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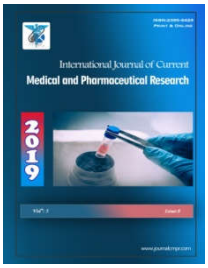

EVALUATION OF LARGE NEUTRAL AROMATIC AMINO ACIDS (LNAA) IN SENILE DEMENTIA

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ABSTRACT

The neurodegeneration disease is a senile dementia characterized by loss of memory, attention and cognitive functions. The Aromatic Amino Acids act as precursors of important neurotransmitters which have important role in attention, cognition and thus have been interest in dementia. Blood levels of Large Neutral Aromatic Amino acids (LNAA) such as Phenylalanine, Tyrosine and Tryptophan were measured using standard methods in 50 patients of dementia and 50 non-demented age and sex matched subjects. There was significant difference observed between healthy controls and Alzheimer dementia, decreased levels of Tryptophan, Phenylalanine and Tyrosine when compared with healthy controls ($p < 0.001$). Average of Phenylalanine, Tyrosine and Tryptophan for healthy Control group were 27.95 with SD. ±6.09,46.52 with SD ±6.99 & 31.9 with SD±4.23. Average of Phenylalanine, Tyrosine and Tryptophan for Alzheimer dementia were 18.28 with SD. ±2.89, ($p < 0.001$), 20.49 with SD ±3.92, ($p < 0.001$) & 25.10 with SD±9.05, ($p < 0.001$). The results suggest that due to decreased concentration of large neutral aromatic amino acids lead to decreased levels of dopamine neurotransmission and serotonergic system in Alzheimer dementia patients and supplementation of aromatic amino acids may improve neuronal functioning and dopaminergic serotonergic activity in Alzheimer dementia.

INTRODUCTION

"Senile Dementia is a neurodegenerative progressive loss of neurons leads loss of cognitive function, or disease of normal brain aging. In the brain there may be loss of memory attention, language, problem solving and disoriented in time, day, week, month, year, place and person in later stages of diseased person''. [(1)]

The diagnosis of dementia clinically made by Diagnostic and statistical manual of mental disorders, 4th edition (DSM IV).[(2)] Dementia can be classified by various types like Alzheimer's disease, vascular dementia, dementia due to head trauma, HIV, Parkinsons disease etc.

The 3.7 million people are affected by dementia at present in India according to ARDSI and it will double by 2030. The estimated cost of dementia patient for taking is huge about 43,000 annually. So we cant ignore challenges posed by dementia on the grounds of health and social issues, despite immensity there is gross of unawareness abandon and lack of services for dementia patients and their families.[(3)]

The main culprit of Alzheimer's disease is Amyloid-β(Aβ), Aβ aggregation amyloidogenesis and deposition of Aβ leads to plaque formation. Aβ induces tau phosphorylation leads in to ROS formation through peptidyl radicals' damages mitochondrial DNA, RNA, lipids and protein that results in to synapse damage and death of neuronal cell. This is taking place at memory centre, the hippocampus of the brain.

Aromatic amino acids has important role and formation of neurotransmitters because they act as precursors, such as catecholamine's and serotonin, which have important roles in attention and cognition and have thus been of interest in dementia. Several studies showed that plasma levels of aromatic amino acids might be act as a regulating factor in brain. [(4)]

Phenylalanine can be metabolized through the same metabolic pathways as tyrosine, but only when tyrosine levels are low. [(5)] The amino acid tyrosine is important for synthesizing catecholamine's like dopamine. Although several studies have found that oral tyrosine administered improves memory and cognitive function. [(6)]

The metabolism of neurotransmitters is an important consideration in the pathology of all neurological diseases. Dopamine is a catecholamine neurotransmitter, which plays several important role in the brain acting via four distinct pathways, the mesolimbic, mesocortical, nigrostatial and tuberoinfundibular pathway. These pathways are responsible for regulating mood, and aiding cognitive and motor function. Impairment of this system potentially causes depression, memory loss and impaired motor control observed in patients with Alzheimer's disease. [(7,8)] The aim of this study to evaluate

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levels and there role of large neutral aromatic amino acids (LNAA) in dementia patients and to understand basic mechanism of nerve degeneration and loss of memory in dementia.

MATERIAL AND METHODS

Aims and Objective

1. To Evaluate levels of Large Neutral aromatic amino acids (LNAA) such as Phenylalanine, Tyrosine and Tryptophan.
2. To understand basic mechanism of nerve degeneration and memory loss in dementia.
3. To understand the role of Large Neutral aromatic amino acids (LNAA) such as Phenylalanine, Tyrosine and Tryptophan.

Study design

This study was designed as randomised controlled study.

Subjects and Method

The total number of subjects included in this study was 100 out of which 50 patients of Alzheimer's type of dementia diagnosed by DSM-IV and 50 age and sex matched controls. The informed written consent was taken from the subjects and study was approved by institute ethical committee.

Inclusion Criteria

- Newly diagnosed cases, not on treatments
- Male subjects, above 50 to 70 years.
- MMSE Score of less than 26.

Exclusion Criteria

- Patients addicted to alcohol or drug abuse.
- Patients suffering from major psychiatric disorder, chronic illness.
- Any other concurrent drug intake

Blood samples from patients and control were collected from anticubital vein, with all aseptic precautions, in plain polythene tubes for the estimation of large neutral aromatic amino acids. Serum was separated by centrifuging the samples at 3000rpm for 10 minutes. These serum samples were preserved in freezer till the laboratory estimation proceeds.

Estimation of Large neutral aromatic amino acids (LNAA)

Estimation of large neutral Aromatic amino acids was done by Spectrofluorometric.

Estimation of Phenylalanine

Deproteinization: Pipette 0.1 ml of serum in eppendorf tube. Add 0.1 ml of series of standards to correspondingly labelled tubes (WS1, WS2, WS3, WS4, WS5). Add 0.1 ml of 0.6 M TCA to all the tubes and vortex. Transfer the tubes to an ice bucket and allow them to stand for 10 minutes. Centrifuge (5000 rpm/10 minutes). Collect the supernatant for the assay of tyrosine.

Procedure: Pipette 50 µl TCA supernatant/extract from previous step in to appropriately labelled test tubes (T and WS1-5). Add 500 µl of Nitrosonaphthol reagent. Incubate at 33^0C for 20 minutes. Add 2.0 ml of water to all tubes. Add 3.0 ml of Ethylene dichloride and vortex the tubes. Centrifuge (3000 rpm/10 minutes). Remove the top aqueous layer allow to stand for 30 minutes at 25^0C and take reading of this aqueous layer. Read the fluorescence (Excitation 460 nm/Emission 570 nm) within 30 minutes. The values were expressed in Units per mole per litre (UM/L).

Estimation of Tyrosine

Deproteinization: Pipette 0.1 ml of serum in eppendorf tube. Add 0.1 ml of series of standards to correspondingly labelled tubes (WS1, WS2, WS3, WS4, WS5). Add 0.1 ml of 0.6 M TCA to all the tubes and vortex. Transfer the tubes to an ice bucket and allow them to stand for 10 minutes. Centrifuge (5000 rpm/10 minutes). Collect the supernatant for the assay of tyrosine.

Procedure: Pipette 50 µl TCA supernatant/extract from previous step in to appropriately labelled test tubes (T and WS1-5). Add 500 µl of Nitrosonaphthol reagent. Incubate at 33^0C for 20 minutes. Add 2.0 ml of water to all tubes. Add 3.0 ml of Ethylene dichloride and vortex the tubes. Centrifuge (3000 rpm/10 minutes). Remove the top aqueous layer allow to stand for 30 minutes at 25^0C and take reading of this aqueous layer. Read the fluorescence (Excitation 460 nm/Emission 570 nm) within 30 minutes. The values were expressed in Units per mole per litre (UM/L).

Estimation of Tryptophan

Deproteinization: Pipette 0.2 ml of serum and water for Blank (B) in eppendorf tube. Add 0.1 ml of series of standards to correspondingly labelled tubes (WS1, WS2, WS3, WS4, WS5) Add 0.2 ml of 0.6 M TCA to all the tubes and vortex. Transfer the tubes to an ice bucket and allow them to stand for 10 minutes. Centrifuge (5000 rpm/10 minutes) Collect the supernatant for the assay of tryptophan.

Procedure: Pipette 100 µl TCA supernatant/extract from previous step in to correspondingly labelled test tubes (T and WS1-5). Add 2.1 ml of 0.6 M TCA to all the tubes. Add 2.2 ml of 0.6 M TCA to reagent Blank (B) tube. Add 0.2 ml of formaldehyde solution to all the tubes and mix. Add 0.1 ml of FeCl3 solution to each of the tubes and mix. Transfer the tubes to boiling water bath and drop them with clean marbles. After 1 hour take out the tubes from boiling water bath and allow them to attain room temperature. Read the fluorescence (Excitation 365 nm/Emission 460 nm). Extrapolate the test (T) readings from the standard curve for calculation of tryptophan content of the serum.

The quantitative data was analysed by SPSS 18 using student 't' test. P value < 0.001was considered significant.

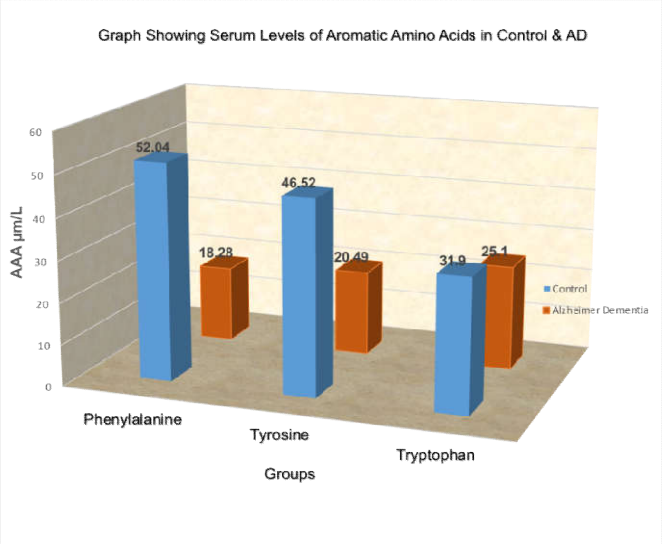
RESULT

The study was done on male subjects with mean age of cases being 73.42 ± 3.72 and that of controls 74.56 ± 4.30. The Phenylalanine levels were lower in Alzheimer's dementia patients (18.28 with SD. ±2.89, P value < 0.001) compared to healthy controls (52.04 with SD. ±6.09). Similarly Tyrosine and Tryptophan levels were lower in Alzheimer's dementia (20.49 with SD ±3.92 & 25.10 with SD±9.05, P value < 0.001) as compared to healthy controls (46.52 with SD ±6.99 & 31.9 with SD±4.23).

Table 1 Serum Levels of Phenylalanine, Tyrosine and Tryptophan (LNAA) in Control and Alzheimer dementia

Sr. No.			Groups		P value
			Healthy Control Mean Age (73.42 ± 3.72)	Alzheimer's Dementia Mean Age (73.42 ± 3.72)	
2	Phenylalanine um/L	Mean/ SD	52.04±6.09	18.28±2.89	< 0.0001
3	Tyrosine um/L	Mean/ SD	46.52±6.99	20.493.92	< 0.0001
4	Tryptophan um/L	Mean/ SD	31.9±4.23	25.10±9.05	< 0.0001

Statistically significant difference was observed between healthy controls and Alzheimer's dementia i.e decreased levels Phenylalanine, Tyrosine and Tryptophan when compared with healthy controls. ($p < 0.001$).



DISCUSSION

Aromatic amino acids has important role and formation of neurotransmitters because they act as precursors, such as catecholamine's and serotonin, which have important roles in attention and cognition and thus have been of interest in dementia.

Plasma levels of aromatic amino acids might be act as a regulating factor in brain by experimental studies. [4] Phenylalanine metabolized by same pathways as tyrosine, but when low levels of tyrosine. The amino acid tyrosine is important for synthesizing catecholamine's like dopamine. The several studies have found that by oral administering the tyrosine improves memory and cognitive function. [5,6]

In our study the Aromatic amino acids such as Phenylalanine, Tyrosine, and Tryptophan were found to be reduced in Alzheimer's dementia than healthy controls. Our results suggest that due to decreased concentration of aromatic amino acids lead to decreased levels of dopamine neurotransmission and serotonergic system or imbalance between serotonergic and dopaminergic noradrenergic neurotransmission process in Alzheimer dementia patients or it may due to neurons of AD brain shows lack glucose metabolism and lack of enzymes for beta-oxidation of fatty acids, so to prevent the neuronal cell death of disease alternative source for energy leads to oxidation of aromatic amino acids, this results in to reduction of aromatic amino acids in neurons and disease is progressed. This could be the one reason behind the decreased levels of all three aromatic amino acids in Alzheimer's dementia and another reason was due to reduced levels of phenylalanine reduced levels of dopamine could reduce the amount of the neurotransmitters released in to the synaptic cleft during synaptic transmission leading to impaired signal transduction this taking place in Alzheimer's dementia.

This study supported by Hashimoto M, Masliah E showed that dopamine is synthesized in two steps of reaction first tyrosine is converted to L-Dopa by tyrosine hydroxylase (TH) and second the L-DOPA is converted to dopamine-catalysed decarboxylase (AAAD). The greatest reduction in dopamine in the middle frontal Gyrus (MFG) was observed in the asymptomatic patients. The activity of both TH and AAAD are inhibited by alpha-synuclein a protein that has been shown to important role in the neurodegeneration diseases, including Alzheimer's disease. [11]

The previous study by Hargreaves KM, Pardridge WM, showed that due to increased levels of both the aromatic amino acid Phenylalanine and tryptophan may lead to brain damage through several mechanisms including competition with the other amino acids for transport by the same carrier at the blood brain barrier, leads to decreased brain protein synthesis and increased myelin turnover.

Our results show the lower concentration tryptophan in the serum of AD patients compared to a control group this is due to reduced levels of tryptophan, reduced levels of serotonin could reduce the amount of the neurotransmitters released in to the synaptic cleft during synaptic transmission leading to impaired signal transduction this taking place in Alzheimer's dementia, agree with those reported in other studies Basun *et al.*1990, Rudman *et al* showed that the lower tryptophan concentration in the serum of AD patients may result in a reduced synthesis of Serotonin neurotransmitter leads to loss of memory. [12,13]

In the brain tryptophan has two different metabolic fates, it can be metabolized in to serotonin or it can enter the kynurenine pathway (KP) where it is degraded to quinolic acid (QA). There is shift of tryptophan degradation to the KP pathway diverts tryptophan from serotonin synthesis pathway. This could deprive the AD brain of serotonin contributing to the pathogenesis of AD and acute tryptophan depletion leads to memory impairment in human. The enzymes involved in the KP are upregulated. Quinolic acid and other metabolic intermediates in the KP pathway cause oxidative damage to the brain. Increased quinolinic acid lead to increased concentration of ROS levels in the synaptosomes and lipid peroxidation in the hippocampus. Amyloid-β production increased the concentration of quinolinic acid more directly to oxidative damage from amino acid metabolites in Alzheimer dementia.

In conclusion our results suggest that due to decreased concentration of aromatic amino acids lead to decreased levels of dopamine neurotransmission and serotonergic system or imbalance between serotonergic and dopaminergic noradrenergic neurotransmission process in Alzheimer dementia patients and supplementation of aromatic amino acids may improve neuronal functioning and dopaminergic serotonergic activity in Alzheimer dementia.

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