Measurement of salivary testosterone in adolescents and young men with Duchenne muscular dystrophy

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Technical advance

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Abstract

Background

Many young adults with Duchenne Muscular Dystrophy (DMD) receive long-term glucocorticoids (GC). GC can cause hypogonadotrophic hypogonadism and adolescents may therefore be candidates for pubertal induction. It is unclear whether men with DMD on or off GC have age-appropriate endogenous testosterone production.

Methods

We undertook a quality improvement project to assess the feasibility of measuring salivary testosterone (SalT) levels in men with DMD at home. A Sal-T sampling kit was sent by post to all patients with DMD, aged 17 and older, registered at the John Walton Muscular Centre in Newcastle (n=75). Submitted Sal-T samples were collected and submitted for analysis.

Results

28 out of 75 patients returned samples (age range: 17-34 years). 6/28 samples were unsuitable for analysis. Overall Sal-T levels (n=22) were significantly lower than in the healthy population (178 ±107 v 287 ±109 pmol/l, p=0.0001). Sal-T was lower in those on GC compared to those off GC (144± 81 versus 218 ±125 pmol/l, p=0.05). 3 patients were unable to collect a sample due to ventilator dependence.

Conclusion

Sal T can provide a useful method of assessing androgen status in DMD patients at home, overcoming barriers such as mobility difficulties or venepuncture. However we only obtained samples in a minority of patients suggesting that Sal-T measurement may not be appropriate or acceptable to everyone. There needs to be a more detailed exploration of the barriers to sample submission and a greater understanding of the implications of testosterone status on physical and psychological health in DMD patients.

Background

The improving health of adolescents and young adults with Duchenne Muscular Dystrophy (DMD) has resulted in changing expectations with many seeking to establish relationships and to lead independent adult lives. The majority of young people with DMD in the UK receive long-term, high dose glucocorticoid (GC) therapy, consistent with the published standards of care (1). GC in pharmacological doses delay or inhibit activation of the hypothalamo-pituitary gonadal axis in adolescence with associated hypogonadotrophic hypogonadism and androgen deficiency. Androgen deficiency is frequently managed with exogenous testosterone replacement in order to promote virilisation and the development of muscle and bone mass. Low testosterone levels are associated with low mood and treatment of hypogonadism in young men is associated with improved well-being.
We routinely discuss the potential benefits of pubertal induction with testosterone (T) in adolescents with DMD (2). However, the androgen status of older individuals with DMD, on or off long-term GC and irrespective of whether they have received earlier androgen therapy is unknown. Few adolescents continue with T supplementation through into adulthood in our experience and there is a cohort of older men with DMD whose androgen status is unknown.

Assessing androgen status in this population is challenging. Adults with DMD often have difficult venous access and don't routinely have venepuncture for other reasons. Furthermore, for mobility reasons, patients often attend outreach clinics in the afternoon and so it is a logistical challenge to obtain a testosterone level in the morning when it is most informative.

Salivary testosterone (Sal-T) assays using liquid chromatography tandem-mass spectrometry (LC-MS/MS) are validated (3) and age-adjusted reference levels for the adult population are available (4). Sal-T measurements potentially provide a non-invasive, stress-free method of capturing androgen status in this population at home, without the burden of attending a hospital appointment.

**Methods**

We undertook a quality improvement project (QI) to assess the feasibility of measuring Sal-T levels in men with DMD without the need to attend hospital. We were particularly keen to assess the androgen status of patients with ongoing GC exposure. A Sal-T sampling kit (Salimetrics®, Carlsbad, CA92008) with instructions showing how to collect a saliva sample was sent by post to all patients with DMD, aged 17 and older, registered at the John Walton Muscular Centre in Newcastle (n = 75). A short questionnaire regarding GC/T usage was also included. A follow-up telephone call was made 2–4 weeks later to address any outstanding questions and encourage sample return.

After discussion with the Ethics Committee chair of NRES Committee North East- Newcastle and North Tyneside, it was concluded that ethical approval was not required for this study as it was considered a QI project (registered with Newcastle upon Tyne Hospitals Foundation Trust, project number 10158) that was intended to evaluate standard care delivered to patients. Consent for publication was therefore not obtained from patients, with only anonymised data included in the publication.

Sal-T levels were collected in the laboratory in Newcastle and sent for batch analysis using LC-MS/MS to South Manchester University Hospital (the same assay platform from which the reference ranges are derived).

Salivary testosterone data was checked for normality and t-tests used to compare the mean Sal-T level to the reference population and evaluate by GC status. Mean ± 1 standard deviation are shown Statistical analysis was performed using Stata v15. A p-value of < 0.05 was considered statistically significant.

**Results**
28 out of 75 patients returned samples by post to our Paediatric Endocrinology Service (age range: 17–34 years). 22 were suitable for analysis (one mislaid, four insufficient and in one instance the kit was inadvertently sent to someone who was too young). Three patients were unable to collect a sample due to poor health with associated ventilator dependence.

Figure I shows that Sal-T was significantly lower than the healthy population of the same age group (178 ± 107 vs 287 ± 109 pmol/l, p = 0.0001) (4) despite 6 patients (17 to 23 years) already being on testosterone supplementation. Sal-T was lower in those on GC compared to those off GC (144 ± 81 versus 218 ± 125 pmol/l, p = 0.05). The mean age of the patients on GC was lower than those not on GC (20 ± 2 years vs 25 ± 2 years for those off GC, p = 0.007). The patient and family doctor were informed of the outcome of Sal-T measurement, highlighting whether this was within the reference range or not.

**Figure I** Mean salivary testosterone levels (+/-1SD) in Duchenne muscular Dystrophy (DMD) patients both on and off glucocorticoids (GC), showing lower levels in those on GC and a significant reduction compared to the healthy male population of the same age (*** signifies p < 0.001 when comparing DMD men to healthy population)

**Discussion**

This project has provided information about the androgen status of young adults with a rare disease, DMD, as well as providing information about the feasibility of assessing androgen status by measuring salivary testosterone in the home environment. This project suggests that Sal-T can be used to screen and monitor androgen status in some but not all patients with DMD. Although we contacted a relatively large number of young people with DMD we were only able to obtain data on a minority of patients. The relatively low response (37% of samples returned) may reflect the complexity of our DMD population including a wide age range, varying degrees of disability and issues such as moving address. The 3 patients who were unable to send a sample represent those with the worst pulmonary function. Older, sicker patients and those in residential care appeared to be less able or willing to submit samples. The fact that 14% of the samples sent were insufficient highlights the importance of using the correct technique to ensure that enough saliva is obtained, that contamination with blood is avoided and that sufficient time has elapsed since mouth hygiene.

The salivary testosterone levels in patients with DMD on GC were lower than those off-GC and compared to age-matched reference data, even though some of the patients on GC were already on testosterone supplementation. Patients on GC were younger and it is unclear the extent to which GC prevent or simply delay progress through puberty in this patient group. What impact testosterone supplementation will have on the physical and psychological well-being of adult patients on GC therapy is also unknown.

**Conclusions**
To our knowledge this is the first attempt to use home Sal-T sampling as a means of improving patient care. Our desire was to reduce inconvenience and cost for a group of patients with complex, chronic health needs. Before this technique can be used more widely there needs to be a better understanding of the implications of testosterone status in these adolescents and young men and a more detailed exploration of the barriers to sample submission and analysis.

**Abbreviations**

- **DMD** Duchenne muscular dystrophy
- **GC** Glucocorticoids
- **LC-MS/MS** Liquid chromatography mass spectrometry
- **QI** Quality improvement
- **Sal-T** Salivary testosterone
- **T** Testosterone

**Declarations**

**Ethics approval and consent to participate**

The QI project was discussed with the Chair of NRES Committee North East- Newcastle and North Tyneside, who felt that ethical approval was not required for the project. Consent for publication was not obtained from patients due to the QI nature, with only anonymised data included in the publication.

**Consent for publication**

Not applicable

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

There are no competing interests to be declared

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**Author’s contributions**

VS, TC and CW designed the project. YAS, CB, TC and CW undertook data collection/analysis. All authors read and approved the final draft of the manuscript.

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**Figures**
Figure 1

Mean salivary testosterone levels (+/-1SD) in Duchenne muscular Dystrophy (DMD) patients both on and off glucocorticoids (GC), showing lower levels in those on GC and a significant reduction compared to the healthy male population of the same age (*** signifies p<0.001 when comparing DMD men to healthy population)