*International Journal of Innovative Research and Review ISSN: 2347 – 4424 (Online)*
*An Online International Journal Available at http://www.cibtech.org/jirr.htm*
*2015 Vol. 3 (3) July-September, pp.15-21/Wankar et al.*
***Research Article***

# SUBCLINICAL MITOCHONDRIAL DYSFUNCTION IN STROKES OF UNDETERMINED ETIOLOGY: A PILOT STUDY

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## ABSTRACT
Possibility of a cryptogenic stroke always surfaces when one encounters an ischemic cerebral event with well controlled risk factors. Melas is well documented in paediatric age group and young adults. It was suggested that mitochondrial dysfunction (mtdx) may present oligosymptomatically with strokes alone. Forearm aerobic exercise test (faet) and magnetic resonance spectroscopy (mrs) are tools for screening for mtdx. The aim of the study was to assess the presence of mtdx in patients with strokes of undetermined etiology. It was a pilot study including nine patients with stroke of undetermined etiology. Patients were screened for mtdx using faet and mrs. Faet was performed as per previously described protocol in 11 healthy volunteers. Mrs using a point resolved spectroscopy (press) sequence with protocol as described by Jose *et al.*, (2008) was done for all cases, earliest after 10 days of ictus. Mrs was performed in 15 healthy volunteers the prevalence of mtdx in studied population was analyzed by percentage calculation. Significance of difference of means for po2 and lactate was identified between 0, third and tenth minute after exercise protocol by using student t-test. Out of nine cases included in the study three were found to have impaired oxygen utilization on faet. These patients were relatively younger with mean age of 35.66 years. The impaired oxygen extraction in these patients was statistically significant on faet but not lactate. Lactate levels were noted at the tenth minute after exercise remained high. None of the patients or controles had a lactate peak on mrs. It may be concluded from the study that mitochondrial dysfunction may oligosymptomatically present with stroke and all patients with cs should be worked up for mtdx using faet. Mrs may not a sensitive tool for screening these patients for mtdx.

***Keywords:*** *Cryptogenic Stroke, Forearm Aerobic Exercise Test, MR Spectroscopy, Mitochondrial Dysfunction*

## INTRODUCTION
Stroke being the second commonest cause of death and fourth leading cause of disability worldwide is a global health problem which is potentially preventable (Strong, 2007).
Effectiveness of interventions for secondary stroke prevention depends on identification of underlying process. But, despite extensive investigations, in 30-40% of the cases the etiology remains undermined (Finsterer, 2010). This number is even more in patients those under 50 years of age. Current guidelines do not approach specifically the strategies for secondary prevention of these cryptogenic strokes (Putaala, 2014).
Mitochondrial dysfunction (mtdx) is known to present with stroke as a part of a syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes (melas) in paediatric population and young adults (Lorenzo, 2009). It is known that mtdx other then melas can present with sroke at any age (Walker, 2014; Conforto, 2007; Martínez-fernández, 2001; Mjjamaa, 1997). Still in older adult population mtdx manifesting as solely stroke is rarely considered.
Forearm exercise test, aerobic (faet) and ishchemic (fiet) are used to screen for mtdx. Skeletal muscles are the highest consumer of oxygen in the body, furthermore, mitochondrial oxidative phosphorylation is found to increase up to 100 fold from rest to exercise in healthy muscles (Jensen, 2002). Faet, with sensivity comparable to muscle biopsy, evaluates the oxygen extraction capability of exercising muscles. Mtdx results in paradoxical increase of venous oxygen pressure (vpo) in venous blood drawn from exerciseing arm (Taivassalo, 2002; Jensen, 2002).

Robust evidence is available in literature to suggest persistence of lactate in brain in patients with mtdx (Jose, 2008; Cross, 1993; Linn, 2003). We report our experience of nine patients with cs who were screened with faet and mrs for subclinical mtdx in them.

## MATERIALS AND METHODS

Patients of ischemic strokes with normal transthorasic two dimentional echocardiography, cardiac rhythm, renal parametres, hemogram, homocystine levels and normal cerebral angiography on mri presenting to the department of neurology of this institute, between august 2014- February 2015 were included in study. Patients with mild hypertention, well controlled dibetes mallietus and mild dyslipidemia were also included for evaluation. Those smoking more than ten cigarettes were excluded. After obtaining an informed consent nine (five males, four females) patients and eleven healthy volunteers (nine males and two females) were tested with faet as per the previously described protocol (Meulemans, 2007).

### *Faet Protocol*

Maximal voluntary contraction (mvc) was determined using a hand dynamometer thirty minutes before taking up the exercise protocol. After thirty minutes of rest patients performed submaximal (40% of mvc) exercise of forearm with one second contraction and one second relaxation for three minutes. Samples were collected in a heparinized syringe from an intravenous catheter placed in the median cubital vein before the initiation of aerobic exercise, at three minutes of exercise and at ten minutes of the termination of exercise. Labeled samples were transported within twenty minutes to the biochemistry lab in a ice pack and were analyzed for vpo2, lactate and $so_2$ (roche diagnostics usa cobas b 221 blood gas auto analyzer).

### *Mr Spectroscopy*

All the patients were subjected to mr spectroscopy after tenth day of stroke. Fifteen healthy subjects (four male and eleven female) between ages 19 to 68 years, also underwent mrs.

Mr spectroscopy was done using a point resolved spectroscopy (press) sequence using 1.5 tesla mri system (seimens magnetom symphony). Single voxels were applied on the lesion and frontal horns with te 30ms and tr- 1500ms. Csi (chemical shift imaging) through bilateral basal ganglia and body of the lateral ventricles at te-135ms and tr 1500ms.

### *Statistics*

The prevalence of mtdx in study population was analyzed by percentage calculation. For all controles the means and standard deviation (sd) were assessed for $po_2$, lactate and their differences during the test. The upper and lower limits were calculated using 95% confidence intervals (2sd), on either side of the mean. Significance of difference of means for $po_2$ and lactate identified between zeros, third and tenth minute after exercise protocol by using student t-test. The study protocol was approved by institutional ethics committee.

## RESULTS AND DISCUSSION

Out of nine cases included in the study three were found to have a paradoxical rise in venous partial pressure of oxygen after three minutes of exercise in faet implying impaired oxygen utilization by tissues. These cases were classified as smt. Strokes with a normal oxygen utilization as described by Taivassalo *et al.*, (2002) were classified as sm0. The mean age of eleven controles for forearm exercise test was 35.72 years. The mean age of 15 controles for mrs 39 years.

### *Demographic Data of Smt Group*

**Table 1: Shows the demographic data of the SMt group showing a relatively younger group**

| | Age/ Gender | Diabetes | Hypertension | Drugs |
|---|---|---|---|---|
| Case 1 | 28/F | No | No | No |
| Case 2 | 34/M | No | No | No |
| Case 3 | 45/M | No | No | No |
| Mean | 35.67 | | | |
| Std Dev | 8.621678104 | | | |
| p Value | 0.0674 | | | |

***Demographic Data of Sm0 Group***

**Table 2: Demographic data of SM0 group shows patients in SM0 group showing a higher mean ages as compared to the patients with MtDx**

| | Age/ Gender | Diabetes | Hypertension | Drugs |
|---|---|---|---|---|
| Case 1 | 50/F | No | No | No |
| Case 2 | 70/M | No | No | No |
| Case 3 | 70/F | No | No | No |
| Case 4 | 78/M | Yes | Yes | Ramipril 5mg OD<br>Glimepride 2mg OD |
| Case 5 | 39/M | No | No | No |
| Case 6 | 42/F | No | No | No |
| Mean | 58.166 | | | |
| Std Dev | 16.546 | | | |

***Changes in Partial Pressure of Oxygen in the Three Groups***

**Table 3: Shows the mean changes in partial pressure of oxygen and changes in lactate levels at the beginning of FAET, at 3 minutes and at 10 minutes of completion of exercise in the 3 groups. The p value is statistically significant for means of differences of partial pressure of oxygen between start and 3rd and 3rd and 10th minute in SMt group and controles. However in the same time the differences between the means of lactate between the groups was not statistically significant**

| Parameter | | N | Mean | SD | P |
|---|---|---|---|---|---|
| vPO0- vPO3 | Controles | 11 | 9.08182 | 4.21469 | |
| | SMt0 | 6 | 7.366 | 2.71563 | 0.3854 |
| | SMt | 3 | -7 | 5.31131 | <0.0001 |
| vPO10- vPO3 | Controles | 11 | 12.73818 | 3.51232 | |
| | SM0 | 6 | 7.616 | 3.20338 | 0.0098 |
| | SMt | 3 | -6.1 | 1.4 | <0.0001 |
| vLac3- vLac0 | Controles | 11 | 1.89091 | 1.21913 | |
| | SM0 | 6 | 1.3833 | 1.33778 | 0.4397 |
| | SMt | 3 | 1.8333 | 1.51767 | 0.9458 |
| vLac3- vLac10 | Controles | 11 | 1.60909 | 1.18893 | |
| | SM0 | 6 | 1.233 | 0.99067 | 0.5211 |
| | SMt | 3 | 0.66 | 1.10604 | 0.2388 |

*vPO0- Venous partial pressure of oxygen before starting exercise protocol*
*vPO3- Venous partial pressure of oxygen after 3 minutes of exercise*
*vPO10-Venous partial pressure of oxygen after 10 minutes of stopping the exercise*
*vLac0- Venous lactate before starting exercise protocol*
*vLac3- Venous lactate after 3 minutes of exercise*
*vLac10- Venous lactate after 10 minutes of stopping the exercise*

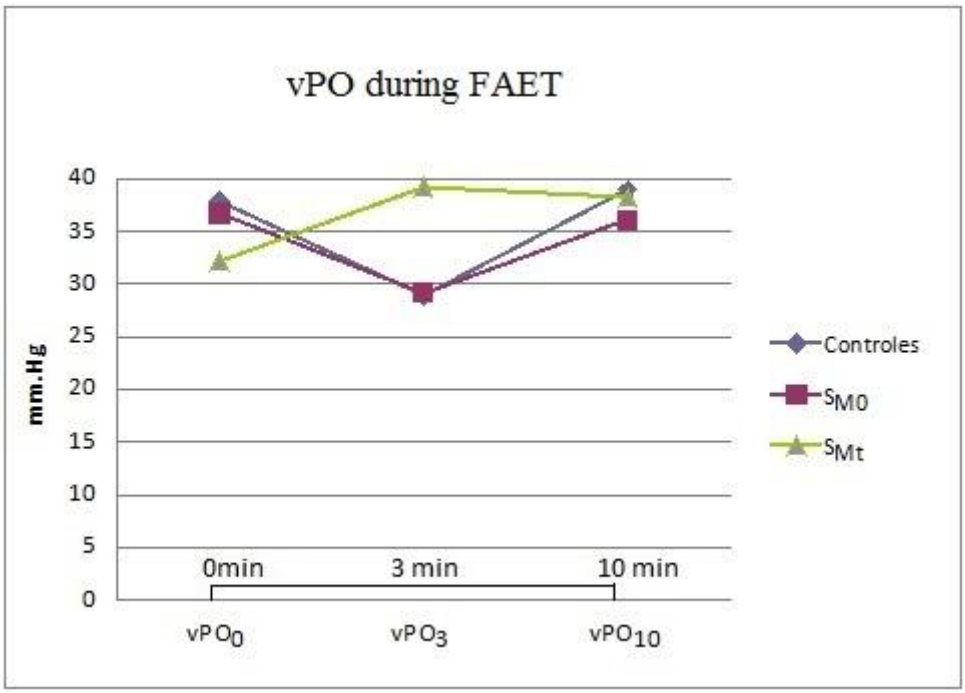


**Figure 1: Mean partial pressures of oxygen between groups**

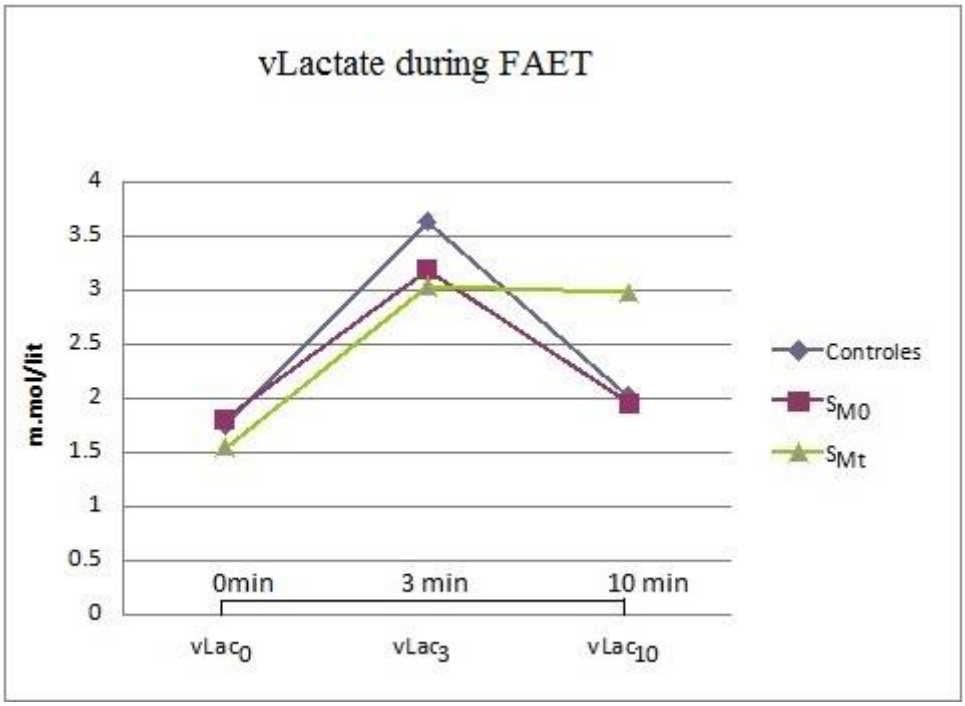


**Figure 2: Mean lactate levels between groups**

In general, symptoms in mitochondrial disorders are related to involvement of tissues with high oxidative metabolism. Stroke-like episodes are considered to be caused by energy failure due to mitochondrial dysfunction in neural tissue (Goto, 1992) or by mitochondrial angiopathy caused by mitochondrial dysfunction in small cerebral blood vessels (Ohama, 1987). Stroke may be the expression of either an oligosymptomatic type of a well-known mitochondrial disease or the initial expression of another disease,

so far unknown. The study was undertaken with an aim to assess the presence of mitochondrial dysfunction among patients with strokes of undetermined etiology.

In present study the patients with strokes of undetermined origin were selected and worked up for mtdx. Our study shows a 33% prevalance of mitochondrial dysfunction in this group which is comparable to previous study by Martinez-lopez *et al.,* (2001). The former authors concluded a prevalance of 33% of the patients to have biochemical evidance of a mitochondrial disease. Martinez *et al.,* (2001) screened the patients with fiet and established mitochondrial cytopathy on histopathological analysis of muscle biopsy and molecular analysis of mitochondrial dna. In our study no muscle biopsies or genetic testings were not done. Jensen *et al.,* (2002) have found results of faet comparable to that of muscle biopsy. Hence non invasive faet and mrs were more the selected tools for mtdx in this study. We also observed the patients who were screened for mtdx were younger. Thus a possibility of a mitochondrial dysfunction should be raised among the patients of cs especially who are young.

***Mr Spectroscopy***

Lin *et al.,* (2003) observed a good correlation between high lactate levels detected by mrs and other markers of mitochondrial disease. In mrs, the lactate doublet peak is located at a chemical shift of 1.33 ppm, and needs to be clearly visible above the noise background for its detection. Cross *et al.,* (1993) and Kingslay *et al.,* (2006) have showed a sensitivity of lactate peaks in mitochondrial disease to be 18–27%. Absence of a lactate peak similarly does not rule-out mitochondrial disease, as the specific tissues involved in mitochondrial disease vary, as does the location of brain involvement. Mrs specificity for mitochondrial disease is further limited by the possibility of other conditions giving rise to lactate peaks like pml, lymphoma, hypoxia, wilsons disease etc (Kingsley, 2006). This low sensitivity and specificity of mr spectroscopy specially after the acute stage may explain the absence of lactate peak in any of the patients in smt group. However, in the scenario of clinical and biochemical suspicion for mitochondrial disease, identification of a lactate peak would have added weight to the possibility of mitochondrial dysfunction.

***Forearm Exercise Test***

Faet is a sensitive tool to detect impaired muscle oxidative metabolism in patients with mitochondrial myopathies (Taivassalo, 2002; Jensen, 2002). In normal subjects, the level of oxygen in venous blood decreases during moderate exercise (Greenwood, 1965; Barcroft, 1963). This phenomenon may be explained by mitochondrial oxygen demand for electron transport chain and generation of atp from adp leading to oxygen extraction from blood, leading to a fall in partial pressure of oxygen after exertion.

Post ten minutes of completion rhythmic contractions, an overshoot of o2 on vbg is noted among our controles.

This overshoot may be attributed to the increased blood flow to the exercising muscle (Greenwood, 1965). As per ann Meulemans *et al.,* (2007) oxygen extraction is a key factor for screening for mtochondrial disorder. When the oxidative phosphorylation is blocked, the ability of muscle to increase the rate of extraction of oxygen from blood is impaired. As a result the venous partial pressure of oxygen and oxygen saturation remain high during exercise because the higher supply of oxygen to the tissues which remains unutilized. Thus, by measuring blood gases before, during and after the aerobic exercise, the mitochondrial function can be evaluated.

Jensen *et al.,* (2002) showed the test to have a sensitivity of 92% and comparable to that of muscle biopsy for mitochondrial dysfunction. However, it cannot discriminate between patients with a mitochondrial defect or another muscle disease (Taivassalo, 2002).

In our study among the controls, mean baseline partial pressure of oxygen (vpo) was 37.97± 7.61 mm.hg which fell to 28.89 ±6.10 mm. Hg at three minutes of aerobic exercise as compared to previous study by Taivassalo *et al.,* (2002) who showed vpo to fall from 27.2± 4.2mmhg to 24.2±2.7mmhg at third minute of aerobic exercise among healthy controles. In our patients in smt group vpo increased from 32.2±4.95 mm.hg to 39.2±9.87mm.hg which is similar to previous observations Taivassalo *et al.,* (2002) who demonstrated an increase in vpo from 27.2±4.0mmhg to 38.2± 13.3mmhg among patients with mitochondrial myopathy. The minor difference in mean vpo levels may be explained by the racial

differences. However the trends of vpo in controles as well as patients with mtdx are similar in both studies.

In present study the changes in lactate levels did not show any sensitivity for mtdx similar to the observations in previous studies (Martínez, 2001; Meulemans, 2007). Lactate was persistently elevated among the patients with mtdx. This observation may be explained by the basic metabolism of lactate in mitochondria rich tissues like skeletal muscles. Lactate in the skelatal muscles is metabolized by the oxphos shuttle in the two $co_2$ and water (Phybers, 2006). Jacobs *et al.*, (2002) have proposed the utilization of lactate as a substrate for respiration by the skeletal muscle mitochondria. A persistently elevated lactate after rest indicates a decreased mitochondrial lactate metabolism and may be used as a potential surrogate marker of mtdx.

The observations in present study suggest that stroke may be the initial or only manifestation of a mitochondrial dysfunction.

This may be useful for work up of strokes of undetermined etiology. However from present study mr spectroscopy did not show any abnormality in patients of mtdx in our study. Therefore it seems that mrs may not be a sensitive tool to screening for mitochondrial disorders where they oligosymptomatically present with stroke.

Present study is also associated with certain limitations. The study is a pilot study of a larger ongoing study and recruited of small number of patients in each group. However, in view of observations from the present study demonstrates the presence of mtdx among patients of cs demonstrable with faet.

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