6.1 CONCLUSIONS

- The above outlined enzymatic and molecular methods are relatively simple to diagnose TSD patients with 98% sensitivity and 100% specificity. The clinical profile identified in present study suggest that presence of cherry red spots in macula, abnormal muscle tones, seizures, hyperacusis, with neuroregression should be considered for assessment of TSD.
- Mutations c.1385A>T (p.E462V), c.964G>T (p.D322Y), and c.1278insTATC (p.Y427IfsX5) are possibly the commonest ones (~41%) detected for TSD in the Indian population (*refer chapter 4; figure 4.8*) and can be used as a part of common mutation screening program. Except for this mutation, the rest of the mutations were non-recurrent. The mutations responsible for TSD in Indian patients are mostly novel with nearly 62% mutations clustering in between exons 5 to 12. In addition, patients originating from Gujarat could be screened for the founder mutation c.1385A>T (p.E462V). Developments of ARM PCR and RFLP-PCR for two common mutations prevalent in Indian population are more cost effective and reliable for the mass screening of common mutations in the populations.
- All of our cases represented the infantile form of TSD. We did not have any cases with Juvenile and adult form. Two of our cases have shown deficiency in GM2 activator protein which is very rare and only 9 cases so far reported in the world literature. It can be concluded from this case that children with global developmental delay, hyperacusis, presence of cherry red spot in macula and unmyelinated white matter with normal Hexosaminidase (Hex-A and Total-Hex) study should be further investigated for GM2 activator protein deficiency.
- This study also demonstrates that for storage disorders like Tay-Sachs enzyme analysis from CT and/or AF provide highly reliable information for prenatal study and can be used as with high sensitivity and specificity for PD of the disease where index case has confirmed diagnosis proven by enzyme activity or by molecular analysis. Moreover mutation identification helps in early and accurate diagnosis in families at risk.

6.2 SUMMARY AND FUTURE PROSPECTS

- Overall, 79 children have been and identified as GM2 gangliosidosis using biochemical and molecular method that includes 48 with Tay-Sachs including 2 with GM2 activator protein deficiency and 31 with Sandhoff disease. Furthermore 8 carrier couples have been also identified in present study demonstrating the usefulness of enzymes study in carrier detection at least for TSD.
- Overall, we have enrolled 56 cases (48 affected individual and 8 carrier couples) for molecular analysis. Furthermore, 8 cases of prenatal diagnosis were also enrolled for molecular analysis.
- Present study identified 36 mutations in 53 cases (34 mutations in *HEXA* gene and 2 mutations in *GM2A* gene), 23 (63.8%) of which were novel and 13 (36.1%) known, that include 18 (50%) missense, 6 (16.6%) nonsense, 7 (19.4%) splice site, 3 (8.3%) deletion and 2 (5.5%) insertion. In 3 cases mutation was not identified.
- The *HEXA* gene mutations responsible for TSD in Indian population are unique. The most common mutation detected in 21/51 individuals [~41%] were c.1385A>T (p.E462V) in 11, c.1278insTATC (p.Y427Ifs5) in 6 and c.964G>T (p.D322Y) in 4 patients. This be used for targeted mutation analysis in the affected individuals and mass screening at large. Rests of the mutations were indentified different exons as mentioned earlier.
- For the first time, the mutation c.1385A>T (p.E462V) mutation was observed in eleven unrelated patients with one consanguineous and remaining non-consanguineous parentage of the same ethnic group form Gujarat and c.964G>T (p.D322Y) was observed in all four Muslim patients.
- In *HEXA* gene ~ 62% (21/34) mutations that have been identified in exon 5 to exon 12 and that can be used as a hot spot region for sequencing in absence of common mutations (*refer figure 5.6*).
- Nearly 80% of variants occurred in C-terminal domain (Domain-II) comprising a core TIM barrel fold ($(\beta,\alpha)_8$-barrel) that cover active pocket while remaining 20% of variants

were arise in N-terminal domain (Domain-I) of *HEXA* gene. We have also observed that nearly 37% (19/51) of cases had shown variants at αArg178, αAsp322, and αGlu462 which are functionally the active residues of *HEXA* gene. Furthermore, seven of the 29 point mutations, or ~25%, occur at CpG dinucleotides which are generally known to be mutational "hotspots".

- Identification of novel mutational spectrum in Indian TSD patients which will be helpful in offering precise genetic counseling and prenatal diagnosis. Additionally, identification of these novel mutations provides further insight to the molecular understanding of the *HEXA* gene in Indian subjects.
- Prenatal diagnosis and genetics counseling were provided in 8 pregnancy and we have found 2 pregnancies affected with TSD, 1 carrier and 5 normal pregnancy.
- We have also demonstrated that all *HEXA* mutation for Indian patients is associated with severe phenotypes and pseudo-deficient or adult onset alleles are highly unlikely in TSD patients from India.
- This database can be used as a tool for carrier screening of larger Indian population and this information can promote development of the chips and therapeutic model development.