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Correlation of Testosterone levels with progression of Amyotrophic Lateral Sclerosis in Males: A Cross-Sectional Study

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ABSTRACT

The known higher incidence of Amyotrophic Lateral Sclerosis (ALS) in men and older age suggests a role of sex steroidal hormones in the disease process. Animal models of ALS have shown lower levels of plasma testosterone. Testosterone is known to exert neuroprotective and neurotrophic actions on neurons. Our objective was to study the association of total testosterone (TT) levels with disease severity on ALS Functional Rating Scale-Revised (ALS FRS-R) scale and King's Staging. This cross-sectional study included 64 males with definite/probable ALS. Patients' morning plasma TT levels were tested, and ALS FRS-R and King's Staging was marked. Standard score was used to compare the deviation of patients' TT levels from average TT levels of age matched healthy males. A scatter plot was constructed, and correlation analysis was performed using Spearman's Rank Correlation. 39/64 patients (60.9 %) had TT levels that were lower by 1/more standard deviation, than age matched average levels in healthy men. There was a statistically significant positive correlation of TT levels with ALS FRS-R (r=.326, p=0.009) and negative correlation with King's Staging (r=-.312, p=0.012). Thus, with declining function and disease progression, standard scores of TT levels decreased. Maintaining plasma TT levels, as close to age matched average levels, may be explored as an adjuvant therapy in ALS.

Keywords: Amyotrophic Lateral Sclerosis, King's Staging, Testosterone, Amyotrophic Lateral Sclerosis Functional Rating-Revised.

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I. INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult onset, fatal neurodegenerative disease primarily affecting the motor neurons. The disease is characterized by skeletal muscle weakness, atrophy and paralysis, which eventually leads to respiratory failure [1]. The incidence and mortality rate of the disease has been increasing since the time it was first discovered [2]. The global incidence is around 1–2.6 per 1,00,000 persons every year [3]. Moreover, despite extensive research, ALS continues to greatly affect the quality of life of the affected individuals and is still fatal, with a mean survival of approximately 3-5 years from the time of onset [4]. Several studies have shown a higher risk of ALS in males than in females with a male to female ratio of 1.5:1 [5]–[9]. Also, studies have demonstrated that peak incidence of the disease is between 70-79 years [5],[10],[11], while it is rare before the age of 40 years [12]. Our previous studies showed better prognosis of cell therapy in premenopausal women and in younger male and female patients (under 50 years of age) compared with older male and female patients (above 50 years of age) suggesting a possible beneficial role of sex steroidal hormones [13],[14]. Other studies have also reported sex steroidal hormone dysregulation, as a possible factor in the etiopathogenesis of the disease [15],[16]. Militello et al demonstrated decrease in serum levels of free testosterone levels in patients with ALS and suggested a possible role of androgens in the pathophysiology of ALS [15]. However, levels of testosterone during different stages of disease

severity and its possible role in disease progression has not been studied. The objective of this study was to evaluate the deviation of total testosterone (TT) levels in ALS patients from mean age matched levels in healthy men and to characterize its association with disease severity on ALS Functional Rating Scale-Revised (ALS FRS-R) and King's Staging.

II. MATERIALS AND METHODS

A. Study Design, protocol and patient selection

This is a cross-sectional study including 64 males, diagnosed with clinically definite/probable ALS as per Revised El-Escorial Criteria, from March 2015 to April 2019. A written informed consent was obtained from each patient. Plasma TT levels were tested in the morning. All the patients had been examined clinically on ALS FRS-R and King's Staging at the time of testosterone testing.

B. Inclusion criteria

Male patients above 18 years of age with a clinically definite or probable ALS as defined by Revised El-Escorial diagnostic criteria; and patients with normal blood sugar and Hemoglobin A1c (HBA1c) levels, liver and renal functions were included in the study. Also, the included patients' records demonstrated absence of cancer, cardiac failure, coronary heart disease, glucocorticoids, antidepressants, sedatives or antipsychotic medicines. All patients were non-smokers/tobacco users, occasional alcohol consumers.

C. Exclusion criteria

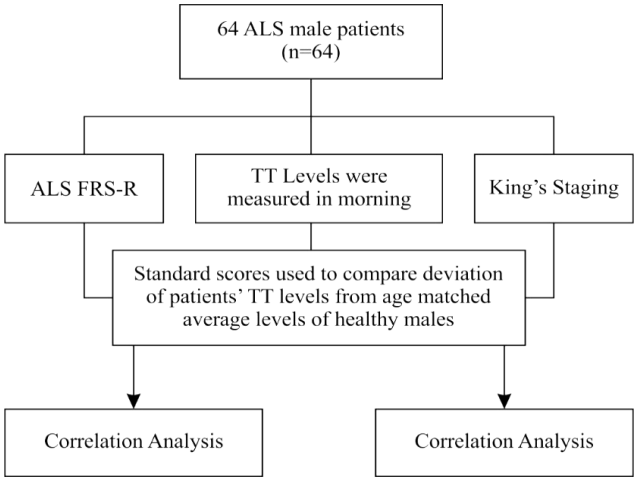
Neuromuscular disorders that mimic the symptoms of ALS, acute respiratory or systemic infections, Human Immunodeficiency Virus infection, Hepatitis B/C viral infections, bleeding disorders, uncontrolled hypertension and uncontrolled thyroid disorders.

D. Procedure

Plasma TT levels (n=64) had been tested in the morning. Chemiluminescent microparticle immunoassay (ARCHITECT 2nd generation Testosterone Assay, Abbott Diagnostics) for the quantitative determination of plasma levels of testosterone (unit: ng/dl) was used. Each of the patients had been marked on King's Staging and ALS FRS-R scales by experienced doctors.

Standard score was used to compare the deviation of patients' TT levels from the average TT levels of age matched healthy males in the reference data [17] [Appendix I]. Standard scores of TT levels were then compared with disease severity on ALS FRS-R scale and King's Staging (Fig 1).

Figure 1: Study protocol and outcome measures



E. Demographic assessment

Demographic data of the patients was gathered. Distribution of the patient population by age, ALS FRS-R scale and King's staging was computed. Also, the plasma TT levels of the patients in relation to the ALS FRS-R score was plotted.

III. STATISTICAL ANALYSIS

ALS disease severity was examined through ALS FRS-R scale and King's Staging and was tested for statistical correlation with standard scores of TT levels. Levels below one standard deviation (-1SD) from the mean age matched TT levels, were considered deviated from mean on the lower side and levels above one standard deviation (1SD) were considered deviated from the mean on the higher side. For assessing the relationship between the standard score of TT levels and the ALS FRS-R scale and King's Staging a scatter plot was plotted. Spearman's Rank Correlation analysis was used to compare the standard scores of plasma TT levels with disease severity as measured on ALS FRS-R scale and King's Staging. Statistical significance was considered with p value less than 0.05. SPSS (version 23.0) and excel was used for the analyses.

IV. RESULTS

A. Demographic Description

All the patients were males ranging from 25 to 71 years of age. The mean age was 48.9 (10.2) years. The demographic description has been shown in Table 1 and Table 2. Also, the plasma TT levels of the patients in relation to the ALS FRS-R score is shown in Figure 2.

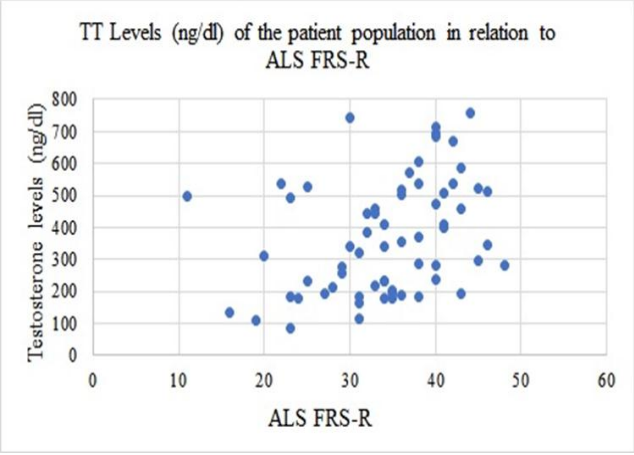
Table 1: Demographic data of the study population

	Mean (SD)	*Minimum*	*Maximum*
Age (years)	*48.9 (10.2)*	*25*	*71*
ALS FRS-R	*34.2 (7.9)*	*11*	*48*
King's Staging	*2.5 (0.8)*	*1*	*4*

Table 2: Number of patients, mean TT levels by 10-year age groups

Age group (Years)	Mean Plasma Testosterone (ng/dl)	Standard Deviation	Number of patients included
25-34	368.14	155.71	5
35-44	387.38	154.12	19
45-54	374.62	199.09	23
55-64	315.35	154.39	14
65-74	455.05	241.63	4

Figure 2: TT levels of the patients in relation to ALS FRS-R



B. Comparison of progression of the disease on ALS FRS-R and King's Staging with standard score of TT levels

We found that 56/64 patients (87.5%) and 39/64 patients (60.9 %) had TT levels that were lower than mean or 1 standard deviation/more, than age matched average levels in healthy men respectively.

We found a monotonic relationship between disease severity on ALS FRS-R and standard scores of plasma TT levels; and between King's Staging and standard scores of plasma TT levels. There was a statistically significant positive correlation between ALS FRS-R and standard scores of plasma TT levels (r=.326, p=0.009) (Figure 3).

There was a statistically significant negative correlation between King's staging and standard scores of plasma TT levels (r=-.312, p=0.012) (Figure 4).

Figure 3: There was a statistically significant positive monotonic correlation between ALS FRS-R score of the patients and standard scores of plasma TT levels; r=0.326, p=0.009

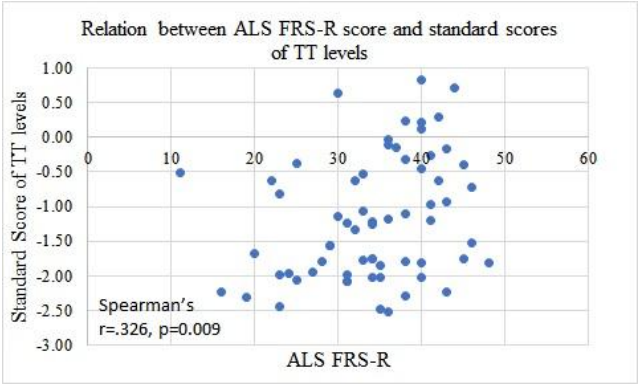
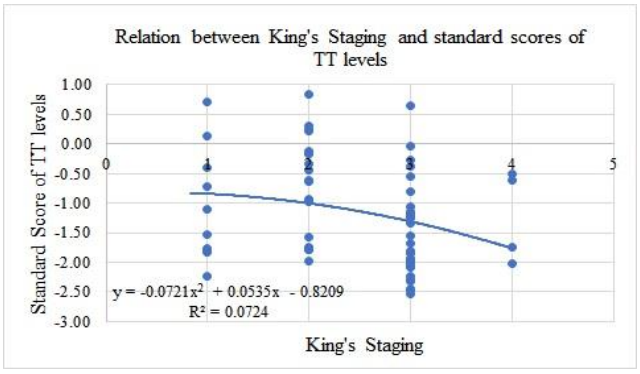


Figure 4: There was a statistically significant negative monotonic correlation between King's staging and standard score of TT levels, r=-0.312, p=0.012.



V. DISCUSSION

In this study, it was found that 87.5% and 60.9% had TT levels that were lower than mean or 1 standard deviation/more, than age matched average levels in healthy men respectively. In addition, we have demonstrated a statistically significant positive correlation between ALS FRS-R scales and standard scores of plasma TT levels and a statistically significant negative correlation between King's Staging and standard score of plasma TT levels. Thus, with increasing disease severity as measured on ALS FRS-R and King's Staging, there was a further decline in standard scores of TT levels.

ALS is characterized by the loss of Betz cells and of anterior horn cells in the motor cortex and the spinal cord respectively and/or of the lower cranial motor nuclei of the brainstem [18]. It begins with involvement of the limb or bulbar muscles and then involves the adjoining and eventually the respiratory myotomes. At cellular level, studies have demonstrated mitochondrial dysfunction to play a central role which induces a cascade of events including oxidative stress, glutamate excitotoxicity, increase in intracellular calcium, and cell death [19]. Excitotoxicity, might trigger in dying forward mechanism, distal degeneration characterized by NMJ remodeling,

distal axon damage and NMJ retraction [20]. Early in the disease, the distal motor neuron segments that are resistant to excitotoxicity demonstrate compensatory plasticity by establishing new connections through collateral branching, reinnervating the motor units because of which larger motor units are formed [21,22]. Comley et al demonstrated selective death of motor neurons, with a loss of neuronal innervation and shrinkage of motor end plates in a mouse model of ALS [23]. Reinnervation was noted with disease progression reflected as partially occupied end plates. However, as the disease further progresses, damage within motor neurons is increased by damage caused by non-neuronal neighboring cells such as macrophages/microglial cells, by an inflammatory response that hastens disease progression [1]. Later in the disease, the sprouting and enlargement of the intact motor units becomes progressively compromised with a progressive loss of functional motor units [22]. Incidentally, these changes, of progressive motor unit loss, reinnervation of the denervated motor end plates and recession of nerve terminals (though at a rapidly accelerated rate in ALS) are also seen as a natural occurrence of aging and these may be related to an age-related decline in testosterone levels [22],[24]. One of the less known actions of testosterone is neuroprotection which involve salvaging, recovery or regeneration of the nervous system, its cells, structure and function [25]. The lower standard scores of testosterone levels with increasing disease severity that were evident in our study, may be the reason for the hampering of the intrinsic neuroprotective mechanism and increase in susceptibility of neurons to damage. Also, sex steroids are known to modulate dendritic growth and dendritic spine density [26],[27]. Since innervation is driven by sex steroids, the lower than average testosterone levels in ALS may impair the reinnervation of denervated end-plates and the natural compensatory mechanisms in the affected tissue, contributing to exacerbation of symptoms and disease progression. Lower levels of testosterone may also cause decrease in dendritic length which may in turn hamper synaptic connectivity and neuronal function [19]. Finally, lower levels of testosterone are associated with a reduction in muscle mass and strength which may further contribute to muscle weakness and atrophy seen in ALS [28],[29].

Only two human studies have investigated plasma TT levels in ALS [15],[30]. Militello et al demonstrated that there was no difference in TT levels and controls [15]. But, in this study, control group included patients with other neurological disorders which can influence testosterone levels. In the second study, TT levels did not decline with increasing age in ALS patients. However, in this study both genders' levels were analyzed together and influence of gender on testosterone levels has not been considered [30].

Animal models of ALS have reported sex steroidal hormone dysregulation, as a possible factor in the etiopathogenesis of ALS [16]. Sar and Stumpf, reported high concentration of androgen receptors in selective motor neurons of spinal cord and cranial nerves [31]. Based on their observation of variability in the concentration of androgen receptors that coincided with the neurons that are involved or spared in ALS, Weiner et al hypothesized that androgens may be important for motor neuron function and repair processes following axonal injury [32]. Daniselle et al demonstrated deficiency of testosterone synthesis along with its precursor ADIONE and metabolite 5α-DHT in the testes of animal model of ALS [16]. Further, the low plasma testosterone levels correlated to spinal cord testosterone levels, indicating that low plasma testosterone levels reflect low testosterone levels in the spinal cord [16]. In normal individuals, low testosterone levels cause activation of the Hypothalamus Pituitary Gonadal axis, to release luteinizing hormone [33]. But, in the study gonadotropin releasing hormone and luteinizing hormone levels were also found to be lower, demonstrating dysregulation at all the levels of Hypothalamus Pituitary Gonadal axis. The study suggested a possible involvement of androgens in the pathophysiology of ALS and dysregulation of the Hypothalamus Pituitary Gonadal axis at the central level [16]. Similarly, administration of anti-androgen in mouse model of mice accelerated disease onset and motor deficits implicating the role of androgens in the disease [34].

Maintaining plasma TT levels as close to age matched mean plasma TT levels may be of importance in ALS patients. Administration of exogenous androgens may exert therapeutic benefits through their impact on the survival of neurons, promoting neuronal plasticity and regeneration of damaged axons and dendrites. Enhancement of axon regeneration was found in rats and this may be of critical significance in treatment of ALS [35],[36]. Neuronal differentiation and increase in neurite outgrowth has been observed in cultured neural cells after stimulation of the androgen pathways [37],[38]. Since low levels of testosterone are associated with reduced muscle mass and strength, administration of androgens may also act by reducing muscle weakness and atrophy [35]. The quality of life of patients with ALS is largely affected by muscle-related symptoms, targeting the muscles may provide therapeutic benefits to patients [15]. In a preclinical study, treatment with dihydrotestosterone (DHT) significantly increased muscle weight, cross sectional area and muscle fiber area in the gastrocnemius and tibialis anterior muscles in mouse model of familial ALS [39]. Androgen mediated increased muscle mass has been shown to be correlated with increase in muscle strength [39]. To reduce muscle weakness and atrophy, administration of androgens could be a potential treatment strategy.

Our results demonstrated a correlation between disease severity and decreasing standard scores of TT levels in patients with ALS. Therefore, maintaining TT levels closer to mean age matched TT levels in healthy males may provide neuroprotection and salvage the neurons from accelerated damage. One of the limitations of the study includes unavailability of body mass index.

VI. CONCLUSION

In our study, 56/64 patients (87.5%) and 39/64 patients (60.9 %) had TT levels that were lower than mean or 1 standard deviation/more, than age matched average levels in healthy men respectively. It was found that standard scores of TT levels further decline with increase in

disease severity as seen on ALSFRS-R and King's staging. Low levels of TT may expose the neurons to risk of damage and may contribute to the etiopathogenesis of the disease. Thus, maintaining testosterone levels close to age matched average levels maybe of importance and testosterone replacement may be explored as an adjunctive therapeutic strategy in ALS. Larger studies to clarify the role of decline in testosterone levels with disease severity, as a cause or effect in the etiopathogenesis of ALS are needed.

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APPENDIX-I

Average Plasma Total Testosterone levels (ng/dl) in healthy males by 10-year age groups.

Age	Mean Plasma Testosterone (ng/dl)	Standard Deviation
25-34	616.14	170.03
35-44	666.86	212.10
45-54	605.76	212.39
55-64	561.67	194.52
65-74	523.05	196.83
75-84	470.32	168.59

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