Conclusions

The present study was aimed to resolve the clinical picture of pediatric patients with suspected mitochondrial encephalomyopathies at biochemical and molecular level and to study the consequences of biochemical defect upon cell function in terms of accumulation of reactive oxygen species and apoptosis.

Mitochondrial encephalomyopathies are relatively common neurometabolic disorders in Indian populations. The male-female ratio was 3 to 7. Infantile onset was frequent, and the course in these cases was often severe. Complex I deficiency was the most common biochemical defect. The complex I deficiency was severe enough to cause a declined ATP synthesis and loss of mitochondrial membrane potential. In addition, combined complex I plus III, and complex IV deficiencies were also evident (21.4% cases). Mitochondrial genome sequencing allowed us to detect both novel and already known variants. Because of the unique features of mitochondrial genetics and clinical heterogeneity of mitochondrial disorders, it is not easy to establish a pathogenic role to a mtDNA variant. The two novel non-synonymous variants T11916A and A9194G were fulfilling most of the pathogenic criteria and hence could be cause of disease in these families. Variant T11916A possibly affected complex I assembly and may result in partially assembled complex. The other known mutation A4136G is known to associate with LHON. In this study, we identified this variant in a family with clinical signs differing considerably from the classical symptoms of LHON. These results suggest that the phenotype caused by this mutation to be more variable and not specific to LHON.

Complex I deficiency was related to mtDNA mutations in 42% (6/14) patients, out of which tRNA mutations were present in 28% cases. We were able to identify a tRNA mutation G5783A previously found in a single family with encephalopathy and cardiomyopathy. We identified this variant in 13% of patients with variable clinical symptoms, suggesting that this variant to be considered a confirmed mutation and at genetic level can be a cause of many
childhood encephalomyopathies. Our results are correlating with earlier studies, that tRNA mutations can be the commonest cause of majority of early childhood encephalomyopathies. Hence, tRNA mutations can be used for molecular diagnosis in child encephalomyopathies. The remaining 58% cases could have mutations in nuclear genes encoding OXPHOS proteins or assembly factors.

A part from tRNA mutations, the contribution of sequence variants elsewhere in mtDNA (e.g. T3866C, T3394C, G4812C, and C4640A) cannot be underestimated. Patients harboring these variants did show a complex I deficiency, we believe that the enzyme deficiency is not due to these mtDNA variants but may be due to mutations in nuclear genes encoding complex I subunits or assembly factors. However, the interaction of nuclear background or environmental factors with mtDNA variants and thereby helping in the expression of disease cannot be ignored. Although to some extent mutation T3866C alters the hydropathy of ND1 subunit, it can be hypothesized that it may lead to altered stability of complex I when associated with some specific haplogroup background.

Our findings and other recent studies emphasize a more frequent contribution of mtDNA mutations to the aetiology of pediatric OXPHOS deficiencies. Screening for the mtDNA mutations such as A3243G, A4136G, G5783A, T10158C, T10191C, T11916A, C11777A, G13513A, A13514G, T14487C and T14709C should be carried out when assessing the underlying genetic defect in children with mitochondrial encephalomyopathies.

Defective complex I caused increased ROS and lower ATP levels in patient cells, the further consequences of which resulted in a change of status of antioxidant enzymes. There is an increase in majority of the antioxidant enzymes at transcription or translational level, suggesting compensatory mechanisms to overcome the consequences of oxidative damage. But the activities of major cytosolic and peroxisomal antioxidant enzymes such as catalase, GPx and GST are impaired, being ROS sensitive. The activity of SOD probably MnSOD remained to be higher in patient derived OXPHOS deficient cell lines, most expressed and active in cells having severe complex
I deficiency compared to those having a partial defect. These results suggest that MnSOD seems to be the first line of defense against mitochondrial oxidative stress and a relatively stable enzyme compared to other antioxidant enzymes.

A defective antioxidant system and lower ATP pool caused typical apoptosis and not necrosis. The patient cells undergo apoptosis either by energy deficit or by oxidative stress. The observations from our findings could also have profound implications for therapy of mitochondrial diseases by using anti-apoptotic drugs, in addition to most current therapies, which are focused on the use of antioxidant vitamins and bioenergetic-supporting drugs. But more in vivo studies are required to support these results.