ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Examination, Screening and Statistical Analysis with respect to Prevalent Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disease, caused by accumulation of abundant senile plaques and neurofibrillary tangles in certain brain regions. It is an irreversible and progressive disease that slowly destroys memory, thinking skills and, eventually the ability to perform simple day to day tasks. This project was to investigate the prevalence of Alzheimer's disease (AD) in Vellore and Chennai, India by conducting an epidemiological survey of 120 individuals from dementia club and old age homes and carrying out certain blood tests on patient's sample and comparing the results with the controls. Patient's cognitive function like orientation, registration, attention, calculation, recalling, and language were graded by minimal mental state examination (MMSE). Caregivers of the patients were interviewed to confirm the familial history and progression of the disease in the patients. The blood test results were correlated with that of MMSE. ESR, blood glucose and lithium ion concentrations were shown to be significant and presence of oxidative stress was seen in majority of the patients. Hence it is suggested that these factors can be quantified to confirm the presence or absence of disease. The in situ hybridization of blood samples of the patients yielded no result as there is no presence of amyloid-β protein, and can be avoided as a confirmatory test.

Keywords: Alzheimer's disease, amyloid-β protein, Minimal mental state examination, senile plaques, neurofibrillary tangles

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ISSN: 0975-8585

INTRODUCTION

AD, named after Dr. Alois Alzheimer, a German doctor who in 1906 described changes in the brain tissue of a woman who died of a strange mental sickness. He reported the formation of abnormal clumps and tangled bundles of fibers now considered to be hallmarks of AD, called amyloid plaques and neurofibrillary tangles respectively. Plaques [1] are made up of amyloid-β, a protein fragment cleaved from amyloid precursor protein (APP) [2] that is associated with the neuron membrane. It is cleaved by two types of enzymes- β secretase and gamma secretase, both of which present potential targets for drug therapies. Also inhibiting their upregulation by oxidants and cytokines respectively, suggest of a number of nutraceutical or lifestyle measures as a potential for prevention [3]. Neurofibrillary tangles are insoluble twisted fibers that build up inside the nerve cell. These are formed when a protein tau, involved in stability of microtubule structure inside neurons, is chemically changed. This causes microtubules to disintegrate, collapsing the neuron's transport system. The other pathophysiology associated with AD include widespread fatty deposits in small blood vessels, disruption of communication between neurons due to reduced production of neurotransmitters, dramatic shrinkage of the brain, especially cerebral cortex region; the outer layer involved in memory, thinking, judgment, consciousness, attention and speech and even dying of brain cells.

According to recent estimates, as many as 2.4 million to 4.5 million people have AD and this number is thought to double in the next ten years. It is not a normal part of aging but is the most common cause of dementia, decline in cognitive function that interferes with day to day activities. This makes it different from Mild cognitive impairment (MCI), a condition in which a person has memory problems greater than those expected for their age however they do not have personality changes or cognitive problems that characterize AD. It is thought that people with MCI go on to develop AD. It starts in a region of the brain that affects recent memory, and gradually spreads to other parts of the brain progressing through five stages- preclinical AD, mild cognitive impairment, mild AD, moderate AD and severe AD.

Early-onset AD (familial AD) is thought to be caused due to genetic mutations on chromosome 1, 14 and 21 that lead to formation of abnormal preseniline 2, preseline 1 and APP respectively. While incidence of late-onset AD is thought to be connected with apolipoprotein E (APOE) gene found on chromosome 19 which is involved in the formation a protein helpful for carrying cholesterol in bloodstream. Also mutation or decrease in levels of SORLI, a gene involved in transporting APP within cells is known to increase amyloid-β levels and harm neurons [4]. Furthermore, free radicals being very active injure neurons by attacking their membrane or DNA [5]. The resulting molecules set off chain reaction releasing more free radicals that enhance damage. This oxidative damage and in turn development of AD [6] can be counteracted by providing anti-oxidants from dietary supplements or food. Risk of AD is seen to be increased with elevation in levels of an amino acid homocysteine, a risk factor for heart disease and reduced by statins, the most commonly prescribed drug for lowering of cholesterol. Increasing the intake of folic acid and vitamin B6 and B12 can help reduce blood levels of homocysteine. It is thought that amyloid-β has binding sites for zinc, lithium and copper ions that increases its resistance to breakdown by enzymes and promotes its tendency to clump together to form plaques. This

ISSN: 0975-8585

can be combated with Clioquinol a chemical that binds these metal ions. High blood pressure and other stroke risk factors can damage blood vessels in the brain and reduce the brain's oxygen supply which may disrupt nerve cell circuits thought to be important for decision making [7]. Too much insulin in the blood, which happens as a result of insulin resistance, may encourage inflammation and oxidative stress both of which contribute to damage in AD [8]. Physical activity [9], social engagement [10] and involvement in intellectually stimulating activities also decrease the risk of dementia. The most frequent cause of death for people with AD is aspiration pneumonia. This type of pneumonia develops when a person cannot swallow properly and takes food or liquids into the lungs instead of air.

MATERIALS AND METHODS

Blood samples were collected (Fig 1).

Erythrocyte Sedimentation Rate (Fig 2): blood samples are collected with anticoagulants and mixed. Distance travelled by blood cells in 20 min is noted and reported in units of mm/hour

Catalase activity test (Fig 3): sample is smeared on a microscope slide and a drop of hydrogen peroxide is placed on the smear. Copious bubbles liberated in the hydrogen peroxide, indicates presence of catalase.

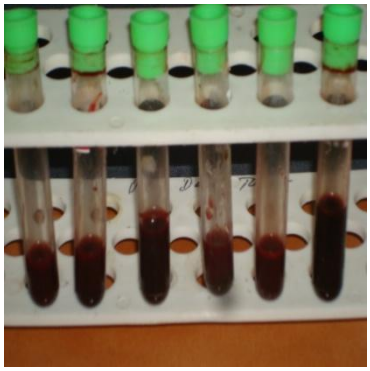

Fig 1. Blood samples collected

Fig 2. Erythrocyte sedimentation rate

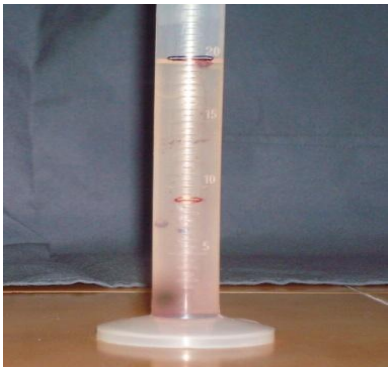

Fig 3. Catalase activity test

Lithium concentration quantified using flame photometry (Fig 4).

Estimation of Cholesterol (Fig 5): Test serum (0.1ml) and ferric chloride (99ml) are mixed, allowed to stand for 15-20 min then centrifuged at 2500rpm for 15min (room temperature). 5ml of clear supernatant is added to 3ml concentrated sulfuric acid. Cholesterol undergoes dehydration to form a red complex which is quantified after 30min at 560 nm.

Blood glucose measurement (Fig 6): Done using commercially available kit. A drop of blood is put on the strip which flows in through capillary action. There the glucose in blood reacts with enzyme glucose oxidase to create gluconic acid. This reacts with another chemical in the strip (ferricyanide) to give ferrocyanide which influences current which can be measured and hence concentration of blood glucose.

ISSN: 0975-8585

Fig 4. Lithium ion conc estimation

Fig 5. Cholesterol estimation

Fig 6. Blood glucose

Enumeration of WBC and RBC done using haemocytometer (Fig 7 and 8)

Fig 7 and 8. WBC and RBC estimation using heamocytometer

In Situ Hybridisation [11] is done to localize amyloid-β protein using radiolabelled probes, complementary to its mRNA [12] sequence and quantified using flurescent spectroscopy. The hybridization solution contains dextran sulphate to reduce amount of hydrating water for dissolving nucleotides, formamide and dithiothreitol to reduce thermal stability of bonds, NaCl and sodium citrate to decrease electrostatic attraction between two strands, EDTA to remove free divalent cations from the solution, ssDNA, tRNA, polyA and denhard's solution.

Caregivers were interviewed to find out various things about the patient, like patients experience having problems related to memory, problems doing familiar tasks, with the language, disorientation in time and place, deterioration in judgment etc

MMSE (Mini Mental State Examination): consists of a series of questions and test each of which fetches a score on being answered correctly. First section consists of orientation, ability to five correct date and location. Second section tests short term memory. Names of three objects are given to remember, ability to recall is tested. Third section tests attention and calculation. In fourth section patients are asked to recall the items from section 2. Fifth and last section tests language, writing and drawing.

Out of the 120 patients selected for the MMSE and survey 16 females and 14 males (30 patients in total) were randomly selected to do the biomarker study with the following blood tests (Table1). MMSE scores were recorded (Table 2) and then compared it with

ISSN: 0975-8585

respect to the biomarker tests (Table 3). The selected patients were such that they had similar profiles in the MMSE tests so the correlations with the blood tests can be linearised.

Results for patients:
Average MMSE score (Females) is 18.5
Standard Deviation is 2.25
Average MMSE score (males) is 18
Standard Deviation is 2.38

Results for the controls:
Average MMSE score (Females) is 27.4
Standard Deviation is 1.14
Average MMSE score (males) is 27.4
Standard Deviation is 1.51

Table – 1: Test results

Sr. No.	Age	Profile No.	WBC (10^3/µl)	RBC (10^6 /µl)	ESR (secs)	Cholestrol (mg/dL)	Blood Glucose (mg/dL)	Catalase activity	HIV	Li conc.
1	84	**A12303**	8.01	4.44	5	137	220	(-ve)	(-ve)	559
2	57	**A12306**	7.2	5.12	6	113	270	(-ve)	(-ve)	612
3	62	**A12307**	7.7	5.04	6	145	190	(+ve)	(-ve)	634
4	62	**A12308**	8.1	4.98	6	182	193	(-ve)	(-ve)	712
5	72	**A12310**	7.15	4.48	6	161	189	(-ve)	(-ve)	645
6	61	**A12311**	7.92	4.7	5	155	156	(-ve)	(-ve)	590
7	76	**A12312**	7.1	4.59	5	148	182	(+ve)	(-ve)	598
8	73	**A12314**	7.82	4.63	5	163	322	(+ve)	(-ve)	669
9	53	**A12315**	8.85	4.86	6	151	253	(-ve)	(-ve)	610
10	51	**A12319**	7.55	5.09	6	106	285	(+ve)	(-ve)	722
11	69	**A12321**	8.3	5.3	6	139	276	(+ve)	(-ve)	654
12	73	**A12322**	8.31	5.39	6	174	245	(-ve)	(-ve)	612
13	42	**A12326**	7.67	5.12	5	149	231	(+ve)	(-ve)	690
14	82	**A12327**	8.56	6.44	7	145	271	(-ve)	(-ve)	598
15	71	**A12328**	7.76	3.12	5	154	245	(+ve)	(-ve)	643
16	69	**A12329**	8.44	5.92	6	144	273	(+ve)	(-ve)	754
17	69	C3	7.9	4.73	4	121	103	(-ve)	(-ve)	233
18	66	C4	8.3	5.24	4	153	92	(-ve)	(-ve)	330
19	66	C7	9.1	5.13	4	135	104	(-ve)	(-ve)	321
20	64	C9	9.5	4.93	3	156	99	(-ve)	(-ve)	359
21	62	C10	8.93	5.44	3	193	95	(-ve)	(-ve)	473
	66.06/ 65.4	**Avg (patient/ control)**	7.9 / 8.746	4.95125 / 5.094	5.68 / 3.6	147.87 / 151.6	237.5625 / 98.6			643.87 / 343.2
	11.44/ 2.607	**S.D (patient/ control)**	0.5 / 0.640	0.716 / 0.27	0.608/ 0.547	19.2.2 / 27.14	45.70 / 5.128353			53.74 / 86.47

ISSN: 0975-8585

Table - 2: MMSE Scores

Sr.No.	Females			Males		
	Age	Profile No.	MMSE	Age	Profile No.	MMSE
1	84	A12303	20	59	A12301	14
2	57	A12306	16	64	A12302	19
3	62	A12307	19	59	A12304	21
4	62	A12308	23	70	A12305	20
5	72	A12310	20	54	A12309	18
6	61	A12311	16	72	A12313	19
7	76	A12312	18	75	A12316	16
8	73	A12314	17	62	A12317	17
9	53	A12315	16	59	A12318	19
10	51	A12319	19	66	A12320	16
11	69	A12321	21	21	A12323	14
12	73	A12322	19	61	A12324	19
13	42	A12326	22	58	A12325	18
14	82	A12327	17	83	A12330	22
15	71	A12328	16	61	C1	28
16	69	A12329	17	71	C2	29
17	69	C3	27	59	C5	28
18	66	C4	29	67	C6	27
19	66	C7	26	67	C8	25
20	64	C9	28			
21	62	C10	27			

Table - 3: Laboratory test results (biomarker study) - T Test (p value)

	vs. MMSE score (Females)	vs. MMSE score (Males)
AGE	1.84×10^{-11}	2.11×10^{-8}
WBC	1.95×10^{-12}	1.75×10^{-10}
RBC	8.84×10^{-15}	1.96×10^{-11}
ESR	5.32×10^{-14}	1.06×10^{-12}
CHOLESTROL	2.4×10^{-14}	4.1×10^{-10}
BLOOD GLUCOSE	5.4×10^{-12}	4.2×10^{-07}
LITHIUM CONCENTRATION	1.1×10^{-17}	2.4×10^{-15}

ISSN: 0975-8585

RESULTS

Survey Results

- Most caregivers of the Tamil Nadu regions, aged between 21 and 86 believe that the responsibility lies with those closely related to the patient, to notice signs and symptoms and take action.
- 65.4 % respondents of the survey were the sole person providing care for the patient with AD. Of these 72.7% were females with 90.9 % of them married.
- 50 % of these work full time, 33.3 % part time and 16.7 % are retired or not working.
- 92.3 % of them agree that if they thought a loved one had AD, they would encourage him or her to find out as early as possible.
- Whilst, 21.4 % of the patients are taken care of at the caregivers home the majority (57.1 %) are taken care at the patients home itself. The remaining 21.4 % are taken care of at assisted living facilities.
- 57.6 % of the patients are directly related to the patients as relatives (either spouse/partner, Parents, In-laws, grandparents). An interesting fact here lies in that, of all the respondents not a single caregiver was the patient's brother/sister.
- The caregivers have been providing care for an average 36.1 months (3 years), data ranging from 2 months up to 108 months.
- The caregivers specify that the patient has been experiencing memory related problems for an average 43 months (3.5 years).
- On average, the caregivers provide support to the patients for about 5.25 days/week, though 61.5 % provide care everyday.
- 35.8 % of the caregivers feel that the patients very much depend on them for their everyday activities including bathing, dressing, preparing meals [10] etc., while 21. 4 % each think, that the patients depend on them somewhat, little and not at all.
- 8.7 % of the caregivers feel that taking care of the patient effects their relation with other family and friends in a negative way; while 13.1 % feel it affects their health, finances and time. While 26.1 % find the care giving stressful, 21.6 % feel that it affects their work in a negative way [11].
- 63.1 % of the respondents said that they have emotional support from their families and friends who help them cope up with any stress/help they need.
- The internet is the prime source of information for 32 % of the caregivers, followed by 24 % from physicians, 12 % from family members, and the remaining 32 % are from other sources like co-workers, societies, clubs, television, journals etc.

Statistics From Caregivers

66.18% of the caregivers experience patients having memory related problems. 60.6% experience problems doing familiar tasks. 65.9 % experience problems with the language. 66.6% with disorientation in time and place. 83.3 % with deterioration in judgment. 66.6 % having difficulty in abstract. 80.2 % with misplacing things. 48.18 %

having changes in behaviour and mood. 57.7 % having problems with changing personality. 60.14 % experience patients becoming more passive.

Patients Prospective – The Mmse Statistics

49.50% of the patients successfully cleared the general analysis test, where questions pertaining to everyday life like date, time, season, places were asked. 51.61% cleared the short term memory test, where they were asked to repeat 3 sets of words in continuity over several periods of time. 41.18% cleared the orientation test, where they were asked to spell a word backwards, and count the number in multiples backwards. 81.81% cleared the object identification test. 62.26% cleared the listening and following instructions test. 58.33% of the patients successfully cleared the visual instructions test.

DISCUSSION

The survey throws light on the importance of increased education and awareness about Alzheimer's disease and its symptoms. The correlation between MMSE results with the variables shows that only ESR, blood glucose and Li concentrations are significant and can be quantified to confirm the presence or absence of the disease (Table 4). Presence of oxidative stress is also seen in majority of the patients and can also be regarded as one of the important test to identify the condition. The in situ hybridization of blood samples of patients yields no result as there is no presence on amyloid-β protein and can be avoided as a confirmatory test.

Table - 4: Significance values

	Difference between sample value and control value		Significance	
	Males	Females	Males	Females
WBC	- 0.76686	- 0.2435	Insignificant	Insignificant
RBC	0.84643	0.45725	Insignificant	Insignificant
ESR	2.25714	2.0875	Significant	Significant
Cholesterol	- 46.9143	- 3.725	Insignificant	Insignificant
Blood glucose	108.143	138.9625	Significant	Significant
Lithium concentration	198.3714286	300.675	Significant	Significant

ACKNOWLEDGEMENT

We would also like to thank our honorable Chancellor, Dr. G Viswanathn for his guidance. We would like to thank all the patients who helped me perform tests on them and their caregivers for spending time with us and making sure this project turns out a success with all their possible help.

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