Chapter-6

Results & Discussion

6.1 Alzheimer’s disease and Diabetes Mellitus

Alzheimer’s disease (AD) and Type 2 Diabetes Mellitus (T2DM) are conditions that affect a large number of people in developed and developing countries. Both conditions are on the increase, and finding novel treatments to cure or prevent them are a major aim in research. Somewhat surprisingly, AD and T2DM share several molecular processes that underlie the respective degenerative developments. Tahirovic I et al (2007) performed the role of oxidative stress in the pathogenesis of metabolic diseases like diabetes mellitus and its complications, as well as in neurodegenerative disorders like AD and reported that the oxidative stress alterations in the brain of STZ-induced rats and humans with AD could be useful in the search for new drugs in the treatment of AD that have antioxidant activity. The misfolding of proteins plays an important role in both diseases,

Diabetes mellitus is associated with changes in cognition. In type 1 diabetes mellitus this association is shown by a mild to moderate slowing of mental speed and a diminished mental flexibility. (Brands AMA et al. 2005) In type 2 diabetes cognitive changes mainly affect learning and memory, mental flexibility, and mental speed.2–4 Several large longitudinal population-based studies have shown that the rate of cognitive decline is accelerated in elderly people with type 2 diabetes. (Allen
The determinants of this accelerated cognitive decline, however, are less clear; some researchers suggest that hypertension could be an important mediator, whereas others have found associations with glycaemic control. Although the association between diabetes and these modest changes in cognition is now well established, the relation between diabetes and dementia is an area of controversy. Early studies that reported a low rate of diabetes in patients with Alzheimer's disease, suggested that diabetes and Alzheimer's disease might not coexist. However, a more recent study suggested that type 2 diabetes or impaired fasting glucose might be present in up to 80% of patients with Alzheimer's disease. These opposing results clearly indicate that studies of the prevalence of diabetes in people with established dementia are unlikely to provide reliable data for the risk of dementia in individuals with diabetes.

These differing outcomes may be the result of methodological issues, such as survival bias of nondiabetic patients with Alzheimer’s disease and the possible effects of Alzheimer’s disease itself on glucose metabolism, which might obscure the relation with diabetes in more advanced cases of dementia. Population based studies that compare the incidence of dementia between patients with and without diabetes provide more reliable risk estimates than studies on patients with established dementia.

AD and T2DM occur with increasing frequency as age advances. Besides, the development of one increases the risk of the other.
Epidemiological studies have shown an association of diabetes mellitus and Alzheimer's disease. A population-based historical cohort study estimated that the risk of Alzheimer's disease increased with adult onset diabetes mellitus (Leibson CL. et al, 1997) A longitudinal study of 1,262 elderly subjects without dementia at baseline, adjusted relative risk of Alzheimer's disease among persons with diabetes was 1.3 (Luchsinger JA et al, 2001). In a more recent community-based study among 1301 dementia-free persons aged 75 and above, diabetes mellitus was associated with subsequent development of Alzheimer's disease (Qiu CX et al, 2005). Similarly patients with Alzheimer's disease were more vulnerable to developing impaired fasting glucose and type 2 diabetes mellitus (Janson J. et al 2004). A variety of mechanisms has been postulated in the risk of Alzheimer's disease and type 2 diabetes mellitus: metabolic abnormalities of insulin resistance (dyslipidemia, hypertension), hyperglycemia per se or insulin, by disturbing synaptic plasticity, learning and memory (Biessels G. et al, 2005).

Recent studies suggest that diabetes and pre-diabetes are associated with about a 75% increased risk of developing Alzheimer's disease (AD). It has previously shown that insulin plays a role in normal memory function and modulating levels of proteins that accumulate in the brains of people with AD. Further, it was demonstrated that hyperinsulinemia increases levels of inflammatory markers and neurotoxic peptides in the central nervous system. There were two pathways through which conditions associated with insulin dysregulation, such as type 2 diabetes, may increase the risk of AD in older adults. The research demonstrated that adults with insulin resistance show
a pattern of reduced cerebral glucose metabolism characteristic of patients with early Alzheimer's disease. Twenty-three newly diagnosed individuals with impaired glucose tolerance or type 2 diabetes, and 11 healthy older adults – all cognitively normal – underwent PET scanning of their brains, and their brain glucose metabolism was compared. Reduced glucose metabolism was observed in brain regions associated with early Alzheimer's disease in the insulin-resistant adults. The pattern was characteristic of that seen in people with Alzheimer's years before they show any clinical symptoms of dementia, and it is considered a pattern of vulnerability to dementia.

Butyrylcholinesterase (BChE) is increased in the cerebral cortex of Alzheimer's disease (AD) patients, particularly those carrying e4 allele of the apolipoprotein E gene (ApoE) and certain BChE variants that predict increased AD risk and poor response to anticholinesterase therapy. Darreh-Shori, et al (2006). Measured BChE activity and protein level in CSF of eighty mild AD patients in relation to age, gender, ApoE e4 genotype, cognition and cerebral glucose metabolism (CMRglc). BChE activity was 23% higher in men than women (p < 0.03) and 40–60% higher in ApoE e4 negative patients than in those carrying one or two e4 alleles (p < 0.0004). CSF BChE level correlated with cortical CMRglc. Patients with high to moderate CSF BChE showed better cognitive function scores than others. They hypothesize that CSF BChE varies inversely with BChE in cortical amyloid plaques.
Figure 6.1: Alzheimer's Disease & Diabetes Mellitus: Mechanisms
Thus, low BChE in a patient's CSF may predict extensive incorporation in neuritic plaques, increased neurotoxicity and greater central neurodegeneration.

Acetylcholinesterase and butyrylcholinesterase activities emerge in association with plaques and tangles in Alzheimer's disease. These pathological cholinesterases, with altered properties, are suggested to participate in formation of plaques (Mariam F. Eskander. et.al.2005). It is evident from the preceding discussion that acetylcholinesterase and butyrylcholinesterase are present in various regions of the brain and are increased in the brains of patients with Alzheimer's disease. Furthermore, the activities of these two enzymes seem to be closely associated with the disease activity itself. Thus, higher the activity of acetylcholinesterase and butyrylcholinesterase, more severe the manifestations of Alzheimer's disease and increasing number of cortical and neocortical amyloid-rich neuritic plaques and neurofibrillary tangles(Guillozet A.et.al.1997, Greig NH.et.al.2005).

It is interesting to note that changes in the activities of acetylcholinesterase and butyrylcholinesterase have also been reported in other diseases Acetylcholinesterase was found to be about an order of magnitude higher in islets of Langerhans than in the exocrine tissue in rat pancreas. This difference in activity was found in rats made diabetic with streptozotocin as well as in the controls (Godfrey DA .et.al.1975). Abbott et al (1993) reported that the activity of serum butyrylcholinesterase was significantly elevated in both type 1 (8.10 ± 3.35 units/ml) and type 2 (7.22 ± 1.95 units/ml) diabetes compared with the control subjects (4.23 ± 1.89 units/ml) (P < 0.001). In addition, serum butyrylcholinesterase activity correlated with serum fasting
triacylglycerol concentration and insulin sensitivity in patients with type 1 and type 2 diabetes. On the other hand, in non-diabetic subjects with butyrylcholinesterase deficiency serum triacylglycerol levels were in the normal range. These results suggested that butyrylcholinesterase might have a role in the altered lipoprotein metabolism in hypertriglyceridaemia associated with insulin insensitivity or insulin deficiency in diabetes mellitus (Sanchez-Chavez G. et al. 2000).

In contrast, streptozotocin diabetes did not affect acetylcholinesterase activity in the retina but increased its activity in the cerebral cortex (100%) and in serum (55%), and decreased it by 30-40% in erythrocytes. The butyrylcholinesterase activity was decreased by 30-50% in retina and hippocampus and to a lesser extent in retinal pigment epithelium from rats treated with streptozotocin for one week. The changes noted in cholinesterase activities were not correlated with the fasting blood glucose concentration. These results suggest that diabetes might influence a specific subset of cells and isoforms of cholinesterases that could lead to alterations associated with diabetes complications (Sanchez-Chavez G. et al. 2001). It was also reported that the butyrylcholinesterase K variant allele was more common among Type II diabetic subjects than non-diabetic subjects suggesting that the close association of the butyrylcholinesterase gene (3q26) with Type II diabetes could be related to an identified susceptibility locus on chromosome 3q27 but independent of islet function (Sanchez-Chavez G. et al. 2001).

Since elevated serum butyrylcholinesterase activity is elevated in the diabetic rat, mouse and humans, Dave and Katyare studied the source of the increased level of
butyrylcholinesterase and reported that in alloxan-induced diabetic animals both the serum and cardiac butyrylcholinesterase activities were increased 2.2- to 2.8-fold with almost no significant change in the activity of the enzyme after insulin treatment compared with controls (Dave KR, Katyare SS.et.al.2002). Furthermore, correlation analysis showed that butyrylcholinesterase activity was positively correlated with age, sex, body mass index, hypertension and diabetes, as well as with triglycerides, total cholesterol, low-density lipoprotein cholesterol and apolipoprotein B (Apo B), whereas a step-wise multiple regression analysis revealed that the only risk factors for coronary heart disease that showed independent correlations with butyrylcholinesterase activity were, in descending order of importance, Apo B, triglycerides, and diabetes. These findings reinforce the idea that butyrylcholinesterase activity is associated with lipoprotein synthesis, hypertension, and diabetes (Alcantara VM .et.al.2002).

6.2 Key Reports

There is increasing evidence from recent studies involving a variety of disciplines revealed that Alzheimer's disease is an inflammatory condition. It was reported that plasma and cerebrospinal fluid levels of pro-inflammatory cytokines, extracellular acetylcholine in the hippocampus suggesting that increased concentrations of interleukin-1 (IL-1) in patients with Alzheimer’s disease could be responsible for lowered cerebral acetylcholine levels seen in this condition. In addition, IL-1 stimulates the beta-amyloid precursor protein promoter, which is processed out of the larger amyloid precursor protein (APP), which is found in the
form of Amyloid plaques in the brains of Alzheimer’s diseased patients [Donnelly RJ et al. 1990, Blume AJ et al. 1989]. There is evidence to suggest that low-grade systemic inflammation occurs in metabolic syndrome X [Das UN et al. 2002, Ford ES et al. 2003]. Plasma levels of C-reactive protein (CRP), TNF-\(\alpha\), and IL-6, markers of inflammation, are elevated in subjects with obesity, insulin resistance, essential hypertension, type 2 diabetes, and CHD [Das UN et al. 2002].

As patients suffering from these problems usually present with more than one of its component disorders, management of these complications are more complicated than when treating a single disease. So, in applying pharmacological treatments to this group of patients, their effect on both should be considered.

The present research was performed to identify underlying proteins that are likely to cause AD in individuals with T2DM and their root connection as in Table 6.1. The role of several proteins that are involved in the pathobiology of AD in individuals with T2DM by employing Multiple Sequence Alignment (MSA) using ClustalW tool and constructed a Phylogenetic tree as in figure 6.2 using functional protein sequences extracted from NCBI. Phylogenetic tree was constructed following Bioinformatic approach using Neighbor Joining Algorithm.

Results of this bioinformatics study revealed that BChE and AChE are closely associated with AD in individuals with T2DM. Studies have linked BchE with the pathogenesis of Alzheimer's disease and diabetes mellitus. BchE may modify the risk of Alzheimer's disease either alone, or in synergy with apoE-epsilon 4. The present bioinformatics study strengthens such a role for BChE and AChE in AD and T2DM.
and further implicates these genes/proteins could be of value in the prevention and treatment of AD and T2DM basing on the our result.

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<td>AAB59546</td>
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<td>707aa</td>
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<td>AA126151</td>
<td>241aa</td>
<td>Pooled, cerebellum, kidney, placenta, tests, lung, colon, liver, heart, thyroid, bladder, uterus, PCR rescued clones</td>
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</tbody>
</table>

Table 6.1: List of proteins Involved in AD and T2DM

Cladogram tree

![Cladogram tree](image)

Figure 6.2: shows the relationship between the proteins by using cladogram tree between Alzeimers and type 2 Diabetes
6.3 Discussion

Bioinformatics analysis of functional protein sequences of genes and related proteins that are involved in AD in individuals with T2DM revealed a high degree of homology between BChE and AChE. It is evident from the preceding discussion and results of the present bioinformatics study that BchE may be involved in the pathogenesis of type-2 diabetes either by way of amyloid fibrils or by modifying other risk factors of insulin resistance. The biochemical deficits of Alzheimer’s are reduced levels of Ach because of substantial reduction in the activity of choline acetyl transferase, reduced activity of AchE, and by contrast, increased activity of BchE. Both AchE & BchE; accumulate within amyloid plaques and tangles.

In view of the close the classical action of AchE is to catalyze the hydrolysis of Ach within cholinergic synapses of the brain and autonomic nervous system. Although BchE shares some of these functions; its role in brain remains unclear. The ratio of BchE to AchE changes dramatically in cortical regions affected by Alzheimer’s disease from 0.2 up to as much as 11 (Giacobini E. et al 2003). Clearly, this altered ratio in Alzheimer’s disease brain could modify the normally supportive role of BchE in hydrolyzing excess Ach only. Selective BchE inhibition may therefore be useful in ameliorating a cholinergic deficit, which likely worsens in Alzheimer’s disease due to increased activity of BchE.
References


the brain or insulin-induced amyloid pathology? Biochem Soc Trans 2005, 33:1041-4


