HIGH RESOLUTION NMR BASED ANALYSIS OF SERUM LIPIDS IN DUCHENNE MUSCULAR DYSTROPHY PATIENTS AND ITS POSSIBLE DIAGNOSTIC SIGNIFICANCE

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Introduction: Duchenne Muscular Dystrophy (DMD), the most rapidly progressive and fatal form of dystrophy, is also the most frequent variety, having an occurrence of 1 in 3500 live male births [1]. Most children with DMD have proximal muscle weakness by the age of 5 years and inability to walk by 8-12 years. Measurement of serum CPK is a simple and inexpensive diagnostic test for muscle diseases EMG (Electromyography) is important for establishment of the myopathic nature of dystrophy and for exclusion of neurogenic causes of weakness, including peripheral nerve disorders. Genetic studies can be performed for the establishment of diagnosis in most of the cases. In the present study, we estimate the triglycerides (TG), phospholipids (PL), free cholesterol (CHOL) and cholesterol ester (CHOLest) in serum of 31 genetically proven and 10 cases of negative gene deletion test of DMD patients vs. normal individuals by ^{1}H NMR spectroscopy.

Material & Methods: A total of blood samples from forty-one patients and twenty two controls were collected from out patient clinics and inpatients wards of the Neurology Department of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. All the samples from each patient were collected directly in sterilized tubes.EDTAblood- blood was used for the gene mutation study. Serum was used for CPK analysis & NMR based lipid study.Quantitation of lipid components were carried out by taking the integrals of -CH_2- signals of glycerol moiety at 4.2 ppm, -CH_2- signals of glycerol moiety at 3.8 ppm, C19 methyl signal at 1.02 ppm and at 1.01 ppm relative to the TSP signal at 0.0 ppm, for the triglycerides, phospholipids, cholesterol ester & free cholesterol, respectively.NMR experiments were performed on a Bruker Avance 400 MHz spectrometer (Bruker Biospin, Zurich, Switzerland) at 25°C temperature.

Results: Clinical symptoms & signs were common in all these patients with positive Gower's & valley signs (Fig-1.) [1, 2].CPK was 8-15 times higher and EMG was myopatheic in all the cases**.** Gene deletion study was positive in thirty-one cases (Fig-2) but it was negative in ten cases due to point mutation. ^{1}H NMR based quantitative analysis of the lipid constituents- triglycerides, phospholipids, free cholesterol, cholesterol ester and total cholesterol showed significant difference (Fig-3) and the ratios of CHOL/CHOLest was also significant ($p < 0.001$) from the normal vs. disease serum. Lipid profile of serum of thirty-one cases with positive gene deletion & ten with negative showed the similar NMR based lipid profile. Among all these variables, phospholipids were most consistently increased in DMD patients compared to controls with discriminatory index of 87.5 % and come close to 92 % with the ratio of PL/CHOL.

Fig-1. Arrow showed the clinical sign (valley sign) in DMD.

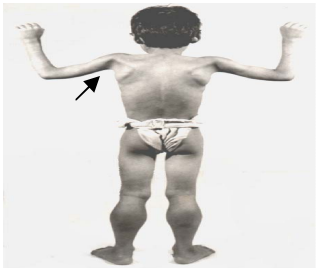


Fig-2. Electrophoretic pattern showed the gene deletion.

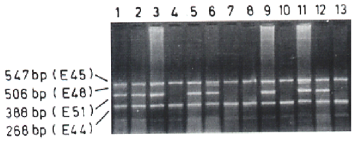
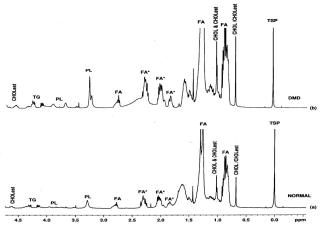


Fig-3. ^{1}H NMR spectra of the lipid extract of the serum.



Discussion: Importance of lipid metabolism in muscular dystrophy was already reported in prehistoric Indian medical treatise "Charak Samhita" [3]. Elevated quantities of all the lipids constituents are occurring due to altered metabolism of phospholipids, triglycerides and cholesterol [4]. Phospholipids are required for the synthesis of cell membrane whereas cholesterol is required for cell proliferation because there is a continuous degeneration and re-generation of the muscle cell persists, which is responsible for altered synthesis of the lipid components. Increase turnover of the phospholipids also increases the synthesis rate of triglycerides. Phospholipids levels with phospholipids to cholesterol ratios were the most consistent findings; these observations may form the basis of our future studies to discriminate DMD from other neuromuscular disorders

References:

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