II. REVIEW OF LITERATURE

Child neurology was recognized as a board-certified specialty only 40 years ago, but its true beginnings can be traced to the 16th and 17th century with classical descriptions of chorea, hydrocephalus, spina bifida, and poliomyelitis (Finger, 1994; Ashwal and Rust, 2003). However, the true scientific foundation in clinical and scientific advances in child neurology and pediatrics was created at the end of the 19th century. Like other pediatric disciplines, child neurology evolved into a distinct clinical and scientific specialty early in the 20th century (Millichap and Millichap, 2009).

Remarkable advancement in the neurosciences, particularly in the fields of genetics, molecular biology, metabolism, immunology and nutrition have greatly advanced our understanding, towards brain development and its responses to environmental influences. Advancement in neuroimaging, electromyography, electroencephalography, neuropharmacology, muscle histology, and biochemistry has considerably improved our ability to evaluate and treat children with neurological disorders. (Ashwal et al., 2003).

Mental retardation has been the traditional foothold of psychologist and psychometricians, apart from clinicians and biologists.

2.1 MENTAL RETARDATION (MR)

Mental retardation has been recognized since biblical times, but its scientific investigation, development of psychological testing and causes were critical under stood in 20th century. Introduction of the Bimet-Simon test for children (1905), introduction of the term “intelligence quotient(I.Q)” by Terman (1916), and the work of Thurston describing factors of intelligence (1938) led to an explosion of developmental and psychological test instruments that examined virtually all aspects of infant and childhood development (Ashwal and Rust, 2002).

The first edition of Diagnostic and Statistical Manual (1952) statistically defined category of mental retardation (MR) delineated by IQ level. It includes only those cases which were formerly known as familial or "idiopathic" mental deficiencies. Thereafter,

The most accepted definition of MR is a significant sub average general intellectual functioning (criterion A) that is accompanied by limitations in adaptive functioning in at least 2 key skill areas (criterion B) and the onset must occur before age 18 years (criterion C). General intellectual functioning is defined by the intelligence quotient, IQ. Adaptive functioning refers to how effectively individuals cope with common life demands (WHO, 1992; APA, 1994; AAMR, 1992; Stevenson et al., 2000; Chelly and Mandel, 2001; Ashwal and Rust, 2003; Zalfa and Bagni, 2004; Raymond, 2010)

Early onset cognitive impairment commonly referred to as mental retardation or more recently, as intellectual disability (ID) (Schalock et al., 2007), is the leading socio-economic problem of health care, at least in Western countries (Center for Disease Control and Prevention, U.S., 2003; Salvador and Bretelli, 2008). ID is the most costly of all diagnoses listed in the International Classification of Disease (ICD-10) (Center for Disease Control and Prevention, U.S., 2003; Polder et al., 2002), more costlier than dementia and far more than cancer. Still, ID has received very little public attention, even in psychiatry, partly because many health care professionals, nongovernmental organizations and parent organizations do not perceive it as a health condition but as a social or educational issue (Salvador and Bretelli, 2008). This is also reflected by the recently launched WHO Mental Health Gap Action Plan (http://www.who.int/mental_health/mhgap/en/), which does not list ID as one of its priorities. Similarly, a recently published authoritative overview on global mental health emphasizes that neuropsychiatric conditions such as uni- and bipolar affective disorders, schizophrenia, dementia, drug abuse, and epilepsy account for up to 25 % of the disease burden worldwide but fails to mention ID altogether (Ropers, 2010).


2.2 CLASSIFICATION OF MR

A classification of mental disorders in the United States was the need to collect statistical information. The first official attempt was made in 1840 census, which used a single category, "idiocy/ insanity". In 1917, a Committee on Statistics from what is now known as the American Psychiatric Association (APA), together with the National Commission on Mental Hygiene, developed a new guide for mental hospitals called the "Statistical Manual for the Use of Institutions for the Insane".

In 1949, the World Health Organization published sixth revision of the International Statistical Classification of Diseases (ICD) which included a section on mental disorders for the first time and forwarded to Diagnostic and Statistical Manual (DSM-1) first edition categorized mental disorders. A two-fold scheme of classification of MR has been presented by various researchers as endogenous or genotous and exogenous or acquired (Ireland, 1877), congenital and acquired (Shutteworth, 1895), primary and secondary or so called nature vs. nurture cases, pathological and subcultural or physiological (Lewis, 1933; Grossmen, 1977; Luckasan et al., 2002).

Conventionally, genetic forms of MR are subdivided into two major categories-syndromic MR characterized by associated clinical, radiological, metabolic or biological features, and non-syndromic (or non-specific) MR forms in which cognitive impairment represents the only manifestation of the disease. Although this distinction remains very useful for clinical purposes, recent phenotype – genotype studies and detailed clinical follow-up of patients are indicating that the boundaries between syndromic and non-syndromic MR forms are vanishing, and some of the latter forms could be recognized as syndromic forms (Chiurazzi et al., 2004; Ropers and Hamel, 2005; Chelly et al., 2006).

Murphy et al., (1987) and Tylenda et al., (2007) reported in their study four levels of mental retardation: mild (IQ of 50-70), moderate (IQ of 35-49), severe (IQ of 20-34), and profound (IQ <20), in the 3rd edition of Diagnostic and Statistical Manual of Mental Disorders. This is the most commonly used categorization of MR.

2.3 PREVELANCE OF MR

Mental retardation is one of the most common disabilities in childhood, with a prevalence rate of 2 % or greater in most populations (Crinic and Chase, 1968). Developmental delay is a broader label, often used during preschool years before a
diagnosis of mental retardation is made. Based on data from the National Health Interview Survey, it has been estimated that 16.8% of children under 18 years old in the United States have at least one developmental disability (Dobbing, 1968).

Mental retardation is one of the most frequently encountered, and most distressing, disabilities, among children in industrialized (Kiely, 1987; Chen and Simeonsson, 1993) and developed countries (Mitchell et al., 1989; 1972; Durkin et al., 1998; Hazmi et al., 2003). According to 11 edition of AAIDD (2010) to Intellectual disability (ID) is a prevalent form of cognitive impairment affecting 2 – 3% of the population in the industrialized world. The incidence of MR in developing countries is about 2-3 times more as compared to developed countries. In developed countries, reported estimates of prevalence are 0.3 - 0.5% for moderate and severe MR and 1 – 3% for mild MR. Durkin and Coworkers in 1998 reported an extra ordinarily high prevalence estimates in the range of 12 – 24/1,000 in selected population in Pakistan and India. Males are found to be more affected than females. The risk of mental retardation is found to be higher in children with congenital structural defects (Decoufle et al., 2001, Mahajan et al., 2011). MR prevalence is also influenced by a variety of factors including social, economic, cultural rational, ethnic, environmental, age and gender (Ayoglu et al. 2008).

2.4 ETIOLOGY

The cases of MR are extremely heterogeneous. A number of environmental, genetic or multiple factors can cause mental retardation (Armatas, 2009). It is also believed that behavioral or social factors such as poverty, malnutrition, maternal drug and alcohol use can contribute to MR. Environmental factors include exposure to toxins, infectious, trauma or perinatal anoxia. But in a significant percentage of cases, the underlying cause remains unknown (Faradz, 2003). In addition, multiple problems before birth (prenatal) during birth (perinatal) or after birth (post natal), may also culminate in MR. (Winnepenninckx et al., 2003 and Armatas, 2009). In many cases, secondry mental retardation sets in as a part of complex syndrome comprising developmental brain abnormalities such as microcephali, lissencephali, neuronal heterotopias, agenesis, poly microgyria and schizencephali which results in a cerebral cortex that lacks the normal pattern of organization (Chelly et al., 2006).
The most common preventable cause of ID in the Western World, particularly in the United States is fetal alcohol syndrome (FAS). FAS consist of characteristic facial dysmorphic features, growth deficiency and central nervous dysfunction (Niccols, 2007). In these subject IQ’s scores rage between 40 and 80, with a mean of 60 to 65 and remained remarkably constant from infancy to adulthood (Spohr et al., 2007). Causes of mental retardation can be roughly grouped into several categories:

### 2.4.1 INFECTIONS

Stern et al., (1969) reported a significant association between cytomegalovirus infection and microcephalic mental deficiency. This virus account for 10% of such cases, while rubella and toxoplasmosis together were responsible for about 2 – 3 % of all cases of mental deficiency. Numerous studies during the past 30 years have reported associations between urinary tract infections during pregnancy and cognitive function of the infant has been explored (Alleyne, 1977; Bacola et al., 1966; Bacola et al., 1966; Bass, 1970). General vaccination of school girls (age 12, grade 6) was introduced in 1975. After 20 years with 90 % vaccinated and 1% loss of immunity/ year-this program was calculated to have reduced rubella embryopathies by two/thirds, but not, however, to have eradicated them. Recently, therefore, a more effective program was started comprising both sexes and with a combined vaccine (measles-mumps rubella), with a first shot at 1 year and a second one at 12 years of age (Fitzhardinge and Stevans, 1972). This leads to eradication of rubella of embryopathies in the Swedish society within 10 years.

### 2.4.2 CHROMOSOMAL ABNORMALITIES

As many as one fourth of person with mental retardation have a detectable chromosome abnormality (Batshaw and Perret, 1992). Some chromosomal abnormalities are inherited from a parent but most occur de novo (McKusick, 1994). A numerical chromosome abnormality is caused by additional (polyploidy) or missing (monosomy) chromosomes from the normal set of 46 chromosomes.

Chromosomal translocations also cause aberrations in the number of chromosomes are easy to detect by counting the chromosomes in spreads obtained from blood cells under a microscope. Reported live-born autosomal chromosome polyploidies are restricted to trisomy 13 (Patau’s syndrome), 18 (Edwards’ syndrome) and 21 (Down syndrome). Chromosomal abnormalities occur in 0.1–0.2 % of live-born infants. The incidence of
trisomy 13 live births has been reported to range from 1/5,000 to 1/30,000 (Rasmussen et al., 2003; Parker et al., 2003). Trisomy 13 was first described by Patau, et al., in 1960.

The common clinically associated anomalies include brain defects, midline facial defects, cardiac anomalies, genitourinary brain, craniofacial defects, heart defects, limb defects, and severe mental retardation (Ishikiriyama et al., 1984).

Trisomy of 18th chromosome was originally described by Edwards et al. in 1960. Its prevalence is estimated to range from 1 in 3600 to 1 in 8500 in newly born infants (Carey, 2005). It is frequently associated with neurologic abnormalities, including hypotonia in infancy, mental retardation, central apnea, and epilepsy (Gozal, 2004).

Cry du chat syndrome is the result of chromosomal deletion in the short arm of 5th chromosome while William’s syndrome is the result of a micro-deletion on chromosome 7.

Fragile X syndrome is the leading inherited cause of mental retardation (Sherman, 2002). It is caused by a mutation in a single gene (FMR1) on the X chromosome (Brown, 2002). The prevalence of affected individuals is 1 in 4000 males and 1 in 8000 females (Crawford et al., 2001).

2.4.3 NUTRITIONAL

Although the deleterious effect of protein or protein calorie malnutrition on the postnatal growth and development of infants have been known for a long time, the concept of in utero under nutrition has received little attention until fairly recently. Primarily, because the developing fetus was considered to be a perfect parasite capable of with drawing any nutrition it needed from the mother even if her nutritional status was poor. A number of investigations in human have revealed that inadequate prepartum nutrition, as reflected in low maternal weight gains, were related to both the low birth weight and mortality rates of the offspring from these pregnancies (Rosso and Cramoy, 1979; Zamenhof, 1981). It is possible that this form of protein malnutrition in man may be accompanied by some form of neurological alteration which appears to be the manifestation of minimal brain dysfunction (MBD) in children.

2.4.5 METABOLIC

Congenital hypothyroidism, hypoglycemia Reye syndrome hyperbilirubinemia can result in mental disability of medical care is inadequate. Maternal iodine deficiency
restricts brain growth and leads to a hypothyroidism than to mild impairment of
intelligence (Rachel et al., 2007).

2.4.6 TRAUMA (BEFORE AND AFTER BIRTH)

Intracranial hemorrhage before or after birth, lack of oxygen to the brain and severe
head injury can also be the cause of MR in early childhood.

2.4.7 INBORN ERRORS OF METABOLISM

A century ago, Sir Archibald Garrod in 1909 was the first to introduce the term
inborn errors of metabolism (Garrod, 1909). He pointed out In IEMs single gene defects are
responsible for the abnormalities in the synthesis or catabolism of proteins, carbohydrates
or fats by way of defective enzymes or transport proteins, resulting in a block of metabolic
pathway. IEMs are individually rare but are collectively common. The male to female ratio
is 1:1 for autosomal dominant and autosomal recessive. It is also 1:1 for X-linked dominant
if transmission is from mother to child. Environmental factors may trigger the onset and
severity of disease. It also depends upon the degree of accumulation of toxic substances
before the metabolic block e.g. diet, intercurrent, infections, fasting, drugs etc (Choudhuri
et al., 2006).

Until the mid-20th century, treatment did not exist, destiny would take its course,
and genetic counselling about recurrence risks was all that could be offered. Phenylketonuria
was then shown by Horst Bickel to be a treatable "genetic" disease in
which early diagnosis and dietary treatment prevented mental retardation. Subsequently,
several other inborn errors became manageable in a similar way, i.e., with substrate
derprivation strategy in maple syrup urine disease, urea cycle defects, galactosemia,
fructosemia, tyrosinemiastype 2, homocystinuria, and some others etc (Hoffmann et al.,
2006).

In 1999, the World Health Organisation (WHO) announced genetic and orphan
diseases as a major challenge to future health followed by similar initiatives of the
European Union (EU). In March, 2006 the Dimes Birth Defects Foundation issued the first
comprehensive global report on all geneticbirth defects ranging from 82 to 39.7 per 1000
live births worldwide (Christianson et al., 2006).
However, the total number of known neurometabolic disorders is today confusingly large. The contribution of inborn errors of metabolism to SMR and MMR is 4 – 5 % and ≪ 1 % in recent Swedish series (Hagberg and Kyllerman, 1983).

Verma (2000) screened of 4400 cases of mental retardation and reported that 5.75 % (256 cases) were due to various inherited metabolic disorders in India. He further reported that there are 25 million births in India annually; 8 lakhs are born with congenital malformation; 3.5 lakhs with glucose 6 phosphate deficiency (G6PD); 25,000 with metabolic disorders; 20,000 with Down Syndrome, 15,000 with congenital hypothyroidism, 14,000 with thalassemia and 5,000 with sickle cell anemia. Several inborn errors of metabolism that present the feature of neurodevelopmental delay include disorders related to MAO, OCT, CBS, PAH and GALPUT enzymes.

2.4.7.1 MENTAL RETARDATION DUE TO MAO

Monoamine oxidase (MAO) (EC 1.4.3.4) is an oxidoreductase. They are categorized in a family of flavin containing enzymes which catalyze oxidative deamination of monoamines and variety of other neutroamine. The enzyme is located in the human plasma, serum, brain, and periphery and also in the outer mitochondrial membrane of all mammalians species (Shih et al., 1999). Human plasma and serum MAO convert benzylamine to benzaldehyde as recently demonstrated (Mihalik et al., 2011; Reyes and Parada, 2005; Mcewen, 1964).

MAO catalyses the oxidatively deamination of many exogenous, endogenous and biogenic sympathomimetic amines (primary, secondary and tertiary) such as noradrenaline (NA), serotonin (5-HT), phenylethylamine, benzylamine, dopamine, tyramine, tryptamine and phenylethylamine ingested in the diet. It also plays a vital role in the regulation of neurotransmitters in the mammalian central nervous system including regulation of synaptic other catecholaminergic neurotransmitters. The concentrations of these neurotransmitters contribute to the regulation of mood, movement, memory and arousal (Abell and Kwan, 2001; Berry et al., 1994; Weyler et al., 1990). Monoamine oxidases that occur in the plasma of mammals have been intensively studied (Mcewen, 1964).
MAO
\[
\text{RCH}_2\text{NH}_2 + 0_2 + \text{H}_2\text{O} \rightarrow \text{RCHO} + \text{NH}_3 + \text{H}_2\text{O}_2
\]

Subsequent studies elucidated the structural configuration of MAO-A and MAO-B genes, revealing that they are located on the locus Xp 11.23, in a tail to tail arrangement, with the 3’-coding sequences separated by about 50 kb (Ozelius et al., 1988; Lan et al., 1989a, b; Levy et al., 1989; Chen et al., 1992). These genes encoded for two proteins of 527 and 520 amino acids, with molecular weights of 59.7 and 58.8 kDa (Breakefield et al., 1976; Murphy, 1976). The enzyme encoded by these genes exists in two forms, MAO-A and MAO-B. These functional proteins MAO-A and MAO-B thought to consist of two identical subunits each with molecular weights of 59 and 58 kDa respectively (Shih et al., 1999). Lenders et al (1996) in their study find that monoamine oxidase (MAO) exist as two isoenzymes forms and plays a central role in the metabolism of monoamine neurotransmitter. These two isoenzymes can be separated electrophoretically (Shih and Eidskon, 1969 and Youdim et al., 1969).

MAO has important physiological functions: First, different neuroamines are principally inactivated by oneform and not the other. For example, norepinephrine and, in many tissues, serotonin, are primarily inactivated by MAO-A, while phenylethylamine is an MAO-B-selective substrate; dopamine, however, is a substrate for both the MAO-A and MAO-B forms. Second, inhibitors of high specificity for the MAO-A or MAO-B forms have been developed and have recently begun to be evaluated as adjuncts in the treatment of Parkinson’s disease, depression and other disorders, in the hope that more selective clinical effects and/or lesser toxicity might be found. Third, increasing information on the differential localization of MAO-A and MAO-B in various tissues, brain areas, cell lines and sub-cellular preparations offers the possibility of better understanding the functional roles of MAO in detoxification, in the regulation of cellular endogenous and exogenous amine levels and amine synthesis and the considerable research in the recent years (Avshalom et al., 2002).

Decreased MAO activity has been reported in many case studies. Sandier et al (1974) have reported the marked reductions in MAO activity has been found to be the
significant element in diverse disorders as migraine, the Lesch-Nyhan syndrome, alcoholism and various psychiatric disorders such as schizophrenia and bipolar affective disorders (Breakefield et al., 1976; Murphy, 1976; Sandier et al., 1974). In one more study conducted by Nunez and Medina (2011) it was revealed that MAO inhibitors prevent the oxidative catabolism of amines, thus maintaining high levels of neurotransmitters such as dopamine, serotonin, tryptamine, and phenylethylamine (Nunez and Medina, 2011). The clinical implication of MAO was rekindled by a number of reports on the implication of its deficiency in typical deficiency Norrie disease (ND) patients. The recessive X-linked disease is caused by loss of functional mutations of NDP (Norrie disease pseudogoliama) gene which encode for norrin, a protein involved in the development and vascularization of the retina and inner ear. In affected males, total norrin deficiency result in congenital blindness, cataracts and progressive deterioration of the iris.

To date, research has linked the low-activity MAO-A alleles to various psychopathologies, maladaptive behaviors, cognitive dysfunctions, and criminal behaviors. In particular, the deletion of MAO –A and MAO – B in ND patient is conducive to severe mental retardation, growth failure, alterations of sleep pattern, and autistic – like symptoms (Lan et al., 1989a, b; Sims et al., 1989a, b; Murphy et al. 1990; Collins et al., 1992).

Brunner et al (1993) identified Brunner syndrome, which is an X-linked disorder characterized by impulsivity, heightened aggressiveness, mild mental retardation, and serious criminal behaviors including and sexual assault caused by MAO-A deficiency (Brunner et al., 1993). Like MAO B, it actively oxidisesbenzylamine but metabolises other monoamines poorly (McEwen, 1972). Nies and co-workers (1974), Landowski (1975), Edward (1978) and Mann (1979) compared platelet MAO activity inbipolar and unipolar subgroups patients, they have shown significant elevated platelet MAO activity compared with control. High values have been noted in depressed schizophrenics (Orsulak et al., 1978). Murphy and his co-worker found a 10% increase, which did not reach statistical significance value, in unipolar patients. No case of unipolar depression has observed a decrease in platelet MAO activity (Murphy and Weiss, 1972). The reported results about the studies of platelets MAO activity in bipolar depression patients is more complex. The activity of this enzyme in this group has reported to be significantly decreased (Landowski
et al., 1975; Mann 1979; Murphy and Weiss, 1972; Leckman et al., 1977), increased, (Nies et al., 1974; Belmaker et al., 1976) or not significantly different "(Berrettini et al., 1979) from control values.

Independent studies using various assay procedures on material from different patient populations show a similar trend, and the findings become more convincing. Out of the six independent studies (Sicuteri et al., 1972; Sandler et al., 1974; Glover et al., 1977) of migraine, five noted a reduction in platelet activity, significant in four, and in none was there an increase; all five published independent studies, and unpublished observations demonstrated an increase in platelet MAO activity (Landowski et al., 1975; Edwards et al., 1978; Murphy and Weiss, 1972). Mann and Chiu (1978) and Belendiuk (1979) in their studies carried out platelet MAO activity in Huntington’s chorea. They have observed a significantly raised in platelet MAO activity. Out of these studies, the former reported a 20% increase in MAO activity was observed for the whole group, although the values were significantly different from controls only in male patients. In two subjects, improvement in clinical condition was paralleled by a fall in enzyme activity (Mann and Chiu, 1978; Belendiuk et al., 1979).

Sullivan (1978) also noted that platelet MAO activity (using tryptamine as substrate) is lower in chronic alcoholics than in controls. This was observed on each of three abstinent intervals over a 12-month time period, suggesting that such low activity is a stable characteristic of this illness, regardless of alcohol consumption (Sullivan et al., 1978). However, both Takahashi and Brown found that activity returns to normal as the acute episode of alcoholism subsides (Takahashi et al., 1976; Brown, 1977). Major and Murphy reported that 99 healthy male alcoholics, in varying stages of abstinence, had significantly lower mean activity than controls; there was no correlation with severity or chronicity of drinking or with duration of abstinence, nor was there a rise to normal values during abstinence, as Wiberg had found alcoholics having a first degree relative with this disease had lower activity than those with a negative family history (Major and Murphy, 1978). Alcohol itself, in the rat at least, does not appear to affect tissue MAO activity in blood concentrations of the order found in human alcoholics. Nor does acetaldehyde cause a change in platelet MAO activity in vitro in the highest concentrations reported to occur in Vivo. On balance, the evidence thus far suggests that low platelet MAO activity occurs in at
least some alcoholics as a stable trait and is not an artifact of ethanol consumption or with
drawal. Wiberg et al., 1977; Sandler 1974; Glover 1977 and 1980 in their studies have
compared platelet MAO activity in patients with a history of dietary migraine and in those
without. Migranous patients have lower platelet MAO activity than controls. Anselmi and
co-worker (1976) reported that a group of 31 hypertensive patients have platelet MAO
enzyme activity values about 40 % lower than those of a control group. Mean platelet MAO
activity in patients with iron deficiency anaemia appears to be about 30 % lower than that
of normal controls (Callender et al., 1974; Youdim et al., 1975). Hanington and co-worker
(1970 and 1978) in their study on migraine patients might account a significant reduced
platelet MAO activity with control. In their study of migraine patient after consuming
tyramine-containing foods (Blackwell et al., 1967) such as cheese (which is a rich source of
tyramine) to initiate, hypertensive response to initiate attacks.

Several independent studies made by Sicuteri et al (1972), Sandler et al (1974) and
Bussone et al (1977) using different methods, have shown that migraine patients have a
significantly reduced platelet MAO activity to about 50 % compared with controls (Sicuteri
et al., 1972; Sandler et al., 1974; Bussone et al., 1977). Friedhoff and co-worker (1978)
have reported a change in specific MAO activity these two diseases i.e. autoimmune
thrombocytopenic purpura and reactive thrombocytosis. In autoimmune
thrombocytopenic purpura platelet count and platelet protein density are both reduced by
more than 50 %. Specific MAO activity is also reduced by about half. In reactive
thrombocytosis, platelet count and platelet protein density both became doubled, and
specific MAO activity is also considerably increased in this disease.

2.4.7.2 MENTAL RETARDATION DUE TO PAH

Phenylalanine hydroxylase (PAH) (EC 1.14.16.1) is an iron-containing
enzyme that catalyzes the hydroxylation of L-phenylalanine to L-tyrosine (Fisher et al.,
hydroxylase consists of two isoenzyme, each of which is capable of existing as a monomer
(51,000 to 55,000), a dimer (110,000) and a tetramer (210,000). In their study
phenylalanine hydroxylase isoenzyme during electrophoresis in polyacrylamide-gel. When
electrophoresis is carried out 0° (0°-2°) two closely migrating bands, the same as a single
band were observed. When electrophoresis was carried out at 30°, two faster migrating
band were observed. The tetrameric form of PAH is more prevalent (Kaufman, 1985); Comparison of the cloned human and rat enzymes shows that their amino acid sequences differ by less than 8% (Kwok et al., 1985; Dahl and Mercer, 1986). Phenylalanine hydroxylase is also the prototype for the class of enzymes known as pterin-dependent hydroxylases, which includes the less studied but neurochemically important enzymes tyrosine hydroxylase and tryptophan hydroxylase (Kaufman, 1987a). The enzyme shows an absolute requirement for a tetrahydropterin cofactor (Kaufman, 1958a). This natural cofactor isolated from rat liver (Kaufman, 1963).

The first step in phenylalanine metabolism was discovered by Folling in 1934. The irreversible conversion of phenylalanine to tyrosine is the initial and rate-limiting step in the only pathway in which mammals can catabolize this aromatic amino acid to carbon dioxide and water (Milstien and Kaufman, 1975).

Reaction:

$$\text{PAH} \quad \text{Tetrahydropterin} + \text{phenylalanine} + \text{O}_2 \rightarrow \text{quinoid dihydropterin} + \text{tyrosine} + \text{H}_2\text{O}$$

Since 1934, there has been an enormous flood of work on all aspects of the condition-its genetics, the primary biochemical defect and the secondary biochemical effects, and most of all the relation between the abnormal biochemistry and the mental and neurological aspects of the disease. An inborn error in metabolism is transmitted in humans due to the absence of phenylalanine hydroxylase results in the disease known as phenylketonuria (Jervis, 1947). The disorder is transmitted in an autosomal recessive pattern and is the most common inborn error of amino acid metabolism in the white population, with an average incidence of 1/10,000 (Scriver et al., 1995). This disease is caused by a complete or near-complete deficiency of PAH activity, and is associated with profound and irreversible mental retardation. (Ledley et al. 1985; Grenet et al., 1987). The reports from different countries around the world show that 1–3% of the patients in the institutions for mentally retarded suffer from the PKU disease. Accumulation of phenylalanine generates a brain damage and consequently irreversible mental retardation (Matalon and Rouse, 1998; Pietz et al., 1998). Mental retardation is attributed to a toxic effect of excess phenylalanine on brain development and functions. Neurological problems...
increase with age of diagnosis, profound mental retardation, epilepsy, spasticity, and severe behavioural disorders are typical across the disorder's life course (Blaskovics and Nelson, 1971; Yalaz et al., 2006; Brenton and Pietz et al., 2000).

2.4.7.3 MENTAL RETARDATION DUE TO CBS

Cystathionine β-synthase (CBS) (EC 4.2.1.22) (L-serine hydrolyase [adding homocysteine]), is a pyridoxal 5'-phosphate-dependent enzyme in the mammalian trans-sulfuration pathway which catalyses the condensation of L-homocysteine (L-Hcys), a toxic metabolic intermediate of L-methionine (L-Met) metabolism, to produce L-cystathionine (L-Cth). Mammalian CBS is the only enzyme known to contain both pyridoxal 5’-phosphate (PLP), the catalytic cofactor, and heme (Kery et al., 1994), which is bound by the 70-amino acid, N-terminal domain (Meier et al., 2001; Taoka et al., 2002). Israelsson and co-worker (1988) human CBS is a homotetramer composed of 63 kDa subunits each 551 amino acids in length. Each subunit has a modular structure consisting of three domains: an N-terminal heme binding domain, a highly conserved pyridoxal-5'-phosphate (PLP)-binding catalytic core, and a S-adenosyl-L-methionine (AdoMet)-binding C-terminal regulatory domain. PLP serves in the catalytic chemistry of CBS via a well-established mechanism (Miles et al., 1986; Kery et al., 1999; Banerjee et al., 2003). CBS catalyzes the following reaction
The gene encoding CBS is located on the 21st chromosome in humans and is linked to the genetic disorders of homocystinuria and Down syndrome (Munke et al., 1988; Kraus et al., 1999; Kamoun, 2001). The primary translation product of the human and rat gene is a Mr 63,000 polypeptide. However immuno precipitation of synthase from fresh liver extracts with monospecific antisynthase antiserum yield two forms of the enzyme: a predominant form (95%), a tetramer of Mr 48,000 subunits (Skovby et al., 1984). It has been shown that the smaller form of the enzyme is derived from the larger form by the action of specific protease.

Deficiency of CBS is an autosomal recessive disease, one of the known enzymatic deficient conditions and is the most prevalent inborn errors of methionine metabolism leading with clinical manifestation such as ectopialentis, skeletal abnormalities, premature arteriosclerosis, thrombosis, cerebral atrophy, epileptic seizures, psychiatric disorders and mental retardation (Leo, 1999; Kraus et al., 1999; Kamoun, 2001; McCully, 2005). Its deficiency is characterized by elevated plasma L-Homocysteine levels, as the substrate of transmethylation and transsulfuration pathways, L-Hcys is situated at a metabolic branch point. The flux of L-Hcys through these competing pathways is regulated, via allosteric regulation, by the ubiquitous methyl donor S-adenosylmethionine (SAM) (Selhub and Miller, 1992; Finkelstein et al., 1975). Human CBS is activated 2–3-fold by SAM, thereby increasing the flux of L-Hcys through the transsulfuration pathway when the cellular methionine pool exceeds the level required to maintain homeostasis (Finkelstein et al., 1975; Janosik et al., 2001; Prudova et al., 2005).

Helga, (2004) reported that the most common cause of homocystinuria CBS deficiency. CBS deficiency results in markedly elevated blood levels of homocysteine and methionine (Mudd et al., 1995). Martignoni (2007) reported that in the trans-sulfuration pathway, homocysteine condenses with serine to form cystathionine, through an irreversible reaction catalyzed by the pyridoxal-5′-phosphate (vitamin B6)-dependent enzyme, cystathionine β- synthase. Finally, cystathionine is hydrolyzed to form cysteine, which may be incorporated in glutathione or excreted in the urine (Stead et al., 2004). Homocysteine is involved in numerous transmethylation mechanisms playing pivotal roles in the biochemistry of the human body, with SAM acting as the main donor of methyl
groups in reactions targeting DNA, RNA, proteins, phospholipids and neurotransmitters (Stead et al., 2004; Mattson and Shea, 2003; Selhub, 1999).

Mudd and Levy, (1983) characterized early onset of atherosclerosis and thromboembolism as an IEM. It has been also proposed that homocysteinemia might be a risk factor for the development of atherosclerosis (Mudd and Levy, 1983; Brattstrom et al., 1984; Israelsson et al., 1988; Fowler et al., 1978) have shown an association between vascular disease and homocysteinemia either in the fasting state and/or diagnosed by a methionine load. These patients are considered heterozygotes for CBS deficiency and have reduced enzyme activity to less than 50 % of normal (Schneider and Mitsui, 1976; Norris and Shock, 1966; Linn, 1975; Mudd and Levy, 1983).

Israelsson (1988) reported that the CBS deficiency is hereditary disease or, in rare cases, due to defects in certain enzymes of folate and vitamin B, metabolism. In its homozygote form, CBS deficiency is associated with the development of arteriosclerosis and frequent thromboembolic episodes at an early age (Mudd and Levy., 1983). Heterozygosity for CBS deficiency is also a possible risk factor; in one recent study it was commonly found among patients with premature peripheral and cerebral occlusive arterial disease, but not among those with coronary artery disease (Bores et al., 1985).

Chadefaux (1985) measured CBS activity in the trisomic cells, was significantly greater than in normal cells with respectively 23.54 ± 3.12 nmol/mg/h and 14.2 ± 2.03 nmol/mg/h (mean, SD) (p < 0.001). The mean ratio of trisomy 21 to normal (T/N) was 1.66. A 1.04 ratio was found in 21q21→21pter monosomy; a 1.04 and 0.99 ratio was found in two 21qter→21q22 monosomies; a 1.14 ratio in 21qter→21q22 monosomy; a 0.89 ratio in a 21q21→21pter trisomy; an excess of CBS activity was found in a 21q22.1→21q21 trisomy with a 1.57 ratio. It was also reported that the 1.66-fold increase in mean CBS activity in fibroblasts obtained from inditiiuals with trisomy 21 is quite close to the expected gene dosage effect of 1.5. The CBS enzymatic activity in several cases of partial monosomies and trisomies 21 suggest the assignment of human CBS locus between 21q22.1 and 21q21(Chadefaux, 1985).

Abbott et al (1987) marked that CBS deficiency in aldoscent and adult is manifests with mild mental retardation, lens dislocation, marfan like appearance and thromboembolic complications. Psychiatric complication occurs in as many as 51 % of
adult patients (Abbott et al., 1987; Sedel et al., 2007). Abbot et al (1987), Mudd et al (1985), Ryan et al (2002) in their study found that CBS deficiency have been reported in schizophrenia or psychotic episodes (Abbot et al., 1987; Mudd et al., 1985; Ryan et al., 2002; Sedel et al., 2007).

Nordström and co-worker (1992) found that the CBS activities and reported the results as nmol/h per mg protein. The highest values were found in embryonic cells (EC); mean value without and with pyridoxal 5'-phosphate (PLP) added to the assay mixture (S.D.) was 14.45 (f 5.1) and 25.72 (f 9.6) nmol/h per mg. Lower enzyme activities were seen in the other groups: controls (C) having CBS-activities of 4.48 (3.9) and 9.85 (9.5); Down’s syndrome without and with PLP 3.35 (f 1.7) and 7.34 (f 4.1); patients with atherosclerotic vascular disease (P) 1.50 (f 1.2) and 2.90 (f 2.4); obligate heterozygotes (HeZ) 0.88 (f 0.7) and 2.31 (f 2.0) and patients homozygous for CBS deficiency (HoZ) 0.00 and 0.04 nmol/h per mg protein Nordström et al. (1992) also reported that CBS activity was significantly lower in the atherosclerotic patients as compared to control subjects. In further studies in cell culture systems are needed to investigate if young patients (< 45 years old) with atherosclerotic disease could be identified by low CBS activity in fibroblast cultures as indicated by this study. Marked deficiency of cystathionine β-synthetase activity has been demonstrated in liver, skin fibroblast and phytohemagglutinin-stimulated lymphocytes from patients with the most common form of inherited homocysteinuria (Mudd and Levy, 1983).

2.4.7.4 MENTAL RETARDATION DUE TO GALPUT

Galactose-1-phosphate uridylyltransferase (GALPUT) (EC 2.7.7.12) is one of the three enzymes of the galactose metabolic pathway i.e. galactokinase, galactose-1-phosphate uridylyltransferase (GALT), and uridine diphosphate (UDP) galactose-4-epimerase, that are responsible for the rapid conversion of galactose to glucose in the liver after ingestion of dietary lactose or the breakdown of endogenous galactose containing compounds. Although a deficiency of any of the three enzymes can lead to galactose accumulation in plasma but the term galactosemia strictly refers to GALT deficiency, the most common of the three enzyme deficiencies in the newborn period. Absence of the catalytic activity of this enzyme in men results in intolerance of galactose and in the so-called “transferase deficiency galactosemia,” which is inherited as an autosomal recessive trait.
Tedesco et al (1972) reported a molecular weight of about 30,000 for the subunit of the transferase and a molecular weight of 90,000 for the native enzyme, suggesting that it was a trimer. The normal transferase consists of subunits, probably identical, of 31,000 molecular weight, but many observations suggest that the native enzyme is a dimer and not a trimer. All three enzymes have been associated with inborn errors of galactose metabolism. Hammersen et al (1975) reported that starch gel electrophoresis of transferase from fetal erythrocytes showed two brightly fluorescing isoenzyme bands and a single poorly fluorescing and slower moving third isoenzyme band. This electrophoretic pattern differs from the normal postnatal erythrocyte transferase in the presence of three rather than only two bands. However the pattern of three bands is identical to that found in the individuals with reticulocytosis and confirmed by the author. There was no difference in electrophoretic mobility of transferase from fetal and adult fibroblasts. Fibroblast lysates from both source showed four activity bands, each slower moving than any erythrocyte band. In one more study conducted by Won et al (1969), starch gel electrophoretic system showed a single transferase activity isoenzyme band is obtained with hemolysates from normal persons from galactosemia/normal heterozygotes. For such individuals, the results are similar to those with the phosphate system at pH 7.0 of Mathai and Beutler (Mathai and Beutler, 1966)

The most common initial clinical signs of GALPUT deficiency are poor feeding, poor growth, irritability, lethargy, vomiting, cataracts and mental retardation. Jaundice may be present in the first few weeks of life and can persist. (Hsia, 1969; Dale et al., 1976). Groppet (1917) reported first case of galactosemia, characterized with hepatomegaly, jaundice, failure to thrive, and urinary excretion of albumin and sugar. The patient suffering of galactosemia may also be mentally retarded. The frequency of clinically significant galactosemia is estimated at 1 in 60,000 births in United States, but would be higher if the more frequent partial deficiencies are considered. While Fateen (2004) reported the incidence rate of galactosemia in the range of 1:35,000-73,296 and 1:30,000-191,000 in Canada and USA was respectively. Cheung et al (1996) reported that the incidence in a Chinese population was approximately 1:400,000. Newborn screening and other surveillance programmes in Europe and North America have established newborn rates of
1:30,000-50,000 in white Caucasians (Murphy et al., 1999; Applegarth et al., 2000; Suzuki et al., 2001).

The clinical syndrome of transferase-deficiency galactosemia has changed since the advent of newborn screening. In instances of the rapid availability of newborn screening results (3 to 4 days of life) patients rarely require hospitalization. In the past, a severe multi-organ toxicity syndrome was a much more common occurrence which was associated with unlimited intake of lactose in the proprietary formula or breast milk. Initially the hyper-bilirubinemia may be indirect and is only later associated with an elevation of the direct component as well. With continual lactose ingestion, multiorgan toxicity syndrome ensues, which is associated with liver disease that can progress to cirrhosis with portal hypertension, splenomegaly, ascites, renal tubular dysfunction, and sometimes full-blown renal fanconi syndrome. Anemia, primarily caused by decreased red blood cell (RBC) survival, and lethargy; brain edema associated with a bulging fontanel can also occur. On the other hand, cataracts may be evident in the first few weeks of life. However, some infants are born with congenital cataracts that are associated with abnormalities of the embryonal lens; they are central in nature and require slit-lamp examination for documentation. Following is the reaction catalyzed by GALPUT

\[
\text{Galactose l-phosphate} + \text{UDP-glucose} \xrightarrow{\text{GALPUT}} \text{glucose l-phosphate} + \text{UDP-galactose}
\]

Joseph (1974) reported partially purified galactose l-phosphate uridylyltransferase from red blood cells of six different galactosemic patients, heterozygote, and normal human subjects while Wang and Desforges (1966) reported Galactose 1-phosphate Uridyl Transferase enzyme activity in the philadelphia chromosome or Ph chromosome. Wang and co-worker (1966) showed the results of the leukocyte GALPUT enzyme assay 10 patients with philadelphia (Ph group) chromosome and 3 controls and reported a mean variation of 0.12 units/10^8 WBC with a range of 0 to 0.37 Units. The mean enzyme activity in the Phgroup was 1.22 Units/10^8 WBC with a mean control value of 1.34. The difference between these two means is not statistically significant (p > 0.1). The enzyme appears even to be increased in the red cells of patients with the Ph chromosome when compared to normal individuals. The reasons for this finding are not immediately clear but may be due
to a younger population of red cells in the patients with CML. Red cell count was not usually performed at the time of the study, but polychromacia of red cells was present in 11 of our 16 Ph – positive patients (Wang and Desforges, 1966). In order to study its relation to chromosome No. 21, elevated activity of the enzyme Galactose 1-phosphate Uridyl Transferase has been reported in the blood of patients with mongolism (Brandt et al., 1963).

2.4.7.5 MENTAL RETARDATION DUE TO OCT

Ornithine transcarbamylase (OTC) (ornithinecarbamoyltransferase; EC 2.1.1.3.3) is a liver enzyme of one round of Urea Cycle which condenses two molecules of toxic ammonia and one molecule of bicarbonate to form a molecule of urea that is nontoxic and is readily excreted in the urine. These two reactions are responsible for citrulline synthesis, occur in the mitochondrion. The next three reactions occur in the cytoplasm after citrulline is transported out of the mitochondrion. Because citrulline is the product of the carbamyl phosphate synthetase type 1 (CPS-I) and OTC reactions and is the substrate for argininosuccinate (ASA) synthetase, the transport of ornithine back into the mitochondrion and the shuttling of aspartate to the cytoplasm are clearly imperative and account for the two other genes that cause urea cycle defects. The urea cycle disorders (UCDs) are inherited as autosomal recessive traits. Majority of these UCDs occur in the newborn period and in infancy; they are carbamyl phosphate synthetase I deficiency, ornithine transcarbamylase (OTC) deficiency, argininosuccinate (ASA) synthetase deficiency (citrullinemia), and ASA lyase deficiency (argininosuccinicacidemia). N-Acetylglutamate, the product of the first enzyme in the cycle, is an obligatory activator of carbamyl phosphate synthetase.

In aggregate, disorders of the urea cycle may be as frequent as 1 in 25,000 births or more. The main laboratory finding in the UCDs is a plasma ammonium elevation. In newborn-onset N-acetylglutamate synthase (NAGS), CPS-I and OTC deficiencies, plasma citrulline concentrations may be undetectable and are always low. With OTC deficiency, there is increased urinary orotate excretion secondary to carbamyl phosphate accumulation and pyrimidine synthesis. With NAGS or CPS-I deficiency, carbamylphosphate production is decreased or absent, and orotate excretion is decreased. In citrullinemia, the eponymous amino acid citrulline has markedly elevated
concentrations. With argininosuccinic aciduria, plasma citrulline concentration is moderately elevated, in the range of 100 to 300 μmol/L, and can be readily detected during a study of plasma by amino acid analysis. Clinical presentation in the newborn period is similar for all these defects, almost all the infants are well in the first 12 to 24 hours of life until they begin to feed poorly, vomit, hyperventilate, become irritable and lethargic, and become comatose, usually with seizures. All children in whom coma lasted 5 days or longer were severely mentally retarded when these diseases are not treated aggressively and are almost always fatal. With the characteristic abnormality hyperammonemia is respiratory alkalosis caused by the effect of ammonia on the respiratory control centers in the brainstem (Brusilow and Horwich, 1995; Leonard, 2006).

Hata et al., (1988) reported that OCT is a homo trimer of 36-kD subunits, OTC is localized to the mitochondrial matrix and is found exclusively in two tissues: the liver and small intestine. (Hata et al., 1988). Kalousek et al (1977) in their study also found that OCT must be added to the small list of mammalian enzymes which exist as trimers of identical subunits. On the other hand, Baron and Buttery (1972) reported that human and pig ornithine carbamoyltransferase from liver extract both shows one band each but have different mobility. A mixture of both was distinguishable as two discrete bands. With the recent cloning of the human OTC gene, the gene was found to be a 73-kb gene containing 10 exons (Hata et al., 1988). An X-linked disease, a deficiency of OTC is the most common inborn error of ureagenesis in humans and is a disease with frequent new mutations (Maddalena et al., 1982; Nussbaum et al., 1986; Jeannin T lee, 1989).

The outcome for patients with severe newborn-onset CPS-I and OTC deficiencies is poor. Sometimes dialysis therapy cannot rescue severely affected boys with X-linked OTC deficiency in the first few days of life (Enns et al., 2008). Hemodialysis (or extracorporeal membrane oxygenation) is the most effective way of reducing plasma ammonium levels, because it affords the greatest clearance of ammonia (Rutledge et al, 1990). Even after institution of successful therapy, the morbidity and mortality rates are high in these severely affected patients and mental retardation is common in survivors (Brusilow and Horwich, 1995). There is a significant correlation between the duration of newborn hyperammonemonic coma and the developmental quotient score at 12 months of age (Msall et al., 1984). Prospectively administered alternative pathway therapy in conjunction with
high-calorie fluids usually prevents death and severe hyperammonemia in patients known from family studies or prenatal diagnosis to be at risk. This therapy is done best in collaboration with an expert in the treatment of urea cycle disorders.