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Association of Cerebrospinal Fluid Tau Protein in Patients with Alzheimer's and Non Alzheimer's Dementias in a Tertiary Level Hospital in Bangladesh

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Abstract Background: Alzheimer's disease is one of the common causes of dementia in our country. The growth of the elderly population, together with the rising incidence of dementia requires immediate attention. There is very limited data regarding how CSF Tau protein correlates with this group of people's cognitive function. Objectives: To evaluate association of CSF Tau protein in different types of dementia patients (AD and non-AD) and to find out the correlation of CSF Tau with severity of dementia and duration of disease. Methods: This cross sectional analytical study was conducted in dementia clinic (OPD) and inpatient department of Neurology, BSMMU from March'2013 to September'2015. 48 both male and female adult subjects were included in this study. Then they were divided into 3 groups: Alzheimer's disease (AD) group (n=15), non-AD other dementias (OD) group (n=18) and subjects having neither AD and/or OD were included as control (n=15). CSF Tau protein was measured and compared between 3 groups. Results: Mean age of dementia in AD group was 68.2±9.33 years and in OD group was 67.72±12.74 years. Mean MMSE score in AD group was 13.47± 4.72, in OD group 15.83±3.31 and in control group 28.60±1.12. This study showed that CSF Tau protein was highly elevated in AD group (315.30±279.68 pg/ml) than in OD (57.08±27.41pg/ml) and control (39.23±12.21) groups. Conclusion: The study found that CSF Tau levels are elevated in AD patients in comparison to non AD other dementias. So, CSF Tau protein can be an early biomarker of Alzheimer's disease.

Keywords: *Alzheimer's disease, ANOVA, CSF Tau protein, Dementia*

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1. Introduction

Dementia is an acquired and persistent compromise in multiple cognitive domains that is severe enough to interfere with everyday functioning [1]. As life expectancy increases, the worldwide number of demented patients is projected to grow from 24.3 million in 2001 to 81.1 million in 2040 [2]. 10% of all above 70 years has memory impairment, of them 50% have AD [3]. The most common forms of dementia are Alzheimer's disease (AD) and vascular dementia (VaD), with respective frequencies of 70% and 15% among all dementias [4]. The classic clinical features of Alzheimer's disease (AD) are an amnesic type of memory impairment deterioration of language and visuospatial deficits. Motor and sensory abnormalities, gait disturbances, and seizures are uncommon until the late phases of the disease [5]. Dementia is commonly recognized with use of the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) [6]. Neuroimaging plays an important role in the diagnosis of Alzheimer's disease and is particularly helpful in excluding alternative causes of dementia. Diagnosing AD is especially difficult at the beginning of the disease since early symptoms are often nonspecific and can as well be present in a variety of other disorders. Therefore, there is an increasing need to develop objective tests to aid in the diagnosis of early AD and to assess the efficacy of therapies. Furthermore, follow-up of the pathologic progression in patients with AD or in persons at risk of developing the disease is impossible without specific biologic markers. Accumulation of neurofibrillary tangles (NFTs) intraneuronally is one of the pathological hall marks of AD [7,8,9]. In more than 80% of AD patients the most consistent finding protein is microtubule associated protein i.e. tau in cerebrospinal fluid (CSF) which has direct contact with brain, where the change or modification in the protein levels can be monitored biochemically [7]. Currently 'tau' is the most

investigated marker in the diagnosis of AD. Vandermeeren et al. 1993 [10] first showed increased CSF tau concentration in AD. Since then many studies have confirmed the increase of total CSF tau protein concentration in AD patients compared to controls [11,12,13]. Therefore, CSF Tau levels may be considered as potential biochemical marker for AD because it represents directly to the neuronal (axon) degeneration. Previous studies revealed that with the CSF Tau estimation, AD could be differentiated from other dementias such as fronto- temporal dementia (FTD) and Lewy body disease with dementia (DLB) [9]. So, based on these findings we hypothesized that CSF Tau levels reflects the degree of neuronal damage in dementia. This hypothesis was tested among 15 AD, 18 non-AD other dementia and 15 neurological control patients who provided CSF samples during their clinical work up. The objectives of the present study were to estimate and compare CSF Tau protein levels among AD patients, non AD other dementias (OD) and age and sex matched non dementia neurological controls who were admitted in department of Neurology, BSMMU.

2. Materials and Methods

After approval of the Institutional Review Board of BSMMU, informed consent was obtained from each patient or their caregivers. Altogether 48 patients were enrolled over 3 years in three groups.

2.1. Alzheimer's Disease (AD) Group

Inclusion criteria:

Fifteen (15) AD patients, of either sex, above 18 years of age were recruited. Patients underwent extensive clinical neurologic examination including neuropsychological tests, Mini-Mental State Examination (MMSE), EEG, brain CT or MRI, routine laboratory tests, and CSF analysis.

Exclusion criteria:

The patients who were less than 18 years with AD, doubtful AD, AD overlapping with other dementias and with other neurological diseases were excluded from this group.

2.2. Non-AD Other Dementias (OD) Group

Inclusion criteria:

Eighteen (18) patients, of either sex, more than 18 years, with dementias other than AD, like Vascular Dementia (VaD), Parkinson's disease with Dementia (PDD), Normal Pressure Hydrocephalus (NPH), Frontotemporal Dementia (FTD), Progressive Supranuclear Palsy (PSP) and Dentatorubropallidoluysian atrophy (DRPLA). Patients underwent extensive clinical neurologic examination including neuropsychological tests, MMSE, EEG, brain CT or MRI, routine laboratory tests, and CSF analysis.

Exclusion criteria:

The patients who were less than 18 years and with other neurological diseases were excluded in this group.

2.3. Neurological Controls (NC) Group

Inclusion criteria:

Fifteen (15) NC group of either sex, above 18 years, with other neurological diseases for which dementia is not a component like polyneuropathy, motor neuron disease (MND), demyelinating diseases like multiple sclerosis, amyotrophic lateral sclerosis (ALS), meningitis, encephalopathy, cervical myelopathy, myopathy etc.

Exclusion criteria:

Neurological diseases with dementia as a component, hypothyroidism, cerebral palsy, psychomotor patients were excluded from this group.

2.4. Study Procedure

Patients having features of AD and non-AD other dementias were included according to the DSM-IV criteria. All patients were submitted to a diagnostic workup investigation, including clinical history, physical and neurological examination, and appropriate blood tests to find out different causes of dementia. CT and/or brain MRI and other complementary exams if necessary were done. In every case, the clinical diagnosis was made before CSF examination. The diagnosis of probable AD was based on the DSM-IV & NINCDS-ADRDA criteria5. For the AD and non-AD group, the severity of dementia was classified as mild, moderate or severe. The diagnosis of fronto-temporal dementia (FTD) was made according to the Lund/Manchester criteria [14]. Vascular dementia (VaD) patients were selected according to probable VaD criteria of NINDS-IREN [15]. A total of 33 dementia patients were taken as sample. They were first diagnosed to have dementia by DSM-IV and then divided into two groups: patients with Alzheimer's Dementia (AD group) and non-Alzheimer's dementia (non-AD group). Another 15 non dementia control subjects admitted in department of Neurology who undergone LP and CSF study due to diseases other than dementia were included as neurological control (NC group) to compare with AD and non-AD group. The cognitive impairment was assessed by Bengali version of MMSE [16] (Mild 21-24, Moderate 10-20 and Severe <10) at the time of recruitment. CSF Tau was measured and compared between 3 groups.

2.5. CSF Analysis

CSF was obtained by lumbar puncture with all aseptic technique between the L3/L4 or L4/L5 intervertebral space, using a 23-gauge needle, and collected in 10-mL polypropylene tubes. Patient were fasted overnight, CSF collected between 08.00 to 10.00 am .Within 2 hours, CSF samples were centrifuged at 1500 rpm for 10 minutes at 4°C. A small amount of CSF was used for routine analysis. Aliquots of each sample were immediately frozen at -70°C until further analysis. CSF T-Tau concentrations were determined using commercially available sandwich ELISA kits (Innotest, hTau, Innogenetics, Ghent, Belgium) in the department of Biochemistry, BSMMU, Dhaka.

2.6. Statistical Analysis

The clinical diagnoses, CSF Tau levels in different types of dementia were compared and analyzed. Patients with Alzheimer's disease was defined as AD and non AD dementias were collectively defined other dementias (OD).

An optimal cut off line will be calculated comparing AD vs OD. Based on the cut off line the percentage of patients with a CSF AD profile was calculated. The comparison between AD, OD and neurological control groups were made by the ANOVA test; Post-hoc analysis was performed using the Bonferroni adjustment, where appropriate. In the AD group, the correlation between CSF Tau levels and severity of dementia, MMSE scores and duration of the disease was made by the Pearson and Spearman's rank correlation test. Statistical significance was set at $p < 0.05$.

3. Results

The demographic profile has been depicted in Table 1. Mean age of AD patients were 68.2 years, OD patients 67.72 years and that of the neurological controls were 48.27 years. In AD group 9(60%) were male and 6(40%) were female, in OD group 14(77.78%) were male and 4(22.22%) were female. Among the control group, 8(53.33%) were male and 7(46.67%) were female. Among the cases, mean disease duration in AD group was 2.9 years and OD group 4.67 years. There was no significant difference in age and sex between dementia cases and non demented neurological controls. Distribution of respondents by MMSE score shows mean MMSE score in AD group was 13.47 (range 6-10) with a standard deviation of ± 4.72, in OD group it was 15.83 (range 8-21) with a standard deviation of ± 3.31 and in NC group was 28.60 (range 27-30) with a standard deviation of ± 1.12. The concentrations of CSF Tau in AD, OD, and NC are presented in Table 2, which clearly depicts the increase in the concentration of CSF Tau in AD group in comparison with the OD and NC. The mean ± SD values for AD was 315.30 ± 279.68, in OD group it was 57.08 ± 27.41and in control group was 39.23 ±12.21 (Figure 1). The P value of AD with respect to the OD or NC is statistically significant (0.001) (Table 3). We obtained a significant correlation in the AD group, between levels of CSF Tau level and MMSE scores (r=-0.552, P<0.05) (Figure 2) and duration of disease (r=+0.652, P<0.01) (Figure 3).

Table 1. Demographic profile of the study subjects (n=48)

	AD	OD	NC
Number of subjects	15	18	15
Age	68.20±9.33	67.72±12.74	48.27±11.27
Sex(M:F)	9:6	14:4	8:7
Disease duration (Years)	2.90±2.54	4.67±3.70	0.84±1.28
MMSE Score	13.47±4.72	15.83±3.31	28.60±1.12

AD = Alzheimer's dementia, OD = Non-AD other dementia, NC = Neurological controls (No Alzheimer's and/or other dementia), MMSE =Minimental state examination.

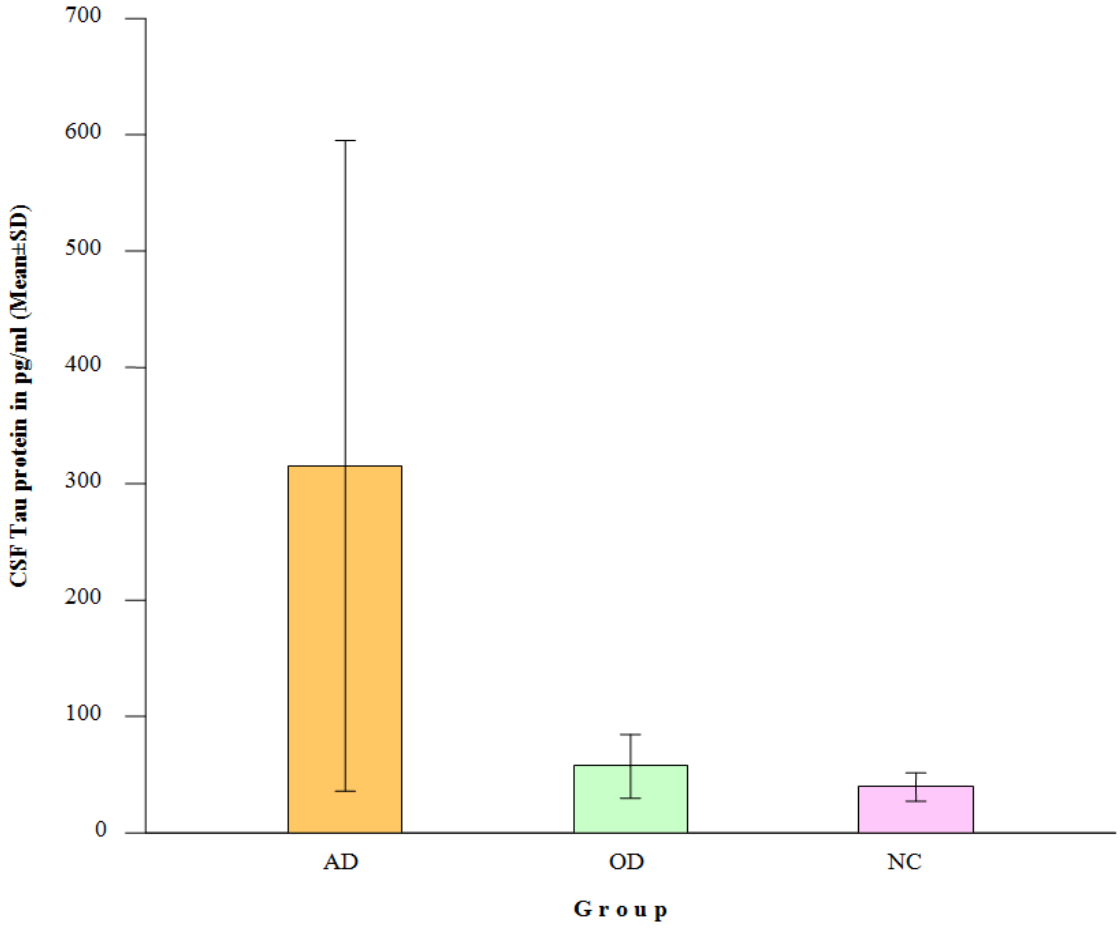

Figure 1. CSF Tau protein levels in study groups

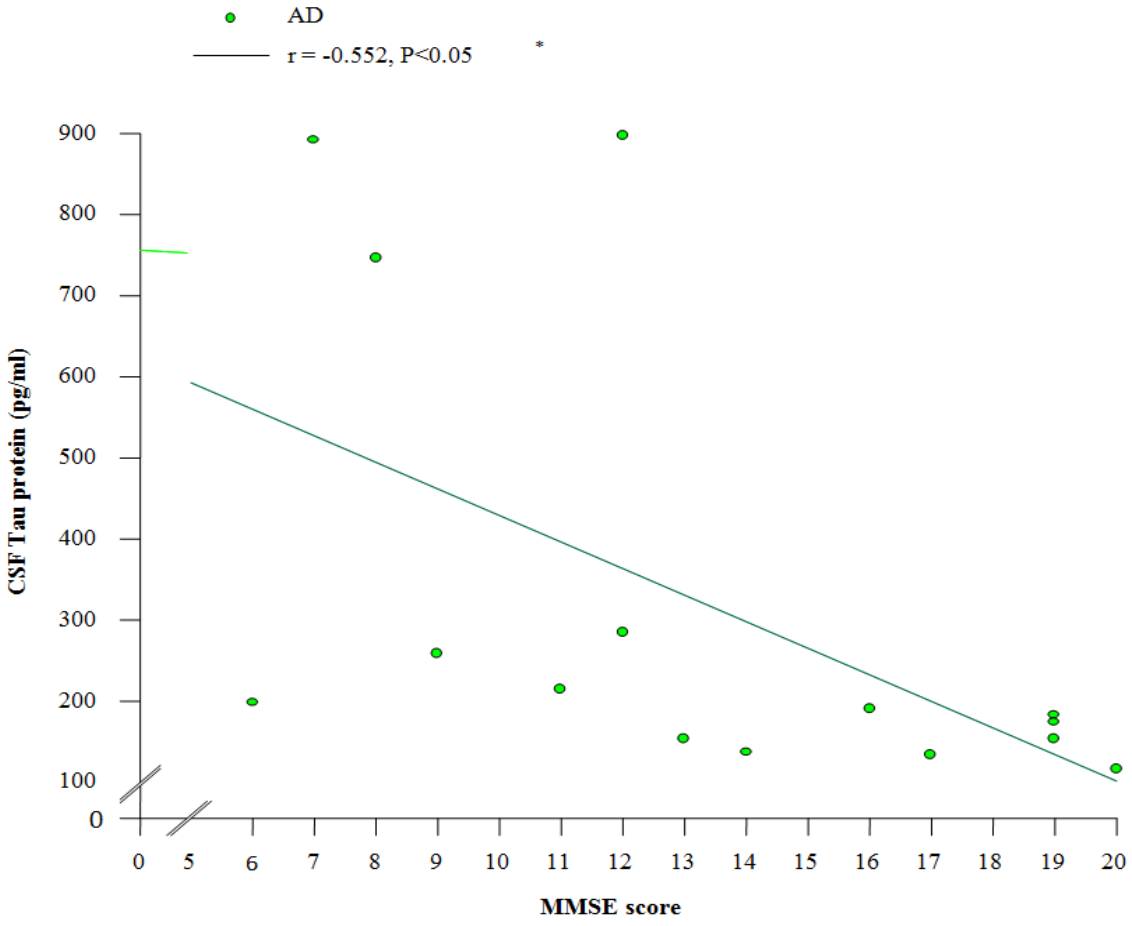

Figure 2. Relationship between MMSE score and CSF Tau protein in AD patients (n=15)

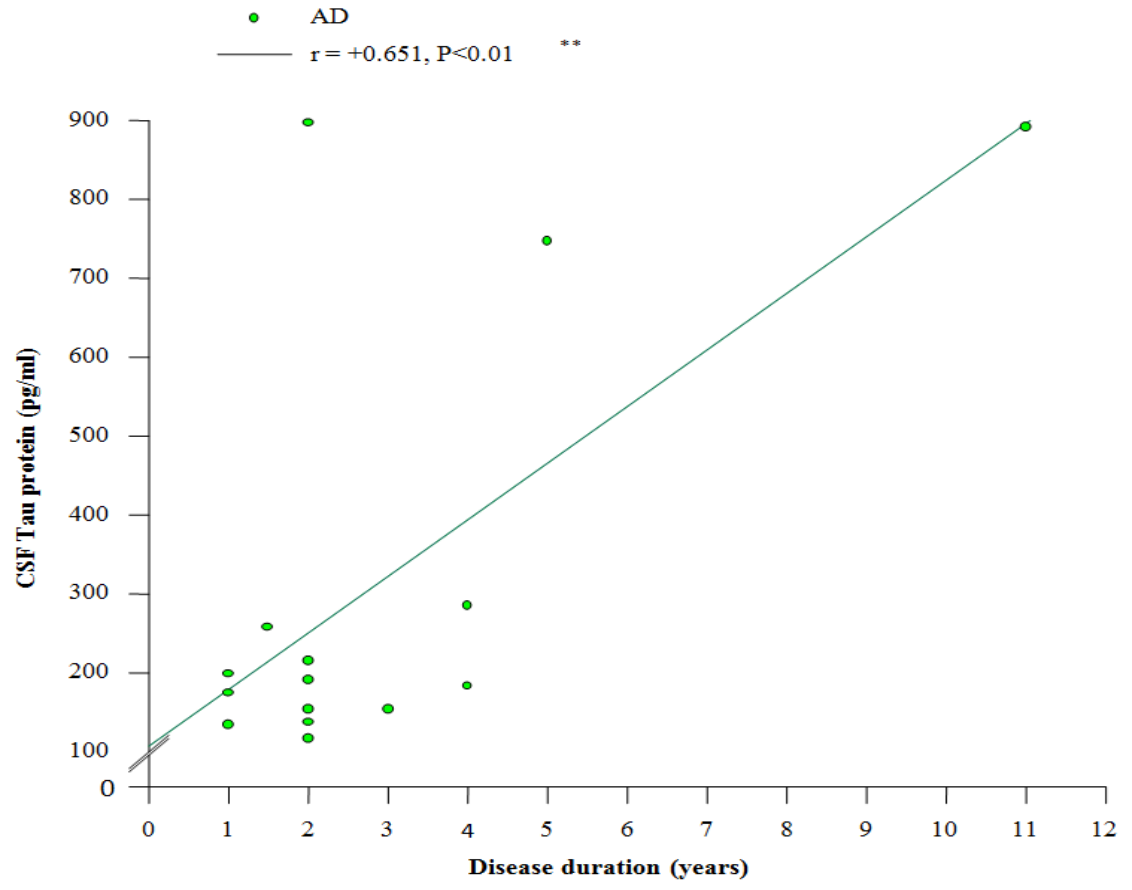

Figure 3. Relationship between disease duration and CSF Tau protein in AD patients (n=15)

Table 2. Distribution of respondents by CSF Tau protein level (n=48)

Groups	Range	Mean±SD
AD (n=15)	115.50-896.75	315.30±279.68
OD (n=18)	21.76-109.25	57.08±27.41
NC (n=15)	21.76-58.28	39.23±12.21

AD = Alzheimer's dementia, OD = Non-AD other dementia, NC = Neurological controls (No Alzheimer's and/or other dementia), MMSE = Minimental state examination.

Table 3. Comparison of CSF Tau protein between groups (n=48)

Parameter	AD (n=15) Mean±SD	OD (n=18) Mean±SD	NC (n=15) Mean±SD
CSF Tau Protein (pg/ml)	315.30±279.68	57.08±27.41	39.23±12.21
			P value
AD vs OD			<0.001***
AD vs NC			<0.001***
OD vs NC			1.000ns

AD = Alzheimer's dementia, OD =Non-AD other dementia, NC =, Neurological controls (No Alzheimer's and/or other dementia). One-way ANOVA (PostHoc, Bonferroni) test was performed to compare between groups. ns = Not significant, P<0.001 = Significant.

4. Discussion

Dementia is not a specific disease. It's a general term that describes a wide range of symptoms associated with a decline in memory or other thinking skills severe enough to reduce a person's ability to perform everyday activities. CSF is an ideal source for developing viable biomarkers in AD as it directly interacts with the extracellular space in the brain, thus potentially reflecting the associated biochemical/pathologic changes [17]. As a result, CSF biomarkers have been become accepted and adopted to varying degrees for the clinical diagnosis of AD in different countries. This cross sectional analytical study was conducted in the department of Neurology, BSMMU, Dhaka from March 2013 to September 2015 to evaluate the association of CSF Tau protein in AD and non-AD other dementias. In this study 15 cases were found to be of AD, next to it is the mixed dementia (MD) and then vascular dementia (VaD). In Bangladesh, according to the WHO report 2011, AD ranked 28th in the world in respect of age adjusted death rate 10.96 per 100,000 of population. Another study on prevalence of dementia in Bangladesh showed that AD is common after vascular dementia [18]. In our study, mean age of AD patients were 68.2 years, OD patients 67.72 years. In UK, the prevalence of dementia varied little by age or gender, increasing from 55.6% among those aged 65–69 to 64.8% in those aged 95 and over [19]. A lesser age for dementia in our country may be due to life expectancy which is in male 68.75 years and in female 72.63 years with average of 70.65 years [20] whereas in UK it is 81 years and in the USA it is 79 years [21]. A similar result was found and reported that cases of dementia are increasing due to longer life expectancy and 10% of all above 70 years has memory impairment and 50% above the age of 85 years have dementia [3]. The distribution of study population according to sex was recorded in this study. It shows, among AD group 9 (60%) were male and 6(40%) were female, in OD group 14 (77.78%) were male and 4(22.22%) were female. Among the control group, 8(53.33%) were male and 7 (46.67 %) were female. Similar result was reported by Hoffman et al. 1991 [22] and mentioned that in subjects under 75 years, the prevalence of dementia was slightly higher in men than in women. But Schmidt R 2008 [23] reported that the prevalence of AD is higher in women than in men. According to Ruitenberg et al. 2001 [24] overall, dementia incidence is similar for men and women (rate ratio women versus men: 1.00, 95% CI: 0.80-1.24). The distribution of respondents by MMSE score was recorded. Mean MMSE score in AD group was 13.47 (range 6-10) with a standard deviation of ± 4.72, in OD group it was 15.83 (range 8-21) with a standard deviation of ± 3.31and in AD group was 28.60 (range 27-30) with a standard deviation of ± 1.12. The severity of the disease was classified according to MMSE score. MMSE score above 27 was considered normal. In this study neurological control (NC) group having no dementia fell in this category. MMSE score 21-24 was considered to have mild dementia, score 10-20 moderate dementia and score below 10 as severe dementia. In this study, most of the cases in both AD and OD groups had moderate dementia (MMSE score 10-20). The explanation may be attention and seriousness of the caregivers was delayed initially due to lack of knowledge and awareness which led them not to come during mild stages of the disease. Due to difficulties in obtaining CSF samples from normal controls and as to the best of our knowledge there is no previous study done on CSF Tau protein we have been unable to establish a standard value for this study. To overcome this, we took CSF samples from non dementia neurological control patients who were admitted in inpatient department of Neurology, BSMMU and undergone LP for other purposes. Mean CSF Tau level in AD group was 315.30 pg/ml; that is far higher than that of OD group 57.08 pg/ml and NC group 39.23 pg/ml. This finding was similar to previous reports [25,26]. These findings also agreed with those of Vandermeeren et al.1993 [10] although their values are higher than ours. Comparison was done to see significance of CSF tau variation between 3 groups. Post Hoc ANOVA shows a significant difference between CSF Tau levels of AD vs OD groups and AD vs Control groups ($p< 0.001$). No differences were observed between CSF Tau levels of OD and Control groups ($p=1.000$). The statistical analysis also showed that there was significant difference in AD patients according to duration of the disease ($p<0.01$,

severity of dementia ($p<0.05$) and MMSE scores ($p<0.05$) like other previous results [25,26,27]. There was strong correlation, in the AD group, between levels of CSF Tau levels and severity of dementia ($r=+0.523$; $p<0.05$). Previous study (Vickers et al. 1994 [28]) suggests that there are changes in the cytoskeleton of hippocampal neurons associated with age and they might be potentiated in Alzheimer's disease, leading to neurofibrillary tangle formation and cellular degeneration. This neuronal degeneration could justify the high CSF tau concentrations found in this study in patients with moderate Alzheimer's disease. We found it of interest to study if CSF-tau changes with time during the disease process. There was a strong correlation between levels of CSF tau and duration of dementia ($r= +0.651$, $p<0.01$). Although most previous studies have not found any significant correlations between CSF-tau and duration of dementia [29] but Tato et al. 1995 [30] found a positive correlation between CSF-tau concentrations and duration of dementia. We also tried to see the correlation between CSF tau concentrations and MMSE scores and it was found that there was negative correlation between them ($r=- 0.552$, $p< 0.05$). Most previous studies have not found any significant correlations between CSF-tau and severity of disease/MMSE scores [29] although Tato et al. 1995 [30] found a decrease in CSF-tau with higher MMSE score.

5. Conclusion

The present study found that CSF Tau levels are consistently elevated in AD patients in comparison to non AD patients. In AD group, there was strong positive correlation of duration of disease and dementia severity with CSF Tau level but negative correlation between MMSE score and CSF Tau protein level. So, CSF Tau could be an initial screening marker for the detection of AD from other non-dementing neurodegenerative disorders.

6. Limitations

Sometime the recruited patients disagreed to have undergone LP as it is an invasive procedure. AD cases were diagnosed clinically, not always supported by radiology due either to unavailability of CT or MRI with dementia protocol. Sample size was small in relation to huge number of population of Bangladesh. The study was done in limited time of span, respondents were collected from only one center hence it may not represent the whole population of the country.

7. Recommendations

Broad based studies involving multiple hospitals/ institutions of different parts of the country would be done. As phosphorylated tau (p-Tau) is more specific for AD diagnosis, so further research to see the association of p-Tau with AD should be done. Research should now focus on mild cognitive impairment (MCI) without dementia, which could be a preliminary stage of AD. Finally, epidemiological studies are also necessary to assess the evolution over time of the burden of Alzheimer's and non-Alzheimer's dementias at the community level in Bangladesh in order to have a better knowledge of the need in terms of resources and its progression over time.

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Competing Interests

None.

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