Do people with HIV have decreased muscle regeneration?

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1. Aims

A. To better understand muscle pathology in older people living with HIV (PLWH).
B. To determine the extent of reduced physical function and HIV.

2. Introduction

Some **people living with HIV (PLWH) appear to be ageing quicker** than the general population, despite successful viral suppression through treatment of anti-retroviral therapy (ART). ART stops the virus replicating in the body and so suppresses and stops the HIV disease. This means that these **PLWH are more likely to develop age-related diseases such as cancer or diabetes and have a higher rate of mortality.** The exact causes for this are still unknown, but it is thought that mitochondrial abnormalities are involved.

Mitochondria are organelles in the cell responsible for a range of processes, but most importantly they are where energy (in the form of ATP) is produced. We know that mitochondria are damaged in some PLWH.

As skeletal muscle has high energy requirements and is very important to physical function, it is vital to try to better understand if and how mitochondrial abnormalities can affect muscle in older PLWH.

Therefore, we wanted to investigate muscle pathology in a cohort of older PLWH and age matched HIV-negative individuals. To do this we have performed haemotoxylin & eosin (H&E) histochemistry on muscle samples to quantify the proportion of muscle fibres that have regenerated and that are degenerating.

3. Methods

Patient cohort:

- 30 HIV+
- 15 HIV-
- 2 mitochondrial disease patients (POLG mutation)
- All patients were >50 years old and male.
- Of the HIV+ and HIV- subjects, 37 were recruited at Newcastle RVI and 8 were recruited at St. Marys in London.

Muscle Biopsy Technique:

Tibialis anterior (TA) needle biopsy taken from patient and immediately snap frozen in isopentane for preservation of muscle tissue.

10µm sections were then cut onto glass slides on a cryostat in our lab and immediately stored at -80˚c until ready to use.

H&E Staining:

- This is the best method to show degenerating and regenerating muscle fibres.
- This stain shows nuclei as blue and cytoplasm as red-pink [1].
- Any fibres with **central nuclei are regenerating.**
- **Degenerating fibres appear shrunk and are surrounded by fibrotic tissue and several nuclei [2].**

Imaging and Analysis:

1. Stained sections were imaged using the brightfield channel on a widefield microscope at 20x magnification.
2. The number of fibres in each section were counted.
3. The number of regenerated and degenerated fibres in each section were counted.

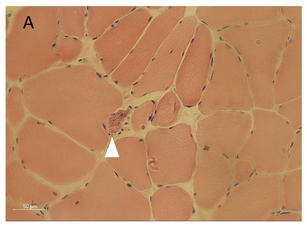

Figure 1 – Example images of (**A**) a degenerating fibre, and (**B**) a regenerating fibre

4. Results

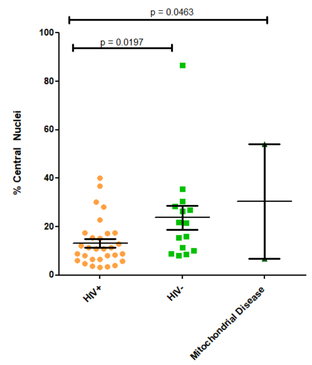

Figure 2 – Proportion of myofibres with centralised nuclei.

The HIV group had a significantly lower proportion of fibres with a centralised nuclei compared to the HIV- (p = 0.0197) and mitochondrial disease groups (p = 0.0463).

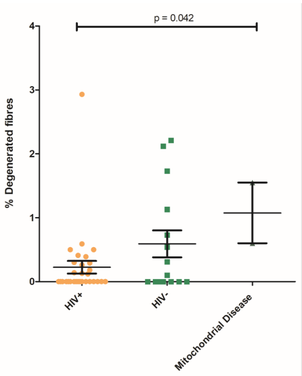

Figure 3 – Proportion degenerated fibres.

The HIV group had a significantly lower proportion degenerated fibres compared to the mitochondrial disease group (p = 0.042). There was no significant difference between the HIV+ and HIV- group.

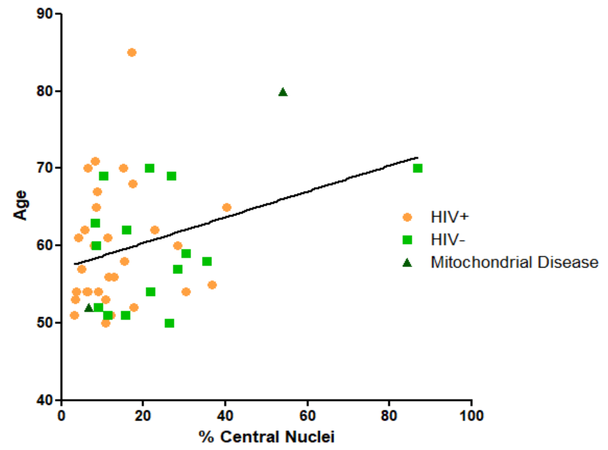

Figure 4 – Graph of the correlation between age and % regenerating fibres.

Next, we wanted to investigate whether the proportion of regenerated or degenerated fibres correlated with any factors associated with ageing and muscle function, such as age; grip strength (kg); % lean tissue and % fat tissue.

We found that of those factors, only **age was significantly associated to the % of regenerated fibres** (R = 0.099, p = 0.031).

5. Conclusions and future work

Interestingly, we have shown that **older PLWH have a significantly lower proportion of regenerating fibres** compared to age-matched HIV-negative individuals. This would indicate that these PLWH have a **lower capacity for muscle regeneration**, which may **contribute to accelerated ageing and reduce muscle function** in these individuals.

In the immediate future we will look to investigate more factors which are suspected to contribute to muscle pathogenesis, such as fibre type proportions, fibrotic tissue and the prevalence of satellite cells (muscle stem cells).

We aim to investigate whether any of these factors are significantly different in PLWH compared to age-matched HIV- individuals, and then evaluate whether mitochondrial defects are contributing to muscle pathology.

6. References

1. Ruegg M, Meinen S. Histopathology in Hematoxylin & Eosin stained muscle sections [Internet]. 2019 [cited 8 October 2019]. Available from: https://treat-nmd.org/wp-content/uploads/2016/08/cmd-MDC1A_M.1.2.004-68.pdf
2. Wang C, Yue F, Kuang S. Muscle Histology Characterization Using H&E Staining and Muscle Fiber Type Classification Using Immunofluorescence Staining [Internet]. 2019 [cited 8 October 2019]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5526362/