REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

2.1 Lysosomal storage disorders (LSDs)

Lysosomal storage disorders (LSDs) are considered to be a rare metabolic disease for the national health forum, clinicians, and scientists (Sheth et al., 2014).They result from the inherited deficiency of 1 or more of the many catabolic enzymes that are located within the lysosome. This group of inborn errors of metabolism encompasses 50 different storage diseases, each characterized by the accumulation of specific substrates (Staretz-Chacham et al., 2009; Beaudet et al., 1995; Wraith, 2002; Futerman and Van Meer, 2004 and Vellodi, 2005). All LSDs are inherited in an autosomal recessive fashion, except for Fabry, Hunter (mucopolysaccharidosis type II [MPS II] and Danon diseases, which are X-linked. Some disorders are more prevalent in certain geographic areas or among particular population groups (e.g., Gaucher, Tay-Sachs, Niemann-Pick type A, and mucolipidosis IV are more common in Ashkenazi Jews), largely as a result of ancestral founder mutations (Mistry, 1999; Natowicz and Prence, 1996; Schuchman ad Miranada, 1997).

Apart from selected populations presenting a high prevalence for specific diseases, such as the Ashkenazi Jewish population at high risk for GD (Beutler, 2001), Tay – Sachs disease and Niemann – Pick disease (Vallance and Ford, 2003); the Finnish population with its high incidence of aspartylglucosaminuria (Arvioet al., 1993) and infantile/juvenile neuronal ceroid lipofuscinosis (Santavuori, 1988), as far as we know, prevalence data on LSDs, as a group, have only been reported in Greece (Michelakakiset al., 1995), the Netherlands (Poorthuiset al., 1999), Australia (Meikleet al., 1999), Portugal (Pintoet al., 2004) and the Czech Republic (Poupetovaet al., 2010). As a group, overall incidence of LSDs is estimated at around 1:5,000–1:8,000 (Filocamo and Morrone, 2011).

The study for different storage disorders was carried in India and among them glycolipid storage disorders was found with high prevalence followed by mucopolysaccharide disorders and defective sulfatide degradation of the children. Less common defects were glycogen degradation defect and protein degradation

defect, lysosomal trafficking protein defect and transport defect. This study demonstrates higher incidence of GD followed by GM2 gangliosidosis that includes Tay-Sachs disease and Sandhoff disease and mucopolysaccharide disorders among all LSDs (Sheth et al, 2014).

2.2 Gaucher disease (GD)

2.2.1 Biological relevance

GD is one of the most common genetic disorders in Ashkenazi Jews, with a frequency of 1 in 855 live births (Staretz-Chacham et al. 2009). The study carried out in India also demonstrates glycolipid storage disorders as one of the most common LSDs similar to that observed in Portugal, Australia, and Czech Republic (Pinto et al. 2004; Meikle et al. 1999; Poupetova et al. 2010) with higher frequency of GD (Sheth et al, 2013). The high prevalence of GD (16 %) is in accordance with previously reported study (14.4 %) from northern India (Verma et al. 2012) and Mappila Muslims from southern India encompassing Kerala (Feroze et al., 1994).

This being an autosomal recessive genetic disorder the carrier rate in general population is expected to be approximately 1 in 100. It presents with varied clinical manifestationsoften with some uncommon features. Therefore, it is very important to diagnose the disease early so as to give the best treatment and prevent further progress of the disease, as early onset of clinical symptoms and signs predispose patients to severe phenotype with irreversible complications.

Nonneuronopathic GD is the most prevalent form (94%) and is differentiated from the acute neuronopathic (1%) and chronic neuronopathic (5%) forms by the absence of central nervous system involvement (Beutler and Grabowski, 2001). The manifestations of nonneuronopathic GD include splenomegaly, hepatomegaly, anemia, thrombocytopenia, bone disease (ie, bone marrow infiltration, Erlenmeyer flask deformity of the distal femur, osteopenia, osteoporosis, infarction, avascular necrosis, and pathologic fractures), and growth retardation (Beutler and Grabowski, 2001; Zevin et al., 1993; Kaplan et al., 1996). Bone pain is common, manifesting as a mild to moderate intermittent pain or more severe acute “bone crises” accompanied by periosteal elevation, leukocytosis, and fever, which may cause debilitation for several days and require narcotic analgesics. Clinical expression of GD is highly variable

among patients (Beutler and Grabowski, 2001), but, particularly when manifested during childhood, the natural history of GD is that of a progressive, multisystemic, and debilitating disorder.

The most common signs and symptoms noted were splenomegaly (95%), hepatomegaly (87%), radiologic bone disease (81%), thrombocytopenia (50%), anemia (40%), growth retardation (34%), bone pain (27%), and bone crisis (9%). Anemia and more severe splenomegaly and hepatomegaly were observed more frequently in younger patients. Skeletal manifestations were found more often in older children (Kaplanet al., 2006).

2.3 Plasma chitotriosidase as screening marker

Plasma chitotriosidase originates from activated macrophages and this elevation is secondary to the basic defect in GD. It was estimated for different 24 lysosomal storage disorders. In 11 different diseases increased chitotriosidase activity in plasma was found (in 28% of the patients). None of these diseases showed elevations as high as in GD (Guo et al., 1995).

It was observed that moderately raised activity of ChT could be utilized as a positive predictive test for certain LSD's. Those with marked elevated ChT have confirmed GD and making it a strong screening marker (Sheth et al., 2010).

It was found that plasma chitotriosidase levels can serve as a new diagnostic hallmark of GD and should prove to be useful in assessing whether clinical manifestations of GD are present and for monitoring the efficacy of therapeutic intervention (Hollak et al., 1994). Several studies have shown this biomarker to be employed to monitor effectiveness of thetherapeutic intervention (Cox et al., 2000; Hollak et al., 1994, 2001; Mistry and Abrahamov 1997). Chitotriosidase is, a chitinase encoded by the chitotriosidase gene (CHIT1; MIM 600031). In state of Minas Gerais Brazil, ChT activity was found to beuseful as therapeutic monitoring in 82% of GD patients (Adelinoet al., 2012).

Monitoring of plasmachitotriosidase activity in GD patients during progression and therapeutic correction of their disease is useful to obtain insight aboutchanges in body burden on pathological macrophages (Aguileraet al., 2003).

2.4 β-Glucosidase Activity as a Diagnostic Index of Gaucher's Disease

β -glucosidase (EC 3.2.1.21) is a glucosidase enzyme that acts upon β1->4 bonds linking two glucose or glucose-substituted molecules(i.e., the disaccharide cellobiose). It is an exocellulase with specificity for a variety of beta-D-glycoside substrates. It catalyzes the hydrolysis of terminal non-reducing residues in beta-D-glucosides with release of glucose.

In 1968, Ockermann and Kohlins (Ockerman and Kohlin, 1968) demonstrated a decrease in β-glucosidase activity in the spleen and liver of Gaucher patients as measured on an artificial substrate 4-methylumbeliferryl-β–D-glucopyranoside. This substrate offers many advantages; it is commercially available and it is hydrolysed to give 4-methylumbelliferone, which is highly fluorescent, thereby giving the assay enhanced sensitivity. Encouraged by the observation of Ockerman and Kohlin (Ockerman and Kohlin, 1968) that extracts of liver and spleen from patients with GD were deficient in their capacity to hydrolyze glucose from MUG (Figure 2.1), Beutler and Kuhl (Beutler and Kuhl, 1970) in 1970 used this fluorogenic substrate in developing a technique to estimate the relative glucocerebrosidase content of human leukocytes. They were the first to show that the specific enzymic lesion of GD could be demonstrated by using nonphysiologic 13-D-glucosides as substrates and leukocytes as sources of enzyme. Assays at pH 4.0, a condition far below the pH optimum of glucocerebrosidase (Peterset al., 1976), allowed optimum differentiation between leukocytes of patients with GD and those of control subjects.

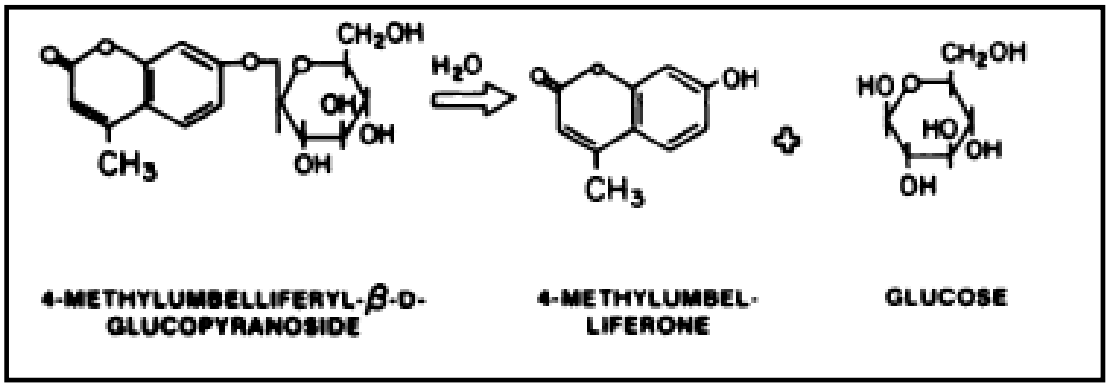

Figure 2.1: Structure of nonphysiological β-glucosidase substrates & their reaction products (Daniels and Glew, 1982)

Although the “acid β -glucosidase” assay has proven useful in identifying patients and some carriers of GD in some laboratories (Beutler and Kuhl, 1970; Beutler and Kuhi, 1970), many studies have found the “acid β -glucosidase” assay generally unsatisfactory in identifying heterozygotes for GD when leukocytes serve as the source of enzyme activity(Chiaoet al., 1980; Raghavanet al., 1980; Wengeret al., 1978).

Beutler and Kuhl (Beutler and Kuhl, 1970) reported a bimodal pH-activity curve for human leukocyte 4MU- β-glucosidase with optima at pH 4.0 and 5.3. They found that in GD, only the activity at pH 4.0 is severely depressed. On the basis of this observation, assay conditions have been developed for diagnosing GD using leukocytes and fibroblasts (Beutler et al., 1971; Hoet al., 1972; Hultberget al., 1973; Klibanskyet al., 1974; Peterset al., 1975). However, these conditions are often not reliable nor reproducible for detecting heterozygous carriers of this genetic disease. Although use of the natural glycolipid substrate, glucocerebroside, would avoid ambiguity, the synthesis of this compound with a radioactive label in the glucose portion of the molecule is very difficult and expensive (Klibansky, 1974; Kampineet al., 1967; Mc Master and Radinns, 1977). Consequently, the natural substrate is available in very few laboratories. If the glycolipid is labeled at positions other than the sugar residue, the assay procedure is a tedious one requiring chromatographic isolation of the product (Peterset al., 1976) and is unsuitable for the screening of large samples for diagnosis.

In clinically affected GD patients there is a significant increased in plasma concentration of glucosylceramide (Ullman and Mc Cluer, 1977; Dawsonet al., 1982; Nilssonet al., 1982; Strasberget al., 1983). The average increase is about twofold, being far less spectatular than that in tissues. More pronounced elevations are usually noted for the more severely affected neuronopathic GD patients. The measurement of plasma glucosylceramide concentration is tedious and requires considerable expertise. As an alternative the measurement of glucosylceramide content of erythrocytes rather than plasma is preferred by some researchers (Eriksonet al., 1993). Glucosylceramide levels in erythrocytes and plasma are usually elevated to a similar extent. Although it is very probable that the elevation in plasma (and erythrocyte) glucosylceramide is related to the presence of storage cells in tissues and organs, there is no insight into the precise nature of the involved lipid exchange mechanism.

The gold standard for diagnosing GD is measurement of glucocerebrosidase enzyme activity in leucocytes or skin fibroblasts on a skin biopsy (Hoet al., 1972). Enzyme activity in heterozygote carriers and normal individuals may show overlap and therefore enzyme analysis by itself cannot be used to differentiate carriers from normal individuals. With the availability of the diagnostic enzyme test, there is no indication to histologically examine the bone, liver or spleen for diagnosis. Bone marrow examination may be needed if the splenomegaly does not regress on treatment or patient develops enlarged lymph nodes or B symptoms to suggest development of a lymphoma (Nagral, 2014).

2.5 Molecular Pathology of GD

The gene for glucocerebrosidase (*GBA*; MIM] 606463) was localized to 1q21 (Barneveld et al., 1983) and subsequently cloned and sequenced (Ginns et al., 1984; Horowitz et al., 1989; Sorge et al., 1985b). The length of the glucocerebrosidase cDNA is approximately 2kb. Several different mRNA species have been detected on Northern blots, which can be attributed to splice variants, alternate polyadenylation sites, or transcription of the pseudogene (Graves et al., 1988; Reiner et al., 1988).

To date, over 250 mutations have been reported in *GBA*. These include 203 missense mutations, 18 nonsense mutations, 36 small insertions or deletions that lead to either frameshifts or in-frame alterations, 14 splice junction mutations, and 13 complex alleles carrying two or more mutations in cis (Figure 2.2). Recombination events with a highly homologous pseudogene downstream of the *GBA* locus also have been identified, resulting from gene conversion, fusion, or duplication (Hruskaet al., 2008).

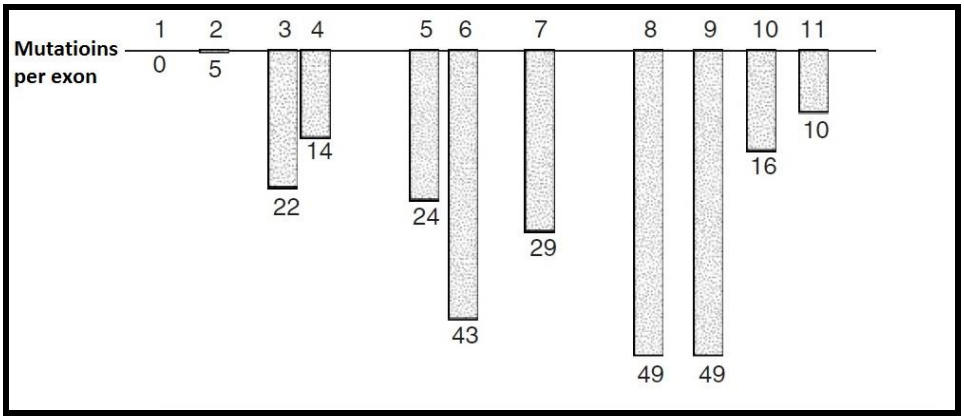

Figure 2.2: *GBA* structure and mutation distribution. Number of reported

substitution, deletion, insertion, and splice-sitemutations per exon (Hruska et al., 2008).

The first two *GBA* mutations were described in the late 1980s (Tsuji et al., 1987, 1988). These two alleles, L444P (c.1448T>C) and N370S (c.1226A>G), are still the prevalent mutant alleles encountered in most populations. Initially, investigators screened patient cohorts for the presence or absence of these two single nucleotide changes using Southern blotting, and then PCR. Subsequently, a wide range of mutations, including the missense, nonsense, frameshift, and splice-site mutations, as well as the insertions, deletions, and recombinant alleles. The identification of mutant *GBA* alleles can be problematic, as primers must be designed to discriminate between the functional gene and the pseudogene. Initially, laboratories used different PCR-based screening techniques to identify a limited panel of two to seven known mutations. Among type 1 patients of Ashkenazi Jewish ancestry; this was a fairly efficient approach, detecting approximately 90% of mutant alleles with a screen for five to six mutations (Beutler et al., 1993b; Grabowski, 1997; Koprivica et al., 2000). In non-Jewish populations, however, this screening strategy did not identify a significant portion of mutations, especially in patients with neuronopathic GD. The wider incorporation of automated sequencing to genotype Gaucher patients has lead to the identification of many new *GBA* mutations.

The frequency of specific mutant allele varies in different populations. In Ashkenazi Jewish ancestry, four mutations, N370S (c.1226 A>G), L444P (c.1448 T>C), 84insG (c.84dupG) and IVS2+1 G>A (c.115 + 1 G>A), account for nearly 90% of the disease alleles. Whereas in non-Jewish patients, these mutations account for about 50–60%, and there is a broad spectrum of other mutations in different ethnic groups (Grabowski, 1997; Alfonso et al., 2007). In Indian population, L444P(c.1448 T>C)seems to bethe most common mutation in GD patients (Ankleshwaria et al., 2014). Though larger study data will be required to understand the molecular pathology of GD.

2.5.1 Recombinant and Complex Alleles

A prevalent class of mutant alleles encountered in patients with GD is due to recombination events (Eyal et al., 1990; Hong et al., 1990; Latham et al., 1990, 1991; Tayebi et al., 2003; Zimran et al., 1990b). Such alleles have been designated as

‘‘complex’’ alleles (Latham et al., 1990), ‘‘pseudopattern’’ or ‘‘psi’’ (Hong et al., 1990), ‘‘rec’’’ for ‘‘recombinant’’ (Eyal et al., 1990), ‘‘fusion’’ alleles (Zimran et al., 1990b), and ‘‘chimeric’’ alleles (Sarria et al., 1999). Recombination within the glucocerebrosidase locus appears to be enhanced by the high degree of sequence identity and the close physical proximity of the *GBA* pseudogene. While one specific recombinant allele, traditionally referred to as RecNciI (Latham et al., 1990], is often screened for by detection of the pseudogene-derived alterations L444P (c.1448T>C) and A456P (c.1483G>C), this approach does not accurately describe the many different possible recombinant alleles that include these point mutations.

The most prevalent complex alleles are Rec*Nci*I, including amino acid changes L444P (c.1448 T>C)and A456P (1483G>C), and the silent nucleotide change at codon 460 V460V(497G>C), and RecTL, with the same changes as Rec*Nci*I plus mutation D409H (1342G>C) (Cormandet al., 1998).

The mutation profile and mutation frequencies differ in Jewish, non-Jewish (Caucasian) and Japanese populations. In the Ashkenazic-Jewish population, the N370S (c.1226A>G) mutation accounts for approximately 72% of mutant alleles. The 84insG mutation is the second most common mutation among Ashkenazi Jewish patients, accounting for 11% of mutant alleles. Mutation L444P (c.1448T>C), IVS2 (+1) G>A(c.115 + 1 G>A), recTL, and recNciI represent another 6% of alleles. Only 11% of mutant alleles are occupied by rare or unknown mutations (Horowitz and Zimran, 1994; Grabowski, 1997). No non- Jewish patients with mutation 84insG have been reported (Beutleret al.,1991).

In non-Jewish (Caucasian) populations, common mutations (N370S (c.1226A>G), L444P(c.1448T>C), recNciI, recTL, D409H (1342G>C), and IVS2 (+1) A(c.115 + 1 G>A)) account for about 60–70% of mutant alleles, the rest are rare or private mutations. The L444P (c.1448 T>C) and N370S (c.1226A>G) are the most prevalent, occupying about 20% and 15% of mutant alleles, respectively in Caucasian (Grabowski, 1997). In Japanese population, L444P(c.1448 T>C) is the most prevalent mutation, while no alleles carrying N370S have been found (Ida, 1995 and 1997). Very few reports are available about the mutation analysis in Gaucher patients from Slavic populations. In the Polish Gaucher population there is a high incidence of type 3 patients with severe visceral involvement, who are homozygous for the L444P

mutation (Tylki-Szymanska et al., 1996).

The prevalence of different mutations has been extensively studied and some useful genotype – phenotype correlations have been inferred in type-I and type-3 GD patients(Sidranskyet al., 1994; Koprivikaet al., 2000). Although the predictive value of these correlations is generally limited, their study in different ethnic groups had a significant impact on genetic counseling and on the better understanding of the molecular aspects of GD (Druganet al., 2002).

A few genotype-phenotype correlations have been established. The p. N409S (N370S, traditional nomenclature) allele has been associated with absence of neurological involvement. To date, it has always been present either in homozygous or heterozygous state in patients with type 1 disease where homozygotes exhibit a less severe phenotype (Sibille, 1993; Diazet al., 2000). A high correlation has been found between L444P (c.1448T>C) homozygosity and Gaucher type 3 in Swedish (Norrbottnian) patients (Dahlet al., 1990 and Sidransky, 1992), associating this allele to the neuronopathic form of GD. Similar phenotypes have been described in Japanese patients homozygous for the aforementioned allele (Idaet al., 1999).

The known β-glucocerebrosidase gene mutations have been divided into three groups according to their phenotypical effect (null, severe and mild mutations) (Beutler and Gelbart, 1998 and 1997). Patients carrying at least one mild mutation, most frequently N370S (c.1226A>G), have non-neuronopathic disease (type 1), while patients carrying two severe mutations or a severe and a null mutation usually develop neurological symptoms (types 2 and 3). Fetuses with two null alleles are non- viable. Although the patients sharing the same genotype tend to have similar phenotypes, there is a considerable variability that weakens the predictive value of the genotype and is well documented by the wide phenotypic range (mild to severe) observed in patient's homoallelic for the N370S (c.1226A>G) mutation (Hodanova, 1999).