Muscle mitochondrial function and contemporary anti-retroviral therapy

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1. Clinical characteristics

<table>
<thead>
<tr>
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<th>Naive</th>
<th>Older NRTIs</th>
<th>Contemporary NRTIs</th>
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<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Age (y)</td>
<td>36.9 ± 10.6</td>
<td>57.7 ± 8.7</td>
<td>48.4 ± 13.3</td>
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<tr>
<td>Months since HIV diagnosis</td>
<td>74 ± 58</td>
<td>193 ± 60</td>
<td>100 ± 86</td>
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<td>Months on treatment</td>
<td>0</td>
<td>171 ± 42</td>
<td>34 ± 16</td>
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<td>CD4 lymphocyte count (cells/μL)</td>
<td>834.7 ± 437</td>
<td>513.2 ± 179</td>
<td>512 ± 200.7</td>
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<tr>
<td>NADH CD4 lymphocyte count (cells/μL)</td>
<td>414.6 ± 226.1</td>
<td>163.5 ± 132.2</td>
<td>249.6 ± 114.2</td>
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<tr>
<td>First load (copies/mL)</td>
<td>11533.1</td>
<td>&lt;40</td>
<td>&lt;40</td>
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2. Multiplex immunofluorescence for assessing mitochondrial defects

- Multiplex immunofluorescence assay developed in our lab enables the quantification of mitochondrial respiratory chain complexes I and IV along with a mitochondrial mass marker and cell marker.
- Complex I (CI) was detected by using an antibody for accessory protein NDUFB8.
- Complex IV (CIV) was detected using antibody for mitochondria-encoded protein MTOC1.
- Mitochondrial mass was quantified using VDAC1 antibody for outer mitochondrial membrane channel porin, and laminin was used to label myofibres boundaries.
- Muscle fibres were classified into categories based on Z scores of CI and CIV deficiency and severe deficiency (Z between -3SD and -6SD) and ‘normal’ (Z > 3SD).

Summary

- Patients exposed to older NRTIs have the highest levels of mitochondrial defects in skeletal muscle, despite no longer being treated with these medications.
- Surprisingly, patients exposed only to contemporary ART had intermediate levels of mitochondrial defects. Further work is needed to define the mechanisms behind this.
- Mitochondrial defects predominantly affected complex I, which could be of relevance for future novel therapeutic interventions.

3. CI and CIV deficiency in NRTI treated individuals

- CI deficiency and severe deficiency is significantly higher in both NRTI-treated groups compared to the NRTI naive group.
- No significant difference in CI deficiency and severe deficiency between NRTI treatment groups.
- Subjects exposed to older NRTIs had significantly higher CIV deficiency and severe deficiency than NRTI naive subjects, unlike subjects in the contemporary NRTI group.

4. Correlation between mitochondrial deficiency and clinical characteristics

- Correlation between severe CI/CIV deficiency and COX defect. This validates the reliability of the multiplex assay as COX/SDH IHC is an established and comprehensively validated tool for assessing mitochondrial deficiency.
- Association between severe CI deficiency and months on ART, but not severe CIV deficiency.
- Association between severe CI deficiency and age, but not severe CIV deficiency and age.
- No association between mitochondrial deficiency and current CD4 count, nadir CD4 count or months since diagnosis.

Background

- Anti-retroviral therapy (ART) eliminates viral replication and restores immune function BUT it may be associated with premature molecular ageing.
- In particular, older nucleoside reverse transcribe inhibitors (NRTIs) cause dysregulation of mitochondrial maintenance, by inhibiting mitochondrial polymerase-γ leading to the clonal expansion of pre-existing mitochondrial DNA (mtDNA) mutations.
- Mitochondrial defects contribute to premature ageing in ART-treated patients, increasing frailty and the susceptibility to acquiring age-associated comorbidities.

Methods

- Tibialis anterior biopsies were obtained, in which a range of molecular assessments were performed on 10µm transverse sections. These include:
  - COX/SDH immunohistochemistry (IHC).
  - Multiplex immunofluorescence for mitochondrial mass and respiratory chain complexes I and IV, with automated analysis.

References