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Association of Electrophoretically Resolved Low Molecular Weight Protein Fractions with Senile Dementia in Elderly People

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Abstract.- The study was designed to investigate the relationship of serum protein fractions with senile dementia in elderly people (>65 years of age; n=36). A Mini Mental State Examination (MMSE) was conducted to screen and categorize the subjects, on the basis of MMSE score, as normal, mild, moderate and severe dementia subjects. Severe dementia subjects were receiving multivitamins and protein supplements since three months. Protein fractions, resolved by Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis, were quantified and analyzed, statistically, in finding the enhancement/reduction and appearance/disappearance of particular protein fractions for comparison amongst the groups. Eight protein fractions (ranging 66-14kDa) were detected in all of the groups. Significant reductions were observed in 66, 54, 20 and 14kDa protein fractions in mild, moderate and severe dementia subjects as compared to controls. No significant alterations in 40, 33 and 25kDa protein fractions were observed in mild dementia, however, the fractions declined significantly in moderate and severe dementia subjects as compared to control group. Fraction of 23kDa showed significant elevations in mild and severe dementia subjects with no variations in moderate dementia group as compared to control group. There was a progressive decline in most of the serum protein fractions with advancing stage of senile dementia but in severe dementia group, protein profile was closer to control group. It might be due to the supplemented diet provided to severe dementia people, indicating that well balanced diet if provided for longer time periods might be helpful either in treating dementia or preventing its manifestation before its onset.

Key words: Senile dementia, protein profile, elderly people.

INTRODUCTION

Dementia is characterized by a progressive deterioration of cognitive skills that leads to decline in the ability to perform daily activities (Canadian Study of health And Aging Working Group, 1994). It is a highly frequent disorder among elderly persons. Its prevalence increases exponentially with age to more than 30 percent among persons of 85 years and over (Ott *et al.,* 1995). Dementia diminishes the individual's ability to live independently and adversely affect the quality of life. Therefore, it would be of great advantage if modifiable risk factors for dementia could be identified (Heyman *et al.,* 1984; Katzman *et al.,* 1989; Yoshimasu *et al.,* 1991).

Alzheimer's disease (AD) is a common neurodegenerative disorder that causes senile dementia. The pathological characteristics are the appearance of neurofibrillary tangles comprising abnormally phosphorylated tau and senile plaques composed of amyloid beta-protein depositions (Suzuki *et al.,* 2006). A characteristic symptom of AD dementia is associated with dysfunctions of cognitive memory such as calculation, space orientation, and speech impairment (Brunden *et al.,* 2008).

There is growing evidence that some kind of mental exercise can help dementia (Farlow and Cummings, 2007). Physical activity prevents cognitive decline in older community-dwelling women (Yaffe *et al.,* 2001).

The pineal hormone, melatonin, is known to decrease in normal aging, and are specially low in Alzheimer's disease (Skene *et al.,* 1990). Hypoalbuminemia is a risk factor for dementia in hemodialysis patients (Huang *et al.,* 2004). Low albumin, low haemoglobin and low BMI are independently associated with poor cognitive performance in community-living older adults (Te-Pin Ng *et al.,* 2008).

Several strategies for drug intervention in both the treatment and prevention of AD has been pursued, but so far there is no fully effective cure without side effects. Transplantation of nerve cells and genetic therapy are looked upon as new

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perspectives (Brunden *et al.*, 2008). People with mild cognitive impairment do not always develop dementia. However, when dementia does occur, it usually gets worse and often decreases quality of life and lifespan (Farlow and Cummings, 2007).

The study was, therefore, designed to correlate the stages of senile dementia with changing serum protein profile in elderly so as to identify the marker protein/s associated with particular stage of dementia. Marker protein/s, if identified, may be helpful in early prediction of dementia with progressing age, long before its manifestation. Employment of preventive measures like diet and exercise therapy at an earlier stage might be helpful in prevention or slow progression of the disease.

MATERIALS AND METHODS

Subjects

The study was approved and conducted at Ghurki Trust Teaching Hospital, Lahore Medical College, Lahore with the involvement of a registered psychatrist. A proforma, based on the criteria of the study, was designed. The volunteers fulfilling the criteria and agreeing to participate were finally selected. The purpose of investigation was explained to volunteers and an informed consent was obtained from each of the participants before his/her recruitment for the study. Serum blood samples of volunteers with more than 65 years of age were collected with the help of registered technician. A Mini Mental State Examination (MMSE) was conducted to screen the senile dementia. Later on, these subjects were categorized on the basis of MMSE score into four groups *i.e.* normal, mild, moderate and severe dementia subjects. Severe dementia subjects receiving multivitamins and protein supplements during the past one month were also included in the study.

Sampling and procedures

Blood (5ml) was drawn out and added to acid wash test tubes. The serum was isolated by centrifugation at 3000 rpm for 15 minutes and were stored at -20ºC until used for analysis. For SDS-PAGE, the serum samples were diluted with loading dye and distilled water and proteins were denatured by heating with loading dye (1.54 g dithiothreitol, 2 g sodium dodecyl sulfate, 8 mL of 1.0 M Tris HCL; pH 6.8, 10 mL of glycerol and 20 mg of bromophenol blue dye) in boiling water bath for two minutes before loading on the gel. Lyophilized mixture of 7 proteins (Sigma SDS7-IVL; 124K6045) for low molecular weight proteins (66-14.2 kDa) were used as molecular weight markers. It was reconstituted in 1.5ml of sample buffer (0.0625M Trizma HCL pH 6.745, containing 2% SDS, 5% mercaptoethanol, 10% glycerol and 0.001% bromophenol blue), heated in a boiling water bath for 2 minutes and stored at -20^{o}C.

Polyacrylamide gels (15%) were prepared (Laemmli, 1970). Protein markers and each of the samples were loaded in wells and gels were electrophoresed at a current supply of 20mA and voltage of 200 volts in a cooling chamber maintained at 4°C. Electrophoresis was stopped, immediately, after dye seemed to diffuse in the buffer in the lower chamber. Following electrophoresis, the 15% gels were stained with coomassie brilliant blue for two hours. After staining, the gels were destained until the clearance of blue background. Protein fractions of different molecular weights were visible in the form of blue bands on a transparent background. Gels were photographed and their image were saved for protein quantification by Gene Genius Bio-imaging Gel Documentation System that provides the data of molecular density and the total area covered by each fraction.

Statistical analyses

The data was analyzed statistically using Student 't' test and employed in finding the enhancement or reduction and appearance or disappearance of particular protein fractions for comparisons among the demented and the control subjects.

RESULTS AND DISCUSSION

Eight protein fractions ranging 66-14kDa were detected in all of the study groups. No new fractions were detected in demented group when compared to control group (Fig. 1).

The average percent raw volume covered by 66kDa protein fraction was found to be 31.84±0.23 in controls, 28.88±0.25 in mild dementia, 26.08±0.13 in moderate dementia and 30.64±0.40 in severe dementia subjects indicating a highly significant decrease of 9% (p=0.0000) in mild, 18% (p=0.0000) in moderate and 4% (p=0.024) in severe dementia subjects when compared to controls (Fig.1a). These results are in agreement with observations of many researchers who have suggested that albumin is a potent inhibitor of amyloid beta (Aß) polymerization and has been implicated in Alzheimer's disease (AD) since it can bind to and transport Aß, the causative agent of AD (Quinlan *et al.,* 2005). It is also suggested that low albumin, low haemoglobin and low BMI are independently associated with poor cognitive performance in community-living older adults (Te-pin-ng *et al.,* 2008).

Protein fraction of 54kDa exhibited average percent raw volume of 14.90±0.13 in controls, 14.17±0.16 in mild, 8.93±0.13 in moderate and 12.04±0.19 in severe dementia subjects indicating a significant decrease of 5% (p=0.0032) in mild, 40% (p=0.0000) in moderate and 19% in severe dementia subjects when compared to controls (Fig.1b). These results are in line with many studies in which protein fraction of 54kDa is selectively decreased in AD and normal pressure hydrocephalus revealing the potential of this protein to be used as additional biomarker in the neurochemical differential diagnosis of AD (Gloeckner, 2006). Studies also revealed that protein fraction of 54kDa, binds amyloid-ß (Aß) and prevents Aß fibril formation (Wati *et al.,* 2004).

Forty kDa protein fraction was found to be at 5.86±0.27% in controls, 6.00±0.14% in mild, 4.39±0.19% in moderate and 4.64±0.31% in severe dementia subjects. The fraction did not vary significantly (p=0.66) in mild, however, a significant decrease of 25% (p=0.0006) in moderate and 21% (p=0.0096) in severe dementia subjects was observed as compared to controls (Fig.1c).

The average percent raw volume covered by 33kDa protein fraction was found to be 2.87±0.14 in controls, 2.69±0.05 in mild dementia, 2.12±0.15 in moderate dementia and 2.13±0.14 in severe dementia subjects. The fraction did not vary significantly (p=0.25) in mild, however, a significant decrease of 26% (p=0.0024) in moderate and 26% (p=0.0017) in severe dementia subjects was observed as compared to controls (Fig.1d). It is suggested that Alzheimer's disease is characterized by plaques consisting of the peptide beta-amyloid. This protein fraction enhances proteolytic break-down of this peptide, both within and between cells, so its level is gradually decreased with progression of the disease (Jiang *et al*., 2008).

Twenty five kDa protein fraction was found to be at 2.42±0.11% in controls, 2.35±0.05% in mild, 1.39±0.06% in moderate and 1.80±0.10% in severe dementia subjects indicating the fraction did not vary significantly (p=0.58) in mild, however, a significant decrease of 43% (p=0.0000) in moderate and 26% (p=0.0009) in severe dementia subjects was observed as compared to controls (Fig.1e). Studies revealed that 25kDa protein fraction is a normal plasma constituent that is observed in senile plaques and neurofibrillary tangles in brains of Alzheimer's disease (AD) patients and its level in the AD group is significantly lower than that of the control group (Nishiyama, 1996).

Protein fraction of 23kDa exhibited average percent raw volume of 8.52±0.10 in controls, 9.00±0.17 in mild, 8.53±0.13 in moderate and 9.77±0.23 in severe dementia subjects indicating a significant increase of 6% (p=0.029) in mild, 15% (p=0.0005) in severe and the fraction did not vary significantly (p=0.98) in moderate dementia subjects when compared to controls (Fig.1f). The average percent raw volume covered by 20kDa protein fraction was found to be 3.30±0.26 in controls, 2.23±0.10 in mild, 2.47±0.20 in moderate and 2.06±0.19 in severe dementia subjects indicating a significant decrease of 32% (p=0.0031) in mild, 25% (p=0.023) in moderate and 37% (p=0.0017) in severe dementia subjects when compared to controls (Fig.1g).

These results are in correlation with earlier investigations where 20kDa protein fraction showed the highest expression in the normal control group. Studies also revealed that this protein fraction is observed to gradually decrease or absent according to disease progression (Sousa *et al.,* 2007).

Protein fraction of 14kDa exhibited average percent raw volume of 2.46±0.13 in controls,

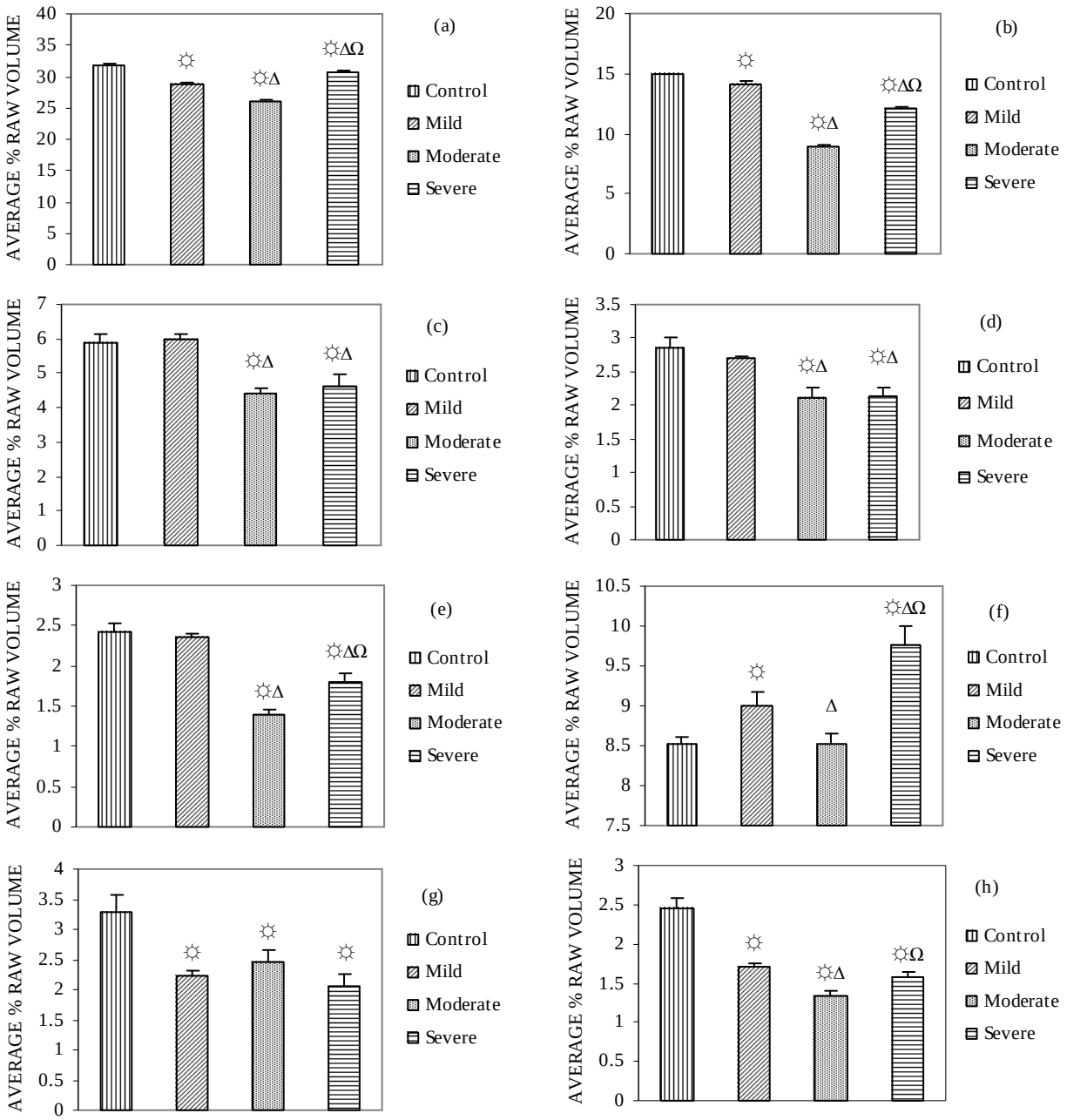

Fig. 1. Average % raw volumes exhibited by 66kDa (a), 54kDa (b), 40kDa (c), 33kDa (d), 25kDa (e), 23kDa (f), 20kDa (g) and 14kDa (h) protein fractions in control, mild, moderate and severe dementia subjects. Values are Mean ± SEM. ☼ΔΩ significance ($p<0.05$) in relation to control, mild and moderate dementia subjects.

1.72±0.03 in mild, 1.34±0.05 in moderate and 1.57±0.07 in severe dementia subjects indicating a significant decrease of 30% (p=0.0004) in mild, 46% (p=0.0000) in moderate and 36% (p=0.0001) in severe dementia subjects when compared to control (Fig.1h). It has been found that increased 14kDa activity in the brain protects against the development of Alzheimer disease (AD). Lower

14kDa protein fraction is associated with higher risk of AD independent of age and also its low levels precede clinically manifested Alzheimer disease (AD) in elderly men free of dementia at baseline and may be a marker of future risk of AD. These findings strengthen the evidence for a role of 14kDa in the development of clinical AD (Sundelof, 2008).

The results of present study indicate that there is a progressive decline in most of the serum proteins with advancing stage of senile dementia but in severe dementia group, protein profile is closer to control group. It may be due to the supplemented diet and mental exercises suggested to severe dementia people, indicating that well balanced diet and mental exercise, if provided for longer time periods, may be helpful either in treating dementia or preventing its manifestation before its onset.

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