CHAPTER 5

SUMMARY AND CONCLUSION

Oxidative stress plays a vital role in the pathogenesis of AD. The Aβ plaques and NFTs are the major source of ROS which is both a cause and consequence for the development of oxidative stress. The trace element Se and selenoproteins act as antioxidants in scavenging the excess ROS. Limited studies are available on the levels of these proteins in plasma and CSF of AD patients. The present study focussed on analysing the levels of Se, selenoproteins, antioxidants and lipid peroxidation in CSF and plasma of AD patients and correlating their status with the pathological markers of AD viz Aβ42 and total tau. These parameters were comparatively analysed in AD, VD and controls for identification of AD specific plasma biomarkers. The study also evaluated the RAGE-Aβ interaction and the influence of polymorphisms in the ligand binding domain of RAGE on its interaction with Aβ using an In-silico analysis.

Blood samples from AD (n=45), VD (n=45) and age matched controls (n=55) and CSF samples from AD (n=24) and controls (n=22) were used for the study. Levels of Se, selenoproteins, Aβ42, tau and antioxidants were analysed using suitable methods. The results in plasma and CSF are summarized below:
5.1 PATHOLOGICAL MARKERS OF ALZHEIMER’S DISEASE

Plasma Aβ42 level was significantly higher in AD patients compared to controls, while CSF Aβ42 was significantly reduced in AD patients indicating that the excess Aβ42 produced in brain was transported to blood. Since Aβ42 level is influenced by Apo E polymorphism, the frequency of APO E ε4 was analysed in subjects and was found to be higher in the patient group compared to controls. The levels of Aβ42 in both plasma and CSF was also observed to be similar in both Apo E ε4 and non-ε4 carriers. Plasma tau was significantly lower in AD patients when compared to controls while the CSF tau was significantly higher in CSF as a result of excessive neurodegeneration. Correlation analysis indicated that plasma and CSF levels of Aβ and tau were not correlated.

Since the parameters were analysed in a limited sample size, a meta analysis was performed to validate the use of plasma Aβ and tau as markers for AD. The results indicated that no significant difference was observed for plasma Aβ42 and tau between AD and controls indicating that plasma amyloid and tau may not serve as a reliable marker for AD independently. However, our results indicate that tau-to-amyloid ratio in plasma and CSF was reliable in differentiating AD from controls with ROC derived cutoff values of <3.35 pg/mL (sensitivity 71%, specificity 65%) in plasma and >0.88 pg/mL (sensitivity 79.2%, specificity 72.7%) in CSF. Hence the study suggests that tau-to-amyloid ratio may be used as a reliable marker to diagnose AD.

5.2 IN-SILICO ANALYSIS OF RAGE-Aβ INTERACTION

The results of the In-silico analysis indicated that the amino acids Q24, K37, K39, K43, K52, R104, N105, R98, Q100, E108, and K110 in the
ligand binding domain of RAGE were mainly involved in binding with Aβ42 peptides and the fibril form of Aβ42 peptide bound more efficiently with RAGE. Compared to wtRAGE, the G82S variant showed higher affinity whereas the R48Q variant showed lesser affinity to all three forms of Aβ42, evidenced from the binding scores. The R77C variant showed significant decrease in affinity compared to wtRAGE when docked with 1IYT, and increased affinity with the other forms. The results of the study indicate that G82S polymorphism could possibly increase the affinity of RAGE towards Aβ42 thereby causing an increased transport of Aβ42 from blood to brain and augmenting the inflammatory response which could account for its association with AD pathology.

5.3 SELENIUM, SELENOPROTEINS AND REDOX STATUS IN ALZHEIMER’S DISEASE PATIENTS

Se levels in plasma and CSF did not vary significantly between AD and controls indicating that Se levels are maintained for proper functioning of the brain. However, plasma SeP level was significantly increased in AD patients indicating induced expression of the protein in disease condition to counter the excessive oxidative stress. ROC analysis indicated that SeP can fairly differentiate AD and VD from controls with a cutoff value of > 7.85 μg/mL. SeP is also reported to be a survival factor for neurons. The observed decreased in CSF SeP level in the present study, may augment AD pathology by increasing neurodegeneration. The results emphasise the role of SeP in maintaining the normal function of the neurons. The levels of SeP in plasma and CSF were not correlated indicating a differential regulation of SeP in expression in both brain and blood. Hence, measurement of plasma SeP should always be matched with the corresponding level in CSF to use SeP as possible biomarker for AD diagnosis. The frequency of SePP exon 5-Ala234Thr and 3’ UTR-G/A gene polymorphism were similar in both AD and
controls and no association was observed with AD pathology. However, a large co-hort study is warranted to validate the frequency and association of these SNPs with AD and the use of plasma SeP as a reliable diagnostic marker for AD diagnosis.

Among the peroxidative enzymes studied, the activity of SOD was significantly elevated in both CSF and plasma of AD patients indicating the increased production of ROS in disease condition which is substantiated by the increase in MDA level. Concomitant increase in the activity of GPx was observed in both AD and VD compared to controls, with a decrease in TR, GR and G6PD activity thereby depleting GSH level in both plasma and CSF. GSH was significantly reduced in all the three compartments (Plasma, RBC and CSF). Mandal et al (2012) also reported that GSH levels were reduced in AD brains. Hence, plasma GSH could possibly reflect the change in redox status of the brain in AD patients and may be used a marker for AD diagnosis.

Thus the present study reports an alteration in the redox status of AD patients as a results of excessive oxidative stress. A thorough clinical evaluation with age, sex and APOE correlation, in a larger co-hort would help in validating the use of these proteins as oxidative stress markers of AD.

The significant findings of the study, illustrated in Figure 5.1, is summarised as below:

- In plasma, Aβ42 level was higher and total tau was significantly lower in AD compared to controls, whereas a reverse trend was observed for these markers in CSF, indicating accumulation of these proteins in brain, leading to neurodegeneration. The study reports for the first time that Tau/amyloid ratio in plasma was effective in differentiating
AD from controls, and may be used as an effective biomarker.

**Figure 5.1** Status of selenium, selenoproteins and antioxidants in plasma and CSF of Alzheimer’s disease patients

- The molecular dynamics study revealed that RAGE binds to fibril form of Aβ42 peptide more efficiently and G82S RAGE polymorphism in the ligand binding domain increased the affinity of RAGE towards Aβ42 peptides, leading to enhanced inflammatory response and thereby augmenting AD pathology.

- The study pinpoints the enhanced plasma SeP expression, which could be an indicator of increased oxidative stress. SeP level in CSF of AD patients was quantified for the first
time and the observed decrease in CSF could augment AD pathology by increasing the oxidative stress. Plasma and CSF GSH levels were significantly lowered in AD patients indicating altered redox status in AD.

5.4 FUTURE DIRECTION

The present study suggests the possible use of plasma tau-to-amyloid ratio as possible marker of AD along with GSH and plasma/CSF SeP. A thorough study in a larger cohort with age, sex and APOE correlation, would help in validating the use of these proteins as biomarkers for clinical diagnosis of AD.

In-silico analysis of RAGE-Aβ42 interaction indicated that fibrillar form of Aβ42 showed increased interaction and G82S polymorphism in the ligand binding domain enhanced the RAGE-Aβ42 interaction. In-vitro and In-vivo studies will be helpful to validate these factors and would be effective in analyzing the actual role of RAGE in the pathology of Alzheimer’s disease.