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ORIGINAL ARTICLES

Association of Serum Homocysteine level with Amyotropic lateral Sclerosis

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Abstract

Background: *Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive neurodegenerative disease characterized by dysfunction and loss of motor neurons in the brain and or spinal cord. Potential biomarkers like homocysteine (Hcy) that are sensitive to the progression of disease, which might enhance the diagnostic algorithm and provide new drug targets, are now being on trial. Several studies found elevated serum Hcy level with ALS than in normal people. Elevated Hcy is associated with duration of illness and also severity of illness.* ***Objective:*** *Aim of this study was to determine whether plasma Hcy levels were elevated in ALS patients than healthy control. Methods: This case control study was done with 42 patients of ALS fulfilling the inclusion and exclusion criteria and similar number of age and sex matched healthy person were taken as comparison. Result: In this study the mean age of the cases and control were 41.55±14.9 years and 42.78±14.87 years respectively. Maximum respondents were in between 30-50 years age group (Table-1). Higher frequency of ALS patients was found in male (78.6%) than female (21.4%). Among the cases majority 34(80.95%) had the onset of ALS by spinal/limb involvement and bulbar onset were 8(19.05%). Majority of patient (73.81%) had weakness of all four limbs. Study variable analysis revealed that the mean Hcy level in ALS cases was higher 14.06 mg/dl with SD 7.02 than in control 10.43 mg/dl with SD 2.85 and p value was <0.003 which explains Hcy level significantly elevated in ALS. The frequency of hyperhomocysteinemia (>15µmol/L) among case and control Group were found 33.3% versus 7.1% respectively which is statistically significant (p =<0.003). The odds ratio for hyperhomocysteinemia between case and control is 6.5(95% CI: 1.71-24.78).The Odds ratio was 6.5, indicating that the homocysteine higher than >15µmol/L increases the risk of ALS by 6.5 times.* ***Conclusion:*** *The present study demonstrated that HCY levels are significantly elevated in the serum of ALS patients compared to well-matched controls. Eleveted homocysteine level increases the risk of ALS. Since most of the ALS cases reveal delayed symptoms; therefore, HCY levels could be considered a biomarker of disease progression in the early phase in ALS patients.*

Keywords: *Homocysteine, Amyotrophic lateral sclerosis, hyperhomocysteinemia.*

Introduction:

Motor neuron disease (MND) is a progressive neurodegenerative disease characterized by progressive muscle paralysis determined by degeneration of motor neurons in the motor cortex, brainstem and spinal cord. First described by Neurobiologist Jean Martin Charcot in 1870s as Charcot's Sclerosis. MND is subdivided into several subtypes on the basis of symptoms and signs which include Amyotrophic lateral sclerosis (ALS),

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Progressive bulbar palsy (PBP), Progressive muscular atrophy (PMA), Primary lateral sclerosis (PLS), Flail arm syndrome (Vulpian-Bernhardt syndrome), Flail leg syndrome (Pseudopolyneuritic form) and ALS with multi-system involvement (*e.g.*, ALS with, frontotemporal dementia, autonomic insufficiency, parkinsonism, supranuclear gaze paresis, and/or sensory loss). Lord Russell Brain proposed the term Motor neuron disease (MND) to incorporate these conditions into a single spectrum of disorder[1].

ALS is the most common and severe form of adult onset MND with an incidence of about 1/100000[2]. ALS is a progressive disease, 50% patients die within 30 months of symptom onset and 20% of patients survive between 5 years and 10 years of symptoms[3]. As many as 10% of patients with ALS survive for more than 10 years, indicating that certain individuals harbor some form of protective factors[4] .

The full pathogenesis of ALS is not well understood but several important factors can be noted including: (1) Genetics (2) Excitotoxicity (3) Oxidative stress (4) Mitochondrial dysfunction (5) Impaired axonal transport (6) Neurofilament aggregation (7) Protein aggregation, (8) Inflammatory dysfunction and contribution of non-neuronal cells (9) Deficits of neurotrophic factors and dysfunction of signaling pathways and (10) Apoptosis [1].

Homocysteine an amino acid generated from dietary Methionine has multiple potential neuro pathologic mechanism of actions including free radical & cytosolic calcium accumulation, mitochondrial dysfunction, apoptotic pathway activation, increased production of â-Amyloid aggregates & excitotoxicity by stimulating NMDA receptor.The neurotoxic and vasculotoxic effect of homocysteine has been widely studied. These neurotoxic mechanisms have also been shown to be relevant in the pathogenesis of ALS & have been linked to damage in the motor neuron[5,6] . Elevated plasma homocysteine is an established risk factor for vascular disease such as stroke, coronary artery disease and venous thrombosis etc.[5]. Elevated plasma homocysteine level have been also observed in patient with Alzheimers disease, PD patient treated with levodopa and recently ALS[5,7]. However, Experimental data showed- even a modest increase of homocysteine may induce oxidative stress and DNA damage and support the hypothesis that oxidative stress, which is increased by homocysteine accumulation, may play a role in ALS pathogenesis[5]. There are some uncertainity whether elevated serum homocysteine levels are a cause or consequence of this neurodegenerative disease, but most studies found its causal relationship.

Several studies found elevated serum homocysteine level with ALS than in normal people[5,6,7,8]. Homocysteine levels were strongly correlated with shorter interval onset diagnosis. ALS cases with shorter time to diagnosis presented higher homocysteine levels, suggesting that higher homocysteine may be linked to faster progression of the disease[5].

Few studies regarding ALS were done in Bangladesh. MND is homogenously distributed with no known etiology, ALS variety was the highest (61%), male to female ratio was 1.79:1, mean ± SD age for ALS was 53±7.18[9].

Elevated homocysteine is associated with duration of illness and also severity of illness defined as progression rate of the disease (ÄFS). The progression rate of the disease (ÄFS) from symptom onset to the time of examination was calculated as follows ÄFS= 48- ALSFRS-R score at the time of examination /duration between symptom onset and time of examination (months)[11]

Measuring serum homocysteine is affordable, accurate, and minimally invasive thus representing an appealing biological marker for Amyotrophic lateral sclerosis. These all studies support the hypothesis that oxidative stress is an important mechanism in ALS and that homocysteine exerts oxidative stress and potentiate disease activity.

A recent small sample double blind clinical trial conducted on 24 Japanese ALS patients found that-short term (4wks) high dosage (.5mg/day) administration of methylcobalamine was effective in improving compound motor action potential (which is used as an indicator of reduced spinal

motor neuron number). Patient with good response to treatment showed slower disease progression[5].

Administration of vitamin B6, B9, B12 or other antioxidant to decrease oxidative stress produced by homocysteine could be a potential therapeutic or modulator agent for ALS and that might play role in slower disease progression and prolong survival. Many studies yet give some confusing results. So the exact role of homocysteine in neurodegenerative process, including ALS, and even whether the elevated homocysteine levels are a cause or a consequence of disease, is not clear. More studies are required to clarify the meaning and possible therapeutic implications

Methods:

The study was conducted at Neuromuscular disorder clinic, inpatient and outpatient department of Neurology, BSMMU, Dhaka from April- 2018 to September- 2019. 42 patients diagnosed as ALS at Neuromuscular disorder clinic, inpatient and outpatient department of Neurology, BSMMU, Dhaka were taken as study population and another 42 age and sex matched healthy subjects were taken as control. Non random purposive sampling technique was followed to collect sample. The Revised El Escorial diagnostic criteria was used to diagnose cases of ALS. Multiple sclerosis, Dementia, Parkinson's disease, familial ALS and other neurodegenerative disorders and systemic conditions which interfere with serum homocysteine level were excluded from the study. After ethical clearance from institutional review board (IRB), all subjects were selected according to the revised El Escorial criteria, and fulfilled the criteria for possible, probable, probable—laboratory-supported or definite ALS. The ALS Functional Rating Scale-Revised (ALSFRS-R) was used to assess the patients 'functional status (severity). Informed written consent was taken from each patient. After taking proper history, physical and neurological examination, NCS and EMG (for cases only,done in our electrophysiology Lab) fasting serum homocysteine level and other relevant investigations were done. The serum Homocysteine level was estimated by using AUTOMATED ANALYZER ARCHITECT PLUS ci4100.The normal ranges of the serum homocysteine level was based on the reference used for serum homocysteine level in the laboratory of Department of Biochemistry & Molecular Biology, BSMMU, Dhaka, Bangladesh. After completion of data collection, data were analyzed with SPSS software properly. Continuous variables (e.g., age, BMI, and serum Homocysteine level) were presented as mean ±SD values. Comparisons between ALS patients and control subjects regarding demographic and laboratory characteristics was performed using Student's t-test and chi-square tests. The correlations between serum Homocysteine levels and the variables were calculated using Pearson's correlation. The cutoff for statistical significance was set at $p<0.05$ for all of the data analyses. Statistical analyses carried out using SPSS version 22.

Results:

Total 42 patients of ALS were studied in this study and similar number of age and sex matched healthy control were taken as comparison. The mean age of the cases and control were 41.55±14.9 years and 42.78±14.87 years respectively. Maximum respondents were in between 30-50 years age group (Table-I). Higher frequency of ALS patients was found in male (78.6%) than female (21.4%).

Table-I

Comparison of age between two groups (n=84)

Age group (years)	Case (n=42) No. (%)	Control (n=42) No. (%)	p-value
<30	11(26.2%)	10(23.8%)	
30-50	18(42.9%)	17(40.5%)	
≥ 50	13(31.0%)	15(35.7%)	
Total	42(100.0%)	42(100.0%)	
Mean±SD	41.55±14.9	42.78±14.87	
Range	(16-78) yrs	(17-70) yrs	0.705^{ns}

Data were expressed as frequency, percentage and mean±SD, Unpaired t-test was done, ns= not significant

In this study involved anatomical site at the onset were identified by interviewing the cases. Among the cases majority 34(80.95%) had the onset of ALS by spinal/limb involvement and bulbar onset were 8(19.05%) (Figure -1).

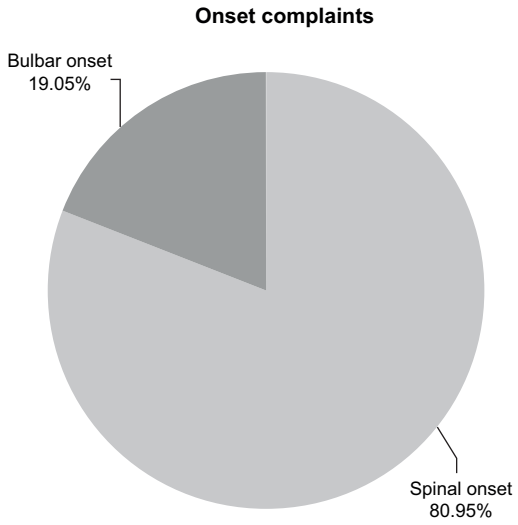

Fig.-1: *Distribution of cases by involved anatomical site at onset:* *n=42

In this study presenting complaints or symptoms were assessed. Among the symptoms, majority of patient (73.81%) had weakness of all four limbs. Among other presenting complaints, twitching of muscles were most common,41(97.62%); 39(92.86%) cases had small muscle atrophy, 33(78.57%) cases had muscle cramp and walking difficulty and 2 cases had neck dropping an unusual presentation of ALS (Table II). Severity of the disease state of the ALS patients was categorized according to revised ALS functional rating scale. The severity scale was mild (>40), moderate (39-30), severe (<30) and advanced (<20). The mean score was 40.02±4.93. Among the cases 41 were in mild to moderate state, 1 case in severe state and none was in advance state of the disease (Table III).

Study variable analysis revealed that the mean Homocysteine level in ALS cases was higher 14.06 mg/dl with SD 7.02 than in control 10.43 mg/dl with SD 2.85 (Table-IV). Where the P value was <0.003 which explains Homocysteine level elevated significantly in ALS.

Study variable analysis revealed that the mean of HCY in bulbar onset ALS is not significantly elevated than spinal onset ALS cases (p value is 0.717) (Table -V).

In this study the frequency of hyper-homocysteinemia (>15µmol/L) among case and control group were found 33.3% versus 7.1% respectively which is statistically significant (p =<0.003). The odds ratio for hyperhomo-

Table-II

Distribution of cases by presenting complaints (n=42)

Presenting complaints	Frequency	Percentage
Weakness in all 4 limbs	31	73.81
Weakness in upper limbs	6	14.29
Weakness in lower limbs	2	4.76
Weakness in single limb	1	2.38
Tongue atrophy	18	42.86
small muscle atrophy	39	92.86
Difficulty in walking	33	78.57
Twitching of muscle	41	97.62
Muscle cramps	33	78.57
Sensory complaints	1	2.38
Speech difficulty	16	38.10
Difficulty in deglutition	16	38.10
Nasal regurgitation	9	21.43
Foot drop	3	7.14
Dropping of head and camptocormia	2	4.76

Table-III

Distribution of ALS cases by severity on the basis of ALS-FRS (revised) (n=42)

Severity of ALS	Frequency	Percentage
Mild (>40)	22	52.4
Moderate (30-39)	19	45.2
Severe (<30)	1	2.4
Advance (<20)	0	0.0
Total	42	100.0
Mean±SD		40.02±4.93

Table-IV

Association serum homocysteine with ALS (n=84)

Serum homocysteine	Case (n=42) No. (%)	Control (n=42) No. (%)	p-value
Elevated Hcy (>15ìmol/L)	14(33.3%)	3(7.1%)	
Normal Hcy (<15ìmol/L)	28(66.7%)	39(92.9%)	
Total	42(100.0%)	42(100.0%)	
Mean±SD	14.06±7.02	10.43±2.85	0.003*
Range	(5.60-44.36) μmol/L	(4.19-17.25) μmol/L	

Data were expressed as frequency, percentage and mean±SD P-value was reached from Unpaired student t-test, *P<0.05 was considered as significant.

Table-V

Comparison of serum homocysteine between spinal onset and bulbar onset ALS (n=42)

	Homocysteine (μmol/L)		P value
	Frequency	Mean ± Std. Deviation	
Spinal onset	34(80.95%)	13.8679 ± 7.50667	0.717
Bulbar onset	8(19.05%)	14.8850 ± 4.72887	

P value was reached from unpaired student t-test, The mean difference is not significant.

Table-VI

Frequency of hyperhomocysteinemia among case and control group (n=84)

Serum homocysteine	Case (n=42) No. (%)	Control (n=42) No. (%)	p-value	OR 95% CI
Elevated Hcy (>15ìmol/L)	14(33.3%)	3(7.1%)	0.003*	6.50(1.71-24.78)
Normal Hcy (<15ìmol/L)	28(66.7%)	39(92.9%)		

OR= Odds Ratio, Data were expressed as frequency & percentage. Unpaired t-test was done, *significant

1cysteinemia between case and control is 6.5(95% CI: 1.71-24.78).The Odds ratio was 6.5, indicating that the homocysteine higher than >15μmol/L increases the risk of ALS by 6.5 times (Table-VI).

Discussion:

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of the motor neurons of motor cortex, brainstem, and spinal cord. The

present case-control study was targeted to find out the association of serum Homocysteine level with ALS. For the research, total 42 patients (ALS cases) were selected of all age group along with control group. They were interviewed by specific questionnaire to find out the association.

Respondents were taken from all age group irrespective of sex and religion. The mean age of the cases was 41.55±14.9 years and mean age of the control was 42.78±14.87 years, which resembles, the case and control were taken from same population.) research identified the mean age of onset of ALS varies from 50 to 65 years in a study done by Logroscino et al[1]. (2008. Only 5% of the cases have an onset <30 years of age with the median age of onset was 64 years. In our study mean age of ALS was found low in comparison to other studies.

Out of 42 cases 33 (78.57%) were male and 9 (21.43%) were female. Among the control population 33 (78.57%) were male and 9 (21.43%) were female. The age-adjusted incidences of 1.27 per 100000 person-years in males and 1.03 per 100 000 person-years in females were lower than recent rates in the northern US, Canadian, and northern European studies but higher than rates in southern European studies.

In this study the involvement of anatomical sites at onset were identified by interviewing the cases. Among the cases majority 34 (80.95%) had the onset of ALS by spinal involvement and Bulbar onset 8 (19.05%) were prominent. According to the result about 19% of cases, muscles of the bulbar region are affected first. In approximately 25% of patients, weakness begins in bulbar-innervated muscles (bulbar-onset ALS)[13]. Because motor neurons in the part of the brain stem called the medulla oblongata (formerly called the “bulb”) start to die first along with lower motor neurons “bulbar onset”.

In this study presenting complaints or symptoms were assessed. Following the symptoms, majority cases 34 (81%) had weakness in four limbs. Among other presenting complaints, Twitching of muscles were most common, 41 (97.62%). 39 (92.86%) cases had small muscle atrophy, 33 (78.57%) cases had Muscle cramps.

Homocysteine (HCY) is considered to be one of the most important oxidative stressor in the blood, while elevated Homocysteine levels are found in many disease states, elevated Homocysteine levels are reported in many neurodegenerative diseases, including Alzheimer’s disease, Parkinson’s disease, Multiple sclerosis, and Amyotrophic lateral sclerosis (ALS). Various studies showed consistently that ALS patients have elevated serum Homocysteine levels than healthy individuals, more prominently in cases with longer disease duration.

In our study, variable analysis revealed that the mean of Homocysteine in ALS cases is elevated 14.06 mg/dl with SD 7.02 than in control 10.43 mg/dl with SD 2.85. Where the P value is <0.003 which explains Homocysteine level elevates significantly in ALS.

Several studies found elevated serum homocysteine level with ALS than in normal people[5,6,7,8]. Elevated homocysteine is associated with duration of illness and also severity of illness defined as progression rate of the disease (DFS)[5].

Study variable analysis revealed that the mean of Homocysteine in ALS cases is significantly elevated than healthy control (P value is <0.003). Similar report was found in[5,6,7,8]. In our study we have found no significant difference of mean of Homocysteine between bulbar and spinal onset ALS which is in contrast to the result of Zoccolella et al[13].

The current study design does not allow us to conclude the causal relationship between elevate Homocysteine level and ALS but increased serum levels of Homocysteine in ALS patients compared to control might be the outcome of an oxidative stress-related process in the central nervous system. If the elevated Homocysteine concentration is a secondary event, a pharmacological decrease in the Homocysteine level might contribute to the decreasing oxidative stress, and thus slow down disease progression.

Conclusion:

HCY is an excitatory amino acid, which markedly enhances the vulnerability of neurons to oxidative injury. The present study demonstrated that HCY

levels are elevated in the serum of ALS patients compared to well-matched controls. Since most of the ALS cases reveal delayed symptoms; therefore, HCY levels could be considered a biomarker of disease progression in the early phase in ALS patients. It also supports the hypothesis that oxidative stress is involved in the pathogenesis of ALS, and (HCY might increase disease activity by producing oxidative stress). This pathogenetic pathway could lead to new directions for future therapeutic interventions.

Ethical issues

All patients gave written informed consents and the study was approved by Institutional Review Board of Bangabandhu Sheikh Mujib Medical University.

Conflict of interests: None

References:

1. Wijesekera, L. C. & Leigh, P. N. 'Amyotrophic lateral sclerosis' *Orphanet journal of rare diseases. 2009;* 4, 3, 3.
2. Zarei, S., Carr, K., Reiley, L., Diaz, K., Guerra, O., Altamirano, P. F., Pagani, W., Lodin, D., Orozco, G. & Chinea, A. A comprehensive review of amyotrophic lateral sclerosis. *Surgical neurology international. 2015;* 6.
3. Talbot K. Motor neurone disease, Postgrad Med J. 2002;78:513-519.
4. Goodall, E. F. & Morrison, K. E. Amyotrophic lateral sclerosis (motor neuron disease): proposed mechanisms and pathways to treatment. *Expert reviews in molecular medicine. 2006;* 8, 1-22.
5. Zoccolella, S., Simone I.L. Samarelli, V, Tortetelli, R. Elevated plasma homocysteine levels in patients with ALS. Neurology. 2008; 70, 222-5.
6. Levin, J., Botzel, K., Giese, A., Vogeser, M & Lorenzl, S. Elevated levels of mythylmalonate and Hcy in Parkinsons disease, PSP and ALS, Dement Geriatr Cogn Disord. 2010; 29,553-559.
7. Valentino, F., Bivona, G., Butera, D., Paladino, P., Fazzari, M.,Piccoli, T & Ciaccio, M. Elevated CSF and plasma homocysteine levels in ALS, Europian Journal of Neurology. 2010; 17: 84-89.
8. Hong-li, W. & sheng, F. D.The relation between plasma homocysteine level and ALS, chin J Med. 2012; 51(04): 308-310.
9. MA HAYEEa, M NUSRATULLAH. Plasma and CSF Aluminium in Sporadic Motor Neuron Disease (MND). J Bangladesh Coll Phys Surg. 2005; 23 : 101-106
10. Kimura, F., Fujimura, C., Ishida, S., Nakajima, H., Furutama, D., Uehara, H., Shinoda, K., Sugino, M. & Hanafusa, T. Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. *Neurology. 2006.* 66; 265-267.
11. Logroscino, G., Traynor, B.J., Hardiman, O., Chio, A., Couratier, P., Mitchell, J.D. 'Descriptive epidemiology of amyotrophic lateral sclerosis: New evidence and unsolved issues', *J NeurolNeurosurg Psychiatry*. 2008;79, 6-11.*
12. Daroff, R. B., Jankovic, J., Mazziotta, J. C. & Pomeroy, S. L. *Bradley's neurology in clinical practice*, 7th edition, Elsevier Health Sciences. 2015; 1508.
13. Zoccolella ,S., Bendotti, C., Beghi, E & Logrocino, G. Homocysteine levels and ALS: A possible link, Amyotrophic Lateral sclerosis. 2010. 11; 140-147.