

Cytogenetic Analysis and Screening of ApoE and Neurotransmitters in Alzheimer's Patients in Tamil Nadu Population

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ABSTRACT Alzheimer's disease (AD) is the leading neurodegenerative disorder and the cause of dementia in the elderly people. The current research investigated the relevance of AD individuals challenged with copper exposure and the disease onset through cytogenetic and molecular analysis. The research was performed on 70 AD patients categorized into two groups as the early-onset with age below 65 (n=31) and the late-onset aged above 65 (n=39). The chromosomal aberrations (CAs) were examined in both the groups showing higher CAs in the late-onset and the anomalies were seen in the chromosomes 10q, 9p, 9q, 14p and 19q in 21 patients and the micronucleus (MN) assay was also found to be higher in the late-onset patients. Assessment of ApoE gene showed a definite significant difference between the two groups. In this study, the exposure of copper did not reveal any significant changes in the two groups, conversely, the biochemical parameters serotonin, GABA, dopamine and homocysteine were analyzed and a higher level of homocysteine was seen in the late-onset cases. Consequently, from these findings, it is pertinent to believe that, this is the first report in the Tamil Nadu region where the researchers predicted ApoE to be a molecular marker to diagnose AD.

INTRODUCTION

Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disorder which leads to dementia (Lee et al. 2014). Its incidence affects 35 million people worldwide (Querfurth and Laferla 2010) with approximately two percent seen in the population of the industrialized countries (Mattson 2004). Age is a greatest risk factor and after the age of 65 its prevalence doubles every five years (Querfurth and Laferla 2010). The factors associated with AD are high cholesterol level, obesity, diabetes, coronary artery disease (CAD), low density lipoprotein receptor-related protein-1 (LRP-1) and ApoE (Stewart and Liolitsa 1999). The disease inception is categorized into two types, early-onset AD affects peo-

ple who are younger than the age of 65 and the late-onset AD affects people older than 65. The brain of AD patients exhibits atrophy of the neocortex resulting from the synaptic loss, including entorhinal cortex, hippocampus, basal forebrain and amygdala (Mattson 2004). As these symptoms are same as with other neurological disorders, diagnosis substantiation of AD requires the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) in the postmortem examination of the brain (Hung et al. 2010), as these are the two pathological hallmarks of AD. ApoE differentially regulates A β aggregation and the clearance in the brain. Proteolytic degradation of A β by ApoE was seen in the study by Jiang et al. (2008).

Genetic and environmental factors are involved in the disease occurrence and progression (Eva et al. 2014). Relevantly APP, PSEN1 and PSEN2 were seen on the chromosomes 21, 14 and 1 respectively (Hardy 1997) in which mutations resulted in the alteration of A β production that have been seen in the early-onset cases (Eva et al. 2014). For the late-onset, ApoE ϵ 4 allele has been considered as a risk factor and

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identified to be associated with the rare, autosomal dominant form of AD (Dursun et al. 2008; Feulner et al. 2010) seen on chromosome 19 (Farmer et al. 1997). ApoE accounts for about fifty percent of the genetic variation in the late-onset AD. In ApoE, there were two SNPs (single nucleotide polymorphisms) seen on exon 4 which were non-synonymous and resulted in the amino acid change from Cys to Arg and Arg to Cys (Kim et al. 2014). ApoE carried three alleles namely $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ and it is a unique, well established susceptibility gene for the late-onset AD with a frequency of 8.4 percent, 77.9 percent and 13.7 percent worldwide (Liu et al. 2013). Not only A β peptides and tau/phospho tau protein were also assessed as biomarker, ApoE was also used as a biomarker (Holtzman 2011).

Apart from the amyloid plaques and the NFTs, the other important factor involved in the pathogenesis of AD was the imbalance of the metal homeostasis in the brain (Bush 2013). Generally, metals such as copper, iron and zinc are necessary for neurotransmission, oxidative phosphorylation, synaptogenesis, neurite outgrowth, neurogenesis, neurotransmitter biosynthesis and oxygen transport. The association of ApoE $\epsilon 4$ mechanism with AD is yet to be known; though, the relation between the metals and the ApoE may provide evidence. The ApoE isoforms binds with zinc, copper and iron, with the affinity being greater for copper than for iron and zinc (Miyata and Smith 1996). The probability supported that A β is protected by ApoE from zinc-induced precipitation in the order of ApoE $\epsilon 2 >$ ApoE $\epsilon 3 >$ ApoE $\epsilon 4$ Moir et al. (1999). It is also hypothesized that the binding capacity of copper in ApoE4 is reduced due to the lack of cysteine residues (Hung et al. 2013).

The neuropathology of AD indicated the dyshomeostasis of iron (Fe), copper (Cu), zinc (Zn) and APP/A β /metal redox interactions Huang et al. (2004a). The cerebral homeostasis of Cu, Fe, and Zn were closely associated with AD. Many reports showed that at the neuronal synapse both the lipid peroxidation and copper promotes amyloid- β aggregation to be a contributing factor of the copper toxicity (Paoletti et al. 2009; Viles 2012). It would be expected that the neurotoxicity would be a function of the copper concentration. Moreover, amyloid- β with the copper solutions was less toxic than amyloid- β with the nanomolar concentration of copper which was 50 times more concentrated (Sarell et

al. 2010). Although the dysregulation of copper, iron and zinc in maintaining the redox balance in the brain lead to oxidative stress. Oxidative stress produced the OH radical through a Fenton reaction mediated by H₂O₂ and commenced a variety of reactions together with the post-translational protein modification, DNA damage and lipid peroxidation Markesbury (2007). Accordingly, the metal based therapeutic strategies further supported the development and progression of AD (Adlard et al. 2008; Lannfelt et al. 2008).

Biogenic monoamines were the source of nitrogen and precursor for the synthesis of the hormones, alkaloids, nucleic acids, proteins and amines. Certain monoamines played a vital role in the cognitive dysfunction of the neurodegenerative disorders. Recent neurochemical studies have indicated an abnormality in the cholinergic system and the down-regulation of choline acetyltransferase, enzyme responsible for the acetylcholine (ACh) was responsible for causing AD (Tabet 2006). Further, the other neurotransmitters have contributed in the pathogenesis of AD only in a few studies. A homologue of cysteine amino acid called homocysteine was seen in higher concentration in people with AD but there are some controversies whether elevated plasma homocysteine (tHcy) is an AD risk factor or merely a biomarker (Seshadri 2006). The relation between plasma tHcy level and AD have recently been reviewed, which showed that overall the majority of the retrospective studies that reported an increase in plasma tHcy in AD patients (Zhuo et al. 2011). So far, there is not much data on neurotransmitters with AD since genetic studies have been given preference. For AD diagnosis, circulating biomarkers A β peptides and tau/phospho tau protein were used (Kim et al. 2014). The clinical trials have been focusing on A β which is a treatment benefit in the symptomatic stage of the disease. The lack of efficacy is that the treatment may be administered too late in the disease process. Indeed, AD pathophysiology is thought to begin several years, probably decades, before the emergence of the clinical symptoms (Holtzman et al. 2011; Clark et al. 2012; Fleisher et al. 2012).

Objective

The focal aim of the study was to analyze the copper effect in causing AD by performing cytogenetic analysis to identify the chromosom-

al alterations (CA) using Trypsin G banding, micronucleus assay by cytokinesis B technique and the biochemical parameters serotonin, GABA, homocysteine and dopamine levels were assessed using HPLC and the genetic polymorphism of the ApoE was investigated using the PCR – RFLP technique. The current study has been designed and accomplished to reveal the genetic characteristics of the Alzheimer's disease patients among the age groups so as to enable a better treatment and counseling for a healthy society.

MATERIAL AND METHODS

Subject Recruitment and Sample Collection

Samples of AD patients who were exposed to copper were selected from various hospitals from amongst the Tamil Nadu provinces. In the present research, totally 70 AD patients were selected and categorized into two groups namely early-onset ($n=28$) and late-onset ($n=42$) AD patients. At the time of blood collection the AD patients were interviewed personally and an open questionnaire was directed towards them for getting the relevant clinical details of the etiological factors such as age, status, gender, diet, height, weight, medical and obesity, history of alcohol, smoking status, area of residence were noted down and family history was conducted by performing a pedigree analysis. Samples were collected in pedigree analysis heparin tube and were brought to the culture laboratory in sterile and tightly covered ice packed plastic containers for carrying out chromosomal, genotypic and biochemical analysis. The ethical review boards at each institution approved the study, and all the participants had provided informed consent.

Cytogenetic Analysis

Cultures of leucocytes were obtained from the peripheral blood following (Moorhead et al. 1960) method. 0.5ml blood was added to 4.5ml RPMI 1640 medium supplemented with ten percent fetal bovine serum, 2mM l-glutamine, one percent streptomycin-penicillin, 0.2ml reagent grade phytohemagglutinin, and was incubated at 37°C for 72nd hours. At the 71st hour, cultures were treated with 0.1g/ml colcemid to block the cells in the metaphase stage. Lymphocytes were harvested after the 72nd hr by centrifuging the

cells to remove the culture medium (800 -1000 rpm). Hypotonic solution (KCl 0.075 M) was added and incubated at 37°C for 20 minutes to swell the cells, then the cells were treated twice with Carnoy's fixative (3:1 ratio of methanol: acetic acid). The lymphocytes were then added onto the slides carefully and dried on a hot plate (56°C, 2 min). Finally, the slides were stained using the Trypsin - Giemsa technique. For the CA analysis, 100 metaphases in the first cell cycle were evaluated per subject under a microscope (100X) to identify the numerical and structural chromosomal abnormalities. Observations were recorded on the master tables and later transferred to a computer file.

Micronucleus (MN) Assay

The MN assay was performed using the cytokinesis B technique (Fenech and Morley 1985). Lymphocytes were cultured in the same manner as described above. Cytokinesis B (6 µg/ml) was added at the 44th hour. After the total incubation time of 72nd hour at 37°C, cells were harvested by centrifugation, rinsed and treated with mild hypotonic solution followed by immediate fixation with methanol: Acetic acid. The slides were prepared according to the standard cytogenetic procedures and stained with four percent Giemsa. Slides were coded and scored by a light microscopy at 400X magnification. For each experiment, 1000 binucleated lymphocytes with well-preserved cytoplasm were scored. MN was identified according to the criteria of Fenech et al. (2003).

Biochemical Analysis

Blood

About 7-10 ml of fresh blood was collected and transferred immediately into 10 ml EDTA (7.2 mg) tube containing sodium meta-bisulphite (20µL, 2M) as an antioxidant and kept at 4°C. The plasma was separated by centrifugation (4°C, 2000 rpm) for 30 minutes. The plasma was deproteinated with 50µl of 4M HClO₄, centrifuged (4°C, 5000 rpm) and the supernatant was collected for the analysis

Extraction of Plasma Monoamines

The deproteinated plasma was added to 1.5 ml of Tris-HCl buffer with pH 8.6 and then ad-

sorbed onto the acid-boiled dry alumina (25 mg) kept at 65°C. The alumina was washed thrice with millipore water containing 0.001 EDTA and 0.1M sodium meta-bisulphite. The monoamines were eluted from the alumina (dried) with 100µl of 0.6M H_3PO_4 , mixed for 5 minutes, then centrifuged for 5 minutes (4°C, 2000 rpm). The elute was transferred to a micro centrifuge tube for the HPLC analysis (Rao et al. 1989).

Determination of Plasma Monoamines by HPLC

The protein in the sample was denatured by storage in the frozen state and separated by centrifugation. 2ml of the solution was taken in 15ml capacity centrifuge tube and treated with 200µl of internal standard solution, 400µL of 0.5M Tris-HCl, pH-8.6. This was followed by the addition of 20 mg of activated alumina. The content of the tube was shaken gently for 15 min in a spiral mixture. Tube was centrifuged at 600xg for 2 min. The supernatant was removed and the alumina was washed three times with a buffer solution by centrifugation each time for 2 min. Dopamine, GABA, homocysteine and serotonin were eluted from the alumina and the elute was centrifuged and 20µl of supernatant was collected and injected into the chromatograph and compared with the injected reference standard solution of the neurotransmitters.

Genotyping

Genomic DNA was isolated from the buffy coat using a Blood Spin DNA purification kit by Qiagen. The DNA was amplified by the primers used in (Moon et al. 1997) and the obtained PCR products were characterized by gel electrophoresis. Five microliter of the PCR product was digested with 10U of *HhaI* (New England Biolab. Co., Beverly, MA, USA) restriction enzyme for 4h. The digested fragments were separated by electrophoresis using one percent agarose at 150V for few hours. After electrophoresis the polymorphic patterns were analyzed. Electrophoresis was performed with the digested fragments and the polymorphic patterns were analyzed. For the restricted patterns from each Apo alleles, nucleotide sequence analysis was performed using the PC/GENE computer program (Intelli-genetics Inc., Mountain View, CA, USA).

Statistical Analysis

All the statistical analyses were performed using the software SPSS, version 17 to assess the group statistics for subjects such as Mean $\pm$ SD. For the statistical data inference, the t-test for independent variables and ANOVA were used to compare the mean values of the quantitative variables. The comparison of the genotype frequencies and the deviation of genotype frequencies from expected subjects were examined by the chi-square test. Multiple regression analysis was carried out to determine the correlation of the continuous variables, by using them as dependent variables, with several independent variables. In all the analyses, the significance level 0.05 was adopted.

RESULTS

In the current research totally 70 subjects were recruited who were exposed to copper which included n=28 early-onset AD patients (30 to 60 yrs of age) and n=42 the late-onset AD (> 60 yrs of age) patients. Table 1 shows the general characteristics of the AD patients. Furthermore, in the present study patients were classified based on their smoking habits as smokers (n=12) 42.85 percent and non smokers (n=16) with 57.14 percent in the early-onset and in the late-onset the smokers (n=30) 71.42 percent and the non smokers (n=12) were found with 28.57 percent. Pedigree analysis was conducted for each and every subject and it was found that 85.71 percent (n=24) were familial and 14.28 percent (n=4) were sporadic in the early-onset and in the late-onset 19.04 percent (n=8) were familial and 80.95 percent (n=34) were found to be sporadic.

The detailed chromosomal aberration (CA) and frequency of micronucleus (MN) for the individual samples were analyzed. Table 2 depicts the comparative analysis of CA for the early-onset AD patients and the late-onset AD patients and the mean $\pm$ SD values of the age groups. The mean $\pm$ SD values of chromatid type aberrations (CTAs) and chromosomal type aberrations (CSAs) for early-onset and late-onset AD patients were 1.51 ± 0.50 , 2.89 ± 0.85 , 0.77 ± 0.61 and 1.53 ± 0.68 respectively. The mean age of the late-onset AD patients was 71.97 ± 5.73 yrs and early-onset AD patients was 45.38 ± 6.93 yrs. The late-onset AD patients showed a

Table 1: General characteristics of Alzheimer's disease patients

Group	Particulars		No. of subjects (n = 70)	Percentage (%)
EOADn = 31	Gender	Male	14	45.16
		Female	17	54.83
	Smoking	Smokers	12	38.70
		Non smokers	19	61.29
	Family history	Familial	24	77.41
		Sporadic	7	22.58
LOADn = 39	Gender	Male	16	41.02
		Female	23	58.97
	Smoking	Smokers	20	51.28
		Non smokers	19	48.71
	Family history	Familial	8	20.51
		Sporadic	31	79.48

EOAD - Early-Onset Alzheimer's disease, LOAD - Late-Onset Alzheimer's disease

higher CA level (4.43 ± 1.46) when compared to the early-onset AD patients (2.25 ± 0.96). The MN frequencies were found to be higher in the late-onset AD patients (1.64 ± 0.95) than in the early-onset AD patients (0.54 ± 0.50). Higher degree of MN frequency was observed in the smokers of late-onset AD (1.85 ± 1.08) when compared to the early-onset AD (0.54 ± 0.45). Also, the MN frequency in the non smokers was significant in the late-onset AD (1.42 ± 0.68) when compared to the early-onset AD (0.42 ± 0.50) at $P < 0.05$ level.

Table 3 depicts the karyotypic results of the AD patients. Totally 21/70 patients were seen with chromosomal anomalies. The aberrations were: 2/21 (9.52%) supernumerary markers from chromosome 15 and 17p; 9/21 (42.85%) deletions of 13p22.33, 19q, 22q, 19q21.2, 22q, 19q, 10q22., 13q, 12q; 5/21 (23.80%) duplications of 13q, 17p, 14q, 9p, 19q; 2/21 (9.52%) translocations of two balanced t(5;11)(q25;q31.2), t(10;14)(q12;q22.4); 3/21 (14.28%) inversions of 19q, 17q, 19p.

In Table 4, the ApoE genotype frequencies for the early-onset AD and the late-onset AD patients were compared. Genotypes of the late-onset AD patients were 47.6 percent (n=20) for $\epsilon 3/\epsilon 4$, 30.9 percent (n=13) for $\epsilon 3/\epsilon 3$, 9.5 percent (n=4) for $\epsilon 2/\epsilon 3$, 9.5 percent (n=4) for $\epsilon 4/\epsilon 4$, 2.3 percent (n=1) for $\epsilon 2/\epsilon 4$ and 0 percent for 2/2. The ApoE allele frequencies were 0.100 for $\epsilon 2$, 0.543 for $\epsilon 3$ and 0.357 for $\epsilon 4$. The ApoE genotype frequencies for the early-onset AD subjects were 42.8 percent (n=12) for $\epsilon 3/\epsilon 3$, 25 percent (n=7) for $\epsilon 3/\epsilon 4$, 14.2 percent (n=4) for $\epsilon 2/\epsilon 3$, 7.1 percent (n=2) for $\epsilon 2/\epsilon 4$ and 7.1 percent (n=2) for $\epsilon 2/\epsilon 2$ and 3.5 percent (n=1) for $\epsilon 4/\epsilon 4$, and allele frequencies

were 0.763 for $\epsilon 3$, 0.137 for $\epsilon 4$ and 0.100 for $\epsilon 2$. A comparison of the frequencies for ApoE genotypes among the the early-onset AD and the late-onset AD subjects demonstrated a significant difference in the late-onset Apo $\epsilon 3/\epsilon 4$ with 47.6 percent and for the early-onset in Apo $\epsilon 3/\epsilon 3$ with 42.8 percent. The allele frequencies $\epsilon 4$ were also significantly different between the late-onset AD (0.357) and the early-onset AD (0.13).

The levels of serotonin, GABA, and dopamine in plasma of the late-onset AD subjects were found to be low with $0.20 \pm 0.02 \mu\text{mol/L}$, $83.12 \pm 4.21 \mu\text{mol/L}$, $305.97 \pm 40.19 \text{ pmol/L}$ respectively and for the early-onset it is $0.26 \pm 0.03 \mu\text{mol/L}$, $95.61 \pm 4.97 \mu\text{mol/L}$, $421.87 \pm 47.22 \text{ pmol/L}$. The level of homocysteine in plasma of the late-onset AD was higher $18.02 \pm 1.12 \mu\text{mol/L}$, when compared to the early-onset AD 15.06 ± 0.65 (Table 5).

DISCUSSION

Alzheimer's disease accounts more than seventy percent of dementia cases hence it is necessary to recognize the symptoms in advance before it gets worse. Recent research on cytogenetics was less and the screening of polymorphisms has been given importance in Alzheimer's disease. Cytogenetic analysis on the AD patients has been carried out in a number of laboratories which reported an evidence for linkage at 9p21.3, 9q22.33, 10q21.2 and 19q13.32 chromosomes (Marian et al. 2007). There was a significant evidence to support a susceptibility locus on 10q21.2, with a strong indication that this locus operated in a number of populations,

and the most likely location of the risk gene(s). Interestingly, in current research, abnormalities were seen in 21 patients and a high degrees of anomalies were seen in chromosomes 10q, 9p, 9q, 14p and 19q. Some chromatid type aberrations (CTAs) have also been observed and it has been already reported in the previous studies by Sillen et al. (2006). The effect of copper has no unusual effect on the chromosomes rather commonly known aberrations were seen. In the research investigation the Mean $\pm$ SD values of the total CA showed a higher level in the late-onset AD patient, when compared to the early-onset AD patient. Moreover, families with a rare autosomal dominant form of AD begins before the age of 50 years that have been linked to either chromosome 14p or chromosome 21, but these individuals represent less than one percent of all patients with AD (Van et al. 1992). The study investigated the MN frequencies which were found to coincide with the results of Petrozzi et al. (2002) which indicated a high level of spontaneous micronucleus binucleated MN in the late-onset AD patients.

In the current study, there are no de novo mutations seen in the ApoE due to the exposure of copper. Hence, the frequencies of ApoE obtained in the previous studies were matched with the present work. The ApoE ϵ^4 allele has received attention as a genetic risk factor and increase of developing AD. The pathology of the late onset AD is unknown and the majority of the cases are sporadic and heterogenous. In the existing study, the ApoE genotype frequencies of late-onset AD (Apo ϵ^3/ϵ^4 = 46.1%) and early-onset AD (Apo ϵ^3/ϵ^3 = 45.1%) were significantly different from allele frequency of the late-onset AD (0.357) and the early-onset AD (0.317). Recently, ApoE ϵ^4 carriers showed age related changes in the cerebrospinal fluid phosphorylated tau, total tau and amyloid- β 1-42 (Toledo et al. 2015). Several studies showed an increased frequency of the ApoE ϵ^4 allele in the persons with AD when compared with the controls (Corder et al. 1993). Previous studies on ApoE polymorphisms on Indian cases reported subsidiary higher ϵ^4 frequencies in the subjects aged 55 years or older (Chandra et al. 1998). Corder et al. (1993) revealed that the two ApoE ϵ^4 alleles were 8.1 times likely to develop AD rather than one allele. Not all persons with ApoE ϵ^4 alleles will develop AD, nor do all persons with AD carry an ApoE ϵ^4 allele. Therefore, ApoE is neither necessary nor

sufficient for the development of AD. There is also an increased risk of developing AD when associated with the increased copies of the ApoE ϵ^4 allele. However, relatively low frequency of the allele in the general and AD populations limits the utility of ApoE. Earlier studies have reported the association of ApoE ϵ^4 allele for both late-onset familial AD and sporadic AD (Strittmatter et al. 1993; Tsai et al. 1994).

Besides the genetic association of AD, the neurotransmitters played a key role in the pathogenesis of AD. Generally, AD is affected by the neurotransmitter acetylcholine due to the lack of choline acetyltransferase regulation and its association with AD had been studied in many researches so, for a change, in this research, the four biochemical monoamines serotonin, dopamine, GABA and homocysteine were chosen for investigations. Association of the cardiovascular factors (vascular dementia) and Alzheimer's have an increased level of plasma homocysteine, which is a major biochemical for the vascular risk factor. The plasma homocysteine (tHcy) level to the development of dementia and AD elevated to 5 μ mol per liter which increased the multivariable-adjusted risk of AD by forty percent (Sudha et al. 2002). Homocysteine has the potential to produce ROS by reducing Cu (II) to Cu (I). In a study, neuronal cell death occurred during cell-free Cu (I) detection assay which correlated with the Hcyc/Cu specific potentiation (Anthony et al. 2001). Hcyc is involved in the over activation of the ionotropic glutamate receptors to induce neuronal damage in the susceptible neurons (D'Emilia and Lipton 1999; Kim 1999). Interaction between Hcyc and Cu in the brain has the potency to cause Cu neurotoxicity and lead to a neuronal degeneration Anthony et al. (2001). Elevated plasma homocysteine levels are associated with carotid atherosclerosis and an increased risk of stroke (Bostom et al. 1999). From the precedent findings the current research revealed a higher level of homocysteine in the late-onset rather than in the early-onset cases. The occurrence of psychologic disturbances closely related to the disruption of the serotonergic system (Lancot et al. 2001). Loss of neurons in the serotonergic raphe nuclei (Mann and Yates 1983) and dysfunction of its nerve terminals in the neocortex (Palmer et al. 1987) have been reported in AD. Many evidences support the serotonin (5HT) deficiency theory concerning the psychobehavioral symptomatology of

AD, as examined in the postmortem (Assal et al. 2004) and the pharmacotherapeutic studies (Petracca et al. 1996). There is always a combination of serotonergic and cholinergic disturbances which play a role in the cognitive impairment in AD with serotonergic dysfunction responsible for the behavioural aspects of the disease (Carolyn et al. 1998). The comorbidity of intrinsic depression in the patients with AD may be a confounding factor in a study of in vivo serotonergic changes in AD (Kepe et al. 2006; Truchot et al. 2008). Similar to the previous studies on serotonin dysfunction, present findings were also responsible in causing AD and showed the least presence when compared to the normal level 0.28-1.14 $\mu\text{mol/L}$.

In spite of serotonin, other neurotransmitters also took part in the pathogenesis of AD. In the 2012 study in a mouse model, a decrease in the hippocampal GABAergic neurons was seen by the overproduction of A β 42 (induced for 2-4 months) and in vitro the GABAergic neurons were susceptible to the A β 42 neurotoxicity which showed that the GABAergic neuron dysfunction was seen in later the stages of Alzheimer's (Krantic et al. 2012). The loss of GABA in normal aging and AD contrast sharply with the long standing accepted the view that GABA receptors were fairly well spared in the senescent (Allen et al. 1983) and the AD brains (Owen et al. 1983). The decline of GABA was consistent with the soma shrinking (Hoff and Morrison 2004) and marked regressions of dendritic arbors and neurophil complexity observed in the cortical areas of the aged brains (Dickstein et al. 2007). The other pathological hallmark of AD was the dopamine (DA) reduction. Dopaminergic neurons had an unclear relationship with A β pathology and their contribution to AD was still indistinct (Trillo et al. 2013). Dopamine dysfunction was by apathy and the EPS (extrapyramidal symptoms) and it progresses in individuals with mild cognitive impairment, as well as in AD patients (Iqbal et al. 2000; Mitchell et al. 2011). When compared with the earlier results, the current outcome showed that the GABA seems to be less when compared to their normal levels 101-150 $\mu\text{mol/L}$ and dopamine level did not show any variation from their normal value <475 pmol/L.

The science ruins the flawed and more research needs to be done, however professionals are discovering new role of genes in AD. In

general, the existence risk of getting Alzheimer's is estimated between ten and twelve percent. Awareness of depression in the differential diagnosis of AD is essential for the genetic counselors seeing patients at risk for an early-onset familial AD (EOFAD). Besides, research has demonstrated that testing individuals for Apo E can be valuable and safe in certain milieu. However, because of the intricate genetic nature of the disorder, few clinicians are prepared to address the genetic risks of AD with their patients. Given the increased awareness in family history, the increasing incidence of AD, and the availability of direct consumer testing, patient requests for information is increasing. This practice guideline provides clinicians with a framework for assessing their patient's genetic risk for AD.

CONCLUSION

The researchers concluded that AD is a neurodegenerative disease involved interaction between the biological and environmental factors. The exposure of copper did not show any de novo changes in ApoE or in the neurotransmitters. The cytogenetic and molecular analyses are essential for the detection of AD. In previous researches, there is not much research in cytogenetics hence the information obtained by the techniques would provide the basis for determining the risk of recurrence of abnormalities and deciding clinical treatment and genetic counseling. The accuracy of clinical diagnosis could be improved using the fluorescence in situ hybridization (FISH) and other complementary molecular approaches. Molecular studies on ApoE were done using PCR-RFLP and their strong association with the late-onset AD patients was seen. Biochemical analysis was used as a biomarker study which showed the severity of AD. Therefore, future work will be critical for confirming the current findings in the larger cohorts of patients and broad range of AD using the GWAS (genome wide association screening) studies, for an accurate differentiation of gene expression studies in AD patients based on sex and age, precise onset age and family history.

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