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Association of Serum Ferritin with Amyotrophic Lateral Sclerosis: A Case Control Study

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Abstract:

Background: *Despite conflicting results from prospective studies, substantial evidence is accumulating suggesting that high Serum Ferritin was associated with Amyotrophic Lateral Sclerosis.* ***Objective:*** *Our aim was to find out the association of serum ferritin with Amyotrophic Lateral Sclerosis.* ***Methods:*** *This was a retrospective, case-control study done in the Neurology, Department of Bangabandhu Sheikh Mujib Medical University on 76 subjects with equal number of cases and controls with their age ranging from 15 to 70 years. Results: The analysis revealed that serum ferritin were significantly high in ALS patients 158.9 ± 16.6 and p= 0.004 as compared to control 104.8 ± 7.9. The proportion of patients below 40 years of age was a bit higher. Predominance in male 73.7% and female 26.3%, predominantly workers or housewife 65% followd by day labourers 26%. A large proportion of patients in both groups were non-smoker (73.7% in case and 78.9 in control group).* ***Conclusions:*** *High Serum ferritin is an important risk factor for ALS. A strong positive correlation was also observed between high serum iron and ALS in our study.*

Key words: *Serum ferritin serum iron and ALS.*

Introduction:

Amyotrophic lateral sclerosis, or ALS, were reported as early as 1824 by Charles Bell and others[1,2]. However, Charcot was the first to describe the condition in detail, based on careful clinico-pathological correlation and he coined the term amyotrophic lateral sclerosis[3]. Motor neuron disease, or MND, as used by Brain in the first edition of this textbook[4], is the name used for the disease most commonly in the United Kingdom and represents an umbrella term encompassing amyotrophic lateral sclerosis and three other disorders that are considered its clinical variants: primary lateral sclerosis, progressive muscular atrophy, and progressive bulbar palsy. In the USA, amyotrophic lateral sclerosis is sometimes known as Lou Gehrig's disease after the famous Yankee baseball player who developed the disease at the peak of his sports career and died in 1941. Amyotrophic lateral sclerosis is sometimes referred to as LAS/MND.

The incidence of ALS reported from recent epidemiological studies ranges from 1 and 3 per 100 000, with point prevalence rates of 6-8 per 100

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000[5,6]. Most of the studies have been conducted in developed countries and relatively little is known of the incidence and prevalence in developing countries, or in specific racial or ethnic groups. Pockets of high incidence are described amongst the Chamorro indigenous population of the Western Pacific Island of Guam, on the Kii peninsula of Japan and amongst the Auyu and Jakia people of Irian Jaya[7,8]. The explanation for the strikingly increased incidence and prevalence of motor neurone disease in these geographical foci remains uncertain. Although the prevalence remains high in Guam compared to typical populations in western countries, there has been a substantial decrease over the last half century.

The disease usually begins with weakness and wasting of hand muscles, associated with cramping and fasciculations in the arm muscles and then shoulder girdles. Less often, the symptoms begin in one leg as a foot drop, soon followed by weakness of plantar flexor and other leg muscles. Before long, the triad of atrophic weakness of the hands and forearms, slight spasticity of the legs, and generalized hyperreflexia with Babinski and Hoffman signs- all in the absence of sensory changes- leaves little doubt as to the diagnosis. Early or late in the illness, dysarthria, dysphagia, and dysphonia set in, and the tongue may wither and fasciculate; or a spastic bulbar paralysis (pseudobulbar palsy) may become prominent.

Diagnosis of ALS is clinical and based on diagnostic criteria. The EL Escorial diagnostics criteria is established since 1994. These criteria were subsequently revised and the key feature of the revised criteria are summarized[9]. Electrophysiological study demonstrating neurogenic changes that cannot be explained by a single nerve, root, or plexus lesion[10]. Assessment of the thoracic paraspinal muscles can be particularly valuable in differentiating amyotrophic lateral sclerosis from multilevel spondylotic radiculomyelopathy[11]. In ALS pathology has been considered traditionally a pure motor disorder now regarded as multi system disease in which motor neurons tend to be affected earliest and most severely[12].

In ALS patients will typically have lost 50 per cent of the lower motor neurons in the limb enlargement areas of the spinal cord at autopsy[13]. Many of the remaining lower motor neurons show atrophic and basophilic changes that are likely to represent part of the spectrum of an apoptosis, programmed cell death pathway[14]. The depletion of lower motor neurons is accompanied by diffuse astrocytic gliosis in the spinal grey matter. There is relative preservation of motor neurone in the nucleus of Onufrowitz, Onuf's nucleus, in the sacral spinal cord[15], which innervates skeletal muscles of the pelvic floor, and in the cranial motor nuclei of the oculomotor, trochlear, and abducens nerve which control eye movements.

The primary pathogenetic processes underlying amyotrophic lateral sclerosis are multifactorial and the precise mechanisms underlying selective cell death in the disease are at present incompletely understood. There may be complex interplay between multiple mechanisms including genetic factors, oxidative stress, excitotoxicity, and protein aggregation as well as damage to critical cellular processes, including axonal transport and organelles such as mitochondria[16,17,18]. Recently there has been growing interest in the role played in motor neuron injury by neighboring non- neuronal glial cells and in dysfunction of particular molecular signaling pathways. The relative importance of these different pathways may well vary in different subgroups of patients.

The cause of ALS is unknown. Many hypothesis have put forward, including that of viral infection, activation of the immune system, exogenous toxins, and hormonal disturbances. However, there has been insufficient evidence to implicate any of these as the major cause of motor neuron degeneration in ALS patients.

The only proven risk factors with the development of ALS disease are gender, a positive family history, and increasing age. Search for linked with environmental, occupational, or physical factors have been done but not yet established. Recently several studies show high serum Ferritin associated with neurodegenerative disease. Several studies also revealed that there was a association of high serum

Ferritin with ALS patient. Elevated levels of Ferritin also have been reported in the spinal cord of ALS patients compared to controls and in Guamanian ALS patient brain samples. High Ferritin promotes oxidative agent in ALS patient. As we know abundant neurofilaments are present in the cytoskeleton of motor neuron, where they are vital for bidirectional axonal transport, oxidative stress causes slow axonal transport, ultimately neurofilament injury. Higher serum Ferritin also causes uncontrolled release of iron, which donate electron for the generation of the super oxide radical and can participate in the generation of hydroxyl radicals via the Fenton reaction (Fe (II) + H2O2'!Fe (III) + OH- + OH) and generation of such reactive species directly damage DNA, lipids and proteins leading to profound cellular toxicity specially motor neuron cell[19].

Materials and Methods: Study populations: This was an observational, retrospective, case control study done in the Department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka, from January 2010 to December 2011. Study population was patient of 15 to 70 years of age. Age and sex was matched apparently healthy person and cases.

Statistical Analysis: All data were recorded systematically in preformed data collection form. Unpaired 't' tests were used to compare group means and Chi square test, Odds ratio with 95% confidence interval were done to evaluate differences between groups for other variable. Risk factors analysis was performed by computer based software Statistical Package for Social Science (SPSS for windows version 16.0). Probability value <0.05 was considered as minimum level of significance.

Results and Observation:

A total 76 subjects were studied and out of them 38 were ALS patients and 38 were normal healthy individuals. In this study the age range was 15 to 70 years with mean (±SD) 37.1 ± 12.9 in case and 39.9 ± 13.5. in control group.

Table I demonstrates that the proportion of patients below 40 years was more than 60% in case group compared to 52.6% in control group. However, the mean ages of case and control groups were almost identical (p = 0.480).

Table-I

Comparison of age between two groups

Age# (years)	Group		p value
	Case (n = 38)	Control (n = 38)	
**<40	23(60.5)	20(52.6)	
40 – 60	12(31.6)	13(34.2)	
e" 60	3(7.9)	5(13.2)	
Mean ± SD	37.1 ± 12.9	39.2 ± 13.5	0.480^{NS}

NS- Not significant

Figures in the parenthesis denote corresponding %;

Data were analysed using Student's t-Test and were presented as mean ± SD.

**Most of the people of our country are illiterate and they have no clear idea about there age.

Males demonstrated their predominance in male 28(73.7%) and female 10(26.3%) in cases group. In male 29(76.3%) and female 9(23.7%) in control groups. The groups were almost identical in terms of sex distribution (p= 0.791).

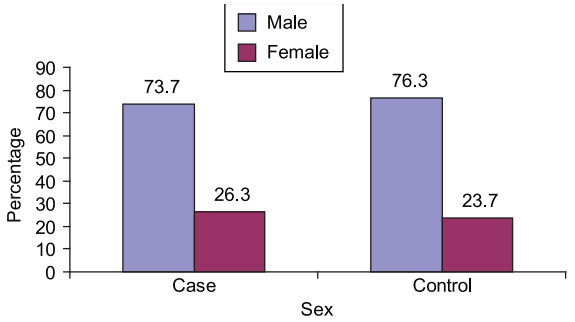

Fig.-1: *Comparison of sex between groups*

The study subjects in both case and control groups were predominantly workers/ housewife (65%) followed by day laborers (26%). Very few were students and service holders. There was no significant difference between groups in terms of occupation (p = 0.392).

Table-II

Comparison of occupation between two groups

Occupation	Group		p value
	Case (n = 38)	Control (n = 38)	
Student	3(7.9)	1(2.6)	
Service	00	2(5.3)	0.392^{NS}
Workers/House wife	25(65.8)	25(65.8)	
Day labourers	10(26.3)	10(26.3)	

NS- Not significant

Figures in the parenthesis denote corresponding %; c^2 Test was employed to analyse the data.

Figure 2 shows that a large proportion of patients in the both groups were non-smoker (73.7% of cases and 78.9% of controls). Approximately 11% of patients in case group and 5.3% patients in control group were ex-smoker. Current smoker was equal in the both case and control groups (each of 15.8%) (p = 0.692).

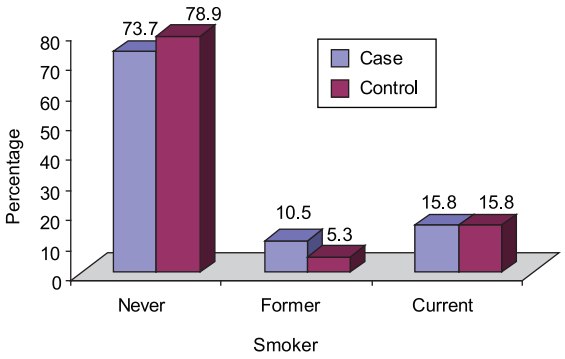

Fig.-2: *Comparison of smoker between groups*

Half (50%) of the patients in case group was habituated of chewing betel nut compared to 44.7% of patients in control group (p = 0.646). All of the patients in the both groups had usual food habit. Majority of the patients in case and control used to drink tube-well water (94.7% vs. 97.4%, p = 0.500).

Table-III

Comparison of behavioral factors between two groups

Behavioral factors	Group		p value
	Case (n = 38)	Control (n = 38)	
Betel nut-chewing*	19(50.0)	17(44.7)	0.646^{NS}
Food habit (usual)	38(100.0)	38(100.0)	-
Source of drinking water			
Tube-well	36(94.7)	37(97.4)	0.500^{NS}
Ponds	2(5.3)	1(2.6)	

NS- Not significant
*Chi-square (c^2) Test was employed to analyse the data.
Data were analysed using Fisher's Exact Test.

Biochemical investigations of the patients demonstrated that the mean serum Ferritin and serum total iron were significantly higher in patients of case group than those in patients of control group (158.9 ± 16.6 vs. 104.8 ± 7.9 µgm/L, p = 0.004 and 111.1 ± 9.2 vs. 81.3 ± 9.3 µgm/dL, p = 0.029 respectively).

Table-IV

Comparison of biochemical investigations between two groups

Biochemical investigations	Group		p value
	Case (n = 38)	Control (n = 38)	
Serum Ferritin (µgm/L)	158.9±16.6	104.8±7.9	0.004
Serum total iron (µgm/dL)	111.1 ± 9.2	81.3 ± 9.3	0.029

Data were analysed using Student's t Test and were presented as mean ± SD.

A staggeringly higher proportion of cases 14(36.8%) had elevated serum Ferritin as opposed to only 2(5.3%) of the control group (p = 0.001). Serum iron was also significantly higher in the former group 11(28.9%) than that in the latter group 2(5.3%) (p = 0.006). The likelihoods of having raised serum Ferritin and serum iron in patients with Amyotrophic lateral sclerosis were observed to be 10.5(95% of CI = 2.2 – 50.4) and 7.3(1.5 – 35.8) times higher respectively compared to their control counterpart (Table V).

Table-V

Risk of increased serum Ferritin and iron in Amyotrophic lateral sclerosis

Iron parameters	Group		p value	Odds Ratio (95% CI of OR)
	Case (n = 38)	Control (n = 38)		
Serum Ferritin (µgm/L)				
Raised	14(36.8)	2(5.3)	0.001	10.5(2.2 – 50.4)
Normal	24(63.2)	36(94.7)		
Serum iron (µg/dL)				
Raised	11(28.9)	2(5.3)	0.006	7.3(1.5 – 35.8)
Normal	27(71.1)	36(94.7)		

Figures in the parentheses indicate corresponding percentage;
Data were analysed using Chi-square (Ç2) Test.

Discussion:

The study was carried out in the Department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka from January, 2010, December 2011 for the duration of two years. A total of 76 participants were allocated into two groups– case group 38 (28 male and 10 female) with ALS and control group 38 (29 male and 9 female) to evaluate the association of serum Ferritin with ALS. Patients

of ALS and age & sex matched normal participants in the above mentioned place were included the study population. The demographic variables were age, sex and occupation. The behavioral factors were smoking, alcoholism, betel nut chewing, food habit and source of drinking water. The biochemical investigations were serum Ferritin, serum total iron, Hb%, TC, DC, ESR and CRP. The test statistics used to analyze the data were Chi-square (c2) tests, Fisher's Exact Test and Student's t-Test. For all analytical tests, the level of significance was set at 0.05 and p <0.05 was considered significant. The proportion of patients below 40 years of age was a bit higher. In our study demonstrated their predominance in male 28(73.7%) in cases group. And 29(76.3%) in control groups. The study subjects in both case and control groups were predominantly workers/ housewife (65%) followed by day laborers (26%), few students and service holders. A large proportion of patients in both groups were non-smoker (73.7% of cases and 78.9% of controls). Approximately 11% of patients in case group and 5.3% patients in control group were ex-smoker. Current smoker was equal in the both case and control groups (each of 15.8%). Half (50%) of the patients in case group was habituated chewing betel nut compared to 44.7% of patients in control group. All of the patients in both groups had usual food habit. Majority of the patients in case and control group drink tube-well water. The mean serum Ferritin and serum total iron were significantly higher in patients of case group than those in patients of control group (158.9 ± 16.6 vs. 104.8 ± 7.9 µgm/L, and 111.1 ± 9.2 vs. 81.3 ± 9.3 µgm/dL respectively).In our study we have excluded inflammation, infection, iron overloading condition and found that out of 38 ALS patients 36.8%(14) had higher serum Ferritin level and 28.9%(11) had higher serum iron level. Out of 14 ALS patients with high serum Ferritin 10 were male and 4 were female (in 10 male ALS patients serum Ferritin level was >300¼gm/L and in 4 female ALS patients serum Ferritin level was >120¼gm/L). Out of 11 ALS patients with higher iron level 8 were male and 3 were female (in all cases total serum iron level was >150¼gm/dL). Our study also found that the proportion of patients below 40 years of age was a bit higher and this maybe due to most our patients were illiterate and they have no idea about their age.

But in a previous study in Qureshi et al 2008[20] series, in Neuromuscular Clinic at the Massachusetts General Hospital (MGH) about 321 patients of ALS over ten years period between January 1994 and December 2003 and found serum Ferritin levels elevated in ALS patients and the majority of participants were male (59%) and the mean age was 56.3 years (mean ± SD =13.0). The mean serum Ferritin level in the ALS population was significantly higher (ANOVA: males p=0.037, females p=0.032). The mean serum Ferritin level in the 17 males with ALS was 269.9 ng/ml (±126.4 SD) compared to 164.1 ng/ml (±142.2 SD) in 14 healthy controls. The mean serum Ferritin value in 13 female with ALS was 183.5 ng/ml (±186.9SD) compared to 71.3 ng/ml (±60.4 SD) in 16 female control subjects. The other series Goodall, 2008[21] associates demonstrated that serum Ferritin level was increased in ALS patients. In their series 60 patients of ALS (41 male and 19 female) and 44 age-matched controls (14 male, 30 female) were studied. Serum Ferritin levels were significantly elevated in ALS patients compared to controls (p<0.001).

In another study Molfino and colleagues findings of 84 patients (40 male, 44 females) mean age of 62.38± 10.19 (range of 32-79 years) those presented with dysphasia. In their nutrition Unit for nutritional evaluation and treatment found that 34(40.4%) patients had increased levels of serum Ferritin and in 36 (43%) serum Ferritin was above the upper level of normality and rest (16.6%) are normal.

Comparison of the results of our study with those from previous studies, our study revealed similar to their study report[22] and 40.4% of ALS patients had high levels of serum Ferritin vs our study that found 36.8% had higher serum Ferritin level although selection criteria, methodology and examination were also same in our and pervious study. In previous study they were unable to do genetic study and environmental factors but they concluded that increased serum Ferritin levels in their cases could reflect the ongoing muscle degradation. or greater storage of iron of patients

In our study we found that Ferritin level were increase in ALS patients and this could signify a greater storage of iron of ALS patients or reflect the ongoing muscle degeneration characteristics of the disease, like previous study, as we also unable to do genetic study and environmental factor study. So, high serum ferritin in our study indicating that high serum ferritin was associated with amyotrophic lateral sclerosis.

Conclusion:

In the present study serum Ferritin was found to be elevated in patients with ALS indicating that high serum Ferritin was associated with amyotrophic lateral sclerosis (ALS) which was consequence of disease process itself. This increase may be due to iron overloading conditions like environmental or genetic factors which were not ruled out by concomitant investigations and limitations of study.

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