

A Cross-Sectional Study of serum Brain Derived Neurotropic Factor (BDNF) concentrations in a Saudi Population and changes associated with the use of Selective Serotonin Reuptake Inhibitors (SSRIs) in patients with Alzheimer's disease.

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**With loving memory of my darling Grandmother, Latifa Menahi Al-Remal,
(1928-2018)**

Abstract

Alzheimer's disease (AD), which results in disability, is one of the most prevalent neurological conditions in the elderly. The symptoms of disability are includes deterioration in memory, thinking, behavioural and the ability to perform everyday activities. While there is no cure for the symptoms of AD, pharmacological management and non-pharmacological support may help to improve cognitive and behavioural symptoms. Brain derived neurotrophic factor (BDNF), which is a protein and a member of the neurotrophin family of growth factors, supports the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses. There is evidence that patients with AD have decreased BDNF concentrations; however, serotonin selective reuptake inhibitors (SSRIs) elevate BDNF. Additionally, there are known BDNF gene Val66Met polymorphism (rs6265/G196A) responsible for BDNF synthesis that impact BDNF function. During the first phase of the research, 123 healthy young, middle ages (25-59 years old) and elderly (more than 60 years old) participants were selected from the King Salman Social Centre (KSSC), to determine whether there were differences in their serum BDNF with a commercially available enzyme-linked Immunosorbent assay (ELISA) kit. The evidence suggested that healthy participants experienced a decrease in serum BDNF concentrations as their age increased. Also, during this phase, a correlated comparison was conducted that evaluated how BDNF concentrations were affected by different variables such as age, body mass index and diabetes mellitus. Throughout the second phase of the study, the patients were recruited from the King Fahad Medical City (KFMC) in Riyadh. The levels of the serum BDNF concentration in AD patients (n=27) was compared to the serum BDNF concentration of healthy elderly participants (n=48). Then, the correlation between the changes in concentrations and cognition functions with assessments tools (Clinical Rating Scales and Mini Mental examination tests) was studied. During the third phase of the study, the impact of SSRIs on serum BDNF concentration and cognition function for both AD patients (n=13) and healthy elderly participants (n=17) were assessed.. The results of this study indicated that the use of SSRIs stimulated BDNF concentrations for both groups ($p=0.001$), positively improved cognitive function in elderly participants ($p>0.001$) and did not improve cognitive function in AD patients ($p=0.1$).

The final phase of the study investigated whether the BDNF gene Val66Met polymorphism (rs6265/G196A) in both AD and elderly is associated with cognitions functions changes. The genotyping for the association study was performed in 40 patients and 140 controls of Saudi origin by real-time PCR using Taqman chemistry in the ABI Prism 7900HT Sequence Detection System. The findings demonstrated that the BDNF Val66Met genotype distributions and allele frequencies showed significant association with cognition performance for both the elderly control group and AD patient's .The main findings showed that GG homozygotes (Val/Val) have superior cognition performance among AD patients and elderly control groups.

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Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other University for a degree, and does not incorporate any material already submitted for a degree.



Jawza Fahad Al-Sabhan

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Publications

- Jawza Fahad Alsabhan, Paul Gard, Greg Scutt, Norah Abanmi 'A cross-sectional study of serum brain derived neurotrophic factor (BDNF) concentration in a Saudi population and Alzheimer's Disease, Alzheimer's and Dementia the Journal of Alzheimer's Diseases, July 2017 Volume 13, Issue 7, Supplement, Page P332.
- Jawza Fahad Alsabhan, Paul Gard, Greg Scutt, Norah Abanmi Impact of age, gender, body mass index, platelet counts and glucose levels on Brain-derived neurotrophic factor concentration among Saudi population , Research & Reviews in Pharmacy and Pharmaceutical Sciences.2016 Volume 5,Issue 4

Conferences

- Alzheimer's Association International Conference (AAIC 2017), United Kingdom, London 2017
- Global Pharmacy and Healthcare Research Symposium (GPHRS 2016), United Arab of Emirates, Dubai

1. Chapter One: Introduction

This chapter includes the introduction, theoretical framework, statement of the problem, research questions, hypothesis, significance of the study and the definition of terms used.

1.1 Foreword

Alzheimer's disease (AD) is the primary neurodegenerative disease responsible for dementia, and it is characterised by the accumulation of amyloid plaques and neurofibrillary tangles in the brain, with impairment of specific immunological defence mechanisms as a contributing factor (Marx et al., 1998). AD is one of the most common neurological conditions leading to disability in the elderly. It is characterised by loss of memory or other thinking skills, diminished ability to concentrate and a decline in communication during regular activities. While the cause of AD has still not been identified, several factors are known to increase the risk of developing the condition. Ageing is the single most significant factor in the development of AD. The World Health Organization (WHO) defined ageing as a natural process that can be described in two dimensions. Primary ageing is the gradual inevitable process of ageing, and it is unavoidable. It occurs throughout the years, even with efforts and technology used to help slow the ageing process. Secondary ageing is avoidable, as it results from disease and abuse. For instance, having a healthy lifestyle might help reduce the risks of entering secondary ageing or delay it. According to human development biologists Papalia et al. (2009), there are three groups for older adults' ages: "young old", "old old" and "oldest old". Young old is suggested as the age from 65 to 74; old old as the age from 75 to 84; and oldest old as the age of 85 and above. Inherited genes can contribute to the risk of developing AD (WHO, 2015).

Furthermore, severe head injuries have been found to increase the risk of developing this disease. Research has also shown that several lifestyle factors and conditions associated with cardiovascular disease can increase the risk of AD (Grand *et al.*, 2011). This degenerative disease typically progresses slowly in three general stages: mild (early stage), moderate (middle stage) and severe (late stage). Since AD affects people

in different circumstances, each individual will experience symptoms or progress differently throughout the stages of AD (Perl, 2010).

Alzheimer's disease is named after Dr Alois Alzheimer. In 1906, Dr Alzheimer noticed changes in the brain tissue of a woman who had died of an unusual mental illness. Her symptoms included memory loss, language problems and unpredictable behaviour. After she died, he examined her brain and found many abnormal clumps (now called amyloid plaques) and tangled bundles of fibres (now called neurofibrillary, or tau, tangles).

The disease is diagnosed through a complete medical assessment, including medical history, mental status testing, physical and neurological exams and brain imaging. There is no cure for the symptoms of AD. However, pharmacological management and non-pharmacological support may help improve cognitive and behavioural symptoms (Cerejeira *et al.*, 2012; Bishara *et al.*, 2015; O'Brien *et al.*, 2017).

AD is currently ranked as one of the leading cause of death in the United Kingdom, and for females, dementia and AD are the most common causes of death, followed by heart disease. For both males and females over 80 years old, dementia and AD are the leading causes of death (Alzheimer's Association, 2017).

According to recent statistical reports regarding the prevalence of AD patients, there has been an increase worldwide, especially in the Asian population. Studies conducted in developed countries have consistently shown that AD is the most common type of dementia (Zahir, 2013). Therefore, research on disease prevention, diagnosis and treatment is critically important. The main area of this research has been in AD patients in the Saudi population. Recently, a cross-sectional study was conducted using the Montreal Cognitive Assessment (MoCA) test to determine the prevalence of cognitive impairment (mild cognitive impairment (MCI) and dementia) among elderly patients above 60 years old in a community-based setting in Riyadh, Saudi Arabia. The results showed that the prevalence of cognitive impairment among 171 Saudi patients was 45%, with the prevalence of MCI at 38.6% and the prevalence of dementia at 6.4%. The study also assessed the risk factors for cognitive impairment, including age, low level of education, hypertension and cardiovascular disease (Alkhunizan *et al.*, 2018).

There have been many studies conducted to investigate biomarkers, indicators of biological conditions that could potentially be utilised in the early detection of AD (Ahmed *et al.*, 2014). Most of these studies have focused on neuroimaging and cerebrospinal fluid biomarkers. However, there is a growing need to search for blood-based biomarkers that are less invasive and less expensive for use in developed countries.

A few studies have attempted to explain the role of brain-derived neurotrophic factor (BDNF) as a biomarker for AD. BDNF is a protein and a member of the neurotrophin family of growth factors. It is found in the brain and peripheral tissue; it mainly helps support the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses (Perovic *et al.*, 2013). In the brain, BDNF is active in the hippocampus, cortex and basal forebrain, which are areas for learning, memory and higher thinking (J. Y. Lim *et al.*, 2015). It is also expressed in the retina, motor neurons, kidneys, salivary glands and prostate (Mandel *et al.*, 2009). BDNF concentrations tend to decline with age (Budni *et al.*, 2015). Post-mortem studies of AD patients showing decreased messenger ribonucleic acid (mRNA) BDNF in brain regions commonly affected by AD have ignited an interest in BDNF as a potential marker (Connor *et al.*, 1997).

1.2 The research theoretical framework

1.2.1 BDNF concentrations and age

The earlier studies have been done to test the hypothesis that serum BDNF concentrations affected by age due to morphological changes of the brain and that these factors reduce the expression of BDNF in the hypothalamus area area to be discussed deeply in literature review chapter 2. Evidence from previous studies has proved that the hippocampus is sensitive to the ageing process (Rosenzweig & Barnes, 2003; Von Bohlen und Halbach, 2010). The pathological hypothesis can be explained by the reduction in the hippocampal volume and morphological changes of the cortex in the brain during ageing (Balcombe & Sinclair, 2001). As a contributing factor, this age-related volume reduction is accompanied by behavioural, cognitive and functional deficits in hippocampus-dependent learning and recall tasks (Tapia-Arancibia *et al.*, 2008). Interestingly, this age-related volume reduction of the hippocampus is not a consequence of an age-related loss of hippocampal neurons alone but is accompanied by different morphological changes, including two different patterns first, is a reduction in the innervation pattern of the hippocampus changes in the branching pattern of distinct neuronal populations within the hippocampus and second, is a reduction of spine densities of fibres projecting into the hippocampus (Von Bohlen und Halbach, 2010). It is very unlikely that a single factor or a single class of molecules is responsible for all these age-related morphological changes in the hippocampus. However, the positive role of neuropeptides possibly has a contributing effect on age-related changes. In light of this, growth factors may play an important role in the maintenance of the postnatal hippocampal construction. Von Bohlen und Halbach (2010) hypothesised that BDNF is a factor critically involved in the regulation of age-related processes in the hippocampus (Von Bohlen und Halbach, 2010). Also, the BDNF-trkB system has been suggested as being sensitive to ageing in the hippocampus, and this mechanism can be explained as the levels of trkB mRNA diminish over the life span (Webster *et al.*, 2006).

The current study will investigate whether the concentrations of circulating serum BDNF change with the ageing process in healthy (young and elderly) Saudi subjects.

1.2.2 BDNF concentrations and confounding variables

The lack of normative BDNF serum concentration values limits the usefulness of BDNF in clinical settings. This is complicated by the confounding variables that influence the interpretation of BDNF serum concentration. For example, Bus et al. (2012) showed that BDNF concentrations decline with age in women, whereas in men, the levels remain stable (Bus *et al.*, 2012). Concurrently used medications are an important variable. High BDNF serum levels were found in patients treated with mood stabilisers, antiepileptic medications and L-DOPA, while low BDNF concentrations were found in patients treated with benzodiazepines (Ventriglia *et al.*, 2013). After 15 months of treatment with donepezil, a cholinesterase inhibitor, BDNF serum concentrations in AD patients increased from a pretreatment level of 19.2 +/- 3.7 ng/ml to 23.6 +/- 7.0 ng/ml, as compared to healthy controls who were at a steady level of 23.2 +/- 6.0 ng/ml, showing no significant between the samples (Laske et al., 2006). This can be explained by the neuroprotective effects of mood stabilisers (Post, 2007). Both lithium and valproate can increase BDNF through their active effects on the mitogen-activated protein (MAP) kinase mechanism, which play key roles in neuronal development, neuronal survival and long-term neuronal plasticity (Einat *et al.*, 2003). Moreover, extensive-studied researches have explained that lithium regulates neurogenesis through the extracellular signal-regulated kinase (ERK) pathway that increases the expression of additional cell survival factors, such as B-cell lymphoma protein-2 (Bcl-2) in the frontal cortex of the brain, and inhibits the production of cell death factors, such as the protein levels of proapoptotic Bax and p53 that bind to specific DNA sequences, function as transcriptional activators and promote the expression of the proapoptotic gene that leads to cell death. Bax has been shown to bind to mitochondrial membranes, thereby, causing the release of cytochrome c, which, in turn, leads to the activation of caspases and degradation of specific protein substrates that contribute to the fate of a living cell (Chen *et al.*, 1999; Hao, 2004).

Nettiksimmons et al. (2014) measured serum BDNF near baseline in 912 elderly individuals and repeatedly conducted cognitive assessments using the modified Mini-Mental State Examination (MMSE) and the Digit Symbol Substitution test over 10 years. They did not find evidence of a longitudinal association between serum BDNF and subsequent cognitive tests, but after simultaneous adjustments in a multivariate model, they found that race, depression and platelet count were significant predictors of BDNF concentrations, suggesting that future studies should control for these potential confounders (Nettiksimmons *et al.*, 2014).

Broadly speaking, different influences, such as age, weight and gender, have been offered as factors that affect the stored and circulating BDNF concentrations in peripheral blood (Lommatzsch *et al.*, 2005). Another confounding variable is the diagnosis of major depression (MD). Accumulating evidence suggests that low serum concentrations of BDNF indicate an abnormal state that is evident during depression and which normalises during remission. Molendijk et al. (2010) found BDNF serum concentrations to be low in depressed patients who were antidepressant-free, compared to controls and depressed patients who were treated with an antidepressant. BDNF concentrations of fully remitted persons (whether unmedicated, untreated or treated with an antidepressant) were comparable to those of controls. In addition, the results showed that BDNF levels were unrelated to the core clinical features of depression, such as its severity or the first versus recurrent episodes (Molendijk *et al.*, 2011). This finding was replicated by another study, which found that depressed patients with an index episode over one year long were more likely to have significantly lower serum BDNF levels compared with patients who had a shorter index episode (Birkenhäger *et al.*, 2012).

The current study will focus more on the circulating protein BDNF. Different variables, such as age, body mass index (BMI), diabetes mellitus and the use of antidepressant medication, can alter this protein concentration in serum. Further theoretical explanations to be discussed in the literature review in Chapter 2. This study will focus on the correlations between this variable and BDNF concentrations.

1.2.3 BDNF concentrations and AD

Consequently, the age-related decline in BDNF could contribute to age-related changes seen in conditions of normal ageing, whereas further disturbances in the BDNF system may be related to pathological changes in the brain. Moreover, evidence has suggested that disturbances in the BDNF system also affect hippocampal dysfunctions related to pathological changes in the brain (Douglas *et al.*, 2010). For example, the pathological cause of AD is the substantial hippocampal cell loss that leads to a reduction of the hippocampal volume (Perl, 2010). In the last decade, it has been demonstrated that BDNF plays a role in AD. In 1991, Phillips *et al.* (1991) first established decreased BDNF expression in hippocampus samples from AD donors, suggesting that this decrease may contribute to the progressive cell death characteristic of AD. This finding has been replicated in a number of studies (Connor *et al.*, 1997; Webster *et al.*, 2006), with decreased BDNF also observed in the frontal and parietal cortices of AD patients (Carvalho *et al.*, 2008). In addition to post-mortem studies, recent genetic investigations have studied the association between BDNF genetic polymorphisms and the cause of sporadic AD and support the suggested role of BDNF in AD pathogenesis (Tanzi, 2012). In 2007, a study assessing the serum levels of BDNF in different neurological diseases found significant decreases in BDNF serum concentrations in AD, frontotemporal dementia (FTD), Lewy body dementia (LBD) and vascular dementia (VAD) patients compared with controls. In contrast, significant increases in BDNF serum concentrations were found in Parkinson's disease (PD) patients (Ventriglia *et al.*, 2013). Another study compared the differences between the BDNF concentration levels in AD patients (n=28) and healthy older participants (n=27) and found a significant decrease of BDNF serum concentrations in AD patients (18.6 ng/ml) compared to the healthy controls (21.3 ng/ml). However, BDNF serum concentrations did not correlate with age or MMSE scores in AD patients (Laske *et al.*, 2007).

A further aim of this study is to test the hypothesis regarding the mechanism of BDNF concentrations among the Saudi AD and elderly populations.

1.2.4 BDNF concentrations and cognition functions in AD patients

From previous researches, the decrease of BDNF has also been observed to affect the cognition functions in AD patients (Fumagalli *et al.*, 2006). The theory about cognitive impairment has been studied in MD patients (Diniz *et al.*, 2014), with these individuals suffering increased risk for development of AD (Hall, 2011). Furthermore, depressive symptoms are common in AD patients. This obvious combination suggests pathogenic influences common to AD and MD. To explain more, a decrease in BDNF, a member of the neurotrophic factor family, is related to both AD and MD, and many researches have concluded that BDNF could be a strong bond between AD and MD, which explains both the depressive symptoms in AD and the cognitive impairment in MD (Tsai, 2003). The mechanism of BDNF involvement in learning and memory skills has been tested in animal studies through both pharmacological and genetic deprivation of BDNF (Linnarsson *et al.*, 1997; Aarse *et al.*, 2016). Also, a further theory, in response to water-maze training, explained that the role of hippocampal BDNF mRNA expression is associated with memory performance (Psotta *et al.*, 2015). Additionally, a recent large cross-sectional study was conducted by Shimada *et al.* (2014) on 4,463 elderly people and showed that low serum BDNF was associated with lower cognitive test scores and MCI. The study also concluded that future prospective studies should establish the discriminative value of BDNF serum concentrations for the risk of MCI (Shimada *et al.*, 2014). Despite this, there is conflicting evidence in the literature regarding the association of BDNF concentrations with cognitive decline and dementia. Angelucci *et al.* (2010) measured BDNF serum concentrations in AD patients with different degrees of severity, from MCI patients to healthy subjects. They found that BDNF serum levels were significantly increased in MCI and AD patients when compared to healthy subjects; this increase in AD patients was neither dependent on illness severity nor on treatment with acetyl cholinesterase inhibitors and/or antidepressant medication, supporting the hypothesis of an up regulation of BDNF concentrations in both the preclinical phase of dementia (MCI) and clinical stages of AD (Angelucci *et al.*, 2010).

A more important objective of this study is to test the hypothesis that indicates the mechanism of BDNF concentration in regard to cognition function among the Saudi AD and elderly populations. Additionally, this study will also explore the association of cognitive performance and AD severity with the circulating BDNF serum concentrations in Saudi AD patients.

1.2.5 BDNF concentrations and SSRI use in relation to cognition functions in AD patients

One of the cornerstone aims of the thesis is cognition improvement through the BDNF system's role in AD by building on the previous studies that have reported the connection between BDNF in MD and antidepressant mechanisms. Preclinical investigations using animal models of depression have demonstrated that centrally administered BDNF produced antidepressant-like activity (Siuciak *et al.*, 1997; Shimada *et al.*, 2014). In the last decade, two animal experimental studies by Nibuya *et al.* (1995, 1996) have demonstrated that chronic administration of several types of antidepressants, including selective serotonin reuptake inhibitors (SSRIs), in rats increases BDNF expression in the hippocampus (Nibuya *et al.*, 1995; Nibuya *et al.*, 1996). Furthermore, a post-mortem study demonstrated a trend towards increased cerebral BDNF expression in antidepressant-treated MD sufferers in comparison to depressed patients untreated at the time of death (Chen *et al.*, 2001). Several studies have shown that the use of SSRIs could stimulate the BDNF concentration, and as a result of the increase in the serum BDNF concentration, cognitive function is improved in patients with major depression. For instance, Matrisciano *et al.* (2009) studied BDNF serum concentrations in healthy subjects and depressed patients at baseline and after five weeks and six months of antidepressant therapy either sertraline, escitalopram or venlafaxine. The study found that BDNF concentrations were lower in depressed patients compared with patients treated with antidepressant. Patients on sertraline showed increased the BDNF concentrations after five weeks and six months, while escitalopram revealed increased BDNF concentrations only after six months during the duration of treatment. However, non SSRI antidepressant such as venlafaxine did not change the concentrations of serum BDNF in depressed patients. This study indicated that SSRIs have an effect on BDNF serum concentrations (Matrisciano *et al.*, 2009a).

Another study, with a duration of SSRI treatment that was two months less than the previous study, revealed significant increases in BDNF serum levels with 30 unmedicated depressed subjects who were treated with escitalopram (n=16) or sertraline (n=14) for eight weeks, as compared to 28 healthy controls. Serum for BDNF

assay was obtained at baseline in all subjects and after eight weeks of treatment in the depressed subjects (Wolkowitz et al., 2011). In contrast, only one study, which investigated the effect of fluoxetine on BDNF levels, has explored the effect of antidepressant therapy in patients with vascular Alzheimer's disease (VAD). A significant increase in the mean BDNF serum concentrations from baseline between the fluoxetine group and control groups was proved. Additionally, the MMSE score significantly increased from baseline to the 12-week follow-up in the fluoxetine group (Xuan Liu et al., 2014). Therefore, it appears likely that early and sustained antidepressant treatment for AD, especially in aged patients, may decrease cognitive impairment and prevent the progression of AD in these individuals. In addition, experimental studies have discovered that BDNF produces antidepressant effects in behavioural models of depression (Li et al., 2011). Moreover, activation of trkB receptors is induced by antidepressant drugs, and this receptor activation is required for antidepressant-induced behavioural effects (Saarelainen et al., 2003). Since BDNF is capable of increasing dendritic spine densities, it is likely that BDNF has a beneficial effect on the disturbed neuronal plasticity seen in depression. Antidepressant treatments may, through enhanced BDNF signalling, improve the ability of critical brain circuits to respond optimally to environmental demands, a process that may be critical in the recovery from depression (Kim et al., 2017), and drugs that selectively stimulate the production of neurotrophins could represent a new generation of antidepressants.

However, after the previous review concerning the importance of applying BDNF to improve cognitive functions in AD patients, it can be seen there is a need to use BDNF directly, but several animal experimental studies focusing on the modulation of blood glucose and insulin levels after BDNF administration have discussed the disadvantage of BDNF administration due to unsuitable pharmacokinetic profiles (i.e., poor blood brain barrier (BBB) penetrability, short half-lives and low bioavailability), and previous unsuccessful experiences question its current clinical utilisation (Xuan et al., 2008). Additionally, the route of administration of BDNF can cause serious side effects, and intraventricular and intrathecal injections have failed to provide reliable effects (Boyuk et al., 2014a).

There is a need to test the hypothesis regarding the mechanism of SSRIs on BDNF concentrations among the Saudi elderly population and AD patients. More specifically, the current study will investigate the differences in circulating BDNF serum concentrations and cognitive performance in AD patients receiving and not receiving antidepressant medications. Additionally, the main goal of this project is to test the hypothesis that the use of SSRIs in AD may delay the symptoms of dementia through BDNF concentration increase.

1.2.6 BDNF concentrations and association of serum BDNF and Val66Met BDNF gene in AD patients

Concerning the possible role of BDNF in AD, there is a controversy as to whether polymorphisms in the BDNF gene are associated with AD. Thus, there are reports indicating that an association exists between BDNF gene polymorphisms and AD. Many clinical research studies in humans have established strong compelling evidence that the Val66Met polymorphism of the BDNF gene could serve as a reliable predictor for genetic predisposition to major depressive disorder (Pei *et al.*, 2012; Caldwell *et al.*, 2013), geriatric depression (Hwang *et al.*, 2006), schizophrenia (Rowbotham *et al.*, 2015), bipolar depression (Vincze *et al.*, 2008), anxiety (Frustaci *et al.*, 2008), reduced cognition and memory (Nagata *et al.*, 2012; Ward *et al.*, 2017) and suicide (Youssef *et al.*, 2018). Also, animal models have supported the evidence of the correlation among the Val66Met polymorphism of the BDNF gene, the genetic predication for depression and reduced cognition performance (Metsis *et al.*, 1993; Egan *et al.*, 2003). The pathological theories for this correlation can be explained by the fact that BDNF Val66Met polymorphism reduces the shape of cortical plasticity (Antal *et al.*, 2010), diminishes synaptic transmission in medial pre-frontal cortical (Pattwell *et al.*, 2012), reduces activity of synaptic plasticity in the dorsolateral striatum, reduces hippocampal volume (Lee *et al.*, 2016), reduces white matter connectivity and impairs intracellular survival signalling and spike-timing synaptic plasticity (Pang, 2011). In regard to the correlation between the BDNF Val66Met polymorphism gene and BDNF serum concentration, Lang *et al.* (2009) established that the Met allele of BDNF Val66Met polymorphism is associated with higher BDNF concentrations in the serum due to the compensated mechanism of a defective intracellular protein signalling (Lang *et al.*, 2009). Additionally, several researches conducted a correlational study that showed positive relationship between BDNF concentrations in the central nervous system (CNS) and in the blood (Klein *et al.*, 2011). This will supports the view that measures of serum BDNF concentrations reflect brain-tissue (hippocampus) BDNF concentrations.

also, a significant relationship between episodic stress and depression, moderated by the Val66Met allele, was established in one meta-analytic study (Hosang *et al.*, 2014). Regarding the association between the Val66Met allele and hippocampal volume changes in predicting depression, meta-analyses have hypothesised no significant genotype effect regarding this single nucleotide polymorphism, brain morphological changes and the BDNF plasma concentration (Kim *et al.*, 2015). In addition to mood disorders, and consistent with the role of BDNF in putative neuronal survival and neuroprotective effects (Phillips, 2017), the Met allele of BDNF is associated with decreased recovery following several forms of brain damage, such as subarachnoid haemorrhage (Siironen *et al.*, 2007) and stroke (Kim *et al.*, 2016). Recently, a review article discussed the importance of BDNF genetics and neuropsychiatric disorders, as they are complex diseases that can be caused by many genetic and environmental factors (Tsai, 2018). Regarding the role of the Val66Met polymorphism of the gene for BDNF in AD", a meta-analysis research by Fukumoto *et al.* (2010) showed that the Met allele of Val66Met increased the risk of AD in women but not in men (Fukumoto *et al.*, 2010). Also, Lin *et al.* (2014) found that the Met allele of the BDNF gene increased the risk of AD in Caucasian females (Lin *et al.*, 2014). However, Ji *et al.* (2015) found a lack of association between the Met allele of Val66Met and AD (Ji *et al.*, 2015). In regard to the association between cognition performance and the Val66Met polymorphism of the gene for BDNF, Kambeitz *et al.* (2012) reviewed 32 clinical studies and found that the Met allele carriers of the BDNF gene performed worse in cognition function than the Val allele homozygotes (Kambeitz *et al.*, 2012), while Mandelman and Grigorenko (2012) studied 23 publications that included 7,095 healthy and neuropsychiatric participants aged 18–70 years old, with the results revealing a lack of association between the cognition performance and the Val66Met polymorphism of BDNF (Mandelman & Grigorenko, 2012). To close the gap regarding this theoretical conflict there is a need to test the hypothesis regarding the association between cognitive performance and polymorphism of the Val66Met BDNF gene in the Saudi population.

1.3 Statement of the research

As shown in the previous introductory review, the effect of SSRIs on cognition changes among AD patients has not been studied, nor has the correlation of the distribution of Val66Met polymorphism and antidepressant response in the Saudi population. In this study, the concentrations of BDNF in different age groups will be assessed then correlated with changes in cognition among MCI and AD patients. The target population will be Saudi Arabians. It is argued here that Saudi Arabians have specific genotypes, so it will be informative to study the polymorphism of the BDNF Val66Met gene and its association with circulating BDNF and cognition improvement through antidepressant therapy.

Therefore, this thesis aims to assess the role of serum BDNF during SSRI treatment and its role in cognition improvement outcomes. Taking recent findings into consideration, the thesis hypothesised that BDNF serum concentration is lower in the AD patients as compared to the healthy control subjects. Additionally, this thesis also predicted that serum BDNF increases in association with positive cognition in response to SSRIs used for AD patients. Finally, this thesis also predicts that serum BDNF and the BDNF Val66Met polymorphism are associated with each other and with the severity of cognitive symptoms in AD.

1.4 Purpose of the study

This cross-sectional research design is a quantitative study. The research variables are serum BDNF concentrations and cognition changes. The enzyme-linked immunosorbent assay (ELISA) technique was used to assess the BDNF concentrations, while the Clinical Dementia Rating (CDR) scale and the MMSE test were used to assess cognitive performance. The independent variables included age, gender, BMI, DM and the use of medications. Also, a real-time polymerase chain reaction (real-time PCR) was used to study the polymorphism of the Val66Met gene.

The populations in this study were healthy volunteers and patients with both MCI and AD. The sample was selected randomly from two different government institutes after obtaining official ethical approval. The healthy volunteers were selected from the King Salman Social Centre (KSSC), while the patients were recruited from the King Fahad Medical City (KFMC) in Riyadh. Most of these factors will be discussed in detail in the methodology chapter.

1.5 Significance of the study

This study investigates the possible effects of SSRI on BDNF concentrations and the improvement of cognition in Saudi AD patients while taking into consideration confounding factors such as age, gender, BMI and current drug therapy. The possible contribution of BDNF gene polymorphism to cognitive improvement associated with SSRIs will also be investigated. This study is important because of the class effect of SSRIs on BDNF levels being examined in a setting with patients diagnosed with major depression disorder; however, the correlation between BDNF alteration and the SSRI effect has not been well investigated in AD patients.

1.6 Primary research questions

The primary research questions for the current study are as follows.

1. Do BDNF serum concentrations decrease in the elderly Saudi population compared to the young population?
2. Are BDNF serum concentrations related to gender in the general Saudi population and AD patients?
3. Are BDNF serum concentrations affected by BMI in the general Saudi population and AD patients?
4. Are BDNF serum concentrations affected by glycated haemoglobin (HbA1c) in the general Saudi population and AD patients?
5. Are BDNF serum concentrations altered in Saudi AD patients and those with MCI?
6. Do SSRIs increase BDNF serum concentrations and improve cognitive function in Saudi AD patients?
7. Is there a relationship between BDNF Val66Met polymorphism and cognition in Saudi AD patients? Do AD patients with the Met allele carrier have better cognition performance than those with the Val allele carrier?

1.7 Hypotheses

The following hypotheses are presented in response to the corresponding research questions presented in the previous section:

1. BDNF serum concentrations will decrease with age in the elderly group rather than in the healthy young group.
2. BDNF serum concentrations will be decreased in female for both the healthy Saudi population and the AD patients
3. BDNF serum concentrations will decreased with increasing BMI for both the healthy Saudi population and the AD patients
4. BDNF serum concentrations will increased or decreased with alteration of HbA1c for both the healthy Saudi population and the AD patients
5. Decreased BDNF serum concentrations are associated with impaired cognition in both elderly and AD patients.
6. Cognitive performance will improve more in AD patients receiving SSRIs than in the elderly group.
7. BDNF Val66Met polymorphism and circulating BDNF have associated effects on cognition improvement in AD patients undergoing SSRIs antidepressant therapy.

1.8 Research design

A cross-sectional study was conducted at KFMC and KSSC. The study duration was 12 months, from January until December 2016. Ethical approval was obtained from the Institutional Review Board at KFMC and KSSC. The first six months of the study period were for recruiting healthy participants from KSSC after they signed the informed consent form and filled in the demographic data form. Patient recruitment was the next stage of this study, which took place at the Memory Disorder and Neurology Clinics at KFMC. The informed consent, which encompassed the detailed descriptions of the study process, was signed by all patients and/or their substitute decision maker. The demographic data for each healthy volunteer included the participant's name (optional), age, gender, weight, height, medical history and medication history. The study included healthy volunteers from Saudi participants older than 25 years of both the male and female genders. The volunteers needed to be able to communicate verbally and provide their consent for study participation. The exclusion criteria for healthy participants included any history of psychiatric disorders and any current use of antidepressants, anxiolytics, mood stabilisers, antiepileptic medications or cholinesterase inhibitors.

1.9 Overview of the Study

This thesis consists of eight chapters within four main parts. Part I (Chapters 2 and 3) focuses on the prior research related to the study and presents the methodology. Chapter 2 intensively discusses the literature review regarding AD and the theoretical history of BDNF. This includes a critical review of the historical context, current practice and the role of BDNF in practice as well as issues relevant to their role in improving cognitive functions in regard to SSRI use in AD patients. In this regard, a cross-disciplinary approach is used to illustrate areas including the role of BDNF during the ageing process; the association between BDNF and confounding factors, such as gender, BMI and diabetes in Saudi young healthy and elderly participants; the role of BDNF in AD; the role of BDNF in cognition improvements; the role of SSRIs regarding BDNF; and the association between BDNF gene polymorphisms and AD. Combining these insights, Chapter 2 argues for the need to investigate the role of SSRIs in delaying the progression of AD symptoms in the Saudi population. Based on this, the most pressing gaps in the literature are identified and research questions are posed accordingly. Chapter 3 deals with the methodological issues and research design and provides the philosophical foundations and the theoretical and procedural descriptions of instruments used in the study to collect, present and analyse data. Part II (Chapter 4, 5 and 6) presents the results of the data analysis regarding the correlation between serum BDNF in young healthy and elderly Saudi participants; serum BDNF in AD; and the association between serum BDNF and cognition function for both elderly and AD patients. Part III (Chapter 7) presents the expanded genetic methodology used in this part and the results of the association between BDNF gene polymorphisms and Saudi AD patients. Finally, Part IV (Chapter 8) contains the conclusions and reflective evaluation of each part of the study as well as the study's limitations and recommendations for future research.

1.10 Definition of term

ACh: acetylcholine

AD: Alzheimer's disease

AD-D: Alzheimer's disease-related depression

ADL: activities of daily living

AMPA: a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

BBB: blood brain barrier

BDNF: brain-derived neurotrophic factor

CaRF: Ca²⁺ response factor

CAT: computed axial tomography

CDRs: clinical dementia rating scale

CNS: central nervous system

CREB: cyclic AMP response element binding protein

CSF: cerebrospinal fluid

CT: computed tomography

DMTs: disease-modifying therapies or treatments

EEG: Electroencephalogram

ELISA: enzyme-linked immunosorbent assay

EOAD: early onset Alzheimer's disease

ER: endoplasmic reticulum

FDA: Food and Drug Administration

FTD: frontotemporal dementia

GRP: glucose-regulated protein

HbA1c Haemoglobin A1c, glycated hemoglobin

HWE: Hardy–Weinberg equilibrium

HT: serotonin, 5-hydroxytryptamine

KFMC: King Fahad Medical City

KSSC: King Salman Social Centre

LBD: Lewy body dementia

LOAD: late-onset Alzheimer's disease

LTP: long-term potentiation
MAP: mitogen-activated protein
MCI: mild cognitive impairment
Met: methionine
Min: minutes
MMSE: Mini Mental Status Examination
MRI: magnetic resonance imaging
NGF: nerve growth factor
NIH: National Institutes of Health
NINCDS-ADRDA: National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association
NMDA: *N*-methyl-D-aspartate
PD: Parkinson's disease
PI3K: phosphatidylinositol- 3' OH-kinase
PKC: protein kinase C
PLC- g: phospholipase C-g
PS-1: presenilin 1
PS-2: presenilin 2
RT: room temperature
SNP: single nucleotide polymorphism
SSRI: selective serotonin re-uptake inhibitors
TCAs: tricyclic antidepressants
TGN: trans-Golgi network
Trk: tropomyosin-related kinase
VAD: vascular dementia
Val: valine
WHO: World Health Organization

2. Chapter Two: Literature review

2.1 Alzheimer's disease review

2.1.1 Alzheimer's disease statistics

According to the World Alzheimer's Report (2015), researchers have estimated that the world's older population currently comprises nearly 900 million people. Most of them live in relatively poor countries. Mortality rates among older people are falling, and life expectancy from the age of 60 continues to increase in all the world regions. It has been argued that between 2015 and 2050, the number of older people living in high-income countries will increase by just 56% compared with 138% in upper-middle income countries, 185% in lower-middle income countries and 239% (a more than three-fold increase) in low-income countries (World Bank Classification, 2015). With this longevity comes an increased prevalence of chronic diseases like dementia.

Recent reviews completed by the World Alzheimer's Report (2017) estimate that 46.8 million people worldwide were living with dementia in 2015. These numbers will almost double every twenty years, reaching 74.7 million in 2030 and 131.5 million in 2050. The global incidence of dementia is estimated to be over 9.9 million new cases of dementia each year worldwide, implying one new case every 3.2 seconds. The geographical distribution of new dementia cases is 4.9 million (49% of the total) in Asia, 2.5 million (25%) in Europe, 1.7 million (18%) in the Americas and 0.8 million (8%) in Africa (World Alzheimer's Report, 2017). In Europe and the Americas, peak incidence is among those aged 80 to 89 years; in Asia, between 75 and 84, and in Africa between 65 and 74 years.

In the United Kingdom (UK), the estimated number of dementia patients was around 850,000 in 2015, which cost £26 billion. According to the type of dementia, AD is the most common form of dementia, making up 62% of all dementias, followed by vascular dementia at 17%, mixed dementia at 10%, Lewy-body dementia at 4%, frontotemporal dementia at 2% and Parkinson's dementia, also at 2%. Moreover, according to AD figures in UK, 17% of over 80 years old, 4% between age 70-79 years old, 1% 65-69 and 0.1% below 65.

In the Middle East, the population is ageing gradually; the percentage of elderly will double over the next ten years. There is no clear estimate in the region of Alzheimer's disease prevalence, although it seems likely that it is similar to other developed countries despite the lack of studies in this area. There are few sources on AD statistics in the Middle East. None of the health authorities in the region has released public figures on the number of people with dementia or AD, and there are no Alzheimer's associations or advocacy groups collecting data. The recent dementia report from the World Health Organization (WHO) (2016) claims that the Middle East and North Africa will have a 125% increase in cases by 2050, and it estimates that almost six per cent of Arab populations over 60 years of age suffer from AD. However, the extrapolated prevalence estimates for AD in the Middle East showed that around 265,000 patients in Syria, 83,000 people in Jordan, 55,500 in Lebanon, 37,000 in the United Arab of Emirates and 33,200 in Kuwait suffer from the disease. These estimates for AD represent population percentages of approximately 1.15%, 1.28%, 1.22%, 0.41%, and 0.94% for Syria, Jordan, Lebanon, the UAE and Kuwait respectively. The interesting aspect is the low values for the UAE and Kuwait since they are both below the UK percentages.

The Saudi population projections show a sharp increase in the number of AD patients in the elderly population of over 60. According to recent statistics from the Central Department of Statistics and Information (Ministry of Health, Saudi Arabia, 2013), the elderly population represents 4.3% of the total population. In addition, the WHO has published the life expectancy for Saudis: the female life expectancy is 77.3, while for males it is 73.7, and the total life expectancy is 75.3 years old. The incidence of dementia was estimated by the WHO to be about 0.2% in the Saudi general population. The estimated incidence is very low compared to other Arab countries. The predictable costs of dementia in Saudi Arabia were estimated to be around US \$628.9 million yearly to treat 41,700 patients.

The Ministry of Health Portal (2015) in Saudi Arabia reported that there are no official statistics on the spread of AD, but experts estimate that there are at least 50,000 patients living in the country with this disease. In 2016, the Saudi Alzheimer's Disease Association indicated that the chances of getting the disease doubles every five years among individuals 65 and above, while half of those over 85 were found to be affected by the disease (Ministry of Health Portal, 2015). This is much higher than the UK figures as there are over 40,000 people with early-onset dementia (before the age of 65) in the UK (Dementia UK: Update, 2014). Furthermore, Amr et al. (2013) have been conducting a study at the King Faisal University Hospital in Al-Hafof city, eastern Saudi Arabia, to determine the prevalence of dementia among 485 patients older than 50 attending the Neurology Outpatient clinic. The study revealed that the majority of patients (approximately 87%) diagnosed with dementia were aged between 60 and 85 years old. However, around 6% of the patients diagnosed with dementia were younger than 60 (Amr *et al.*, 2013). Another study implemented at the university hospital in Riyadh city showed that the prediction of Alzheimer's patients was 19.3/100,000. The mean age on presentation of the disease was 74.6. The types of dementia were: AD (51.9%), vascular dementia (VD) (18.2%), mixed cases (15.6%), dementia with PD (7.8%) and treatable dementia (5.2%) (Al-Rajeh *et al.*, 1999).

Additionally, the demographic characteristics and the diagnostic profile of geriatric patients attending the psychiatry and neurology outpatient clinics of a tertiary hospital in Saudi Arabia revealed that the prevalence of dementia was 29.6%. The majority (57%) had AD, whereas 25% had vascular dementia (VD) and 18% mixed AD and VD (Amr *et al.*, 2013). According to the previous figures regarding the prevalence of Alzheimer's in the Saudi population, and in contrast to the world-wide statistics, there is a need for further investigation into the biological causes of AD among Saudi patients. AD does not receive a great deal of attention because families are not aware of the disease. This is also confounded by the lack of knowledge among the health-care teams in how to deal adequately with the disease due to an absence of training in the field. In addition, in the region, there are limited geriatricians and incomplete geriatric teams within hospitals and health centres, both at the governmental and private levels. Considering this, it is necessary to raise awareness about the disease through this research.

2.1.2 Alzheimer's disease pathology

AD is a progressive neurodegenerative brain disorder that causes significant disruption of the normal brain structure and function. At the cellular level, AD is characterized by a progressive loss of cortical neurons, especially pyramidal cells, which mediate higher cognitive functions (Stelzmann *et al.*, 1995).

Extensive evidence suggests that AD causes synaptic dysfunction early in the disease process, disrupting communication within neural circuits important for memory and other cognitive functions. AD-related degeneration begins in the medial temporal lobe, specifically in the entorhinal cortex and hippocampus. Damage to the brain structures results in memory and learning deficits that are classically observed with early clinical manifestations of AD (Burns & Iliffe, 2009). The degeneration then spreads throughout the temporal association cortex and to the parietal areas. As the disease progresses, degeneration can be seen in the frontal cortex, and eventually throughout most of the remaining neocortex (Karran *et al.*, 2011). Of note is the fact that AD causes pronounced damage to multiple components of the limbic system, including the hippocampal formation and the major fibre tracts that connect it to the cerebral cortex, amygdala, cingulate gyrus, and thalamus. This widespread pattern of neurodegeneration, affecting both limbic and neocortical regions, correlates closely with the array of cognitive deficits and behavioural changes that AD patients exhibit. In addition to cognitive impairment across multiple domains (memory, language, reasoning, executive, and visuospatial function), patients with AD show an impaired ability to perform activities of daily living and often experience psychiatric, emotional, and personality disturbances (Cerejeira *et al.*, 2012).

It has been theorized that the neuronal damage seen in AD is related to the deposition of abnormal proteins, both within and outside of neurons. These are the hallmark pathological lesions of AD known as plaques *and* tangles. The abnormal proteins are deposited in the cerebral cortex following a stereotypical pattern of spread along neural pathways that mediate memory and other cognitive functions (Lim *et al.*, 2015). Senile plaques are extracellular accumulations of amyloid protein, and consist of insoluble amyloid-beta protein (Ab). Normally, cells throughout life release soluble AB after a cleavage of the amyloid precursor protein (APP), a cell surface receptor. AD involves abnormal cleavage of APP that results in the precipitation of Ab into dense beta sheets, and the formation of senile plaques. It is believed that microglia and astrocytes then mount an inflammatory response to clear the amyloid aggregates, and this inflammation likely causes destruction of adjacent neurons and their neuritis (axons and dendrites) (Binder *et al.*, 2005). Neurofibrillary tangles (NFT) are intracellular aggregates of abnormally hyper-phosphorylated protein tau, which in normal form serves as a microtubule stabilizing protein and plays a role in intracellular (axonal and vesicular) transport. It is possible that the NFT interfere with the normal axonal transport of components necessary for proper neuronal function and survival (e.g., synaptic vesicles with neurotransmitters, neurotrophic factors, and mitochondria), eventually causing neurons to die or be destroyed (Reitz, 2012). Substantial evidence supports the idea that amyloid formation and deposition in the cerebral cortex is one of the earliest pathological processes in AD, preceding the clinical onset of the disease by 10-20 years (Korolev, 2014). Despite this, the temporal sequence of events in the deposition of amyloid plaques and the formation of NFT during the development of AD remain open to debate. In fact, a recent study suggests that the initial formation of NFT may occur in the brainstem rather than the medial temporal lobe, and may precede the appearance of the first amyloid plaques in the neocortex.

2.1.3 Alzheimer's disease diagnosis

2.1.3.1 Initiation of a Dementia Evaluation

Evaluations for dementia are initiated under different circumstances. Most often, family members bring their relatives who complain of neural dysfunction. Patients who often lack insight due to their central nervous system (CNS) disease (or psychological defences) are unlikely to recognize the need for such an evaluation. Increasingly, patients themselves seem to be sharing concerns with their physicians about problems with forgetfulness, word-finding difficulties, or slowness in retrieving names. Some of these patients will be in the early stages of a dementing illness (Korolev, 2014). Others may be particularly sensitive to the cognitive changes that are associated with 'normal' ageing, or be suffering from depression (MukaetovaLadinska *et al.*, 1995).

Workup of a potential dementia patient is a multidimensional process with two major branching points. The first major step involves establishing whether or not an individual fits the criteria for being clinically demented. The second major step occurs after the establishment of a diagnosis of dementia, and involves a workup to evaluate possible underlying conditions that fall within the differential diagnosis (American Academy of Neurology practice parameters algorithm).

Establishing a diagnosis of dementia relies principally on a detailed history and mental state assessment. Identifying the most likely underlying causes of dementia relies on recognizing the salient patterns of cognitive decline as revealed by the history and mental state examination, and obtaining appropriate diagnostic studies that look for potential contributors to the deterioration in the patient's cognitive or behavioral status (U.S Department of Health and Human Services, 2017).

2.1.3.2 Diagnostic Criteria

According to both DSM-V and NINCDS-ADRDA, diagnostic criteria for dementia require a decline in memory and other cognitive processes, such as language, visual-spatial abilities, or executive functions. DSM-V criteria explicitly state that such cognitive deficits must “cause significant impairment in social or occupational functioning and must represent a decline from a previous level of functioning”. This criterion is not explicitly included in the NINCDS-ADRDA formula (Table 2.1) (Hugo & Ganguli, 2015; American Psychiatric Association., 2013)

DSM-V Criteria	NINCDS-ADRDA criteria
<p>Insidious onset with progressive decline of cognitive function resulting in impairment of social or occupational functioning from a previously higher level</p>	<ul style="list-style-type: none"> • Probable AD – Deficits in two or more domains of cognition – Progressive decline of memory and other cognitive functions – Preserved consciousness – Onset between the ages 40 and 90 – Absence of systemic or other brain disease that could account for symptoms
<p>Impairment of recent memory and at least one of the following cognitive domains:</p> <ul style="list-style-type: none"> – Aphasia – Apraxia – Agnosia – Executive functioning (planning, organizing, sequencing, abstraction) 	<ul style="list-style-type: none"> • Possible AD – Atypical onset, presentation, or clinical course of dementia – Presence of another illness capable of producing dementia
<p>Cognitive deficits are not due to other neurological, psychiatric, toxic, metabolic, or systemic diseases</p>	<ul style="list-style-type: none"> • Definite AD – Clinical criteria for probable AD – Tissue diagnosis by autopsy or biopsy
<p>Cognitive deficits do not occur in the setting of delirium</p>	

Table 2.1: Summary of DSM-V and National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s disease and Related Disorders Association (NINCDS-ADRDA) criteria for the diagnosis of Alzheimer’s disease (AD) adapted from Lewis G. DSM-V (2013).

2.1.3.3 Past Medical History

Past medical history and ongoing medical conditions may also provide clues about processes contributing to a decline in cognitive functioning. Specifically, the clinician wants to inquire about a history of cerebrovascular disease, systemic illness, and risk factors for infections. Also, current and past medication history use, a history of alcohol or substance abuse, major head trauma, depression or other psychiatric illness, poor nutritional status, and potential exposure to toxins should be explored. Finally, one wants to identify if there is a family history of dementing illness or other diseases that can affect the central nervous system. If so, what was the age of onset of dementia in the family member, the clinical characteristics, and was there an autopsy that confirmed the suspected underlying pathology (Piemontese, 2017).

2.1.3.4 Mental state evaluation

A mental state examination is an essential feature of a dementia assessment. This may be the most variable aspect of the evaluation among clinicians. There is no consensus among neurologists, psychiatrists, or primary care physicians of the best mental state screening examination or testing strategy to practice. It is important for clinicians to have a means of estimating whether a patient's performance falls within the age-appropriate norms. There are several standard mental state screening tools that clinicians use, including the Mini Mental State Exam (MMSE). A very poor performance on a mental state screening test certainly can help identify patients suffering from a dementing illness. If there is a discrepancy between an informant's observations of cognitive and behavioural functioning and the patient's performance on mental state tests, it suggests the need for close follow-up and further investigation, with more extensive neuropsychological testing (Kukull *et al.*, 1994).

2.1.3.5 Sensorimotor examination

The sensorimotor neurological examination does not contribute to making a diagnosis of dementia. However, the pattern of neurological abnormalities often points to likely underlying diseases that may be contributing to the dementing process. For example, a clinician should look for evidence of upper motor neuron signs (e.g. hemiparesis, asymmetric deep tendon reflexes, extensor plantar responses) that would suggest the possibility of stroke or structural lesion. Extrapyramidal signs would raise the question of Parkinson's disease, progressive supranuclear palsy, or Lewy body dementia. Abnormalities of gait may be associated with cerebrovascular disease, and can cause a risk of dementia (Albers *et al.*, 2014).

2.1.3.6 Laboratory tests

The practice parameters of the American Academy of Neurology recommend that a workup include the following: a complete blood count, electrolytes, calcium, glucose, BUN, creatinine, liver function tests, thyroid function tests, B12, and syphilis serology.

Many would also include Erythrocyte sedimentation rate, urinalysis, and chest radiograph (Igarashi *et al.*, 2015). A patient's history should help guide other tests that may need to be ordered. For example, a patient with a long history of smoking should have a chest radiograph if none has been done recently because smoking can increase cardiovascular risk, as a consequence of which cardiovascular risk factors have been linked to an increased risk of dementia (Peters *et al.*, 2008). Individuals with a history of high-risk sexual behaviour or exposure to intravenous drugs should have HIV testing. Patients who may have been exposed to industrial toxins at work should be considered for 24-hour urine collection for heavy metals. However, the acquisition of ApoE genotyping is not recommended for routine evaluations (Cupples *et al.*, 2009).

2.1.3.7 Neuroimaging

Traditionally, neuroimaging computed tomography (CT) scan or magnetic resonance imaging (MRI) has been used to rule out potential structural abnormalities that may be causing or contributing to a decline in cognitive functioning. Specifically, the clinician is looking for any evidence of tumour, subdural hematoma, hydrocephalus, large and small vessel strokes, and white matter disease. An MRI is much more sensitive than a CT in detecting abnormalities in white matter (Albers *et al.*, 2014), while the clinical significance of such white matter changes is often uncertain. Atrophy is common in degenerative dementias such as Alzheimer's disease. However, such a finding is not diagnostic and cannot clearly distinguish demented patients from those undergoing normal ageing (Ahmed *et al.*, 2014).

2.1.3.8 CSF Evaluation

A lumbar puncture with cerebrospinal fluid (CSF) analysis is no longer part of the routine evaluation of dementia. This procedure is appropriate if there are concerns about any of the following: CNS infection (e.g. fever, headache), carcinomatous meningitis, reactive syphilis serology, subacute onset, or other atypical presentations of dementia, or if dementia occurs under the age of 50 (Blennow, 2017).

In addition, a lumbar puncture is indicated when there is evidence that a patient may be suffering from an inflammatory or vasculitic process, or when the patient is immunosuppressed. A recent report suggested that the diagnosis of Creutzfeldt-Jakob disease could be confirmed with reasonably high sensitivity and specificity in patients with dementia without a history of recent infarction or encephalitis, who were found to have the protein in their CSF (Uflacker *et al.*, 2016).

2.1.3.9 Electroencephalogram (EEG)

An EEG is also not currently part of a standard dementia evaluation. Although the EEG of patients with dementia often reveals a slowed background, this pattern lacks specificity. It can also be seen in 'normal', ageing and be found in a variety of dementing illnesses. A quantitative EEG analysis has pointed to patterns of abnormal electrical activity that are seen more commonly in Alzheimer's disease than in normal ageing. However, to date, such analyses have not yielded sufficient sensitivity and specificity to justify the routine use of such tests in the diagnostic evaluation of dementia (Engedal *et al.*, 2015).

2.1.3.10 Cerebral Biopsy

Currently, a brain biopsy in patients with dementia is not frequently followed for diagnosis because of the mortality and morbidity risks; in experienced centres, the mortality rate for the cerebral biopsy is less than 1%, and the postoperative morbidity is around 70 %. However, most clinicians would not recommend such an invasive procedure unless the results were to lead to a change in the therapy or management of the individual patient. Unfortunately, 20–25% of cerebral biopsies for dementia do not yield a specific diagnosis (Ahmed *et al.*, 2014; Blennow, 2017).

2.1.4 Alzheimer's disease clinical symptoms

2.1.4 .1 Alzheimer's Diseases Symptoms

Alzheimer's disease is a chronic progressive neurodegenerative disease characterized by three primary groups of symptoms.

The first group (cognitive dysfunction) includes memory impairment (declarative and procedural memory problems), aphasia (either receptive or expressive language difficulties), apraxia (inability to carry out directed coordination of movements despite intact sensory and motor nervous systems), agnosia (the inability to recognize specific elements of an individual's environment or self), attentional difficulties (including sustained and divided attention), and executive functioning impairment (including difficulties with abstraction, cognitive flexibility, inhibition, planning, organizing, and adaptation to novel stimuli) (Grand *et al.*, 2011).

The second group comprises psychiatric symptoms and behavioural disturbances that are called non-cognitive symptoms, for example: depression, hallucinations, delusions, and agitation. The third group includes difficulties with performing activities of daily living activities such as driving, shopping, eating, and dressing (Burns & Iliffe, 2009).

Memory loss is universal and is the first symptom in the vast majority of cases. The gradual onset of memory loss means that it may (understandably) be misattributed to normal ageing, and is often recognised only in retrospect as the onset of Alzheimer's disease. The onset is insidious, emerging with mild loss of memory and difficulty with word finding, symptoms that are common in everyday life to varying degrees (Feldman & Qadi, 2006).

2.1.4.2 Alzheimer's Diseases Staging

The symptoms of Alzheimer's disease progress from mild symptoms of memory loss to very severe dementia (Table 2.2).

Clinical Stages	symptoms
Mild cognitive impairment	Complaints of memory loss, intact activities of daily living, no evidence of Alzheimer's disease.
Mild Alzheimer's disease	Forgetfulness, short term memory loss, repetitive questions, hobbies & interests lost, impaired activities of daily living
Moderate Alzheimer's disease	Progression of cognitive deficits, dysexecutive syndrome, further impaired activities of daily living, transitions in care, emergence of behavioural and psychological symptoms of dementia
Severe Alzheimer's disease	Agitation, altered sleep patterns, assistance required in dressing, feeding, bathing, established behavioural and psychological symptoms of dementia
Very severe Alzheimer's disease	Bedbound, no speech, incontinent, basic psychomotor skills lost

Table 2. 2: Symptoms progression of Alzheimer's disease. Adapted from Feldman et al. (2006)

2.1.4.3 Alzheimer's disease risk factors

AD is the most common form of dementia, accounting for an estimated two-thirds of dementia cases in individuals aged 65 or older. Age is the most consistent risk factor for AD, presumably due to lifelong exposure to various forms of neural damage, including minor vascular events, white matter disease, and inflammation (Buscemi *et al.*, 2012). Other potential risk factors include: level of education (higher education potentially conferring increased brain and cognitive reserve), gender (increased risk for the female sex), family history of dementia particularly for first degree relatives, genetic factors (familial forms of AD are associated with mutations in three autosomal dominant genes: presenilin 1 (PS-1), presenilin 2 (PS-2), and amyloid precursor protein (APP), which are thought to work through increased production of A β peptides; and polymorphisms of apolipoprotein E, particularly Apo ϵ 4, which is associated with 2 to 3 times (heterozygotes) and 6 to 8 times (homozygotes) increased risk of developing AD), vascular risk factors (including hypertension, hypercholesterolemia, diabetes mellitus), mood disorders (particularly depression), and psychosocial lifestyle factors (including reduced intellectual, physical, recreational, and social engagement) (Iadecola *et al.*, 2016).

2.1.5 Alzheimer's disease pharmacotherapy

2.1.5 .1 Pharmacological Management

The important roles of current AD managements are to moderate the cognitive decline symptoms in AD patients. The dominant character of these drugs is to stabilize, and thus minimize, the disruption of two key neurotransmitters: acetylcholine (ACh) (the cholinergic hypothesis of Alzheimer's disease), and glutamate. AChE inhibition is used to protect the cholinergic neurons and glutamate (Evans et al., 2004; Seow et al., 2007).

The cholinergic drugs that target the first key transmitter, acetylcholine (ACh), are donepezil, rivastigmine and galantamine; these three compounds work on the basis of AChE inhibition. All three compounds are efficacious in reversing and improving memory and global cognition in mild to moderately demented patients (Piemontese, 2017; Taylor *et al.*, 2018).

Another key transmitter aimed at is glutamate, the primary excitatory neurotransmitter in the brain. The interaction of glutamate with the *N*-methyl-D-aspartate (NMDA) receptor is important in the mechanisms of memory and the learning process. In Alzheimer's disease, an increase in glutamate activity results in NMDA receptors being excessively activated, which may lead to neurodegeneration (Yiannopoulou & Papageorgiou, 2013). Memantine, an NMDA antagonist, is used to counter the loss or damage of NMDA receptors due to excess glutamate excitation in Alzheimer's disease patients. Memantine was approved in February 2002 by the European Medicine Agency for use with primarily moderate to severely demented patients (Evans *et al.*, 2004). Memantine is considered to overall reduce the burden of care, as well as clinically reverse and improve memory and global cognition, reducing behavioural instabilities, and improving the quality of life (Bishara et al., 2015).

According to the clinical practice review and experts` evidence, there are a number of treatments used to improve non-cognitive neuropsychiatric symptoms, including antidepressants, antipsychotics, anticonvulsants and benzodiazepines. For comorbid depression in AD dementia with behavioural disturbance, SSRIs are largely considered to be among the most efficient antidepressants of choice to control these symptoms. Also, antipsychotics are essentially used to overcome agitation, aggression, and psychosis in patients with AD dementia (O'Brien *et al.*, 2017). Treatment regimens with anticonvulsant mood stabilizers have shown promising results and seem to be beneficial for some dementia patients (Cerejeira *et al.*, 2012). Additionally, benzodiazepines are used to reduce agitation and anxiety over the short-term in AD patients with acute agitation. However, they can also trigger further agitation and are associated with rapid cognitive and functional decline in AD patients (Bishara *et al.*, 2015; Taylor *et al.*, 2018).

On the other hand, other pharmacological interventions can help to provide clinical improvements for AD, such as selegiline, an inhibitor of monoamine oxidase (MAO-B). There has been some evidence of improvement in cognition function with short term use by AD patients (Evans *et al.*, 2004; Ngo & Holroyd-Leduc, 2015). Recently, several clinical trials were conducted to evaluate the use of anti-inflammatory drugs and AD treatments, but the results were not promising (O'Brien *et al.*, 2017). Estrogen replacement also had questionable results in women with AD (Ngo & Holroyd-Leduc, 2015). Finally, several observational studies have identified that the statin group of drugs have a possible protective effect against dementia (Wu *et al.*, 2015; Geifman *et al.*, 2017).

A variety of other agents have been used to manage AD, including Gingko Biloba, vitamin E, folic acid, vitamin B, ginseng and omega-3, but their clinical efficacy remains inadequately evidenced (Dipiro *et al.*, 2009).

2.1.5.2 New approaches to management (Disease-Modifying Agents)

The pharmaceutical industry faces a new challenge for the prevention and treatment of neurodegenerative diseases. The disease-modifying agents are still under extensive clinical research for modifying AD. These compounds are responsible for slowing the progress of the stages of the disease through action on its pathological substrate: extracellular amyloid β ($A\beta$) plaques and intracellular neurofibrillary tangles (NFTs) (Yiannopoulou & Papageorgiou, 2013).

There are currently four main therapeutic approaches: (a) reducing the generation of $A\beta$, (b) facilitating the clearance of $A\beta$, (c) preventing the aggregation of $A\beta$ and destabilizing $A\beta$ oligomers, and (d) drugs targeting tau. Drug classes include active and passive immunization directed against $A\beta$, compounds that interfere with the secretases regulating $A\beta$ generation from APP, drugs to prevent $A\beta$ aggregation and destabilize $A\beta$ oligomers, and drugs targeting tau protein through modulation of tau phosphorylation with inhibitors of tau-phosphorylation kinases and compounds that inhibit tau aggregation and/or promoting aggregate disassembly (Reitz, 2012; Korolev, 2014).

A recent review of the AD drug development pipeline in 2017 shows that there are about 105 active agents, of which 25 are being tested in 29 trials in phase I, 52 are in 68 trials in phase II, and 28 are in 42 clinical trials in phase III. That the trials include a wide range of clinical trial populations was determined from the information on clinicaltrials.gov as a comprehensive resource for the study of clinical trials governed by the US Food and Drug Administration (FDA) or the National Institutes of Health (NIH). The mechanisms of action include symptomatic agents or disease-modifying therapies or treatments (DMTs). Most of the developmental drugs have failed to show promising effects as treatment options for AD patients (FDA, 2013; Cummings *et al.*, 2017).

Recently, in September 2017, the CEO of biotech startup Axovant Sciences announced that Axovant, one of the most promising drugs, had failed to treat AD, although it had passed 70 clinical trials in Japan. The drugs industry has spent billions over the past decade-plus developing pills and injections that might reverse the course of the disease, or at least arrest its progress. All showed early promise; all eventually failed, leaving Alzheimer's patients with few options in the management of their condition (Galvin, 2017).

2.1.5.3 Non-pharmacological interventions

Non-pharmacological interventions for AD patients have been categorized into the following groups (i) cognitive/emotion-oriented interventions (reminiscence therapy, simulated presence therapy, validation therapy); (ii) sensory stimulation interventions (acupuncture, aromatherapy, light therapy, massage/touch, music therapy, transcutaneous electrical nerve stimulation); (iii) behavior management techniques; and (iv) other psychosocial interventions such as animal-assisted therapy and exercise (Gitlin *et al.*, 2012). Unfortunately, despite efforts in investigating these interventions, consistent evidence about the efficacy of the various psychosocial therapies is lacking (Gates *et al.*, 2011). Benefits from psycho-educational interventions for caregivers have been documented to be long-lasting, especially when delivered individually (Cerejeira *et al.*, 2012).

Some experts believe that eating a healthy diet prevents or slows down Alzheimer's progression. This sort of diet mainly includes a lot of vegetables, fruits, legumes, nuts, olive oil, whole grain products, fish and poultry (Olazarán *et al.*, 2010).

A recent systematic review decided that a Mediterranean diet, in particular, is supposed to have a positive effect on memory and cognitive abilities and may protect against cognitive impairment and dementia (Petersson & Philippou, 2016). Having said that, there is still a need for large randomized clinical trials to prove or support the argument that eating a Mediterranean diet can prevent or slow down Alzheimer's symptoms (Petersson & Philippou, 2016).

Physical activity can be used as a therapeutic approach in a wide range of target populations with dementia. The benefits of physical activity have been demonstrated in terms of mood, quality of life, falls, cardiovascular function, and disability rates (Grand *et al.*, 2011).

2.2 Brain-derived neurotrophic factor (BDNF) review

2.2.1 History

BDNF, one of the neurotrophic family members, was discovered for its trophic effects on the dorsal root ganglion neurons in 1982. It was isolated from a pig brain and named brain-derived neurotrophic factor (BDNF) (Barde *et al.*, 1982). Remarkably, BDNF is the neurotrophin showing the most widespread expression in the development of the adult mammalian brain, and has been implicated in numerous studies in the mechanisms of synaptic plasticity and morphology of the brain (Zuccato & Cattaneo, 2009).

2.2.2 BDNF synthesis and release

Since 1982, a lot of research has investigated the gene structure of the BDNF protein, which is 240–260 amino acids in length. Chromosome 11 band p13 is the location of the gene that codes for BDNF (IP *et al.*, 1992). The BDNF gene in humans has four 5' exons (exons I-IV), including its own promoters, and it is combined with one 3' exon (exon V), which encodes the mature BDNF protein; this combination yields eight distinct transcribed mRNAs, with transcripts containing exons I-III expressed predominantly in the brain and exon IV found in the lungs and the heart (Pruunsild *et al.*, 2007). Each of these mRNAs is expressed in a tissue-specific and developmentally regulated manner (Metsis *et al.*, 1993). Exon V codes for pre-pro-BDNF proteins, the initial precursor of BDNF, which are found in the endoplasmic reticulum (ER) of the brain nucleus. The pre-BDNF is cleaved off immediately after sequestration of the nascent protein into the ER. Following the cleavage of the signal peptide, proBDNF is transported to the Golgi for sorting into either constitutive or regulated secretory vesicle pathways through the trans-Golgi network (TGN). ProBDNF buds off from the TGN in immature secretory granules containing a distinct set of protein convertases (Lessmann *et al.*, 2003). Mature BDNF is excised in the secretory granules en route to the plasma membrane to yield secretory granules (Pang *et al.*, 2011) (see figure 2.1).

Synthesis, storage, and release of neurotrophins

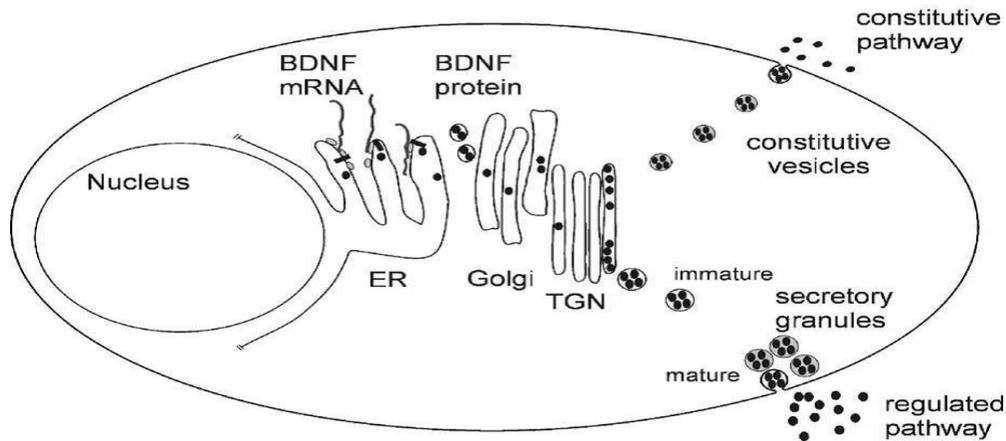


Figure 2.1: BDNF synthesis, storage, and release. Adapted from Lessmann et al. (2003)

The transcription process of BDNF mRNA can be regulated by neuronal activity via Ca^{2+} influx, through Ca^{2+} permeable glutamate receptors (mainly N-Methyl-D-aspartate [NMDA] receptors) and voltage-gated Ca^{2+} channels. Preceding work has revealed that Ca^{2+} initiates the binding of transcription factors such as the cyclic AMP response element binding protein (CREB) and the Ca^{2+} response factor (CaRF) to the BDNF promoters (Murray & Holmes, 2011).

There is abundant evidence that BDNF undergoes anterograde transport in the brain. Firstly, the BDNF protein is localized to nerve terminals, and pathway transection or axonal transport inhibition abrogates this terminal expression. Secondly, higher resolution studies have shown that BDNF is associated with dense-core vesicles, which are the primary site for neuropeptide storage and release from nerve terminals. Thirdly, further functional studies have supported the anterograde transport hypothesis.

Fourthly, pro-BDNF is shuttled from the trans-Golgi network into secretory granules where it is cleaved by prohormone convertases (Farhadi *et al.*, 2000).

Following the synaptic release, BDNF binds with high affinity to a tropomyosin-related kinase receptor (TrkB), a member of the family of tyrosine kinase receptors. Ligand-induced receptor dimerization results in kinase activation; subsequent receptor auto-phosphorylation of multiple tyrosine residues creates specific binding sites for intracellular target proteins, which bind to the activated receptor via SH2 domains. BDNF can also bind to the p75 neurotrophin receptor, although with lower affinity (Meeker and Williams, 2015). BDNF and TrkB mRNA have a widespread distribution in the central nervous system (CNS). BDNF and TrkB protein immunoreactivity is also widespread as with BDNF mRNA, the constitutive BDNF protein expression is particularly high in the hippocampus (Binder and Scharfman, 2004). Many studies have established a critical role for BDNF-TrkB in synaptic plasticity mechanisms. TrkB receptors are expressed both pre- and post- synaptically, and BDNF has been shown to regulate neurotransmitter release as well as post-synaptic responses (Madara & Levine, 2008).

2.2.3 BDNF signalling

BDNF binding to TrkB can regulate at least three intracellular signalling pathways (Park & Poo, 2012). One pathway involves phospholipase C-g (PLC- g) leading to protein kinase C (PKC) activation. A second involves mitogen-activated protein (MAP) kinase, which can activate Ras leading to downstream effects. A third signalling pathway involves phosphatidylinositol- 3" OH-kinase (PI3K) that can activate the AKTmTOR pathway. BDNF facilitates high-frequency activity-induced long-term potentiation (LTP) in the Schaffer collaterals of the hippocampus by enhancing pre-synaptic neurotransmitter release (Cunha *et al.*, 2010). Thus,

it seems likely that LTP plays an important role in learning and memory (Duman & Voleti, 2012).

Additionally, BDNF increases the post-synaptic response by increasing the conductance of NMDA receptors (Levine et al., 1998). BDNF has been shown to increase α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) responses by enhancing AMPA receptor translation and cell surface expression (Fortin et al., 2012). Furthermore, BDNF can facilitate excitatory transmission indirectly by attenuating inhibitory neurotransmission by, for example, reducing the surface expression of GABA_A receptors (Jovanovic et al., 2004) (see figure 2.2)

Moreover, LTP-induced enlargement of hippocampal dendritic spine volume is dependent on BDNF signalling and local protein translation (Tanaka et al., 2008). Recent studies have also identified a key role for BDNF in synaptic potentiation seen after a sustained blockade of NMDA receptor activity where BDNF action appears to up-regulate postsynaptic AMPA receptors (Heise *et al.*, 2014).

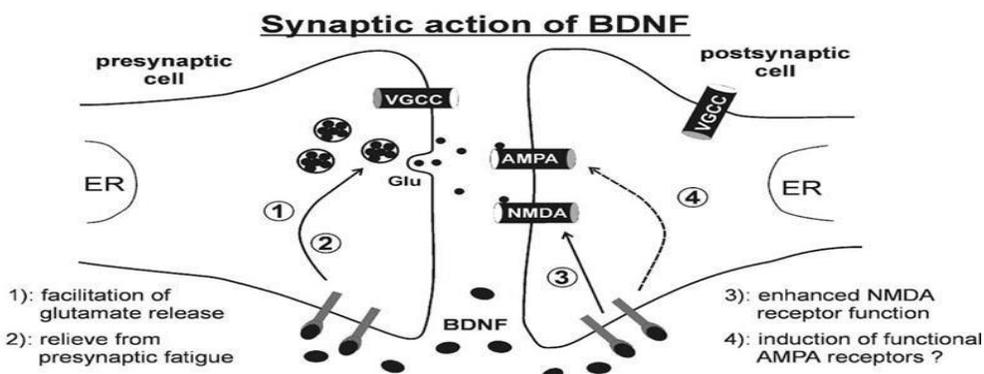


Figure 2. 2: Synaptic function of BDNF in the hippocampus. Adapted from Lessmann et al. (2003)

2.2.4 BDNF function

A primary function of neurotrophins is to promote peripheral and CNS neuronal growth, differentiation and survival (Porter & Kaplan, 2011). Immature proBDNF has been found to prune existing neurons by binding to the p75NTR and initiating cell death. Mature BDNF activates cell growth, survival and regeneration through the TrkB receptor (Teng et al., 2005). The BDNF function of helping to grow new neurons is the reason for the antidepressant effect, according to the neurotrophic hypothesis of depression (Duman and Monteggia, 2006).

2.2.5 BDNF circulation and measurement

BDNF freely crosses the blood-brain barrier (BBB) (Pan et al., 1998). Pre-clinical evidence from mammals has found positive associations between brain levels of BDNF and that circulating in blood samples (Klein et al., 2011). Other sources of circulating BDNF include muscles (Sakuma and Yamaguchi, 2011), vascular endothelial cells and platelets (Nakahashi et al., 2000). Interestingly, the ability for BDNF to cross the BBB has been investigated by Pan et al. (1998) after an injection of conjugated labelled BDNF; the result showed that BDNF was stable in blood for up to sixty minutes after the intravenous injection. By ten minutes, most of the BDNF sequestered by the cerebral cortex was associated with the parenchyma rather than with the endothelial cells, demonstrating a complete passage across the BBB.

Measurement of BDNF in the periphery can be achieved through serum or plasma samples, using an enzyme-linked immunosorbent assay (ELISA) (Engvall & Perlman, 1971). There has been a study that has provided evidence of reliability with little variability for BDNF measurement in serum or plasma with an ELISA (Elfving et al., 2010). While serum and plasma samples both provide reliable ELISA measures, serum samples of

BDNF are estimated to be higher than the levels in plasma, which may be due to the release of BDNF from platelets (Fujimura *et al.*, 2002). Regardless of these differences, researchers generally find serum and plasma BDNF levels follow a similar pattern as, for example, evidenced by studies indicating individuals with depression have lower levels of serum and plasma BDNF than healthy control subjects (Brunoni, Lopes and Fregni, 2008).

2.2.6 BDNF and neurogenesis

BDNF has also been found to enhance neurogenesis in rats. For example, intraventricular infusion of BDNF or adeno-viral-induced BDNF activity increases the number of neurons in the adult olfactory bulb, striatum, septum and thalamus (Pencea *et al.*, 2001), which can be potentiated by concurrent inhibition of glial differentiation of subependymal progenitor cells (Chmielnicki *et al.*, 2004).

2.2.7 BDNF and memory formation

Since BDNF appears to be involved in activity-dependent synaptic plasticity, there is great interest in its role in learning and memory formation. The hippocampus, which is required for many forms of long-term memory in humans and animals, appears to be an important site for BDNF action. In humans, a valine to methionine polymorphism at the 5' pro region of the human BDNF protein was found to be associated with poorer episodic memory; *in vitro*, neurons transfected with met-BDNFGFP exhibited reduced depolarization-induced BDNF secretion (Egan *et al.*, 2003). Further detail about memory formation and BDNF, explained by induction of long-term potentiation (LTP) that is defined as an activity induced sustained increase in synaptic strength, is the best studied form of synaptic plasticity and is considered as a cellular correlate of learning and memory. The induction of LTP is associated with the activation of a large number of signalling cascades, including the ones activated by BDNF (Cunha *et al.*, 2010).

Additionally, Kafitz et al. (1999) showed that a low concentration of BDNF causes membrane depolarization of hippocampal, cortical and cerebellar neurons within a few milliseconds, leading to the firing of an action potential (Kafitz *et al.*, 1999). Since then, substantial evidence has accumulated to indicate a critical role for BDNF in LTP induction, not only at hippocampal synapses at both the Schaffer collateral → CA1 synapse (Kang *et al.*, 1997) and in the dentate gyrus (Kuipers *et al.*, 2016). Thus, BDNF can activate multiple signalling pathways that may act in a concerted approach to regulate downstream cellular effects required for synaptic plasticity and memory formation. The interaction between each of these intracellular pathways has to be further resolved, but the extent to which each of them is activated and selective biological responses initiated is likely to depend on the levels of BDNF and TrkB, the temporal pattern of BDNF stimulation and whether the signalling is activated pre- or postsynaptically (Cunha *et al.*, 2010).

2.2.8 BDNF and neurological diseases

The idea that degenerative diseases of the nervous system may result from an insufficient supply of neurotrophic factors has generated great interest in BDNF as a potential therapeutic agent. Many reports have documented evidence of decreased expression of BDNF in neurological disease (Murer *et al.*, 2001). Selective reduction of BDNF mRNA in the hippocampus has been reported in AD specimens (Phillips *et al.*, 1991; Ferrer *et al.*, 1999).

Single nucleotide polymorphisms (SNPs), have been identified in the BDNF gene, which has provided an opportunity to study individuals carrying these SNPs (Petryshen *et al.*, 2010). The best characterized SNP in the BDNF gene is located in the pro-BDNF region, changing codon 66 from a valine (val) to methionine (Met); that is, val66met. Individuals with the val66met SNP have reduced episodic memory and aberrant hippocampal function that is believed to be due to disturbed intracellular trafficking and activity-dependent secretion of BDNF (Egan *et al.*, 2003). The val66met SNP has also been suggested as playing a role in the vulnerability to several psychiatric disorders and traits, including mood disorders and impaired cognition (Lu *et al.*, 2012; Petryshen *et al.*, 2010).

2.2.9 BDNF and Alzheimer's disease

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays an essential role in the progress, maintenance and utility of neurons. Several research studies have shown that BDNF plays an important role in the pathogenesis of Alzheimer's disease. Furthermore, several studies conducted using animal models have provided evidence to support the role of the BDNF mechanism in AD pathophysiology (Perovic *et al.*, 2013; Angelucci *et al.*, 2011).

According to a review article published in 1994, studies of AD patients showed reduced levels of BDNF mRNA in the hippocampus and parietal cortex, and decreased protein levels of BDNF in the entorhinal cortex, hippocampus, temporal, frontal and parietal cortex, indicating that there is a link between BDNF levels and the cause of AD (Hellweg & Jockers-scheriibl, 1994). These findings support the hypothesis that alterations to BDNF levels are involved in neurodegenerative mechanisms, and suggest that low BDNF levels are non-specific markers of neurodegeneration common to several cognitive disorders (Ventriglia *et al.*, 2013).

The relationship between cognitive deficits in AD patients and the reduction of brain and cerebrospinal fluid levels of BDNF has been widely investigated (Peng *et al.*, 2005; Ginsberg *et al.*, 2006). The study conducted by Peng *et al.* (2005) focused on the two forms of BDNF - the precursor BDNF forms and mature BDNF - in association with the cognitive function in an AD patient's brain. The results confirmed that the reduction of both forms of BDNF occurs in the pre-clinical stages of AD, and is associated with the loss of cognitive function (Peng *et al.*, 2005). While there are inadequate studies on the influence of peripheral BDNF on cognitive functions in AD patients, a study by Alvarez *et al.* (2014) evidenced that BDNF serum concentrations were significantly reduced in those AD patients with poorer cognitive performance (Alvarez *et al.*, 2014). In addition, the findings of a Framingham heart study show that higher BDNF

serum levels are associated with a lower risk of developing AD (Weinstein, 2013).

More recently, a study conducted in Brazil showed that, compared to a control group, the level of BDNF increases in AD patients regardless of the use of antidepressants and acetylcholine esterase inhibitors (AChEI) (Faria *et al.*, 2014). The authors of this study argued that this was due to the compensatory mechanism related to inflammation in the early stages of neurodegenerative diseases (Faria *et al.*, 2014).

All evidence to date relating to the association between the cause of AD and the reduction of serum BDNF seems to support the hypothesis that interventions that enhance peripheral BDNF signalling could represent an effective option for delaying AD onset and/or cognitive decline. Confounding variables may explain some of these discrepancies. Several studies examining associations between BDNF and cognitive status used basic tests of group differences with minimal controls for confounding factors, and only a few have examined the longitudinal association between BDNF and cognitive change. This explains the need for an elderly control group and an elderly AD group for potential confounders.

There is therefore a need to link the variation in BDNF levels and ageing in a large sample size to examine whether the higher serum concentration in BDNF levels in cognitively healthy younger and elderly subjects can help protect against the future risk of dementia in Saudi Arabian patients.

2.2.10 BDNF Serum concentrations and the response to antidepressants

Antidepressants, such as selective serotonin reuptake inhibitors (SSRIs) or tricyclic antidepressants (TCAs), mediate their antidepressant effect by modulating the extracellular levels of monoamines, mainly serotonin or norepinephrine. It is generally thought that these drugs enhance the extracellular levels of monoamines quickly: within hours. However, the antidepressant response is delayed and typically requires weeks of treatment before a sufficient antidepressant response is obtained. Therefore, it is believed that other mechanisms downstream of the enhancement of the extracellular level of monoamines are mediating the antidepressant response. BDNF-stimulated signalling cascades have also been implicated in depression and treatment response (Duman & Voleti, 2012).

Several experimental studies have been carried out to link the BDNF to the mechanistic action of antidepressants (Duman & Voleti, 2012). Furthermore, a recent review article explained in detail the role that BDNF plays as a key transducer of antidepressant effects (Björkholm & Monteggia, 2016). In rodent models, infusion of the BDNF protein directly into the midbrain produced an antidepressant-like effect, mediated by increased activity in monoaminergic neurons (Siuciak *et al.*, 1997). When ECT was induced, it showed a promising effect in the treatment of depression through the increased expression of BDNF and TrkB mRNA in the rodent frontal cortex and hippocampus. This expression could have beneficial effects on neuronal function and survival (Nibuya *et al.*, 1995). These basic research findings, coupled with brain-imaging studies reporting the decreased volume of the limbic brain regions, have led to a neurotrophic hypothesis of depression, and a wide range of basic and clinical studies of BDNF in depression. Some of the key questions regarding this hypothesis are whether decreased BDNF underlies the deleterious effects of stress and depression and, conversely, whether induction of BDNF mediates the beneficial effects of antidepressants. The results indicate that BDNF is sufficient to produce an

antidepressant response in behavioural models of depression, and that genetic deletion or blockade of BDNF blocks the effects of antidepressant treatments. From this point of view, the BDNF mechanism involved can be as important as that of both antidepressants and ECT in depression pathology and treatment.

Several human studies have examined the effects that ECT and different classes of antidepressants - such as serotonin selective reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), and tricyclic antidepressants (TCAs) - have on the peripheral BDNF in major depressive disorder (MDD) patients (Molendijk *et al.*, 2011; Mikoteit *et al.*, 2016). The first studies to implicate BDNF in antidepressant responses showed that conventional antidepressant drugs, as well as electroconvulsive therapy (ECT), enhanced BDNF and TrkB mRNA expression in the hippocampus and cortical regions in a timeframe similar to the onset of the antidepressant-like responses (Nibuya *et al.*, 1995). Focusing more strongly on the association between the serum BDNF concentrations and the effects of antidepressants, research undertaken by Shimizu *et al.* measured the serum BDNF concentrations in three different groups, including antidepressant-naive patients with MDD, antidepressant-treated patients with MDD and normal control subjects. The serum BDNF concentrations were assessed using the ELISA technique. The antidepressant drugs administered for treatment included different classes: milnacipran, fluvoxamine, trazodone, amoxapine, amitriptyline, clomipramine, imipramine, and mianserin, at various doses. The results showed that serum BDNF was significantly lower in the antidepressant-naive group than in the treated group p value=0.001 or in the control group p value=0.002 (Shimizu *et al.*, 2003). A recent meta-analysis reviewed 20 trials on the effects of differential antidepressant drugs on peripheral BDNF concentrations in MDD. The review determined that treatment using antidepressants had a significant effect on BDNF levels during the treatment period. While both SSRIs and SNRIs could increase the BDNF levels after a period of antidepressant medication treatment, sertraline was superior to the other three drugs (venlafaxine, paroxetine or

escitalopram) in the increase of BDNF concentrations with depression patients (Zhou *et al.*, 2017).

Other studies have reported conflicting effects of antidepressants on the BDNF concentrations. For example, Matrisciano *et al.* found that both sertraline and venlafaxine possibly increased BDNF concentrations, while escitalopram (an SSRI) did not affect BDNF concentrations during treatment (Matrisciano *et al.*, 2009b). Another study reported that fluoxetine increased the serum BDNF concentration while venlafaxine could not affect the serum BDNF concentration in depressed patients after 6 weeks of treatment (Başterzi *et al.*, 2009).

In different psychiatric diagnoses, such as pain in psychosomatic disorders, other research found that serum BDNF concentrations were increased regardless of antidepressant treatment. For example, Laske *et al.* assessed the serum concentrations of BDNF in fibromyalgia (FM) patients. Their findings indicated that the mean BDNF serum concentrations in FM patients were significantly increased when compared to healthy controls. But comparisons of FM patients with and without recurrent major depression, as well as with or without antidepressant medication, revealed no statistical significant differences (Laske *et al.*, 2007).

When considering the effects of antidepressants on serum BDNF concentrations in AD patients, there is little evidence for the use of SSRIs and their effects on serum BDNF concentrations. However, recent research found an association between cognition improvement and an increase of serum BDNF in AD patients treated with cerebrolysin and donepezil (Alvarez *et al.*, 2016). This conflicts with a previous study suggesting that BDNF serum concentration may not be a reliable diagnostic biomarker to identify neurodegenerative diseases as BDNF serum concentrations show no relationship with any diagnostic group or medication status in neurodegenerative disease (Woolley *et al.*, 2012).

The role of antidepressants in improving the cognitive function in AD patients in association with BDNF function could be expanded because of the evidence suggesting that a decrease in BDNF concentrations could be associated with AD pathogenesis, and because of the involvement of BDNF in the mechanism of the action of drugs that improve cognitive deficits in both animal AD models and AD patients (Fumagalli *et al.*, 2006).

2.2.11 BDNF serum concentrations and age changes

There is some evidence for the hypothesis that brain-derived neurotrophic factor (BDNF) has an effect on the hippocampus area of the brain during age-related processes (Von Bohlen und Halbach, 2010). It is now well-established from a variety of studies that the hippocampus is sensitive to ageing, and that there is a decrease in hippocampal volume during the ageing process. Behavioural and functional deficits in hippocampus-dependent learning and memory tasks are a consequence of this volume reduction (Tapia-Arancibia *et al.*, 2004). The possible explanation for this age-related volume reduction of the hippocampus is not a consequence of an age-related loss of hippocampal neurons. The morphological changes associated with ageing include reductions in the branching pattern of dendrites, as well as reductions in spine densities, reductions in the densities of fibres projecting into the hippocampus, and declines in the rate of neurogenesis. It is unlikely that a single factor or a class of molecules is responsible for all these age-related morphological changes in the hippocampus (Tapia-Arancibia *et al.*, 2008). However, up until now, a significant proportion of research has been descriptive in nature, indicating that growth factors may play an important role in the maintenance of the postnatal hippocampal architecture. Moreover, evidence suggests that disturbances in the BDNF system also affect hippocampal dysfunctions, major depression and AD (Mariga *et al.*, 2017).

BDNF is required in the postnatal brain, playing an important role in the maintenance of the brain construction. Reductions in BDNF, seen either during normal aging or in pathological conditions, are related to declines in neuronal plasticity and changes in the morphology of hippocampal neurons. These alterations could result in hippocampal dysfunctions and could contribute to hippocampal atrophy, seen during normal ageing or in AD. A number of studies suggest that neurotrophins such as BDNF play a critical role in regulating neuronal morphology and neuronal plasticity in depression. Alterations in neuronal plasticity, accompanied by changes in the construction of the hippocampus, are also observed during normal ageing. Since there is an age-related decline in the expression of BDNF and since alterations in BDNF

levels have effects upon the morphology of hippocampal neurons as well as on hippocampal neuronal plasticity, BDNF may represent a candidate that plays a critical role in age-related changes, at least in the hippocampus.

Several additional efforts applied to restore plasticity and/or cognitive functions in aged animals have shown some promise. The subjects of these avenues of inquiry include brain-derived neurotrophic factor (BDNF).

In humans, there are many studies reporting that serum BDNF concentration decreases with advanced age. A Japanese study investigated the correlation between serum BDNF and its relation to age changes in healthy controls compared to autism cases. The results showed that the serum BDNF concentration increased in healthy controls, then slightly decreased after reaching the adult level (Katoh-Semba *et al.*, 2007). Furthermore, in a large cohort study carried out by Ziegenhorn *et al.* (2007) that assessed serum BDNF concentrations in both healthy and diseased individuals (aged 70 and over; n=516), findings confirmed that there was a negative correlation between serum BDNF levels and age in healthy elderly adults (Ziegenhorn *et al.*, 2007).

Another explanation for the influence of BDNF through the ageing process in the hippocampus is the link to BDNF val66met polymorphism (rs6265) which influences activity-dependent secretion of BDNF in the synapse and crucial for learning and memory. Individuals who are homozygous or heterozygous for the met allele have lower BDNF secretion than val homozygotes and may be at risk for reduced declarative memory performance, but it remains unclear which types of declarative memory may be affected and how the ageing of memory across the lifespan is impacted by BDNF val66met polymorphism. For example, a cross-sectional study investigated the effects of BDNF polymorphism on multiple indices of memory (item, associative, prospective, subjective complaints) in a lifespan sample of 116 healthy adults aged 20–93. Advancing age showed a negative effect on item, associative and prospective memory, but not on subjective

memory complaints. For item and prospective memory, there were significant age BDNF group interactions, indicating that the adverse effect of age on memory performance across the lifespan was much stronger in the BDNF met carriers than for the Val homozygotes. BDNF met carriers also endorsed significantly greater subjective memory complaints, regardless of age, and showed a trend toward poorer associative memory performance compared to Val homozygotes. These results suggest that genetic predisposition to the availability of brain-derived neurotrophic factor by way of the BDNF val66met polymorphism exerts an influence on multiple indices of episodic memory. In some cases, this is in all individuals, regardless of age (subjective memory and perhaps associative memory), and in others as an exacerbation of age-related differences in memory across the lifespan (item and prospective memory) (Mandelman & Grigorenko, 2012).

Numerous studies have attempted to explain the changes of serum BDNF concentrations relating to the ageing process. For example, Murer et al. stipulated that changes in the concentration of BDNF might be attributed to the shrinkage of the hippocampus in late adulthood (Murer *et al.*, 2001). A further explanation is that BDNF is a molecule that is highly concentrated in the hippocampus, which, according to Tanila et al. (2017), is important in synaptic plasticity and is thought to contribute to neurogenesis in the dentate gyrus (Tanila et al., 2017). However, its concentration declines in late adulthood (Lommatzsch *et al.*, 2005).

There is therefore a need to examine the link between the variation in BDNF concentrations and ageing, using a large sample size, to establish whether or not the higher serum concentrations of BDNF levels in cognitively healthy younger and elderly people can protect against the future risk of dementia in the Saudi Arabian population.

2.2.12 BDNF serum concentrations and gender

Previous studies have explored the relationships between gender and serum BDNF concentrations, many of them establishing that gender can affect BDNF concentrations. In a cohort study that assessed BDNF as a potential gender-dependent marker for depression, a significant positive correlation was detected between baseline BDNF and disease severity in female subjects ($p = 0.001$), but not in male ($p = 0.52$) (Kreinin *et al.*, 2015). However, this conflicts with another study, which found that cognitive impairments relating to immediate and delayed memory were greater in male schizophrenia patients than in female, and that the males also had lower BDNF serum levels (Zhang *et al.*, 2014).

Interestingly, in a Brazilian study, serum BDNF concentrations were observed to be significantly decreased in transsexual Brazilian women when compared to cis-sexual men (Fontanari *et al.*, 2013). Furthermore, another study found that BDNF serum concentration is decreased in transsexual men when compared to both cis-sexual men and women (Fontanari *et al.*, 2016).

These variations can be attributed to several causes. The first can be answered through the study that attempted to establish that the impact of gender change is caused by alteration in brain morphological structures due to management roles (Fuss *et al.*, 2015). In an investigation into the influence of exposure to high levels of cross-sex hormones on brain structures in adulthood, Pol *et al.* (2006), found that male-to-female subjects treated with anti-androgen-estrogen treatment showed decreased brain volumes towards female proportions, while androgen treatment in female-to-male subjects revealed increased total brain and hypothalamus volumes towards male proportions (Pol *et al.*, 2006). Lifetime psychiatric comorbid conditions and treatment plans could be another explanation for gender influences on serum BDNF concentrations. A considerable amount of literature has been published on the relationship between gender identity and BDNF

concentrations with emotional and psychiatric conditions. For example, Fontanari et al. believed that the low BDNF concentrations found in transsexual individuals could be related to emotional trauma in childhood (Fontanari *et al.*, 2016), while Kauer et al. found that low BDNF concentrations detected in bipolar individuals were associated with traumatic life events (Kauer-Sant'Anna *et al.*, 2007).

Alternatively, for further explanation of the correlation between BDNF function and gonadal hormones (progesterone, estrogens, and testosterone) with relevance in clinical application, research studies have shown that BDNF mRNA levels are significantly reduced in practically all hippocampal layers and the cortex after ovariectomy in rats (Da Silva Moreira *et al.*, 2016). In addition, it has been reported that during the oestrous cycle, the concentrations of BDNF mRNA fluctuated significantly in different parts of the hippocampus. The highest levels were detected on the morning of diestrus 2, when progesterone levels are relatively low; the lowest levels were detected on the afternoon of pro-oestrus, when progesterone levels were highest (Gibbs, 1999). Furthermore, estrogen treatment of ovariectomised rats has also been reported to improve their learning acquisition in a radial maze task, and in their performance in a working memory task (Heikkinen *et al.*, 2002), suggesting that the protective effect of estrogens on those cognitive functions linked to the hippocampus could be mediated by BDNF ((Pluchino *et al.*, 2013). Gibbs et al. also confirmed that in response to hormone replacement, BDNF mRNA levels increased throughout the hippocampus in ovariectomized rats, particularly when estrogen plus progesterone was administered. The increase in BDNF mRNA in the hippocampus occurred concomitantly with a decrease in BDNF protein in this region (Gibbs, 1999). However, a small number of studies have evaluated the effect of testosterone on BDNF with relation to gender difference. Yang et al. reported that treatment with testosterone and BDNF presented an interactive effect on the regulation of androgen receptor expression ((Yang & Arnold, 2000), while testosterone administration was shown to increase BDNF protein levels in castrated male rats (Verhovshek *et al.*, 2013). A further group of studies

indicated that BDNF mediates the effects of testosterone on neuronal survival (Verhovshek *et al.*, 2013).

Recently, Chhibber *et al.* (2017) correlated the cause of menopausal depression with the inhibition of the estrogen receptor. Moreover, findings implicate that the disruption in estrogen homeostasis during menopause leads to dysregulation of BDNF–5-HT_{2A} signalling and weakened synaptic plasticity, which together predispose depression symptoms (Chhibber *et al.*, 2017). In conclusion, there is a need to evaluate the effect of gender difference on the serum BDNF concentrations for our research.

2.2.13 BDNF serum concentrations and body mass index changes

Energy balance and food intake are controlled directly by hypothalamic nuclei that receive and integrate peripheral stimuli arising from the gastrointestinal tract. Peripheral factors external to the gastrointestinal tract, such as leptin from adipose tissue, are involved in the regulation of food intake via complex processes. A number of orexigenic and anorexigenic molecules are involved, including BDNF, which plays an essential role.

BDNF is believed to act primarily within the ventromedial hypothalamus (VMH), where it is highly expressed, regulating energy intake downstream of the leptin proopiomelanocortin (POMC) signalling pathway (Figure 2.3) (Rosas-Vargas *et al.*, 2011). It has been shown that the hyperphagic and obese phenotype caused by disrupted melanocortin-4 receptor (MC4R) signalling can be partially reversed by the infusion of exogenous BDNF. Concomitantly, it was determined that BDNF expression in the VMH is regulated by the nutritional state and by MC4R signalling. This is due to the BDNF expression reduction in VMH that is seen in food-deprived wild-type mice, a reduction which could be reversed through administration of the MC3/4R agonist melanotan II (MTII) (Ziegenhorn *et al.*, 2007). Confirming these findings, it was later reported that selective BDNF deletion in the VMH of mice elicited hyperphagia and obesity. All these data suggest that MC4R signalling controls BDNF expression in the VMH, supporting the hypothesis that BDNF is an important effector through which MC4R controls energy balance. Upstream MC4R, the adipokine leptin, plays an important role in food intake regulation through the modulation of both orexigenic and anorexigenic signals. Its binding to the leptin receptor (LepRb) triggers multiple signalling cascades, and the physiological effects are translated into appetite inhibition and the positive regulation of body weight. Supporting evidence is provided by studies showing that rodents and patients lacking leptin or functional leptin receptors developed hyperphagia and obesity. Also, peripheral leptin administration results in decreased food

intake and body weight loss. The precise mechanism that mediates BDNF expression by leptin is still to be made clear; however, two apparent alternatives have been proposed. One possibility is that the leptin-LepRb interaction directly triggers a signal transduction cascade that induces BDNF in the VMH. The second option involves the leptin-mediated production of the melanocortin precursor alpha-melanocyte stimulating hormone (alpha-MSH) in the arcuate nucleus (ARC), which activates BDNF in the VMH via MC4R. In parallel to leptin, it has been shown that insulin stimulates PI3K in the POMC neurons of the ARC, integrating both stimuli downstream and exerting anorexigenic effects, while insulin binding to the insulin receptor (InsR) at the neuropeptide Y(NPY)/agouti-related peptide(AgRP)-expressing neurons in the ARC elicits an orexigenic outcome that is opposite to the blockage produced by leptin.

NPY is one of the most potent counterbalancing molecules of hypothalamic anorexigenic signals, including those mediated by BDNF. This brain-abundant neuropeptide is produced by the NPY/AgRP cells in the ARC and is induced by low leptin levels, hypoglycemia and hypoinsulinemia associated with fasting. Mammalian NPY receptors Y1, Y2, Y4 and Y5 are expressed in the hypothalamic nuclei where NPY binds with a higher affinity to Y1, Y2 and Y5 receptors and to a lesser extent to the Y4 receptor. The VMN expresses a high density of Y1 receptors that are activated by NPY binding, suppressing the anorexigenic output of the VMN. This effect involves a NPY-BDNF interaction that has been evidenced by the results of Wang et al. (Wang *et al.*, 2012). They demonstrated in a murine model that a VMN-BDNF infusion significantly inhibits NPY-induced feeding and body weight gain via TrkB signalling, and that it also produces an increase in energy expenditure by elevating both the resting metabolic rate and physical activity.

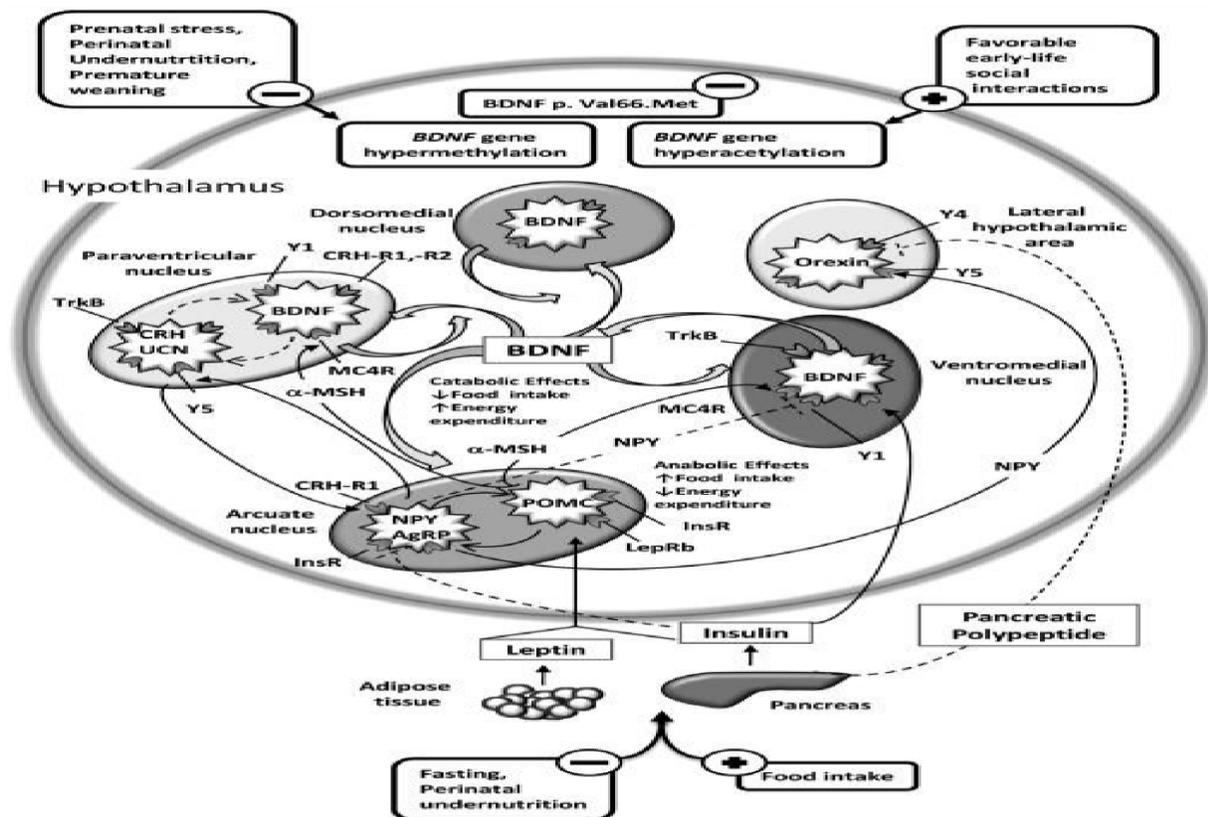


Figure 2. 3: Model of BDNF-mediated hypothalamic food intake regulation. Action of peripheral leptin and insulin on the leptin receptor (LepR) and insulin receptor (InsR) stimulate the secretion of α -melanocyte-stimulating hormone (α -MSH) from proopiomelanocortin (POMC) neurons in the arcuate nucleus (ARC). α -MSH binds to the melanocortin 4 receptor (MC4R) and activates BDNF production in both the ventro medial nucleus (VMN) and the paraventricular nucleus (PVN). Secreted BDNF interacts with the tyrosinekinase receptor B (TrkB) at neurons in the PVN, dorsomedial nucleus (DMN), ARC and VMN, producing feedback stimulation (ribbon arrows) that promotes food intake reduction. Neuropeptide Y (NPY) secreted from ARC binds to Y1 and Y5 receptors at the hypothalamic nuclei and activates pathways that fire an orexigenic response, and inhibits BDNF production. NPY signalling activates orexin production in the lateral hypothalamic area (LHA), which promotes a food intake increase that is counter-regulated by the pancreatic polypeptide (PP) that binds to Y4 receptors. Corticotropin-releasing hormone (CRH) and urocortin (UCN) activate CRH-R1 and CRH-R2 receptors at the PVN and establish a negative feedback with BDNF (dashed arrows). CRH/UCN output is reinforced through a positive feedback with NPY signaling from the ARC. Dashed lines indicate an inhibitory effect. Genetic and environmental positive (+) and negative (-) BDNF regulators accounting for molecular and epigenetic effects are indicated.

In the mammalian brain, BDNF and its TrkB receptors are extensively spread in hypothalamic nuclei, and are responsible for regulating appetite. Several attempts have been made to demonstrate that the BDNF/TrkB system contributes to food intake and body weight control (Xian Liu *et al.*, 2014). Recently, investigators have examined the effects of BDNF and its TrkB receptors on body mass index. Montalbano *et al.* (2016) assessed the concentrations for both BDNF and its receptor TrkB in the brain and gastrointestinal tract of an experimental model of diet-induced obesity in zebra fish. The results revealed an increased weight and body mass index, as well as an accumulation of adipose tissue in the visceral, subcutaneous and hepatic areas. These changes were concomitant with decreased concentrations of BDNF mRNA in the gastrointestinal tract and increased expression of TrkB mRNA in the brain (Montalbano *et al.*, 2016).

In humans, low levels of circulating BDNF have been found in individuals with obesity and type 2 diabetes (Krabbe *et al.*, 2007). Additionally, an opposite association between the concentration of peripheral BDNF and body mass index (BMI) in children and adults has been confirmed (Lommatzsch *et al.*, 2005). The etiological cause for obesity is due to gene mutations. Some evidence has shown that certain types of obesity or related eating disorder problems are due to BDNF gene mutations (Thorleifsson *et al.*, 2009). The BDNF Val66Met is by far the main SNP linked to an increased risk of obesity that share bingeing episodes as a common characteristic.

Defined as an excessive accumulation of body fat, obesity is a serious health problem that is increasing within populations of developed and developing countries. The traits of obesity and being overweight are becoming increasingly common in the Saudi population, with an overall obesity prevalence of 33%. Reducing the incidence of obesity and being overweight is of considerable importance to public health (Alenazi *et al.*, 2015), and for this reason, there is a need to investigate the correlation between BDNF concentrations and body mass index.

2.2.14 BDNF serum concentrations and glycated haemoglobin (HbA1c)

There are various potential mechanisms that connect the BDNF action and the development of type 2 diabetes mellitus (T2DM). Glycated haemoglobin (HbA1c) of 48mmol/mol (6.5%) is recommended as the cut-off point for diabetes diagnosis. Animal experiments illustrate how BDNF levels play an important role in the management of insulin resistance and dyslipidemia (Barbosa-da-silva *et al.*, 2012) ; and both animal experiments and clinical research have shown that BDNF levels play a key role in T2DM. For example, administration of BDNF to obese and diabetic rodents significantly suppressed their blood glucose levels, attenuated body weight gains and food intake, and enhanced their levels of energy and glucose metabolism. Several studies were conducted to research the association between BDNF concentration and the risk of T2DM, including a study in China that found serum levels of BDNF were significantly lower and associated with cognitive deficits in T2DM patients when compared to the control group (Zhen *et al.*, 2013). Another Chinese study correlated the role of BDNF in the development of diabetic retinopathy as a risk factor in T2DM (Liu *et al.*, 2016).

Because insulin sensitivity and BDNF may both affect brain function, Arentoft *et al.* (2009) assessed the plasma level of BDNF in middle-aged and elderly women with impaired insulin function. Results revealed that individuals with impaired insulin function had lower BDNF levels in comparison to matched non-insulin-resistant controls. However, a compensatory mechanism was found, whereby BDNF concentrations increased in women with severe insulin resistance-related endothelial dysfunction (Arentoft *et al.*, 2009). It is likely that higher concentrations of BDNF are created as a compensatory response to the brain impairment caused by progressing stages of insulin resistance (Gold *et al.*, 2007).

A report published in 2009 by an International Expert Committee on the role of HbA1c in the diagnosis of diabetes recommended that HbA1c can be used to diagnose diabetes, and that the diagnosis can be made if the HbA1c level is $\geq 6.5\%$.

Some studies have been conducted to show the impact on BDNF concentrations by antidiabetic drugs *in vitro*, and in animal and human models. In an animal model, subjects were given a high-fat diet for five weeks, followed by oral administration of metformin or metformin with glimepiride once a day for 3 weeks. Both treatments resulted in decreased weight gain and food intake. Furthermore, with the high-fat diet vehicle-treated group, BDNF protein levels were significantly reduced in the dentate gyrus, which is an input region of the hippocampus, compared to the low-fat diet-treated group. The administration of metformin or metformin with glimepiride in the high-fat diet group prevented the reduction of BDNF levels in the dentate gyrus. A recent article reviewed the effects of BDNF on antidiabetic drugs, and its association with T2DM. The review concluded that the BDNF mechanism of action enhances energy expenditure, ameliorates systemic glucose balance, and improves insulin sensitivity, and that it may be useful in the prevention and management of T2DM (Eyileten *et al.*, 2017).

2.2.15 BDNF gene

A genetically identified form of AD that usually has an onset before the age of 65 has been recognised, and it accounts for 0.1% of cases. The current thinking is that there are sporadic/late onset and familial/early-onset cases of AD.

There have been many genetic polymorphisms described as being associated with AD, such as the amyloid precursor protein (APP), presenilin-1 (PSEN1), presenilin-2 (PSEN2) (Campion *et al.*, 1999), apolipoprotein E (APOE), and sortilin-related receptor 1 (SORL1) (Ward *et al.*, 2017). The BDNF gene is also thought to participate in the development of AD (Lukiw & Rogaevev, 2017). Autopsy studies found reduced mRNA expression of BDNF in the hippocampus of patients with AD (Connor *et al.*, 1997), which implicates the possible participation of BDNF in the pathogenesis of the condition. Moreover, the BDNF gene represents an interesting potential genetic mechanism for the risk of late-onset AD (Zuccato & Cattaneo, 2009).

The most common BDNF polymorphism is the Val66Met polymorphism (rs6265, G196A). This single nucleotide polymorphism (SNP) substitutes valine (Val) for methionine (Met) at codon 66. Val66Met is a substitution of amino acids within the BDNF protein; G196A is a substitution of a Guanine for Adenine at position 196 of the gene. It is this change in nucleic acid that codes for the different amino acid in Val66Met. This substitution interferes with intracellular trafficking of BDNF and activity-dependent BDNF secretion. The Met66-BDNF protein has been shown to be associated with reduced transport of BDNF from the Golgi region to the appropriate secretory granules in neurons, compared with the Val66-BDNF protein (Toro *et al.*, 2006). Regarding the A allele of rs6265, it is related to poorer episodic memory, abnormal hippocampal activation and lower hippocampal n-acetyl aspartate (NAA) in human subjects (Egan *et al.*, 2003).

Many polymorphisms of the BDNF gene have been studied, for example rs11030104, rs16917204, rs7103411, rs6265 and rs2030324. However, only the last two polymorphisms have been widely studied; these studies yielded no evidence of a link between the genes and the diagnosis of AD. Moreover, the results from these studies are inconsistent, and individual studies have relatively little power in confirming such an association. For example, the G allele of rs6265 confers the risk effect for AD in subjects in Japan (Matsushita et al., 2005), but no significant association was found among Italians (Boiocchi et al., 2013). A meta-analysis conducted in 2014, including twenty-nine articles about rs6265 and twenty-two articles about rs2030324, found that there was no evidence for an association between rs2030324 and AD. Having said that, rs6265 has been found to lead to an increased risk of AD in Caucasian females. However, this is not the case for Asians. Furthermore, the A allele of rs6265 has been found to contribute significantly to the increased risk of AD in female late-onset AD patients.

In the USA, there are several studies that correlate the effect of BDNF polymorphism with BDNF protein levels in AD. Lee et al. (2005) demonstrated a marked decrease of BDNF protein levels in an AD temporal neocortex, which tended to be associated with neuropathological measures of disease severity. AD diagnosis and brain BDNF protein levels have been considered largely independent of reported BDNF polymorphisms, suggesting that the two SNPs (BDNF 196 G allele and BDNF 270 T carrier ship) are not strong genetic risk factors for AD or the primary determinants of BDNF protein levels in AD (Lee *et al.*, 2005)

Another cohort study conducted by Desai et al. (2005) compared American white versus American black (n= 995) late-onset AD (n=671), and control white American versus American black (64 AD cases and 45 controls). The study examined the association of Val66Met (G196A) polymorphism with quantitative measures of AD progression, including age at onset (AAO), disease duration and MMSE scores. The study revealed that no significant difference in allele genotype frequencies was observed

between AD cases and controls within the American white and black cohorts for G196A. However, the frequency of the 196A allele was significantly lower in American black subjects compared to the white subjects. Moreover, the BDNF polymorphism did not affect AAO or disease duration measurements in American whites or blacks. Additionally, the study result does not support an association between BDNFVal66 Met polymorphism and the risk of sporadic late-onset AD among American whites or blacks (Desai et al., 2005).

In China, He et al. (2007) demonstrated that there was no association between BDNF gene Val66Met polymorphism and AD; the study results indicated that the distribution of BDNF genotypes and alleles did not differ significantly. Similar results were observed when the AD and control groups were stratified by age, age at onset and sex. Additionally, this study presented data that revealed no significant effect of the genotypes on the age at onset for developing AD and no significant association between the genotypes and the severity of the disease (He *et al.*, 2007). In a north Indian population, there was no significant difference obtained between the BDNF genotype (val66met polymorphism) and BDNF serum levels between AD patients with amnesic mild cognitive impairment versus control subjects (Sonali et al., 2013)

Genotype and allele frequencies for BDNF Val66Met polymorphism in a sample of 102 AD cases and a 168 sample of Colombian AD patients were stratified by family history and sex. It was found that that BDNF polymorphism was not significantly associated with A (Met) carrier genotypes or A allele (A versus G) in the total sample in late-onset AD female patients or in APOE4 carrier patients. However, there was a significant association between A carrier genotypes and familial AD (Diego et al., 2006).

A further study examined the potential association between single nucleotide polymorphisms (SNPs) of the BDNF gene (G11757 C, C270T, G196A, G-712A) and Alzheimer's disease-related depression (AD-D) (Liu *et*

al., 2014). Participants included 336 patients with AD; 128 of these patients had AD-D. A response to an eight-week paroxetine treatment was also assessed. The frequency of the 11757 C allele was significantly higher in AD-D than in the AD patients without depression. Further, the 196A allele occurred with significantly higher frequency in AD-D patients versus AD non-depressed patients. Carriers of the A allele of G196A responded better to the paroxetine treatment. These findings support an important role of the BDNF polymorphism in AD-D (Lu *et al.*, 2012).

A systematic review conducted by Yan *et al.* (2014) to evaluate the efficacy of antidepressants in correlation with BDNF Val66Met polymorphism in Asian populations found that the Met carriers had a better response rate than the Val/Val carriers. In Asians, the Met carrier was positively associated with the response rate in the SSRI group and with treatments of ≥ 6 weeks. The authors suggested that the effect of antidepressants may depend on ethnic origin because BDNF had a lesser influence on response in mixed-race studies. It was also found that the duration of the treatment plays an important factor in the response and remission rate of the antidepressant efficacy (Lin *et al.*, 2014). However, BDNF Val66Met polymorphism influences antidepressant response and remission in Caucasian patients in a different manner for SSRI and SNRI/TCA. Val/Val patients have a higher probability of a three-month response to SSRI compared to SNRI/TCA. Carriers of the Met allele have a higher probability of a six-month remission rate with SNRI/TCA in comparison to SSRI. This effect is not related to antidepressant side effects. Thus, the results of the study suggest that SSRI could be recommended for Val/Val patients and that, conversely, SNRI/TCA could be beneficial to Met patients (Colle *et al.*, 2015). Based on the previous reviews, it can be said that there was a lack of association between the BDNF gene Val66Met polymorphism and AD. The distribution of the Val66Met polymorphism is unknown in Saudi Arabia. Essentially, to support the association between the uses of SSRI in Saudi AD patients there is a need to study the association between the BDNF gene Val66Met polymorphism and AD.

2.3 AD cognitive assessments review

The assessment of AD patients' cognition in clinical trials is commonly measured using various item numeric scales. The numbers generated by these scales must be measures of the variable of interest (such as the cognitive function). Cano et al. (2007) claim that the important criteria for scales are that the items contained within the scale represent the variable they intend to measure; this means the numbers that are generated are values that actually measure the variables of interest, and are not just numbers. To determine the stages for an AD measure, it is necessary to understand the cognitive domains relevant to AD, and the impact the disease has on them.

Traditionally the perception of the patient and caregiver has not featured in the development of staging levels. Reasonably, researchers and clinicians select tests or items to be included based on their particular knowledge of AD. Whilst these measures are developed with sound scientific underpinnings, their clinical meaningfulness is lacking (Rockwood, Fay et al., 2006). Given that clinical meaningfulness is fundamental for regulatory approval, it is essential that any newly developed measure meets this criterion. Frank et al. (2011) point out that there are some aspects of the disease for which the clinician's judgment is likely to be most accurate; however, at the earliest stages, subtle yet significant impairments may be accessible only to the patient or caregiver. Interviews with patients and caregivers have highlighted a dissociation between cognitive test scores and functional impairment; that is to say that those scoring highly on cognitive tests still experience a number of difficulties with activities of daily living (Morrison et al., 2012). There are several scales useful in assessing cases in research or clinical service. Each of these scales assess a specific area in AD. For example, the Mini-Mental State Examination MMSE is a reliable, widely and frequently used tool for the screening of dementia by examining the cognition in easy and quick techniques (Galea & Woodward, 2005). However, the MMSE is misinterpreted as a diagnostic test due to a limitation

in sensitivity (NICE, 2006). The most commonly used assessment of AD stages is the Clinical Dementia Rating scale (Morriss, 1993). This allows more reliable staging of dementia than the MMSE. It is based on caregiver accounts of problems in daily functional and cognitive tasks and classifies people with dementia into questionable, mild, moderate and severe (Sheehan, 2012).

The Arabic version of the Mini-Mental State Examination has undergone various validation assessments. Firstly, a preliminary assessment method was conducted on elderly Saudi participants in University Hospital by Al-Rajeh et al. (1999). They concluded that the Arabic version of the MMSE test needed larger studies before it could be recommended for cognitive impairment and dementia screening in an Arab population (Al-Rajeh *et al.*, 1999). Following that, another preliminary study conducted on 200 Arab-American participants supported the validity of the Arabic version of the MMSE as a predictor of informant-reported cognitive decline (Wrobel & Farrag, 2007).

Recently, a study was conducted by Albanna et al. (2017) on Arabic populations to validate the Arabic version of the translated standard version of the MMSE following the back-translation method. The research for validation included 134 elderly Arabs aged over 60 who had been diagnosed with Alzheimer's disease; the participants were from different Arab countries, including Saudi Arabia. The result proved that the sensitivity and specificity of the MMSE test was 71.4%. This means that the Arabic versions of the MMSE is a good screening tool for cognitive impairment in Arabs (Albanna *et al.*, 2017).

The CDR Arabic version is a reliable and valid tool to be used in clinical and research settings among Arabic-speaking populations. A recent review article assessed different scales of questionnaires that were used to evaluate AD patients; the tools included Arabic versions of the eight-item Alzheimer's Dementia test, the Alzheimer's Questionnaire, and Clinical Dementia Rating scales. It also assessed the Arabic version of Katz Activities of Daily Living, and the Neuropsychiatric Inventory. The results revealed that the Arabic

Clinical Dementia Rating (A-CDR) – sum of boxes was superior to the regular ACDR score in detecting dementia cases among the study sample. The A-CDR showed similar characteristics as the original version (Karam *et al.*, 2018).

2.4 Enzyme-linked Immunosorbent Assay review

The ELISA method was invented by Eva Engvall and Peter Perlman in 1970. The first assay used cellulose as a particulate immunosorbent, but quickly was switched to plastic due to the tedious phases of centrifugation. The application of the antibodies to the plastic resulted in a simple, robust, inexpensive, and efficient test. The test was initially tested in simple conditions in East Africa. After the primary launch of the method, its application has been cited in numerous publications (Engvall & Perlman, 1971).

Immunoassays are used as analytical methods to detect analytes with antibodies. Enzyme immunoassays use enzymes attached to one of the reactants in an immunoassay to allow quantification. The quantification of analytes is done through the development of colour or luminescence, in addition to an enzyme-specific substrate or chromogen.

The most commonly used ELISA method is the so-called sandwich ELISA. The basic principle of the method is as follows: an antibody is attached to the bottom of an ELISA plate. Next, the target protein is added, which then attaches to the antibody. The secondary antibody, which has been labelled with an enzyme, attaches itself to the target protein. Finally, an enzyme-specific substrate is added which produces a detectable reaction. If the reaction is colourimetric, the reaction can be measured visually or by using a spectrometer. For quantification, a standard curve is analysed along with the analytes to determine the concentration of the analyte.

The advantages of the sandwich ELISA are its high specificity, suitable for complex samples, its flexibility and its sensitivity. The high specificity comes from the two antibodies that are used. This allows the antigen being captured to have a higher chance of being the correct analyte compared to other methods. The suitability of this method for complex samples is made

possible by the fact that the samples do not need any purification prior to the analysis. The flexibility of the analysis is achieved due to the many possible detection methods. The main disadvantages of the ELISA method are the manual labour phases, the margin for error, and the time taken for the analysis to be complete. The method often involves pipetting samples individually into wells which entails a lot of work if bigger batches of samples need to be analysed. The margin of error increases as the number of phases of the method increases. The possibility of human error increases with every phase that involves human interference. The reliability of the materials used also affects the margin for error of the method. When the method is conducted correctly, the ELISA method is a valuable and affordable method in the laboratory.

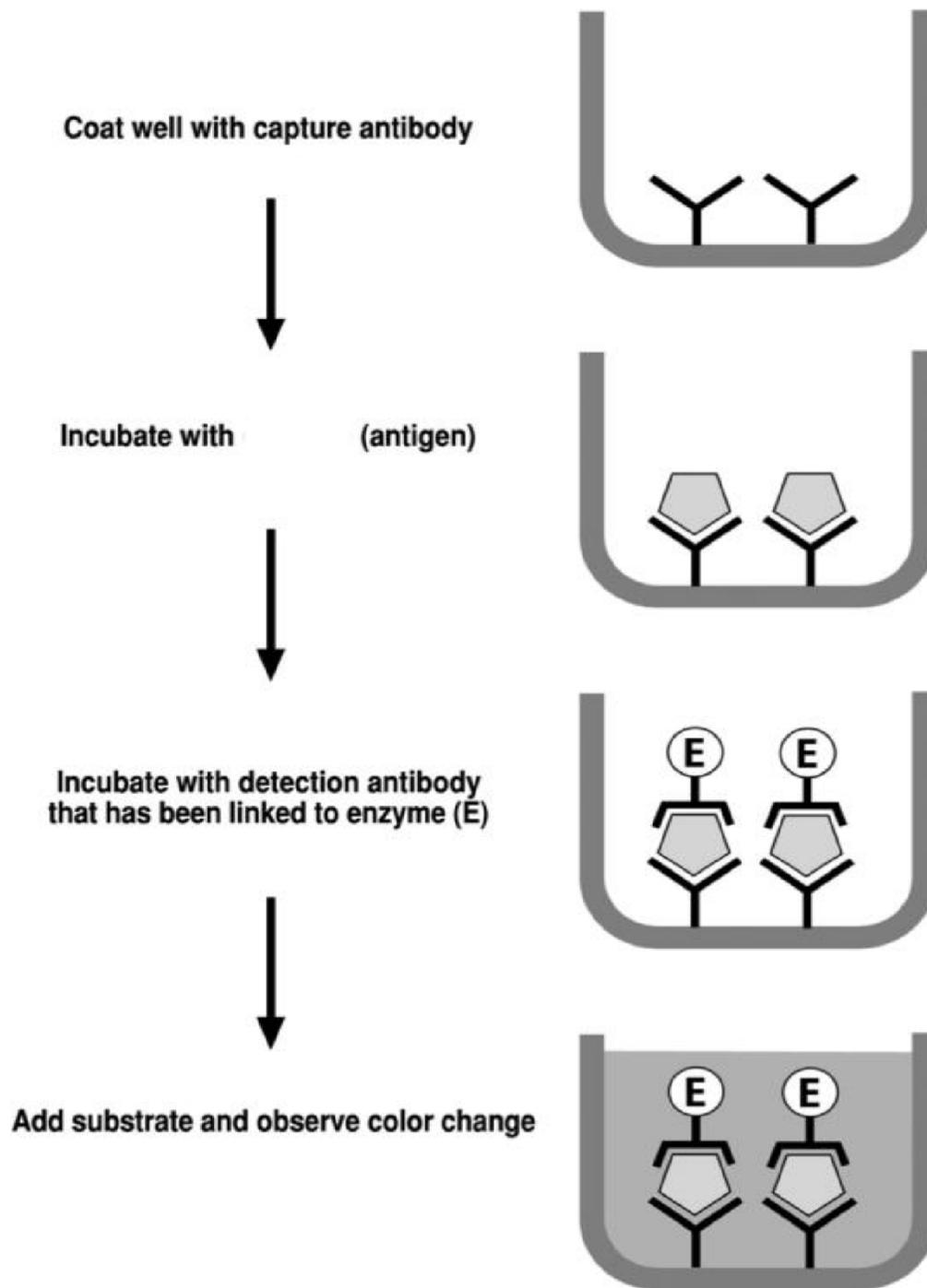


Figure 2. 4: Mechanism of Sandwich ELISA. Adapted from Engvall & Perlman (1971).

2.5 Summary

This chapter has offered a literature review, outlines for the study, highlighted a major area in the study of Alzheimer's disease, a review of brain-derived neurotrophic factor (BDNF), an appraisal of AD cognitive assessments and a review of the Enzyme-Linked Immunosorbent Assay employed as an analytical method to assess BDNF serum concentration. The next chapter will focus on the methodological part of the research.

3. Chapter Three: Methodology

This chapter presents the research methodologies used in the study. This includes the research design, sources of data, data gathering procedure and the statistical analysis of data.

3.1 Research setting

The research setting for this study was KFMC a tertiary hospital in Riyadh city, which is part of the Ministry of Health in Saudi Arabia. The patients enroll through the psychiatric clinic in the outpatient clinics. The clinic for newly diagnosed AD patients is held two days a week on average, and sees four to eight patients per day. Therefore, the total number of patients who visit the clinic is approximately sixteen patients per month. Moreover, the other setting of the research is KSSC, located in Riyadh city; the concept of this center is to provide social and health services especially for old people, including social, cultural and sports activities. It also provides different healthcare services to their members and visitors of varying age groups.

3.2 Participants

3.2.1 Healthy participants

The healthy participant group was obtained from both the settings: KFMC and KSSC in Riyadh city, Saudi Arabia. The study participants included 123 young and elderly participants. All volunteers signed the informed consent of the study after full explanations about the study objectives. The inclusion criteria for the healthy volunteers group were that they had to be male and female Saudis aged between 25 and 90 years old, without any major complicated medical conditions such as diabetes, cardiovascular diseases or asthmatic conditions. The exclusion criteria for this group included volunteers who had a history of head injuries,

neurological diseases, psychiatric disease, using antidepressants or antipsychotic medication, smokers or those involved in drug abuse.

3.2.2 Patient participants

The patient group was obtained from those who attended the outpatient psychiatric clinic's "newly diagnosed clinic" in KFMC. The informed consent was gained from all patients and/or their substitute decision maker by describing the study process and through obtaining their voluntary participation in the study. Those who agreed to participate signed the approved consent form to undergo the required research protocol in the research clinic. The patient inclusion criteria included any 60 year plus, male or female Saudi clinically diagnosed with AD. Additionally, the patients needed to be able to communicate verbally and provide their consent for the study participation. Alternatively, a substitute decision maker supplied their consent for participation.

The patient exclusion criteria were: (1) patients diagnosed with comorbidity of primary psychiatric (such as schizophrenia and major depression onset before the AD onset) or neurological disorders (including stroke, PD, seizure disorders or head injuries with loss of consciousness within the past year); (2) patients who used antidepressant medications, anxiolytic agents, mood stabilizers/antiepileptic or cholinesterase inhibitor medications; (3) patients with a known suspected history of alcoholism or drug abuse; (4) patients complaining of haematological disorders and patients receiving anticoagulation medications.

3.3 Research design

3.3.1 Healthy volunteers

The demographic data sheet (Appendix 10.5) was filled in to rule out any excluded criteria. This sheet contained the following information: participant names, age, and gender, level of education, weight, height, medical history and medication history. Prior to enrolment, the volunteers had to sign the informed consent after a detailed explanation about the aim of the research, and all previous lab tests were checked for normal results if applicable. Further to this, a 5ml blood sample was drawn from the participants to assess their BDNF levels. A 3 ml blood sample was also taken to apply a biochemical analysis used for the assessment of haemoglobin A1c, sodium levels, potassium levels, liver function test, and blood analysis (white blood cells, red blood cells and platelets).

3.3.2 Patient recruitment

Firstly, the patients attended the out-patients" psychiatric clinic; a consultant examined the physical and cognitive levels of the participants in this study. Their demographic details were taken alongside a complete medical history. Patients were also screened by the Hamilton Depression Rating Scale (HDRS) to rule out the onset of depression. The clinician explained to the patients and caregivers about the research objectives, and then the informed consent was signed and participants agreed to being recruited for the research. Following this, the staging process of AD was completed by using an Arabic version of the clinical dementia rating scale; this staging was carried out by qualified clinicians through semi-structured interviews for a twenty- to thirty-minute period.

The final step involved taking the blood samples. The blood was collected (5 millilitres) from each participant by venipuncture and placed into an anticoagulant-free vacuum tube. The samples were allowed to clot and then centrifuged at 1000 X g for 15 minutes. Serum aliquots were stored at -20°C until they were assayed and thawed immediately prior to the measurement of biochemical parameters. For the chemistry variables, one ml was used directly after being centrifuged. The other serum was aliquoted in Eppendorf tubes already labelled with the subject's information and date (400 mL/Eppendorf, ~ 3-4 aliquots depending on the serum volume/subject). All serum aliquots were stored at -20°C . For the EDTA tube, 3 millilitres of whole blood was collected by venipuncture into a vacutainer in K3-EDTA; 3 ml of anticoagulant (purple top tubes) were used directly for haematology for complete blood counts (CBC) and other differential counts.

3.4 Research instruments

3.4.1 Cognition test

The tool used for the assessment of the patients' cognition performance was The Arabic version of the Clinical Dementia Rating (CDR) scale for AD (See Appendix 10.7). This instrument was developed by the Memory and Aging Project at the Washington University School of Medicine in 1979 for the evaluation of staging severity of dementia. The CDR is a 5-point scale used to characterize six domains of cognitive and functional performance applicable to AD and related dementias: memory, orientation, judgment and problem solving, community affairs, home and hobbies and personal care. The necessary information to make each rating is obtained through a semi-structured interview of the patients and care providers.

In rating each of these domains, the assessment needed to be on the patient's cognitive ability to function in these areas. If they were limited in performing activities at home because of physical frailty, this did not affect their scoring on the CDR, which was rated on their cognitive ability alone. To best determine distinctions between various severity levels, the information collected during the data collection became important. During the scoring process, the idea was to use all information available and make the best judgment through semi-structured interviews of patients and informants, and cognitive functioning is rated in 6 domains of functioning: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. Each domain is rated on a 5-point scale of functioning as follows: 0, no impairment; 0.5, questionable impairment; 1, mild impairment; 2, moderate impairment; and 3, severe impairment (personal care is scored on a 4-point scale without a 0.5 rating available). The global CDR score is computed via an algorithm. Herein, each global score was calculated using the Washington University online algorithm (<http://www.biostat.wustl.edu/~adrc/cdrpgm/index.html>). Domain scores were entered into the online algorithm independently by 2 different research assistants, and discrepancies in computed global CDR scores were double

checked for entry errors and resolved. The CDR-SOB score is obtained by summing each of the domain box scores, with scores ranging from 0 to 18. Each category was marked in a box according to how they have declined from the person's usual level due to cognitive loss alone, not due to other factors, such as physical handicap or depression.

Aphasia is taken into account by assessing both language and non-language functions in each cognitive category. If aphasia is present to a greater degree than the general dementia, the subject is rated according to the general dementia.

The global CDR is derived from the scores in each of the six categories as follows: Memory (M) is considered the primary category and all others are secondary. $CDR = M$ if at least three secondary categories are given the same score as memory. Whenever three or more secondary categories are given a score greater or less than the memory score, $CDR =$ score of majority of secondary categories on whichever side of M has the greater number of secondary categories. When three secondary categories are scored on one side of M and two secondary categories are scored on the other side of M, $CDR=M$. When $M = 0.5$, $CDR = 1$ if at least three of the other categories are scored one or greater. If $M = 0.5$, CDR cannot be 0; it can only be 0.5 or 1. If $M = 0$, $CDR = 0$ unless there is impairment (0.5 or greater) in two or more secondary categories, in which case $CDR = 0.5$.

Although applicable to most AD situations, these rules do not cover all possible scoring combinations. Unusual circumstances occur occasionally in AD and may be expected in non-Alzheimer dementia" these are scored as follows:

- With ties in the secondary categories on one side of M, choose the tied scores closest to M for CDR (such as M and another secondary category = 3, two secondary categories = 2, and two secondary categories = 1; $CDR = 2$).

- When only one or two secondary categories are given the same score as M, CDR = M as long as not more than two secondary categories are on either side of M.
- When M = 1 or greater, CDR cannot be 0; in this circumstance, CDR = 0.5 when the majority of secondary categories are 0.

3.4.2 Assessment of depression

All patients were accurately screened by a consultant psychiatrist for the onset of depression after the onset of cognitive symptoms of dementia using HDRS. Subjects whose depression onset preceded the onset of dementia were excluded. The HDRS is a multiple item questionnaire used to provide an indication of the severity of depressive symptoms in adults by probing mood, feelings of guilt, suicide ideation, insomnia, agitation or retardation, anxiety, weight loss and somatic symptoms. It is composed of 17 items scored individually on a range from 0-4 based on clinical observation. A total score of 0-7 is considered to be normal. A score of 8-13 = mild depression, 14-18 = moderate depression, 19-22 = severe depression, more than 23 = very severe depression. (See Appendix 10.4)

3.4.3 Serum BDNF measurement technique

Circulating serum levels of BDNF were determined with a commercially available enzyme-linked Immunosorbent assay (ELISA) kit, with a sensitivity < 2 pg/ml and an intra-assay variation < 5% (Piockine® Human BDNF ELISA Kit, Booster Biological Technology, Valley Ave, Pleasanton, USA, Cat. #: EK0307).

Figure 3.1 shows a typical standard concentration curve illustrating the sensitivity down to low concentrations and a linear relationship to 2,000 pg/ml of standard concentration.

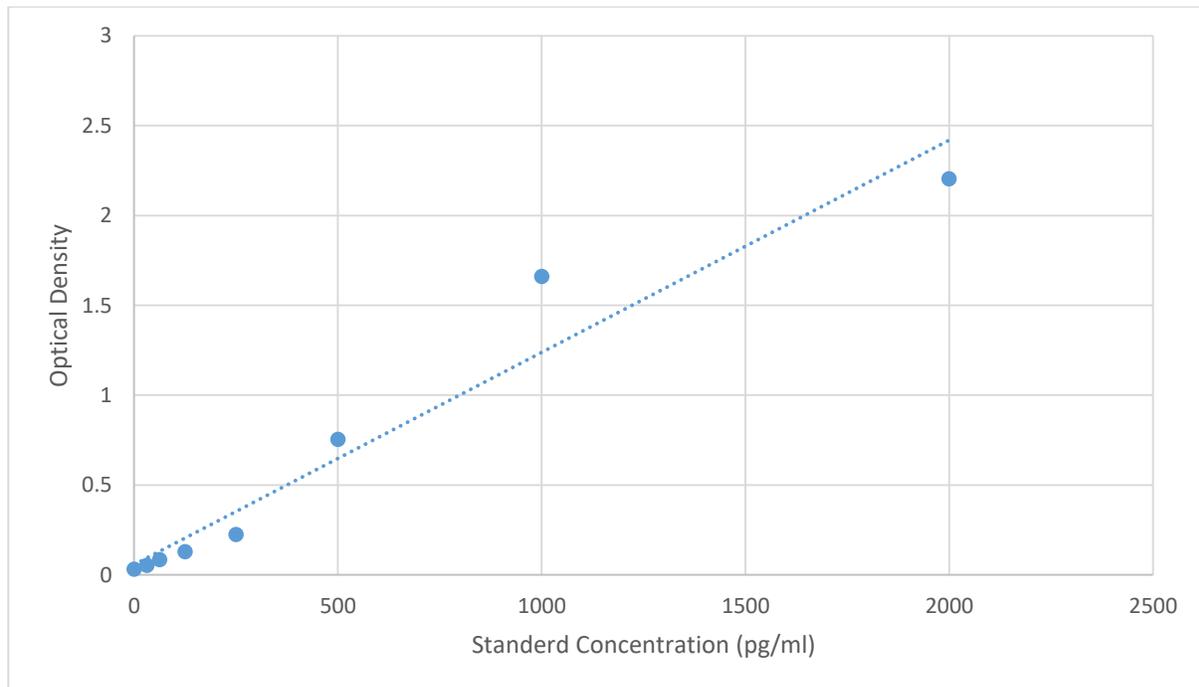


Figure3. 1: BDNF standard curve using a commercial ELISA kit.

Serum samples were diluted in a ratio of 1:10. Sample BDNF concentrations were then determined by non-linear regression analysis derived from the standard curves of BDNF concentration. Measurements were performed in duplicate. A standard curve was run and calculated with each assay after multiply the results for the serum x10 to account for the dilution.

In brief, the assay technique was conducted according to the commercial manufacturing instructions. First, a blank and standard well were prepared by adding 0.1 ml of a range of BDNF standard solutions (31.25 pg/ml–2,000 pg/ml) into the pre-coated 96-well plate. The plate was then incubated for 90 minutes to allow the binding of the BDNF to the antibody at 37°C. Second, a biotinylated detection specific for BDNF was added to each well and the plate was incubated for another 90 minutes to form a BDNF complex at 37°C. The plate was then washed with a TBS buffer; it was washed three times with the buffer solution. Third, the Avidin-Biotin-Peroxidase Complex working solution was added to each well and the plate was incubated at 37°C for 30 minutes. Subsequently, the plate was washed with the buffer to remove the unbound conjugates and incubated in the dark at 37°C for 25 to 30 minutes. Fourth, a colouring agent, to visualize the reaction, and acidic stop solution, to begin the reaction, were added to the wells. Once the colour changed from blue to yellow, the density of the yellow matter is proportional to the human BDNF sample amount captured in the plate. Finally, the plate was read using a microplate reader at 450 nm and the data was entered into curve expert 1.4. This software calculated the serum BDNF with unknown concentrations from categorized data including the absorption of the blank well (0 pg/ml), absorption of known standard concentrations and absorption of samples.

3.5 Ethical approval

Ethical approval for this study was sought and obtained from both the University of Brighton (UoB) (see Appendix 10.3) and the King Fahad Medical City (KFMC) in which this study took place, in January 2016. Also, during this process, institutional permission to access the research area was obtained from the King Fahad Medical City (KFMC) and the King Salman Social Centre (KSSC) (see Appendix 10.1, and Appendix 10.2) for consent form and ethical approval letters).

3.6 Data analysis

All statistical analyses were performed using the statistical analysis software package Minitab 18. The mean, standard deviation, media and skew of the data are presented in the findings. The mean was used in the case of normally distributed data to measure the central tendency. If the frequency of distribution for the data was skewed, the median was considered a better measure of central tendency than the mean. When the data was perfectly normal, the mean, median and mode were identical. Moreover, they all represented the most frequent value in the data set. However, as the data becomes skewed, the mean loses its ability to provide the best central location for the data because the skewed data causes it to move away from the central value. However, the median better retains this central position because it is not as strongly influenced by the skewed values. The skewed distribution section explains this rationale in detail later. For example, section 5.4, the skewed distribution section, explains this rationale in greater detail. The significance for the results was set at $p < 0.05$. Continuous variables were tested for normal distribution with the Kolmogorov–Smirnov test. A bivariate correlation analysis (Pearson test and Spearman-Rho test) between age, BMI, HbA1C, platelet count, use of medication and BDNF serum levels for the healthy group and the different patient groups was conducted. The Mann–Whitney U test was used to assess differences between the AD patients and elderly group in cases of non-normal distribution. The two-tailed t test was used to assess differences between the two groups in cases of normal distribution. However, the Kruskal-Wallis test for analysis of the variance was used for data which are not normally distributed.

3.7 Power analysis and sample size calculation

For healthy and AD patients, the sample size was calculated based on the mean BDNF concentrations found in a previous study for healthy and Alzheimer patients that took into consideration the divided group process. For example, suppose that in previous experiments the mean of serum BDNF was 130.84 pg/ml, with a standard deviation of 59.81 pg BDNF/mL (Boyuk *et al.*, 2014a). Assume also that the previous study would like to detect a 6.5 pg BDNF/mL reduction in serum BDNF between the control group and the treated AD group with a power of 80% and a significance level of 5%. Using a computer program called Creative Research Systems that calculated and expected the current sample size with suggestion of 13 individuals per group or roughly 54 individuals are required for the study. <https://www.surveysystem.com/sscalc.htm>

Other method for calculation the sample size depend on polymorphism was calculation based on the following equation, the sample size was calculated based on a power of 80% using the assumption that SNPs are informative when they are present in 5% of the sample against the one sided alternative with a critical region size of 5%.

The null hypothesis is represented as $H^0:P^0$ and the alternative hypothesis is $H^1:P^1$.

The sample size is calculated by applying the following formula developed by Fliess (1981):

$$n = \left\{ \frac{Z\beta\sqrt{p^1 q^1} + Z\alpha\sqrt{p^0 q^0}}{p^0 - p^1} \right\}^2$$

Where, $P^0 = 0.10$ (the prevalence of the genotype or allele in the null hypothesis), $P^1 > 0.5$ (the prevalence of the genotype for the alternative hypothesis), $q = 1-P$, $Z\alpha = 1.64$ (the cut-off point of the standardized normal distribution at α level = 5%) and $Z\beta = 0.84$ (the normal distribution cut-off for a power of 80%). Thus,

$$n = \left\{ \frac{0.84\sqrt{(0.22)(0.78)} + 1.64\sqrt{(0.05)(0.95)}}{0.05 - 0.22} \right\}^2 .$$

Therefore, using this calculation method, an adequate sample size for the study was determined to be 60 individuals.

3.8 Hardy-Weinberg Equilibrium Calculations

3.8.1 Hardy-Weinberg Equilibrium Calculations for all participants

BDNF genotype distributions were different from the Hardy-Weinberg equilibrium among all of the participants (n=180) (P-value=0.002) (Table7.2)

$$p + q = 1$$

$$p^2 + 2pq + q^2 = 1$$

p Is the allele dominate frequency

q Is the frequency of the recessive allele

p^2 Is the frequency of individuals with the homozygote dominate genotype

2pq Is the frequency of individuals with the heterozygote genotype

q^2 Is the frequency of individuals with the homozygote recessive genotype.

Calculations steps

Step 1: Find the frequency of GG, the homozygous recessive genotype (q^2):

1. Add up all individuals to calculate the total population (72+60+48=180).

2. Frequency of individuals = $\frac{\text{Individuals}}{\text{Total population}}$

3. Frequency = $\frac{48}{180} = 0.26$

4. $q^2 = (0.26)$

Step 2: Find q by calculating the square root of q^2 :

1. $\sqrt{q^2} = \sqrt{0.26}$

2. $q = 0.5$

Step 3: Use the first Hardy-Weinberg equation $p + q = 1$ to solve for p:

1. $p + q = 1$

2. $p = 1 - q$

3. $p = 1 - (0.5)$

4. $p = 0.5$

Now that the allele frequencies in the population are known, solve for the remaining frequency of individuals by using $p^2 + 2pq + q^2$.

Step 4: Square p to find p^2 :

1. $p = 0.5$
2. $p^2 = (0.5)^2$
3. $p^2 = 0.25$

Step 5: Multiply $2 * p * q$ to get $2pq$:

1. $2pq = 2 * (0.5) * (0.5)$
2. $2pq = 0.5$

Therefore:

The frequency of the dominant alleles: $p = 0.5$

The frequency of the recessive alleles: $q = 0.5$

The frequency of individuals with the dominant genotype: $p^2 = 0.25$

The frequency of individuals with the heterozygous genotype: $2pq = 0.5$

The frequency of individuals with the recessive genotype: $q^2 = 0.26$

Step 6: Multiply the frequency of individuals p^2 , $2pq$, and q^2 by the total population to get the number of individuals with that given genotype:

$$p^2 \times \text{Total population} = 0.25 \times 180 = 45 \text{ AA genotype}$$

$$2pq \times \text{Total population} = 0.5 \times 180 = 90 \text{ AG genotype}$$

$$q^2 \times \text{Total population} = 0.26 \times 180 = 47 \text{ AG genotype}$$

	AA	AG	GG	P-value
Actual	72	60	48	0.002
Expected	45	90	47	

Table3. 1 Hardy-Weinberg equilibrium expected genotype among all of the participants (n=180)

3.8.2 Hardy-Weinberg Equilibrium Calculations for Healthy Participants

The BDNF genotype distributions were, in the Hardy-Weinberg equilibrium, among the young and middle age healthy group (n=75) (P-value=0.072) (Table7.3):

$$p + q = 1$$

$$p^2 + 2pq + q^2 = 1$$

p Is the allele dominant frequency

q Is the frequency of the recessive allele

p^2 Is the frequency of individuals with the homozygote dominant genotype

$2pq$ Is the frequency of individuals with the heterozygote genotype

q^2 Is the frequency of individuals with the homozygote recessive genotype

Calculations steps

Step 1: Find the frequency of GG, the homozygous recessive genotype (q^2):

3. Add up all individual to calculate the total population (24+25+26=75).

4. Frequency of individuals = $\frac{\text{Individuals}}{\text{Total population}}$

5. Frequency = $\frac{26}{75} = 0.34$

6. $q^2 = (0.34)$

Step 2: Find q by taking the square root of q^2 :

7. $\sqrt{q^2} = \sqrt{0.26}$

8. $q = 0.58$

Step 3: Use the first Hardy-Weinberg equation $p + q = 1$ to solve for p:

9. $p + q = 1$

10. $p = 1 - q$

11. $p = 1 - (0.58)$

12. $p = 0.42$

Now that the allele frequencies in the population are known, solve for the remaining frequency of individuals by using $p^2 + 2pq + q^2$.

Step 4: Square p to find p^2 :

13. $p = 0.42$

14. $p^2 = (0.42)^2$

15. $p^2 = 0.17$

Step 5: Multiply $2 * p * q$ to get $2pq$:

16. $2pq = 2 * (0.42) * (0.58)$

17. $2pq = 0.48$

Therefore:

The frequency of the dominant alleles: $p = 0.42$

The frequency of the recessive alleles: $q = 0.58$

The frequency of individuals with the dominant genotype: $p^2 = 0.17$

The frequency of individuals with the heterozygous genotype: $2pq = 0.48$

The frequency of individuals with the recessive genotype: $q^2 = 0.34$

Step 6: Multiply the frequency of individuals p^2 , $2pq$, and q^2 by the total population to get the number of individuals with that given genotype:

$p^2 \times \text{Total population} = 0.17 \times 75 = 13 \text{ AA genotype}$

$2pq \times \text{Total population} = 0.48 \times 75 = 36 \text{ AG genotype}$

$q^2 \times \text{Total population} = 0.34 \times 75 = 47 \text{ GG genotype}$

	AA	AG	GG	P-value
Actual	24	25	26	0.072
Expected	13	36	26	

Table3. 2: Hardy-Weinberg equilibrium expected genotype among healthy young and middle age group participants (n=75)

4. Chapter Four: Factors Affecting BDNF serum concentrations of Healthy Population

This chapter presents the gathered data for the factors affecting BDNF serum concentrations in healthy Saudi participants, providing an analysis and interpretation of the findings based on the results from the applied statistical treatment. The data are organised in chronological order based on the problem statement in Chapter 1.

4.1. Introduction:

In this chapter, the effect of serum BDNF concentrations among healthy participants and their association with other factors such as ageing, gender, BMI, and DM are tested and examined. These factors have been thoroughly discussed in the literature review (2.2.11, 2.2.12, 2.2.13 and 2.2.14). The research hypothesis included important questions regarding variables such as age, sex, BMI and incidence of DM and their contributions as confounding factors that determine the serum BDNF concentrations (Shimada et al., 2014). The aim included testing the hypothesis that gender significantly affected serum BDNF concentrations. Additionally, obesity is a serious and growing health problem among the Saudi population; there is a need to investigate the correlation between BDNF concentrations and BMI. Moreover, it would be relevant and interesting to investigate the associations between BDNF concentrations and the development of diabetes in Saudi Arabia. This section focus on the demonstrable effects of serum concentrations among healthy participants compared with other confounding variables.

4.2 Methodology

4.2.1 Participants

A total of 123 healthy participants were included in this study. All the participants were enrolled from KFMC tertiary hospital and KSSC in Riyadh city, Saudi Arabia. The investigation was approved by the ethics committees of both the University of Brighton (UoB) and KFMC, where the investigations were conducted.

The inclusion criteria for the participants were as follows:

- (a) aged between 25 and 60
- (b) belonging to either gender;
- (c) in possession of normal clinical laboratory test results

The following persons were excluded from participating:

- (a) persons diagnosed with a co-morbidity involving a primary psychiatric disorder (such as schizophrenia or major depression) with onset before the AD onset or a neurological disorder (including stroke, seizure disorders, or head injuries with loss of consciousness within the past year);
- (b) persons who used anxiolytic agents, mood stabilisers/antiepileptic medications or cholinesterase inhibitors;
- (c) persons with a known or suspected history of alcoholism or drug abuse; and
- (d) persons complaining of haematological disorders.

4.2.2 Measurement of BDNF concentrations

Circulating serum concentrations of BDNF were analysed in duplicate using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, exhibiting sensitivity < 2 pg/ml and intra-assay variation < 5% (Piockine Human BDNF ELISA Kit, Booster Biological Technology, Valley Ave., Pleasanton, USA, Cat. #EK0307; see Chapter 3 for more details).

4.2.3 Measurement of other chemistry variables

For the assessment of chemistry variables, 1 ml was used directly after being centrifuged. The other serum was aliquoted in Eppendorf tubes already labelled with the subject's information and date (400 mL/Eppendorf, ~ 3-4 aliquots depending on the serum volume/subject). All serum aliquots were stored at -20°C. For the EDTA tube, 3 ml of whole blood was collected by venipuncture into a vacutainer in K3-EDTA; 3 ml of anticoagulant (purple top tubes) were used directly for haematology for complete blood counts (CBC) and other differential counts

4.3 Data analysis

All statistical analyses were performed using the statistical analysis software package Minitab 18. The mean, standard deviation, median and skew of the data are presented in the findings. The mean was used in the case of normally distributed data to measure the central tendency as all serum BDNF concentrations for healthy participants were normally distributed. A bivariate correlation analysis (Pearson test) between age, BMI, HbA1C, platelet count, use of medication and BDNF serum levels for the healthy group was conducted. The significance level for the results was set at $p < 0.05$.

4.5 Results

4.5.1 General data results

The characteristics of the participants are presented in Table 4.1. The healthy group included 123 participants and constituted three groups: a group of younger healthy subjects, with their ages ranging between 25 and 35 ($n = 34$); a middle aged group, with ages ranging from 36 to 59 ($n = 41$); and an elderly healthy group who were aged above 60 ($n = 48$).

	Healthy subject Young (N=34)	Healthy subject Middle aged (N=41)	Healthy subject Elderly (N=48)	p-value
Age (years)	30.0 ± 2.77	47.8±6.63	69.8±6.45	<0.001
Gender M, F (%)	M=25 (73.5) F=9 (26.4)	M=27(65.8) F=14(34.2)	M=12 (25) F=36(75)	0.045 <0.001
BMI (kg/m ²)	25.9±3.19	28.1±4.33	29.7±5.30	0.878
HbA1c (%)	5.3±0.67	5.8±0.92	7.9±10.09	0.740
White Blood Cell Count (x 10 ⁹ per liter L)	8.0±1.56	7.6±1.78	8.0 ±2.45	0.993
Red Blood Cell Count (10 ⁶ /μL)	5.1±0.45	4.9±0.44	4.3±0.46	0.964
Haemoglobin level (gm/dL)	15.3±1.36	14.8±1.33	13.1±1.40	0.912
Platelet count (10 ⁹ /L)	268.6±51.93	268.0±57.29701	270.8±61.51	0.684
Use of medication (%)	2.9%	17%	50%	<0.001
Serum BDNF (pg/ml)	338.9±124.30	289.4±103.09	80.3±27.84	<0.001

Table 4. 1: The clinical characteristics of the groups of participant

4.5.2 BDNF serum concentrations of distribution

There was a wide range of BDNF concentrations in the serum of healthy participants ($N = 123$) (Table 4.2). To some extent, the serum BDNF concentrations in the healthy control group were normally distributed according to the Kolmogorov-Smirnov (K-S) test, with a p -value ≤ 0.01 .

	BDNF concentrations (pg BDNF/ml)		
	Young	Middle aged	Elderly
Mean	338.9	289.4	80.3
Standard Deviation	124.30	103.09	27.84
Median	323.9	251.616	79.3
Minimum	143.1	179.2	30.7
Maximum	589.6	594.1	167.8
Skewness	0.4	1.4	0.5

Table 4. 2: The BDNF concentrations in human serum of healthy participants

4.5.3 BDNF serum concentrations and age

The serum BDNF concentration values were inversely correlated with the age of the healthy group. Because of the nature of the data distribution, the test that was used to determine correlation was Pearson's correlation coefficient. There was a statistically significant correlation between the serum BDNF concentrations and age. This was a strong negative correlation, ($r = -0.70$; $p \leq 0.001$) (Figure 4.5 A).

The scatterplot representing this correlation can be found in Figure 4.6 A. As the plot shows, there was an approximate four-fold lowering in serum BDNF values for participants who were more than 60 years old (80.3 ± 27.84 pg /ml).

4.5.4 BDNF serum concentrations and gender

When evaluating the concentrations for the subjects in the healthy group ($n = 59$ females and $n = 64$ males), the differences between the genders with regard to serum BDNF concentrations were significant ($p < 0.001$), as seen in Figure 4.1. However, there were no significant differences between the females ($n = 36$) and males ($n = 12$) in the elderly participant group ($n = 48$). The Mann-Whitney test revealed a p-value = 0.63. The serum BDNF concentrations were not normally distributed (Figure 4.2).

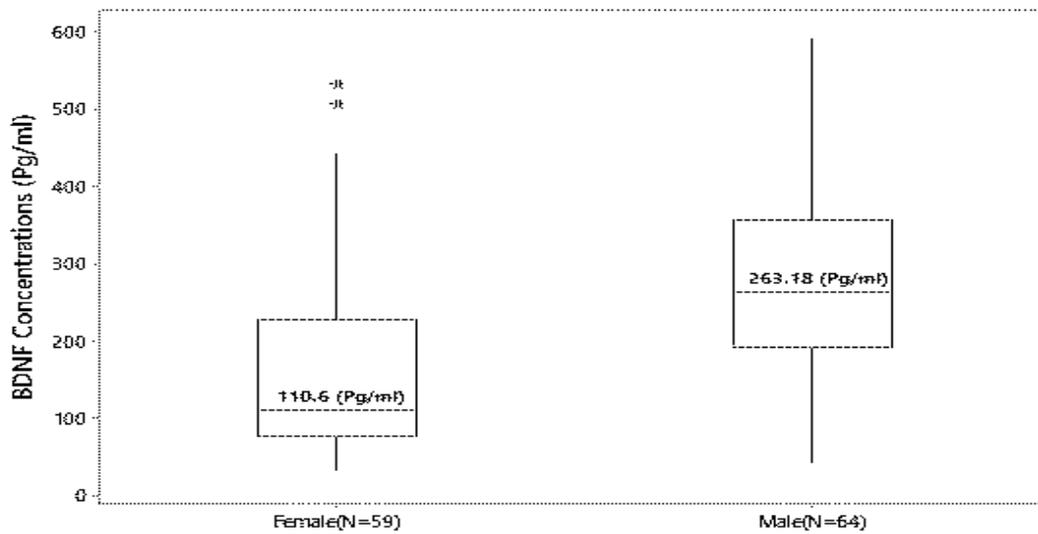


Figure4. 1 : The box plot showing the serum BDNF concentrations for both genders in all healthy participants (N=123)

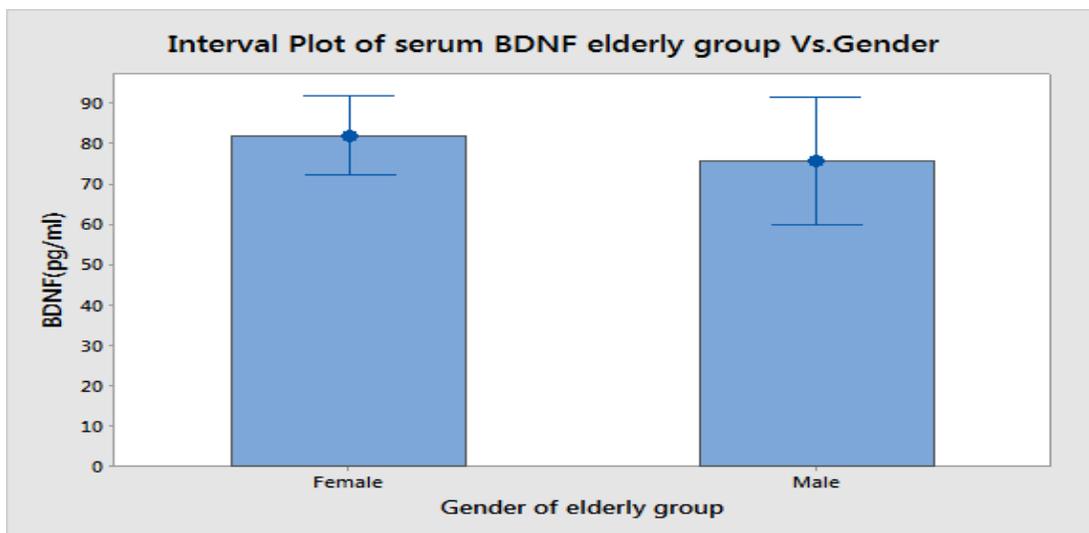


Figure4. 2: The serum BDNF concentrations for both genders among the elderly participants.

4.5.5 BDNF serum concentrations and body mass index

The BMI records revealed a correlation effect when using the Pearson correlation coefficient test. It demonstrated that there was a slightly negative correlation ($r = -0.2$, $P = 0.02$) between the serum BDNF concentrations and BMI among the control groups. On the other hand, the elderly control group displayed no significant correlation with BMI values ($r = 0.23$, $p = 0.10$) when using the Pearson correlation coefficient test (Figure 4.5 B).

4.5.6 BDNF serum concentrations and glycated haemoglobin (HbA1c)

The correlation coefficient according to the Pearson correlation coefficient test revealed a negative correlation between serum BDNF concentrations and the stored level of glucose. The results demonstrated that increased HbA1c was associated with decreasing serum BDNF ($r = -0.43$, $P < 0.001$) (Figure 4.5 C).

Interestingly, the ANOVA which was used to assess the difference among the means of the glycated haemoglobin concentrations and serum BDNF concentrations revealed no significant difference between the groups ($F = 5.4$, p -value = 0.06). Moreover, the results revealed that 14 individuals (8.9%) with an HbA1c above 7.5, which is indicative of poor glycaemic control (diabetes mellitus), all had BDNF values below 175.5 pg /ml (Figure 4.3).

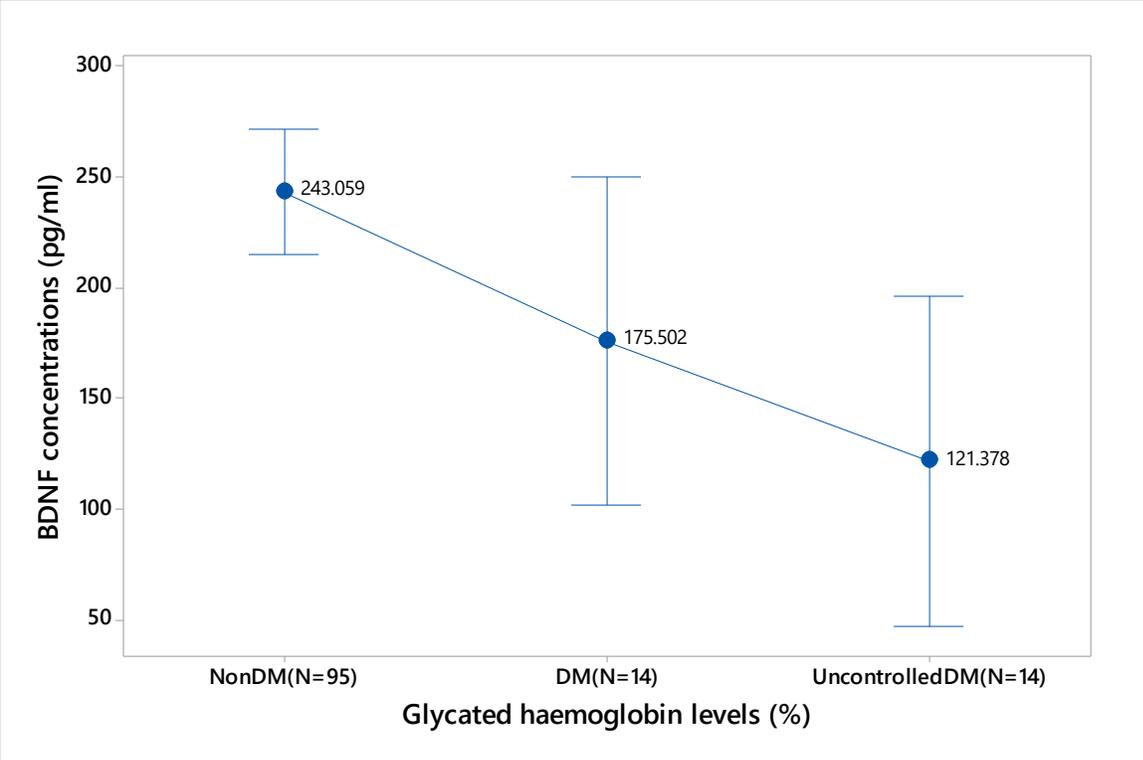


Figure4.3: The interval plot showing the differences between BDNF levels (Pg/ml) and the levels of glycated Haemoglobin (%) in healthy (N=123) participants

4.5.7 BDNF serum concentrations and blood cell counts

Previous literatures have suggested a correlation between platelet counts and serum BDNF concentration levels. Therefore, in this study, we sought to verify these correlations. The results of the serum BDNF values were correlated with white blood cells, red blood cells and platelet counts. No relationship was apparent for white or red blood cells. Interestingly, the serum BDNF displayed no significant correlation with the number of platelets in peripheral blood ($r = 0.014$, $p = 0.861$) according to Pearson correlation testing (Figure 4.5 D).

4.5.8 BDNF serum concentrations and the use of medication

Most of the participants did not use any medication ($n = 93$, 75.8%), while approximately 30 of the participants (24.2%) did. A comparison of the mean of the two groups, using the t-test, revealed a statistically significant effect for medication use for serum BDNF ($t = 2.003$, $p = 0.00017$) (Figure 4.4).

The medication used included (antihypertensive medication ($n = 5$), oral hypoglycemic agents ($n = 9$), insulin ($n = 3$), thyroid supplements ($n = 3$), lipid-lowering agents ($n = 5$), vitamin D3 supplements ($n = 3$) and aspirin ($n = 2$).

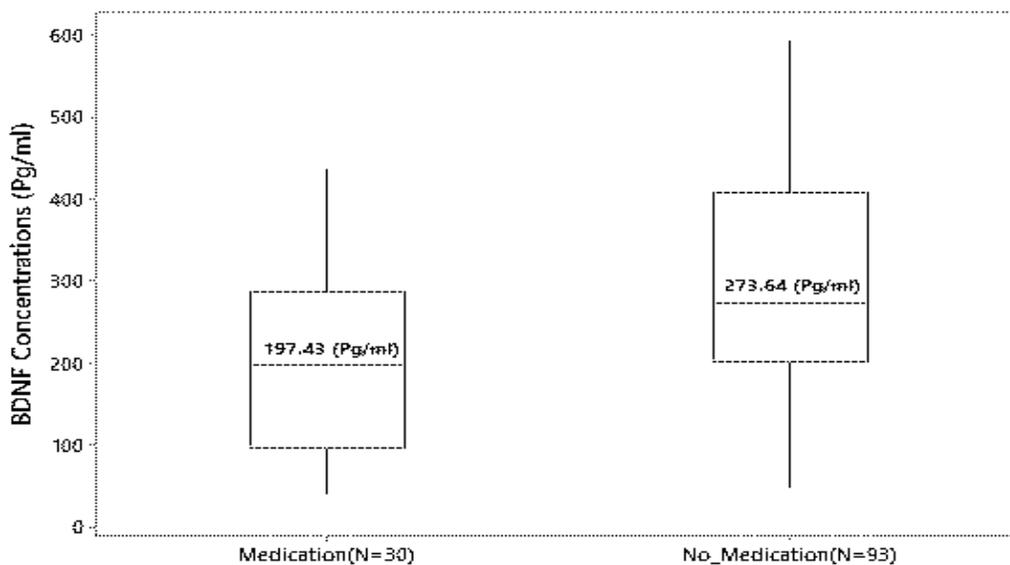


Figure4. 4:Box plot showing the changes of serum BDNF concentrations with the use of medication in healthy control participants (N=123)

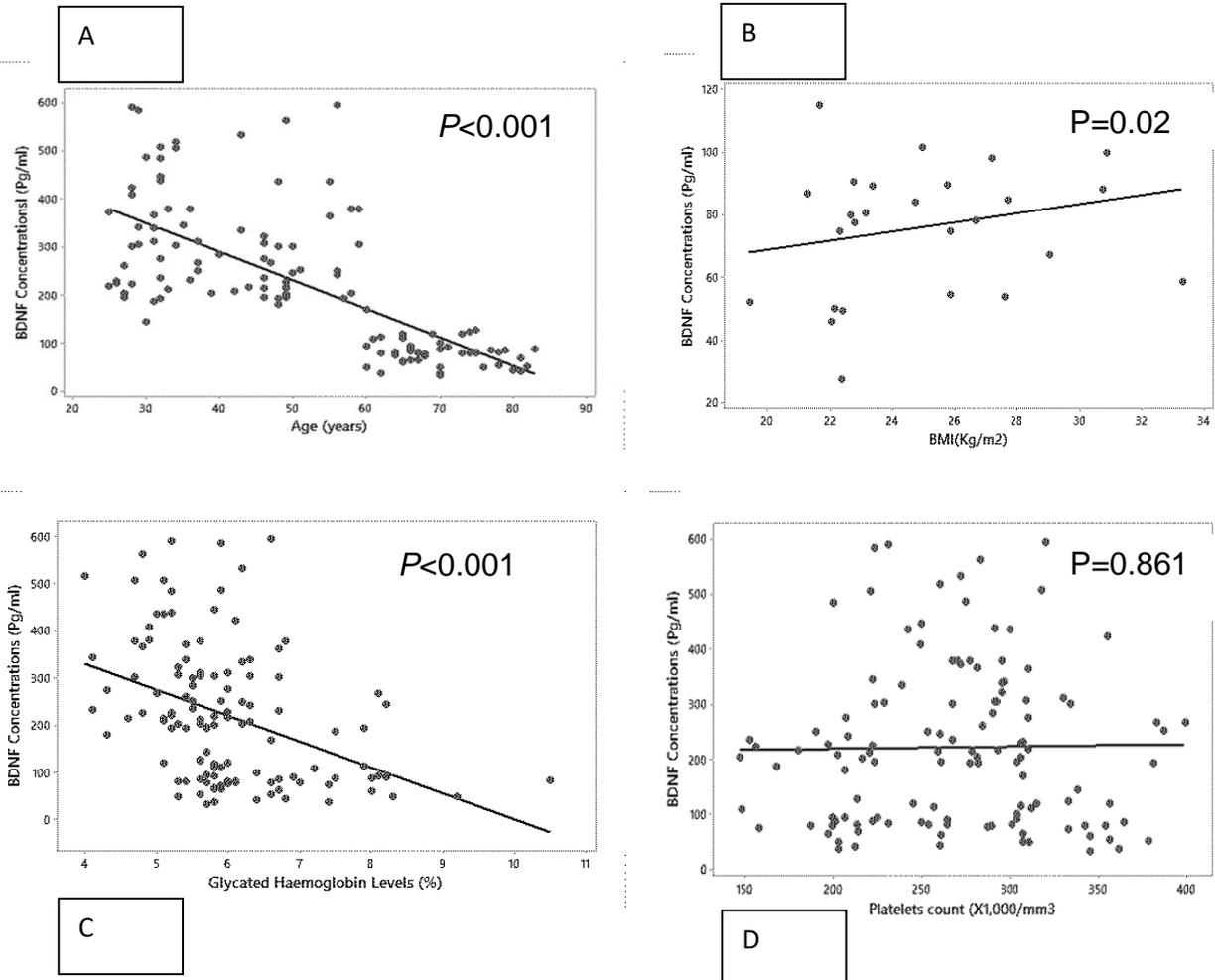


Figure 4. 5: Scatter plots showing the relationship between the serum BDNF concentrations with (A) age, (B) body mass index, (C) glycated haemoglobin, and (D) platelets counts for healthy participants (N=123)

4.6 Discussion

Among the healthy participants, the mean BDNF concentrations were found to be 221.5 ± 145.58 pg/ml. These values are supported by other relevant studies. For example, Boyuk et al. (2014) recently reported that the serum BDNF concentrations of their population was 206.81 ± 107.32 pg /mL in healthy control subjects, although, Nettiksimmons et al. (2014) reported values of 15.1 ± 3.6 ng/ml, approximately 100 times greater than our results.

According to a clinical review that compared different measurement methods for BDNF in cases of major depression, the best technique to use was an Enzyme Linked Immunosorbent Assay (ELISA) (Teche et al., 2013). This review indicated that BDNF in serum samples could be detected within an ng/ml range, while the plasma samples were usually measured within a pg/ml range. This explains why the literature review observed that three studies measuring plasma values had results for BDNF concentration in pg/ml (Faria et al., 2014). In contrast, a second review (Angelucci *et al.*, 2010) reported serum samples and detected BDNF levels in a control group of (5166.9 pg/ml), MCI (6215 pg/ml), and AD mild (6061.6 pg/ml), AD moderate to severe (6072.7 pg/ml). In addition, another longitudinal study that assessed the level of plasma BDNF in individuals with late-life depression and MCI revealed concentrations in the control group of 18.3 pg/ml. The results for groups in late-life depression and for groups displaying no cognitive decline were 16.2 pg/ml; with the late life depression with mild cognitive impairment group being 15.5 pg/ml (Diniz *et al.*, 2014).

The average serum concentrations of BDNF was more than 100-fold greater than the plasma concentration because during the clotting process, there is degranulation of platelets which contain large amounts of BDNF protein. Thus, the difference between serum and plasma BDNF levels seem to reflect the amount of BDNF stored in the circulating platelets (Teche et al., 2013). Additionally, BDNF is found in serum, platelets or plasma, although previous research has suggested that BDNF values vary depending on the method of measurement (Polacchini et al., 2015). However, recent research has also revealed that that BDNF concentrations can be reliably measured in

human serum and that these concentrations were quite stable over one year (Naegelin et al., 2018). Further details about BDNF variations are discussed in Chapter 8.

This research has provided evidence that the serum BDNF concentrations of healthy participants decreases with ageing. This result answered the first research question. Recognizing the sample characteristics, the result shows that the serum BDNF levels of the healthy younger group (25 to 35 years old) and middle aged group (36 to 59 years old) are approximately equal; however, the serum BDNF levels of the elderly group (more than 60 years old ($n = 48$)) was approximately one quarter of the healthy younger group's. These results support those of Lommatzsch et al. (2005) which suggested a decrease in plasma BDNF for age range of 20 to 60 years old. Nettiksimmons et al. (2014) however, using serum, discovered no significant correlation between serum BDNF levels and age in 910 healthy participants, although the age group included was aged 70 and above only (Nettiksimmons et al., 2014). As our healthy participants group started at the age of 25, our outcomes could be deemed more accurate, when compared with results in studies which only include older participants. Overall, the significance of the current results in a Saudi population highlights that BDNF levels decline in late adulthood, an important consideration when comparing values with those of AD patients. Our results match the theoretical explanation of the reduction in BDNF concentration in the elderly that appear as the sum of different morphological changes, all of which lead to hippocampal volume reduction in the brain (Von Bohlen und Halbach, 2010). The morphological changes associated with aging include reductions in the branching pattern of dendrites, as well as reductions in spine densities, reductions in the densities of fibers projecting into the hippocampus as well as declines in the rate of neurogenesis. It is very unlikely that a single factor or a single class of molecules is responsible for all these age-related morphological changes in the hippocampus. Von Bohlen und Halbach (2010) hypothesized BDNF was a factor critically involved in the regulation of age-related processes in the hippocampus. Moreover, evidence suggests that disturbances in the BDNF-system also affect hippocampal dysfunctions (Von Bohlen und Halbach, 2010).

When evaluating the role of gender with regard to the serum BDNF concentrations for the healthy group, the current results display that females have lower serum BDNF concentrations than males—approximately 50%. Interestingly, the research discovered that there was no significant difference between females and males in the serum BDNF of the elderly healthy group. However, Nettiksimmons et al. (2014) stated that their female participants had higher BDNF concentrations than males; importantly, all of the subjects in this 2014 study were over 70 years of age; that is, post-menopausal (Nettiksimmons et al., 2014). These results suggest that the female reproductive hormones increase serum BDNF concentrations (Nettiksimmons et al., 2014).

This mechanism had been previously explained with the idea that estrogen modulates BDNF expression through four different mechanisms. First, estrogen directly induces BDNF expression by activating the endoplasmic reticulum (ER) of the brain nucleus, which induces *BDNF* transcription by initiating the ER/DNA tethering. Second, estrogen modifies the activity of the BDNF promoter epigenetically because ovariectomy-induced estrogen depletion causes an increase of methylation on BDNF organizers IV and V in the hippocampus that enhances response element binding protein (CREB) activity (Matyi et al., 2017). Third, ER regulates the activity of CREB through nongenomic activity. In cultured hippocampal neurons, estradiol induces an influx of extracellular Ca^{2+} , leading to the activation of CREB (Chhibber et al., 2017). Given that CREB is a major transcription factor that controls *BDNF* expression in neurons in response to Ca^{2+} concentration change, it is reasonable to conclude that the ER/CREB axis may modulate the expression of *BDNF* (Rosenzweig & Barnes, 2003). Finally, ER controls BDNF expression indirectly through interneuronal activity that leads to estrogen increasing GABA activity. Because GABA is an inhibitory neurotransmitter of BDNF expression, the reduced GABA production in the interneurons results in an elevation of BDNF transcription in the target cells (Metsis et al., 1993).

However, several studies have suggested that testosterone, the major component of androgens, had a positive regulator of BDNF expression and production in the motor neurons of spinal nucleus of the bulbocavernosus, hippocampal CA1, and brain homogenate after middle cerebral artery occlusion (Yang & Arnold, 2000; Pol et al., 2006). Progesterone, another important sex steroid produced mainly in the ovary, can also increase the production of BDNF in cortex explant and glial cells (Pluchino et al., 2013).

In our current findings, diversity in body weight and composition, hormonal status, and enzyme activity between male and female interact and contribute to these differences between gender and serum BDNF concentrations. There is evidence that the average BMI in Saudi Arabia is higher than in other countries: One third of the population suffer from obesity in Saudi Arabia (33%) compared to 27% in the United Kingdom, according to a 2016 report from the World Health Organization. This trend is of particular interest as the current study has shown that serum BDNF decreases with increasing BMI. These results support the hypothesis that mutations of the BDNF gene may be related to certain types of obesity or other forms of eating disorders (Rosas-Vargas et al., 2011).

Similarly, the prevalence of Type II diabetes mellitus is greater in Saudi Arabia than other comparable populations. For example, according to a recent World Health Organization report in 2016, the prevalence of diabetes in Saudi Arabia is around 14%. Because of this, diabetes was not an exclusion criterion in the recruitment of healthy volunteers. The current results demonstrate that serum BDNF decreases with increasing glycosylated haemoglobin, indicative of diabetes mellitus. These results support the work of Fujinami et al. (2008), who reported that serum BDNF concentrations were significantly lower in patients with advanced Type II diabetes mellitus compared to control subjects (Fujinami et al., 2008). In contrast, Boyuk et al. (2014) found that the serum BDNF concentrations in newly diagnosed Type II diabetes mellitus patients were significantly higher than that of the control subjects (Boyuk *et al.*, 2014b). Consequently, both longer diabetes duration and higher glucose levels are believed to be related with decreased BDNF levels in T2DM. The results also support

the theoretical explanation that BDNF may have a role in the treatment of diabetes. Different studies have reported the antidiabetic effects of BDNF. Regarding the mechanism of antidiabetic medications on BDNF and its association with diabetes mellitus, a recent review article concluded that BDNF may enhance the energy expenditure, ameliorate systemic glucose balance, and improve insulin sensitivity. Furthermore, it may be useful in the prevention and management of T2DM (Eyileten et al., 2017).

5. Chapter Five: Comparing serum BDNF concentrations of Alzheimer's disease patients and elderly participants in Saudi population

This chapter presents the data gathered for the AD patients and the elderly control group participants as well as the analyses and interpretations of the findings based on the results of the applied statistical treatments. The data are organized in chronological order based on the statement of the problem in Chapter 1.

5.1 Introduction

A further aim of this study was to examine the hypothesis about the changes of BDNF concentrations in Saudi AD patients at different stages as compared to the healthy elderly participants. Based on the literature review given in section 2.2.9, it appears that BDNF has been found to play an important role in AD pathogenesis. In addition, more evidence has accumulated as to the mechanism of BDNF in AD pathophysiology based on both animal and human models (Angelucci *et al.*, 2011; Perovic *et al.*, 2013). Laske and colleagues (2006) were the first to hypothesize that serum BDNF concentrations could be related to AD stages after finding that an early increase in serum BDNF and its subsequent reduction could depend on disease progression. Their initial up-regulation had been hypothesized to be a compensatory mechanism directed at counteracting β -amyloid accumulation, providing trophic support to offset the neuronal loss, and/or promoting tau dephosphorylation or, alternatively, a reflection of the increased choline acetyltransferase activity which characterizes the stage preceding neurodegeneration (Laske *et al.*, 2006; Laske *et al.*, 2007; Leyhe *et al.*, 2008). On the other hand, the belief in an association between BDNF and AD being found in different AD stages is shared by some researchers and rejected by others (Angelucci *et al.*, 2010; Perl, 2010). Many findings from previous studies reporting the levels of BDNF in patients with AD and individuals with mild cognitive impairment (MCI) have been conflicting. Therefore, in the experimental test discussed in this chapter, the focus was on the serum BDNF concentrations among AD patients at different stages regardless of the effects of cholinesterase inhibitors, mood stabilizers, or SSRI medications, as all classes of medications could contribute to increasing BDNF concentrations (Chen *et al.*, 1999; Leyhe *et al.*, 2008; Xuan Liu *et al.*, 2014). Also,

because depressive symptoms have been shown to have a negative effect on cognitive performance and have, therefore, been referred to as pseudo-dementia (Diniz *et al.*, 2014), in this part of the research, the effect of depression on BDNF concentrations was excluded, as all participants were free from depressive symptoms.

In Chapter 4, it was shown that BDNF concentrations decreased with advanced age in the Saudi elderly population. Therefore, in this part of the research, one of the objectives was to compare serum BDNF concentrations in the AD group with the elderly control group. Both the normal ageing process and AD are characterized by a progressive deterioration of memory functions. This strong relationship between ageing and AD is a key element to consider in discovering the point at which they overlap since neurotrophic factors such as BDNF play roles in the pathophysiological mechanism in both cases. Weinstein *et al.* (2013) found that higher peripheral BDNF levels protected older adults against AD. This was explained by considering that when BDNF concentrations were higher by one standard deviation, the risk for AD or dementia was lowered by 33% (Weinstein, 2013). Several researchers have investigated the association between cognitive impairment and serum BDNF concentrations in AD patients compared to elderly participants without AD (Peng *et al.*, 2005; Ginsberg *et al.*, 2006).

This part of the present study additionally aimed to compare cognitive function by using the validated Arabic versions of the Clinical Dementia Rating (CDR) scale, which is considered a more reliable tool for AD staging, for the AD patient group and the Mini Mental State Examination (MMSE) test for the assessment of cognition in the elderly control group. The main focus of this part of the study was to test whether decreased serum BDNF concentrations are associated with impaired cognition in both the elderly control group and in the AD patients. This chapter will also address assessment of other potential contributors to the discrepancies in findings, such as differences in the recruitment process, diagnostic criteria, stages of the disease, age, MMSE scores, or sex (Angelucci *et al.*, 2010), as well as the correlation between serum BDNF concentrations and other variables such as sex, body mass index (BMI), and glycated haemoglobin in AD patients to determine if other factors contributed to the differences in serum BDNF between AD patients and the elderly control group.

5.2 Methodology

5.2.1 Participants

A total of 27 AD patients and 48 elderly control group participants were enrolled in this phase of the study. All AD patients were registered for this study at the KFMC tertiary hospital in Riyadh city, while the control group participants were enrolled at King Salman Social Centre in Riyadh city, Saudi Arabia. The investigation was approved by the ethics committees from both the University of Brighton (UoB) and the KFMC, where the investigation was conducted. All subjects provided written informed consent and agreed to participate in the investigation following a short explanation of the investigation's goals by the researcher.

Inclusion criteria for the elderly control group were:

- a) Age 60 years or older
- b) Either gender
- c) Good medical health or normal clinical laboratory test results

Inclusion criteria for the AD patients were

- a) Age 60 years or older
- b) Either gender
- c) Clinically diagnosed with AD

Exclusion criteria for both the AD patient group and the elderly control group were:

- a) Patient/elderly diagnosed with comorbidity of primary psychiatric disorder (such as schizophrenia or major depression) with onset before the AD onset or neurological disorders (including stroke, seizure disorders, or head injuries with loss of consciousness within the past year).
- b) Patient/elderly who used antidepressant medications, anxiolytic agents, mood stabilizers/antiepileptic medications, or cholinesterase inhibitors.
- c) Patient /elderly with a known or suspected history of alcoholism or drug abuse.
- d) Patient/elderly complaining of haematological disorders and patients receiving anticoagulation medications.

5.2.2 Measurement of BDNF concentrations

Circulating serum concentrations of BDNF were analysed in duplicate via a commercially available enzyme-linked immunosorbent assay (ELISA) kit, exhibiting sensitivity <2 pg/ml and intra-assay variation <5% (Piockine Human BDNF ELISA Kit, Booster Biological Technology, Valley Ave, Pleasanton, USA, Cat. #: EK0307). See Chapter 3 for more details.

5.2.3 Clinical Dementia Rating (CDR) score

The CDR instrument (Morris, 1993) (Arabic Version) was used in the evaluation of the AD patients' cognitive performance. The CDR utilizes a five-point scale for characterization of six domains of cognitive and functional performance pertinent to AD and associated dementias. The six domains are titled Memory; Orientation; Judgment & Problem Solving; Community Affairs; Home & Hobbies; and Personal Care. Application of the CDR enabled the AD sample patients to be further subdivided into groups based on the severity of cognitive impairment. The required information to inform each score was acquired via a semi-structured patient interview in conjunction with a reliable informant or collateral source (see Appendix 10.7).

5.2.4 The Mini Mental State Examination (MMSE)

The MMSE technique can be employed to provide a detailed and systematic assessment of mental status. It takes the form of an 11-question test that evaluates five regions of cognitive function: orientation, registration, attention and calculation, recall, and language. The maximum score is 30; a score of 23 or below indicates cognitive impairment. The MMSE only takes 5–10 minutes to complete and, consequently, represents a simple practical tool suitable for routine and repeated application (see Appendix 10.6).

5.3 Data analysis

All statistical analyses were performed using the statistical analysis software package Minitab 18. The median and skew of the data are presented in the findings. As all serum BDNF concentrations for participants are non-normally distributed. A bivariate correlation analysis (Spearman Rho test) applied to assess correlation between factors such as ages, BMI, HbA1C, platelet count; use of medication and BDNF serum levels

for the healthy group was conducted. The significance level for the results was set at $p < 0.05$.

5.4 Results

5.4.1 General data

The statistical descriptions of BDNF serum concentrations and CDR scores in patients with mild AD, moderate to severe AD, and MCI are shown in Table 5.1.

The Alzheimer's patients were all over 60 years old (n = 27) and were categorized according to CDR scores into: MCI, n = 4 (14.8); mild dementia, n = 6 (22.2); moderate dementia, n = 13 (48.1); or severe dementia, n = 4 (14.8). Comparisons of the clinical physical characteristics of the AD patients (N = 27) and elderly control group participants (N = 48) are provided in Table 5.2.

	MCI (n = 4)	Mild Dementia (n = 6)	Moderate Dementia (n = 13)	Severe Dementia (n = 4)	MCI (n = 4)	Mild Dementia (n = 6)	Moderate Dementia (n = 13)	Severe Dementia (n = 4)
AD Patients BDNF level (pg/ml)					CDR Scores			
Mean	95.10	89.10	71.80	53.00	3.60	8.00	13.70	17.60
Median	105.40	89.20	77.30	49.60	3.60	7.90	14.50	17.80
Standard deviation	28.10	6.40	19.30	9.50	0.23	0.55	1.40	0.54
Skewness	-1.60	0.40	-0.96	1.70	1.10	0.90	-1.20	-1.80
Minimum	54.50	79.90	27.10	45.90	3.50	7.50	10.50	16.80
Maximum	115.00	99.80	101.40	67.20	4.00	9.00	15.20	18.00

Table 5. 1The statistical characteristics of Alzheimer's disease patients, showing BDNF serum concentrations and CDR scores in patients with mild AD, moderate AD, severe AD, or MCI.

	Alzheimer's patients (N=27)	Elderly control (N=48)	p-value
Age (years)	78.9 ± 5.280	69.8 ± 6.45	0.450
Gender M, F (%)	M = 14 (51.8) F = 13 (48.2)	M = 12 (25) F = 36 (75)	0.960 0.001
BMI (kg/m²)	25.1 ± 3.472	29.7 ± 5.30	0.530
HbA1c (%)	6.7 ± 1.177	7.9 ± 10.09	0.750
White blood cell count (x 10⁹/L)	6.4 ± 2.030	8.0 ± 2.45	0.670
Red blood cell count (10⁶/μL)	4.6 ± 0.090	4.3 ± 0.46	0.920
Haemoglobin level (gm/dL)	14.0 ± 0.270	13.1 ± 1.40	0.860
Platelet count (10⁹/L)	274.0 ± 10.300	270.8 ± 61.51	0.890
Use of medication (%)	55.6%	50%	0.620
Cognition test	CDR Score 9.3 ± 4.360	MMSE Score 28.0 ± 1.25	–
Serum BDNF (pg/ml)	76.3 ± 21.640	80.3 ± 27.84	0.750

Table 5. 2: Comparison of the clinical features of AD patients and elderly control group participants (mean ± SD).

5.4.2 Comparison of BDNF serum concentrations among AD patients with elderly control group participants

The median values of BDNF concentrations were compared between the AD patients (n = 27) and the elderly control group participants (n = 48) using the Mann-Whitney test. With a p-value of 0.75, the results showed no significant differences between the groups (Figure 5.1).

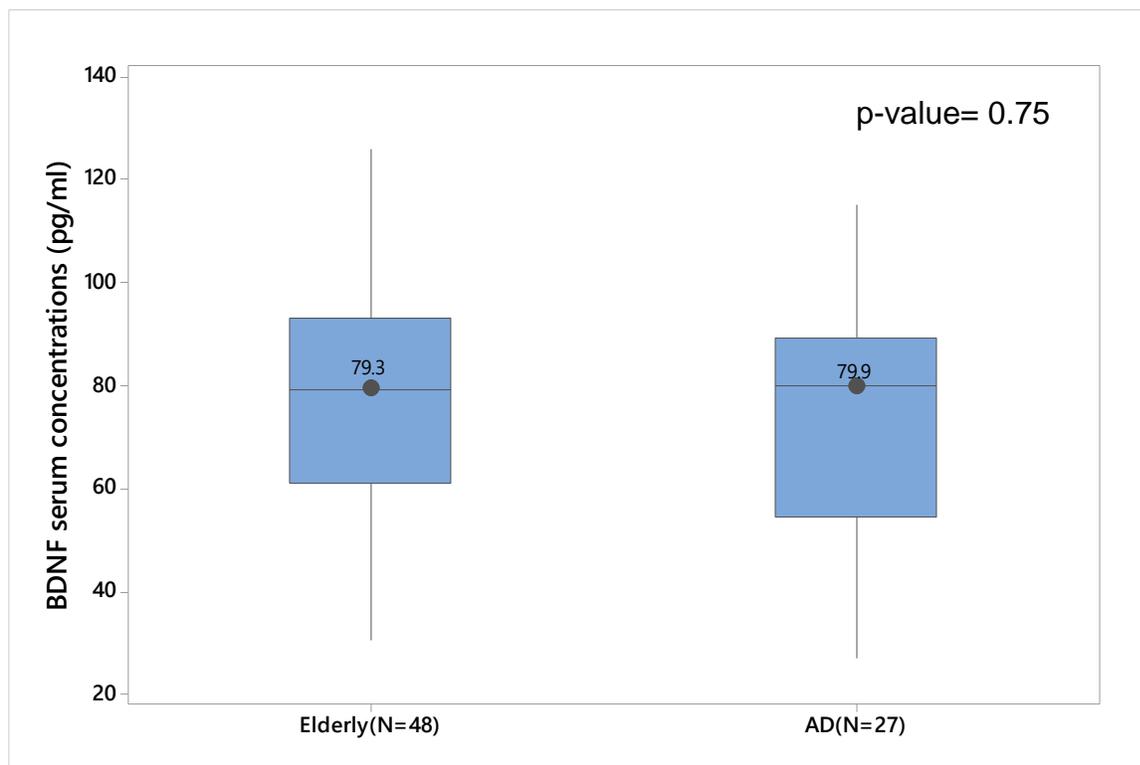


Figure5. 1: Differences in serum BDNF concentrations between the elderly control group (N = 48) and the AD patients group (N = 27).

5.4.3 BDNF serum concentrations among AD patients

The BDNF serum concentrations in patients with mild AD (N = 6), patients with moderate AD (N = 13), patients with severe AD (N = 4), patients with MCI (N = 4), and participants in the elderly control group (N = 48) are shown in Figure 5.2.

A comparison of the serum BDNF concentrations in patients with mild AD, patients with moderate AD, patients with severe AD, patients with MCI, and the elderly control group participants using the non-parametric analysis of variance Kruskal-Wallis test revealed significant differences in the severity of the disease and serum BDNF concentrations in AD patients compared to the elderly control group ($H = 10.63$, $DF = 4$, $p\text{-value} = 0.031$).

Using the Mann-Whitney test revealed that there were no significant differences in BDNF concentrations between MCI patients (N = 4), and moderate dementia patients (N = 13), with a p-value of 0.10.

Overall, these results indicate that when the disease progresses to the severe stage, the concentration of serum BDNF gradually decreased. However, the median of serum BDNF concentration for the elderly control group (79.3 pg/ml) was lower than the median for the MCI patients (105.4 pg/ml). Interestingly, this was a significant difference, with a p-value <0.01 .

On the other hand, no significant difference was observed between the moderate AD group and the healthy elderly control group (Figure 5.2).

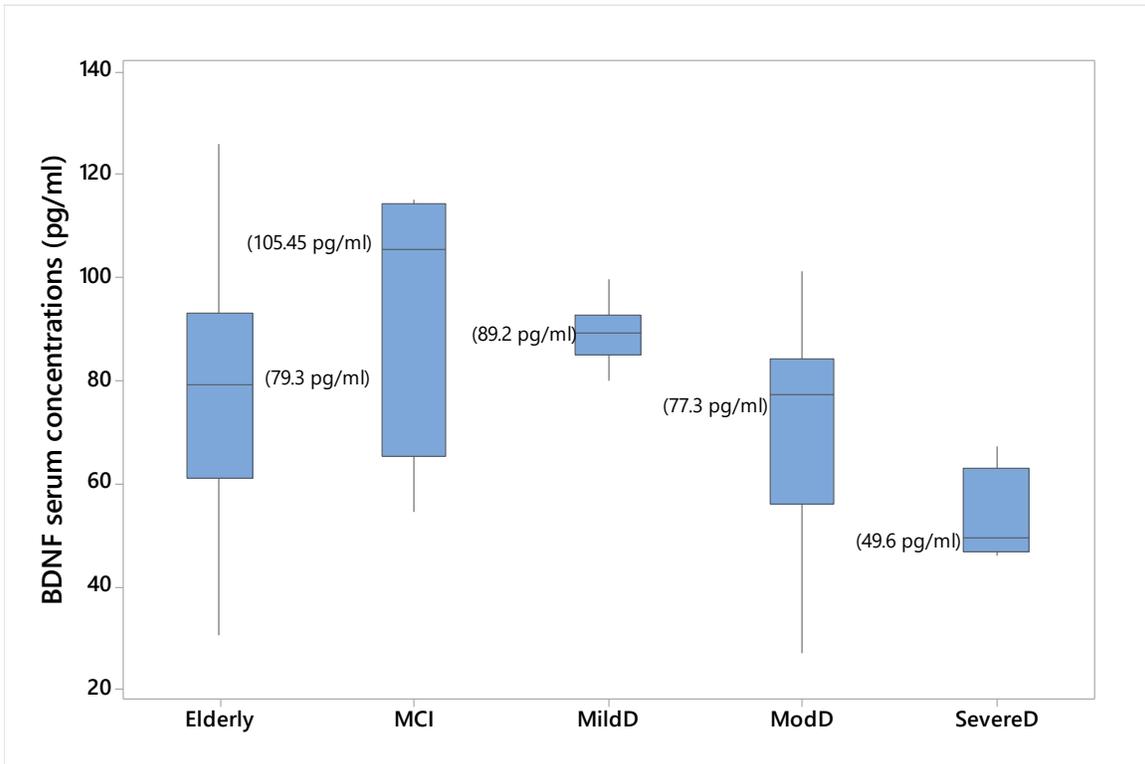


Figure5. 2: The median of serum BDNF concentrations among patients at various AD stages (N = 27) compared to the elderly control group participants (N = 48) p-value <0.01.

5.4.4 BDNF serum concentrations and CDR scores for AD patients

The sum of the CDR scores characterizes the stages of AD patients, and the correlation coefficients between the serum BDNF and the CDR score for patients at the different stages of AD were statistically calculated using the Spearman Rho test. The results revealed a negative correlation between BDNF serum concentrations and CDR scores ($r = -0.6$, $p\text{-value} < 0.001$) (Figure 5.3)

Moreover, the results of the correlation revealed that the serum BDNF concentrations decreased in proportion to increases in the CDR scale score of AD patients. Further, as can be seen in Figure 5.3, the correlation of serum BDNF concentrations with CDR scores when comparing serum BDNF levels of MCI patients (low scores) and severe dementia patients (high scores) revealed that the serum levels of MCI patients were higher than the levels of the severe AD patients. In addition, our results showed that there was a significant increase in the serum BDNF levels in moderate stage patients as compared to the levels of severe stage patients.

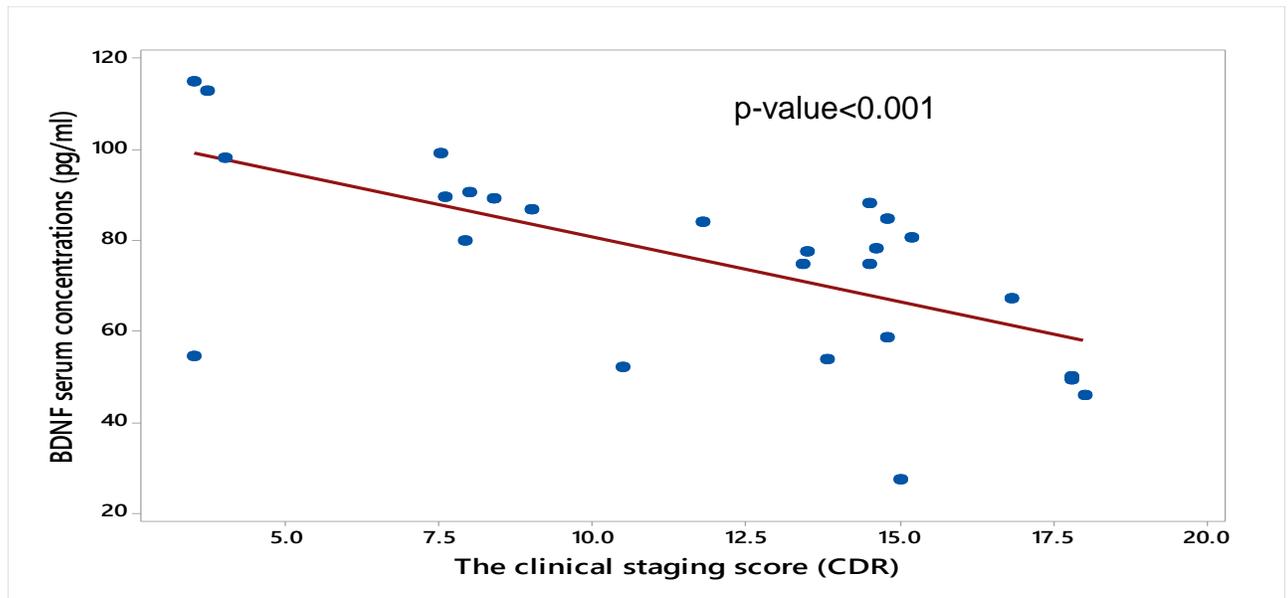


Figure 5. 3: The serum BDNF concentrations of AD patients (N = 27) in relation to CDR scores.

5.4.5 BDNF serum concentrations and MMSE scores for the elderly control group

The serum BDNF concentrations in the elderly control group were examined in relation to that group's scores on the MMSE using the Spearman Rho test. The results revealed no correlation between the BDNF serum concentrations and the MMSE ($r = 0.26$, p -value = 0.06) (Figure 5.4).

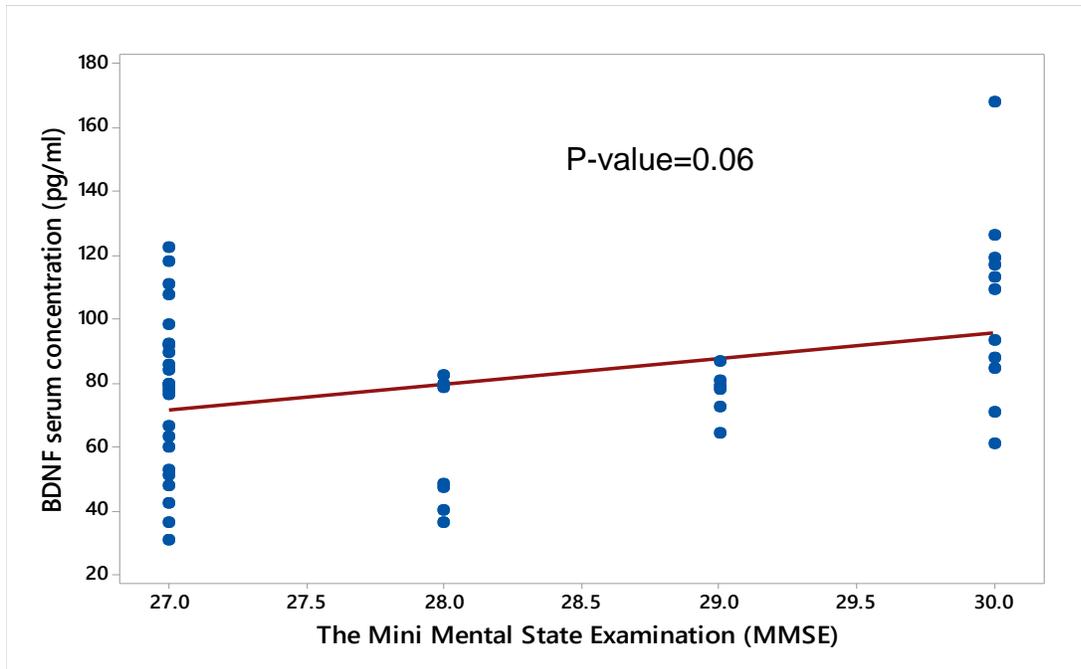


Figure 5. 4: Serum BDNF concentrations among the elderly control group (N = 48) in relation to the scores on the MMSE.

5.4.6 BDNF concentrations of AD patients and the effects of other variables

5.4.6.1 BDNF concentrations and sex of patients

There were no statistically significant differences when comparing the serum BDNF concentrations of male AD patients (n = 14) and female AD patients (n = 13) using Mann-Whitney test (W = 196.0, p-value = 1.00) (Figure 5.5).

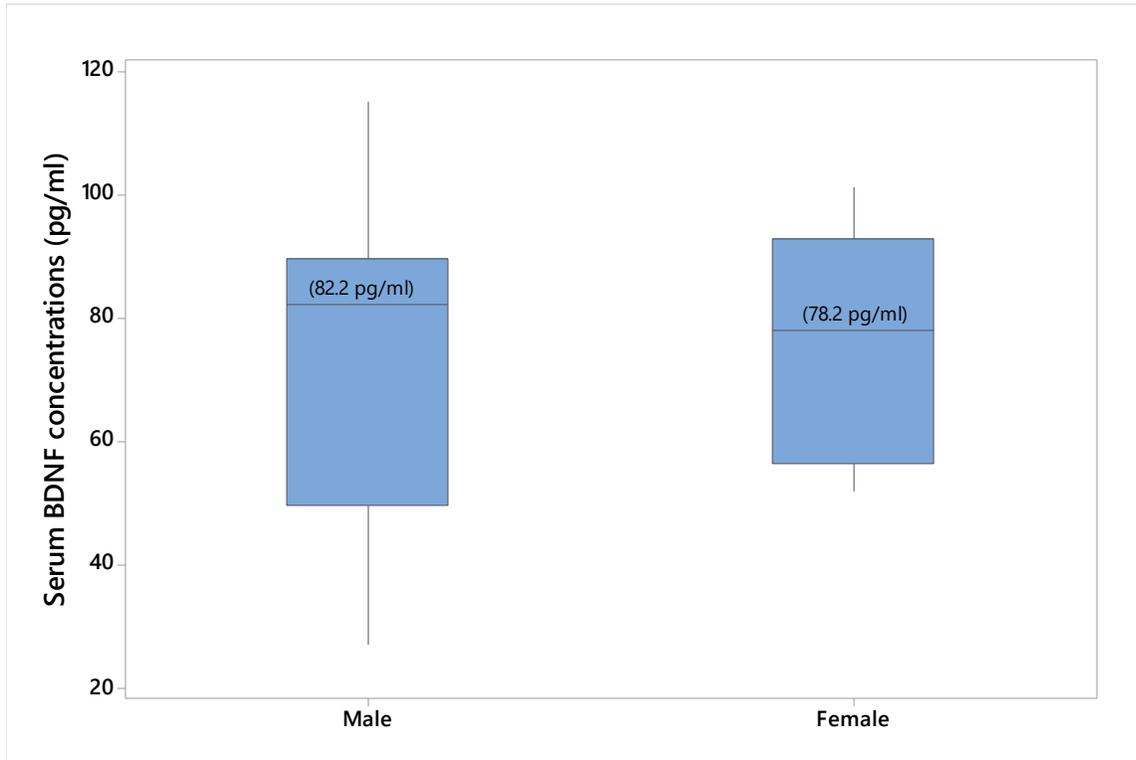


Figure 5. 5: Serum BDNF concentrations in male and female AD patients (N=28).

5.4.6.2 BDNF concentrations and body mass index (BMI)

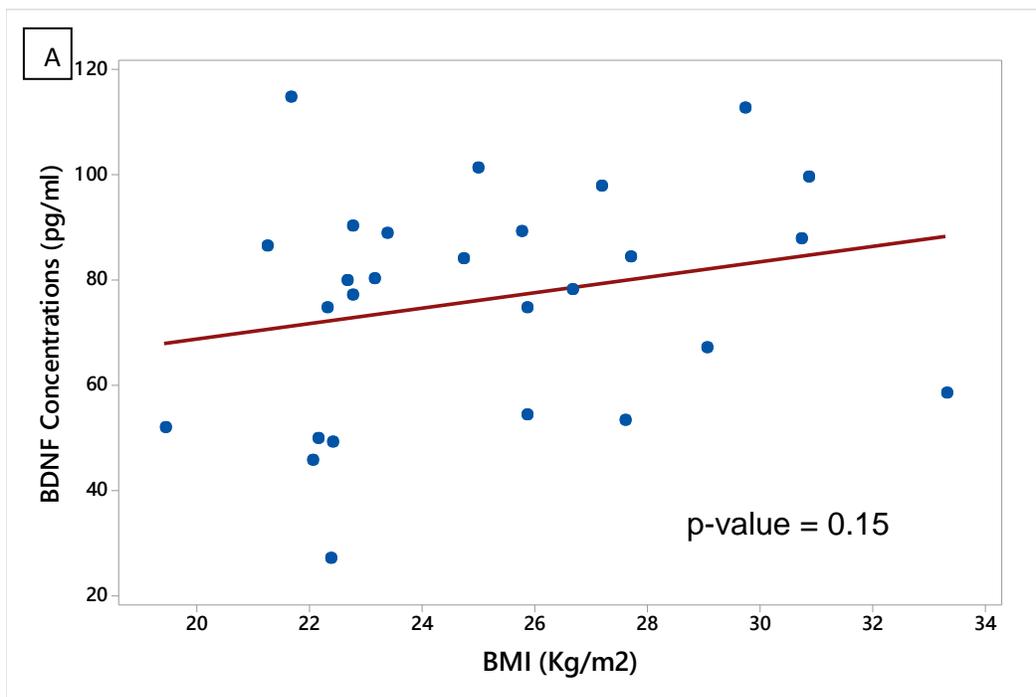
The results of the Spearman's Rho correlation coefficient test revealed no statistically significant correlation between a patient's BMI and serum BDNF concentrations ($r = 0.28$, $p\text{-value} = 0.15$) (Figure 5.6 A).

5.4.6.3 BDNF concentrations and glycated haemoglobin (HbA1c)

The Spearman's Rho correlation coefficient test showed no correlation between serum BDNF levels of AD patients and glycated haemoglobin (HbA1c) ($r = 0.059$, $p\text{-value} = 0.77$) (Figure 5.6 B).

5.4.6.4 BDNF concentrations and blood cell counts

The Spearman's Rho correlation coefficient test of serum BDNF concentrations of AD patients and platelet counts revealed no statistically significant correlation ($r = 0.262$, $p\text{-value} = 0.187$) (Figure 5.6 C).



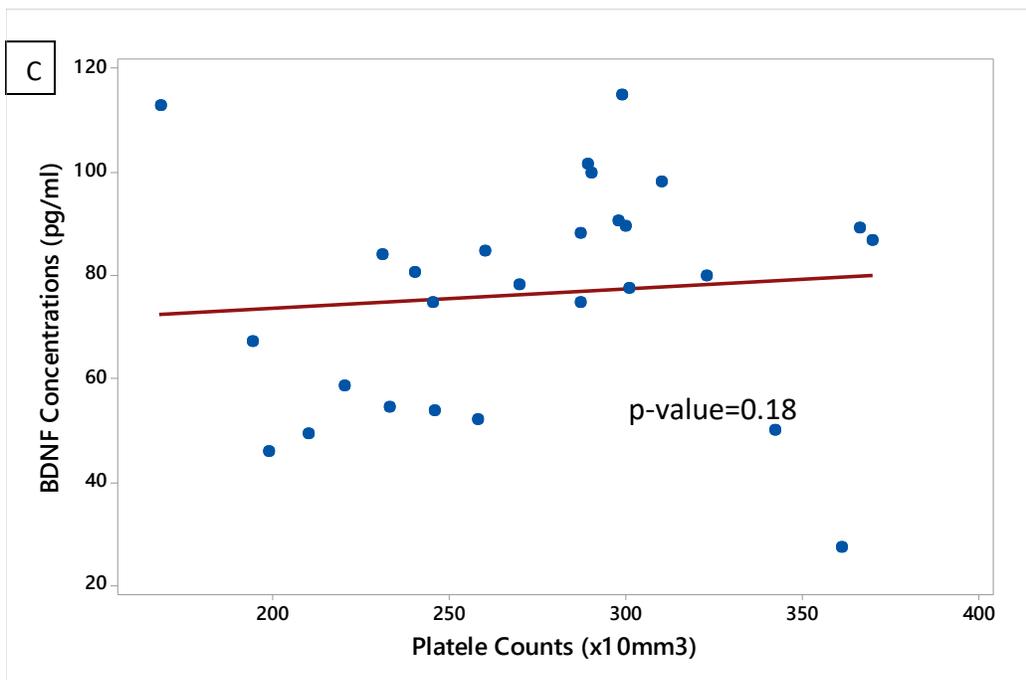
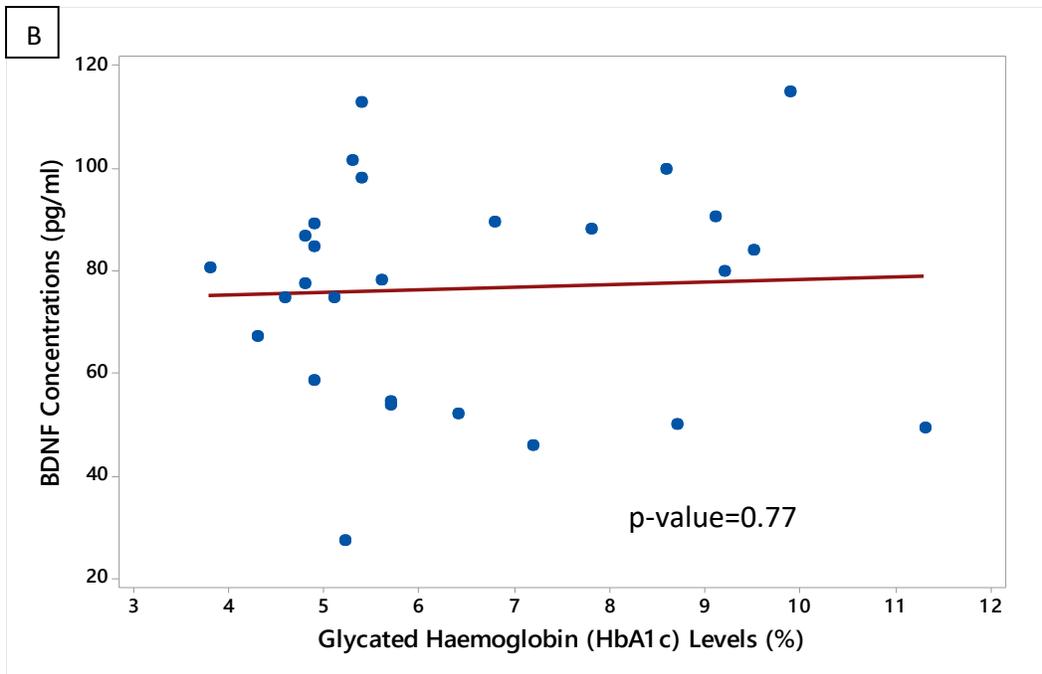


Figure 5. 6: Scatterplots showing the relationships of serum BDNF concentrations with (A) BMI, (B) glycated haemoglobin, and (C) platelet counts in AD patients (N = 27).

5.4.6.5 BDNF levels and use of medications

Around 16 of the 27 AD patients (59.2%) had been receiving different classes of medications (aspirin, vitamin supplements), but there were no statistically significant differences in the median serum BDNF concentrations (86.6 pg/ml) when compared with the median of the patients who did not take any medications (76.0 pg/ml) ($W = 196.5$, $p\text{-value} = 0.34$) (Figure.5.7).

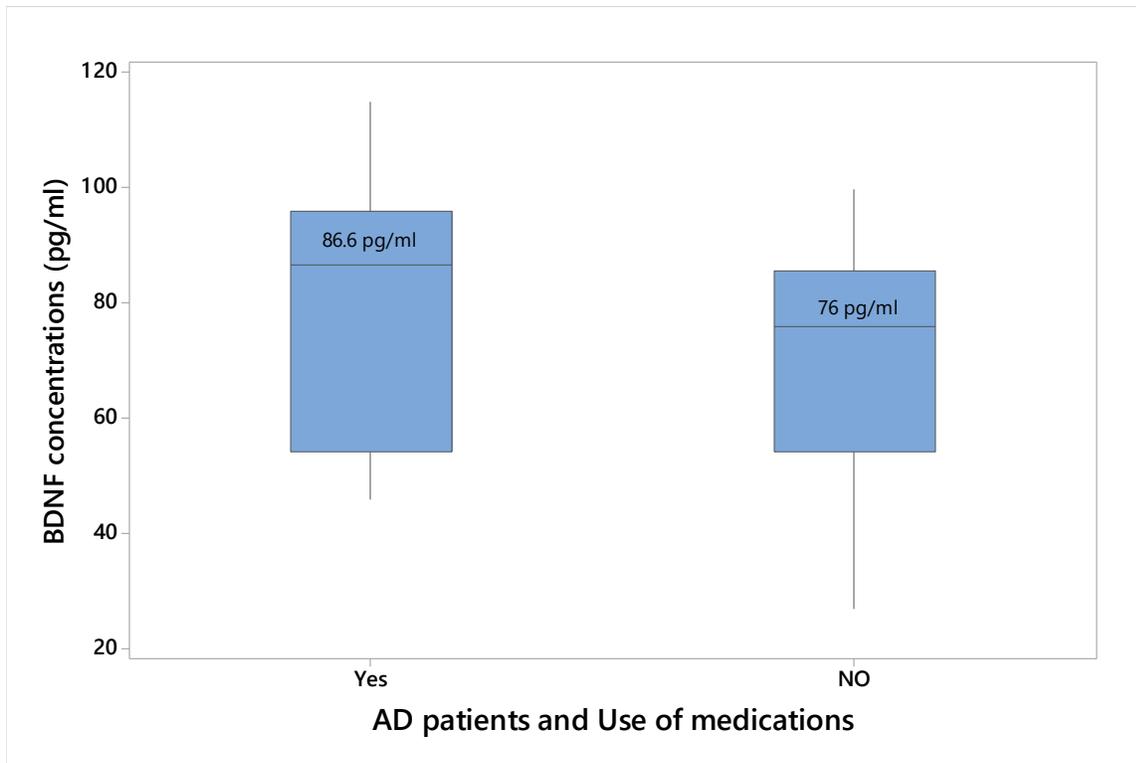


Figure5. 7: Graph of serum BDNF concentrations in AD patients (N = 27) with the use of medications.

5.5 Discussion

A comparison of serum BDNF concentrations in AD patients and the elderly control group participants showed low concentrations in both groups with no significant difference between them. The additive value for this result is that no patients in either group were receiving any medications that contribute to enhancing or decreasing BDNF concentrations, such as acetylcholinesterase inhibitors, mood modulators, or antidepressants. The mechanism behind lower serum BDNF concentrations in the elderly control group was discussed in Chapter 4. With regard to AD patients, the degeneration of cholinergic neurons of the forebrain in AD has been described by Murer et al (2001). as resulting in reduced acetylcholine concentrations consequently leading to cognitive deterioration and preclinical implications of BDNF in AD (Murer, et al., 2001). Such neurodegeneration is correlated with insufficiency of trophic support in the cholinergic neurons, as an exogenous application of trophic factors (or nerve growth factor) eliminates these deficiencies. BDNF is a neurotrophin which, through interaction with its high-affinity receptor TrkB B, is critical for the continued survival and maintenance of a variety of neurons. Furthermore, BDNF is responsible for facilitation of synaptic plasticity and various cognitive processes. A number of recent studies have proposed that AD results from a failure of neuroplasticity, leading to a loss of synaptic contacts which can consequently lead to the observed neuropathological and clinical presentations. As far as AD is concerned, BDNF has demonstrated abilities to facilitate survival and differentiation of basal forebrain cholinergic neurons. It is noteworthy that in these neurons, BDNF stimulates secretion of the neurotransmitter acetylcholine, which is acknowledged to be deficient in AD patients. These preclinical observations imply that deficiencies of BDNF synthesis might be partly responsible for the decline of cellular homeostasis resulting in AD. Recently, a systematic review research compared serum BDNF concentrations of all three groups of subjects, AD, MCI, and healthy control that concluded the BDNF concentrations in the serum of AD patients were significantly lower than healthy controls (15 studies, n = 2067) (Kheng *et al.*, 2019). Also, this review supports the findings of the current thesis.

Regarding the variation in BDNF serum concentrations in Saudi patients with AD and those with MCI, in particular, the findings revealed that among the AD patients, serum BDNF concentrations were significantly decreased only in patients with severe AD as compared to other stages of AD. Therefore, the results of the current study support the hypothesis that a reduction of BDNF concentration is associated with deterioration in cognitive function with AD. Similarly, Yasutake *et al.* found that serum BDNF concentrations were significantly lower in severe AD patients than in either healthy participants or patients with vascular dementia. Further, the findings in the current research showed that serum BDNF concentrations in MCI patients were higher than serum BDNF concentrations of severe dementia patients. In patients with mild to moderate AD, there were no significant differences in serum BDNF concentrations when compared to the other stages of AD or the elderly control group participants. These results match with the hypothesis that this change is an up-regulation of BDNF concentrations in both the preclinical phase of dementia (MCI) and early clinical stages of AD (Angelucci *et al.*, 2010), regardless of the use of acetylcholinesterase inhibitor agents. For example, the acetylcholinesterase inhibitor agent donepezil showed neuroprotective effects by increasing serum BDNF concentrations (Leyhe *et al.*, 2008). Conversely, in recent systematic review, there were no significant differences between either the AD group or the MCI group when compared to the control group but, they assumed that changes in serum BDNF concentrations cannot be detected significantly in individuals with MCI (Kheng *et al.*, 2019). These differences in findings probably represent the use of the CDR scale to assess the cognitive skills of AD patients (Williams *et al.*, 2013).

In this study, the correlation between cognitive impairment and changes in serum BDNF concentrations was evaluated and compared between AD patients and the elderly control group. The findings showed normal cognitive function with low serum BDNF concentrations in the elderly control group participants, while the AD patients showed low serum BDNF concentrations with deterioration of cognitive function, especially in the severe AD stage. According to recent systematic review studies, neurotrophic factors such as BDNF are associated with the pathophysiology of AD. In addition, it has been reported that the deposition of β -amyloid protein is involved in the synthesis of BDNF and signal transduction, resulting in a blockage of synaptic function and accelerated neuronal degeneration (Y. Y. Lim *et al.*, 2015). Such results support the hypothesis that a reduction of BDNF concentration is involved in the aetiology of AD.

A possible explanation for this variation is that serum BDNF concentrations are low in the elderly control participants with normal cognitive function due to the potentiation of LTD rather than LTP in the memory formation process, or it may be due to a genetic impact of BDNF polymorphism. For LTD the pro BDNF played an important role to potentiate LTD rather than LTP. Furthermore explanation, that proBDNF acting through its preferred receptor that often contrasting effects to mBDNF. Thus, conversion of proBDNF to mBDNF through proteolytic cleavage has emerged as an important regulatory mechanism. Indeed, pharmacological and genetic studies have revealed that tPA/plasmin-mediated, extracellular conversion of proBDNF to mBDNF is necessary and sufficient for late-phase LTP. Moreover, proBDNF-p75 signalling has been shown to facilitate LTD in young hippocampal slices *in vitro* and perhaps during stress in adults *in vivo*. Activity-dependent proBDNF→mBDNF conversion appears to play an important role in synaptic competition/elimination during development. These findings form the foundation of the “yin-yang” hypothesis. A second major breakthrough for variation in cognition function in elderly group was the identification of human Val/Met polymorphism, which impacts selectively on activity-dependent, but not constitutive, BDNF secretion. This provides an unprecedented opportunity to study the function of BDNF in cognitive function and dysfunction in humans. Therefore, the final stage of the present study was to explore the distribution of Val/Met polymorphism in the Saudi population in order to ascertain

whether changes in serum BDNF and cognitive impairment may be dependent on genotype and to explore the possibility that particular genotypes may be protected against the development of AD.

There have been conflicting data on peripheral BDNF concentrations in the different stages of AD. Therefore, there was a need to compare AD patients' serum BDNF concentrations with other variables in order to fix the confounder variable. The finding that increased BMI and glycated haemoglobin were not associated with decreased BDNF concentrations is important and novel. Such a finding suggests that decreased BDNF concentrations associated with age and neurodegeneration cannot be further decreased by other factors. Thus better control of BMI and blood glucose in elderly patients is unlikely to have any particularly beneficial effects on BDNF levels and, therefore, possibly no effect on disease progression.

This study provided pilot data which concluded that there was, indeed, decreased BDNF in the serum of patients with severe AD. However, the study observed no significant difference in the serum BDNF levels of patients with AD and healthy controls. Differences in the CDR scores of patients with AD and the MMSE scores in the elderly control group contributed to the heterogeneity in the findings of the studies that were included in the literature review for this study. One of the implications of this study is that decreased peripheral BDNF concentrations in AD could serve as a baseline for future trials to examine the effectiveness of treatment options such as SSRIs in improving peripheral BDNF levels and observation of any concomitant improvements in cognitive domains. Chapter 6 will focus on this approach.

6. Chapter Six: The effect of selective serotonin reuptake inhibitors on the serum concentration of brain-derived neurotrophic factor in Alzheimer's disease patients

This chapter presents the gathered data for the effect of SSRIs on serum BDNF and cognition in elderly participants and AD patients, providing an analysis and interpretation of the findings based on the results from the applied statistical treatment. The data are organised in chronological order based on the problem statement in Chapter 1.

6.1 Introduction

The previous results reported in Chapter 5 showed the correlation between serum BDNF and cognition in AD patients. The severe AD stage exhibited low serum BDNF with poor cognition functions, and the literature review in Chapter 2 (Section 2.2.6) revealed extensive published research clearly indicating that BDNF serum concentrations in AD patients exhibit a protective measure against the development of AD (Weinstein *et al.*, 2014). Adjustment of the BDNF concentrations can help to slow the prognosis of AD. For example, in one meta-analysis, the researchers studied the effect of physical activity on serum BDNF concentrations and found that aerobic training interventions increased resting BDNF concentrations in peripheral blood that enhances cognition and relieves psychiatric symptoms (Dinoff *et al.*, 2016a).

Administration of anti-depressants may reduce the risks associated with AD, and recent research on this issue has revealed potentially modifiable psychosocial risk factors for depression in AD (Burke *et al.*, 2017). Numerous studies have also connected serum BDNF and anti-depressant treatment, reporting low hippocampal and serum BDNF concentrations in unmedicated patients diagnosed with depression; they have also shown that such serum BDNF concentrations can be enhanced through the administration of anti-depressant treatment (Nibuya *et al.*, 1995; Shimizu *et al.*, 2003; Ventriglia *et al.*, 2002). In addition, previous animal simulations of depression have provided further evidence for the neurotrophin hypothesis by indicating that intra-cerebro-ventricular and intra-hippocampal injections of BDNF elicit similar behavioural

effects to those brought about by conventional anti-depressants (Hoshaw *et al.*, 2005). Especially, when emphasizing on the mechanism of SSRIs that inhibit the reuptake of the postsynaptic receptor 5-hydroxytryptamine (5-HT) in the pre-synaptic context. This leads to the enhanced availability of serotonin in the synaptic cleft to bind to 5-HT, a neurotransmitter that influences multiple processes, including autonomic function, motor activity, hormone secretion, cognition and complex processes associated with affection, emotion and reward (Wells *et al.*, 2009).

The mechanism between BDNF and 5-HT can be explained in that both are known to regulate synaptic plasticity, neurogenesis and neuronal survival in the adult brain. These two signals co-regulate one another such that 5-HT stimulates the expression of BDNF, while BDNF enhances the growth and survival of 5-HT neurons (Mattson *et al.*, 2004). In addition, BDNF signalling at the synapses improves long-term potentiation (LTP), a process of synaptic strengthening associated with learning and memory; the effect of BDNF on LTP is apparently mediated by cAMP-response-element-binding (CREB) protein, which regulates the expression of genes involved in LTP and memory formation (Bathina & Das, 2015). A role for 5-HT in neurogenesis has been suggested by studies showing that SSRI anti-depressants can stimulate adult hippocampal neurogenesis (Puranen *et al.*, 2017). 5-HT can also promote the survival of neurons in the adult brain, as demonstrated by the abilities of a 5-HT receptor agonist and SSRI to protect neurons against excitotoxic and ischemic injury in animal models (Semkova, 1998). Illustrating this point, 5-HT and BDNF often function in a cooperative manner for regulating neuronal plasticity and survival. Activation of 5-HT receptors coupled to cAMP production and CREB activation can induce transcription of the BDNF gene. Conversely, BDNF can stimulate the growth and sprouting of 5-HT neuron axons innervating the cerebral cortex, thereby apparently increasing the number of 5-HT synapses in this brain region (Park *et al.*, 2014).

5-HT and BDNF often activate sets of genes that serve complementary functions in neuronal plasticity and survival. For example, SSRIs induce expression of the activity-regulated cytoskeletal-associated protein, an effector immediate–early gene that has been implicated in LTP and other forms of neuroplasticity, suggesting a potential mechanism whereby 5-HT facilitates learning and memory. Furthermore, a human study by Wolkowitz *et al.* verified the presence of low serum BDNF concentrations in untreated depressed patients and found that anti-depressant administration leads to elevated BDNF levels; however, the researchers proposed that the mechanism of action is likely to be more intricate than the simple anti-depressant correction of BDNF deficiency (Wolkowitz *et al.*, 2011a). A systematic review in 2013 of 28 reports evaluated the BDNF concentrations in major depressive patients; the results showed that BDNF concentrations varied greatly when initial trials were compared, and the BDNF levels increased following treatment for major depressive disorder in 50% of the trials. Only a single clinical trial reported reduced concentrations of BDNF values following administration of anti-depressants, while 12 reports testified to a lack of statistically significant variations of BDNF values with different classes of anti-depressants (Teche *et al.*, 2013). However, the action of BDNF in the pathophysiology of depression may not be related to its part in influencing the mode of action of anti-depressants (Groves, 2007). For instance, eradication of hippocampal neurogenesis has no effect on mouse sensitivity to unanticipated mild chronic stress, implying that diminished neurogenesis cannot be the origin of stress-related behavioural deficiencies (Song *et al.*, 2015). Yet, eradication of hippocampal neurogenesis does reduce the anti-depressant-resembling impacts of imipramine and fluoxetine, implying some BDNF action in anti-depressant mechanisms (Xuan Liu *et al.*, 2014).

The part played by anti-depressants in the maximisation of neural plasticity using innovative rehabilitation methods may assist in enhancing the efficiency of this widely available pharmaceutical therapy and extend the utilisation of contemporary anti-depressants towards novel applications. Furthermore, Groves (2007) authored a systematic review article highlighting investigations that promoted the BDNF hypothesis for treatment of depression and argued strongly for the BDNF hypothesis in animal and clinical research. However, the review ultimately indicated that the evidence for BDNF participation in the pathophysiology of depression remains debatable (Groves, 2007).

Only limited data are currently available that study the effects of medications on serum BDNF concentrations in AD patients. Leyhe et al, (2008) studied the correlation between donepezil and serum BDNF concentrations and revealed that the AD patients with AchE inhibitors exhibited an increase of BDNF serum concentration in AD patients reaching the level of healthy controls, while low serum BDNF concentrations have been identified in AD (Leyhe *et al.*, 2008). However, no studies are currently available relating to BDNF levels in AD patients taking SSRIs. Consequently, a comparative investigation of serum BDNF concentrations would appear to be pertinent. The current absence of appropriate evidence relating to the application of cognitive treatment in AD demands a quest for new techniques based on neuroprotective approaches. Recently, an expert review has proposed that available drugs may stimulate neurotrophin signalling and neuronal plasticity (Carrière *et al.*, 2017). In addition, a recent animal study reported on the effects of the co-administration of the anti-dementia drug memantine, a glutamatergic modulator, with the anti-depressant sertraline, an SSRI, in which both were considered to raise the levels of BDNF in AD (Amidfar *et al.*, 2017).

Concerning the effect of *anti-depressant* treatment on cognitive performance, a recent cohort investigation evaluated the influence of anti-depressant administration to 7381 subjects aged at least 65 years over a 10-year period on the five following cognitive domains: verbal fluency, psychomotor speed, executive function, visuospatial skills and global cognition. The findings demonstrated that patients taking tricyclic anti-depressants exhibited lower baseline performances in verbal fluency, visual memory and psychomotor speed, whereas those taking SSRIs exhibited lower verbal fluency

and psychomotor speed. For the two remaining cognitive abilities (executive function and global cognition), no significant variations were found at baseline, regardless of the class of anti-depressants administered (Carrière *et al.*, 2017). However, recent study showed that MMSE increased with citalopram treatment of depression older people as a part of citalopram on mood improvement (Scutt *et al.*, 2018).

A new report recently evaluated BDNF levels and cognitive deterioration in AD patients, demonstrating that AD patients exhibited significantly lower BDNF serum levels relative to MCI patients. In addition, this investigation observed no influence of depression on BDNF serum concentrations, either within a whole group or within each group subject to a separate assessment (Siuda *et al.*, 2017)

It is noteworthy that a 2017 meta-analysis demonstrated conflicting evidence relating to the effects of anti-depressants on cognition described in a number of previous randomised controlled trials (RCTs). Here, AD patients were utilising various anti-depressants (sertraline mirtazapine; imipramine, fluoxetine and clomipramine) versus placebo administration (Orgeta *et al.*, 2017). Some reports described a beneficial influence on cognition performance with the use of anti-depressants in AD patients (Mossello *et al.*, 2008). However, some detrimental influences on cognition following the use of anti-depressants in AD patients were also observed. For instance, Porsteinsson *et al.* (2014) demonstrated that administration of citalopram in AD patients led to poorer cognition performance. In addition, a 2016 review article described 55 investigations concentrating on the effect of exercise on BDNF levels in healthy adults; the findings showed that BDNF levels increased following a single exercise session, irrespective of variations observed in cognitive performances (Dinoff *et al.*, 2016a).

The influence of BDNF on cognitive performance was evaluated in a systematic review article that included seven reports (Piepmeier & Etnier, 2015). This review demonstrated significant evidence for a dynamic action of BDNF in terms of cognitive performance. Furthermore, the outcomes from three of the investigations highlighted that elevated levels of BDNF correlate positively with memory improvements. The present investigation examines the association between BDNF and SSRI effects, effect of SSRIs on BDNF concentrations in AD and elderly patients and correlation between BDNF concentrations and CDR scores of AD. The most crucial element of this research is deemed to be determination of the influence of SSRI use on cognitive performance in elderly and AD patients. The 'aims' of this chapter is evaluating the influence of SSRIs on BDNF concentrations on delayed AD progression, as well as studying the effect of SSRI use on the improvement of cognition among both elderly and AD patients.

6.2 Methodology

6.2.1 Participants

A total of 13 patients diagnosed with AD and 17 elderly patients on SSRI treatment were compared with a total of 27 AD patients and 48 elderly patients who were not using SSRI treatment; their recruitment was described in Chapter 5. All the participants were enrolled at the KFMC tertiary hospital in Riyadh, which constitutes part of the Ministry of Health in Saudi Arabia, for this investigation. The investigation was approved by the ethics committees from both the University of Brighton (UoB) and KFMC, where the investigation was conducted.

The inclusion criteria for the elderly control group were as follows:

- a) Age 60 years or older;
- b) Either gender;
- c) Normal clinical laboratory test results; and
- d) Using SSRIs but not actively depressed.

The inclusion criteria for the AD patients were as follows:

- a) Age 60 years or older;
- b) Either gender;
- c) Clinically diagnosed with AD; and
- d) Using SSRIs but not actively depressed.

The exclusion criteria for both the AD patient group and elderly control group were as follows:

- a) Patient/elderly person diagnosed with a co-morbidity involving a primary psychiatric disorder (such as schizophrenia or major depression) with onset before the AD onset or a neurological disorder (including stroke, seizure disorders, or head injuries with loss of consciousness within the past year);
- b) Patient/elderly person who used anxiolytic agents, mood stabilisers/antiepileptic medications or cholinesterase inhibitors;

- c) Patient/elderly person with a known or suspected history of alcoholism or drug abuse; and
- d) Patient/elderly person complaining of haematological disorders and patients receiving anticoagulation medications.

6.2.2 Measurement of BDNF concentrations

Circulating serum concentrations of BDNF were analysed in duplicate via a commercially available enzyme-linked immunosorbent assay (ELISA) kit, exhibiting sensitivity < 2 pg/ml and intra-assay variation < 5% (Piockine Human BDNF ELISA Kit, Booster Biological Technology, Valley Ave., Pleasanton, USA, Cat. #EK0307; see Chapter 3 for more details).

6.2.3 Clinical Dementia Rate (CDR) score

The CDR instrument (Morris, 1993; Arabic version) was used in the evaluation of the AD patients' cognitive performance. The CDR utilises a 5-point scale for characterisation of six domains of cognitive and functional performance pertinent to AD and associated dementias. The six domains are as follows: Memory, Orientation, Judgment & Problem Solving, Community Affairs, Home & Hobbies and Personal Care. Application of the CDR enabled the AD sample patients to be further subdivided into groups based on the severity of the cognitive impairment. The required information for each score was acquired via a semi-structured patient interview in conjunction with a reliable informant or collateral source (see Appendix 10.7).

6.2.4 The Mini-Mental State Examination (MMSE)

The MMSE technique can be employed for providing a detailed and systematic assessment of mental status. It takes the form of an 11-question test that evaluates five regions of cognitive function: orientation, registration, attention and calculation, recall and language. The maximum score is 30; a score of 23 or below indicates cognitive impairment. The MMSE only takes 5–10 minutes to complete, and consequently,

represents a simple, practical tool that is suitable for routine and repeated application (see Appendix 10.6).

6.2.5 Mapping of MMSE scores on to CDR scores

Pernecky *et al.* (2006) described how MMSE scores can be matched to CDR categories: MMSE scores of 30, 26–29, 21–25, 11–20 and 0–10 map to CDR categories of no, questionable, mild, moderate and severe dementia, respectively (Pernecky *et al.*, 2006).

6.2.6 Hamilton depression rating scale (HDRS)

The HDRS is a multiple-item questionnaire used for providing an indication of the severity of depressive symptoms in adults by probing mood, feelings of guilt, suicide ideation, insomnia, agitation or retardation, anxiety, weight loss and somatic symptoms. It is composed of 17 items scored individually on a range of 0–4 based on clinical observation. A total score of 0–7 is considered to be normal, 8–13 represents mild depression, 14–18 signifies moderate depression, 19–22 represents severe depression and more than 23 signifies very severe depression. In this study, all the participants were accurately screened by a consultant psychiatrist for the onset of depression using HRDS scale with a cut-off point of 7 (see Appendix 10.4).

6.3 Data analysis

Variations in serum BDNF concentrations between groups and variations within subjects were evaluated in independent samples via Mann–Whitney U tests for non-normal distributions. Associations between BDNF concentrations and clinical ratings were further evaluated by means of Spearman correlation coefficients. Fisher's exact test was employed for evaluating the significance of the correlation (contingency) between the control AD group and experimental AD group. The significance level for the results was set at $p < 0.05$.

6.4 Results

6.4.1 General Data

Table 6.1 summarises the primary demographic information, statistical descriptions associated with BDNF serum levels and cognitive scores (CDR) in AD and cognitive test scores (MMSE) of the comparison groups. The primary demographic groups included a group of elderly participants ($n = 17$) and AD patients ($n = 13$), both using SSRI anti-depressants; this group was compared with a control group from the previously collected data (Chapter 5), which included a number of elderly patients ($n = 48$) and AD patients ($n = 27$), none of whom were using SSRI anti-depressant medication.

AD patients and elderly patients also on anti-depressant SSRIs ($n = 13$ and $n = 17$, respectively) were all assessed for cognitive function according to their CDR and MMSE test scores. The elderly group not receiving SSRIs exhibited average scores of 27–30 on the MMSE cognitive function testing. However, the MMSE scores for the elderly group receiving SSRI treatment mapped well to the equivalent CDR test result.

Variables	Elderly without SSRIs <i>n</i> = 48 (%)	Elderly with SSRIs <i>n</i> = 17 (%)	AD without SSRIs <i>n</i> = 27 (%)	AD with SSRIs <i>n</i> = 13 (%)	<i>p</i>
Age (years)	69.8 ± 6.45	79.06 ± 7.49	78.9 ± 5.28	77.62 ± 5.22	0.85
Gender	M = 12 (25) F = 36 (75)	M = 8 (47) F = 9 (53)	M = 14 (51.8) F = 13 (48.2)	M = 6 (46) F = 7 (54)	0.24
Serum BDNF (pg/ml)	80.3 ± 27.84	94.0±10.6	76.3±21.64	183.0± 11.9	<0.001
CDR Score	1.7 ± 1.3	8.21 ± 5.36*	11.570 ± 4.66	9.04 ± 4.63	0.63

Table 6. 1: Statistical characteristics of the demographic data, serum BDNF concentration and cognitive test in the comparison groups.

Note: The CDR scores of the elderly group subject to SSRI administration were calculated from the converted MMSE scores using the mapping scores.

6.4.2 BDNF concentrations in elderly and AD patients receiving SSRI anti-depressants

In the comparison of the BDNF concentrations for both AD patients ($n = 13$) and the elderly control group ($n = 17$) using the Mann–Whitney test, the results demonstrated a significant variation between the two groups in terms of serum BDNF levels p -value 0.003 ($W = 192$). This outcome indicates that the median concentration of BDNF for AD patients (183 pg/ml) is higher than it is for the elderly group (94 pg/ml) when both groups are administered anti-depressant SSRI medications (see Figure 6.1).

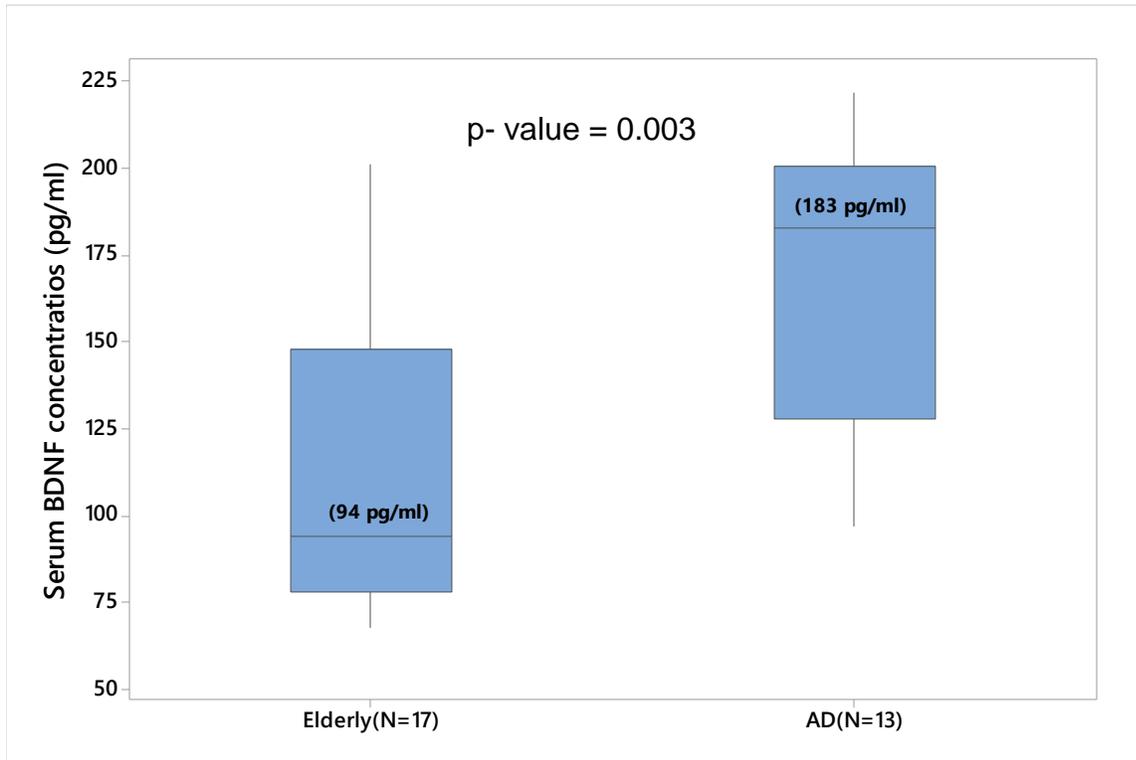


Figure6. 1: Differences between serum BDNF concentrations for the elderly group and AD patients on SSRI anti-depressant medications. Comparison between serum BDNF concentrations for elderly and AD patients on SSRI was performed by the Mann-Whitney Rank Sum test, and p-values are presented above the box plots. Numbers of both groups are shown at the bottom within the graph. Median is presented at the centre inside the graph.

6.4.4 BDNF concentrations with or without the administration of SSRIs

6.4.4.1 Elderly group

Significant variations were observed between the BDNF concentrations of the elderly control group not using SSRIs ($n = 48$) and the elderly group taking SSRI antidepressants ($n = 17$). Comparison testing was conducted between the BDNF concentrations using the Mann-Whitney test, and they indicated significant differences ($W = 746$, $p = 0.005$). The BDNF concentrations exhibited a slightly elevated serum level when SSRIs were administered to the elderly group (94 pg/ml) relative to the control elderly group who were not administered SSRIs (79.3 pg/ml; see Figure 6.2).

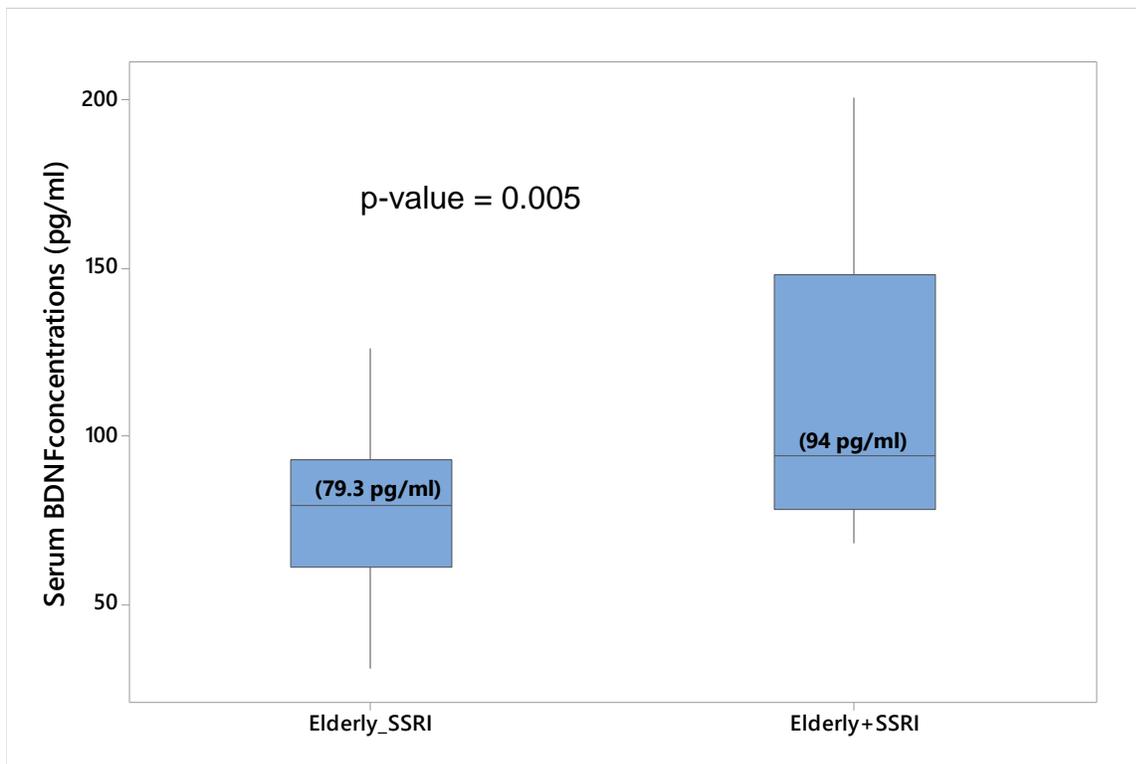


Figure6. 2: Variations of serum BDNF concentrations in the elderly groups with SSRIs used and elderly without SSRIS. The figure displayed a Comparison between serum BDNF concentrations for elderly was performed by the Mann-Whitney Rank Sum test, and p-values are presented above the box plots. Numbers of elderly not on SSRIs (Elderly-SSRIs) ($n = 17$) and elderly on SSRIs (Elderly+SSRIs) ($n = 48$). Median is presented at the centre inside the graph.

6.4.4.2 AD patients

The BDNF serum levels in AD patients who were administered SSRIs ($n = 13$) and control AD patients who were not administered SSRIs (discussed previously in Chapter 5; $n = 27$) are depicted in Figure 3. The comparisons between the medians of the serum BDNF levels for the control patients not administered SSRIs and diagnosed with AD ($n = 27$) and AD patients administered SSRIs ($n = 13$) as the experimental group, with non-parametric analysis of variance testing by application of the Mann–Whitney test, indicated the presence of significant variation between the application of anti-depressant SSRI medications and serum BDNF levels in AD patients ($W = 433$; $p < 0.001$). This finding suggests that application of SSRIs leads to a significant increase in BDNF levels for AD patients receiving SSRIs (203 pg/ml) relative to AD patients not receiving SSRIs (183 pg/ml; Figure 6.3). To conclude, comparisons of the median values of the BDNF concentrations for all the groups using non-parametric analysis of variance tests (Kruskal–Wallis test) indicated a significant variation between the application of SSRIs and the serum BDNF concentrations for AD and elderly patients.

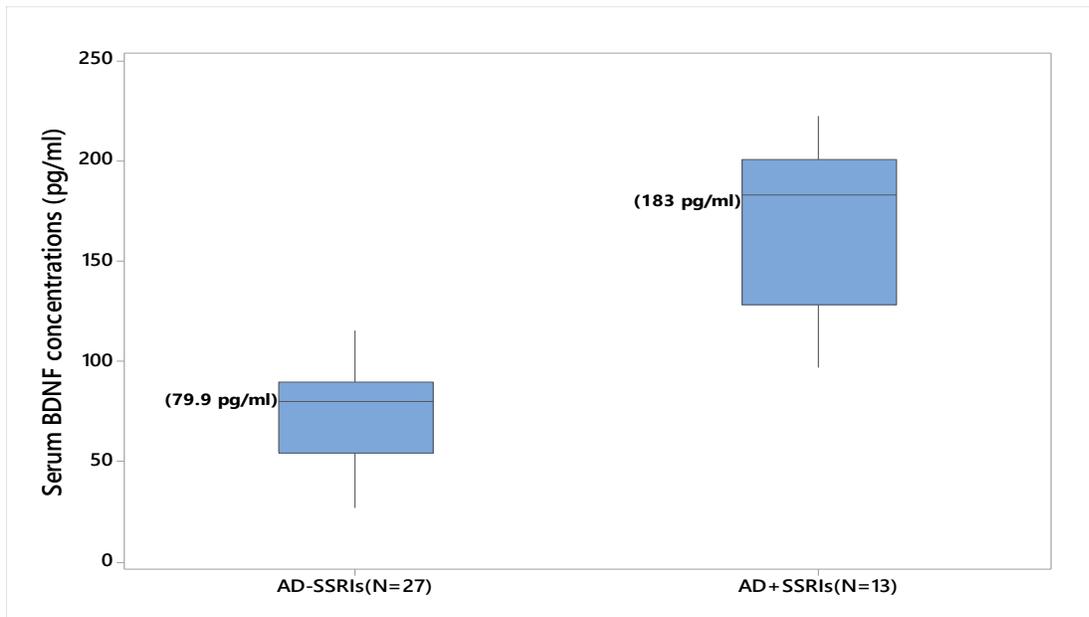


Figure6. 3: Serum BDNF concentrations for AD patients on SSRIs versus AD patients ($n=13$) not receiving SSRI treatment ($n=27$). The figure showed that all AD not on SSRIs had serum concentrations less than 100 pg/ml while AD on SSRI displayed serum concentrations between 100pg/ml-200 pg/ml with median equal to183 pg/ml.

6.4.5 Cognitive assessment

6.4.5.1 Elderly group

Cognitive testing of the elderly group ($n = 48$) with no SSRI anti-depressant treatment produced mean scores of 27–30; when these were mapped to the corresponding CDR scores, values in the range of 3–3.5 were obtained. This indicated that none of the subjects exhibited any cognitive impairment. However, the CDR equivalent values for the elderly group ($n = 17$) receiving SSRI treatment were divided according to different stages of cognition, in line with the MMSE scores, as follows: 11.4% of the subjects exhibited very mild cognitive impairment ($n = 2$), 47.5% exhibited mild cognitive impairment ($n = 8$), 17.6% exhibited moderate cognitive impairment ($n = 3$) and 23.5% exhibited severe cognitive impairment ($n = 4$).

6.4.5.2 AD patients

The clinical rating scores (CDR) for AD participants not receiving SSRI treatment ($n = 27$) were classified in line with the CDR scores as follows: very mild cognitive impairment, $n = 4$ (14.8%); mild dementia, $n = 6$ (22.2%); moderate dementia, $n = 13$ (48.1%); and severe dementia, $n = 4$ (14.8%). Furthermore, the clinical rating scores (CDR) for Alzheimer's patients using SSRIs ($n = 13$) were categorised as follows: mild cognitive impairment, $n = 3$ (23%); mild dementia, $n = 4$ (30.7%); moderate dementia, $n = 5$ (38.3%); and severe dementia, $n = 1$ (8%). Using Fisher's exact test to evaluate the significance of the correlation (contingency) between the control AD group and the experimental AD group in terms of the dementia classification produced a likelihood for chance distribution of 0.032. This outcome explains the number of AD patients with moderate to severe dementia being higher in the control group ($n = 27$) not taking SSRIs. AD patients using SSRIs ($n = 13$) exhibited very mild and mild dementia according to the CDR scores obtained (see Table 6.2).

	MCI	Mild dementia	Moderate dementia	Severe dementia
Control AD patients (<i>n</i> = 27)	<i>n</i> = 4 (14.8%)	<i>n</i> = 6 (22.2%)	<i>n</i> = 13 (48.1%)	<i>n</i> = 4 (14.8%)
AD patients on SSRIs (<i>n</i> = 13)	<i>n</i> = 3 (23%)	<i>n</i> = 4 (30.7%)	<i>n</i> = 5 (38.3%)	<i>n</i> = 1 (8%)

Table 6. 2: Dementia stages according to the CDR scores of AD patients without/with SSRI administration.

6.4.6 BDNF concentrations and MMSE scores of elderly patients on SSRIs

Significant correlations between the MMSE values and BDNF concentrations were found in elderly patients regarding the use of SSRI anti-depressants, as identified by the Spearman rho test, $r = 0.5$, $p = 0.019$ (see Figure 6.4)

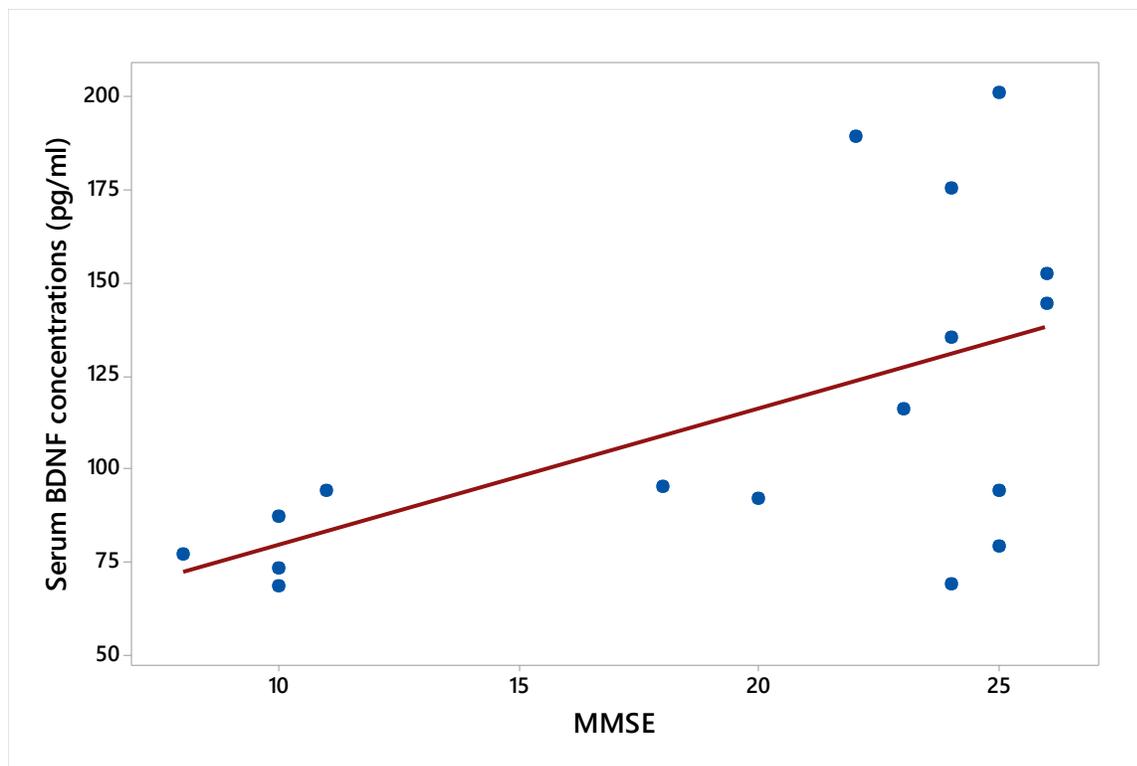


Figure 6. 4: MMSE score scatterplot versus serum BDNF concentrations for elderly patients on SSRIs. Scatter plot illustrating the linear relationship between serum BDNF concentrations and Mini-Mental State Examination Scores for elderly group 17 participants used SSRIs. MMSE score (x-axis) is plotted against its corresponding values of the serum BDNF concentrations (y-axis).

When the MMSE score was mapped to the equivalent CDR score, the results of the correlation between CDR and BDNF concentration indicated the presence of a significant correlation in elderly patients taking SSRI anti-depressants according to the Spearman Rho test, $r = -0.63$, $p = 0.006$ (see Figure 6.5).

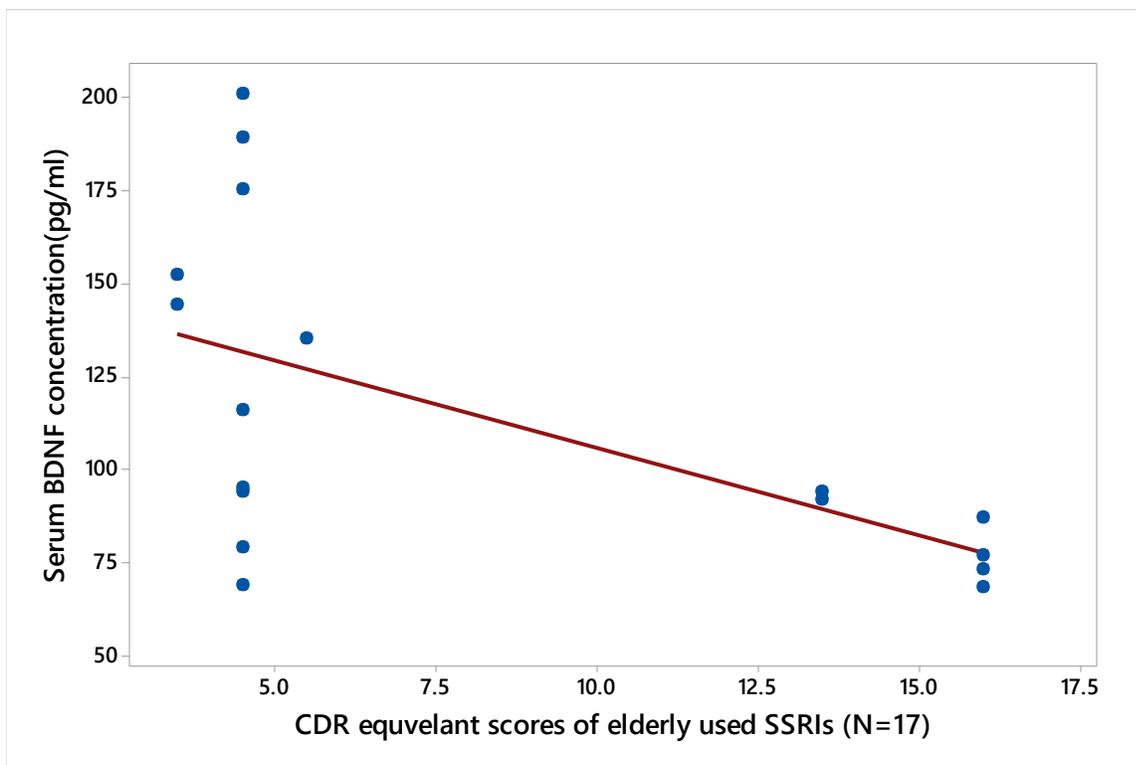


Figure 6.5 Equivalent CDR Scores compared with serum BDNF concentrations for elderly patients using SSRI anti-depressants. Scatter plot illustrating the linear relationship between serum BDNF concentrations and CDR Scores for elderly group 17 participants used SSRIs. CDR (x-axis) is plotted against its corresponding values of the serum BDNF concentrations (y-axis).

6.4.7 BDNF concentrations and CDR scores of AD patients

The correlation coefficients identified between the serum BDNF and clinical staging score (CDR) for patient's subject to anti-depressant SSRI treatment was calculated statistically via the Spearman rho test. The outcomes indicated a lack of any correlation between the serum concentrations and CDR scores, $r = 0.039$, $p = 0.9$ (see Figure 6.6). It is noteworthy that Mann–Whitney test comparisons of the CDR scores between the group of AD patients subject to SSRI anti-depressant medication and the

group of AD patients not taking SSRIs revealed no variation across scores, $p = 0.1$ (Figure 6.7).

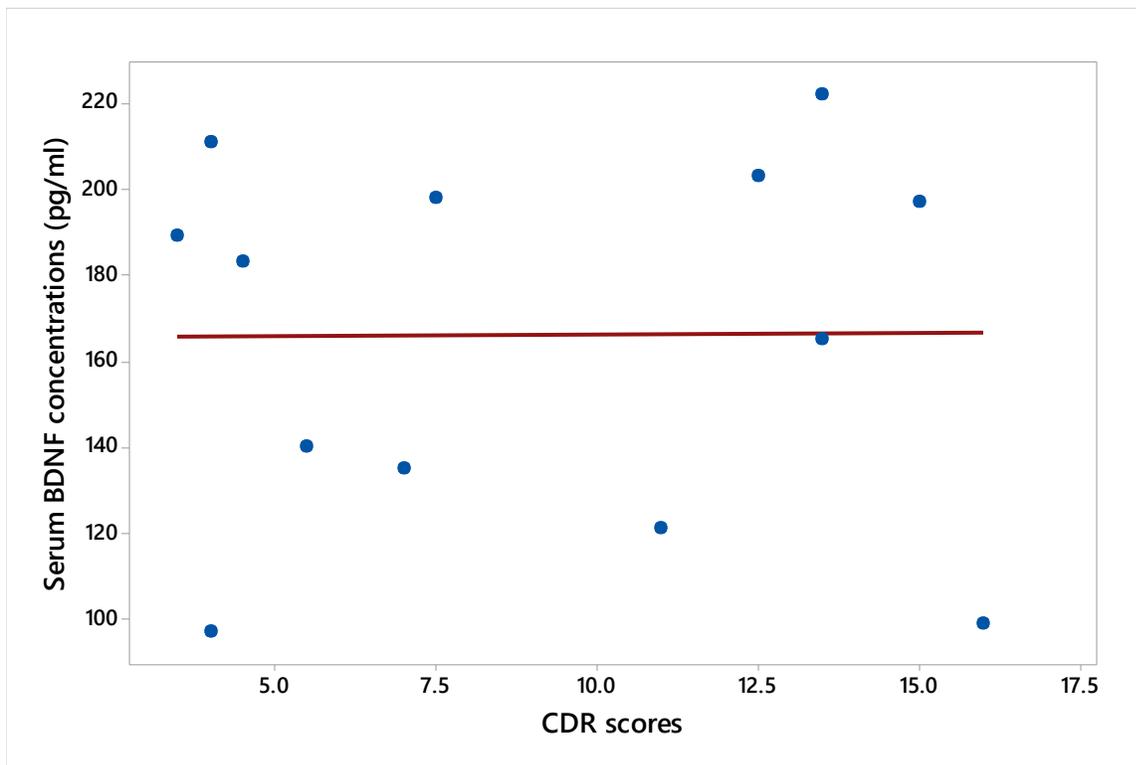


Figure 6. 6: CDR score scatterplots versus serum BDNF levels for AD patients on SSRI anti-depressant medication. Scatter plot illustrating no relationship between serum BDNF concentrations and CDR Scores for 13 AD patient's on SSRIs treatment. CDR (x-axis) is plotted against its corresponding values of the serum BDNF concentrations (y-axis).

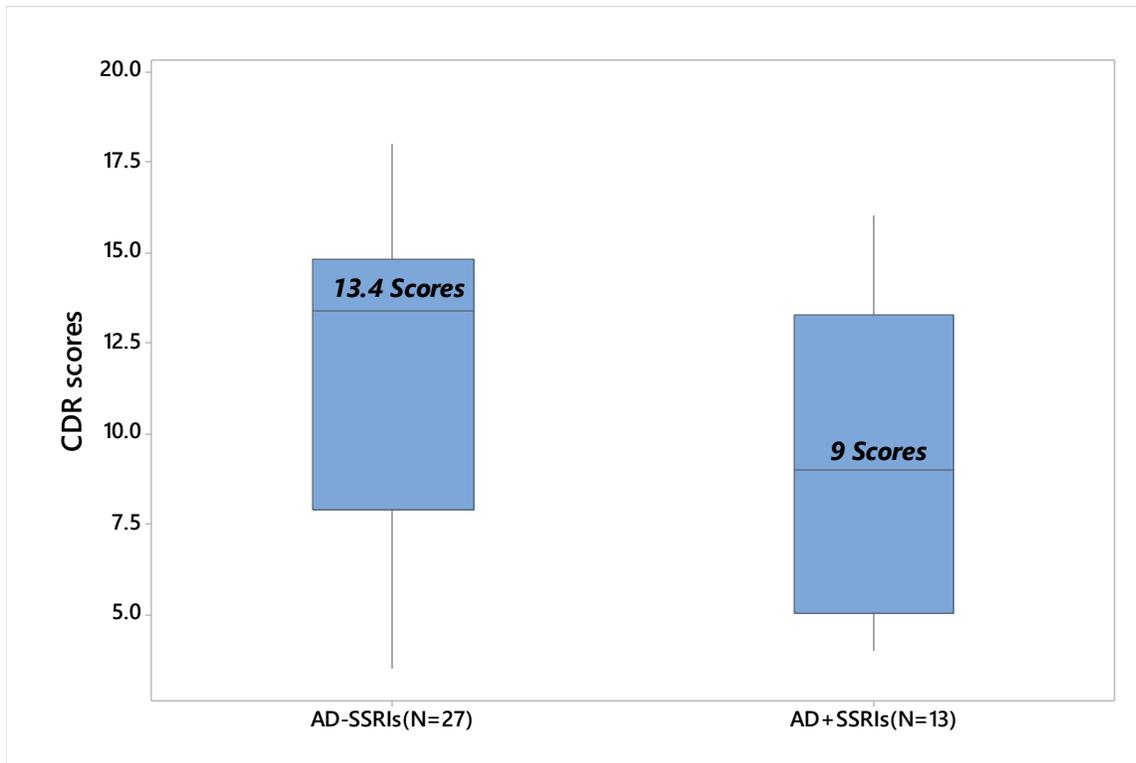


Figure 6.7: CDR scores for AD patients not using SSRIs and AD patients using SSRIs. Comparison of the CDR scores between AD patients was performed by the Mann-Whitney Rank Sum test, and p-values are presented above the box plots. Numbers of AD patients not used SSRIs (AD-SSRIs) and AD patients used SSRIs (AD+SSRIs) participants per cohort are shown at the bottom within the graph. Median is presented at the center inside the graph.

6.4.8 Use of SSRI anti-depressant medications among the elderly group and AD patients

There were three SSRI drugs used in both groups—citalopram, escitalopram and sertraline. Table 6.3 represents the median variations in serum BDNF concentrations in both groups. The BDNF serum concentrations for AD patients on citalopram ($n = 6$) and elderly patients ($n = 10$) are depicted in Figure 6.8. Generally, the BDNF serum concentrations of AD patients among the three different varieties of SSRI medication with non-parametric analysis of variance testing employing the Kruskal–Wallis test indicated no significant variance between the variety of SSRI administered and the serum BDNF concentration for AD patients ($H = 12$; $DF = 12$; $p = 0.44$). Comparisons of median BDNF serum concentrations in patients taking citalopram with the Mann–Whitney test indicated no significant variance between the serum BDNF levels of AD and elderly patients using citalopram and those not using this drug ($p = 0.12$). However, a significant change in BDNF serum concentrations was observed when both elderly people ($n = 4$) and AD patients ($n = 6$) used escitalopram, as shown in the results of the non-parametric Mann–Whitney test ($p = 0.05$; see Figure 6.9). The use of sertraline in the group comprising elderly patients ($n = 3$) and AD patients ($n = 1$) was observed to be less frequent, and consequently, difficult to assess with respect to variations in BDNF serum concentrations. However, it is noteworthy that the median BDNF serum concentration for the elderly group was 77 pg/ml, while in AD patients, it was 165 pg/ml.

Type of SSRI anti-depressants	Serum BDNF concentrations, pg/ml	
	Elderly group ($n = 17$)	AD patients($n = 13$)
Citalopram	125.5 ($n = 10$)	164.5 ($n = 6$)
Escitalopram	94 ($n = 4$)	190 ($n = 6$)
Sertraline	77 ($n = 3$)	165 ($n = 1$)

Table 6. 3: Serum BDNF concentration with different SSRIs used among the elderly group ($n = 17$) and AD patients ($n = 13$)

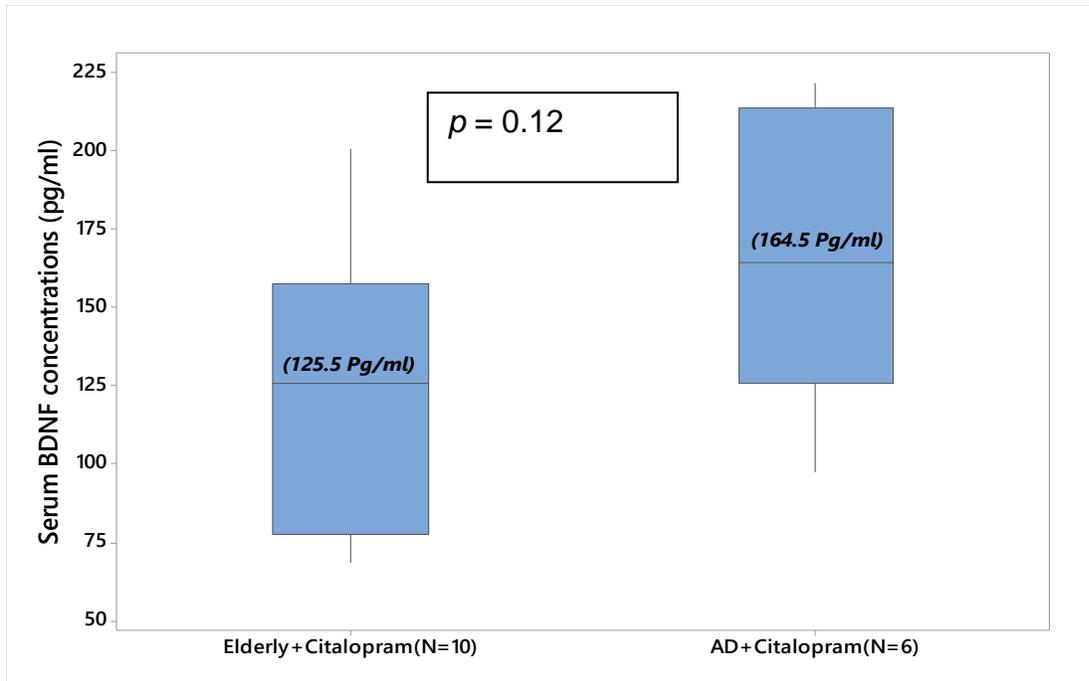


Figure 6.8: Variation in BDNF serum concentrations with the use of citalopram in elderly and AD patients. Comparison of the serum BDNF concentrations between elderly and AD patients was performed by the Mann-Whitney Rank Sum test, and p-values are presented above the box plots. Numbers of elderly and AD patients on citalopram are shown at the bottom within the graph. Median is presented at the center inside the graph.

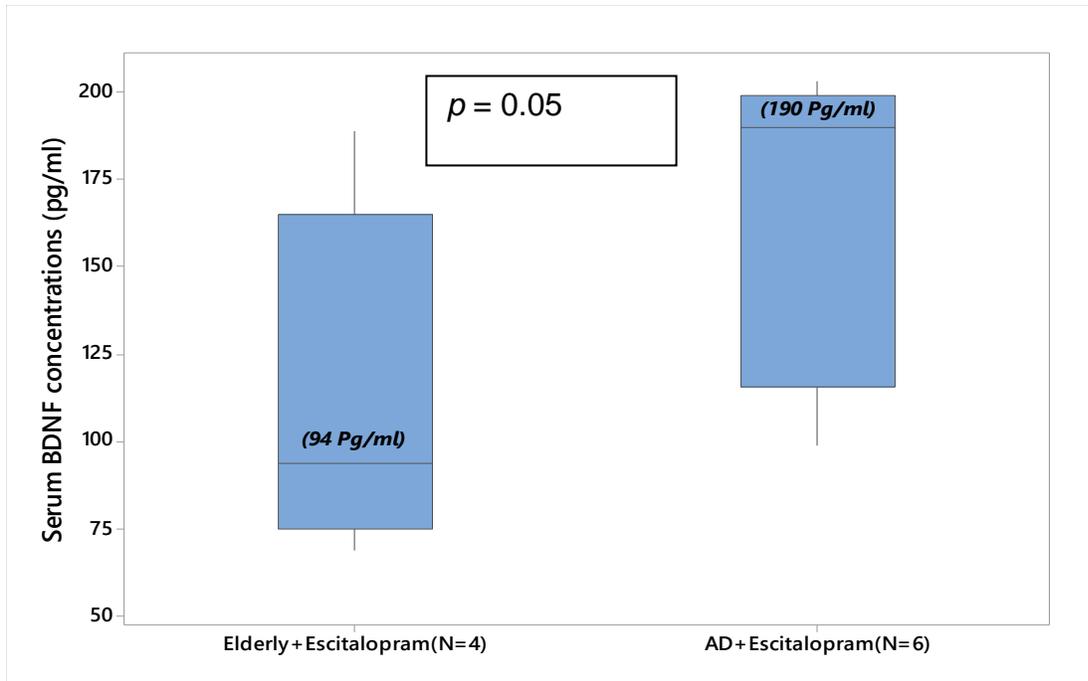


Figure 6.9: BDNF serum concentration variations with the administration of escitalopram in elderly and AD patients. Comparison of the serum BDNF concentrations between elderly and AD patients was performed by the Mann-Whitney Rank Sum test, and p-values are presented above the box plots. Numbers of elderly and AD patients on escitalopram are shown at the bottom within the graph. Median is presented at the center inside the graph.

6.4.9 Duration of SSRI treatment and BDNF serum concentration changes

Comparisons of the BDNF serum concentration of the elderly groups using and not using SSRIs indicated no significant variation according to the Mann–Whitney test for BDNF serum concentration for the duration of treatment in the elderly control group ($p = 0.09$; Table 6.4). However, a significant variation in BDNF serum concentrations was observed with a treatment duration of less than 1 year for AD and elderly patients according to the Mann–Whitney test ($p = 0.002$). The median BDNF serum concentration of AD patients taking SSRIs for less than 1 year ($n = 7$) was 183 pg/ml, which was higher than the BDNF serum concentration of the elderly patient group ($n = 9$), which was 92pg/ml (see Figure 6.10). However, treatment durations of longer than 1 year demonstrated no significant variation in BDNF serum concentrations for the AD and elderly patient groups ($p = 0.13$).

Duration of the treatment	BDNF concentrations (pg/ml)		
	AD patients ($N = 13$)	Elderly group ($N = 17$)	p-value
Less than 1 year	183 ($n = 7$)	92 ($n = 9$)	0.002
More than 1 year	181($n = 6$)	125.2 ($n = 8$)	0.138

Table 6. 4 :Duration of treatment and BDNF concentration change among the elderly group ($n = 17$) and AD patients ($n = 13$).

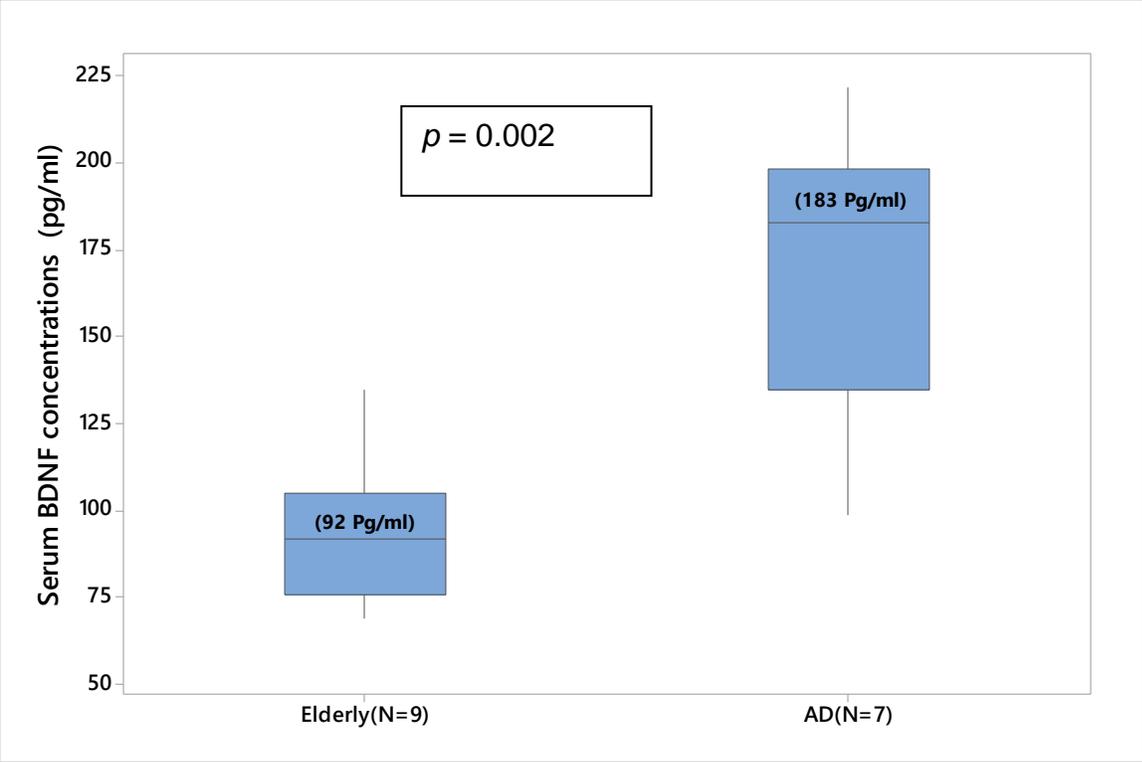


Figure 6.10: BDNF serum concentration variations between AD patients and elderly patients on SSRI treatment for less than 1 year. Comparison of the serum BDNF concentrations between elderly and AD patients was performed by the Mann-Whitney Rank Sum test, and p-values are presented above the box plots. Numbers of elderly and AD patients on SSRI treatment for less than 1 year are shown at the bottom within the graph. Median is presented at the center inside the graph.

6.4.10 Duration of treatment with SSRI medication and corresponding changes in MMSE values

6.4.10.1 Elderly group patients

There was no significant difference between the mean MMSE scores where SSRI was used for less than 1 year (19.1 ± 2.3) and where the SSRI was used for more than 1 year (19.8 ± 2.6), By One-way ANOVA t-test exhibited the following probability score for the elderly group: $p = 0.826$ (DF = 14). In addition, when comparisons of the BDNF serum concentrations were made with MMSE scores for elderly patients using SSRI for less than 1 year, the result obtained from the Pearson correlation test indicated no statistical correlation between variables ($r = 0.291$, $p = 0.448$). Similarly, elderly patients taking SSRIs for longer than 1 year revealed no statistical correlation of MMSE scores with BDNF serum concentrations ($r = 0.587$, $p = 0.126$).

6.4.10.2 AD patients

Overall, no significant variations were observed in CDR scores relating to the duration of treatment in AD patients ($n = 13$) using the Mann–Whitney test ($p = 0.77$). In addition, comparisons of the BDNF serum concentrations with CDR scores for AD patients ($n = 7$) using SSRI for less than 1 year revealed no statistical correlation between variables ($r = -0.1$, $p = 0.81$) according to the Spearman rho test. Furthermore, AD patients ($n = 6$) taking SSRIs for longer than a 1-year treatment period demonstrated no statistical correlation of CDR scores with BDNF serum concentrations of AD patients ($r = 0.870$, $p = 0.024$; Figure 6.11).

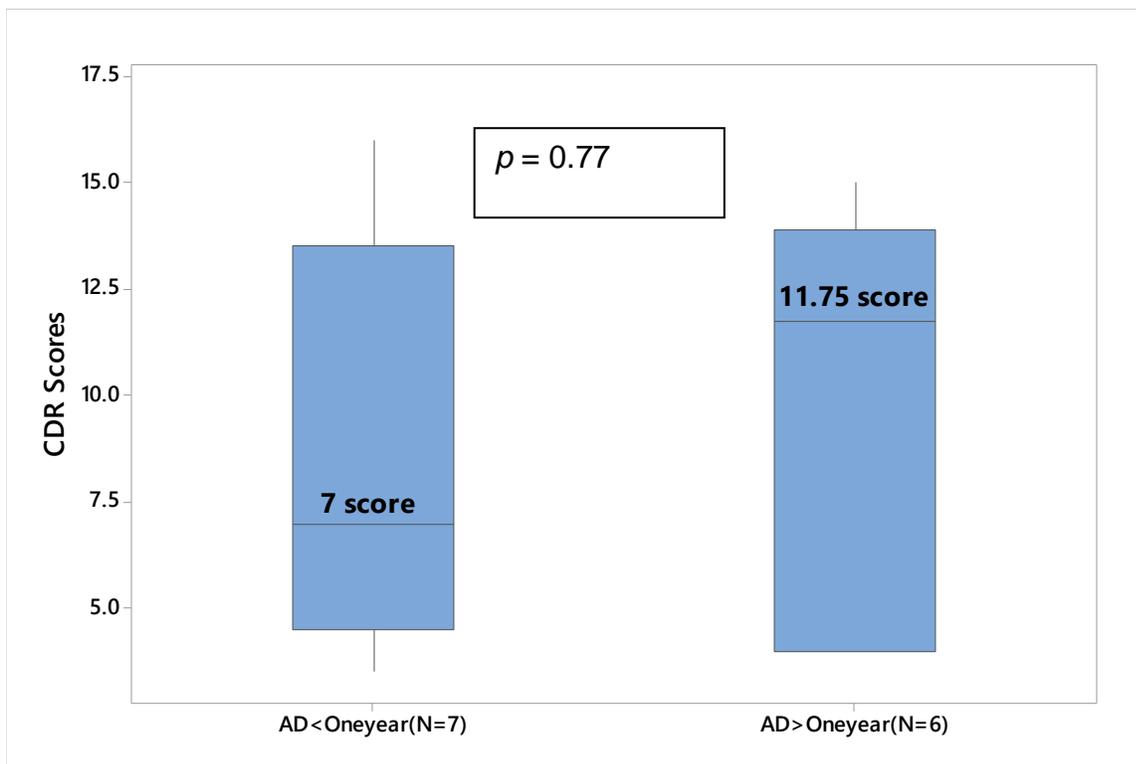


Figure 6.11: Box-plot of CDR scores with duration of SSRI treatment in the AD patients. Comparison of the CDR scores between AD patients was performed by the Mann-Whitney Rank Sum test, and p-values are presented above the box plots. Numbers of AD patients on SSRI treatment for less than 1 year and more than one year are shown at the bottom within the graph. Median is presented at the centre inside the graph.

6.5 Discussion

BDNF is known to be instrumental in regulating synaptic transmission and brain plasticity, and this function is crucial for various brain processes, including learning and memory. Research has highlighted the part played by BDNF in both animal subjects, and patients with AD. Several empirical studies have demonstrated that BDNF offers protection against the beta-amyloid induction of neurotoxicity and may play a role in increasing AB degradation (Song *et al.*, 2015).

This stage of research focussed on the effect of administering SSRI anti-depressants on the BDNF serum levels in AD patients. Clear evidence validating an interaction between serum levels of the BDNF and serotonin neurotransmitters was obtained, with significant evidence that BDNF facilitates both the development and function of serotonergic neurons (Martinowich & Lu, 2008). The current thesis investigation has indicated that serum BDNF levels in AD patients on SSRI treatment increased relative to AD patients not on any type of SSRI treatment. These outcomes are in accordance with those from previous research on human depression, highlighting that BDNF serum levels are higher as a consequence of SSRI administration and enhance the depression prognosis (Owen M Wolkowitz *et al.*, 2011a). Explanations like these serve to support the hypothesis proposing that SSRI therapeutic actions demand induction of adaptive morphological and functional changes at the synaptic cleft (Groves, 2007). Specifically, it has been proposed that underlying such alterations in plasticity may be variations in the expression, secretion or downstream functioning of BDNF as a result of SSRI administration (Björkholm & Monteggia, 2016). Moreover, depression is viewed as a critical risk factor for AD, and it has also been verified that anti-depression therapies may delay AD progression by raising BDNF levels in both the hippocampus of the brain and brain cortex of animal subjects. This is justified by the fact that chronic application of SSRI anti-depressant treatment can result in elevated BDNF transcription in rodents, both in the hippocampal and cortical brain regions (Hoshaw *et al.*, 2005). Here, the mechanism of action involves the co-expression of BDNF and its receptor TrkB in serotonergic neurons in the dorsal and median raphe, with BDNF being

transported in retrograde fashion from 5-HT terminals in the striatum and hippocampus to cell bodies in the raphe nuclei (Diniz *et al.*, 2014).

In contrast to the developments described above, no well-established study has been performed in humans for explaining BDNF's role in ameliorating AD or delaying symptom progression. However, the widening of the role of BDNF neurotransmitters indicates a potential therapeutic use for BDNF in AD patients (Fumagalli *et al.*, 2006), and this supports the primary research outcome of this thesis, which has concentrated on the effect of SSRI anti-depressant administration on BDNF serum concentrations in AD patients. It is noteworthy that the pharmaceuticals clinically employed to target AD, in addition to newly developed therapeutic interventions, share the identical characteristic of modulating brain concentrations of BDNF in brain regions known to directly participate in the pathophysiology of the disease. This research selected the currently established SSRI class of drugs, rather than novel drugs that were only recently developed, for investigating the potential impacts on BDNF levels. It is noteworthy that the outcomes of this research have demonstrated that BDNF serum concentrations are higher in elderly patients receiving SSRI treatment compared with elderly patients not subject to SSRI treatment. This finding indicates that only the administration of SSRI anti-depressants influenced these serum BDNF concentrations among the elderly population.

The administration of SSRI anti-depressants in AD and elderly patients can elevate the BDNF serum concentrations and enhance neural activity (Fumagalli *et al.*, 2006). Thus, there is some justification for a higher usage rate for SSRI anti-depressants to target AD in overcoming the associated anxiety conditions or sleep disorders, symptoms typically associated with AD and elderly patients. This explanation can be taken further to clarify why the rate of adherence to anti-depressant medication is higher for AD patients than it is in elderly people. For instance, a recent investigation conducted at the University of Eastern Finland contrasted data relating to prescription rates for 62 104 community-dwelling subjects with confirmed AD diagnoses with that for the same number of gender- and age-matched controls without AD. Utilising information from the Finnish register-based cohort investigation called Medication Use and

Alzheimer's Disease (MEDALZ), they evaluated data information over a 13-year period, from nine years pre-diagnosis to four years post-diagnosis. The findings from this investigation demonstrated that patients with AD obtained initial anti-depressant prescriptions about twice as often as comparable controls did over the entire study period. The rate was greatest in the 6 months after the AD diagnosis, when it rocketed to five times that of the control comparison group; however, elevated rates of anti-depressant use were noted in the AD test group even nine years before the diagnosis of AD (Puranen *et al.*, 2017).

The severities of the stages of dementia were observed to decrease in AD patients on SSRIs relative to non-medicated AD patients. This observation reveals that the number of AD subjects with moderate to severe dementia was higher in the control group ($n = 27$) not taking SSRIs, whilst in AD patients taking SSRIs ($n = 13$), expressions relating to the extent of dementia according to the CDR scores indicated only very mild and mild dementia. Assessments of dementia severity over both groups indicated that both include mild or moderate dementia, but most of the group receiving SSRI treatment exhibited the moderate stage of AD. In terms of other confounding variables, all the subjects were free from all types of anti-depressant medication (either acetylcholine esterase inhibitors [AChEIs] or SSRIs). The experimental group utilised SSRIs, and this may have been a key contributory factor in reducing the AD severity. However, it was observed that the CDR scores of AD patients on SSRI were lower than those for the control group, but the decrease was not significant, and there was an elevation of serum BDNF concentrations. Thus, it can be seen that there is a lack of correlation between the application of anti-depressants and improved cognitive performance in AD subjects.

A report was recently published that examined only the variations in cognition and BDNF serum levels in AD patients, irrespective of anti-depressant use. The findings reported in our current paper indicate that reductions in dementia severity decline in association with administration of SSRIs due to the up regulation of BDNF levels. Furthermore, the results of a study performed in Poland identified a significant association between BDNF serum concentrations and cognitive deficiencies, significant

associations were identified between BDNF serum concentrations and the various cognitive domains employed in cognitive impairment measures. Direct positive associations were identified between BDNF serum concentrations and episodic memory when measured by number of words memorised and recalled (Siuda *et al.*, 2017).

Robust evidence of the application of anti-depressants and their beneficial effect on cognition with respect to AD was provided by a systematic review article that analysed five research reports and evaluated the part played by anti-depressants in dementia patients. The review proposed that the application of anti-depressants was significantly correlated with improved completion of cognitive tasks for dementia patients younger than 65 years (Moraros *et al.*, 2017). Conversely, the correlation between elderly patients and cognition performance in association with anti-depressant usage in a cohort study performed over 10 years found no evidence for a correlation between anti-depressant usage and cognitive decline (Carrière *et al.*, 2017). The results of the cognition performance testing for elderly patients taking SSRI anti-depressants were mostly categorised as mild cognition impairment according to the MMSE criteria.

It is noteworthy that the age groups for all the samples were similar, with ages in the range of 65–80 years. The published literature strongly supports the suggestion of an increasing burden of dementia with increasing age, especially after 65 years (Bus *et al.*, 2012; Richard *et al.*, 2012; World Alzheimer Report, 2014). Nevertheless, it is transparent that AD/dementia, as a progressive, neurodegenerative condition, is likely to be triggered by events, such as depression, which are initiated well before the commencement of any obvious symptoms (Emery, 2011; Green *et al.*, 2003; Saczynski *et al.*, 2010). As with the general correlation, a more powerful effect of anti-depressants in older respondents may be indicative of a causal effect of anti-depressants on risk. However, other explanations cannot be ignored. For instance, if vascular disease in the elderly results in more frequent anti-depressant prescriptions, subsequent cognition deterioration may be the result of the confounding effects of the underlying vascular disorder and/or anti-depressants.

In relation to types of anti-depressants, the findings of the present paper have revealed that AD subjects receiving escitalopram exhibited elevated serum BDNF concentrations (190 pg/ml) compared with the elderly patient group (94 pg/ml), with no significant variations associated with the application of either citalopram or sertraline across both groups. The finding was similar for the application of escitalopram in depression. The serum BDNF concentrations in patients increased from 115 ± 6 pg/ml at baseline to 160 ± 27 pg/ml, with a significant enhancement of depressive symptoms; however, the MMSE scores were not significantly affected (Martocchia *et al.*, 2014).

A recent preliminary investigation reported raised plasma BDNF levels from the baseline in patients diagnosed with mild neurocognitive conditions due to AD and prescribed 10 mg daily of escitalopram for 2 months. This study revealed significant improvements in cognitive functioning, primarily in terms of the memory domain for mild neurocognitive conditions associated with AD (Levada *et al.*, 2016). It was proposed that the influence of escitalopram was probably mediated by the elevated expression of BDNF in hippocampal and neocortical brain regions, which in turn, resulted in normalisation of the glutamate-dependent mechanism of synaptic plasticity. However, the effect of SSRIs on cognition and serum BDNF concentrations was evaluated in subjects with vascular dementia. This study demonstrated that an elevated serum BDNF concentration in subjects with vascular dementia who are prescribed fluoxetine exhibited enhanced cognitive function, especially executive function (Liu *et al.*, 2014). This is in line with the findings of the present paper, although no AD patients received fluoxetine. This can be explained by the failure in the response of the previous clinical study to successfully evaluate cognitive performance following administration of fluoxetine in AD patients (Geldmacher *et al.*, 1994).

In relation to the timeframe of SSRI administration for AD patients, the findings indicate that increased BDNF serum concentrations are associated with treatments of less than 1 year in duration, relative to more than 1 year, for AD patients ($p = 0.002$). Nevertheless, the cognition status is not enhanced for a duration shorter than 1 year of SSRI treatment in AD patients ($p = 0.126$).

Matrisciano *et al.*(2009) investigated the impact of treatment duration with anti-depressant usage on serum BDNF levels in both healthy and depressed patients at baseline (Time 0) and following 5 weeks and 6 months of sertraline, escitalopram or venlafaxine administration. They indicate that the BDNF levels were lower in depressed patients and that sertraline increased the BDNF concentrations following 5 weeks and 6 months of treatment ($p < 0.001$), while venlafaxine increased the BDNF levels only following 6 months treatment ($p < 0.001$). However, escitalopram did not alter the serum concentration of BDNF. It is interesting that there was no correlation observed between the severity of depressive symptoms at Time 0 and the percentage increase in BDNF concentrations following 5 weeks and 6 months of treatment (Matrisciano *et al.*, 2009a).

A further investigation on depressed patients highlighting the significance of SSRI treatment duration involving escitalopram ($n = 16$) or sertraline ($n = 14$) and the effect of treatment duration on BDNF concentrations; within a 2-month treatment period, significant increases of serum BDNF levels relative to controls were revealed ($p = 0.005$). However the serum BDNF levels were not significantly associated with depression severity ($p = 0.01$). In addition, variations in BDNF serum concentrations with treatment were not associated with improvement according to the standard depression scales. This investigation demonstrated that higher pre-treatment BDNF levels are indicative of superior responses to SSRI anti-depressant treatment ($p < 0.01$; Wolkowitz *et al.*, 2011b).

Preclinical investigations have demonstrated that different classes of anti-depressants display different influences on BDNF transcription, release, receptor activation and secondary messengers. For instance, duloxetine, a serotonergic/noradrenergic re-uptake inhibitor, has been demonstrated to influence BDNF mRNA concentrations in various brain regions of rats, depending on the length of treatment. Chronic administration (3 weeks) of duloxetine raised concentrations of BDNF and mRNA in the prefrontal, entorhinal and parietal cortices but not the hippocampus; however, acute administration did not result in any significant variations (Calabrese *et al.*, 2007). These findings are corroborated by Mannari *et al.* (2008), who recently demonstrated that chronic, but not acute, treatment with duloxetine led to

elevated total BDNF levels in the prefrontal cortex of rats, while only chronic high applications of duloxetine raised total BDNF concentrations in rodent cerebrospinal fluid. Moreover, repeated application of duloxetine, even at the highest dosage, was not capable of altering the total BDNF concentrations, either in the serum or plasma.

Acute and chronic treatments with fluoxetine, citalopram, clomipramine, imipramine, reboxetine and moclobemide have been investigated in mice in terms of TrkB protein concentrations and TrkB autophosphorylation. TrkB is a high-affinity receptor for BDNF, and its autophosphorylation leads to inactivation, representing an indirect signal of BDNF neuronal release (Rantamaki *et al.*, 2007). All the anti-depressants studied significantly elevated TrkB auto-phosphorylation in the mouse anterior cingulate cortex and hippocampal brain regions within 30–60 min of a single injection, but none of the acute applications influenced the expression levels of total TrkB protein (Rantamaki *et al.*, 2007). However, the current research found no significant correlation between AD and elderly that used SSRIs more than one year of durations while less than one year duration showed significant correlation as AD patients had higher BDNF concentration compared to elderly. This is justified by the fact that most of the patients who were administered SSRI for a period of less than 1 year had their medication discontinued as a result of the side-effects profile. Furthermore, recent published literature has correlated the prescription of anti-depressant drugs, especially SSRIs, with raised risks of diverse outcomes, including potential suicide attempts.

It is now well recognised that the use of anti-depressant therapies is associated with increases in serum BDNF. If decreased BDNF is associated with an increased risk of AD, it may be expected that elderly patients using anti-depressants could be at a decreased risk of developing AD, or they may not progress to more severe forms of the disease. This programme of research now explores the relationship of anti-depressant use in elderly patients with cognition and assesses the effects of SSRIs anti-depressant use on serum BDNF concentration in an elderly population (with or without AD). The results of such research may be of value concerning recommendations for the use of anti-depressants in elderly patients as a form of AD prevention.

To conclude, our results suggest that serum BDNF concentrations are higher in SSRI-treated AD patients. The findings indicate that elevated serum BDNF concentrations may be crucial in anti-depressants' modes of action in AD patients. They also suggest that anti-depressant treatment may increase BDNF concentrations not only in elderly people suffering from previous depression but also in AD subjects. However, SSRIs' effects on cognition show no significant correlation in AD. For assessing the detailed mechanism supporting the association between serum BDNF concentrations and cognitive performance during AD, further investigation of relevant genetics will be required, involving assessment of the correlation between cognition performance and BDNF polymorphism.

7. Chapter Seven: The association between BDNF gene Val66Met polymorphism (rs6265/G196A) and cognitive performance with SSRI use in Saudi AD patients

7.1 Introduction

Our understanding of the aetiology of AD pathogenesis can be divided into two periods: pre- and post-identification of gene mutations associated with the development of AD. The past three decades of genetic research in AD have transformed the understanding of its causes and enhanced the innovation and development of novel therapeutics aimed at its treatment and prevention (Tanzi & Bertram, 2005). The modern era of AD research, guided by genetics, began in the 1980s and 1990s with genetic linkage studies and positional cloning efforts that led to the identification of the three EO-FAD genes: amyloid precursor protein APP, presenilin-1 PSEN1, and presenilin-2 PSEN2 (Campion *et al.*, 1999). The late-onset of AD caused by mutation of gene APOE was discovered using a similar strategy but was ultimately validated by genetic association studies (Relkin, 1996). Generally, there seems to be some evidence to indicate that these four genes are responsible for the development of 30%–50% of the inheritability of AD cases (Tanzi, 2012).

Recently, genome-wide association studies identified 11 additional AD candidate genes (Tanzi, 2012). Moreover, it has been proposed that the BDNF gene mutation participates in the development of AD. Autopsy studies found reduced mRNA expression of BDNF in the hippocampus of patients with AD (Connor *et al.*, 1997), which implicates the possible participation of BDNF in AD pathogenesis. Moreover, the BDNF gene represents an interesting, potential, genetic mechanism for the risk of late-onset AD (Matrisciano *et al.*, 2009b). More details about this mechanism were discussed in literature review section (2.2.15).

The most common BDNF polymorphism is the Val66Met polymorphism (rs6265, G196A). This single nucleotide polymorphism (SNP) substitutes valine (Val) for methionine (Met) at codon 66. Val66Met is a substitution of amino acids within the BDNF protein; G196A is a substitution of a guanine for an adenine at position 196 of the gene. It is this change in a nucleic acid that codes for the different amino acid in Val66Met. This substitution interferes with intracellular trafficking of BDNF and activity-dependent BDNF secretion. The Met66-BDNF protein has been shown to be associated with reduced transport of BDNF from the Golgi region to appropriate secretory granules in neurons, compared with the Val66-BDNF protein (Wang *et al.*, 2012). Additionally, the A allele of rs6265 has been related to worse episodic memory, abnormal hippocampal activation and lower hippocampal n-acetyl aspartate (NAA) in human subjects (Egan *et al.*, 2003).

There have been studies of many polymorphisms of the BDNF gene, such as rs11030104, rs16917204, rs7103411, rs6265 and rs2030324. However, only the last two polymorphisms have been widely studied, with no linkage established between them and the diagnosis of AD. Moreover, these results are inconsistent and individual studies have relatively little power to confirm this association. For example, the G allele of rs6265 confers the risk effect for AD in subjects in Japan (Matsushita *et al.*, 2005), but no significant association was found in a Turkish population (Solmaztepe *et al.*, 2017). Moreover, BDNF gene polymorphism (Val66Met, 270C/T, 11757G/C) has been proven to play a potential role in AD pathogenesis, with the Met carrier score related to lower BDNF concentration, certain brain structure atrophy, and impaired cognitive ability (Song *et al.*, 2015).

A meta-analysis conducted in 2014, including 21 articles about rs6265 and 22 articles about rs2030324, found that there was no evidence for an association between rs2030324 and AD (Lin *et al.*, 2014). However, rs6265 leads to an increased risk of AD in Caucasian females, but not for Asians. Moreover, the A allele of rs6265 was found to contribute significantly to the increased risk of AD in female late onset AD patients (Li *et al.*, 2014)

The most studied BDNF variation, Val66Met (rs6265), maps to the pro-domain that is absent in mature BDNF (Figure 7.1) and results in a 30% reduction in BDNF secretion, most likely by partially impairing its packaging into dense core vesicles. The Met66 allele is common in human populations (e.g.~30% heterozygotes and ~4% homozygotes in datasets of European origin). Following early reports proposing that the Val66Met polymorphism confers risk of sporadic Alzheimer's disease (Ventriglia *et al.*, 2002), the presence of this allele has been linked to a wide range of conditions from psychiatric and personality disorders, including depression, substance abuse disorders, eating disorders, schizophrenia and neuroticism, to lower mean intelligence or body mass index (Lu *et al.*, 2012; Martorana *et al.*, 2010). More recently, the involvement of the Val66Met variation in the pathogenesis of neurodegeneration has again been a topic of intense study, following observations of associations with hippocampal atrophy, reduced hippocampal activity and impairments in episodic memory (Lim *et al.*, 2015). The divergence of findings has been attributed to differences between studies in variables such as ethnicity, age, gender, and phenotypic assessment (Hong *et al.*, 2011).

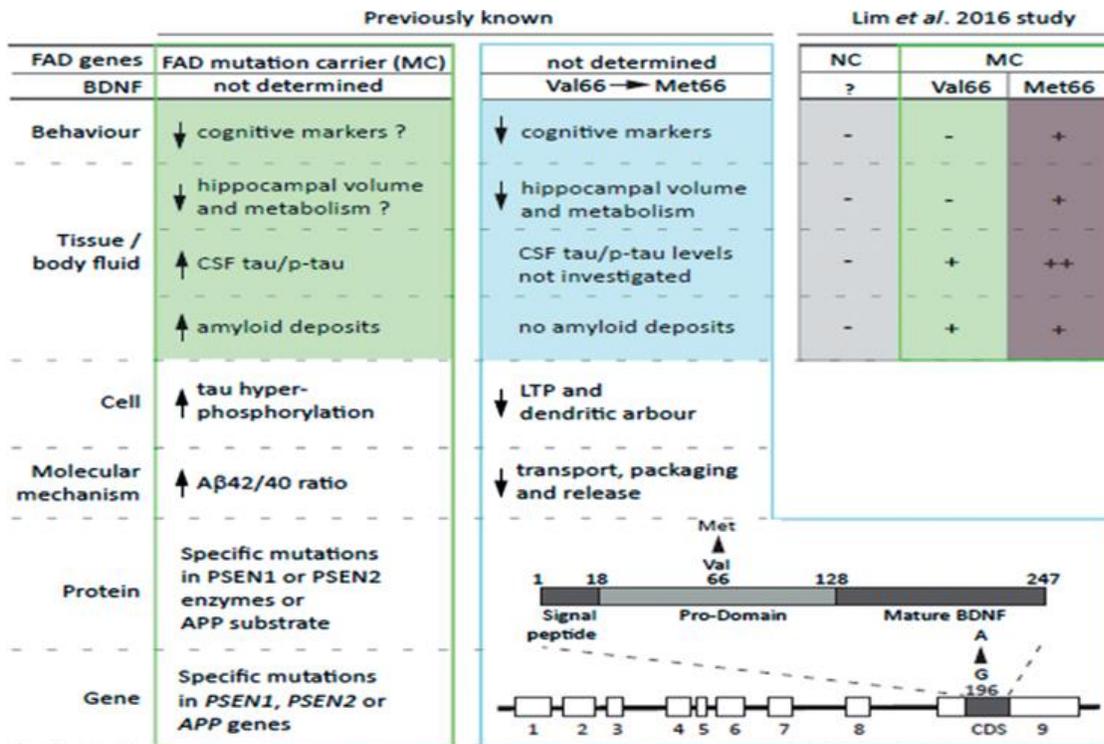


Figure7. 1: Schematic depicting Alzheimer-related endophenotypes affected by familial Alzheimer’s disease-causing mutations and the BDNF Val66Met polymorphism. The green and blue outlined boxes depict previously described observations that emerged in separate studies of familial Alzheimer’s disease mutations and the Met66 allele, respectively. The figure depicts only preclinical Alzheimer’s disease endophenotypes. Background shading is used to highlight phenotypes emphasized by Lim et al. (2016). The box shown on the right depicts key endophenotypes which were either unaffected by BDNF Val66Met variation (amyloid deposition) or were exacerbated by it (CSF tau/p-tau levels, hippocampal volume, and episodic memory). NC = non-carriers.

In the USA, there have been several studies that correlate the effect of BDNF polymorphism with BDNF protein levels in AD and that have demonstrated a marked decrease of BDNF protein levels in the temporal neocortex, which tended to be associated with neuropathological measures of disease severity. AD diagnosis and brain BDNF protein levels were largely independent of reported BDNF polymorphisms, suggesting that the two SNPs (BDNF 196 G allele and the BDNF 270 T carriership) are not strong genetic risk factors for AD or the primary determinants of BDNF protein levels in AD (Lee *et al.*, 2005). Another cohort study, conducted by Desai *et al.* (2005), compared American white (n= 995) versus American black late onset AD (n=671), and control white American versus American black (64 AD cases and 45 controls); the study examined the association of the Val66Met (G196A) polymorphism with quantitative measures of AD progression, including age at onset (AAO), disease duration and MMSE scores. The study revealed that no significant difference in allele genotype frequencies was observed between AD cases and controls within the American white and black cohorts for the G196A. However, the frequency of the 196A allele was significantly lower in the American black subjects compared to the whites. Moreover, the BDNF polymorphism did not affect AAO or disease duration measures in American whites or blacks. Additionally, the study result does not support the association between the BDNFVal66 Met polymorphism and the risk of sporadic late onset AD among American whites or blacks (Desai *et al.*, 2005)

In China, He *et al.*, (2007) identified a lack of association between the BDNF gene Val66Met polymorphism and AD; the study results indicated that the distribution of the BDNF genotypes and alleles did not differ significantly. Similar results were observed when the AD and control groups were stratified by age, age at onset and sex. Additionally, this study presented data that revealed no significant effect of the genotypes on the age at onset for developing AD and no significant association between the genotypes and the severity of the disease (He *et al.*, 2007). Additionally, in a North Indian population, there was no significant difference seen between the BDNF genotype (val66met polymorphism) and BDNF serum levels between AD patients with amnesic mild cognitive impairment versus control subjects (Sonali *et al.*, 2013)

Genotype and allele frequencies for BDNF Val66Met polymorphism in a sample of 102 AD cases and 168 samples of Colombian AD patients were stratified by family history and sex; it was found that that the BDNF polymorphism was not significantly associated with the A (Met) carrier genotypes or A allele (A versus G) in the total sample in late onset AD female patients or in APOE4 carrier patients. However, there was a significant association between A carrier genotypes and familial AD (Diego et al., 2006).

A further study examined the potential association between single nucleotide polymorphisms (SNPs) of the BDNF gene (G11757 C, C270T, G196A, G-712A) and Alzheimer's disease-related depression (AD-D) (Zhang et al., 2011). Participants included 336 patients with AD; 128 of these patients had AD-D. A response to an eight-week paroxetine treatment was also assessed. The frequency of the 11757 C allele was significantly higher in AD-D than in the AD without patients with depression group. Further, the 196A allele occurred with significantly higher frequency in AD-D patients versus AD non-depressed patients. Carriers of the A allele of G196A responded better to the paroxetine treatment. These findings support an important role of BDNF polymorphism in AD-D (Zhang et al., 2011).

In Turkey, Solmaz et al. found that there was no association between AD and BDNF gene Val66Met polymorphism regarding the different variables of age, gender, or AD stage (Solmaz et al., 2017). However, with regard to the effect of serum BDNF and the use of SSRIs in correlation to BDNF gene Val66Met polymorphism in AD this has not been investigated yet. Our aim in this part of the research is to include more suggestions concerning the impact of use SSRIs on both BDNF serum concentration and cognition performance .

With regard to the effect of BDNF Val66Met polymorphism on memory performance, a meta-analysis focused specifically on rs6265 SNP of the BDNF Val66Met gene included 28 articles that have assessed the association between Val and Met carriers in performance during memory tasks using different tools for memory assessment, such as the Wechsler Memory Scale-revised (WMSR), Rey Auditory Verbal Learning Test, Hopkins Verbal Learning Test, and the Recall complex scenes test for healthy participants and individuals with different diagnoses (Schizophrenia, Bipolar Affective Disorder and Depression). The results showed that certainly none of the samples was diagnosed with AD. The results also illustrated that differences in the rs6265 SNP of the BDNF gene have a significant effect on memory performance and on both the structure and physiology of the hippocampus, with carriers of the met allele being harmfully affected. (Kambeitz *et al.*, 2012)

A systematic review conducted by Yan et al. (2014) to evaluate the efficacy of antidepressants in correlation with BDNF Val66Met polymorphism in Asian populations found that the Met carriers had a better response rate than the Val/Val carrier. In Asians, the Met carrier was positively associated with the response rate in the SSRI group and with treatments of ≥ 6 weeks. The authors suggested that the effect of antidepressants may depend on ethnic origin because BDNF had a lesser influence on the response in mixed-race studies. Moreover, the duration of the treatment plays an important factor in the response and remission rate of the antidepressant efficacy (Yan et al., 2014).

However, BDNF Val66Met polymorphism influences antidepressant response and remission in Caucasian patients in a different manner to SSRI and SNRI/TCA. Val/Val patients have a higher probability of a three-month response to SSRI compared to SNRI/TCA. Carriers of the Met allele have a higher probability of a six-month remission rate with SNRI/TCA in comparison to SSRI. This effect is not related to antidepressant side effects. Thus, the results of the study suggest that SSRI could be recommended for Val/Val patients and that, conversely, SNRI/TCA could be beneficial to Met patients (Colle *et al.*, 2015).

In view of all that has been mentioned so far, there is a lack of association between BDNF gene Val66Met polymorphism and AD. Moreover, a recent study that found a relationship between gender difference and the risk of AD in correlation to neurotrophin gene polymorphism recommended examining the role of potential modifying factors, such as diet, exercise, antidepressant medication and hormone-replacement use, in addition to gene–gene interactions, in order to clarify the role of neurotrophins and the risk of AD (Matyi *et al.*, 2017). On the other hand, the distribution of Val66Met polymorphism is unknown in Saudi Arabia. Essentially, to support the association between the uses of SSRI in Saudi AD patients, there is a need to study the association between BDNF gene Val66Met polymorphism and AD.

The experimental work in this part of the research presented here provides one of the first investigations into how to evaluate the relevance of selected single nucleotide polymorphism (rs6265/G196A) with the use of SSRI in Saudi AD patients.

7.2 Methodology

7.2.1. Study patients and blood sampling

The samples investigated involved 40 participants diagnosed with AD (mean age at onset, 78.2 ± 5.2 years), 140 control subjects (mean age, 74.4 ± 6.9 years). All the participants were recruited for this study from KFMC, a tertiary hospital in Riyadh, which is administered by the Ministry of Health in Saudi Arabia. The study was approved by the ethics committees of both the University of Brighton (UoB) and the KFMC.

All the patients were diagnosed and met the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorder Association (NINCDS/ADRDA) criteria for possible AD. The age at onset of obvious cognitive dysfunction, including memory problems, was obtained from spouses or relatives and served to identify the age at onset of AD. Patients with evidence of vascular and "mixed" dementia were excluded. The control group was also recruited from the King Salman Social Center. The controls were given clinical, mental and neurological examinations to rule out cognitive deficiency, and had Mini-Mental Status Examination >27 . All the AD and control subjects were of Saudi nationality.

Exclusion criteria for both groups included recent (within 6 months) alcohol or drug abuse as defined by DSM-IV criteria, concurrent psychotherapeutic interventions, poor medical health or abnormal clinical laboratory results, active suicidality, and the use of medications that could interfere with the study. All subjects were screened for medical illness by completing a medical review sheet, involving cardiopulmonary and physical examinations. All participants gave written informed consent and agreed to be recruited into the study after a brief explanation of its objectives.

The participants' demographic and clinical data were obtained retrospectively through a medical record review or through an interview during the collection of the blood samples.

7.2.2 DNA extraction and quality assurance

Genomic DNA was extracted from circulating blood leukocytes by using Genra Puregene Blood Kits according to the manufacturer's protocol (Qiagen) and was stored at -20°C. The BDNFVal66Met polymorphism (rs6265) was genotyped using TaqMan allelic discrimination, with the ABI Prism®7900HT Sequence Detection System (Life Technologies). The sequence of interest was amplified using the following primers:

5'-CTGTCTTGTGTTTCTGCTTTCTCCCT-3' and reverse primer

5'-ACCCTCATGGACATGTTTGCA-3'. Wild type allele (Val) was detected using a 5'-CTTTCGAACACGTGATAG-3' VIC fluorescent probe. Mutant allele (Met) was detected using 5'-ACTTTCGAACACATGATAGA-3' FAM-fluorescent probe. For both genes, RT-PCR was performed on a TaqMan ABI PRISM® 7000 sequence detection system (Applied Biosystems) for allelic discrimination. Participants were classified into three groups: AA, AG and GG (Val/Val, Met/Val Met/Met genotypes).

7.2.2.1 DNA extraction

Five ml of peripheral blood was sampled from the arm by venipuncture from each AD patient and elderly participant into purple top vacutainer tubes (Beckton Dickenson, Franklin Lakes, NJ USA) containing K2EDTA to a final concentration of 1.5- 2.0 mg/ml blood. The blood was immediately mixed with the EDTA by repeated gentle tilting of the tube in order to prevent the coagulation of blood by binding calcium ions, and then delivered to the lab for DNA extraction.

The samples were then processed immediately or stored at 4 degrees centigrade and the DNA was extracted within 72 hours.

Genomic DNA was isolated from the whole blood using the Genra Puregene DNA Purification Blood Core Kit C (Qiagen Sciences, Germantown, Maryland, USA). Briefly, up-to 3 ml of whole blood were added to 9 ml red blood cell lysis buffer containing 0.15

M ammonium chloride, and incubated for 5 minutes, with continuous mixing, to facilitate the complete lysis of erythrocytes. The mixture was centrifuged at 2000 g for 2 minutes and the supernatant discarded. The leucocyte pellet was re-suspended in 3 ml of cell lysis buffer solution by vigorous vortexing to facilitate complete lysis of the white blood cells. Proteins in the lysate were removed by adding 1 ml protein precipitation solution (containing 3.9mM ammonium sulphate), vortexing for 20 seconds, and centrifuging at 2000 g for 5 minutes to precipitate the protein fraction. The DNA-containing supernatant was added to 3 ml isopropanol in a fresh 15 ml tube and mixed gently to precipitate genomic DNA. The tube was centrifuged at 2000 g for 3 minutes, the supernatant discarded, and the DNA pellet washed twice in 3 ml of 75% ethanol. The DNA pellet was air-dried and dissolved in 250 µl hydration solution (containing Tris-EDTA buffer, pH8.0) at 65 degrees centigrade for 1 hour.

7.2.2.2. DNA Quantification and purity

The concentration and fidelity of the genomic DNA were determined using a Nanodrop ND-1000 spectrophotometer (Wilmington, DE, USA) at 260 nm wavelength. The sample quality was confirmed by ensuring the 260:280 ratio was 1.8 ± 0.1 , and the 260:230 ratio was approximately 2.0 ± 0.2 . Since nucleotides absorb UV light at 260 nm while proteins have a strong absorbance at 280 nm, a value of the 260:280 ratio significantly below 1.8 may indicate protein contamination. Stock samples were then dispensed in 50 µl 74 aliquots in 1.5 ml vials, labeled, and stored at -20°C. Before their use in PCR, stock DNA samples were diluted in a hydration solution to a working concentration of 50 ng/µl.

7.2.3 Association studies

The genotyping for the association study was performed in 40 patients and 140 controls of Saudi origin by real-time PCR using Taqman chemistry in the ABI Prism 7900HT Sequence Detection System (Applied Biosystems Inc. CA, USA). Primers and TaqMan probes were designed using the Primer Express software V2.0 (Applied Biosystems, Foster City, CA, USA). Real Time PCR assay and probe melting point hybridization analyses were used to detect allelic changes. BDNF-F and BDNF-R were used to amplify the 184 bp fragment containing G→A transition responsible for the val66met change. The fluorogenic probes, bearing a suitable reporter dye on the 5'-end and a quencher dye on the 3'-end, were hybridized to the specific complementary sequence bearing the SNP. One probe (for allele 1) was labelled with VIC dye, and the other (for allele 2) with FAM dye at the 5'-primer end. During the primer extension and synthesis of the nascent strand by Taq polymerase, exonuclease activity cleaves the annealed probe, thereby releasing the reporter dye from its proximity to the quencher and allowing emission of fluorescence. Serial dilutions of the probes were run first to determine the optimal working concentration. For each reaction, a 25 µl master-mix was prepared by mixing 5 µl containing 50 ng DNA, 12.5 µl of 2x Universal mix (Eurogentec, Liege Science Park, 4102 Seraing, Belgium), 1.25 µl of 20x probe assay mix (containing primers at 50 µM and probes at 5 µM stock concentrations), and 6.25 µl DNase-free distilled water. Three no-template controls were included in each 96-well plate for normalization of the emission signal. The thermal profile for amplification for the first cycle was set at 50° C for 2 minutes (to optimize AmpErase UNG enzyme activity which prevents nonspecific product carryover), 95° C for 10 min, followed by 40 cycles of 92° C for 15 seconds and 60° C for 60 seconds. The plates were then scanned for FRET signal using the 7900HT sequence detection system, and the data analysed using SDS 2.0 software (Applied Biosystems, Foster City, CA, USA). The sample distribution obtained in the real time-PCR profile for the rs6265 in the NIS gene is given as an example in figure 7.2.

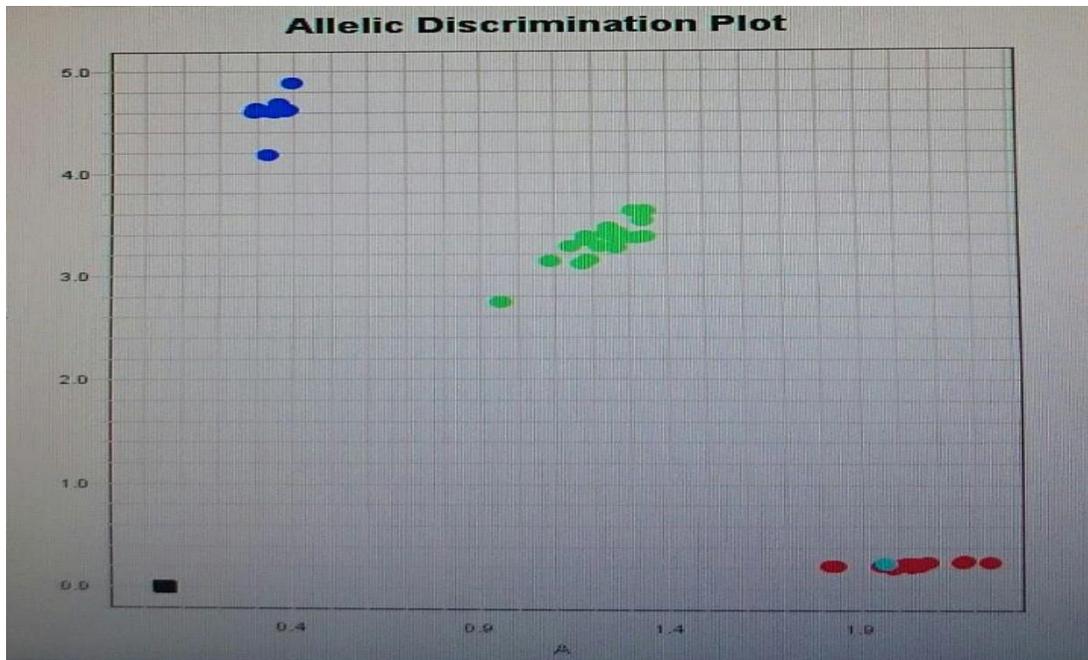


Figure 7. 2: The figure is an allelic discrimination profile obtained for the NIS-(rs6265/G196A) genotypes on a 96 well-plate. The discrimination is indicated by the genotypes being in three different Y zones of the chart. For example, the AA genotype (VIC, red) is found in zone Y<1.0 and X>1.0, the GG (FAM, blue) in the zone Y>1.0 and X<1.0, and the heterozygote AG (both, green) in the zone Y>1.0 and X>1.0. The black symbols indicate three no template controls, while any other symbols outside these zones represent undetermined genotypes.

7.2.4 Fidelity test

Genotyping for randomly selected patients among the population sample was repeated under the same reaction conditions and comparable results were obtained in each case. This indicates that the ABI Prism 7900HT Sequence Detection System (Applied Biosystems Inc. CA, USA) machine used for real-time PCR is reliable.

7.2.5 Statistical Analysis

7.2.5.1 Power analysis and sample size calculation

The sample size was calculated based on the power of 80% using the assumption that SNPs are informative when they are present in 10% of the sample, against a one-sided alternative with the size of the critical region = 5%.

7.2.5.2 Hardy–Weinberg equilibrium

For a population in Hardy-Weinberg's equilibrium: $p^2+2pq+q^2 = 1$

Where p^2 = Frequency of MM genotype; $2pq$ = Frequency of MN genotype; q^2 = Frequency of NN genotype, M is the common allele and N the rare allele.

7.2.5.3 Association statistics

The frequencies of BDNF alleles and genotypes were estimated by gene counting. Comparisons of genotype and allele frequencies were made using the Analysis of Variance (ANOVA) or Student's t-test as appropriate chi square test analyses, and Fisher's exact test, if necessary. Odds ratios (OR) corresponding to 95% CI were used to estimate the association between variables. The interactions between serum BDNF concentrations, cognition stages, age, gender and use of SSRIs with both genotype frequencies and allele distributions were analyzed by the General Linear Model. The statistical significance level was set at $p < 0.05$. All statistical analyses were performed with Minitab 18 software for Windows.

7.3 Results

7.3.1 BDNF Val66Met (rs6265/G196A) genotype frequency and Allele distributions among all participants

The frequency of the BDNF Val66Met genotype among all of the participants (n=180), was divided into 72 (40%) participants who were AA homozygotes (Met/Met), 60 (33.4%) participants with AG heterozygotes (Met/Val) and 48(26.6%) GG homozygotes (Val/Val). The distribution of BDNF Val66Met (rs6265/G196A) alleles among all of the participants (360 alleles in total) were divided into 205 (56.94%) A allele (Met) and 155 (43.06 %) G allele (Val) distribution.

The results showed that age did not affect genotype frequency. The genotype frequency among different age groups included the young age group, 25-35 (N=34); the middle age group, ranging from 36-59 (N=41); and an elderly group aged 60 and above (N=65). There was no significant difference between age and genotype frequency as the p-value=0.231. However, when considering allele distributions (N=360), there was a significant difference between the age groups (p-value=0.048). The frequency of the G allele was lower in the older age group.

Neither gender nor the use of SSRIs showed a significant difference in either genotype frequency. The gender finding for genotype frequency was p-value=0.227 and allele distribution p-value=0.369. Also, SSRIs showed p-value=0.445 for genotype frequency and allele distribution p-value=0.167.

There was a significant difference in genotype and allele frequency between the different cognition groups, p-value= 0.004. Because of these findings, it was appropriate to explore the effect of age, gender and SSRIs use with respect to genotype and cognition status. See table 7.1.

variables	Genotype			p value	Allele		p value
	AA (%)	AG (%)	GG (%)		A (%)	G (%)	
Age	N=180			0.231	N=360		0.048
Young	10(29)	13(38)	11(32)		34(50)	34(50)	
Middle	14(34)	12(29)	15(37)		40(49)	42(51)	
Elderly	48(46)	35(33)	22(21)		131(62)	79(38)	
Gender	N=180			0.227	N=360		0.369
Male	37(40)	35(38)	20(22)		109(59)	75(41)	
Female	35(40)	25(28)	28(32)		96(55)	80(45)	
Use of SSRIs	N=180			0.445	N=360		0.167
No	57(38)	51(34)	42(28)		166(77)	134(23)	
Yes	15(50)	9(30)	6(20)		39(65)	21(35)	
Cognition stages	N=180			0.004	N=360		<0.001
No cognition changes	42(34)	41(33)	40(33)		125(51)	121(49)	
Very Mild	6(38)	3(19)	7(44)		15(47)	17(53)	
Mild	8(53)	7(47)	0		23(77)	7(23)	
Moderate	12(67)	6(33)	0		30(83)	6(17)	
Severe	4(50)	4(50)	0		12(75)	4(25)	

Table 7. 1: The BDNF Val66Met (rs6265/G196A) Genotype frequency (N=180) and Allele distributions (N=360) of all participants

7.3.2 BDNF Val66Met (rs6265/G196A) genotype frequency and Allele distributions among AD patients and the control elderly.

To further investigate the differences of genotype between AD disease status and the elderly age matched group, the data were divided into two groups: AD patients (N=40) and elderly control age matched above 60 years old (N=65). Interestingly, the findings showed that there was no significant difference between the BDNF Val66Met genotype frequency and allele distributions between the AD patients and age matched elderly control subjects (p-value =0.23, p-value =0.54 respectively). See Table7.2.

Groups	Genotype distribution (%)					Allele distribution (%)			
	N	AA	AG	GG	P	N	A	G	P
Elderly	65	27(41.5)	23(35.3)	15(23.2)	0.23	130	77(59.3)	53 (40.7)	0.54
AD	40	21(52.5)	12(30)	7(17.5)		80	54(67.5)	26 (32.5)	

Table 7. 2 : Distribution of Val66Met (rs6265/G196A) genotype frequency and allele distribution for control elderly group (N=65) and AD patients (N=40)

7.3.3 Association of gender with BDNF Val66Met (rs6265/G196A) genotype distributions and allele frequencies in the elderly group and AD patients

Overall, there was no difference between the genotype frequency and/or allele distributions in AD patients and the control elderly group. The association between gender and BDNF A196G allele frequency, when the samples were stratified by gender, showed no association between allele frequency: in the male subgroups, 25% versus 32.5% GG $p=0.459$, and in the female subgroups GG 47.7% versus 32.5% $p=0.104$. Additionally, no significant association between the BDNF genotype frequencies between the male AD cases and the male control groups was detected ($p=0.774$) (Table7.3).

Gender	Groups	Genotype distribution (%)				Allele distribution (%)		
		AA	AG	GG	<i>p</i>	A	G	<i>p</i>
Male	Elderly	11(34.7)	8(32.6)	1(23.1)	0.744	30(75)	10(25)	0.459
	AD	9(45)	9(45)	2(10)		27(67.5)	13(32.5)	
Female	Elderly	16(36)	15(33)	14(31)	0.151	47(52.3)	43(47.7)	0.104
	AD	12(60)	3(15)	5(25)		27(67.5)	13(32.5)	

Table 7. 3 :Gender correlations with Val66Met (rs6265/G196A) genotype and allele in the elderly control group (N=65) and AD patients (N=40)

7.3.4 Association of cognition performance and BDNF Val66Met (rs6265/G196A) genotype distributions and allele frequencies in the control elderly group (age matched) and AD patients

7.3.4.1 Control group Genotype distribution and cognition performance

The genotype frequency for the elderly control group (n=65) and MMSE scores revealed a significant association using the ANOVA test ($F=4.38$ P-value=0.017). The elderly group showed that GG carrier have higher MMSE scores than AA carriers and AG carriers (figure 7.3)

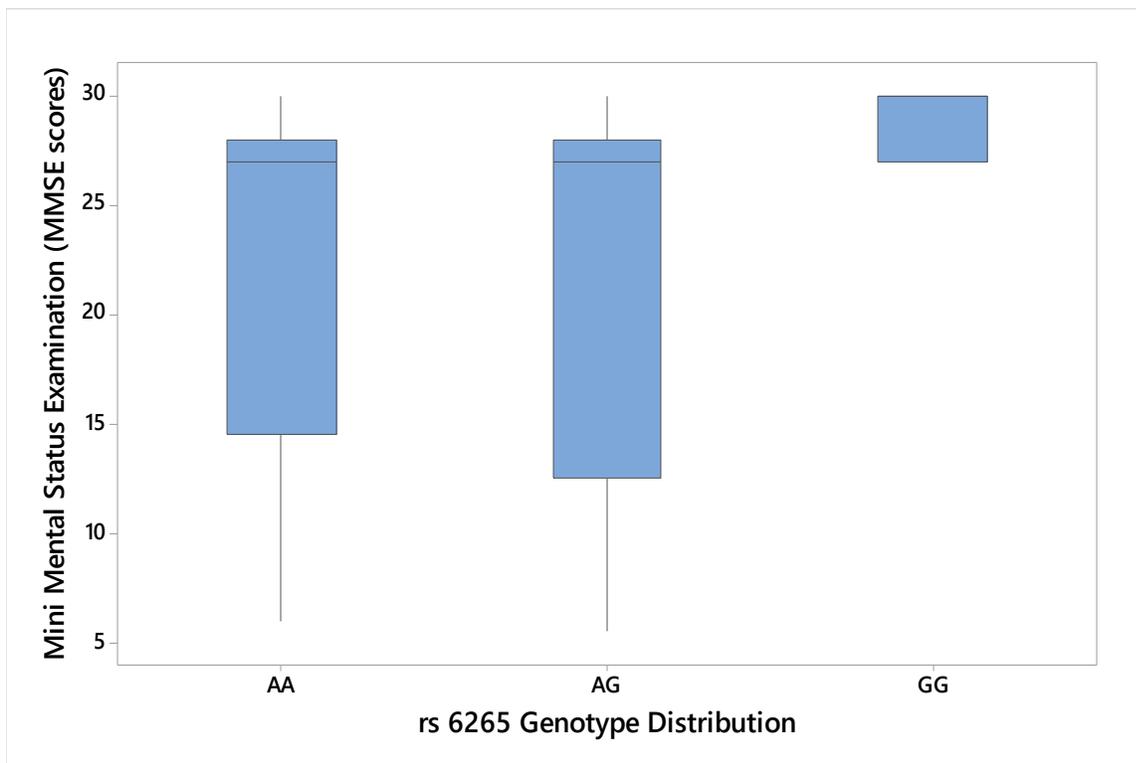


Figure7. 3: Control group genotype distribution and cognition performance (N=65)

7.3.4.2 AD group Genotype distribution and cognition performance

The genotype distributions for AD group (n=40) and CDR scores shows significant association using ANOVA test (F=6.80 p<.001). The CDR scores means for AD with no SSRIs were found to be higher in both AA carrier (12.24±4.13 SD) and AG carrier (10.57±6.47 SD) compared to AD with SSRIs. However, GG carrier shows higher scores for AD compared to the AD with no SSRIs (3.5±0.54 SD).(Table 7.4)

Patients types	Genotype	N	CDR Score			p-Value
			Mean	St Dev	95% CI	
AD with no SSRIs	AA	15	12.24	4.13	(10.13, 14.35)	p<.001
	AG	11	10.57	5.47	(8.11, 13.04)	
	GG	1	12.50	*	(4.32, 20.68)	
AD with SSRIs	AA	6	3.917	1.393	(0.578, 7.255)	
	AG	2	6.00	4.24	(0.22, 11.78)	
	GG	6	3.500	0.548	(0.161, 6.839)	

Table 7. 4: Association of cognition performance and BDNF Val66Met (rs6265/G196A) genotype distributions and allele frequencies for AD patients (N=40)

7.3.4.3 The effects of SSRIs use and genotype on CDR scores

The effects of the use of SSRIs and genotype distributions on CDR scores (after conversion of the MMSE scores into CDR scores) were explored by comparing.

Notably, for elderly and AD patients' groups, the results revealed that the CDR scores can be influenced by the use of SSRIs ($p\text{-value} < 0.001$) regardless of the genotype distributions: $p\text{-value} = 0.301$. There was an interaction between the effects of SSRIs use and genotype. (Table 7.5)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Genotype	2	121.1	60.53	1.21	0.301
Use of SSRIs	1	5015.1	5015.08	100.65	<0.001
Error	101	5032.4	49.83		
Lack-of-Fit	2	546.8	273.38	6.03	0.003
Pure Error	99	4485.7	45.31		
Total	104	10320.1			

Table 7. 5: Two-way ANOVA test between the use of SSRIs and genotype distributions in response to CDR scores for AD patients and elderly group.

7.3.5 The effects of genotype, age, gender, cognition status and use of SSRIs on serum BDNF concentrations

Five variables were tested by ANOVA using the General Linear Model (Stepwise). The relationship between cognition status, age, gender, use of SSRIs and genotype frequencies with serum BDNF concentrations were explored. In chapter six, the serum BDNF concentrations and cognition scores of elderly and AD groups were presented in detail.

The levels of serum BDNF decreased with advancing age, while the use of SSRIs showed a significant increase in serum BDNF concentrations. Age ($p < 0.001$), and the use of SSRIs ($p < 0.001$) were the main effects that showed significant association with serum BDNF concentrations.

The data revealed that gender, cognition stage and genotypes frequency were not associated with serum BDNF concentrations: $p = 0.715$, $p = 0.542$, $p = 0.715$ respectively. See table 7.6.

The analysis was then repeated for allele frequency rather than genotype. A 5-way ANOVA test using the Stepwise General Linear Model demonstrated that age and SSRI use had specific effects on serum BDNF ($p < 0.001$: both cases), but also that cognition status was associated with differences in serum BDNF ($p = 0.007$).

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Age	2	1707328	853664	144.85	<0.001
Gender	1	469	469	0.08	0.778
Use Of SSRI	1	75387	75387	12.79	<0.001
genotype	2	3959	1980	0.34	0.715
Gender*Use Of SSRI	1	1707	1707	0.29	0.591
Gender*genotype	2	2062	1031	0.17	0.840
Use Of SSRI*genotype	2	4843	2422	0.41	0.664
Gender*Use Of SSRI*genotype	2	23464	11732	1.99	0.140
Error	166	978293	5893		
Lack-of-Fit	32	100295	3134	0.48	0.991
Pure Error	134	877999	6552		
Total	179	3223424			

Table 7. 6: The effects of age, gender, use of SSRIs, genotype and cognition status on serum BDNF concentrations (n=180)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Age	2	3228614	1614307	279.94	<0.001
Use of SSRI	1	59364	59364	10.29	0.001
CDR	4	83350	20837	3.61	0.007
Error	352	2029871	5767		
Lack-of-Fit	32	66522	2079	0.34	1.000
Pure Error	320	1963349	6135		
Total	359	6446849			

Table 7. 7: The effects of age, gender, use of SSRIs, allele and cognition status on serum BDNF concentrations (n=360)

7.3.6 Association of BDNF Val66Met polymorphism (rs6265/G196A) with SSRI use

The association between the use of SSRI and BDNF Val66Met genotype distributions and allele frequencies in the elderly group and AD patients was tested. First, the association between the uses of SSRIs and genotype distribution within the elderly group was done by dividing the elderly group into two groups. The first group was the control (n=48) and the second group (n=17) was those who received SSRIs. The Chi-square test revealed significant differences between the two groups regarding both genotype distributions and allele frequencies χ^2 : p=0.031 and p=0.017 (Table 7.8). However, the association between the uses of SSRIs and genotype distribution within AD patients was not significant p=0.32. The healthy elderly almost had an equal BDNF Val66Met genotype distribution. But when comparing the BDNF GG homozygote frequency between healthy elderly and depressed elderly receiving SSRIs, there was a lack of the BDNF GG homozygote distribution in depressed elderly receiving SSRIs, while the higher frequencies BDNF GG homozygote distribution showed with the healthy elderly. However, the AD patients with SSRIs group had a higher BDNF GG homozygote distribution. This means that the AD patients with BDNF GG homozygote distribution are more prone to depression (p-value=0.001) (Table 7.9).

	Genotype distribution (%)					Allele distribution (%)			
	N	AA	AG	GG	P	N	A	G	P
Elderly without SSRI	48	18(37.2)	15(31.2)	15(31.2)	0.031	96	51(53.2)	45(46.8)	0.017
Elderly with SSRI	17	9(53)	8(47%)	-		34	26(76.5)	8(23.5)	
AD without SSRI	27	15(55.5)	11(40.8)	1(3.7)	0.32	54	41(75.9)	13(24.1)	0.950
AD with SSRI	13	6(46.2)	1(7.6)	6(46.2)		26	13(50)	13(50)	
Total	105	48(46)	35(33)	22(21)		210	131(62)	79(38)	

Table 7. 8: Distribution of Val66Met (rs6265/G196A) genotype and allele in the elderly group (N=65) and AD patients (N=40) with/without the use of SSRIs

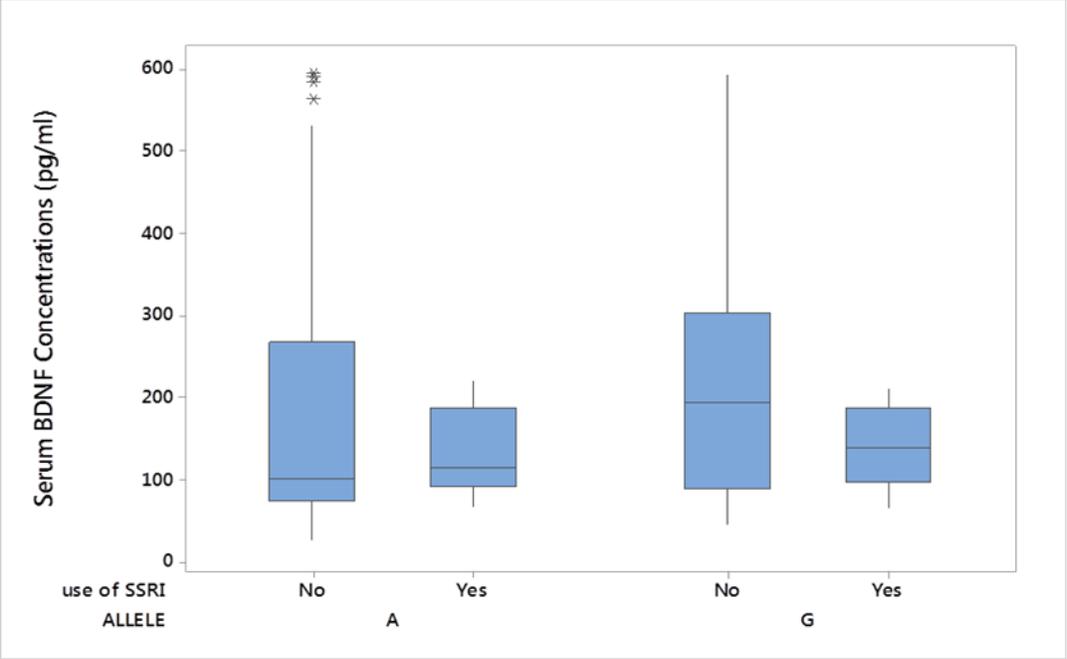


Figure7. 4: Distribution of Val66Met (rs6265/G196A) allele in the elderly group and AD patients with/without the use of SSRIs

	Genotype distribution (%)			Allele distribution (%)		
	GG With SSRIs	GG Without SSRIs	P value	G With SSRIs	G Without SSRIs	P value
Elderly	-	15	<0.001	8	45	0.001
AD	6	1		13	13	

Table 7. 9: Distribution of Val66Met (rs6265/G196A) GG genotype and G allele in the elderly group and AD patients

7.4 Discussion

The results show significant differences in BDNF Val66Met genotype distributions and allele distribution between cognition impairment groups. The research proved that the BDNF Val66Met genotype distribution is a significant contributory factor to the development of AD stages. To focus more, the BDNF Val66Met genotype distributions and allele frequencies showed significant association with cognition performance for both the elderly control group and AD patients using Clinical Rating Scales and Mini Mental examination tests as cognitive test tools. The main findings showed that GG homozygotes (Val/Val) have superior cognition performance among AD patients and elderly control groups. In fact, there was no significant difference between BDNF Val66Met genotype distributions within all participants in the research findings. However, a number of studies suggest that Val66Met BDNF SNP is linked to brain morphology (Tanzi, 2012). Evidence from neuroimaging studies has shown that BDNF A allele (Met) carriers have significantly greater reductions in the dorsolateral prefrontal cortex, caudate nucleus, and frontal grey matter volume, as well as smaller hippocampus volumes, compared to BDNF G allele (Val) carriers (Duijn, 2002; Tanzi, 2012; Lee *et al.*, 2005). The research also found an association between a lower BDNF serum concentration and poorer cognition performance regardless of the BDNF Val66Met genotype distributions. The justification is that the BDNF Val66Met genotype is an important factor in AD pathology as Buchman *et al.* found; i.e. that the effect of AD pathology on cognitive decline was explained by higher brain BDNF expression associated with slower cognitive decline (Buchman *et al.*, 2016). However, Lim *et al.* suggested that BDNF Val66Met gene polymorphism could be a factor in moderating cognitive performance through Amyloid-B in preclinical AD. Lim *et al.* claim that individuals with A allele (Met) carriers can expect to show clinically significant memory impairment after 3 years, whereas BDNF G allele (Val) individuals can expect a similar degree of impairment only after 10 years (Lim *et al.*, 2015). Also, the research findings broadly support the work of other studies in this area linking BDNF Val66Met Polymorphism and cognition performance in Antipsychotic-Naïve Patients with Schizophrenia (Bian *et al.*, 2005).

In this study, the findings found to be a relation between cognition performance and the effects of BDNF Val66Met genotype distributions. First, in depressed geriatric patients, Douglas et al. (2010) revealed that there was no association between cognition performance and BDNF genotypes using cognitive tests of word list learning, prose recall, nonverbal memory, or digit span, i.e. hippocampal functions. Also, there was no association between BDNF genotypes with hippocampal volume change (Douglas *et al.*, 2010). Second, the explanation for the role of BDNF Val66Met genotype distributions and cognition performance was explored by a study conducted recently by Lamb et al. which looked at the genetic influences on normal memory variation in healthy young adults. The findings established that the effect of the BDNF Val66Met genotype polymorphism was specific to hippocampal-dependent forms of memory, such as recall, and demonstrated that the BDNF G allele (Val) benefits recall performance while not influencing familiarity-based recognition performance (Lamb et al., 2015). Third, a systematic review concluded that variations in BDNF Val66Met genotype distributions have a significant effect on memory performance in healthy volunteer and psychiatric diagnoses; none of the reviewed articles included AD diagnosis. The review claims that BDNF Val66Met genotype has an impact on memory functions by acting on the structure and physiology of the hippocampus, with poorer cognition performance in BDNF A allele (Met) carriers (Kambeitz *et al.*, 2012).

It is interesting to note that there was a deviation from the Hardy–Weinberg equilibrium (HWE) found in the genotype distribution of the Val66Met polymorphism for all participants, including healthy participants and AD patients. However, the results showed no deviation from the Hardy–Weinberg equilibrium found in the genotype distribution of the Val66Met polymorphism for healthy participants only. Deviations from HWE are not necessarily due to genotyping error and may be due to chance or genetic factors which include a heterozygous advantage, population admixture/substructure, inbreeding or copy number variants. The ability to detect deviations from HWE depends on the magnitude of the deviation, sample size and α level. When tests for HWE are performed for genotype quality control there is no consensus on which α level should be used, and the p value criterion used to reject the null hypothesis of HWE varies greatly within the literature. Substantial variation was detected in BDNF coding region single-nucleotide polymorphism (SNP) allele and haplotype frequencies between 58 global populations, with the derived Met allele of Val66Met ranging in frequency from 0 to 72% across populations. Within the literature, the frequency of the Met allele has a wide range of values: from 0.55% in Sub-Saharan Africans to 19.9% in Europeans and 43.6% in Asian groups (Petryshen *et al.*, 2010). Within the current results, the distribution of the Met allele for all participants including AD patients was 59.94 %, which is higher than that which is found within the Asian populations. For healthy participants in Europe, it was found that Met allele frequencies were higher than in African populations and lower than in Asian populations (Vulturar *et al.*, 2016).

The most interesting finding in this research was the disappearance of the significant association between BDNF Val66Met (rs6265/G196A) allele and genotype frequencies within AD cases and the controls in the total samples from the Saudi population. Interestingly, the samples illustrated that there was no association between serum BDNF concentrations and the BDNF Val66Met genotype in AD patients or any other age groups. Similarly, Coskunoglu et al. published a study that supports these research findings; they showed lower serum BDNF concentrations in tinnitus patients, but no statistically significant relationship was observed between the polymorphisms of the BDNF gene and the serum BDNF concentration (Coskunoglu et al., 2017). However, the results are different with depressed patients diagnosed with type 2 diabetes; the BDNF serum levels were significantly correlated with the BDNF Val66Met (rs6265/G196A) allele and genotype frequencies (Zhou et al., 2013). Inconsistencies in the association between serum BDNF concentrations and BDNF Val66Met (rs6265/G196A) allele and genotype frequencies may be explained by diagnosis, race and/or age. First, two studies investigating the relationship between genotype and serum BDNF concentrations were performed in depressed subjects (Ozan *et al.*, 2010; Egan *et al.*, 2003); others were performed in healthy subjects. Secondly, two studies were carried out in Asian populations; others comprised Caucasians only. Studying a Caucasian community sample, Li et al. were especially interested in gender effects (Li et al., 2017).

The current results showed no significant difference in serum BDNF concentration between the genders or between BDNF Val66Met (rs6265/G196A) allele and genotypes for AD patients. There was no difference in genotype or allele distribution between the genders, nor were there differences in the serum BDNF. These results contradict the results of a recent study conducted in China that found a female-specific effect of the BDNF Val66Met (rs6265/G196A) allele and genotype frequencies on serum BDNF in AD (Li *et al.*, 2017). On the other hand, in the earlier investigations of this thesis, females were found to have a lower serum BDNF than males. The current results indicate that this effect is not related to genotype. A possible explanation for this might be due to the role of estrogen that rapidly up-regulates BDNF mRNA in the cerebral cortex and the olfactory bulb of ovariectomized animals (Kaphingst *et al.*, 2010). Also, the relative levels of BDNF mRNA and protein in specific regions of the brain were significantly affected by hormone replacement (Gibbs, 1999). In menopausal women, estradiol falls to an undetectable level with aging (Pluchino *et al.*, 2013). Meanwhile, the decreased level of BDNF mRNA in the frontal cortex of females has also been observed in the aging process (Tapia-Arancibia *et al.*, 2008). All this evidence indicated a potential positive correlation between BDNF and estrogen, and the gender-specific effect of rs6265 on AD may be caused by the interaction between BDNF and estrogen (Fukumoto *et al.*, 2010). Recently, sex-specific differences in BDNF genetics, as well as serum BDNF levels, have been demonstrated (Matyi *et al.*, 2017)

Regarding the association of age and allele distribution, there were no significant associations with aging changes. These findings are supported by a recent study that found that there is no association between BDNF polymorphism and age in AD patients (Solmaz *et al.*, 2017). However, another study found a significant interaction between age and baseline whole brain volume and BDNF rs6265 SNP, showing GG homozygote (Val/Val) individuals have lower whole brain volumes with increasing age compared to the AA homozygote (Met/Met) and A allele (Met) carriers (Honea *et al.*, 2013). To elaborate, the results in the current research suggests that the GG homozygote (Val/Val) is associated with early death. This result can be supported by a recent study that found a strong relation between suicide attempts and GG homozygote (Val/Val) individuals in major depression disorders (Youssef *et al.*, 2018). Additionally, this study supports our results that we did not find BDNF serum concentrations associated with genotype distributions. Also, Mirowaska-Guzel *et al.* found that there were no significant differences of allele and genotype distribution between ischemic and hemorrhagic stroke patients, but individuals with a higher risk of developing ischemic stroke have a higher rate (64 %) of GG homozygote (Val/Val) distribution than AA homozygotes (Met/Met) (2%) (Mirowska-Guzel *et al.*, 2012). The possible explanation of early death in the studied population is cardiovascular disease.

Other than early death, another possible explanation of the lack of GG individuals amongst the elderly participants is population changes in Saudi Arabia. In Saudi Arabia, immigration could be a factor to explain the lower prevalence of GG homozygotes (Val/Val) in elderly participants. Most Saudis are ethnically Arabs, the majority of whom are tribal Bedouins. According to a random survey, most would-be Saudis come from the Indian subcontinent and Arab countries. Many Arabs from nearby countries, particularly Egypt, are employed in the kingdom; the Egyptian community has developed gradually from the 1950s onwards. In the 1970s and 1980s, there was also a significant community of South Korean migrant labourers, numbering in the hundreds of thousands, but most have since returned home; the South Korean government's statistics showed only 1,200 of their nationals living in the kingdom as of 2005. There are also significant numbers of Asian expatriates from India, Pakistan, Bangladesh, Indonesia, Philippines, and recently refugees from Syria and Yemen. Petryshan *et al.* report that the G allele is more prevalent in the Asian population than the African (Petryshen *et al.*, 2010). The immigration of Asians into Saudi Arabia could account for an increase in the prevalence of the G allele, as evidenced by higher frequencies in younger populations.

However, the ANOVA test showed that the serum BDNF results were statistically significantly influenced by the use of SSRI and age, but not BDNF Val66Met (rs6265/G196A) allele and genotype frequencies. This finding of reduced BDNF with age was also described by Bian *et al.* (2005) in Chinese Alzheimer's disease patients (Bian *et al.*, 2005), with no influence of genotype. The association between serum BDNF, antidepressant use and cognition is important. This observation may support the hypothesis that serum BDNF can alter without cognition change and progression of AD without any influence of BDNF Val66Met (rs6265/G196A) allele and genotype. According to Kemnitz *et al.*, however, variations in the BDNF Val66Met (rs6265/G196A) allele and genotype frequencies may mediate critical neurocognitive impairments observed in various neuropsychiatric conditions (Kemnitz *et al.*, 2012).

Furthermore, the results found that GG homozygotes (Val/Val) frequency was higher in the female geriatric controls group compared with the female AD cases, which seems to suggest a protection effect by the BDNF GG genotype from AD in females. This could be explained by the work of Egan et al. who found that the BDNF A allele (Met) was associated with poor episodic memory, abnormal hippocampal activation and lower hippocampal n-acetyl aspartate in human subjects (Egan *et al.*, 2003). To focus further, the result shows that in males, the GG genotype frequency was lower than females in both groups of AD patients and the geriatric group. That means in males, neither BDNF A allele (Met) carriers nor BDNF G allele (Val) carriers had any protective effects against AD. This finding contrasts with the hypothesis that the BDNF Val66Met polymorphism may affect susceptibility to regional white matter hyperintensities (WMH) volume and that such a genotype-by-WMH interaction is correlated with cognitive decline in non-demented elderly males, in which the Met allele plays a protective role (Huang *et al.*, 2014).

On the question of BDNF GG homozygote (Val/Val) carriers, this study found that the most important clinically relevant finding was that delayed memory index scores were significantly lower in the BDNF GG homozygote (Val/Val) carriers than the BDNF AA homozygotes (Met/Met) in AD patients with the use of SSRIs. BDNF GG homozygotes (Val/Val) perform better in the aged control group with respect to cognition, while in AD patients, BDNF GG homozygotes (Val/Val) were lacking, suggesting possible early death or depression. In short; these studies indicated that the BDNF Met-66 variant may influence memory in humans, with or without AD.

According to these data, we can infer that BDNF GG homozygotes (Val/Val) genotypes lose their effects with neurodegenerative diseases, i.e. GG is protective in elderly patients but not in AD patients. Together these results provide an important insight into BDNF Val66Met genotype distributions and allele frequencies in the elderly group and AD patients, and their association with the use of SSRI. The AD with BDNF GG homozygote (Val/Val) carriers had more depression symptoms compared with the elderly group, depression being defined as the use of SSRI. In contrast, findings from a meta-analysis of geriatric depression, with AD patients excluded, found an association between geriatric depression and the BDNF Val66Met polymorphism. From five case control studies, it was established that BDNF AA homozygotes (Met/Met) had increased the risk for geriatric depression more than GG. Furthermore, the Met allele was associated with decreased activity of the BDNF system (Pei *et al.*, 2012).

8. Chapter Eight: General Discussion

This chapter offers a summary of the findings, the conclusion and recommendations in accordance with the findings.

8.1 BDNF serum concentrations within the Saudi and other populations

Among the healthy participants ($n=123$), the mean BDNF serum concentration calculated was 221.5 ± 145.58 pg/ml within the 25 to 65-year-old age group. This result is corroborated by other pertinent studies, such as Boyuk et al.'s (2014) research that lately reported that the serum BDNF concentration among healthy control subjects from the Turkish population was 130.84 ± 59.81 pg BDNF/mL (Boyuk *et al.*, 2014a). However, two Brazil-based studies reported serum BDNF values far lower than these current results. The serum BDNF concentration value across 25 healthy participants aged between 18 and 60 years old was 0.747 ± 0.060 pg/ml (Dos Santos *et al.*, 2011). Furthermore, Hauck et al. determined a serum BDNF concentration of 0.25 ± 0.14 pg/ml units among healthy participants.

Additional researchers evidenced a BDNF serum concentration for healthy participants approximately 100 times greater than the present results indicate (Nettiksimmons *et al.*, 2014). However, Nettiksimmons et al. (2014) identified no significant correlation between serum BDNF levels and age among 910 healthy participants, although the age group comprised only of those 70 years old and over. Contrastingly, our healthy participants were aged 25 years and over, which may have helped in reaching an accurate result compared to older participants. Generally, the results for the Saudi population highlights that BDNF concentrations decline in late adulthood, which is an important consideration while comparing values with those of AD patients. Further research shows that the serum BDNF concentration in Italy among healthy participants aged 18-65 was $41,590 \pm 7,820$ (pg/ml), while for 382 healthy control patients (including 237 females) in the Netherlands aged 18–65, the concentration was 9490 ± 3180 ; $p > 0.05$ (pg/ml) (Shimizu *et al.*, 2003).

Heterogeneity in the sample characteristics, study methodology and measures of outcome was determined; essentially, the BDNF protein concentrations were measured as serum, plasma and CSF and various measurement units were used across the studies (pg/ml and pg/ μ g), thus restricting comparability. Preferably, comparing the research reading for serum BDNF concentrations with the previously reported BDNF concentrations records using pg /ml (1ng/ml = 1000 pg/ml or pg/ μ g) should have been undertaken (Table 8.1).

The current research among the elderly Saudi population aged 65 and over determined that the serum BDNF concentration was 80.3 ± 27.84 pg /ml. Comparing the serum BDNF concentration among the elderly from other research, a serum concentration of 130.84 ± 59.81 pg/ml was found for the elderly population above 60 years old (Boyuk *et al.*, 2014a). However, the results here obtained might have been influenced by potential limitations such as the criteria for patient or control recruitment, which frequently do not take into account parameters and/or factors that affect BDNF measurements.

In Saudi AD patients, the serum BDNF concentration was 76.3 ± 21.64 pg/ml; compared to other research, the concentration of serum BDNF was $30,900 \pm 9.172$ pg/ml (Hall, 2011). Lee et al. found that the serum BDNF concentration among Korean AD patients was $22,900 \pm 5000.0$ pg/ml (Lee et al., 2009).

Author	Number of Participants	Age	Serum BDNF concentrations(pg/ml)
Boyuk et al. (2014)	33	Above 60	130.84 ± 59.81
Dos Santos et al. (2011)	25	18-60	0.747 ± 0.060
Hauck et al. (2010)	34	14-65	0.25 ± 0.14
Maina et al. (2010)	24	18-65	$41,590 \pm 7,820$
Molendijk et al. (2011)	50	18-65	$27,700 \pm 11,400$
Shimizu et al. (2003)	382	18-65	9490 ± 3180
Strohle et al. (2010)	12	18-65	$97,450 \pm 7291.15$

Table 8. 1: Comparison of studies into serum BDNF concentrations among healthy control participants

The previous comparisons clearly evidence a significant degree of variability among the BDNF measurements. This can be explained by the different measurement methods. As BDNF is released from platelets during the blood coagulation process, its levels may be readily measured in the serum (Naegelin *et al.*, 2018). However, concerns have been raised following reports that different commercially available ELISA kits for measuring human serum BDNF levels indicate discrepant values for the same tested human serum samples (Polacchini *et al.*, 2015). Moreover, this variability has been explained by problems associated with serum collection process. Possibly, the effect of clotting duration, storage time and temperature may affect the serum BDNF concentrations. Recently, Amadio *et al.* explained that the serum BDNF levels obtained following incubation of blood at 37°C will increase more rapidly compared to samples at room temperature. Specifically, a serum BDNF level obtained at 37°C for 10 minutes, or at room temperature (RT) for 30 minutes (min), reflects the BDNF protein primarily released from platelets. Contrastingly, when serum is obtained after a longer clotting time (>60 min at 37°C or 120 min at RT), its serum levels may reflect the total amount of BDNF produced during thrombus formation *in vivo*. Significantly, BDNF is typically measured in the serum obtained following 1 hour (h) of clotting at room temperature. Additionally, the sample's storage conditions affect the serum BDNF concentrations (Tsuchimine *et al.*, 2014). Significantly, the measurement methods vary according to the detection of antibodies through each kit, enabling the recognition of mature BDNF, pro-BDNF, or both. For example, the blotted BDNF standards were commercial pro-BDNF (Alomone; 10 pg/lane), mature BDNF (1 and 2 from Alomone and Sigma, respectively, both 1000 pg/lane) and the standard BDNF protein included in each kit (Aviscera-Bioscience and Biosensis, 10 pg/lane; Millipore-ChemiKine™, Millipore-Milliplex®- and R&D System-Quantikine®, 100 pg/lane; Promega-Emax®, 1000 pg/lane) (Naegelin *et al.*, 2018; Polacchini *et al.*, 2015).

8.2 BDNF serum concentrations in relation to other variables

The results answered an important question from the research objectives regarding the various variables such as age, sex, BMI and incidence of DM that could have contributed as confounding factors determining the serum BDNF concentrations (Shimada *et al.*, 2014).

Firstly, concerning the relationship between serum BDNF and age, the current thesis evidenced that the serum BDNF concentrations among healthy participants declined with ageing. This result responds to the first research question in the thesis. Acknowledging the sample characteristics, our result shows that the serum BDNF concentrations of the healthy younger group (25-35 years old) and middle age group (36-59 years old) were approximately equal, whereas the serum BDNF concentration of the elderly control group 60 years old and above (n=48) was approximately 75% lower than the younger groups. Generally, the uniqueness of the current results for the Saudi population emphasises that BDNF concentrations diminish in late adulthood, which is a fundamental assumption when comparing values with those of AD patients as both had low serum BDNF concentrations. Consistent with other studies (Lommatzsch *et al.*, 2005; Ziegenhorn *et al.*, 2007), serum BDNF concentrations declined with advancing age. This was explained previously as being due to hippocampal shrinkage as hippocampal volume declined with advancing age, which increased the risk of cognitive impairment (Schneider *et al.*, 2017). Although the current results show lower BDNF concentrations with advanced age, the brain's morphological status in our samples was not measured by magnetic resonance imaging (MRI). However, based on previous evidence, the reduction in serum BDNF concentration was given to be related to both a decline in hippocampal volume and elevated memory deficits. Therefore, the thesis have reasoned that BDNF concentrations might be associated with age-related hippocampal volume loss. Consistent with this hypothesis, we found that increasing age was associated with reduced levels of BDNF, and reduced levels of BDNF were related to the decline in hippocampal volume.

Secondly, as in previous studies, we were encouraged to consider gender as a factor when designing experiments that focus on BDNF concentrations. Our present study found that there was a difference in BDNF concentrations, based on gender, between healthy control groups, but not in AD patients and the elderly control group. Our results showed that a difference exists regarding the relationship between serum BDNF and gender among healthy participants. This can support the hypothesis that claims a significant difference in the brain morphology and anatomy in the genders (Koscik *et al.*, 2009). That may explain how gender affects the expression, signaling and functions of BDNF. Further to this, recent research has suggested that sex hormones or steroids can modulate the activities of BDNF, which may account for the functional discrepancy in the different sexes (Chan & Ye, 2017). Indeed, the cross-talk between BDNF and sex steroids has been known for many years; some sex steroids, like estrogen, have a positive regulatory effect on BDNF expression and signaling (Pol *et al.*, 2006). Previous studies have found differences in the serum BDNF concentrations between depressed men and women. Karege *et al.* (2002) found that serum BDNF concentrations were decreased in women, and also found that women endured a greater severity of depressive symptoms than men (Karege *et al.*, 2002). However, we did not observe a statistically significant association between BDNF serum concentrations and the severity of AD, regardless of gender. This result can be explained by the chronicity of AD as studies have indicated that symptoms worsen with the longer duration of the disease due to high rates of the disease prognosis (Cerejeira *et al.*, 2012). In our study, the target AD population consisted of elderly patients newly diagnosed with AD. Also, among the AD patients and the elderly, all women were known to be post-menopausal; this may explain why there was no evidence of estrogen inducing BDNF expression (Pluchino *et al.*, 2013).

At present, it is not clear why there was a significant difference in BDNF concentrations between males and females only in the control groups, but not in patients. This deserves further investigation. It should be mentioned that not controlling the menstrual cycle in the female patients in the present study is a limitation, despite the fact that in the control group no difference was found between the genders. This limitation should be remedied in future investigations.

Thirdly, the relationship between serum BDNF concentrations and BMI was considered as one of the restricting factors in this research due to the reduction in BDNF expression in the brain, as well as mutations in the BDNF gene and/or of its receptor, being linked to obesity in both human and animal models. For example, BDNF and its receptor (TrkB), are abundantly expressed in hypothalamic regions believed to be important for the maintenance of normal body weight (Ziegenhorn *et al.*, 2007). Also, a recent systematic review found a significantly positive association between BDNF polymorphisms and obesity risk (Akbarian *et al.*, 2018).

Our present study found that there was a decrease in serum BDNF concentrations with an increase in BMI among the healthy control groups, but not in AD patients. The current findings showed that the serum BDNF concentrations decreased with the increase of BMI in the healthy participant group. This can be explained as a positive correlation with the hypothesis of BDNF concentration and BMI that claims the role of food energy intake downstream through the leptin signaling pathway causes the serum BDNF to decline with obesity. Also, it supports the hypothesis positing that BDNF gene mutations may be correlated to certain obesity types or other eating disorders (Xian Liu *et al.*, 2014).

However, a recent meta-analysis, involving 10 studies, carried out a systematic search that assessed the association between obesity and circulating BDNF concentrations. It revealed no significant difference between BDNF concentrations in obese patients and controls. (Sandrini *et al.*, 2018). This review can support the findings regarding AD patients as there was no association between BDNF concentrations and BMI. In addition, the age group can contribute to the explanation as all the AD patients and elderly were over 65 years old and it has been reported that hormonal status influences circulating BDNF (Pol *et al.*, 2006).

Lastly, we consider DM as a contributing factor that has an effect on serum BDNF concentrations. As previously discussed see Chapter 2 for the potential mechanisms that connect BDNF action and the development of type 2 diabetes mellitus. A recent review article explained the effects of antidiabetics on BDNF and its association with Type 2 Diabetes Mellitus and concluded that BDNF may enhance the energy expenditure, ameliorate systemic glucose balance, and improve insulin sensitivity, and it may be useful in the prevention and management of T2DM (Eyileten *et al.*, 2017).

In healthy participants, the present results demonstrated that serum BDNF concentration diminishes with increasing glycosylated haemoglobin, indicative of diabetes mellitus. This finding matches the potential theoretical mechanisms of the link between BDNF concentrations and the development of type 2 diabetes. The analytical theory can be explained as BDNF contributes the same mechanism as leptin in regulating lipid metabolism (Xian Liu *et al.*, 2014). Also, in animal experimental studies, BDNF treatment of obese and diabetic subjects had a positive effect on glucose and lipid metabolism. A study proved that the administration of BDNF subcutaneously in obese mice diminished food intake and ameliorated impaired glucose tolerance (Montalbano *et al.*, 2016).

However, AD patient findings in the current research established that glycosylated haemoglobin in patients was not linked to decreased BDNF concentrations. This has been explained previously by Liu *et al.* (2016). Serum BDNF concentrations vary according to glucose metabolism status, and genetic variants affect BMI and perhaps increase the risk of T2DM (Liu *et al.*, 2016). So, in our AD patients group, the non-correlation between serum BDNF concentrations of AD patients and glycated haemoglobin levels is explained.

8.3 BDNF serum concentrations and AD patients

The important action of BDNF concentration in developing the pathophysiological mechanism of AD has been explained in different sections of the current research. Moreover, a recent meta-analysis study has documented the fact that BDNF serum concentrations are reduced in AD patients relative to controls (Qin *et al.*, 2017). As in our findings, a comparison of serum BDNF concentrations in AD patients and the elderly control group participants showed low concentrations in both groups, with no significant difference between them. The mechanism behind lower serum BDNF concentrations in the elderly control group, as explained, is based on previous research that involved BDNF and age-dependent alterations in the hippocampus for both animal and human models (Kato-Semba *et al.*, 2007; Von Bohlen und Halbach, 2010). Furthermore, evidence suggests that disturbances in the BDNF-system also affect hippocampal dysfunctions, as in major depression or in Alzheimer's disease.

Regarding the variation between BDNF serum concentrations in Saudi patients with AD and those with MCI, the findings showed that the serum BDNF concentrations in MCI patients were higher than the serum BDNF concentrations of severe dementia patients. Such data resemble previous findings and are compatible with our research demonstrating increased BDNF serum concentrations in preclinical phases of AD and lower serum BDNF levels in severely progressed AD patients. These observations can be explained by raised serum BDNF levels mirroring a compensatory repair mechanism during early neurodegeneration which may also possess a neuroprotective function by participating in the degradation of beta-amyloid (Hall, 2011). On the other hand, our findings revealed that the serum BDNF concentrations of those with mild to moderate AD did not differ significantly from those of healthy, elderly control patients. Therefore, these results support the hypothesis of increased BDNF concentrations during both the preclinical phase of dementia (MCI) and AD's clinical stages (Angelucci *et al.*, 2010).

8.4 BDNF serum concentrations and the use of selective serotonin reuptake inhibitors

This section is based on previous research that suggested that BDNF concentrations can be modified. It is possible that BDNF plays a critical role in the effects of exercise on the human brain (Kramer and Erickson, 2007). Several studies have found that higher aerobic fitness levels are associated with larger hippocampal volumes (Erickson et al., 2009) and greater volumes of prefrontal and temporal brain regions (Colcombe et al., 2003). Also, a meta-analysis in 29 studies showed that aerobic exercise increased the resting levels of BDNF in peripheral blood (Dinoff *et al.*, 2016b). Moreover, a recent study discovered that the levels of BDNF were significantly increased after gardening activity. This revealed a potential benefit in gardening activities for the cognitive function in senior individuals (Park *et al.*, 2019). Therefore, improving or enhancing the BDNF concentrations could delay the prognosis of AD stages, and also may improve cognitive performance. For example, Laske et al. (2006) hypothesized that variations of serum BDNF could be related to different AD stages (Laske *et al.*, 2006).

The current thesis investigation has indicated that serum BDNF levels in AD patients on SSRI treatment increased relative to AD patients not on any type of SSRI treatment. These outcomes are in accordance with those from previous research on human depression, highlighting that BDNF serum levels are higher as a consequence of SSRI administration and that they enhance the depression prognosis (Owen M Wolkowitz *et al.*, 2011a). It has been well acknowledged that use of antidepressant therapies is correlated with increased serum BDNF. If lower BDNF is associated with increased AD risk, it may be anticipated that elderly patients using antidepressants may face a diminished risk of developing AD, or may not progress to more severe disease forms. This research programme investigated the relationship of elderly patients' antidepressant use to cognition, assessing the effects of antidepressant use on serum BDNF concentration among the elderly population (with or without AD). These results have significance concerning recommendations for antidepressant use among elderly patients as a form of AD delayed prognosis.

The association between BDNF serum concentration and the use of SSRI antidepressants in the research is based on the hypothesis that explained the mechanism of serotonin reuptake inhibitors. Through increased BDNF expression in the hippocampus, the findings concentrated on the outcomes of SSRI antidepressant use on BDNF concentrations in AD patients. Strong evidence indicated the presence of an interaction between BDNF and serotonin neurotransmitters, with such a mechanism explained in that BDNF promotes serotonergic neuron development and function (Martinowich & Lu, 2008). This can be explained by the theory that supports the mechanism of serotonin reuptake inhibitors, through increased BDNF expression in the hippocampus. This has been explained in detail in chapter 6. The main argument in this thesis is whether SSRIs elevate the serum BDNF concentrations and if this elevation could improve cognition performance among AD patients.

8.5 BDNF serum concentrations and cognitive performance

The correlation between the serum BDNF concentrations and altered CDR scores when comparing the serum BDNF level of MCI patients to that of severe dementia patients showed a greater level in MCI patients compared to severe AD patients. Furthermore, our results showed a significant increase in the serum levels among moderate stage patients compared with severe-level patients. Therefore, differences in serum BDNF were apparent between the MCI group and severe dementia group patients, with the former having the highest and the latter the lowest BDNF serum concentrations. Similar results were uncovered by Laske et al., namely that serum BDNF concentrations are higher during the early stages of Alzheimer's disease. The reason may be the compensatory repair mechanism of AD patients' preclinical stage, which may also contribute to increased beta-amyloid degradation (Laske et al., 2006). Nevertheless, Angelucci et al. determined that serum BDNF concentrations were significantly greater among MCI and AD patients compared to the control subjects. Moreover, the cause of increased BDNF in AD patients was not dependent on treatment with either AchEI or antidepressant drugs (Angelucci et al., 2010). The explanation for reduced BDNF concentrations with disease prognosis may be clarified by the disappearance of the brain's trophic support, alongside the high accumulation of AB in the AD-affected brain during disease progression (Feldman & Qadi, 2006). However, the effects of SSRIs on cognition show no significant correlation in AD. To assess the detailed mechanism supporting the association between serum BDNF concentrations and cognitive performance during AD, further investigation of relevant genetics will be required, involving assessment of the correlation between cognition performance and BDNF polymorphism.

8.6 BDNF gene Val66Met polymorphism (rs6265/G196A) and Serum BDNF concentrations

This study's most interesting finding is the disappearance of the significant correlation between BDNF Val66Met (rs6265/G196A) allele and genotype frequencies within the AD cases and controls in the Saudi population's total samples. Notably, the samples indicated an absence of correlation between serum BDNF concentrations and the BDNF Val66Met genotype among AD patients or any other age group. Supporting the research findings, Coskunoglu et al., (2017) evidenced reduced serum BDNF concentrations in tinnitus patients, although no statistically significant relationship was observed between the polymorphisms of the BDNF gene and the serum BDNF concentration (Coskunoglu et al, 2017). Regardless, the results were different among depressed patients diagnosed with type 2 diabetes as the BDNF serum levels were significantly correlated with BDNF Val66Met (rs6265/G196A) allele and genotype frequencies (Zhou et al., 2013). Inconsistencies in the association between serum BDNF concentrations and BDNF Val66Met (rs6265/G196A) allele and genotype frequencies may be explained by diagnosis, race and/or age. In this regard, two studies investigating the relationship between genotype and serum BDNF concentrations were undertaken involving depressed subjects; others have included healthy subjects. Two studies were carried out on Asian populations, while others comprised of Caucasians only. Investigating a Caucasian community sample, Li et al. were particularly interested in the effects of gender (Li *et al.*, 2017).

8.7 BDNF gene Val66Met polymorphism (rs6265/G196A) and cognitive performance

The results expressed significant differences in BDNF Val66Met genotype distributions and allele distribution between the cognition impairment groups. The research confirmed that the BDNF Val66Met genotype distribution is a significant contributory factor to the AD development stages. More specifically, the BDNF Val66Met genotype distributions and allele frequencies indicated a significant correlation with cognition performance among both the elderly control group and AD patients, implementing the Clinical Rating Scales and Mini Mental State examination test as cognitive assessment mechanisms. The major findings showed that GG homozygotes (Val/Val) have superior cognition performance among AD patients and elderly control groups. Indeed, no significant difference was apparent between the BDNF Val66Met genotype distributions across all participants, according to the research findings. Nevertheless, several studies have indicated that Val66Met BDNF SNP is linked to brain morphology (Tanzi, 2012). Furthermore, evidence from neuroimaging research has shown that compared to BDNF G allele (Val) carriers, BDNF A allele (Met) carriers suffer from significantly greater reductions in dorsolateral prefrontal cortex, caudate nucleus and frontal grey matter volume, while also having smaller hippocampus volumes (Duijn, 2002; Tanzi, 2012; Lee *et al.*, 2005). Moreover, the research found a relationship between reduced BDNF serum concentration and poorer cognition performance, irrespective of BDNF Val66Met genotype distributions. This can be explained given that BDNF Val66Met genotype is a crucial variable in AD pathology, with Buchman *et al.* determining that the effect of AD pathology on cognitive decline can be explained by higher brain BDNF expression, which is linked to slower cognitive decline (Buchman *et al.*, 2016).

However, Lim et al. proposed that the BDNF Val66Met gene polymorphism could be a factor in moderating cognition performance through Amyloid-B in preclinical AD. The researchers suggested that individuals with A allele (Met) carriers can expect to show clinically significant memory impairment after three years, whereas BDNF G allele (Val) individuals may see a similar level of impairment only 10 years on. Furthermore, the research findings broadly support other research in this area connecting BDNF Val66Met Polymorphism and cognitive performance in Antipsychotic-Naïve Patients with Schizophrenia (Bian et al., 2005).

8.8 General Summary and Conclusions

This research was performed by following the research conceptual framework (Figure 8.1). The first part intended to test the hypothesis that serum BDNF concentration diminishes among the elderly Saudi population. Several factors were tested and related to the serum BDNF concentration, identifying an array of variants of interest for future investigation. Several exposed variants were found to be correlated to the serum BDNF concentration. Association studies were then undertaken between the serum BDNF concentration and AD patients' impaired cognition. These results confirmed the hypothesis of an up regulation of BDNF concentrations during both the preclinical phase of dementia and AD clinical stages. Nevertheless, these results were somewhat misleading, given that various preclinical patients were present only among the AD patients, thus confusing the actual presence among the general populations. Furthermore, during this study, it was revealing to test what role SSRI and serum BDNF concentrations played in the cognitive function of Saudi Alzheimer's patients and elderly populations. Significantly, the serum BDNF concentrations in Alzheimer's disease patients receiving SSRI were increased compared to AD patients not treated with any type of antidepressants. Most crucially, this research has comprehensively analysed the relationship of Val66Met polymorphism BDNF and cognition in Saudi Alzheimer's patients. This work was vital for defining the potential role, or lack thereof, of such genes in the development of AD prognosis. Moreover, the results evidenced that GG carriers may have improved cognition, although they face a greater risk of early death as AD patients.

This research has raised several questions concerning the serum BDNF concentration and AD patients' cognitive function. Several approaches can be predicted in attempting to answer some of these questions. Significantly, several associated variants exist with the serum BDNF concentration. One approach is to comprehend the role of SSRIs in terms of the improvements in AD patients' cognition performance. I am interested in further testing their cumulative effect on symptom prognosis.

In conclusion, this research has shown that:

- the GG genotype may lead to enhanced cognition;
- the GG genotype can result in early death among AD patients;
- SSRIs have an effect on elderly patients' cognition performance;
- SSRI antidepressant medications improve AD patients' serum BDNF concentration;
- Increased serum BDNF concentration is linked to AD patients' slow prognosis of the symptoms but not improving cognition functions.

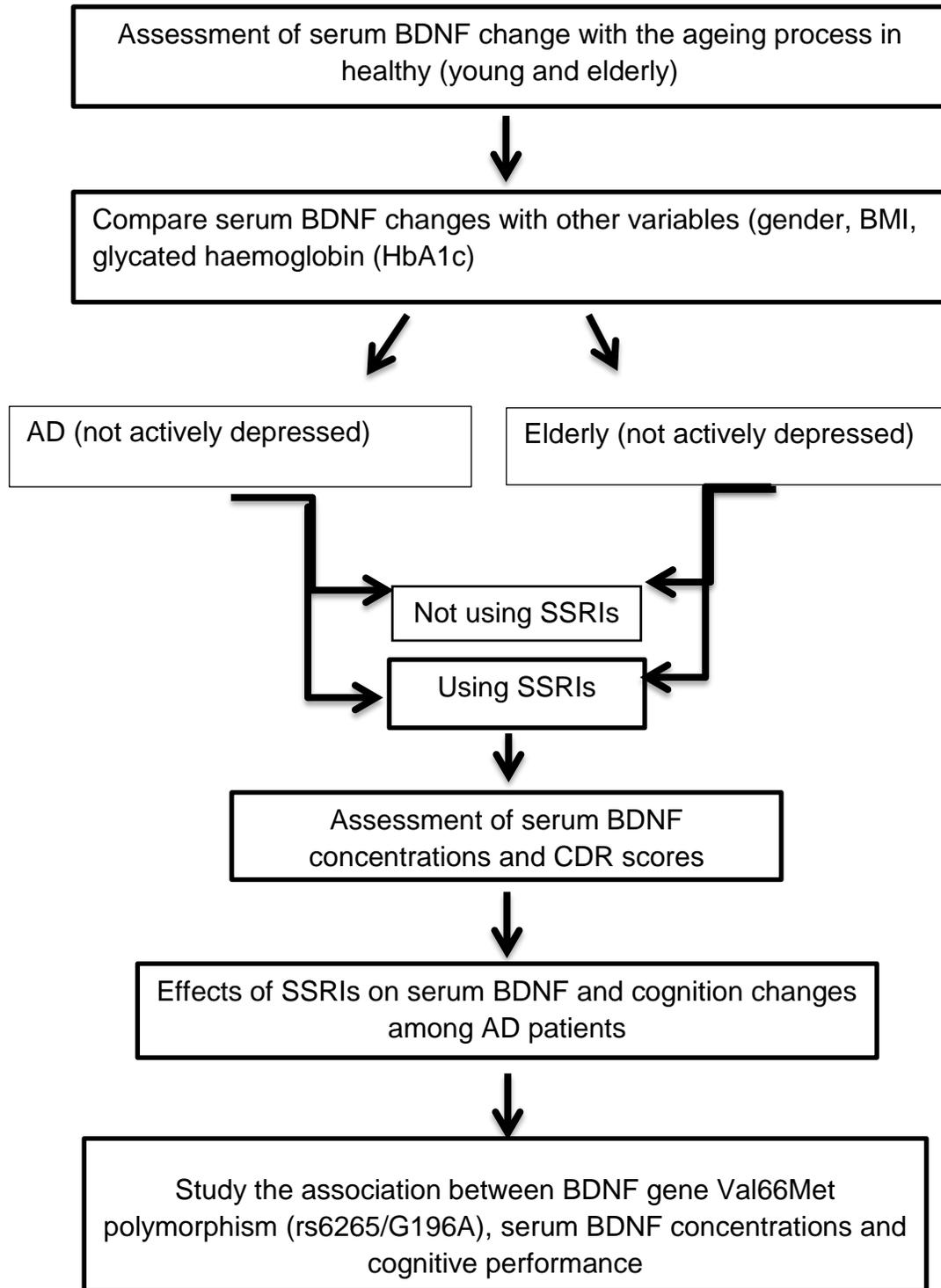


Figure8. 1. Conceptual paradigm showing the steps of the thesis in study the effect of SSRIs on the cognition performance among AD patients.

8.9 Limitations and recommendations for future research

Although much remains to be determined regarding the risks of AD and how cognitive function can be improved in AD patients, this study has produced important results on the effects of SSRIs on AD patients. Nevertheless, in addition to the largely unavoidable limitations of data processing, other limitations of this study remain. Although this research yielded some preliminary findings, its design was not without weaknesses. Therefore, several concerns about limitations must be noted regarding the results of this research, including the patient sample size, place of recruitment, the inclusion criteria for AD, the assessment tools used for cognition, and the duration period of this study.

Based on the current findings, it would be enlightening to investigate the potential role of SSRIs through a clinical trial study, analysing parameters such as serum BDNF concentrations and cognitive performance prior to and following SSRI treatment for the same participants. The sample size is the first important limitation of the current research; of the 123 participants, only 30 represented AD patients. This sample size is too small to provide a general conclusion based on the effects of SSRIs on cognitive performance in terms of elevating AD patients' serum BDNF concentrations; the sample size should be substantially larger. The small numbers of participants also showed a real risk of a type II error; there may have been an effect, but the sample size was too small to be able to detect it. Furthermore, the geographical location of the AD patients in this study was a limitation. The AD patient's samples were taken from a psychiatric clinic at a government tertiary hospital in central Saudi Arabia, in Riyadh City only. Because this investigation was conducted on AD patients in this geographic region only, samples are not necessarily representative of other Saudi regions. In addition, AD patients in other types of hospitals, such as specialised psychiatric and private hospitals in other healthcare sectors, may originate different results from those current samples used in this investigation, assuming that AD patients may receive different forms of care

and support in other types of hospitals. Additionally, this research narrowly focused on the Saudi population alone . The study results were obtained based on these country-specific samples, which may not be generalizable to other populations.

However, the numbers of the AD and control group were estimated based on sample size equation, and the result of the sample size was calculated based on an 80% power test, the results of which revealed that in each group 13 AD patients had used SSRIs, whereas 13 had not. During recruitment, 13 AD patients had used SSRIS, whereas 27 had not. The 13 AD patients were divided according to the stage of the disease they had reached, but they exhibited no significant difference in cognitive performance; this can be explained by the sample size being small and divided according to stages, leading to a loss of test power. Because of this limitation, the research design could be deemed a pilot study that assessed the effects of SSRIs on cognitive performance in AD patients.

Another limitation of the present findings was the past medical history of the individuals recruited during data enrolment to assess the effects of SSRIs on cognitive performance among elderly and AD patients. In such a study, no participant should be actively depressed, particularly because depression symptoms are more prevalent in AD patients, but it was difficult to apply this criterion while investigating the role of SSRIs in both the AD patients and elderly age-matched group. Moreover, several presented evidence that confirmed the diagnostic rate of depression was increased in elderly individuals. For minimising this limitation in this research, the Hamilton rating scale was used; all patients with total scores that higher than 7 (as a cut-off point) were excluded. These criteria for including participants who were not actively depressed made this research particularly novel for assessing the effects of SSRIs on cognitive performance in AD patients.

An additional limitation involved the self-reported data. The cognitive performance outcomes were derived using a qualitative assessment method on AD patients through a CDR tool, whereas MMSE was applied to gather data for the elderly participants. However, the self-reported data were restricted by an independently verified score.

Additionally, a lack of prior research that sufficiently assessed the effects of SSRIs on cognitive function based on the activation of hippocampal BDNF concentrations among AD patients is considered a limitation of the current research. Based on the literature review, all the relevant studies have reported that the use of SSRIs leads to increased BDNF concentrations and improved cognitive performance among depressed patients. However, other studies on AD patients have concluded that the use of an acetylcholinesterase inhibitor increases BDNF concentrations in AD patients and enhances their cognitive function. Moreover, other studies have highlighted the concept of treating neurodegenerative diseases based on synaptic repair therapy, of which BDNF is an exemplar, as it regulates all three aspects of synaptic physiology: it protects and repairs existing synapses; and it stimulates new synapse formation, even in the presence of various toxins.

Finally, the time constraint of this research is considered a limitation because the duration of SSRI administration among the AD patients varied between six months and two years only. Indeed, previous studies have demonstrated that the chronic use of antidepressants but not acute administration enhances hippocampal BDNF expression. However, it seems that the magnitude and duration of the increase in BDNF levels have been insufficient for ensuring TRKB activation. Acute and chronic antidepressant treatment can also increase TRKB signaling. This effect is transient, independent of BDNF, and incomplete. Therefore, future study must be conducted to assess improvement in cognition based on the role of BDNF concentrations in AD patients who received SSRIs for more than two years.

To conclude, a future clinical trial is necessary to determine the effects of SSRIs on BDNF activity as disease-modifying therapy for neurodegenerative disorders with long duration.

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10. Appendices

Appendices10.1: KFMC Ethical Approval

Kingdom of Saudi Arabia
Ministry of Health
King Fahad Medical City
(162)



المملكة العربية السعودية
وزارة الصحة
مدينة الملك فهد الطبية
(١٦٢)

IRB Registration Number with KACST, KSA: H-01-R-012
IRB Registration Number with OHRP/NIH, USA: IRB00008644
Approval Number Federal Wide Assurance NIH, USA: FWA00018774

January 20, 2016
IRB Log Number: 16-012
Department: NNI
Category of Approval: EXEMPT

Dear Dr. Fahad AlWahhabi,

I am pleased to inform you that your submission dated December 21, 2015 for the study titled '**The Association between Serum Brain derived neurotropic factor (BDNF) level and Dementia in Saudi Elderly**' was reviewed and was approved. Please note that this approval is from the research ethics perspective only. You will still need to get permission from the head of department or unit in KFMC or an external institution to commence data collection.

We wish you well as you proceed with the study and request you to keep the IRB informed of the progress on a regular basis, using the IRB log number shown above.

Please be advised that regulations require that you submit a progress report on your research every 6 months. You are also required to submit any manuscript resulting from this research for approval by IRB before submission to journals for publication.

As a researcher you are required to have current and valid certification on protection human research subjects that can be obtained by taking a short online course at the US NIH site or the Saudi NCBE site followed by a multiple choice test. Please submit your current and valid certificate for our records. Failure to submit this certificate shall a reason for suspension of your research project.

If you have any further questions feel free to contact me.

Sincerely yours,


Prof. Omar H. Kasule
Chairman Institutional Review Board--IRB.
King Fahad Medical City, Riyadh, KSA.
Tel: + 966 1 288 9999 Ext. 26913
E-mail: okasule@kfmc.med.sa



Appendices10.2: Consent Form

Form IRB-10.03		KFMC Institutional Review Board
Effective Date:		

King Fahad Medical City
مدينة الملك فهد الطبية
Riyadh, Kingdom of Saudi Arabia
الرياض، المملكة العربية السعودية

CONSENT BY SUBJECT FOR PARTICIPATION IN RESEARCH

إقرار بالموافقة على المشاركة في دراسة بحثية

Protocol Number:	رقم الدراسة:
Name of Subject:	اسم المشارك:
Medical Record Number:	رقم السجل الطبي:
Study Title: The Association between Serum Brain Derived Neurotropic Factor (BDNF) level and Dementia in Saudi Elderly.	عنوان الدراسة: العلاقة بين مستوى عامل التغذية العصبية المستمد من الدماغ (BDNF) في مصل الدم و الخرف عند السعوديين المسنين.
Principal Investigator: Dr. Fahad AlWahhabi	الباحث الرئيس: د. فهد الوهابي
Address: KFMC	العنوان: مدينة الملك فهد الطبية
Telephone: 2889999-24022	رقم الهاتف: 2889999-24022
A member of the research team will explain what is involved in this study and how it will affect you. This consent form describes the study procedures, the risks and benefits of participation, and how your confidentiality will be maintained. Please take your time to ask questions and feel comfortable making a decision whether to participate or not. This process is called informed consent. If you decide to participate in this study, you will be asked to sign this form and will be given a copy for your records. Throughout this consent form, "you" or "participant" will refer to you	سيشرح لك عضو من فريق البحث محتويات هذه الدراسة وتأثيرها عليك. ويصف هذا الإقرار إجراءات الدراسة ومخاطر المشاركة وفوائدها وكيفية الحفاظ على سرية معلوماتك. الرجاء أخذ الوقت الكافي في طرح الأسئلة واتخاذ قرارك ما إذا كنت ستشارك أم لا بكل حرية وارتياح. وعملية أخذ الموافقة هذه تتم بعد الإطلاع الشامل على كافة المعلومات اللازمة المتعلقة بالموضوع. إذا قررت المشاركة في هذه الدراسة سيطلب منك التوقيع على هذا الإقرار وستعطى نسخة لسجلتك، وسيشار إليك باللفظ "أنت" أو "المشارك" ضمن نص هذا الإقرار.
Description of the research study You are invited to participate in this study because you have cognitive impairment. The study aims to assess the association between the serum concentration level of BDNF in relation to age and cognitive /functional status. The expected study duration is 12 months	وصف الدراسة البحثية لقد دُعيت للمشاركة في هذه الدراسة لأنك تعاني من التدهور الإدراكي وتهدف هذه الدراسة إلى تقييم العلاقة بين مستوى تركيز BDNF تبعاً للعمر والحالة المعرفية / الوظيفية لديك. إن مدة الدراسة المتوقعة هي 12 شهراً



Appendices 10.3: UoB Ethical Approval



pabs.ethics@brighton.ac.uk

15 December 2016

APPLICATION FOR ETHICAL APPROVAL FOR PROJECT PROPOSAL

The Association between Serum Brain derived neurotropic factor(BDNF) level and Dementia in Saudi Elderly.

The School Ethics Committee has approved the above application. There are no ethical issues with this proposal.

The End date for your project is **February 2017**. If, towards the end of your project, you realise it will over-run you must apply for an extension, allowing plenty of time for ethics approval.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'Anna Guildford', located below the 'Yours sincerely,' text.

Dr Anna Guildford

Chair, School of Pharmacy and Biomolecular Sciences Research Ethics Committee.

Appendices 10.4: Hamilton Depression Rating Scale

HAMILTON DEPRESSION RATING SCALE (HAM-D)

(Adapted from Hamilton, M. *Journal of Neurology, Neurosurgery, and Psychiatry*. 23:56-62, 1960.)

MRN#: _____ Rater: _____ Date: _____

<p>1. DEPRESSED MOOD (Gloomy attitude, pessimism about the future, feeling of sadness, tendency to weep) 0 = Absent 1 = Sadness, etc. 2 = Occasional weeping 3 = Frequent weeping 4 = Extreme symptoms</p>	<p>2. FEELINGS OF GUILT 0 = Absent 1 = Self-reproach, feels he/she has let people down 2 = Ideas of guilt 3 = Present illness is a punishment; delusions of guilt 4 = Hallucinations of guilt</p>
<p>3. SUICIDE 0 = Absent 1 = Feels life is not worth living 2 = Wishes he/she were dead 3 = Suicidal ideas or gestures 4 = Attempts at suicide</p>	<p>4. INSOMNIA - Initial (Difficulty in falling asleep) 0 = Absent 1 = Occasional 2 = Frequent</p>
<p>5. INSOMNIA - Middle (Complains of being restless and disturbed during the night. Waking during the night.) 0 = Absent 1 = Occasional 2 = Frequent</p>	<p>6. INSOMNIA - Delayed (Waking in early hours of the morning and unable to fall asleep again) 0 = Absent 1 = Occasional 2 = Frequent</p>
<p>7. WORK AND INTERESTS 0 = No difficulty 1 = Feelings of incapacity, listlessness, indecision and vacillation 2 = Loss of interest in hobbies, decreased social activities 3 = Productivity decreased 4 = Unable to work. Stopped working because of present illness only. (Absence from work after treatment or recovery may rate a lower score).</p>	<p>8. RETARDATION (Slowness of thought, speech, and activity; apathy; stupor.) 0 = Absent 1 = Slight retardation at interview 2 = Obvious retardation at interview 3 = Interview difficult 4 = Complete stupor</p>
<p>9. AGITATION (Restlessness associated with anxiety.) 0 = Absent 1 = Occasional 2 = Frequent</p>	<p>10. ANXIETY - PSYCHIC 0 = No difficulty 1 = Tension and irritability 2 = Worrying about minor matters 3 = Apprehensive attitude 4 = Fears</p>
<p>11. ANXIETY - SOMATIC Gastrointestinal, indigestion Cardiovascular, palpitation, Headaches Respiratory, Genitourinary, etc. 0 = Absent 1 = Mild 2 = Moderate 3 = Severe 4 = Incapacitating</p>	<p>12. SOMATIC SYMPTOMS - GASTROINTESTINAL (Loss of appetite, heavy feeling in abdomen; constipation) 0 = Absent 1 = Mild 2 = Severe</p>
<p>13. SOMATIC SYMPTOMS - GENERAL (Heaviness in limbs, back or head; diffuse backache; loss of energy and fatigability) 0 = Absent 1 = Mild 2 = Severe</p>	<p>14. GENITAL SYMPTOMS (Loss of libido, menstrual disturbances) 0 = Absent 1 = Mild 2 = Severe</p>
<p>15. HYPOCHONDRIASIS 0 = Not present 1 = Self-absorption (bodily) 2 = Preoccupation with health 3 = Querulous attitude 4 = Hypochondriacal delusions</p>	<p>16. WEIGHT LOSS 0 = No weight loss 1 = Slight 2 = Obvious or severe</p>
<p>17. INSIGHT (Insight must be interpreted in terms of patient's understanding and background.) 0 = No loss 1 = Partial or doubtful loss 2 = Loss of insight</p>	<p>TOTAL ITEMS 1 TO 17: _____ 0 - 7 = Normal 8 - 13 = Mild Depression 14-18 = Moderate Depression 19 - 22 = Severe Depression > 23 = Very Severe Depression</p>

Appendices10.5: Patients Data Information Sheet (1st Visit) for ALL subjects

Demographic data:

Name : Gender :
 MRN# (if available) : Marital status :
 Age : # Years of formal education :

Medical History:

Hypertension	Diabetes	Congestive heart failure
Bronchial Asthma	Seizure disorder	Schizophrenia
Major depression	Others:	

Prescribed medication:

- Antihypertensive (Name..... Dose.....)
- Hypoglycemic (Name.....Dose.....)
- Use of over the counter medication (Name..... Dose.....)

Related Physical/Cognitive Examination:

Tremors	Yes	No	<u>Aphasia</u>	Yes	No	Disturbance in <u>executive functioning</u>	Yes	No
Rigidity	Yes	No	<u>Apraxia</u>	Yes	No	Psychomotor retardation	Yes	No
Amnesia	Yes	No	<u>Agnosia</u>	Yes	No			

Objective Measurement:

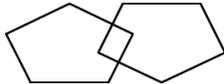
- Hamilton Depression Rating Scale (HDRS):

Appendices10.6: Mini-Mental state Examination

Mini-Mental State Examination (MMSE)

Patient's Name: _____ Date: _____

Instructions: Score one point for each correct response within each question or activity.

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day? Month?"
5		"Where are we now? State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then the instructor asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible.
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, ...) Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.) 
30		TOTAL

Appendices 10.7: Clinical Dementia Rating Scales CDR (Arabic Version)

Subject Initials _____

ورقة عمل التقييم السريري لمرض الخرف

هذه المقابلة شبه مفتوحة. فضلاً أ طرح جميع الأسئلة التالية. اسأل أية أسئلة إضافية تراها ضرورية لتحديد درجة الخرف السريري CDR لدى المريض. الرجاء سجل ملاحظاتك من الأسئلة الإضافية.

أسئلة عن الذاكرة لمقدم المعلومات:

1. هل لديه مشكلة في الذاكرة أو التفكير؟
 نعم لا
1. إذا كانت الإجابة بنعم، هل هي مشكلة دائمة/ ملازمة؟
 نعم لا
2. هل يستطيع تذكر أحداث حصلت مؤخراً؟
 عادة أحياناً نادراً
3. هل يستطيع تذكر قائمة قصيرة من الأشياء (مثلاً مشتريات)؟
 عادة أحياناً نادراً
4. هل كان هناك تدهور بالذاكرة خلال السنة الماضية؟
 نعم لا
5. هل تدهورت ذاكرته إلى الدرجة التي كان من الممكن أن تؤثر على أنشطته اليومية في السنوات القليلة الماضية (أو على أنشطته قبل التقاعد)؟ (بحسب الآراء الأخرى)
 نعم لا
6. هل ينسى حدثاً رئيسياً بشكل كامل (مثلاً رحلة، حفلة، حفلة زفاف عائلية) خلال أسابيع قليلة من ذلك الحدث؟
 عادة أحياناً نادراً
7. هل ينسى التفاصيل المرتبطة بالحدث الرئيسي؟
 عادة أحياناً نادراً
8. هل ينسى بشكل كامل معلومات مهمة من الماضي البعيد (مثلاً تاريخ ميلاد، تاريخ زواج، مكان العمل)؟
 عادة أحياناً نادراً
9. قل لي عن حدث حصل مؤخراً في حياته ويجب أن يتذكره. (من أجل اختبارات لاحقة، أحصل على معلومات مثل موقع الحدث، الوقت، المشاركين، المدة التي أستغرقها، متى انتهى وكيف وصل المريض والمشاركين الآخرين إلى ذلك المكان).

خلال أسبوع 1:

ورقة عمل التقييم السريري لمرض الخرف

أسئلة عن الوعي بالزمان والمكان لمقدم المعلومات:

إلى أي مدى يعرف بشكل صحيح:

1. تاريخ الشهر؟
- عادة أحيانا نادرا لا أعرف
2. الشهر؟
- عادة أحيانا نادرا لا أعرف
3. السنة؟
- عادة أحيانا نادرا لا أعرف
4. اليوم في الأسبوع؟
- عادة أحيانا نادرا لا أعرف
5. هل لديه صعوبة فيما يخص الترتيب الزمني (متى وقعت الأحداث و علاقتها ببعضها)؟
- عادة أحيانا نادرا لا أعرف
6. هل يستطيع أن يجد طريقه في شوارع مألوفة؟
- عادة أحيانا نادرا لا أعرف
7. إلى أي مدى يعرف كيف ينتقل من مكان إلى آخر خارج حارته؟
- عادة أحيانا نادرا لا أعرف
8. إلى أي مدى يستطيع أن يجد طريقه داخل منزل أو مبني مألوف؟
- عادة أحيانا نادرا لا أعرف

ورقة عمل التقييم السريري لمرض الخرف

أسئلة حول إصدار أحكام وحل مشاكل موجهة لمقدم المعلومات:

1. بشكل عام، إذا كان عليك أن تُقيّم قدراته على حل المشاكل في الوقت الحالي، هل تعتبرها:

بنفس الكفاءة التي كانت عليها من قبل

جيدة، ولكن ليس بنفس الكفاءة التي كانت عليها من قبل

متوسطة

ضعيفة

ليس هناك قدرة على الإطلاق

2. قيم قدرته على التعامل مع مبالغ صغيرة من المال (مثال، إعطاء باقي، ترك إكراسية بسيطة):

لم يفقدها

فقد بعضاً منها

فقد الكثير منها

3. قيم قدرته على التعامل مع صفقات تجارية ومالية معقدة (مثال الدفع عن طريق شيكات، دفع فواتير):

لم يفقدها

فقد بعضاً منها

فقد الكثير منها

4. هل يستطيع التعامل مع حادث منزلي طارئ (مثال تسرب من أنابيب مياه، حريق صغير الحجم)؟

بنفس الكفاءة التي كانت سابقاً

أسوأ مما كان عليه سابقاً بسبب اضطراب في التفكير

أسوأ مما كان عليه سابقاً، سبب آخر (لماذا)

5. هل بمقدوره أن يفهم مواقف أو شرح معين؟

عادة أحياناً نادراً لا أعرف

6. هل يتصرف* بشكل لائق [مثلاً، كما كانت (تصرفاته قبل) المرض] في المواقف الاجتماعية وعند التعامل مع الآخرين؟

عادة أحياناً نادراً لا أعرف

* هذا السؤال يقيم السلوك وليس المظهر.

ورقة عمل التقييم السريري لمرض الخرف

أسئلة عن الوضع الاجتماعي لمقدم المعلومات:

العمل

1. هل ما زال المريض يعمل؟
إذا كان السؤال لا ينطبق عليه، انتقل إلى السؤال 4
إذا كان الجواب بنعم، انتقل إلى السؤال 3
إذا كان الجواب بلا، انتقل إلى السؤال 2
2. هل ساهمت مشاكل الذاكرة أو التفكير في قرار المريض بالتقاعد؟ (انتقل إلى السؤال 4)
3. هل لدى المريض صعوبة بالغة في عمله بسبب مشاكل ذات صلة بالذاكرة أو التفكير؟
- نادراً أو أبداً أحياناً عادة لا أعرف

الناحية الاجتماعية:

4. هل سبق له قيادة سيارة؟
- هل يقود المريض سيارة حالياً؟
- إذا كان الجواب بلا، هل هذا بسبب مشاكل الذاكرة أو التفكير؟
5. إذا ما زال يقود سيارة، هل هناك مشاكل أو مخاطر بسبب ضعف التفكير؟
- 6.* هل يقدر على شراء أغراضه بشكل مستقل؟
- نادراً أو أبداً أحياناً عادة لا أعرف
- (يحتاج إلى من يسهل عليه في أي جولة للتسوق)
(يشترى عددًا محدوداً من الأشياء، يشترى أشياء متشابهة أو ينسى أشياء لازمة)
7. هل يقدر أن يؤدي أنشطة خارج البيت بدون مساعدة؟
- نادراً أو أبداً أحياناً عادة لا أعرف
- (يشكل عام غير قادر على القيام بأنشطة دون مساعدة)
(محدودة و/أو روتينية، مثل مشاركة سطحية في فعاليات دينية (مسجد) أو اجتماعات، الذهاب إلى حلق الشعر)
(مشاركة فعالة في أنشطة، مثل التصويت)
8. هل يتم أخذه إلى أنشطة اجتماعية خارج منزله؟
- إذا كان الجواب بلا، لماذا؟
9. إذا رأى شخصاً ماراً بشكل عفوي سلوك المريض هل كان ليظنه مريضاً؟
10. إذا وجد في دار رعاية هل يشارك بشكل جيد في الفعاليات الاجتماعية (تفكير)؟

هام:

هل المعلومات المتوافرة كافية لتقييم مستوى عجز المريض في فعاليات المجتمع؟
إذا (لا)، الرجاء البحث عن معلومات إضافية.

فعاليات المجتمع: مثل الذهاب إلى المسجد، زيارة مع الأصدقاء أو العائلة، فعاليات سياسية، منظمات مهنية، جماعات مهنية أخرى، نوادي اجتماعية، منظمات خدمية، برامج تعليمية.

* فضلاً أصف ملاحظات إذا لزم الأمر لتوضيح مستوى أداء المريض في هذا المجال.

ورقة عمل التقييم السريري لمرض الخرف

أسئلة تتعلق بالبيت والهوايات موجهة لمقدم المعلومات:

- 1أ. ما هي التغيرات التي حدثت في قدراته على أداء الأعمال المنزلية الروتينية؟

- 1ب. ما هي الأشياء التي ما زال بمقدوره القيام بها بشكل جيد؟

- 2أ. ما هي التغيرات التي حدثت في قدراته على ممارسة الهوايات؟

- 2ب. ما هي الأشياء التي ما زال بمقدوره القيام بها بشكل جيد؟

3. إذا وجد في دار رعاية ما هي الأشياء التي لم يعد يقوم بها بشكل جيد؟ (في البيت و هواياته)

الأنشطة اليومية (مقياس بليسيد للخرف)(The Dementia Scale of Blessed):

فقد الكثير منها	0	0.5	1
القدرة على أداء الأعمال المنزلية	لم يفقدها		
فضلاً صيغها:			

5. بأي مستوى يقدر على أداء الأعمال المنزلية الروتينية:
 (اختر إجابة واحدة. لا حاجة لأن يُسأل مقدم المعلومات بشكل مباشر).

- لا يؤدي عملاً له معنى هام (أهمية).
 (يؤدي أنشطة بسيطة، مثل ترتيب الفراش، فقط مع كثير من الإشراف)
- يعمل في إطار أنشطة محدودة فقط.
 (مع بعض الإشراف، يغسل الأطباق بنظافة مقبولة، يرتب الطاولة)
- يعمل بشكل مستقل في بعض الأنشطة.
 (يشغل أجهزة، مثل المكينة الكهربائية؛ يحضر وجبات بسيطة)
- يقوم بأنشطة اعتيادية ولكن ليس بالمستوى العادي.
- أداء طبيعي في الأنشطة المعتادة.

هام:

هل المعلومات المتوفرة كافية لتقييم مستوى عجز المريض فيما يتعلق بالبيت والهوايات؟
 إذا (لا)، الرجاء ابحث عن معلومات إضافية.

الأعمال المنزلية: مثل الطبخ، الغسيل، التنظيف، التسوق لأغراض البقالة، إخراج القمامة، أعمال الحوش، صيانة بسيطة للسيارة، ترميمات منزلية بسيطة.

الهوايات: الخياطة، الرسم، أعمال يدوية، القراءة، التسلية، التصوير، البستنة، الذهاب إلى العروض المسرحية أو الترفيهية، أعمال خشبية، المشاركة في الأنشطة الرياضية.

ورقة عمل التقييم السريري لمرض الخرف

أسئلة تتعلق بالرعاية الشخصية لمقدم المعلومات:

* ما هو تقديرك لقدرته العقلية في المجالات التالية:

بدون مساعدة	يخطئ أحياناً في التزوير ، إلخ.	ارتداء الملابس بالترتيب الخاطئ	عدم القدرة على ارتداء الملابس	إرتداء الملابس (مقياس بليسيد للخرف) (The Dementia Scale of Blessed)
0	1	2	3	
بدون مساعدة	بحاجة إلى تذكير	أحياناً يحتاج إلى مساعدة	دائماً أو تقريبا دائماً بحاجة لمساعدة	ب. الاغتسال والاعتناء بالجسم
0	1	2	3	
نظيف ؛ يستخدم الشوكة/السكين المناسبة	يستخدم الملعقة/يديه في الأكل بشكل فوضوي	يتناول أطعمة صلبة بسيطة(مثل البسكويت)	يجب إطعامه بشكل كامل	ج. عادات الأكل
0	1	2	3	
تحكم كامل طبيعي	في بعض الأحيان يبذل الفراش	عادة يبذل الفراش	عاجز عن التحكم بالبول و البراز	د. التحكم بالعضلات القابضة (الإخراج) (مقياس بليسيد للخرف) (The Dementia Scale of Blessed)
0	1	2	3	

* يُملأ البند رقم 1 إذا تدهورت رعاية المريض الشخصية بالمقارنة مع حالته السابقة، حتى لو لم يحصل على حث/دفع

ورقة عمل التقييم السريري لمرض الخرف

أسئلة تتعلق بالذاكرة موجهة الى المريض:

 نعم لا

1. هل لديك مشاكل بالذاكرة أو التفكير؟

2. قبل لحظات قليلة (شريك حياتك... إلخ) أخبرني عن أحداث مررت بها حديثاً. هل من الممكن أن تقول لي شيء عنها؟ (قم بحته على إعطاء تفاصيل إذا لزم الأمر مثلاً موقع الحدث، الوقت، المشاركين، المدة التي استغرقتها ، متى انتهى وكيف وصل المريض والمشاركون الآخرون إلى ذلك المكان).

خلال أسبوع 1

1.0 – صحيحة بدرجة كبيرة
0.5
0.0 – خطأ بدرجة كبيرة

خلال شهر 1

1.0 – صحيحة بدرجة كبيرة
0.5
0.0 – خطأ بدرجة كبيرة

3. سوف أعطيك اسماً و عنواناً لتتذكره لدقائق قليلة. ردد هذا الاسم والعنوان من بعدي: (كرر العبارة حتى ينطق بشكل صحيح أو كحد أقصى ثلاث محاولات).

العناصر	1	2	3	4	5
أحمد	عمر،	42	شارع صاري،	جدة	
أحمد	عمر،	42	شارع صاري،	جدة	
أحمد	عمر،	42	شارع صاري،	جدة	

(ضع خطأً تحت العناصر التي ردها بشكل صحيح في كل محاولة).

4. متى ولدت؟

5. أين ولدت؟

6. ما هي آخر مدرسة تعلمت بها؟

اسم المدرسة

مكان المدرسة

الصف

7. ماذا كانت مهنتك الرئيسية (أو عمل شريك حياتك إذا كنت لا تعمل)؟

8. ماذا كان عملك الرئيسي الأخير (أو عمل شريك حياتك إذا كنت لا تعمل)؟

9. متى تقاعدت أنت (أو شريك حياتك) ولماذا؟

10. ككرر الاسم والعنوان الذي طلبت منك أن تتذكره:

العناصر	1	2	3	4	5
أحمد	عمر،	42	شارع صاري،	جدة	

(ضع خطأً تحت العناصر التي ردها بشكل صحيح).

ورقة عمل التقييم السريري لمرض الخرف

أسئلة عن المقدرة لتحديد الزمان والمكان :

سجل إجابة المريض حرفيا لكل سؤال

1. ما هو تاريخ اليوم؟

صحيح غير صحيح

2. ما هو اليوم؟

صحيح غير صحيح

3. أي شهر هذا؟

صحيح غير صحيح

4. أي سنة هذه؟

صحيح غير صحيح

5. ما اسم هذا المكان؟

صحيح غير صحيح

6. في أي مدينة أو بلدة نحن؟

صحيح غير صحيح

7. كم الساعة الآن؟

صحيح غير صحيح

8. هل يعرف المريض من هو مقدم المعلومات (بحسب تقديرك)؟

صحيح غير صحيح

ورقة عمل التقييم السريري لمرض الخرف

أسئلة حول إصدار أحكام وحل مشاكل موجهة للمريض:

إرشادات: إذا لم تكن الإجابة الأولية من قبل المريض تستحق الدرجة 0، تابع الموضوع مع المريض لمعرفة أفضل فهم لديه عن المشكلة. ضع دائرة حول الإجابة الأقرب.

أوجه الشبه:

مثال: "ما وجه الشبه بين قلم الحبر وقلم الرصاص؟ (أدوات كتابة)

ما هي أوجه الشبه بين الأشياء التالية؟" إجابة المريض

1. الخس.....السيانخ
(=0 خضروات)
(=1 طعام صالح للأكل،أشياء حية، ممكن طبخها، الخ.)
(=2 إجابات غير مناسبة؛ اختلافات؛ تشتتريها)
2. المكتب (طاولة تجلس عليها
لنكتب)..... خزانة الكتب
(=0 أثاث، أثاث مكتبي؛ كلاهما يحمل كتب)
(=1 خشب، أرجل)
(=2 إجابات غير مناسبة، اختلافات)

اختلافات:

مثال: "ما وجه الاختلاف بين السكر و الخل؟ (حلو مقابل حامض)

ما وجه الاختلاف بين الأشياء التالية؟"

3. كذبة..... خطأ
(=0 واحدة عمدا، واحدة بدون قصد)
(=1 واحدة سيئة والثانية جيدة- أو يشرح واحدة فقط)
(=2 أي شيء آخر، أوجه الشبه)
4. بحيرة.....سد
(=0 طبيعي- اصطناعي)
(=2 أي إجابة أخرى)

حسابات:

5. كم هللة في الريال ؟ صحيح غير صحيح
6. كم ربع ريال في 4,25 ريال سعودي ؟ صحيح غير صحيح
7. أطرح 3 من 20 واستمر في طرح 3 من كل رقم جديد بشكل تنازلي حتى النهاية. صحيح غير صحيح

إصدار الأحكام:

8. عند وصولك إلى مدينة غربية، كيف يمكنك أن تجد صديقا تتمنى رؤيته؟
(=0 تستخدم دليل الهاتف، تتوجه إلى شركة الهاتف السعودي للحصول على دليل الهاتف (كتاب فيه أسماء و عناوين)،
تتصل بصديق مشترك)
(=1 تتصل بخدمة 905 (عادة لا يعطي عناوين)
(=2 لم يعط إجابة واضحة)
9. تقييم المريض للعجز والوضع الاجتماعي وفهمه لسبب تواجده في الاختبار (ممكن أن يكون هذا البند قد ذكر، ولكن قيمه هنا):

معرفة جيدة معرفة جزئية معرفة سطحية

التقييم السريري لمرض الخرف (CDR)

3	2	1	0.5	0	التقييم السريري لمرض الخرف (CDR):
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مستوى التدهور					
شديد 3	متوسط 2	خفيف 1	موضع شك 0.5	لا يوجد 0	
فقدان ذاكرة شديد؛ فقط أشياء متناثرة يتذكرها	فقدان ذاكرة شديد؛ يحفظ فقط الأشياء التي تعلمها لسنوات طويلة؛ فقدان المعلومات الجديدة بسرعة	فقدان ذاكرة متوسطة أكثر وضوحاً لأحداث وقعت مؤخراً؛ خلل يؤثر على الأنشطة اليومية	نسيان خفيف متكرر؛ تذكر جزئي للأحداث؛ نسيان معتدل	لا فقدان للذاكرة أو نسيان متقطع خفيف	ذاكرة
تحديد نفسه (المريض) فقط	صعوبة شديدة في تحديد علاقات الزمن؛ صعوبة عادة في تحديد الزمن، صعوبة غالباً في تحديد المكان	صعوبة متوسطة في تحديد علاقات الزمن؛ إمكانية تحديد مكان الاختيار؛ أخطاء ممكنة في تحديد مواقع جغرافية أخرى	تحديد كامل ماعدا صعوبة قليلة مع العلاقات الزمنية	تحديد كامل لمكان وجوده	القدرة على تحديد الزمن والمكان ومكان الوجود
غير قادر على إصدار أحكام وحل مشاكل.	تدهور شديد في التعامل مع المشاكل، والأمور المتشابهة، والاختلافات؛ أحكامه الإجتماعية عادة ضعيفة	صعوبة متوسطة في التعامل مع المشاكل، الأمور المتشابهة و الاختلافات؛ ثبات في إصدار أحكامه الإجتماعية	تدهور ضئيل في حل المشاكل، الأمور المتشابهة، والاختلافات	قدرة على حل المشاكل اليومية وإنجاز المسائل المالية؛ إصدار أحكام بشكل جيد مقارنة بأدائه في الماضي	إصدار الأحكام وحل المشاكل
لا دليل على أداء مستقل خارج المنزل يبدو مريضاً جداً بحيث لا يمكن أخذه لمناسبات خارج بيت العائلة إلى مناسبات خارج بيت العائلة	لا دليل على أداء مستقل خارج المنزل يبدو أن لديه المقدرة على الإشتراك في فعاليات كافية لأخذه إلى مناسبات خارج بيت العائلة	عدم القدرة على الأداء المستقل في تلك الأنشطة رغم أنه لا يزال يتذكر في بعض مناه؛ يظهر كشخص عادي عند النظرة الأولى	تدهور ضئيل في تلك الأنشطة	أداء مستقل على مستوى العمل العادي، التسوق، التطوع والمجموعات الإجتماعية	فاعليات المجتمع
لا يقوم بأعمال ذات معنى هام في المنزل	القيام فقط بالأعمال المنزلية البسيطة؛ الإبقاء على اهتمامات محدودة جداً	تدهور خفيف ولكن واضح في أداء الأنشطة المنزلية، التخلي عن الأعمال الأصعب؛ التخلي عن الهوايات والاهتمامات المعقدة	الحياة في المنزل، الهوايات، والاهتمام بالأمور العظيمة تدهورت بشكل قليل	يحافظ على الحياة في المنزل، الهوايات، والاهتمام بالأمور العظيمة بشكل جيد	المنزل والهوايات
بحاجة إلى الكثير من المساعدة للعناية بنفسه؛ عجز متكرر عن ضبط البول والبراز	بحاجة لمساعدة في ارتداء الملابس، والعادات الصحية والمحافظة على المقتنيات الشخصية	بحاجة إلى تذكير	قادر بشكل كامل على القيام برعاية نفسه		العناية الشخصية

سجل نقاط فقط في حالة التدهور بسبب فقدان المقدرة على التفكير، وليس التدهور بسبب عوامل أخرى.

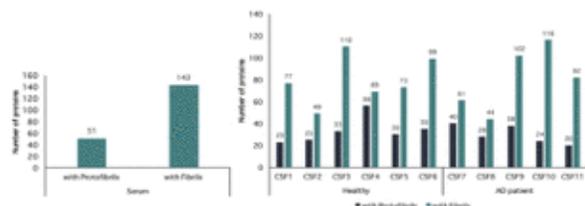
Appendices10.8: BDNF Experiment Preparation



Appendices 10.9 Publications arising from thesis

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Poster Presentations: Sunday, July 16, 2017



Protein name	Healthy						Alzheimer's					
	CSF-1	CSF-2	CSF-3	CSF-4	CSF-5	CSF-6	CSF-1	CSF-2	CSF-3	CSF-4	CSF-5	CSF-6
Neurexin-1												
Neurexin-2												
Neurexin-3												
Agpr1												
Tetraspan-9												
Complement C3a												
Complement C3b												
Neuronal pentraxin-1												
Dccam1												
Protein ADFP												
Calretinin												
Complement C1r subcomponent												
Complement factor D-related protein 1												
ECF containing fibulin-like extracellular matrix protein												
Acetylcholinesterase 1												
Collagen alpha 1(I) chain												
Complement factor D												

(APP) containing vesicles in neuron. Kinesin-1 is a tetrameric protein composed of two heavy chains (KHCs) and two light chains (KLCs). The tetratricopeptide repeat (TPR) domain of KLC1 may be responsible for binding APP either directly or via interaction with C-jun N-terminal kinase-interacting protein 1 (JIP1). However, the binding partners of the TPR domain of KLCs have not yet been fully identified. **Methods:** We used the yeast two-hybrid system to identify the binding proteins that interact with the TPR domain of KLC1. The binding affinity was quantified by measuring β -galactosidase activity in liquid cultures of yeast transformed cells. Direct interaction between binding proteins and KLC1 in mammalian cells as well as *in vitro* was assayed using the co-immunoprecipitation with the antibodies. The cellular co-localization in cells was used the immunocytochemistry. **Results:** We revealed an interaction between the TPR domain of KLC1 and dynamin-1-like protein (Dnm1L), also known as dynamin-related protein 1. Dnm1L bound to the six TPR domain of KLC1 and did not interact with KIF5s. Dnm1L interacts with KLC1 through its GTPase effector domain (GED) domain. When co-expressed in HEK-293T cells, co-localized with KLC1 and co-immunoprecipitated with KLC1, but not KIF5B. **Conclusions:** We suggest that, after mitochondrial fission, interaction of Dnm1L with KLC1 may lead to dissociation of kinesin-1 tetramer, allowing KIF5 to interact with miton and transport mitochondria.

P1-231 A CROSS-SECTIONAL STUDY OF SERUM BRAIN DERIVED NEUROTROPIC FACTOR (BDNF) CONCENTRATIONS IN A SAUDI POPULATION AND ALZHEIMER'S DISEASE

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Background: Brain-derived neurotrophic factor (BDNF) is a protein, a member of the neurotrophin family of growth factors. It is found in the brain and peripheral tissues; it mainly helps to support the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses. BDNF concentrations tend to decline with age. Post-mortem studies of AD patients showing decreased mRNA BDNF in brain regions commonly affected by AD have ignited an interest in BDNF as a potential marker. **Methods:** The research variables are the serum BDNF concentrations and cognition changes. The enzyme-linked immunosorbent assay (ELISA) technique was used to assess the BDNF concentrations. While the clinical rating scales were used to assess the cognitive performance. Moreover, there were independent variables such as age, gender, BMI, DM and the use of medications were assessed. **Results:** The total healthy group was 123 participants made up of younger healthy subject's age range 25-35 years (n=34), middle age group range from 36-59 years (n=41) and the elderly healthy volunteer's age was above 60 years (n=48). As the result showed, elderly subjects had a lower mean serum BDNF level than younger age participants (338.9 ± 124.30 vs. 80.3 ± 27.84 pg/ml, $P < 0.001$). Additionally, the Alzheimer's patients were above 60 years old (n=27) and all categorised according to the CDR scores into four categories (very mild cognitive impairment n=4 (14.8%), Mild dementia n=6 (22.2%), Moderate dementia n=13 (48.1%) then severe dementia n=4 (14.8%). **Conclusions:** Our research has evidence that the serum BDNF concentrations of healthy participants decrease with ageing in comparison to younger healthy control group. Within the patient population, serum BDNF concentrations were found to be significantly decreased only in patients with severe AD. Those with mild to moderate did not have serum BDNF concentrations significantly different to those of healthy, elderly controls.

P1-232 HIGH-SPEED ATOMIC FORCE MICROSCOPY REVEALS STRUCTURAL DYNAMICS OF AMYLOID β 1-42 AGGREGATES

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Background: Alzheimer's disease (AD) is characterized by the accumulation of amyloid plaques and neurofibrillary tangles. Aggregation of amyloidogenic proteins into insoluble amyloid fibrils is implicated in various neurodegenerative diseases. This process involves protein assembly into oligomeric intermediates and fibrils with highly polymorphic molecular structures. These structural differences may be responsible for different disease presentations. **Methods:** In order to elucidate the structural features and assembly kinetics of amyloid β -protein (β), we used high-speed atomic force microscopy (HS-AFM) studies of fibril formation and elongation by the 42-residue form of β 1-42, a key pathogenetic agent of AD. **Results:** 1st, our video-imaging visualized the growth manner of individual filament of β 1-42 fibrils: polarized growth and stepwise elongation. 2nd, our data demonstrate two different growth modes of β 1-42, one producing straight fibrils and the other

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30th Sep-1st Oct 2016, Crowne Plaza Hotel, Dubai, UAE

Impact of Age, Gender, Body Mass Index, Platelet Counts and Glucose Levels on Brain-Derived Neurotrophic Factor Concentration among Saudi Population

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¹King Saud University, Saudi Arabia

²University of Brighton, UK

Abstract

Background: Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is a homodimeric protein that has been highly preserved in structure and function during growth. Generally, different influences have been offered as factors that affect the stored and circulating BDNF levels in peripheral blood, such as age, weight, and gender. The aim of the study is explaining the need for clinical research about factors affecting BDNF levels in the Saudi population.

Materials and methods: This was a prospective cross-sectional study, which was conducted at King Fahad Medical City, and King Salman Social Centre Riyadh, Saudi Arabia during the period between 2015-2016. Circulating serum levels of BDNF were determined with a commercially available enzyme-linked immunosorbent assay (ELISA) kit. The assay technique followed manufacturer's instructions. The data analysis for this study was carried out using Minitab 17 software.

Results: The study participants included 110 young and elderly participants. There was a wide range of BDNF concentrations in participant's serum. There was no significant correlation between BDNF levels and age ($P=0.46$). Additionally, when evaluating the whole cohort ($n=38$ female and 74 male), differences regarding BDNF levels in serum were not significantly different between males and females. Moreover, Spearman's correlation showed that there was no significance correlation ($P=0.087$) between BDNF and BMI. However, there was a negative correlation between BDNF levels and blood glucose concentration ($r=-0.22$, $P=0.02$). Finally, serum BDNF showed no significant correlation with the number of platelets in peripheral blood.

Conclusions: The data obtained from this study suggest that parameters such as age, gender or body mass index, and platelets reactivity do not affect the serum BDNF concentrations in healthy volunteers. These factors therefore have little confounding effect when considering the effects of drugs on serum BDNF.

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