test in newborns in whom these infections are common.

(f) **Serologic tests for syphilis:** If fluorescent treponemal antibody with **absorption** (FTA-ABS) test is positive in serum, Venereal Disease Research Laboratory (VDRL) test should be done on CSF if neurosyphilis is suspected. VDRL test is highly specific but lacks sensitivity. Therefore, a positive test rules in but does not rule out the diagnosis of neurosyphilis. Other serological tests for syphilis are not suitable for diagnosis of neurosyphilis in CSF.

Combination of positive FTA-ABS test in serum and reactive VDRL test in CSF is diagnostic of active neurosyphilis

(g) **CSF culture:** Culture of CSF is indicated if bacteria are seen on Gram-stained smear, or **leukocytes or proteins are increased**. Culture remains the gold standard for diagnois of bacterial meningitis. For culture, CSF collected in tube 2 is used.

Sensitivity of culture for identification of bacteria is about 90%. In incompletely treated cases, sensitivity is less. Usually CSF sample is inoculated on **chocolate (heated blood) agar and blood agar.** In newborn infants, sample is also inoculated on McConkey's agar.

In tuberculous meningitis, culture for *M. tuberculosis* is positive in about 56% of cases. If larger volume of CSF is used (i.e. 10 ml) for inoculation, sensitivity for detection increases. In cryptococcal meningitis, culture is positive in 95% of cases.

(h) **Polymerase chain reaction (PCR):** PCR is a highly specific and sensitive tool for diagnosis of infections of CNS. It uses probes to detect genes specific for the infecting organism. The test is rapid and requires only a small amount of CSF. High cost and availability only in a few specialist laboratories are major limitations. CSF PCR is mainly useful for diagnosis of **viral infections of CNS** (e.g. herpes simplex, enteroviruses, herpes zoster, etc.) and of **tuberculous meningitis**. CSF findings in different types of meningitis are shown in Table 6.4.

4. Special Investigations

(a) **CSF protein electrophoresis:** Protein electrophoresis of normal CSF differs from normal serum in (i) presence of a prominent transthyretin band and (ii) an extra transferrin band (called β2-transferrin or tau protein). Protein electrophoresis of CSF is used:

- For identification of oligoclonal bands, and to determine whether fluid submitted for examination is CSF.

1. *Oligoclonal bands:* Agarose gel electrophoresis of concentrated CSF is used for detection of oligoclonal bands. These are two or more discrete bands in the gamma region (Fig. 6.10). Oligoclonal bands have been identified in majority of patients (90%) with multiple sclerosis; however, they are not specific for this disorder. They are also seen in subacute sclerosing panencephalitis, viral CNS infections, neurosyphilis, and Guillain- Barré syndrome.
2. *CSF leakage:* Occasionally, clear fluid leaking through the nose or ear after trauma or surgery is submitted for examination to determine whether it is CSF. Due to the risk of recurrent meningitis, accurate identification of such fluid as CSF is essential. Recommended test for this purpose is protein electrophoresis with immunofixation for transferrin. Protein electrophoresis of CSF shows an extra transferrin band (called as **'tau' protein**), which is absent in other body fluids and secretions. Tau protein is an enzymatically modified transferrin that moves just behind the unaltered transferrin in β region. Identification of two isoform bands of transferrin on protein electrophoresis is a highly sensitive and specific test for identification of fluid as CSF (Fig. 6.11). Other body fluids or secretions do not show the second isoform band.

(b) **Measurement of albumin and immunoglobulin G (IgG):** Comparison of IgG and albumin CSF/plasma ratio can be helpful for diagnosis of multiple sclerosis (high ratio due to intrathecal synthesis of IgG).

Table 6.4: Cerebrospinal fluid findings in different types of meningitis

	Condition	*Appearance*	*Leukocytes*	*Proteins in mg/dl*	*Glucose in mg/dl*	*Additional investigations*
1.	Normal	Clear, colorless	<5/μl (mostly lymphocytes)	15-45	45-80	–
2.	Acute pyogenic meningitis	Turbid or purulent	Increased (>1000/μl); mostly neutrophils	Increased; 50-1500	Decreased; <40	Gram's stain; culture; Latex agglutination test
3.	Tuberculous meningitis	Clear or cloudy	Increased (100-600/μl); mostly lymphocytes or both lymphocytes and neutrophils	Increased; 45-300	Decreased; 10-45	AFB stain; culture; polymerase chain reaction
4.	Viral meningitis	Clear or cloudy	Increased (6-300/μl); lymphocytes	Increased	Normal	Polymerase chain reaction

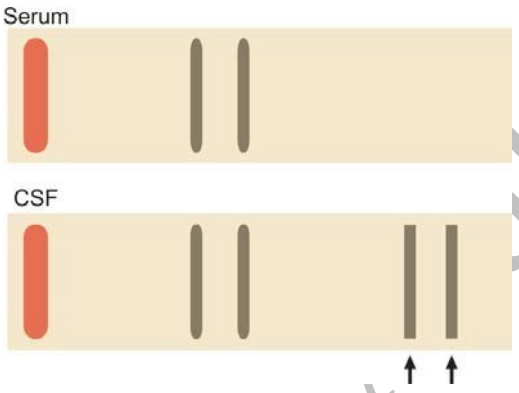

Fig. 6.10: Oligoclonal bands in CSF in gamma region (arrows) in a case of multiple sclerosis

Fig. 6.11: An example of positive tau protein: Lane 1: Normal CSF; Lane 2: Serum; Lane 3: Nasal fluid. (nasal fluid) shows two bands in the same position as normal CSF confirming CSF leakage from nose

Diabetes mellitus (DM) is a metabolic group of disorders characterized by persistent hyperglycemia due to deficiency and/or diminished effectiveness of insulin. There are derangements of carbohydrate, protein, and fat metabolism due to failure of insulin action on target cells. **Typical features of DM are as follows**:

- Fasting hyperglycemia
- Glycosuria
- Symptoms due to marked hyperglycemia: polyuria, polydipsia, weight loss, weakness, polyphagia, and blurred vision
- **Long-term complications** like atherosclerosis (leading to ischemic heart disease, cerebrovascular disease, and peripheral vascular disease) and microangipathy (which can cause **nephropathy** with risk of renal failure; **retinopathy** with potential loss of vision; and

peripheral neuropathy with risk of foot ulcers, amputations, or Charcot joints).

- Acute metabolic complications (hyperglycemic state, diabetic ketoacidosis).
- Susceptibility to infections especially of skin, respiratory tract, and urinary tract.

METABOLIC ACTIONS OF INSULIN

Insulin is the major hormone regulating blood glucose level. Insulin is synthesized by β cells of pancreas as preproinsulin, which is rapidly converted to proinsulin. Proinsulin is a single chain polypeptide. In the Golgi apparatus, proinsulin is broken down into 2 units- insulin (51 amino acids) and C (connecting)-polypeptide (31 amino acids) (Fig. 3.1). Both insulin and C peptide are stored in membrane-bound granules in the cytoplasm of β cells. Upon stimulation (mainly by blood glucose), **both insulin and C peptide are released in circulation**. C peptide is often measured as a marker of activity of β cells. C peptide has no known function.

Insulin acts on various cells (especially those of liver, muscle, and adipose tissue) through receptors.

Important actions of insulin are shown in Box 3.1.

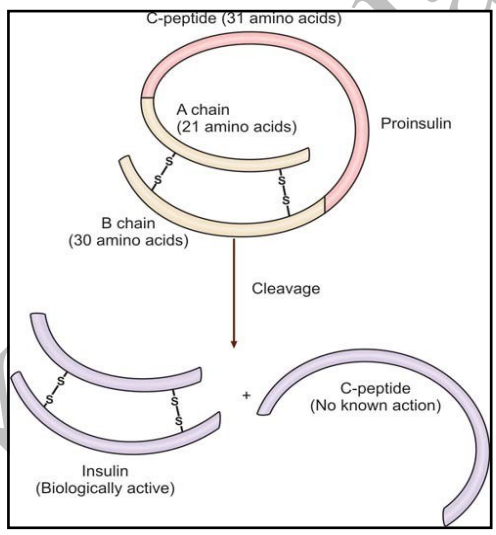

Fig. 3.1: Proinsulin, insulin, and C-peptide. The biochemical cleavage of proinsulin to insulin and C-peptide occurs in Golgi apparatus of β cell. Secretion of insulin is stimulated by glucose, mannose, amino acids, and sulfonylureas

"Stress hormones" like glucagons, glucocorticoids, growth hormone, and adrenaline oppose action of insulin.

CLASSIFICATION OF DIABETES MELLITUS

According to American Diabetes Association (1997), DM is classified into following types:

1. **Type 1** (Absolute deficiency of insulin due to destruction of β cells of pancreas)
   - Immune mediated
   - Idiopathic
2. **Type** 2 (Insulin resistance along with relative deficiency of insulin secretion)

3. Other specific types
4. Gestational DM (onset or first recognition of glucose intolerance during pregnancy).

Type 1 Diabetes Mellitus

It accounts for 5-10% of all cases of DM. This was previously called as insulin-dependent DM or IDDM (because insulin therapy is essential to prevent ketosis), juvenile-onset DM (because it commonly presents during childhood or adolescence), brittle DM, or ketosis-prone DM. It is characterized by **absolute deficiency of insulin secretion.**

Cell-mediated autoimmune destruction of β cells of pancreas is responsible for majority of cases of type 1 DM (immune-mediated type 1 DM), leading to inability of pancreas to synthesize insulin. There is infiltration by cytotoxic CD8+ T lymphocytes in and around islets. It is thought that many cases follow **a viral infection that has damaged the islet cells of pancreas** (Fig. 3.2). **Markers of immune** destruction of β cells, which can be detected in peripheral blood, are islet cell antibodies, **autoantibodies to insulin**, **autoantibodies to glutamic acid decarboxylase (GAD65), and autoantibodies to tyrosine phosphatases** (IA-2 and IA-2b). The disease has strong association with **HLA DR3 and HLA DR4 haplotypes** (Fig. 3.3). This type occurs mainly in children and adolescents, but can occur at any age. These patients are also at risk of other autoimmune disorders like **Graves' disease, Hashimoto's thyroiditis, vitiligo, Addison's disease, pernicious anemia, etc**.

Some cases of type 1 DM do not have any known etiologies or evidence of autoimmunity. These individuals are of Asian or African origin and their disease is strongly inherited. This form of type 1 DM is called as **idiopathic DM**.

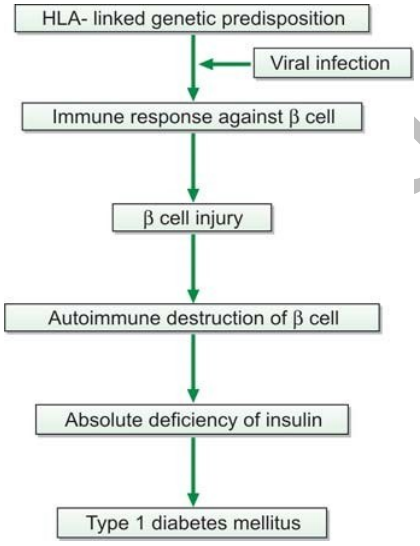

Fig. 3.2: Pathogenesis of type I diabetes

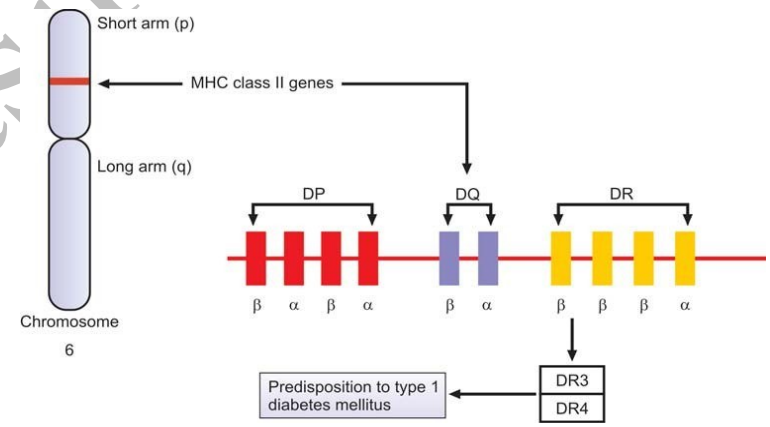

Fig. 3.3: HLA-linked genetic predisposition to type 1 diabetes

Type 2 Diabetes Mellitus

This is the **most common form of DM** comprising about 90-95% of all patients of DM. This was previously called as non-insulin-dependent DM (NIDDM), maturity-onset DM (because onset usually occurs during adult life), stable DM, or ketosis-resistant DM. **It is characterized by insulin resistance along with relative deficiency of insulin secretion** (i.e. **inadequate insulin secretory response to overcome**

peripheral insulin resistance). (Fig. 3.4). **Type 2 DM is not HLA-linked and there is no role of autoimmunity in its pathogenesis.** It has a strong genetic predisposition. Type 2 DM occurs more frequently in individuals with positive family history (parents or siblings with DM), **obesity** (≥ 20% over ideal body weight or body mass index ≥25 kg/m2), **hypertension** (>140/90 mm Hg in adults), **dyslipidemia**, lack of physical activity, pre-diabetes (impaired fasting glucose or impaired glucose tolerance), and prior gestational DM.

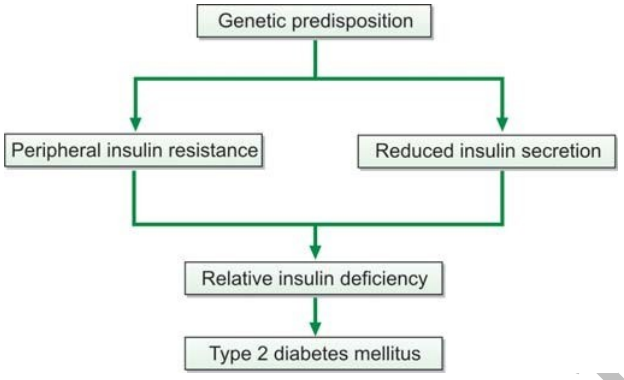

Fig. 3.4: Pathogenesis of type 2 diabetes mellitus

Other Specific Types

There are several forms of DM associated with underlying conditions:

- *Genetic defects of β cell function*: In these disorders, insulin secretion from β cells is impaired. These are called as maturity onset diabetes of the young (MODY). They are inherited in an autosomal dominant manner and they are caused by mutations in genetic loci such as **hepatic nuclear factor**, **glucokinase**, etc.
- *Genetic defects in insulin action*: These result from mutations in insulin receptor gene.
- *Diseases of exocrine pancreas*: Diseases causing generalized pancreatic damage can result in DM. These include cystic fibrosis, hemochromatosis, chronic pancreatitis, trauma, pancreatectomy, and pancreatic cancer.
- *Endocrine disorders*: Several hormones inhibit the action of insulin. Excessive secretion of these hormones will cause DM. Hyperglycemia is corrected following resolution of the primary endocrinopathy. Endocrine disorders associated with hyperglycemia are:

– Acromegaly: Excess growth hormone.
– Cushing's syndrome: Excess cortisol.
– Glucagonoma: Excess glucagon
– Pheochromocytoma: Excess epinephrine.
– Hyperthyroidism: Excess thyroxine

- *Drug*- or chemical-induced DM: Drugs or chemicals can impair insulin secretion or insulin action. Destruction of β cells and formation of islet cell antibodies have also been reported with some drugs. Examples include **thiazide diuretics**, **α-interferon, and glucocorticoids**.
- *Infections*: Certain viral infections (such as Coxsackie virus B, congenital rubella, cytomegalovirus) can cause destruction of β cells.

- Other genetic syndromes sometimes associated with DM: Many genetic syndromes (e.g. Down's syndrome, Klinefelter's syndrome, Turner's syndrome) are associated with increased risk of developing DM.