Evidence to determine Ceruloplasmin as Biomarker for progression of Alzheimer´s diseas

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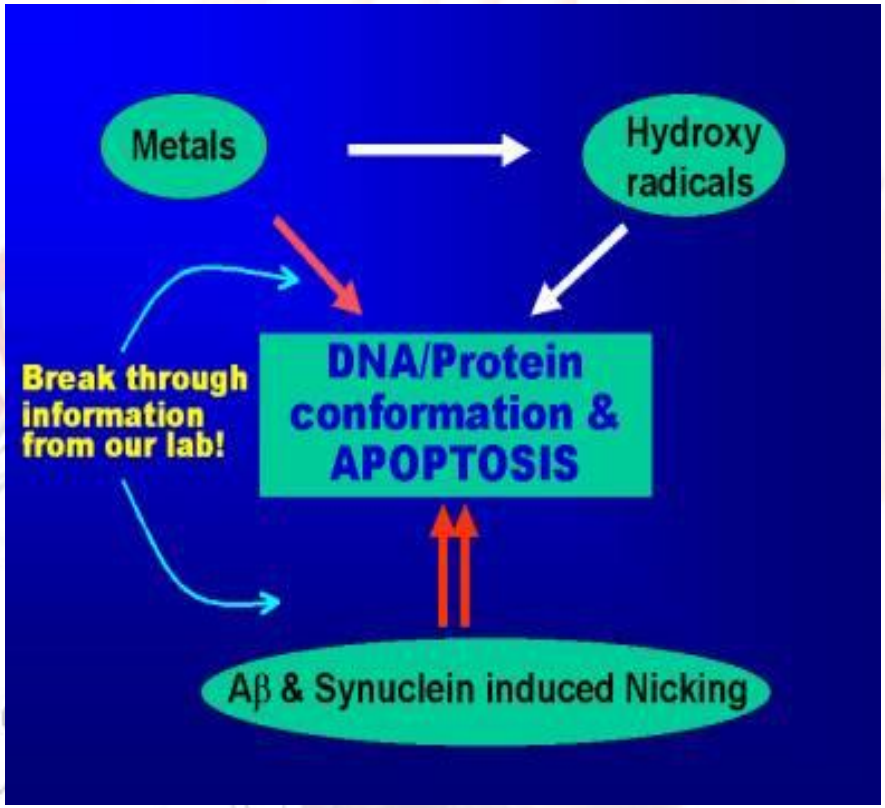
ABSTRACT

Alzheimer's disease (AD) is a complex degenerative brain disease. The etiology of AD is unknown and many theories including the possible role of trace elements such as copper and the related oxidative stress have been debated. The trace metal Copper has been attributed as a major risk factor and therapies have been centered on metal chelation concept. The transporter of copper, ceruloplasmin, is a multifunctional enzyme and sporadic literature in associating ceruloplasmin to neurodegeneration. The increase in brain metal concentration is associated with normal aging and a variety of degenerative diseases including AD. We studied serum copper, Fe and ceruloplasmin and Ferritin levels in 20 early and 15 severe patients with AD, mean age 68, and 30 control samples with age matching. The patient classification as early and severe is done by Psychatrician. Serum metal levels are estimated by ICP-AES and ceruloplasmin by chemoluminisence method and Ferritin by ferro-oxidase technique. We report here differential increase of Copper and iron levels serum samples in early and severe AD associated with increased level of ceruloplasmic only, but not with ferritin. We also hypothesized the role of these metals in on setting redox active iron levels with oxidative stress levels in serum samples. We also noticed that total antioxidant potential of serum is decrease in AD. There is hope to correlate serum copper and iron levels with ceruloplasmin as biomarker to understand the progression of the disease.

Greatest Scientific challenge of 21st century is to understand brain structure-function relationship in human health and disease.

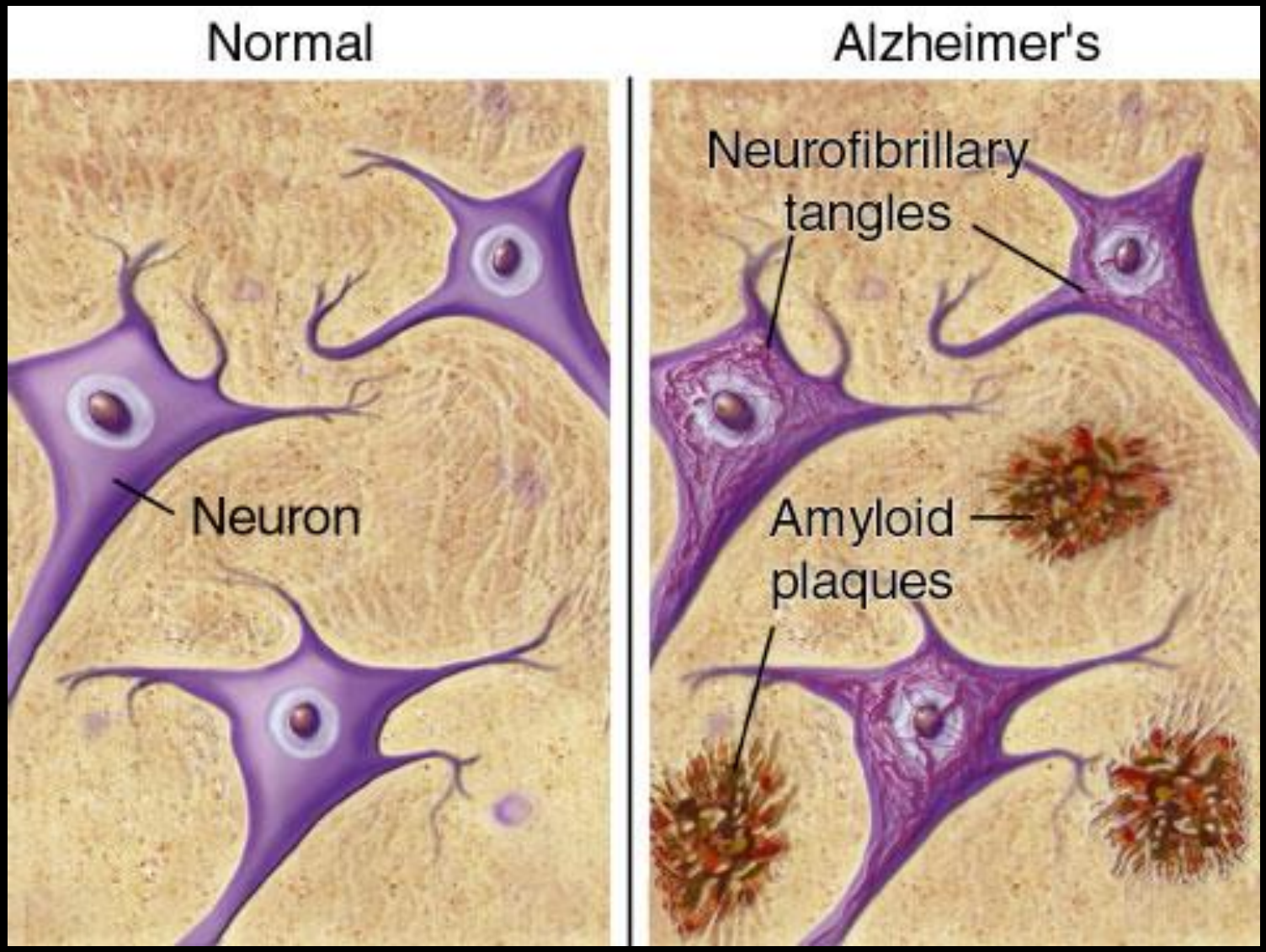
Neurological Disorders (Alzheimer's, Parkinson, Depression etc) account for about one-third of the disease burden in world



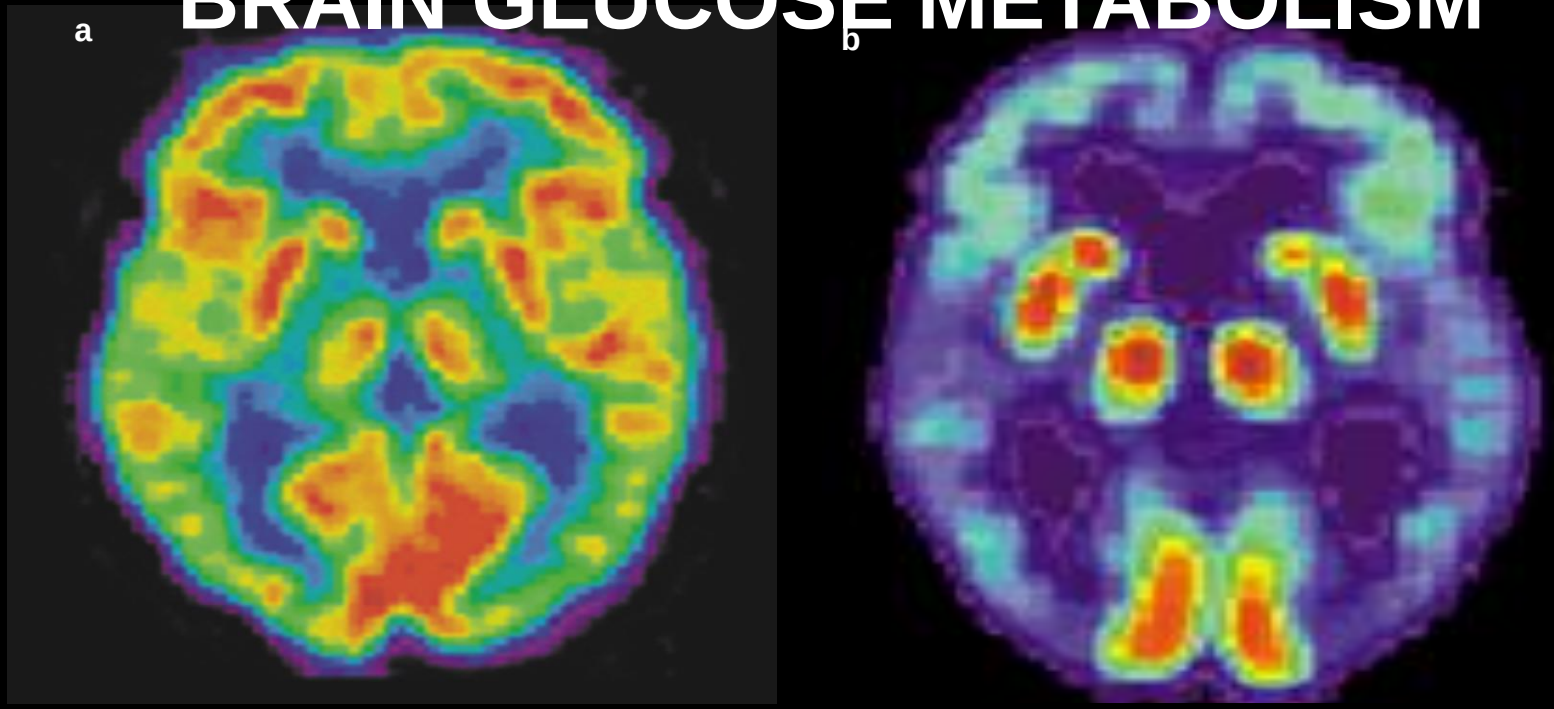
Factors responsible and mechanism of disease is still a challenge

It was shown changes in proteins, trace metals, DNA, neurotransmitters, hormones are prime factors in the disease process.

Characteristics of Alzheimer's Disease



BRAIN GLUCOSE METABOLISM



PET Scan (glucose utilization) of (a) Normal Brain and (b) Alzheimer's Disease Brain. Red and Yellow indicate high level of glucose uptake in a living healthy person and a normal control subject. The Alzheimer’s patient exhibits large decrease in energy metabolism in the frontal cortex (top of the brain) and temporal lobes (sides of the brain).

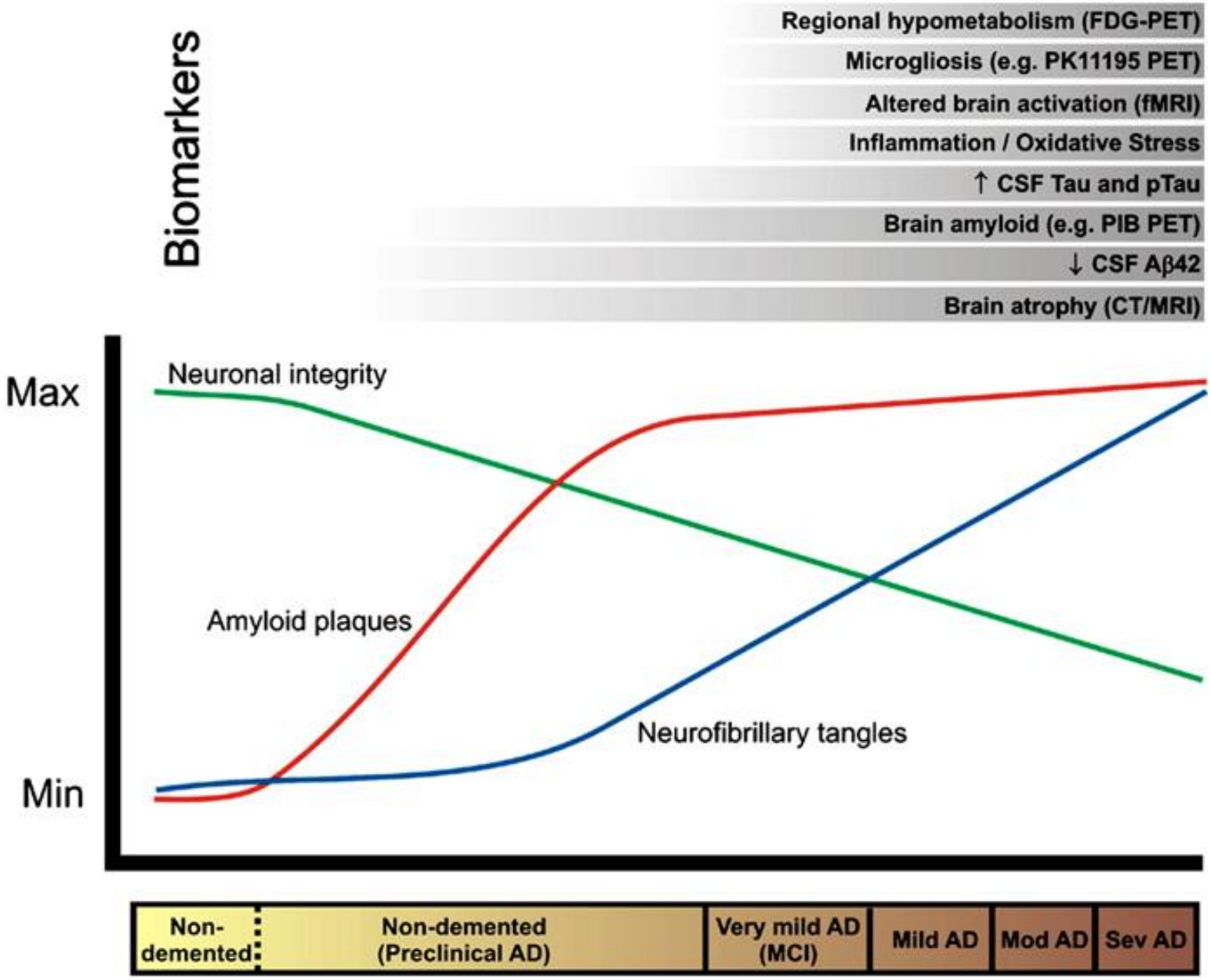
Biomarkers in AD:

There are no definite markers to understand progression or regression of AD

Efforts are made to understand CSF, Serum: Abeta 40, 42, Tau, Cu, etc as biomarkers

Now studies are focused on MRI imaging as possible biomarkers

Still there is lot of debate in identifying good biomarker for diagnosis and drug target



Hypothesized relationship between the time course of changes in various biomarkers in relation to the neuropathology and clinical changes of AD (Martin et al 2010)

Metals play a very significant role in the biology of cell

The significance of metals in nutrition and medicine is gaining substantial significance

The complex biology of metal homeostasis in cell to arrive at significant conclusions

Metal homeostasis as biomarkers

Studies from our lab showed few interesting results. (AD: Alz Reports 1999, ACS book, 2002, PD: JTBM, 2003, Comp Biol Med, 2006, Bipolar and depression: CCA, 2008)

Objective of the study:

To understand the relation between the levels of Cu and Fe to single and double strand breaks in normal ageing brain

To identify whether serum Cu and Fe levels along with Ceruloplasmin and Ferritin can be biomarkers for AD

Materials & Methods for metals and DNA damage in ageing brain:

Brain tissue: Brains are grouped into three groups. Group I: below 40 years, Group II: between 41-60 years and Group III: above 60 years. The two regions namely hippocampus and frontal cortex of normal brains were separated and stored at -80^{o}C until further use. Eight brain samples from each group were included in the study. Human brain samples were collected from the Depression Brain Bank of JSS medical hospital and College, Mysore, India. Autopsies were performed on donors with written informed consent obtained direct next of kin. The control human brains were collected from accident victims, who had no history of long-term illness, psychiatric diseases, dementia, or neurological disease prior to death. We have excluded the subjects who had drug and alcohol abuse. The average postmortem interval between the time of death and collection of brain and freezing was done around 6h. The brain tissue was isolated and stored frozen at -80˚C till the analysis.

Isolation of DNA from brain tissue: Genomic DNA was isolated from hippocampus and frontal cortex of frozen brain tissue by standard 'phenol-chloroform extraction' method after Sambrook et al (1989) with some modifications to prevent DNA fragmentation during isolation. Precautions were taken to prevent in vitro DNA damage during phenol-chloroform genomic DNA extraction. The concentration of DNA was measured using UV/Visible spectrophotometer noting absorbance at 260 nm and purity checked by recording the ratio of absorbance at 260 nm/280 nm which should be ideally between 1.6 and 1.8.

DNA strand breaks by Nick translation assay (Sutherland, 1983; Bhaskar&Rao, 1994; Deng& Wu 1983).

Trace metal analysis by ICP-AES using the following standard protocols

Element	Wavelength nm)	Detection limit*	
		μg/ml (ppm)	μmole/ml
Cu	224.7	0.002	0.00003
Zn	213.856	0.002	0.00003
Fe	259.94	0.005	0.00009

Wavelength and detection limit of the trace elements analyzed in ICPAES

***Detection limit (μ g/ml) for each element was calculated by running a multi-element standard solution containing 500 ng/ml of each of the above-cited elements.**

Trace metals concentrations in frontal cortex and hippocampus regions of aged human brain subjects (concentration in micro/g of wet weight of tissue). Mean +SD of 8 brains in each group. *p> 0.05, ** p> 0.001

Brain regions	Trace metals	Group I (N=8)	Group II (N=8)	Group III (N=8)
Fontal Cortex	Cu	4 .0 ± 1.7	5 .0 ± 1.6*	8.0± 1.3**
	Fe	50.8 ±2.6	60.5±3.5*	75±5.6**
	Zn	7.5 ± 0.5	6.5±1.1*	4.5±0.9**
Hippocampus	Cu	4.0 ± 1.0	4.5±1.1*	5.8±1.3*
	Fe	26.6 ±1.9	30.6±1.6*	45±1.75**
	Zn	6.5 ± 0.5	5.5±0.9*	5.0±0.6*

In both Frontal cortex and hippocampus, the levels of Cu, Fe are increased significantly, while Zn levels decreased.

This indicates the redox active molecules increased, while antioxidant Zn decreased in brain regions during ageing.

Single strand breaks (SSBs (10^6)/µg DNA) frontal cortex and hippocampus regions of aged human brain subjects. *p> 0.05, ** p> 0.001

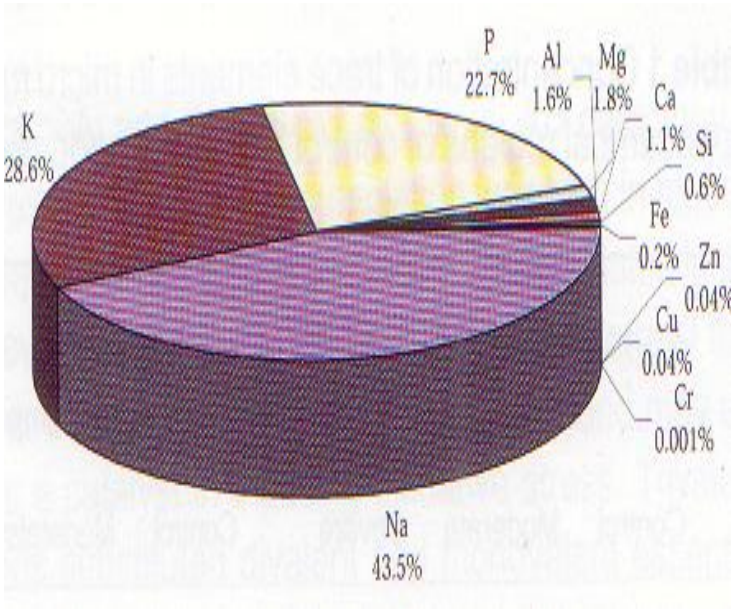
Brain Regions	Group I (N=8)	Group II (N=8)	Group III (N=8)
Fontal Cortex	750	1000*	1750**
Hippocampus	500	600*	850*

Double strand breaks (DSBs x 10^6/µgDNA) frontal cortex and hippocampus regions of aged human brain subjects *p> 0.05, ** p> 0.001

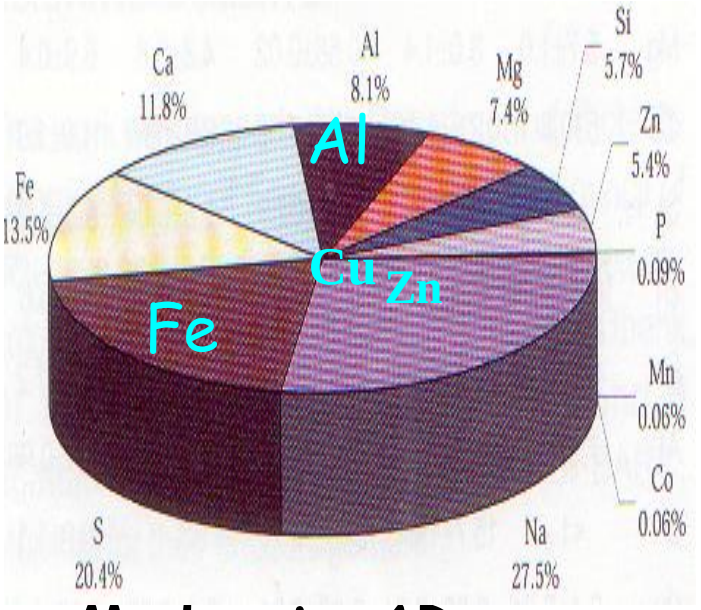
Brain Regions	Group I (N=8)	Group II (N=8)	Group III (N=8)
Fontal Cortex	1000	1500*	2750**
Hippocampus	600	720*	950*

Both single and double strand breaks are more in FC and H and is in correspondence with increasing concentration of redox active Cu and Fe and depletion in antioxidant Zn levels

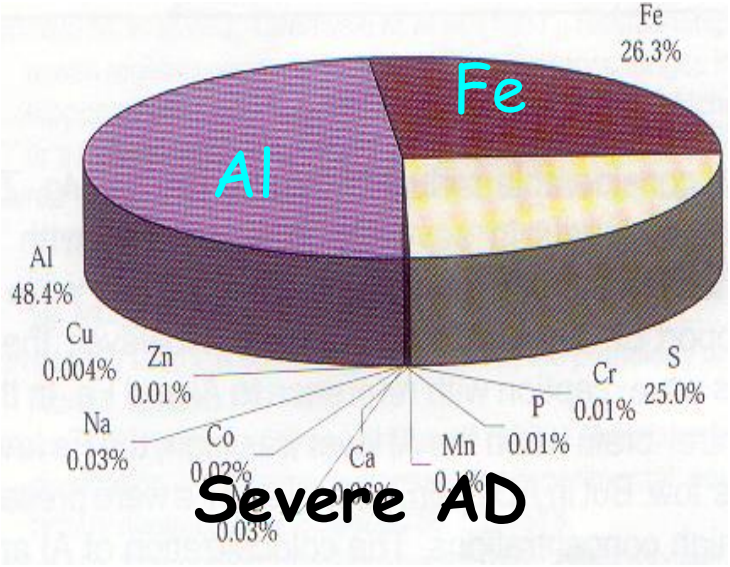
Metals in AD Brain: Fe,Cu & Zn increase in EAD, Al in SAD



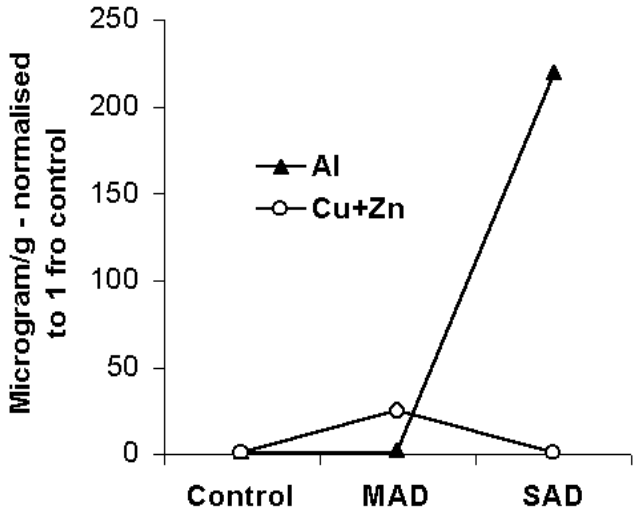
Control



Moderate AD



Severe AD



Methods for biomarker evaluation:

We studied serum Cu, Fe and ceruloplasmin and Ferritin levels in 20 early and 15 severe patients with AD, mean age 68, and 20 control samples with age matching.

The patient classification as early and severe is done by Psychatrician using DSM-IV criteria.

Serum metal levels are estimated by ICP-AES and ceruloplasmin by chemoluminisence method and Ferritin by ferro-oxidase technique.

Ceruloplasmin:

Serum copper and ceruloplasmin levels in early and severe AD
*** Significant compared to control group (p<0.05)**

	Controls (n=20)	Early AD (n=20)	Severe (n=15)
Cu (µg/ml)@	1.07±0.18	1.44±0.35*	1.51±0.3*
Ceruloplasmin (g/L)#	0.34±0.14	0.40±0.12	0.48±0.07
Cu/Ceruloplasmin	0.0015±0.002	0.006.5±0.0015*	0.008±0.0015*

Cu and ceruloplasmin increased significantly in early and severe AD compared to control

Ferritin

Serum Fe and Ferritin levels in early and severe AD
*** Significant compared to control group (p<0.05)**

	Controls (n=20)	Early AD (n=20)	Severe AD (n=15)
Fe (ug/ml)@	1.1±0.22	1.6±0.26*	1.68±0.22*
Ferritin (ng/ml)#	1.5±0.10	1.54±0.28	1.4.8±0.25

Significant increase in Fe levels in early and severe AD compared to control, but Fe levels between early to severe is similar
There is no significant difference in Ferritin levels between control and AD

Major conclusions:

Both single and double strand breaks are more in FC and H and is in

In both Frontal cortex and hippocampus, the levels of Cu, Fe are increased significantly, while Zn levels decreased.

This indicates the redox active molecules increased, while antioxidant Zn decreased in brain regions during ageing.

Correspondence with increasing concentration of redox active Cu and Fe and depletion in antioxidant Zn levels

Metals in AD Brain: Fe,Cu & Zn increase in EAD, Al in SAD

Cu and ceruloplasmin increased significantly in early and severe AD compared to control

Significant increase in Fe levels in early and severe AD compared to control, but Fe levels between early to severe is similar.

There is no significant difference in Ferritin levels between control and AD

Thanks