Section: Miscellaneous

Original Research Article

EVALUATING THE ROLE OF TESTOSTERONE IN AMYOTROPHIC LATERAL SCLEROSIS

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Received *: 10/03/2025*
Received in revised form *: 02/05/2025*
Accepted *: 21/05/2025*

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DOI: 10.70034/ijmedph.2025.4.371

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (4); 2059-2068

ABSTRACT

Background: Dihydrotestosterone [DHT] is implicated in Amyotrophic lateral sclerosis [ALS] pathogenesis. Our previous study found deficiency of DHT in cerebrospinal fluid [CSF] of ALS patients leading us to postulate that DHT was integral to survival of motor neurons. This study explored whether correction of the DHT deficiency using testosterone injections could benefit ALS patients.
Materials and Methods: 9 ALS patients were given intra-muscular testosterone injections with dosages being incremented gradually. As DHT itself does not cross the blood-brain barrier [BBB], CSF and brain levels of DHT can only be increased through administration of testosterone which crosses the BBB and then gets converted to DHT.
Results: Varying degree of reduction in fasciculations was seen in ALS patients during administration of higher doses of testosterone. Stabilization or increments in ALSFRS-R scores were seen in a fraction of treated patients.
Conclusion: Testosterone may have a therapeutic role in ALS, especially for control of fasciculations. However more data from randomised controlled trials and meta-analyses evaluating testosterone in ALS would be required before reaching any further conclusion. We also postulate that Neanderthal-Denisovan DNA introgression plays a role in ALS.
Keywords: Amyotrophic lateral sclerosis, Testosterone, Dihydrotestosterone, Fasciculations, Neanderthals.

INTRODUCTION

Testosterone is implicated by some studies in Amyotrophic lateral Sclerosis [ALS] pathogenesis. A recent paper found markedly low CSF dihydrotestosterone [DHT] levels in ALS patients as compared to normal controls and postulated that deficiency of DHT in brain leads to ALS. Since DHT has very poor blood brain barrier [BBB] penetration, hence almost the entire DHT found in brain is formed from testosterone in the brain. The paper postulated that ALS patients have a BBB which has a low permeability to testosterone [a sort of testosterone resistance] and this model explains the peripheral hyper-androgenic phenotype found in many Amyotrophic lateral Sclerosis /Motor Neuron disease [ALS/MND] patients.[1]

A study using a two sample Mendelian randomization [MR] approach found that higher levels of serum sex hormone binding globulin were associated with an increased risk of ALS.[2] They confirmed this causal effect using sensitivity analyses, including the MR-Egger, weighted median, and weighted mode methods. Higher levels of Sex hormone binding globulin [SHBG] are associated with a decrease in amount of biologically active free testosterone which is the fraction which traverses the Blood brain barrier and is the main contributor to intra-cerebral and cerebrospinal fluid testosterone.

Another study found that maximum concentration of the enzyme 5-alpha reductase were found in the corticospinal tracts amongst all white matter structures of the brain.[3] This leads to the corticospinal tracts having maximum dihydrotestosterone [DHT] concentrations amongst all white matter structures of the brain thereby giving support to the hypotheses proposed by Sawal et al that DHT is essential for proper functioning and survival of the motor cortex and corticospinal tracts.

A study on rat brains found that in the cerebral cortex in layers II, III and V, 5α reductase 2 immunoreactivity was predominantly expressed on

the cell bodies of larger pyramidal neurons again demonstrating that DHT is essential for proper functioning and survival of the motor cortex and corticospinal tracts.[4]

Another study evaluated gonadal hormone levels in both male and female ALS patients and controls.[5] They found that both male and female ALS patients had higher serum testosterone levels than age matched controls with the difference between female ALS patients and female controls being statistically significant. There was no significant difference in serum levels of Sex hormone binding globulin [SHBG] between patients and control subjects. They also found that ALS patients showing higher testosterone levels presented with a more rapid worsening of the monthly forced vital capacity [FVC] values indicating rapid disease progression. They found that levels of total testosterone [TT] and free testosterone [FT]were significantly higher in ALS patients with a monthly FVC decline ≥ 2.5 % than in ALS patients with a monthly FVC decline< 2.5 % [p values being < 0.05 for both TT and FT].

Based on these studies, we can assume that decreased brain DHT may play a causative role in ALS and replenishing brain DHT levels may have a therapeutic role in ALS. Since brain DHT levels can only be increased by administration of testosterone which traverses the BBB, it is plausible that testosterone may have a therapeutic effect in ALS.

MATERIALS AND METHODS

9 patients diagnosed with ALS were included in the study. Inclusion criteria were that patients had to fulfil the diagnosis of clinically definite ALS and clinically probable ALS as per the El Escorial Criteria (EEC) (Brooks, Miller, Swash, & Munsat, 2000).6 Exclusion criteria were history of prostate cancer, symptomatic benign prostatic hyperplasia, serum prostate specific antigen [PSA]values >4.0 ng/ml, male breast cancer, cardiac failure, severe liver or renal dysfunction and active anti-coagulant use. Required clearances from Institutional Ethics Committee was obtained [ECR/28/Inst/PB/2013/RR-19-IEC/OAS/05]. Written informed consent was taken from patients for the study. Patients and their caretakers were informed in detail about the possible side-effects of testosterone and of the doses to be used. Patients were administered testosterone enanthate injections in the ventrogluteal hip region by a trained nurse under aseptic conditions. Testosterone enanthate injections were initiated at a dose of 250 mg per week and then increased accordingly as detailed in table 2. Initial assessment of disease severity and subsequent assessments were done using the ALS Functional rating scale-Revised [ALSFRS-R] by a trained neurologist. Patients were monitored and their serum biochemistry, hemogram, packed cell volume, haematocrit and serum prostate specific antigen [PSA] were measured regularly every fortnight.

RESULTS

Study group comprised of 7 male and 2 female ALS patients. Mean age of patients in study group was 61 years. Patients reported varying degree of improvement in fasciculations with testosterone. Improvement in fasciculations was noticed usually after 3-4 weeks of testosterone therapy. Improvement was seen only with higher doses of testosterone. Minimal response to therapy was seen in bulbar symptoms. Stabilization or increments of ALSFRS-R scores were seen in few patients. Side effects of testosterone observed in the study cohort were self-limiting and non-life threatening. Pain and swelling at the injection site was the most common adverse effect.

Table 1: Clinical details of study patients.

Serial Number Gender, Age	Occupation,Handedness	Clinical presentation	Comorbidities, medications being taken. Substance abuse.	Investigations
1. M,50 y.	Pharmacist.Right handed	Voice change for 4 months, dysphagia for 3 months, right upper shoulder fasciculations for 2 months, distal right arm weakness for 1 month. Total duration of symptoms at presentation – 4 months	DM+, on OHA's for 4 years. Dyslipidemia – on atorvastatin.	MRI Brain/Cervical spine normal. EPS – preganglionic neurogenic localization. Hormonal profile – Normal.
2. F,67.	Housewife. Right handed.	Right Lower limb weakness x 12 months, left Lower limb weakness for 10 months. Florid fasciculations present with atrophy of thighs. Bilateral lower limb spasticity present with upgoing plantars. Distal right arm weakness for 5 months. Left arm weakness for 3 months. Total duration of symptoms at presentation – 12 months	None	MRI Brain normal. MRI Cervical spine - Minor PIVD C6-C7 without any cord compression. EPS – preganglionic neurogenic localization.
3. M,73.	Retired Bank Manager. Right handed.	Difficulty in holding pen and writing/ signatures with right hand since 11 months. Right forearm fasciculations since 11 months. Right thigh fasciculations since 9 months followed by right distal leg weakness in form of slipping of slippers. Atrophy of right hand muscles, right distal leg	HTN +,DM+-on OHA's. Dylipedemia + - on statins.	MRI Brain – Fazekas grade I hypertensive changes. MRI Spine -normal.

		muscles present. Left hand weakness since 6 months followed by left distal leg weakness with wasting. Bulbar symptoms in form of speech dysfunction, dysphagia, drooling of saliva present since past 3-4 months. Widespread fasciculations present.		EPS – preganglionic neurogenic localization.
4. M,70.	Retired Policeman. Right handed.	Speech deficits for 1 year. Initially relatives noticed difficulty in understanding speech on telephonic conversations. Gradually progressed. Right proximal upper limb fasciculations for 6 months. Distal right upper limb weakness affecting only the thumb and index finger for 5 months. Thumb/index finger movements, especially opposition affected. Right first dorsal interosseus wasting present. Fasciculations present in right first dorsal interosseus. Total duration of symptoms at presentation – 12 months	HTN.	MRI Brain – Normal.
5. M,61.	Tea shop owner. Right handed.	Right upper limb weakness [distal followed by proximal] for one and a half year, left upper limb weakness for 1 year Left lower limb distal weakness for 8 months followed by right lower limb distal weakness for 6 months. Atrophy present in all 4 limbs, more marked in distal upper limb musculature. Bulbar symptoms in form of occasional choking for 3 months. Fasciculations present. Flail arm variant with right hand demonstrating split hand syndrome with pronounced wasting of right thumb/index finger musculature. Total duration of symptoms at presentation – 18 months	DM for 6 years, on OHA's. Reformed cigarette smoker.	MRI Brain/Cervical spine normal. EPS – preganglionic neurogenic localization. Hormonal profile – Normal. USG-abdomen- Multiple hepatic cysts, renal cortical cysts present. Paraneoplastic work-up – Normal.
6. M,61.	Lorry driver. Right handed.	Proximal right upper limb weakness followed by proximal left upper limb weakness followed by distal right upper limb weakness for 6 months. Proximal right lower limb weakness for 4 months. Thinning and atrophy present in both upper limbs – proximally as well as distally as well as right proximal lower limb. Fasciculations present. No bulbar symptoms. Total duration of symptoms at presentation – 6 months	DM x 3 years, on OHA's. H/O Bell's palsy 8 years back which improved over 3-4 months. Small left forearm lipoma present.	MRI Brain-mild frontal atrophy. EPS – preganglionic neurogenic localization.
7. F,38.	Housewife. Right handed.	Right proximal and distal upper limb fasciculations for 5 months followed by right upper limb distal weakness f/b left UL distal weakness f/b right upper limb proximal weakness f/b weakness of bilateral lower limbs as well as increase in weakness in upper limbs. Atrophy present in distal upper limb muscles bilaterally. Deep tendon reflexes very brisk. No bulbar symptoms. Widespread fasciculations present over all 4 limbs. Total duration of symptoms at presentation – 5 months	No Co-morbidity. Family history negative for ALS/MND.	MRI Brain normal.
8. M,62.	Retired army veteran. Later set up an hardware shop. Right handed.	Bulbar onset of symptoms. Relatives noticed difficulty in understanding patient's speech on phone which progressed over time. 2 months after onset of speech difficulty, patient developed dysphagia – initially with liquids and later with solid foods. Patient also developed fasciculations over proximal left upper limb f/b fasciculations over right upper limb f/b generalised fasciculations. This was followed by mild left upper limb weakness f/b right upper limb weakness noticed by patients while lifting very heavy iron implements and machine parts at his hardware shop. Total duration of symptoms at presentation – 6 months	None	MRI Brain normal. EPS – preganglionic neurogenic localization
9. M,70	Retired Pharmacist. Right handed.	Right hand weakness followed by fasciculations in right first dorsal interosseus [FDI] f/b left upper limb distal weakness f/b minor proximal weakness in bilateral lower limbs with fasciculations present over all 4 limbs. Atrophy seen in distal limb muscles, hands >> foot/leg muscles. No Dysphagia. Total duration of symptoms at presentation – 15 months	HTN for 5 years. On anti-hypertensive drugs.	MRI Brain – normal. EPS – preganglionic neurogenic localization

Table 2: Evolution of disease in study patients receiving testosterone injections using revised ALS Functional Rating Scale (ALS-FRS-R).

Serial Number	ALS Functional Rating Scale (ALS-FRS-R) at presentation	Details of the therapeutic intervention and duration for which patient received testosterone at our centre.	ALS Functional Rating Scale (ALS-FRS-R) after treatment.
1. M,50 y.	41	Testosterone enanthate injections initiated at dose of 250 mg i.m. once a week, increased after 1 week to twice and then after 1 week to thrice a week and after 1 week to 4 times a week. PSA, serum biochemical and haematological parameters monitored. Duration – 2 months.	41. After 3-4 weeks, patient noticed 60 % decrease in right upper arm fasciculations. No improvement in salivation/drooling. Patient reported 10% improvement in clarity of voice and 10% improvement of dysphagia but not enough to improve ALS-FRS-R score. Patient discontinued follow up at our centre 2 months after initiation of testosterone injections.
2. F,67.	33/44. As patient was illiterate, item 4 of ALS-FRS-R could not be assessed and was excluded from scoring.	Testosterone enanthate injections initiated at dose of 250 mg i.m. once a week, increased after 1 week to twice and then after 1 week to thrice a week. Serum biochemical and haematological parameters monitored. Duration – 3 months.	33/44. After 4 weeks at a dose of 250 mg thrice a week, patient noticed 30-40 % decrease in fasciculations. No other benefit seen. Patient discontinued follow up at our centre 3 months after initiation of testosterone injections
3. M,73.	15	Testosterone enanthate injections initiated at dose of 250 mg i.m. once a week. Dosing and frequency gradually increased. Patient reached dose of 350 mg i.m daily. PSA, Serum biochemical and haematological parameters monitored. Patient was also taking riluzole 50 mg BD since past 10 months. Duration – 2 months.	15. Fasciculations decreased by 50% at dose of 250 mg i.m. daily and by 70% at dose of 350 mg i.m daily. Patient reported further subjective improvement at a dose of 500 mg i.m. daily but no further improvement in fasciculations. No improvement on ALS-FRS-R score. However subsequently patient's PSA increased to 12ng/ml. Multiparametric MRI-prostate was negative for malignancy. Patient also developed hyperbilirubinemia owing to which both testosterone and riluzole were stopped. Fasciculations re-appeared 3 weeks post testosterone cessation.
4. M,70.	43	Testosterone enanthate injections initiated at dose of 250 mg i.m. once a week. Dosing gradually increased. Patient reached dose of 350 mg i.m once a week. PSA, serum biochemical and haematological parameters monitored. Duration – 2 months.	43 At dose of 350 mg once a week, patient noticed 50% reduction in fasciculations. Patient also had some improvement in right thumb and index finger range of motion and in muscle power. Patient could oppose his right thumb and index finger and could make a pincer grasp and do activities involving pincer grasp which he was unable to do earlier. However improvement was not enough to improve ALS-FRS-R score. No improvement in speech dysfunction Patient stopped follow-up at our centre after 2 months.
5. M,61.	31	Testosterone enanthate injections initiated at dose of 250 mg i.m. once a week. Dosing and frequency gradually increased. Patient reached dose of 500 mg i.m daily. PSA, Serum biochemical and haematological parameters monitored. Duration – 8 months.	32. At dose of 350 mg i.m daily, fasciculations reduced by 50-60%. At dose of 500 mg i.m daily, ALS-FRS-R became 32 as dysphagia improved. Patient's walking improved but not enough to improve his ALS-FRS-R score on item 8. Patient maintained his improved ALS-FRS-R score for 3 months. However patient was administered Vit D3 60,000 IU for 2 weeks. His fasciculations worsened and his muscle power in affected muscles deteriorated. ALS-FRS-R score became 30. Vitamin D3 was stopped, testosterone dose was subsequently increased gradually to 700 mg i.m daily leading to improvement in his symptoms with the ALS-FRS-R score improving to 32.
6. M,61.	43	Testosterone enanthate injections initiated at dose of 250 mg i.m. once a week. Dosing and frequency gradually increased. Patient currently on dose of 600 mg i.m daily. PSA, Serum biochemical and haematological parameters monitored. Duration -9 months.	45. At dose of 350 mg i.m daily, fasciculations decreased by 25%. At dose of 500 mg i. daily, fasciculations decreased by 50%. Handwriting improved, patient gained 1 point on item 4, gained 1 point on 5(a), could button and unbutton his clothes (excepting the top collar button)- therefore scored as not having gained one point on item 6, improved on item 9 concerning climbing stairs but not enough to gain one point. At dose of 600 mg i.m daily, fasciculations decreased by 60-65%. No further other gain on ALS-FRS-R.

7. F,38.	36	Testosterone enanthate injections initiated at dose of 250 mg i.m. once a week. Dosing and frequency gradually increased. Patient currently on dose of 500 mg i.m every 3rd day [gap of 2 days between injections]. Serum biochemical and haematological parameters monitored. Duration – 16 months.	48. At dose of 250 mg i.m every 3rd day, fasciculations decreased by 40%. At dose of 350 mg every 3rd day, fasciculations decreased by 70% and at dose of 500 mg every 3rd day, patient was having very occasional fasciculations only in right shoulder region once in 4-5 days lasting only for 1-2 seconds. At dose of 350 mg i.m every 3rd day, patient started having improvement in muscle power and after 4-5 weeks at a dose of 500 mg i.m every 3rd day, all items of ALS-FRS-R were scored at 4 leading to a total score of 48. The activity which was last to recover was eating steamed rice-dal [lentils in curry]. One has to mix the lentil curry with rice using fingers, mix it and then eat it with fingers/hand. It is a dextrous task and patient noticed improvement in this task only at a dose of 500 mg i.m every 3rd day and this deficit was the last to recover. Patient currently says that although she has no clinical deficit but when she does her household work, after 2-3 hours, she has to take rest for 15-20 minutes which was not the case prior to onset of the illness. However there is no household task which she cannot do with pre-illness speed and efficiency.
8. M,62.	38	Testosterone enanthate injections initiated at dose of 250 mg i.m. once a week. Dosing and frequency gradually increased. Patient currently on dose of 700 mg i.m daily. PSA, Serum biochemical and haematological parameters monitored. Duration – 8 months.	41. At dose of 250 mg i.m daily, there was no improvement. At dose of 350 mg daily, fasciculations decreased by 10%, at dose of 500 mg daily, fasciculations decreased by 20% and at dose of 700 mg daily, fasciculations decreased by 40%. At dose of 500 mg daily, patient noticed improvement in swallowing and decreased salivation. The improvement increased at a dose of 700 mg daily. Drooling of saliva decreased by 60% and patient had an improvement of 2 points on item 2 of ALS-FRS-R. Swallowing improved and prior to initiation of testosterone, patient had to eat soft food and take sips of water after every bite but post testosterone injections, patient experienced only mild symptoms. Patient explained that he could now eat 4-5 rotis [a flatbread made of wheat] with dal [lentils in curry] or vegetables and experienced some mild difficulty only at the end of the meal. This lead to improvement of one point on item no 3 of ALS-FRS-R. However there was no improvement in speech at any dose of testosterone. Though not a component of ALS-FRS-R scale, another thing noticed by patient was he had progressive improvement in muscular strength at doses of 500 mg and 700 mg testosterone injections daily. He noticed that earlier he had some difficulty in lifting very heavy iron implements and machine parts at his hardware shop but it lessened with increasing dose of testosterone injections.
9. M,70	41.	Testosterone enanthate injections initiated at dose of 250 mg i.m. once a week. Dosing and frequency gradually increased. Patient currently on dose of 500 mg i.m alternate day [gap of one day between injections]. PSA, Serum biochemical and haematological parameters monitored. Duration – 9 months.	45. At dose of 250 mg i.m alternate day [gap of one day between injections], fasciculations decreased by 10%. At dose of 350 mg i.m alternate day, fasciculations decreased by 25% and at dose of 500 mg i.m alternate day, fasciculations decreased by 50-60%. No improvement in muscle power at 250 mg i.m alternate day. Patient noticed improvement of muscle power in affected muscles at dose of 350 mg i.m alternate day which further increased at a dose of 500 mg i.m alternate day. Patients hand writing improved gradually with alphabets a and r improving in the last [patient specifically had difficulty in writing these 2 alphabets and had to write them frequently since his name included these 2 alphabets]. His ability to break roti to dump it in dal to make a morsel improved gradually. Ability to unbutton clothes was regained earlier than ability to button clothes which he could do independently but slowly and with extreme difficulty with the top collar button. He could post testosterone dose at 500 mg alternate day climb stairs at a normal pre-illness speed. Scores on items 4,5(a),6 and 9 of ALS-FRS-R improved.

Table 3: Adverse effects of testosterone therapy in our study group.

Serial Number. Gender, Age.	Adverse effect
1. M,50 y.	Pain and swelling at Injection site.
2. F,67.	Pain at Injection site. Hirsutism. Irritability. Voice change.
3. M,73.	Pain at Injection site. Slight elevation of serum alkaline phosphatase on testosterone initiation which was clinically asymptomatic and did not increase further on serial monitoring.
4. M,70.	Pain at Injection site.
5. M,61.	Pain at Injection site. Increased irritability, insomnia, anger issues. However these were not disabling and in view of motor improvement, patient chose to continue therapy.
6. M,61.	Pain at Injection site. One episode of superficial tissue infection at injection site which improved with antibiotics/anti-inflammatory agents. Increased anger, irritability.
7. F,38.	Pain at Injection site. Hirsutism. Oligomenorrhoea later evolving to amenorrhoea, voice change, skin hyper-pigmentation with acne requiring topical treatment with anti-seborrheic lotions, shampoos. Increased sexual desire, development of snoring. Patient also developed pica where she felt the strong desire to eat mud/chalk and ate these items to satisfy her craving. At dose of 500 mg every 3rd day, patient also developed secondary polycythaemia. Haemoglobin was 16.8 gm/dl and packed cell volume rose to 56%. Haematology consult was taken and patient initiated on monthly phlebotomy.
8. M,62.	Pain and swelling at Injection site. Increased irritability, stubborn behaviour.
9. M,70	Pain at Injection site. Increased libido. Patient also reported better, sustained penile erections.

DISCUSSION

Results of our study summarised above show that testosterone may provide some degree of therapeutic benefit in ALS/MND. Treatment with testosterone leads to increase in serum testosterone levels with resultant increase in brain testosterone levels. This increased brain testosterone is converted partially to DHT leading to increase in brain DHT levels and this likely resulted in some degree of clinical improvement in our small cohort. There are other studies which indirectly support this hypothesis -

Role of Connexins in ALS -Studies have found that Connexin 43 in astrocytes contributes to motor neuron toxicity in ALS with increasing levels of connexin 43 leading to elevated intracellular calcium levels and cell damage.[7–10]

Other studies have found that Connexin 43 hemichannels mediate spatial and temporal disease spread in amyotrophic lateral sclerosis.[7–10]

Connexins are multimeric proteins that form gap junctions which are communicating junctions between adjacent cells for inter-cellular exchange of metabolites and ions This inter-cellular communication or Gap junctional intercellular communication (GJIC) mediated via gap junctions plays an important role in cellular homeostasis.

It has been found that expression of connexin 43 is upregulated in the motor cortex and spinal cord of patients with ALS. This upregulated expression of connexin 43 is accompanied by increased hemichannel activity and gap junction coupling leading to elevated concentration of intracellular calcium which leads to motor neuron damage.

Androgens regulate a variety of tissues and cell-specific genes including connexins. Castration is associated with a dramatic increase in Connexin 43 mRNA and protein expression, and this coincides with induction of cellular apoptosis. Treatment with testosterone or dihydrotestosterone [DHT] abolishes castration induced Cx43 expression and prevented apoptosis in male Sprague–Dawley rats. Thus deficiency of DHT in the brain of ALS patients probably leads to over-production of connexion 43 leading to neuronal death and resultant clinical symptoms.

Explaining the Vitamin D paradox in ALS through the connexin pathway- A study evaluating survival analyses of ALS patients found a deleterious effect of higher vitamin D concentrations on the prognosis, independently of ALSFRS-R score at diagnosis and BMI, suggestive of the fact that this relation may be direct and independent of the nutritional status.[11]

Another study comprising of 71 ALS patients and 151 healthy controls also found that higher serum vitamin D levels co-related positively with a higher ALS severity score using ALSFRS-R implicating a negative effect of high vitamin D serum concentrations on ALS severity.[12]

A study found that 1-alpha 25-Dihydroxy Vitamin D3 reversed testosterone-induced down-regulation of Connexin 43 in rat testes granulosa cells. Connexin 43 protein expression was markedly decreased when cells were treated with a high dose of testosterone but this effect was blocked by pre-treatment with 1-alpha 25 -Dihydroxy Vitamin D3.[13]

Researchers found a Vitamin D receptor dependent increase in connexin 43 protein levels and connexin 43 mRNA levels.[14] An increase in Vitamin D3 levels increased both Connexin 43 mRNA levels as well as connexin 43 protein concentrations. They postulated that the activated Vitamin D receptor may activate the expression of genes involved in regulation of connexion 43 transcription or of genes involved in regulation of the post-transcriptional stability of connexion 43 mRNA.

Thus vitamin D3 interferes with androgen suppression of connexin 43 levels and also increases connexin 43 levels independently. Increased connexin 43 levels are deleterious in ALS thus explaining this paradox.

Juvenile ALS with mutated Sigma -1 receptor and possible role of dihydrotestosterone - A study on juvenile ALS [ALS with age of onset <25 years] found mutated Sigma-1 receptor to be the causative mechanism of ALS in their patients in Saudi Arabia with an autosomal recessive mode of inheritance Sigma-1 receptor (Sig-1R) is an endoplasmic

reticulum (ER) chaperone that binds a wide range of ligands, including neurosteroids.[15]

Researchers demonstrated that treatment with a Sigma-1 inhibitor prevented 5α-dihydrotestosterone (DHT)-mediated nuclear translocation of the cytoplasmic androgen receptor (AR), induced ubiquitin proteasome mediated degradation of androgen receptor (AR) and suppressed the transcriptional activity and levels of androgen receptor.[16]

We postulate that in the juvenile ALS cases reported by Al-Saif et al with the sigma -1 receptor mutation, the mutation renders the sigma-1 receptor ineffective.15 This leads to cessation of process of 5α-dihydrotestosterone (DHT)-mediated nuclear translocation of the cytoplasmic androgen receptor (AR), increased ubiquitin proteasome mediated degradation of androgen receptor (AR) and decreased transcriptional activity and decreased levels of androgen receptor. This leads to neuronal death in susceptible groups leading to juvenile ALS.

Role of Neanderthal-Denisovan DNA introgression in ALS/MND - Oceanian individuals [which includes Near Oceania, which includes New Guinea, the Bismarck archipelago and the Solomon Islands and the Remote Oceania which includes Micronesia, Santa Cruz, Vanuatu, New Caledonia, Fiji and Polynesia] have the highest levels of combined Neanderthal and Denisovan ancestry worldwide.[17,18]

Various studies have found that incidence of ALS/MND in Australasia/Oceania is second highest in the world.[19,20]

Also there is high-incidence of ALS in the Western Pacific among the Auyu- and Jakai-speaking people of West New Guinea, among the Chamorros of the Mariana Islands and among Japanese in the Kii Peninsula of Japan which no hypotheses proposed till date including the cycad toxin Beta – N-Methyl-Amino L alanine [BMAA] have been able to explain.[21] We hypothesise that increased incidence of ALS in these isolated island communities is owing to higher amounts of Neanderthal-Denisovan genes in them. We further propose that higher amounts of Neanderthal-Denisovan genes predispose to ALS/MND via the androgen pathway through the presence of a blood brain barrier which has lesser permeability to testosterone as compared to blood brain barrier present in normal individuals. Our hypothesis is supported by the following facts.

Neanderthals compared to anatomically modern humans – masculine body: feminine brains: Neanderthals had higher peripheral circulating androgens and a more masculine phenotype. A study proposed that the fossil evidence reflects a significant reduction in androgen-mediated craniofacial masculinity between the modern humans and Neanderthals coincident with archeologically visible increases in human population size and density and with a markedly increased rate of cultural evolution.22 Craniofacial feminization appears to have contributed to modern human population growth and cumulative cultural evolution.

Other studies have also shown that Neanderthals were more strongly built and more heavily muscled than modern humans.[23]

Another study postulated that Neanderthals/Denisovans would have higher steroid hormone levels [including testosterone] than modern humans.[24]

A study on digit ratio found that Neanderthals have lower 2D:4D digit ratios than most contemporary human populations indicating increased androgenization and higher testosterone levels.[25] 2D:4D digit ratio is a commonly used measure which is a surrogate marker for intrauterine testosterone exposure.[26]

Thus we see that data from fossil osseus, genetic, anthropological sources indicate that Neanderthals were more heavily built and had higher serum testosterone levels than anatomically modern humans.

Regional brain volume differences in males and females in anatomically modern humans. Researchers found using high resolution MRI scans with automated tissue segmentation that females have more grey and total volumes of the left occipital lobe and grey volumes of the right occipital lobe as compared to male subjects.[27]

A study evaluated prefrontal volumes of preserved brains and found males had higher right pre-frontal cortical volumes as compared to females.[28]

Researchers using high resolution MRI scans found that males had higher right cerebellar hemisphere volumes than females.[29]

Studies using MRI imaging also found that males had significantly higher cerebellar volumes than females.[30]

A study on 149 healthy male and female volunteers between 10-27 years found that the endogenous testosterone levels in males were positively associated with grey matter volume of the right cerebellar hemisphere.[31]

Researchers used MRI based volumetric evaluation and found that men have higher left inferior parietal lobule volumes than women.[32]

Differences in brains of Neanderthals and anatomically modern humans: Kochiyama et al in their study found that Neanderthals had smaller right cerebellar hemisphere volumes than modern humans.33 They also found that Neanderthals had smaller parietal region volumes than anatomically modern humans(AMH). However Neanderthals had larger occipital regions than modern humans. [Details of fossil samples used in this study - Four Neanderthals -Amud 1- adult male 25 years old, La Chapelle-aux-Saints 1 – mature male, La Ferrassie 1 - adult male around 45 years, Forbes' Quarry 1 – adult female. Four AMH's – Qafzeh 9– likely young male, Skhul 5 – adult male, Mladec 1– likely young female, Cro-magnon 1 – mature male.]

Pearce et al had also demonstrated that Neanderthals had larger orbits and larger visual cortices than modern humans.[34] Richards et al found that

Neanderthals had smaller dorsolateral prefrontal cortices than modern humans.[35]

Thus we see that the Neanderthal brain resembles the brain of an anatomically modern human female more than it resembles the brain of an anatomically modern human male.

Brain atrophy patterns in ALS/MND and the Neanderthal link.

Agosta et al in their MRI based volumetric study of 25 ALS patients [14 males, 11 females] without any signs of fronto-temporal dementia found that patients had significant reduction of grey matter density in the right precentral gyrus.[36]

Tan et al in their MRI based volumetric study of 23 ALS patients without any symptoms of fronto-temporal dementia found that ALS patients had marked atrophy of the inferior lobules of the right cerebellum [lobules VII B, VIII A, VIII B,IX].[37]

A study found that ALS patients had significant atrophy of the right (but not left) precentral gyrus.[38]

Researchers in their MRI study employing voxel based morphometry found that ALS patients had atrophy affecting the left orbitofrontal, bilateral superior frontal and left inferior parietal regions.[39]

Thus we see that the brain regions preferentially affected in ALS are the ones which are less developed in Neanderthals as compared to anatomically modern humans. These preferentially affected brain regions are recent acquisitions from an evolutionary viewpoint.

Many of these brain regions are also less developed in female sex of anatomically modern humans as compared to males. This hints that this better development of these brain regions in males is because of effect of testosterone or dihydrotestosterone. Since Neanderthals have brains more like brains of female sex of anatomically modern humans, it is likely that Neanderthals had lower amounts of brain testosterone and brain dihydrotestosterone as compared to anatomically modern humans. We postulate that patients with ALS/MND have more Neanderthal genes as compared to normal individuals. Thus they have higher peripheral blood testosterone levels but lower brain testosterone/dihydrotestosterone levels. These lower brain testosterone/dihydrotestosterone levels predispose them to ALS/MND.

Since Sawal et al found normal testosterone levels but markedly reduced dihydrotestosterone levels in ALS patients as compared to controls, we postulate that dihydrotestosterone is possibly the androgen responsible for better development and survival of these evolutionary recent acquisitions of the brain.1 These DHT dependent brain structures atrophy in ALS/MND as DHT levels decrease. The split-hand syndrome found in ALS/MND and the differing thumb joint architecture of Neanderthals and anatomically modern humans also supports this.

The split hand syndrome in ALS- It refers to a dissociated pattern of muscle atrophy seen in the hands in ALS, which preferentially affects the musculature around the thenar eminence, mainly the abductor pollicis brevis (APB) and first dorsal interosseous muscle (FDI), with relative preservation of the hypothenar muscles, particularly the abductor digit minimi (ADM). Loss of precise movements involving the thumb and index finger is an early characteristic of split hand syndrome. It is postulated that corticomotoneuronal input to the thenar complex is preferentially affected in ALS.

Corcia et al postulated that the dying forward hypothesis potentially explains that occurrence of the split-hand sign in ALS.[40] The thumb/first finger muscles (APB/FDI) exhibit a greater anatomical and function cortical representation, in part related to recent evolution of specialised activity of these muscles.

Consequently, the corticomotoneuronal projections can effect a greater degree of cortical hyperexcitability and thereby predisposition for neurodegeneration of the spinal motor neurons innervating these hand muscle groups of muscles via an 41anterograde glutamatergic mechanism. They also found that more evidence for a dying forward hypothesis mechanism was provided by clinical observation of relative sparing of the oculomotor and Onuf's nuclei in ALS/MND, which do not directly synapse with the corticospinal tract.

Also studies employing transcranial magnetic stimulation have established cortical hyperexcitability as an early and specific feature of ALS, correlating with patterns of disease spread and the split-hand sign.[41]

The Neanderthal thumb and the explanation of the split hand syndrome - Bardo et al in their work on Neanderthal thumbs found that the Neanderthals possessed trapezial carpometacarpal joints that were flatter and more transversely oriented with extension of their radial and ulnar borders, a trapezial-Mc1 [proximal joint of first metacarpal] joint that was orthogonal relative to the transverse plane, and a small trapezial-trapezoid joint surface.[42] Presence of these features favoured transmission of axial force from the thumb across the radial side of the hand, favouring more extended and adducted thumb movements. These anatomical features favour extended thumb movements, associated with axially/parasagitally-oriented joints. They found that this morphology is consistent with habitual use of a transverse power squeeze grip, in which an object is held transversely across the palm of the hand with strongly flexed fingers and the thumb is extended and adducted to brace against the object.

They also found that modern humans have a trapezio-metacarpal (TMc) complex morphology that favors thumb abduction and that this movement, combined with axial pronation and flexion of the thumb, comprises thumb opposition. An opposed thumb is habitually used by modern humans in strong precision "pad-to-pad" grips, in which the thumb pad opposes the index finger pad, and the joints of the TMc complex are oriented obliquely relative to the transverse plane.

Thus we can infer from these that during evolution, modern humans developed thumb morphology that favoured development of muscles involved in thumb abduction and opposition and for development of strong "pad—to-pad " grips, muscles like first dorsal interossei [FDI] also likely developed better in modern humans as FDI besides causing abduction of the index finger also rotates the index finger at the metacarpophalangeal joint favouring "pad—to-pad " precision grips.

These muscles, being recent phylogenetic acquisitions in human evolution, are preferentially affected in ALS/MND. We propose that the cortical areas sub-serving cortical representation of these muscle groups have been acquired by modern humans relatively recently during evolution. These cortical areas are likely dihydrotestosterone [DHT] dependent for their survival and proper functioning. As ALS sets in, DHT concentrations drop and these areas being sensitive to DHT concentrations get affected and owing to cortical neuronal loss, the entire cortico-motoneuronal circuit is affected leading to the split hand syndrome.

CONCLUSION

Our study demonstrates that testosterone may have a therapeutic role in ALS/MND. However more data from randomised controlled trials and meta-analyses evaluating testosterone in ALS/MND with longer follow up periods would be required before reaching any further conclusion.

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