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Dihydrotestosterone in Amyotrophic Lateral Sclerosis –The missing link ?

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Cover Letter

We, the authors of the research article **"Dihydrotestosterone in Amyotrophic Lateral Sclerosis –The missing link ?"** state our intent to submit our manuscript to Muscle & Nerve for consideration of publication.

We also hereby furnish a disclosure stating that this manuscript is not under consideration elsewhere.

Abstract

Introduction – Testosterone has been postulated to be involved in ALS causation. Increased incidence of ALS is seen in soccer players and jobs requiring physical endurance.

Methods- CSF levels of free testosterone and dihydrotestosterone were measured in 13 ALS patients [7 males , 6 females] and 22 controls [12 males, 10 females].

Results - CSF free testosterone levels did not show any significant differences but there was significant difference in CSF dihydrotestosterone levels between male ALS patients and controls ($p<0.001$) and between female ALS patients and controls ($p<0.001$).

Discussion – Reduced CSF levels of DHT were associated with ALS in our study. DHT might play a role in maintaining integrity of motor neurons. Entire intracerebral DHT is formed from intracerebral testosterone as DHT cannot traverse the BBB. Possible explanation is that in ALS patients, lesser amount of testosterone is able to breach the BBB and enter the central neural axis. There would be inadequate negative feedback suppression of LH at the level of anterior pituitary owing to lesser DHT formed consequently. As a result of higher LH levels , testosterone levels would rise in peripheral testosterone fraction [the fraction outside the BBB] and this would explain the various physical attributes of ALS patients like lower ratio of the index and ring finger lengths (2D:4D ratio) , increased incidence of early onset alopecia etc.

Keywords –

ALS- Amyotrophic Lateral Sclerosis;DHT- dihydrotestosterone; BBB- blood brain barrier;
LH- Luteinizing hormone; CSF-Cerebrospinal fluid;SHBG- sex hormone binding globulin .

Introduction

Amyotrophic Lateral Sclerosis is a progressive neurodegenerative disorder[1] . Androgens may play some role in causation of ALS [2] based on observations like preponderance of male to female patients with ALS, sparing of neurons of cranial nerves III, IV, and VI and Onuf's nucleus in ALS that lack androgen receptors [2] and X-linked spinobulbar muscular atrophy (Kennedy's disease) resulting from a trinucleotide repeat expansion in the androgen receptor gene [3] .

Athletes engaging in sports like Soccer which require high degree of endurance have increased susceptibility to ALS. Studies have found that both male and female athletes in aggressive contact sports have higher testosterone levels[4].

A study [5] found that post-menopausal female ALS patients had significantly higher serum total testosterone and serum free testosterone concentrations than age matched post menopausal controls. Vivekananada et al [6] found that ALS patients had lower ratio of the index and ring finger lengths (2D:4D ratio) in comparison with controls. 2D:4D ratio is dependent on prenatal testosterone levels .

In our study , we measured concentrations of testosterone and dihydrotestosterone in cerebrospinal fluid (CSF) of ALS patients and compared it with normal controls.

Methods

CSF levels of free testosterone and dihydrotestosterone were measured in 13 ALS patients [7 males and 6 females] and in 22 controls [12 males and 10 females] using enzyme-linked immunosorbent assay (ELISA) based kits after taking informed consent. CSF samples were collected in morning between 9:00 AM and 11:00 AM as studies[7] have shown that testosterone concentrations vary during the day . Clearances from Institutional Research and Ethical Committees was obtained (No-IEC/2018/90) .

Statistical analysis - Students t- test was applied to evaluate whether there was any significant difference between the 2 groups.

Inclusion criteria used for patients:

a) Patients fulfilling the diagnosis of Clinically definite ALS and clinically probable ALS as per El Escorial Criteria (EEC)[8].

Inclusion criteria used for controls:

a) Controls were enrolled from surgery, gynecology and obstetrics and orthopedics wards. CSF was obtained from male and female controls undergoing lumbar puncture for spinal anesthesia.

Exclusion criteria used :

a) Subjects not giving consent for study . b) Subjects suffering from cryptorchidism , testicular malignancy or any other testicular pathology . c) Subjects taking anabolic hormones. d) Subjects suffering from pituitary or adrenal disease. e) Female subjects with Polycystic ovarian disease (PCOD) f) Subjects with family history of ALS or any form of motor neuron disease or Frontotemporal dementia [FTD].

Results

Clinical and demographic data and CSF concentrations of Free testosterone and dihydrotestosterone in ALS patients and controls are summarized in tables 1 and 2 and in supplemental tables and Fig 1.

There was no significant difference in CSF testosterone values between male ALS patients and controls [$p=0.344$] and between female ALS patients and controls[$p=0.917$]. However CSF dihydrotestosterone concentrations demonstrated a significant difference between male ALS patients and controls [$p<0.001$] and between female ALS patients and controls[$p<0.001$] with DHT concentrations being significantly lower in ALS patients.

Discussion

In our study we demonstrated significantly decreased CSF dihydrotestosterone levels in ALS patients . Studies on how testosterone and its metabolites exert a negative influence on LH release have demonstrated the following observations –

Radioactively labelled dihydrotestosterone was given intravenously to six rhesus monkeys and it was found that that no radioactively labelled DHT could be found in CSF post-injection thus demonstrating that dihydrotestosterone has minimal penetration across the Blood-brain barrier [BBB][9]. A study[10] on castrated male monkeys found that even in presence of very high, supraphysiological serum levels of DHT , CSF concentrations of DHT stayed very low.

The above findings are in contrast to CSF penetration dynamics of testosterone. Studies [11] have shown that CSF levels of testosterone are equal to those of unbound serum testosterone levels with the entire CSF testosterone being unbound . Also it is known that testosterone crosses the BBB (blood brain barrier) only in the unbound state.

The study [11]also found that a sharp increase in serum total testosterone, unbound serum testosterone and CSF testosterone coincided with an abrupt fall in serum LH (Luteinising hormone) levels . Abbott et al [10] had demonstrated in their study that intracerebral DHT can suppress serum LH levels and also concurred with a previous study by Sholl et al [12] that a majority of DHT within the brain comes from the precursor testosterone.

Patients with 5 alpha reductase type 2 deficiency have high circulating LH levels despite having normal or elevated serum levels of testosterone . However these patients have reduced serum DHT levels[13] but no increased risk of ALS.

Studies[14] suggest that estradiol derived from intracerebral testosterone is the main hormone that provides negative feedback at the hypothalamic level while at the level of the anterior pituitary, both DHT and estradiol, both derived from intracerebral testosterone, are required for negative LH feedback .

Total testosterone and free non-SHBG bound testosterone have been found to have a diurnal circadian variation with highest levels being seen in the morning and lowest in the evening [15,16,17]. A study [15] documented elderly males having reduced testosterone levels as compared to younger males and also an more pronounced difference between the circadian excursion of total serum testosterone levels between the two groups.

Studies [18,19] have shown that both total testosterone and free testosterone concentrations decrease with ageing while SHBG levels increase with ageing. Obesity, insulin resistance, metabolic syndrome and dyslipidemia have a strong association with low serum levels of total testosterone, free testosterone and sex hormone binding globulin (SHBG) [18,19] . SHBG levels play a more important role in the development of insulin resistance/metabolic syndrome than

total or free testosterone does. Many studies [20] have suggested that ALS patients have a lower premorbid body mass index as compared to controls and have been leaner, fitter in their life as compared to controls. In other words , being obese or having a higher BMI is protective against development of ALS in later life. Studies [21] have found that having a higher pre-morbid BMI predicts a better prognosis in ALS.

There is emerging evidence that high carbohydrate/high fat hypercaloric diets may improve survival in ALS patients[22].

Breedlove et al [23] in their study on motor neurons found that following systemic administration of radioactive androgens , DHT accumulated to a greater degree in the spinal motor neuron nuclei than testosterone and unlike testosterone , DHT concentrations in spinal motor neurons did not show any sex difference. This shows that DHT is an essential, integral component of sex steroid machinery in the motor neurons .It was earlier widely believed that the hypothalamic–pituitary–gonadal axis in humans remains quiescent after birth until the onset of pubertal activation and that LH and FSH night-day rhythms begin just before the onset of puberty. However studies using ultra-sensitive assay methods[24] have shown that circulating gonadotrophin concentrations and diurnal Rhythms of Luteinizing Hormone, Follicle-Stimulating Hormone, Testosterone, and Estradiol Secretion exist and are well established in prepubertal children as young as 4.6 years .

Based on data from the above studies , we postulate that DHT or one of its metabolites is probably integral to survival of motor neurons and in ALS, it is lack of DHT in the motor neurons which leads to their death. CSF levels of DHT are probably the final arbiter of LH release at the level of anterior pituitary . Entire DHT fraction in the central neural axis is derived from the testosterone fraction which penetrates the BBB since DHT itself cannot penetrate BBB. When testosterone concentrations breach a certain “critical” threshold, the binding capacity of SHBG (Sex hormone binding globulin) is exceeded and only then does the levels of free , unbound serum testosterone rise sufficiently to cause the rise in CSF testosterone . This CSF testosterone is converted to dihydrotestosterone and the resultant increase in dihydrotestosterone suppresses LH levels by exerting a negative feedback on LH release . We postulate that in patients who are predisposed to develop ALS , there is a sort of “testosterone resistance” at the level of BBB. In these patients , lesser amount of testosterone is able to breach the BBB and enter the central neural axis. As a result , lesser amount of testosterone is available to 5 α

reductase type 2 isoenzyme in the anterior pituitary to be converted to DHT and lesser amount of DHT is generated. As a result , there is inadequate negative feedback suppression of LH at the level of anterior pituitary by DHT or its metabolites like 3-alpha diol. As a result of higher LH levels , testosterone levels rise in the peripheral testosterone fraction [the fraction outside the BBB] and this explains the various physical attributes of ALS patients like the lower Ratio of the index and ring finger lengths (2D:4D ratio) , increased incidence of early onset androgenic alopecia, the increased athleticism in the pre-morbid years , the lower BMI in the pre-morbid years (the "always been lean") phenomenon etc.

Potential Conflicts of Interest – None.

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Data Availability statement - The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Abbreviations used - ALS- Amyotrophic Lateral Sclerosis; DHT- dihydrotestosterone; BBB- blood brain barrier; LH- Luteinizing hormone; CSF-Cerebrospinal fluid; SHBG- sex hormone binding globulin; BMI-Body Mass Index, PCOD-Polycystic Ovarian disease..

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Table 1 – Age distribution of ALS patients and controls

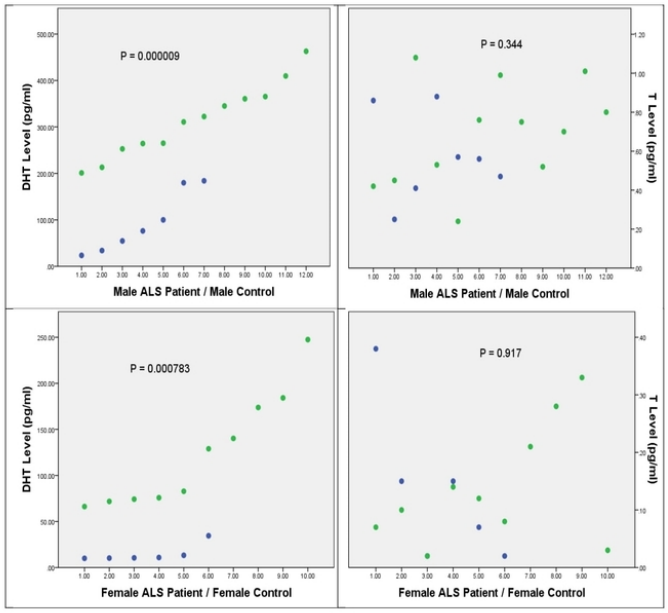
ALS patients		Controls	
	Mean Age and range(years)		Mean Age and range(years)
Males	51.57 (30-60)	**Males**	44.75 (19-62)
Females	59.33 (50-68)	**Females**	46.50 (25-71)

ALS- Amyotrophic Lateral Sclerosis

Table 2 – CSF Dihydrotestosterone and CSF Testosterone concentrations in male and female ALS patients and controls.

CSF Dihydrotestosterone (pg/ml)		CSF Testosterone(pg/ml)	
Male ALS patients	Male Controls	Male ALS patients	Male Controls
76.2	322.4	0.88	0.99
99.9	409.8	0.57	1.01
23.5	310.77	0.86	0.76
54.56	265	0.41	0.24
179.8	252.8	0.56	1.08
33.9	264.31	0.25	0.53
184.02	462.96	0.47	0.8
	345.12		0.75
	365.4		0.7
	201.01		0.42
	213.13		0.45
	360.61		0.52
CSF Dihydrotestosterone (pg/ml)		CSF Testosterone(pg/ml)	
Female ALS patients	Female Controls	Female ALS patients	Female Controls
10.9	82.81	0.15	0.12
10	128.96	0.38	0.08
34.5	184.18	0.02	0.33
13.3	74.21	0.07	0.02
10.5	173.8	0.02	0.28
10.27	71.8	0.15	0.1
	75.8		0.14
	247.47		0.03
	140.2		0.21
	66.2		0.07

pg/ml-picograms per milliliter , CSF-Cerebrospinal fluid, ALS- Amyotrophic Lateral Sclerosis



pg/ml - pico grams per milliliter

DHT-Dihydrotestosterone, T- Testosterone, ALS - Amyotrophic lateral sclerosis.

Blue dots indicate male and female ALS patients,green dots indicate male and female controls.
Plotted on Y axis are levels of Dihydrotestosterone and Testosterone, on X- axis are plotted the study groups- ALS patients and controls.

CSF Dihydrotestosterone(DHT) and Testosterone(T) levels in male and female ALS patients compared with CSF Dihydrotestosterone(DHT) and Testosterone(T) levels in male and female controls.

70x98mm (300 x 300 DPI)