Ann Natl Acad Med Sci (India), **45(2):** 117-129, 2009

Immunohistochemical Study of Aging Rat Brain – Effect of High Aluminium and Restricted Calcium in Diets

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Abstract

Certain environmental factors which accumulate slowly with age could be associated with the pathogenesis and/or progression of neurodegenerative disorders with associated cytoskeletal abnormalities in neuronal cells. In aluminum (Al) induced encephalopathy, abnormally high phosphorylation states of the neurofilament proteins affect crucial interactions between the cytoskeletal components leading to disruption of integrity of architecture. Dietary factors such as deficiency of micronutrients viz iron, magnesium and calcium have been implicated to favor Al absorption and accumulation in the body and contribute towards the etiopathology of Al associated neurological disorders. Therefore, this study was conducted to examine any alteration in the neuronal cytoskeletal components which could arise due to chronic feeding of moderately high levels of Al to experimental rats maintained on low dietary calcium in their diets. This situation simulates the one found in India where Al vessels are commonly used for cooking and storing food and intake of calcium is well below the optimum. The high number of neurofibrillary tangles found mainly in the substantia nigra region in the brains of rats fed moderately high levels of Al merits attention. It also throws light on the contribution of dietary aluminium in the causation of neurotoxicity at the cellular level.

Key words: Aluminium, Cytoskeletal proteins, Low calcium diet, Neurodegeneration, Neuropathology

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Introduction

Most of the neurological disorders like Senile Dementia of the Alzheimer's type (SDAT) and Parkinson's Dementia (PD) where Aluminum (Al) is implicated as one of the risk factors are associated with aging (1,2). This could probably suggest that certain environmental factors which accumulate slowly with age, (like Al) could be associated with the pathogenesis and/or progression of these neurodegenerative disorders which have been shown to reveal defects in cytoskeletal architecture of the neuronal cells (3).

The neuronal cytoskeletal components viz. NeuroFibrillary Proteins (NFPs) and Microtubules Associated Proteins (MAPs) esp. tubulin associated unit (tau) have been shown to be the main targets of Al toxicity (4, 5). Microtubules are involved in various cellular processes viz. chromosomal movement, axonal transport and stabilization of cell shapes. Tau is more abundant in axons and appears to promote tubulin assembly. Neurofilaments (NFs) are the major structural components of the axons, dendrites and neuronal cell bodies. Apart from maintaining the structural integrity of neuronal cells, NFs are also suggested to be involved in axonal transport of lysosomes, enzymes, neurotransmitter molecules and other metabolites. Normal nerve cells and dendrites contain NF in its nonphosphorylated form.

In Al induced neuropathy, selective impairment in the axonal transport of neurofilaments leads to the accumulation of neurofibrillary material in the perikaryon and in the proximal parts of the axons and dendrites (6).This has been viewed as a consequence of abnormal phosphorylation of the neurofilament proteins which might alter certain crucial interactions between the cytoskeletal proteins (7,8). It has also been proposed that specific interactions of Al with the phosphate groups of the cytoskeletal proteins may lead to the aggregation of highly phosphorylated NFs and MAPs in experimental toxic states (9-11). As an alternative hypothesis for the Al induced neurofibrillary degeneration, inhibition of activated proteolytic degradation of neurofilaments by Al has been proposed (10, 12).

Aluminum has been shown to be an important link between tau protein and Alzheimer's disease (AD) (13), where it is suspected to be a cofactor in the formation of "NeuroFibrillary Tangles" (NFTs) by interacting with paired helical filaments - tau (PHF-tau) (4). Al has been shown to cross the blood-brain barrier as L-glutamate

complex and cause neurotoxicity especially in the hippocampus region (14). Aluminum maltolate treated rabbits also have been reported to show neuronal cell loss and neuronal pathology similar to Alzheimer's disease viz. neurofibrillary pathology (15). A study on explant cultures of cortical neurons, established from rat embryos, showed that aluminum enters neurons and induces possible conformational changes in tau as detected by the Alz-50 antibodies (16). Environmental factors have also been implicated in the pathophysiology of most Al associated neurological disorders (17)

Dietary factors such as citrate, lactate, ascorbate etc. and essential nutrients like iron and calcium have been shown to play an important role in modifying the gastrointestinal absorption of aluminium (18). While citrate promotes formation of soluble Al-complexes and thereby increases its absorption, reduced intake of iron or calcium also increases Al absorption and retention in experimental animals (18, 19). The citrate content of the most vegetarian diets is high and calcium requirements are also not generally met in Indians (20). Based on the available evidence, and the potential risk of Al toxicity in the Indian sub-continent a study was conducted to investigate the effects of chronic toxicity of dietary Al coupled with Ca restriction on the neuronal cytoskeletal proteins in the brain.

Materials and Methods

Experimental Design

A total of 24 weanling, male, Wistar (WNIN) rats were randomly divided into four groups of six animals each. The animals were housed in individual plastic cages with stainless steel wire mesh bottom and lid under recommended conditions of 12 hr light/ dark cycle, 25±1°C room temperature and relative humidity of 55 to 60%. Stainless steel diet cups and plastic water bottles with stainless steel nozzles were used. Rats were given diet daily along with double distilled water and the concentration of Al in the drinking water was negligible. The actual food intake was recorded every day and body weights were taken on a weekly basis. Serum Ca levels were also estimated. The animal's dietary regimens were:

(a) Normal Ca diet - Low Al (0.5% Ca, 20-30 ppm Al).

(b) Normal Ca diet - High Al (0.5% Ca, 220-230 ppm Al).

(c) Low Ca diet - Low Al (0.125% Ca, 20- 30 ppm Al).

(d) Low Ca diet - High Al (0.125% Ca, 220 - 230 ppm Al).

The rats were fed on their respective diets for 18 months and then euthanized. The brain samples were removed and subjected to histopathology study as well as tissue Al levels estimation.

Composition of diet used

Diet component	g/kg diet
Starch	350
Sucrose	350
Casein	200
Groundnut oil	50
Salt mixture	40
Vitamin mixture	10

Choline chloride (1g/kg diet) was first added to the vitamin mixture and then mixed with the diet.

One kg of control diet contained 10g $CaCO_3$ (5g Ca/kg diet). Casein provided 20.4 mg Zn/kg diet (the recommended amount for Zn being 12.0mg/kg diet). Therefore, additional Zn was not added in the salt mix. The amount of CaCO3 was adjusted in the experimental diets so as to obtain 1.25g of Ca per Kg diet (75% restriction of Ca). These diets were found to contain 20 to 30 ppm of Al. Additional $Al_2 (SO_4)_3$ $16H_2O$ was added to the diets to achieve 220 to 250 ppm of Al for the high Al diet groups. Ca: P was maintained at 1.25:1 by manipulating the concentration of KH_2PO_4 in diet. All diets had 0.1% citric acid.

Methodology

Brain Al level estimation was done as per the method of D'Haese *et al* (21) and serum Ca level estimation was done as per the method of Zettner *et al* (22)

For histopathological examination of brain, the tissues were fixed in 10% neutral buffered formalin and paraffin embedded 5 μm thick sections were cut uniformly from 3000μm posterior to bregma so as to obtain the hippocampus and substantia nigra regions of the brain in the same section. The sections were stained with Mayer's Hematoxylin-Eosin (H & E) stain as per the conventional staining protocol. Histological alterations if any (granulation / vacuolation), in these regions were observed under a light microscope. Immunohistochemistry was performed on paraffin embedded sections as per the method of Takeda M. *et al* (23). Briefly, antigen specific primary antibody was applied to deparaffinized hydrated tissue sections. Following a brief wash, the section was incubated with a biotinylated secondary antibody. With the addition of Avidin-Peroxidase reagent, a stable avidin-biotin complex is formed with the bound biotinylated secondary antibody. Sites of antibody deposition are visualized by addition of freshly prepared substrate containing hydrogen peroxide and the electron donor chromogen 3-amino-9-ethyl

carbazole (AEC). Bound peroxidase catalyses the oxidation of AEC to form a rose red to reddish brown insoluble precipitate at antigen sites which is observed under a light microscope.

Mouse derived monoclonal MAP anti-tau (clone Tau-2) antibody and anti-neurofilament-200 (NF-200) for phosphorylated and non-phosphorylated protein (clone-N 52) antibody (Sigma SIH) were employed. The quantification of positively stained cells in substantia nigra region was carried out under light microscope. For quantification of positively stained cells for each of the antibody used, the brain section was screened for 100 cells and the percentage of positively stained cells was calculated. The intracellular neurofibrillary tangle bearing neurons were identified using Beilschowsky's silver stain.

Statistical evaluation

The statistical evaluation of the data was done by analysis of variance and the significance was tested by comparing the least significant differences (Snedecor and Cochran, 1967).

Results

There were no differences in the body weights and food intake between any of the treatment groups while serum calcium levels were on expected lines. Increase in brain aluminium levels was observed (Table 1) and was found to be significantly higher (5.5 ± 0.55 μg/g wet weight) in rats receiving Ca- restricted and high- aluminium diet for eighteen months when compared to rats receiving normal Ca and high aluminium diet (3.9 ± 0.36 μg/g wet weight) while low Al levels with and without Ca restriction were comparable. The light microscopic examination of brain sections showed no alteration in the gross histology (viz. granulation/vacuolation) in rats fed on Ca restricted diet with low/high

Al supplementation (Fig. 1) Our earlier short term study of six and twelve months duration of Al exposure

Table 1: Effect of dietary calcium restriction on brain aluminium levels.

SI.No.	Groups (n=6)	Al level (μg/g) wet weight
1.	Normal Ca + Low Al	2.9 ± 0.54^{a}
2.	Normal Ca + High Al	3.9 ± 0.36^{a}
3.	Low Ca + Low Al	2.8 ± 0.45^{a}
4.	Low Ca + High Al	5.5 ± 0.55^{b}

Values with different superscripts are significantly different ($p < 0.01$).

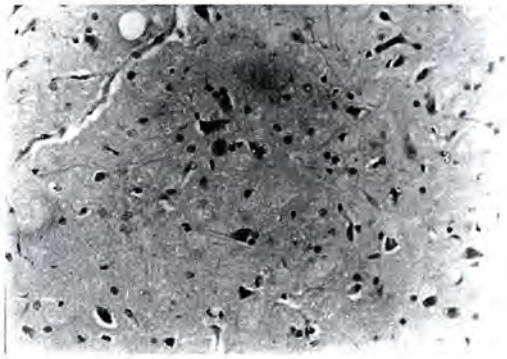

Fig. 1 : Substantia Nigra region of the brain. H & E X 250

(unpublished) showed no cytoskeletal abnormalities in rats fed on calcium restricted diet with low / high Al supplementation. However, the brain Al levels were higher in high Al fed rats and hence it was decided to continue the feeding schedule for 18 months. Ca-restricted rats receiving high Al for 18 months showed an increase in the number of tangle bearing neurons in substantia nigra, as revealed by light microscopic examination of H & E stained brain sections and also confirmed by Bielchowsky's silver stain methods (Fig. 2 and Table 2). No significant changes were observed with respect to any other group of rats.

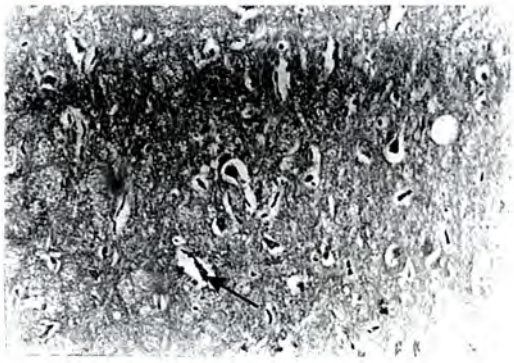

Fig. 2 : Neurofibrillary tangle bearing neurons (arrow) in the Substantia Nigra region - Beilschowsky's stain X 250.

The immunoreactivity to monoclonal antibodies against NF-200 and MAP-Tau was found to be higher in substantia nigra region of rats which received Ca-restricted diets supplemented with high Al. With NF-200

Table 2 : Number of Neurofibrillary Tangle (NFT) bearing neurons in substantia nigra region

Sl.No.	Groups (n=6)	No. of NFT bearing neurons
1.	Normal Ca + Low Al	7.0 ± 1.0^{a}
2.	Normal Ca + High Al	7.0 ± 2.0^{a}
3.	Low Ca + Low Al	5.0 ± 2.0^{a}
4.	Low Ca + High Al	23.0 ± 3.0^{b}

Values with different superscripts are significantly different ($p < 0.01$)

immunoreactivity, the number of positively stained cells in these animals was also comparatively higher than all other groups. However, with respect to MAP tau, both high & low Al exposure with low Ca diets was significantly different for positive cells as compared to normal Ca diets. (Figs. 3-5 and Table 3).

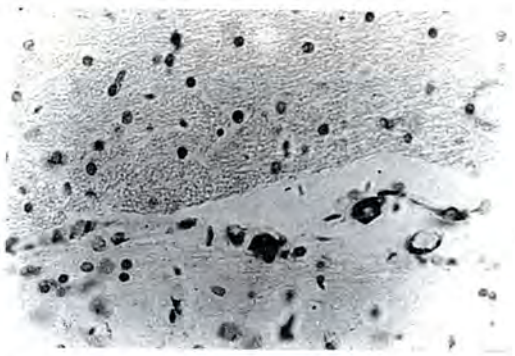

Fig. 3 : Negative control brain section for the immunohistochemical detection of NF - 200 and MAP - Tau X 400.

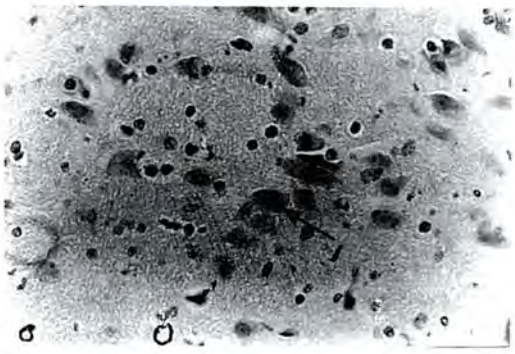

Fig. 4 : NF - 200 positive cells (arrow) in the hippocampus region X 400.

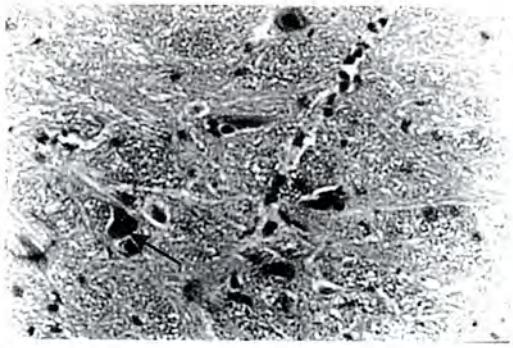

Fig. 5 : MAP - Tau positive cells (arrow) in the substantia nigra region X 400.

Table 3 : Number of Positive cells (%) representing immunoreactivity for monoclonal antibodies to MAP-Tau and NF-200

SI.No.	Groups (N=6)	Positive cells (%)
	Tau	NF - 200
1.	Normal Ca + Low Al	12 ± 3^a 10 ± 4^a
2.	Normal Ca + High Al	11 ± 3^a 9 ± 3^a
3.	Low Ca + Low Al	20 ± 4^b 11 ± 2^a
4.	Low Ca + High Al	25 ± 5^b 21 ± 4^b

Values with different superscripts are significantly different ($p < 0.01$)

Discussion

Abnormalities in the neuronal cytoskeleton in SDAT have been extensively reported in the literature. Cytoskeletal proteins are the potential targets of Al toxicity and hyperphosphorylation of tau and formation of NFTs has been observed in the Al-associated dementias (24, 25). The aggregation of both these protein components in the neurodegenerative disorders with which Al is associated, could be attributed to the specific interactions of Al with the phosphate groups of the highly phosphorylated cytoskeletal proteins-MAPs and NFPs (26, 27).

Earlier studies from our laboratory have shown that age dependent accumulation of Al in the rat brain was influenced by the levels of calcium in the diet (unpublished data). Another study reported earlier showed increased brain Al levels when fed with 200mg/kg/day of $AlCl_3$ by gavage for 8 weeks (28). Also literature evidence indicates enhancement of neurotoxicity potential of Al in states of Ca deficiency (29). Therefore, this study was conducted to understand the effect of moderately high levels of Al on the cytoskeletal components when dietary Ca was low. Earlier histochemical and immunocytochemical procedures adopted on rat brains with Al injections directly into the brain for 5 days, looked into the inflammatory response and cholinergic terminals in the hippocampus (30). We also observed that the changes seen were in line with those reported by other workers, although in different experimental models (29, 31). Moreover, this situation simulates the one widely prevalent in India where Al vessels are commonly used for cooking and storing food and the intake of essential nutrients like iron and calcium is below optimum (20,32). This issue was also stressed in an earlier work by Liu J *et al* (33).

In the present study, high number of NFTs was found mainly in the substantia nigra region, and Al being an integral part of the NFTs has been proved by a study of rabbits (34) and microprobe studies (35). Since Al enters the brain via transferrin endocytosis (36), it is likely that levels of Al could be much higher in areas such as substantia nigra and basal nuclei where the density of transferrin receptors is high. The neurodegeneration in this region is associated with certain neuronal disorders like Parkinson's dementia (24, 37). Earlier Garruto *et al* (31) obtained similar results with regard to Al induced

changes in cytoskeletal components in cyanomolgous monkeys maintained on chronic low dietary Ca supplemented with very high Al levels (150 mg/day). They have observed Al deposition and neurodegenerative changes, comparable to those seen in spinal cord, brain stem, substantia nigra and cerebrum in Amyotrophic lateral neurons. Aluminium binding to MAP-Tau and NF-200 protein and paired helical filaments (PHFs) has been shown *in vitro* also (38, 39). Decrease in total number of synapses was seen in a study done on male rats of 18 months age and fed Al at a dose of 100mg/kg/day for 100 days (40). An important observation in our study is the development of pathological changes even though the dietary Al exposure is not remarkably high as used in earlier studies. The Al-associated neuronal degeneration could probably be due to a longer period of Al exposure in vivo rather than a higher acute exposure. Therefore, Al-Ca interactions at the gut level appear to be important in determining Al-neurotoxicity as suggested by earlier workers also (41). As has been stated that select human populations are at risk of Al neurotoxicity (42), the same could be inferred from our study where long term exposure to Al caused changes in the neuronal milieu.

Acknowledgements

The authors acknowledge the financial support given by the Indian Council of Medical Research for the study. The technical help given by Mr.JS.Acharya and Ms.N.Sharada and Mr.B.Girbabu are also acknowledged

References

1. Mc. Dermott Jr, Smith AI, Iqbal K and Wisnewski HM (1979). Brain aluminum in aging and Alzheimer's disease. *Neurology* **29:** 809-814.
2. Crapper DR, Quittkat S, Krishna SS, Dalton AJ and De Boni U (1980). Intranuclear aluminum content in Alzheimer's disease, dialysis encephalopathy and aluminum encephalopathy. *Acta Neuropathol* **50:** 19-24.
3. Muma N.A and Singer SM (1996). Aluminum induced Neuropathology: Transient changes in microtubule associated proteins. *Neurotoxicology Teratology* **18:** 679-690.
4. Shin RW, Lee VM and Trojanoewski JQ (1995). Neurofibrillary pathology and alumi-

nium in Alzheimer's disease. *Histology and Histopathology* **10:** 969-978.

5. Strong MJ and Jakowec DM (1994). 200 kDa and 160 kDa neurofilament protein phosphatase resistance following in vivo aluminum chloride exposure. *Neurotoxicology* **15:** 799-807.
6. Wakayama I, Nerurkar VR and Garruto RM (1993). Immunocytochemical and ultra structural evidence of dendritic degeneration in motor neurons and aluminum intoxicated rabbits. *Acta Neuropathol (Berl)* **85:** 122-128.
7. Gotow T (2000). Neurofilaments in health and disease. *Med Electron Microsc* **33:** 173-199.
8. Julka D and Gill KD (1996). Involvement of altered cytoskeletal protein phosphorylation in aluminum induced CNS dysfunction. *J Biochem toxicology* **11:** 227-233.
9. Shea TB, Wheeler E and Jung C (1997). Aluminum inhibits neurofilament assembly, cytoskeletal incorporation, and axonal transport. Dynamic nature of aluminum induced perikaryal neurofilament accumulations as revealed by subunit turnover. *Mol. Chem. Neuropathol* **32:** 17-39.
10. Shea TB, Beermann Ml and Nixon RA (1995). Aluminum treatment of intact neuroblastoma cells alters neurofilament subunit phosphorylation. Solubility and proteolysis. *Mol. Chem. Neuropathol* **26:** 1-14.
11. Singer SM, Chambers CB, Newfry GA, Norlund MA and Muma NA (1997). Tau in aluminum induced neurofibrillary tangles. *Neurotoxicol* **18:** 63-76.
12. Cunningham K, Sopper MM and Strong MJ (1997). Enhanced ex-vivo co sedimentation of high molecular weight neurofilament protein (NFH) with microtubules following in vivo aluminum chloride exposure: Inhibition of dephosphorylation dependent dissociation. *Neurotoxicol* **18:** 355-362.
13. Savory J and Garruto RM (1998). Aluminum, tau protein and Alzheimer's disease- an important link? *Nutrition* **14:** 313-14.
14. Deloncle R, Huguet F, Babin P, Fernandez B, Quellard N and Guillard O (1999). Chronic administration of aluminium L - glutamate in young mature rats: effect on iron levels and lipid peroxidation in selected brain areas. *Toxicology Lett* **104:** 65-73.

15. Van Ginkel MF,Vander J, Voet GB, D Haese PC, De Broe ME and de Wolff FA (1993). Effect of citric acid and maltolate on the accumulation of aluminum in rat brain and bone. *J Lab Clin Med* **121(3):** 453-460.

16. Jones KR and Oorschot DE (1998). Do aluminum and / or glutamate induce AIZ-50 reactivity? A light microscopic immunohistochemical study. *J Neurocytol* **27:** 45-47.

17. Martyn CN (1992). The epidemiology of Alzheimer's disease in relation to aluminum. *Ciba Found Symp* **169:** 69-86.

18. Voet GBV (1992). Intestinal absorption of aluminum. *Ciba Found Symp* **169:** 109-122.

19. Oteiza PI, Keen Cl, Han B, Golub MS (1993). Aluminium accumulation and neurotoxicity in Swiss Webster mice after long term dietary exposure to aluminum and citrate. *Metabolism* **42:** 1296-1300.

20. Gopalan C (1994). Nutrition research in south east Asia- the emerging agenda of the future. *WHO regional pub.* Series No.**23.**

21. D'Haese P.C., Vande Vyver FL, De Wolff FA and De Broe ME (1985). Measurement of Al in serum, blood, urine and tissues of chronic haemodialyzed patients by use of electro thermal Atomic Absorption Spectrophotometer. *Clin. Chem.* **31(1):** 24-29.

22. Zettner A and Seligson D (1964). Application of Atomic Absorption Spectrophotometry in the determination of calcium in serum. *Clin. Chem.* **10:** 869.

23. Takeda M., Nishimura T., Kudo T. *et al* (1991). Characterization of intracytoplasmic neurofilament accumulation in hamster brain is caused by Alzheimer buffy coat inoculation: Comparison with experimental neurofibrillary changes produced by aluminium intoxication. *Gerontology* **37 (suppl):** 31-42.

24. Perl DP and Pendlebury WW (1986). Aluminium neurotoxicity - potential role in the pathogenesis of neurofibrillary tangle formation. *Can J Neurol Sci.* **13**: 441-445.

25. Li W, Ma KK, Sun W and Paudel HK (1998). Phosphorylation sensitizes microtubule associated protein tau to Al (3+)-induced aggregation. *Neurochem Res* **23:** 1467-76.

26. Gotow T, Tanaka J and Takeda M (1995). The organization of neurofilaments accumulated in perikaryon following aluminium

administration: Relationship between structure and phosphorylation of neurofilament. *Neurosci* **64:** 553-569.

27. Richard S Jope and Gail WV Johnson (2007). Neurotoxic effects of dietary aluminium. Novartis Foundation Symposia. Derek J Chadwick and Julie Whelan (Eds), 254 - 67

28. Baydar T, Papp A, Sahin G *et al.* (2003). Accumulation of aluminium in rat brain. *Biological Trace Element Research*.**92**, 231 – 244.

29 Taneda M (1984). Effect of aluminium on rat brain. Enhancement by calcium deficiency. *Hokkaido Igaku Zasshi* **59:** 312-337.

30. Bettinni P, Graham F, Gernot R and Zaineb H (2001). Aluminium toxicity in the rat brain: Histochemical and immunocytochemical evidence. *Brain Research bulletin*. **55:** 257-67.

31. Garruto RM, Shankar SK, Yanagihara R, Salazar AM, Amyx HL and Gadjusek DC (1989). Low calcium, high aluminium diet induced motor neuron pathology in cyanomolgous monkeys. *Acta Neuropathol (Berl)* **78:** 210-219.

32. Neelam, Bamji MS and Kaladhar M (2000). Risk of increased aluminum burden in Indian population -contribution from Aluminium cookware. *Food Chem.* **4:** 57-61.

33. Liu J, Nordberg GF and Frech W (1996) Aluminium accumulation in some tissues of rats with compromised kidney function induced by cadmium – metallothionein. *Pharmacology toxicology* **78(5):** 289-95

34. Savory J, Herman MM and Ghribi O (2006). Mechanisms of aluminium induced neurodegeneration in animals, *J Alzheimer's Dis* **10(2-3):** 135-44.

35. Perl DP and Good PF (2007). Aluminium and the neurofibrillary tangle: Results of tissue microprobe studies. Novartis Foundation Symposia. Derek J Chadwick and Julie Whelan (Eds), 217-36

36. Taylor GA, Morris CM, Fairbairn AF, Candy JM, Edwarson JA (1991). Transferrin- gallium binding in Alzheimer's disease. *Lancet.* **338:** 1394-1135.

37. Flaten TP (1990). Geographical associations between aluminium in drinking water and death rates with dementia (including Alzheimer's disease), Parkinson's

disease and amyotrophic lateral sclerosis in Norway. *Environ Geochem and Health* **12:** 152-167.

38. Pierson KB, Evenson MA (1988). 200 KDa neurofilament protein binds aluminium, copper and zinc. *Biochem. Biophysics. Res. Commun* **152:** 598-604.

39. Murayama H, Shin RW, Higuchi I, Shibuya S, Muramoto T, Kitamoto T. (1999). Interaction of aluminium with PHF - tau in neurofilament degeneration, evidenced by desferrioxamine assisted chelating autoclave method. *Am J Pathol.* **155:** 877-885.

40. Colmina MT, Roig JL, Sanchez DJ and Domingo JL(2002). Influence of age on aluminium induced neurobehavioral effects and morphological changes in rat brain .*Neurotoxicology* **23:** 775-81

41. Kawahara M (2005). Effects of aluminium on the nervous system and its possible link with neurodegenerative diseases. *J Alzheimer's Dis* **8 (2):** 171-82; discussion 209-215.

42. Verstraeten SV, Aimo L and Oteiza PI (2008), Aluminium and Lead: molecular mechanisms of brain toxicity. *Rev. Arch Toxicol* **82(11):** 789-802