

**NEUROCOGNITIVE FUNCTION,
RENIN-ANGIOTENSIN FUNCTION
AND POLYMORPHISM IN CHRONIC
KIDNEY DISEASE PATIENTS**

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Neurocognitive function, renin-angiotensin function and polymorphism in chronic kidney disease patients

Uraemic patients demonstrate cognitive deficits, particularly in attention and memory and chronic kidney disease (CKD) is a risk factor for cognitive impairment. Memory enhancing properties of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor antagonists (AIIA) have been reported in rats and mice. In humans, chronic treatment with an AIIA improved cognition in elderly hypertensive patients; ACEIs improve cognition in young, hypertensive patients and acute administration of an AIIA has cognition-enhancing effects in young, healthy volunteers. The aim of this thesis was to investigate possible differential effects of ACEI and AIIA on mood and cognition in comparison to other antihypertensives in CKD patients. To rule out the possible effect of chronic disease on mood and cognition by examining neurocognitive attributes of colon cancer patients in remission, and finally to investigate the possible contribution of renin angiotensin system (RAS) gene polymorphisms to neurocognitive improvement associated with drugs targeted at the RAS.

In the first stage 60 Saudi CKD patients (18- 60 yr) were recruited, 34 patients received antihypertensive drugs acting via the renin angiotensin system (RAA group) and 26 patients were on other types of antihypertensive (non-RAA group). Age and education-matched healthy Saudi volunteers (36 subjects) were also recruited. Cognition was assessed using the Rey Auditory-Verbal Test (learning & memory); Rey-Osterrieth complex figure (ROCF, visuospatial organization and visuospatial memory); semantic verbal fluency (executive function); letter cancellation (attention); digit-symbol (sustained attention, visual searching, visual sequencing). The 36-item Short-Form Health Survey (quality of life, QOL) and the Hospital Anxiety and Depression Scale were also used to assess QOL, anxiety, and depression, respectively. The neurocognitive functions of 32 colon cancer patients in remission were also examined. Lastly a total of 53 (18-60 yr) Saudi dialysis patients and 42 healthy blood donors were examined for RAS gene polymorphisms. RAS polymorphisms tested were AT₁R (A1166C), AT₂R (C3123A), ACE I/D, AGT (M268T), and AGT (T207M) using polymerase chain reaction and agarose gel electrophoresis. Of 53 dialysis patients, 13 patients were using RAA drugs (RAA group) and 40 patients were on other types of antihypertensives (non-RAA group). Dialysis patients were also examined neuropsychologically. The results demonstrated deficits in neuropsychological function in CKD patients, particularly in executive function, visuospatial organization, and immediate recall of visuospatial memory. However, the RAA group had significantly better performance on executive function compared to non-RAA group; colon cancer patients in remission showed good neurocognitive function except in visuospatial memory compared to healthy subjects, and they even showed significantly less anxiety and depression than healthy subjects. The genetic distributions among dialysis patients and healthy control were similar, as were the distributions between dialysis patients receiving RAA and non-RAA medications. Within the dialysis patients, there were no significant differences in neuropsychological scores between the RAA and non-RAA group. The covariables (RAS genotypes, age, gender, education, drug group, DM, and dialysis duration) all significantly contributed to mood, cognition, anxiety and QOL.

These results indicate that CKD patients have impaired neurocognitive function compared to healthy controls, but that when drugs targeted at the renin-angiotensin system were used in the management of their disease there is a significantly better cognitive outcome mainly in executive function than when other antihypertensives are used. The possible mechanisms of action of this effect are unclear, but the results are a preliminary indication of superiority of one class of medicines over another. The neuropsychological deficits were associated with the renal disease rather than with chronic illness was demonstrated by the fact that there was no neuropsychological impairment amongst colon cancer patients in remission. Lastly no neuropsychological benefit of RAA drugs was identified in dialysis patients, however a significant associations between RAS genotypes and cognitive performance in dialysis patients treated with RAA and non-RAA antihypertensives have been identified.

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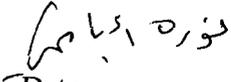
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AUTHOR'S DECLARATION

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.

Signature

Norah Abanmy



Date

03/10/2011

ABBREVIATION LIST

Abbreviation	Full term
A	Adenine
ACE	Angiotensin Converting Enzyme
ACEI	Angiotensin Converting Enzyme Inhibitor
AD	Alzheimer Disease
AGT	Angiotensinogen
Ang I	Angiotensin I
Ang II	Angiotensin II
Ang-(2-8)	Angiotensin III
Ang-(3-8)	Angiotensin IV
Ang-(1-7)	Angiotensin-(1-7)
ANOVA	Analysis of Variance
ARB	Angiotensin II Receptor Blocker
AT₁R	Angiotensin II type 1 Receptor
AT₂R	Angiotensin II type 2 Receptor
AT₄R	Angiotensin II type 4 Receptor
BP	Blood Pressure
C	Cytosine
CAPD	Continuous Ambulatory Peritoneal Dialysis
CKD	Chronic Kidney Disease
D	Deletion allele of ACE gene
D1	Dopamine 1 Receptor
DM	Diabetes Mellitus
DN	Diabetic Nephropathy
DSST	Digit Simple Substitution Test
E4177	AT ₁ antagonist drug (investigational drug name)
eGFR	Estimated Glomerular Filtration Rate
ERPs	Event Related Potentials
ESRD	End-Stage Renal Disease
GABA	Gamma-Aminobutyric Acid

GFR	Glomerular Filtration Rate
HADS	Hospital Anxiety and Depression Scale
Hct	Hematocrit
HD	Hemodialysis
Hgb	Hemoglobin
HTN	Hypertension
I	Insertion allele of ACE gene
ICV	Intracerebroventricular
KAMC	King Abdulaziz Medical City
KFSHRC	King Faisal Specialist Hospital and Research Centre
KKUH	King Khaled University Hospital
Kt/V	An equation used to calculate urea clearance which is a measure of adequacy of dialysis; where K is effective (delivered) dialyzer urea clearance in milliliters per minute integrated over the entire dialysis, t is the time in minutes measured from beginning to end of dialysis, and V is the patient's volume of urea distribution in milliliters; kidney disease outcome quality initiative KDOQI guideline recommend target $Kt/V = 1.2-1.4$ which indicate adequate dialysis.
M	Methionine
MCS	Mental Component Summary
MMSE	Mini Mental State Examination
non-RAA	non-Renin Angiotensin Antagonist
PCR	Polymerase Chain Reaction
PCS	Physical Component Summary
PD123319	AT ₂ antagonist drug name (investigational drug name)
PMP	Per Million Population
PSDC	Prince Salman Dialysis Centre
QOL	Quality Of Life
RAA	Renin-Angiotensin Antagonist
RAS	Renin-Angiotensin System
RAVLT	Rey Auditory verbal Learning Test
RBC	Red Blood Cell

RCF	Rey-Osterrieth Complex Figure
RFLP	Restriction Fragment Length Polymorphism
SCH233390	Dopamine 1 receptor antagonist drug (investigational drug)
T	Threonine

CHAPTER 1. GENERAL INTRODUCTION

This review aims to introduce the contribution of the renin angiotensin system (RAS) to neurocognitive properties in both animals and humans. A review of the latest literature investigating the association between renal impairment, neurocognitive dysfunction and RAS gene polymorphisms will also be given.

1.1 Overview of Renin-Angiotensin System:

This review will initially give a brief overview of the RAS and its involvement in the functionality of different organs.

The importance of renin, the primary rate-limiting enzyme of the RAS, was not appreciated until after the work of Goldblatt in 1934, in spite of the early discovery by Tigerstedt and Bergmann in 1897 (cited by de Gasparo, 2000). In 1940, two sets of workers, Page and Helmer of the United States and Braun-Mendez et al of Argentina both found that renin was a direct pressor or vasoconstrictor substance (Braun-Menendez *et al.*, 1940; Page *et al.*, 1940). Renin, according to their study, acted on a substance present in plasma to produce a heat-stable, short-acting vasoconstrictor substance (de Gasparo et al., 2000).

The new substance was termed "Angiotonin" by Page and Helmer, while the group of Braun-Menendez called it "Hypertensin". With two different terminologies used by separate investigators throughout the world, Page and Braun-Menendez, during a meeting 20 years later at the University of Michigan in 1961 agreed to call the new substance "Angiotensin".

The presence of angiotensin in the blood of animals with experimental hypertension was first demonstrated in 1951 by Skeggs et al (Skeggs et al., 1951). The angiotensin-converting enzyme (ACE) was later discovered. This enzyme catalyses the conversion of angiotensin I (Ang I) to angiotensin II (Ang II) by converting the decapeptide structure of Ang I to octapeptide structure of Ang II (Lentz et al., 1956) whereby removing the two carboxy terminal amino acids. It was also discovered later by this

group that the entire pressor effect of the RAS is a result of the vasoconstrictor action of Ang II (Skeggs et al., 1956), proving that Ang I was in fact inactive.

A South American snake *Bothrops Jaracaca* venom was the initial source of the converting enzyme inhibitor, this venom specifically inhibits the converting enzyme *in vivo* and blocks the pressor action of Ang I (Ferreira et al., 1970; Ng et al., 1970). A humoral factor was discovered by Davis et al (1962) that was responsible for the release of aldosterone from the adrenal cortex. It was later revealed by two other groups that Ang II was the humoral factor which incited the release of the aldosterone (Biron et al., 1961; Laragh et al., 1960). Thus, Ang II acts through the adrenal cortex to cause water and sodium retention in addition to being fifty times more powerful than norepinephrine as a pressor agent in addition to its direct effect in the regulation of cardiovascular homeostasis.

For more than 30 years, the presence of a brain RAS has been assumed. The brain RAS is differentiated from other local tissue RASs given that it is physically separated from the endocrine one (peripheral) by the occurrence of the blood-brain barrier preventing the penetration of angiotensin from the blood into the brain (Joy et al., 1970; Schelling et al., 1976; Volicer et al., 1971). However, through areas deficient in the blood-brain barrier, circulating Ang II may elicit its effects within the brain (Bader et al., 2001). Various neurophysiological reports have established the implication of the brain RAS in the modulation of cardiovascular and fluid-electrolyte homeostasis (Bader et al., 2001), by controlling the activity of the autonomic nervous system (DiBona et al., 1999; Fink, 1997; Gelband et al., 1998), hypothalamic-pituitary axis and vasopressin release (Aguilera et al., 1996), baroreflex sensitivity (Averill et al., 2000), and inciting thirst (Denton et al., 1996; Fitzsimons, 1998).

Moreover, the RAS has been ascribed to brain-specific functions, such as influencing memory, cognition and stress (Baltatu et al., 2000). The cloning of the RAS components was enabled by the development of molecular biology methodologies, which allowed the detection of their synthesis in distinct brain sections. ACE is profuse in separate regions unrelated to local Ang II or angiotensin receptors, which include the basal ganglia, hippocampus and cerebellum. ACE's role is unknown in these areas; however, it may be involved in the processing of other neuropeptides (Mendelsohn et al., 1990).

Subsequent to acute ACE-inhibitor treatment, ACE in the brain regions deficient of blood-brain barrier is inhibited (e.g., circumventricular organs); conversely, with the exception of very high drug dosage, areas protected by the blood-brain barrier are not affected (Sakaguchi *et al.*, 1988a; Sakaguchi *et al.*, 1988b). However, there are some brain penetrating ACEIs e.g. perindopril and captopril that can pass the blood brain barrier and exert an effect (Sink *et al.*, 2009).

Angiotensinogen (AGT) is also produced by the brain (Campbell *et al.*, 1984; Ohkubo *et al.*, 1986; Sernia *et al.*, 1983), and it is the only known source of brain angiotensins. AGT is widely distributed throughout the various regions of the brain, specifically hypothalamus and brain stem where high levels of AGT are found. Bader and his colleagues in 2001 made an extensive review of the main cell type synthesizing AGT that called Astroglia (Deschepper *et al.*, 1986). The conversion of AGT into active products was attributed to the classical enzymes renin and ACE which also have been identified in the brain. Although renin mRNA levels are low or under the detection limit, the renin activity and immunoreactivity in the hypothalamus, pituitary and pineal glands are relatively high (Baltatu *et al.*, 1998b). Areas like the diencephalon, pituitary, and pineal gland which are lacking in blood-brain barrier show a high ACE activity and its mRNA (Baltatu *et al.*, 1997; Saavedra *et al.*, 1982; Strittmatter *et al.*, 1984; Strittmatter *et al.*, 1987).

Furthermore, it is indicated that the biology of the system is more complicated than previously believed with the discovery of several biologically active angiotensin peptides, that also include Ang-(2-8) (AIII) (Reaux *et al.*, 2001), ANG-(3-8) (AIV) (Von Bohlen und Halbach, 2003), Ang-(1-7) (Santos *et al.*, 2000), and enzymatic pathways for their production have been described (Bader *et al.*, 2001; Baltatu *et al.*, 1998a).

Most known effects of Ang II are accredited to the Angiotensin type 1 receptor (AT₁R), which have been identified and typified in the central nervous system (Allen *et al.*, 1999; Bader *et al.*, 2001; Culman *et al.*, 2002). There are two AT₁R subtypes in rodents; the AT_{1A}, which is restricted in brain areas involved in fluid homeostasis and blood pressure control, and AT_{1B} that are primarily in the glandular tissues, e.g., anterior pituitary, pineal and adrenal glands (de Gasparo *et al.*, 2000).

Ang II actions in humans, for instance the control of blood pressure and water-electrolyte balance, have been ascribed essentially to the activation of various signal-transduction pathways modulated by AT₁R. Conversely, the discovery of highly selective peptidic, and nonpeptidic ligands such as GGP42112A and PD123319 led to the discovery of a second subtype angiotensin type 2 receptor (AT₂R) (Inagami *et al.*, 1997; Inagami *et al.*, 1994; Kambayashi *et al.*, 1993; Timmermans *et al.*, 1993; Unger *et al.*, 1996). In adults, AT₂R expression is constrained to the adrenals, uterus, ovary, heart and specific nuclei in the brain. Research on the AT₂R has revealed the antiproliferative effects in several tissues in addition to nerve regeneration, cellular differentiation, and apoptosis. While in the developing fetus AT₂R is expressed at very high level. Primarily, AT₂R cDNA was isolated by expression cloning from PC12 cells and whole fetus (Ichiki *et al.*, 1995; Mukoyama *et al.*, 1993). The AT₂R gene has a physiological role in blood pressure control (depressor) and drinking behaviour in mice (Hein *et al.*, 1995). Furthermore, an AT₂R-mediated inhibitory effect on the growth-promoting signals has been found (Inagami *et al.*, 1997; Unger *et al.*, 1996).

As a result of treatment with AT₁R antagonists, plasma level of Ang II rises and selectively binds to AT₂R which then exerts as yet undetermined effects (Bernstein *et al.*, 1992). Consequently, important pharmacotherapeutic inferences can be achieved by understanding of the AT₂R-mediated pathological actions.

Specific assumed receptors for Ang IV-(3-8) and Ang-(1-7) have been anticipated. Presented evidence implied that the Ang IV-(3-8) receptor (AT₄) is the insulin-regulated membrane aminopeptidase enzyme (Albiston *et al.*, 2001). Moreover, the interaction of Ang II with Ang-(1-7) is possibly as an endogenous ligand for the G protein-coupled receptor (Santos *et al.*, 2003).

Circulating Ang II has a biological effect that is both prevalent and varied, and it plays an essential role in the control of the cardiovascular and renal systems. Ang II is locally produced in the peripheral tissues, for instance, the kidneys, vasculature, adrenal glands and the heart and binds to specific receptors throughout the autocrine or paracrine system and exerts growth-promoting effects on the tissue remodeling process (Paul *et al.*, 1992). By means of isolation and biochemical characterization of the diverse

components of the system, proof of a local renin-angiotensin system in cardiovascular tissues has been obtained.

Angiotensin peptides have been measured in cardiac and vascular tissues of primates and other animals and it has been shown that Ang II concentrations in the ventricles are lower than the atria, and in addition lower in the left than the right atrium (Campbell, 1987; Unger *et al.*, 1990).

ACE has been found in large and small vessels mainly in the endothelial layer and also in the adventitia (Jackson *et al.*, 1988; Sakaguchi *et al.*, 1988a; Yamada *et al.*, 1991). The testes carries the highest tissue concentration of ACE distributed along the seminiferous tubules. Subsequent to treatment with ACE inhibitors, testicular ACE is not inhibited; the drug being excluded from the seminiferous tubule by the blood-testis barrier (Jackson *et al.*, 1988). The role of ACE in the testis is unknown.

The proximal tubule brush border of the kidney shows the highest concentration of ACE within the kidney. ACE's physiological role in this location is unknown. Renal ACE is rapidly inhibited following administration of ACE inhibitors in parallel with changes in plasma ACE activity (Sakaguchi *et al.*, 1988c). Glomerular hypertension has been ameliorated by ACE-inhibitor treatment that is associated with renal protective effects (Anderson *et al.*, 1985; Meyer *et al.*, 1987).

In summary, the RAS is one of the most powerful regulators of arterial blood pressure through Ang II. Ang II target tissues include the adrenals, kidney, brain, pituitary gland, vascular smooth muscle, and the sympathetic nervous system. The cardiovascular and other actions of Ang II are mediated by AT₁ and AT₂ receptors. The development of receptor antagonists has led to major advances in the physiology, pharmacology, and therapy of the renin-angiotensin system.

1.2 Neuropsychological function of RAS:

1.2.1 Animal Studies

In this section a detailed discussion of animal studies will be presented to explore the involvement of RAS in neurocognitive function.

Neurocognitive response can be measured in animals using different types of mazes such as water maze and T-maze. They mainly measure learning and memory and usually animals learn which areas in the mazes can provide food and/or safety. When animals are exposed to these areas later, they recognize and remember them and either avoid danger or go further to find food (Shettleworth, 2001).

The involvement of brain angiotensins in cognitive processing has become widely studied (Allen *et al.*, 1998; Braszko, 1996; Braszko *et al.*, 1997; Mosimann *et al.*, 1996; Okuyama *et al.*, 1999b; Wright *et al.*, 1994). Baranowska in 1983 found that Ang II augmented retention of passive avoidance and facilitated achievement of conditioned avoidance responses (Baranowska *et al.*, 1983). Consequently, various reports described similar effects of intracerebroventricular (ICV) administration of Ang-(2-8) (Ang III) (Braszko *et al.*, 1987), Ang-(3-8) (Ang IV) (Braszko *et al.*, 1988b; Wright *et al.*, 1993; Wright *et al.*, 1999) and Ang-(3-7) (Braszko *et al.*, 1991). Furthermore, Yonkov's group found that male albino rats trained and tested for retention in a shuttle box, improved retention when Ang II was administered. The memory enhancing effect of Ang II was also increased with the combination of gamma-aminobutyric acid (GABA) and Ang II. This may imply a role of GABAergic transmission in the central nervous system in the mechanism of the long-term memory-enhancing effect of Ang II (Yonkov *et al.*, 1987). Moreover, Braszko and Wisniewski found that alpha 1 and alpha 2 adrenergic receptor blockade abolished the increased rate of learning of conditioned avoidance response stimulated by ICV Ang II administration, while alpha 2-receptor blockade eliminated the enhancement of recall of passive avoidance behavior caused by Ang II (Braszko *et al.*, 1990).

Following ICV administration of Ang II and Ang-(3-7) an enhanced discrimination between unfamiliar and the previously seen objects were perceived (Braszko *et al.*,

1995), as well as an enhancement of the aversively motivated learning and increased efficiency of food finding in T-maze (Braszko et al., 1988a).

Assessing the relative involvement of Ang II receptor subtypes in various aspects of cognitive behavior was made possible through the presentation of the selective antagonists of AT₁, AT₂, and AT₄ angiotensin receptors (Chiu *et al.*, 1989; Krebs *et al.*, 1996; Widdop *et al.*, 1993).

An AT₄ angiotensin receptor was discovered (Wright et al., 1993). All cognitive effects of angiotensin were attributed to the AT₄ angiotensin receptor by (Wright et al., 1995). Conversely, the possible involvement of AT₁ (Braszko, 2005; Karwowska-Polecka *et al.*, 1997; Kulakowska *et al.*, 1996) and mainly AT₂ (Ichiki *et al.*, 1995; Okuyama *et al.*, 1999b) receptor subpopulations has substantial evidence. The participation of AT₁ and AT₂ angiotensin receptors in abolishing the behavioural changes produced by Ang II was established in 2002 by Braszko, with the AT₂ antagonist (PD123319) eliminating Ang II increase of the acquisition of conditioned avoidance response in rats (Braszko, 2002), Braszko was able to demonstrate a significant difference in amending the cognitive effects of Ang II by AT₂ (PD123319) and, to a lesser extent, AT₁ (losartan) selective receptor antagonists. Valsartan, another selective AT₁ receptors antagonist gave similar results (Braszko, 2005) as well as saralasin a nonselective Ang II receptor antagonist in crabs (Frenkel et al., 2002).

Trandopril, as ACE inhibitor, also attenuated acquisition of conditioned avoidance response in rats but not consolidation of memory and recall of passive avoidance behavior and object recognition (Braszko et al., 2000).

Pederson et al were able to demonstrate that a particular AT₄ receptor antagonist (Divalinal) blocked the ability of Ang IV to improve the performance of rats undergoing a scopolamine-induced spatial memory deficits (Pederson et al., 2001). Ang IV was also effective in overcoming ethanol-induced long-term potentiation suppression (Wright et al., 2003). While cholinergic potentiation may have a role in cognition enhancement as speculated by Lee and his colleagues (Lee et al., 2001).

Braszko, who was the first to report the cognitive-enhancing properties of Ang IV (Braszko et al., 1988b), has reported that selective blockade of brain D1 dopamine receptors by an effective dose of SCH23390 (a dopamine 1 receptor antagonist) disabled Ang IV and Des-Phe⁶-Ang IV from producing most of their memory enhancing effects in rats (Braszko, 2004).

Most studies support a cognition-enhancing action of the Ang II (Braszko, 2002; Delorenzi *et al.*, 1999; Hein *et al.*, 1995; Ichiki *et al.*, 1995; Kulakowska *et al.*, 1996; Wayner *et al.*, 2001; Wright *et al.*, 1995), but some claim opposite effects. For example, early work (Morgan *et al.*, 1977) showed impaired recall of information and in a later one (Lee et al., 1995) impaired retention was found after intracerebroventricular injection of Ang II. An absence of any influence of Ang II on learning was also reported (Walther et al., 1999). Blockade of induction of long-term potentiation by Ang II (Denny et al., 1991), and blocking the inhibition of long-term potentiation by losartan (Denny et al., 1991) and saralasin (Wayner et al., 1993) was also found. Later, similar outcomes were obtained with losartan (Raghavendra *et al.*, 1998; Tracy *et al.*, 1997; von Bohlen und Halbach *et al.*, 1998) and saralasin, but not with PD12319 (von Bohlen und Halbach *et al.*, 1998). The involvements of cholinergic activity in the memory enhancing properties of losartan (Raghavendra et al., 1998) were also reported. An ACE inhibitor (ACEI) cilazapril and to a minor degree an AT₁ antagonist, E4177, also improved memory dysfunction in a Dahl salt-sensitive rat (Hirawa et al., 1999), and recently, enalapril another ACEI was shown to improve water maze performance, hippocampal long-term potentiation, and hippocampal blood flow in a streptozotocin-diabetic rats (Manschot et al., 2003).

Anxiety- and depression-like behavior were also investigated in animals in relation to ACEI and angiotensin receptor blocker (ARB). An ACEI captopril and SQ29852 (ceronapril) improved the anxiety-like behavior in rodents and marmosets (Costall et al., 1990). Similar results were obtained with losartan as an anxiolytic in mice (Barnes *et al.*, 1990; Kaiser *et al.*, 1992).

In an effort to define the mechanism behind the effect of Ang II as an anxiolytic (Georgiev et al., 1987), Ang II changed exploratory behaviour of male rats but was blocked by saralasin. This effect was amplified by a dopaminergic agonist and reduced

by a dopaminergic antagonist. However, no effects of losartan or PD123177 on anxiety and memory in either rats or mice were found in another study (Shepherd et al., 1996), and there was no change in learning and anxiety-related behavior in mice lacking angiotensinogen (Walther et al., 1999). Anxiety-like behavior exhibited by AT₂R-deficient mice was also reversed by captopril with no depression-like activity and involvement of noradrenergic system (Okuyama et al., 1999a). Moreover, losartan had anxiolytic properties rather than enalapril in renal hypertensive rats which may signify a potential role of AT receptors in the mediation of anxiolysis (Srinivasan et al., 2003).

By means of the forced swim-induced behavioural despair test (The forced swim test (FST) involved two exposures to a cylindrical tank of water where animals cannot touch the bottom of the tank or escape. For the first exposure, the animals were placed in the water for 10 min. Twenty-four hours later, the animals were placed in the water again for 5-min session. The time that the test animal spends without moving in the second trial is measured. This immobility time is decreased by antidepressants), antidepressant-like effects in mice have been assessed and captopril significantly diminished immobility and mimicked the antidepressant effect, this effect was blocked by naloxone which implies a role of brain opioid peptides in this behaviour (Giardina *et al.*, 1989). Utilizing a learned helplessness paradigm test in rats, similar results have been achieved (Martin et al., 1990).

Antidepressant drugs, as determined by Gard et al, were able to lessen Ang II function *in vivo* using angiotensin-induced drinking and *in vitro* by means of contractile response of rat uterus (Gard et al., 1994). Angiotensinogen-deficient mice, as shown by Okuyama et al, also displayed a decrease in depressive-like behaviour (Okuyama et al., 1999c).

There is a large discrepancy in animal studies where Braszko's work suggests that Ang II has cognitive-enhancing properties. On the other hand, ACEI and ARB have been shown to improve memory and cognition. This is might be due to the use of normal healthy animals by Braszko where their RAS is intact and there is no need for memory improvements. Other works used memory-impaired animals where their memory improved upon exposure to ACEI or ARB.

1.2.2 Human Studies

Studies that have examined the neuropsychological response to drugs acting on RAS in humans will be reviewed in this section. However there are a limited number of studies exploring this issue and a lack of consistent findings amongst these studies hence there is no definite conclusion.

Neurocognitive function assessment in humans is usually achieved by applying two types of tests either neuropsychological or neurophysiological.

The most commonly known and used are the neuropsychological tools which include either a measure of global cognitive function such as the Mini Mental State Examination (MMSE), or measures of specific domains of neurocognitive function such as memory (Rey Auditory-Verbal Learning Test), attention (Letter Cancellation), or executive function (Semantic Verbal Fluency) (Lezak et al., 2004).

Neurophysiological examination includes electroencephalogram and cognitive event-related potentials (ERPs). ERPs are an electroencephalogram response to a task-related cognitive function that is generated by a patient's response to auditory, visual, or sensory stimuli. One of the strongest features of the ERP response is a response to unpredictable stimuli. This response, known as the P300 (or simply "P3"), manifests as a positive deflection in voltage approximately 300 milliseconds after the stimulus is presented (Hruby *et al.*, 2003).

Cognitive dysfunction in humans can vary from minimal changes, to mild impairment and severe dementia. At least two domains of cognitive function should be affected to attain such impairment. Domains of cognitive function include: memory, executive function, attention, language, and perceptual motor activity (Knopman *et al.*, 2003; Small *et al.*, 1997).

In an attempt to replicate the animal studies in humans, captopril was administered to 14 healthy volunteers who showed improvement in short-term memory (Currie et al., 1990). However the small sample size weakened the reliability of this study. A pilot clinical trial was done on ceranapril in Alzheimer disease patients to confirm its effect on cognition. Results showed no effect of ceranapril on cognition (Sudilovsky et al., 1993). Whereas Gard et al. presented an antidepressant effect of losartan in animal model but were unsuccessful in obtaining an association between the number of AT₁ receptors and depressed mood in post-partum women treated with either desipramine, fluoxetine, or tranylcypromine (Gard et al., 1999).

Short-term antihypertensive treatment with enalapril was associated with significant little decrease in psychomotor performance and significant small improvement in working memory (Muldoon et al., 2002). Both captopril and enalapril improved cognition and depressive symptoms in young hypertensive patients (Braszko et al., 2003).

Three double-blind randomized controlled trials of the beneficial effect of RAA drugs on cognition have been carried out using losartan, candesartan, and perindopril. Losartan induced a significant improvement in immediate and delayed memory in 120 mild to moderate elderly hypertensive patients (Fogari et al., 2003). Using a global test of cognitive measure (MMSE) in a large number of hypertensive patients, failure to any beneficial effect on cognitive task was reported with both candesartan and perindopril (Lithell *et al.*, 2003; Tzourio *et al.*, 2003). However, participants in perindopril study had cerebrovascular accident before entry into the study.

In a study done on patients with heart failure using enalapril, captopril, lisinopril, ramipril, fosinopril and quinapril, a favorable effect of ACEI on cognition was found in 30% of 446 patients using ACEI versus only 22% of 774 patients not started ACEI ($p=0.001$). (Zuccala et al., 2005).

A review of eight trials enrolling approximately 9000 patients showed that ACE inhibitors (captopril, enalapril, lisinopril, and perindopril) were safe and effective antihypertensive positively influencing cognitive function superior to diuretics and beta

blockers (Amenta et al., 2002). Recently, losartan was found to have cognition-improving properties after acute administration in young healthy volunteers (Mechaeil et al., 2011). Use of ACEIs or diuretics have been shown to improve neurocognitive functions in elderly women without dementia (Yasar et al., 2008).

In spite of the previous favorable effect of ACEI and ARB on cognition, a case report on losartan and enalapril-induced psychosis and depression, respectively have been reported (Ahmed, 1996; Patterson, 1989). The lack of further studies that confirm psychosis and depression as a side effect of ARB or ACEI hinders the documentation of such side effects.

Although the studies on neurocognitive effect of ACEI and ARBs are limited, there is sufficient evidence to support further investigations using more controlled designs. Human studies support a memory enhancing effect of agents blocking the action of RAS. This document the animal studies done on memory-impaired animals where they benefit from drugs that decrease Ang II function.

1.3 Chronic Kidney Disease and Neuropsychological dysfunction:

An exploration of neuropsychological defect in chronic kidney disease patients (CKD) will be given in this section.

CKD is a progressive disease. The etiology of CKD is multifactorial however diabetes mellitus (DM) is considered one of the most common causes of renal impairment. In Saudi Arabia, epidemiological studies documenting the incidence of DM reported an exponential rise in DM yielding different figures. The most recent study showed that 23.7% of Saudi population had DM (Al-Nozha et al., 2004), and diabetes was responsible about 30-45% of patients requiring dialysis (Mitwalli et al., 1997).

Patients suffering from CKD have many complications including anaemia, hypertension (HTN), hyperphosphatemia, hypocalcemia, hyperparathyroidism.

In addition to the previous known complications which are routinely monitored and treated, neurocognitive defect is not routinely tested in CKD patients and not recognized by health care providers; it is thus a hidden burden. This defect may influence patient behaviours and affect their decision regarding medical therapy and dialysis process.

Defects in cognition can also affect many aspects of medical care such as interfering with medication compliance and deteriorating quality of life (QOL) (Long et al., 1998).

A cohort of 51 dialysis patients (25 on hemodialysis (HD) and 26 on continuous ambulatory peritoneal dialysis (CAPD)) older than 70 years were examined with respect to QOL, depression and neurocognitive functioning. Results showed that 30-47% of patients were cognitively impaired, 61% depressed, and 15% had very poor QOL. Furthermore, physicians underestimated the cognitive defect when they were asked to rate this defect (Tyrrell et al., 2005).

Although testing for neurocognitive defects is not routinely performed, there are many studies that have been done on the neuropsychological abilities of renally-impaired patients and proved its implication for CKD patients. Studying cognition in CKD patients has increased dramatically in recent years since it has been considered a public health problem that will affect patient's compliance secondary to inability to learn or remember to take their medications. In a recent study exploring such issues, more than half of the elderly cognitively-impaired HD patients showed evidence of non-adherence (Hain, 2008). Moreover, this can lead to poor physical and mental health and long-term hospitalization (Kimmel *et al.*, 1998; Mittal *et al.*, 2001).

The high prevalence of cardiovascular risk factors among CKD patients such as DM and HTN increases the incidence of cognitive impairment among renally-impaired patients.

There are many older studies documenting neurocognitive impairment among CKD and dialysis patients (Gilli *et al.*, 1983; Marsh *et al.*, 1991; Ratner *et al.*, 1983; Ryan *et al.*, 1980). However utilizing such data might be not precise due to practice changes which include measures of adequacy of dialysis such as Kt/V^1 , urea; aluminum use, and adequate anaemia management. Frequent assessment of neurocognitive function may

¹ Kt/V is urea clearance which is a measure of adequacy of dialysis; where K is effective (delivered) dialyzer urea clearance in milliliters per minute integrated over the entire dialysis, t is the time in minutes measured from beginning to end of dialysis, and V is the patient's volume of urea distribution in milliliters; kidney disease outcome quality initiative KDOQI guideline $Kt/V = 1.2-1.4$.

help such patients in decision making regarding their health and improve medication and dialysis compliance and decrease medical care cost.

It was more than 10 years ago when Brickman et al examined 426 HD patients to investigate their subjective cognitive complaints. There was an increase in subjective complaints more strongly associated with depression and personality measure than demographic, neuropsychological, and medical factors. Whether this finding explains the origin of cognitive complaints in HD patients as mainly psychological or not, it still needs to be fully investigated. This study was the first to explain the etiology of the self-reported memory-concentration defect (Brickman et al., 1996).

The prevalence of cognitive impairment among 336 adult HD patients was examined using the MMSE and showed that 22% had mild impairment and 8% had moderate to severe impairment. This defect was poorly recognized when recognition of such defects by health care providers was investigated. Longer hospital stays and increased need for staff time among poorly cognitive patients were also reported (Sehgal et al., 1997).

Sixteen well-dialyzed, stable, end-stage renal disease patients (ESRD) underwent detailed neuropsychological assessment and were compared to 12 matched chronic disease patients with conditions such as DM, HTN, osteoarthritis, and rheumatoid arthritis. Both groups were mildly neurocognitively-impaired and they were similar which indicated that, in general any chronic diseases can mildly impair neuropsychological function (Pliskin et al., 1996). The authors suggested that early studies (Churchill *et al.*, 1992; Churchill *et al.*, 1991; Hart *et al.*, 1983; Souheaver *et al.*, 1982; Teschan, 1975) that have confirmed the neuropsychological defect of dialysis patients may be related to inadequate dialysis and not directly related to the disease itself. However the small sample size is a major limitation of the study.

It is well known that neuropsychological defects are problematic in CKD patients (Gilli *et al.*, 1983) and anaemia is considered one of the major causes of this defect (Denny *et al.*, 2006; Grimm *et al.*, 1990; Marsh *et al.*, 1991; Pickett *et al.*, 1999). In a recent meta-analysis of two studies addressing the issue of high risk of dementia or cognitive decline among CKD patients with anaemia, the results indicated a significant positive correlation between cognitive defect and anaemia (Peters et al., 2008).

Anaemia is considered one of the early complications of CKD and has silent symptoms. Therapy of anaemia with recombinant human erythropoietin revealed a trend for improvement of neurocognitive function in both predialysis (stage III and IV CKD) and dialysis (stage V CKD) patients, however, the level of improvement did not reach that of healthy volunteers (Singh et al., 2006).

A correlation was found between hematocrit level (Hct), QOL, and neurocognitive function in 56 dialysis patients. Patients were divided into 2 groups according to Hct level, lower and higher than 27.2%, and both groups were identical in most of the demographic and medical conditions. Although the QOL remains the same for both groups, there was a minimal improvement in learning and memory among those achieved a higher Hct level (Lee et al., 2004).

This raises the issue of not only anaemia being responsible for the neurocognitive defect, but of additional factors that need to be focused on and corrected in such a group of patients.

Other causes apart from DM (Gregg et al., 2000), of neurocognitive defect in CKD patients include HTN (Elias et al., 1993), age, education level, cerebrovascular diseases (Kivipelto *et al.*, 2001; Knopman *et al.*, 2001; Whitmer *et al.*, 2005), dialysis efficiency as measured by dialysis dose Kt/V (Eknoyan et al., 2002), frequency of dialysis (Jassal *et al.*, 2006; Vos *et al.*, 2006), and certain drugs that are known to cause such defect such as corticosteroids and antidepressants. However cognitive dysfunction after adjustment of such factors remains significant (Kurella et al., 2005b).

Insufficient studies have linked some additional risk factors for neurocognitive defect in CKD patients such as hyperparathyroidism (Garcia-Maldonado et al., 1991), high level of inflammatory cytokines (Grammas *et al.*, 2001; Yaffe *et al.*, 2003), oxidative stress (Berr, 2002), homocysteine (McIlroy *et al.*, 2002; Parsons *et al.*, 2002; Seshadri *et al.*, 2002), and lipid abnormality (Moroney *et al.*, 1999; Yaffe *et al.*, 2002).

Another issue of major concern is whether HD patients are similar to peritoneal dialysis in neuropsychological defect. It seems that the defect differs between these two groups since CAPD is more efficient in removing larger molecular weight toxins.

In a study done on 42 patients (25 on HD and 17 on CAPD) were neurocognitively evaluated and as suggested CAPD patients performed better. In general, dialysis patients have the same severity of disease but the type of dialysis differ according to certain criteria and CAPD is more efficient in removing big molecular weight toxins. However HD patients performed better 2 hr post-dialysis than 2 hr pre-dialysis, this is explained by the impact of increasing accumulation of uremic toxins, acute intravascular volume loss, and fluid shifts (Tilki et al., 2004). A similar finding was achieved from 20 HD and 10 CAPD patients (Williams et al., 2004).

In a more recent, large study carried out on 338 HD patients older than 55 yr using a battery of 9 neuropsychological tests for 45 minutes, it was found that 50% of the patients were mild to moderately impaired and 37% were severely impaired and only 13% were normal. The most significant factors related to severe cognitive impairment was a history of stroke, and equilibrated Kt/V (>1.2). A random sample of 101 patients from this cohort has been compared to age, sex, and race matched non-dialysis patients. Dialysis patients were 3 times more cognitively impaired than non-dialysis. The persistence of neurocognitive defect despite adequate dialysis indicates contribution of other factors in this defect (Murray et al., 2006).

Kurella et al published a number of studies in this area. The earliest was in 2004 which was a cross sectional study carried out on 80 CKD and 80 HD patients using Modified Mini-Mental State Examination (3MS), Trailmaking Test B (Trail B), and California verbal Learning Trial (CVLT) which examines global cognitive function, executive function, and verbal memory respectively. ESRD patients did worse on global cognitive function, more than double the general population, while CKD patients did worse on verbal memory and executive function. As expected when data were adjusted for the effect of age, education, sex, and comorbidity such as DM and stroke, ESRD patients were more impaired than CKD patients. This documents the direct relationship between stages of CKD and neurocognitive impairment (Kurella et al., 2004).

One year later, the same author applied the same concept on 1015 women younger than 80 yr with early stages of CKD (estimated Glomerular Filtration Rate (eGFR) less than 60 ml/min/1.73 m²) with coronary artery disease. Six neurocognitive tests were applied: 3MS, Trail B, Modified Boston Naming Test, Verbal Fluency Test, World List Memory, and World List Recall tests, in addition to measuring depressive symptoms using the 15-item Geriatric Depression Scale self-administered questionnaire to adjust for such symptoms. There was a direct correlation between severity of CKD and neurocognitive function including global cognition, executive function, language, and memory with a 27% increase in risk of global cognitive impairment with each 10 ml/min/1.73 m² decline in eGFR in menopausal women with coronary artery disease. This defect was independent of age, race, DM, and hypercholesterolemia (Kurella et al., 2005b). Although some studies supported a race contribution to neurocognitive defect which was more pronounced in adult African Americans and Latins than Whites (Albert *et al.*, 1999), this cannot be extended to an Arab population, e.g. Saudi.

Similar results have been achieved by the same investigators applying 3MS on elderly patients (age: 68-80 yr) having early stages of CKD. There was a 1.3-2.4 fold increase in risk of cognitive defect during 4 year of follow-up; however, such risk was greater in subjects with lower eGFR (eGFR <45 ml/min per 1.73 m²) and independent of other risk factors such as age, race, DM, and HTN (Kurella et al., 2005a).

In a further study on cognition and CKD, Kurella et al recruited a large number of participants (23,405) having more advanced CKD and included more women and African-American than the previous studies. Although a very simple test of global cognitive function was used (6-Item Screener) which is less sensitive, the results document the association between CKD and cognitive impairment independent of other risk factors, mainly cardiovascular (Kurella Tamura et al., 2008). Applying computerized neurocognitive tests on adults with moderate CKD (20-59 yr), similar results were attained especially in concentration and attention (Hailpern et al., 2007).

Use of P3 ERP highlighted the same finding in different stages of CKD even after adjustment of confounding factors that are known to exacerbate cognitive dysfunction, to the author's knowledge this is the only study that has examined the neurocognitive

dysfunction in early stages of CKD using a sensitive electrophysiologic test (Madan et al., 2007).

In summary, neurocognitive defect in renally impaired patients is well documented and the pathophysiology is multifactorial and needs to be investigated in greater depth. Such a defect in neurocognitive properties that mainly impairs memory, concentration and executive function has many implications on CKD patients which might affect their ability to give consent for dialysis therapy, compliance to medical therapy since most CKD patients require polypharmacy- and diet modification, in addition to following nurse instructions and education regarding fluid restriction. The high prevalence of such defects during dialysis, which is an important time for advising and educating patients, should be an issue of major concern.

1.4 Neurocognitive dysfunction and RAS in CKD:

As mentioned earlier the neurocognitive defect in CKD patients has many causes. There is no single study that has implied a single cause, and few studies have examined causative factors for cognitive dysfunction in CKD patients such as anaemia as a causative factor.

It is well documented that RAS has an effect on neuropsychological properties (Gard, 2002). Drugs acting on RAS such as ACEIs and ARBs in healthy volunteers have proved effective especially in attention and short-term memory (Currie *et al.*, 1990; Frcka *et al.*, 1988; Olajide *et al.*, 1985). In addition, studies of ACEIs and ARBs in hypertensive patients demonstrate the benefit of these drugs on mood and cognition (Braszko *et al.*, 2003; Fogari *et al.*, 2003).

Patients treated with brain-penetrating ACEI (perindopril or captopril), based on a study conducted on Alzheimer disease (AD) patients with HTN, demonstrated a lower decline in the rate of cognition than with people treated with non brain-penetrating ACEI (enalapril or imidapril) and calcium-channel blockers (nifedipine or nilvadipine) (Ohrui et al., 2004). Recently, this finding has been confirmed in a group of elderly hypertensive patients treated with ACEI that cross the blood-brain barrier (captopril and perindopril) (Sink et al., 2009). A 65% reduction in cognitive decline per year of

exposure to the centrally acting ACEI that cross blood-brain barrier compared to ACEI that do not cross the blood-brain barrier. However, ACEI that do not cross the blood-brain barrier was associated with higher risk of incident dementia. The author's explanation for such effect would be simply that non-centrally acting ACE inhibitors were less helpful in the prevention of dementia than other antihypertensive drugs which support the hypothesis that RAA drugs lower the risk of cognitive decline via mechanism other than blood pressure control. Losartan, the ARB agent that cross blood-brain barrier was also shown to improve neurocognitive function independent of blood pressure control (Tedesco et al., 2002).

Cerebral microvascular abnormalities have been implicated as one of the etiologies of vascular dementia (Baker *et al.*, 2007; Wong *et al.*, 2002). In an attempt to implicate the responsibilities of microvascular abnormalities, as evidenced by albuminuria, for neurocognitive defects, Barzilay et al tested urinary albumin and cognitive properties of 2,389 elderly subjects from Cardiovascular Health Cognition Study (CHCS). There were trends for increasing the association between albuminuria and decline in neurocognitive properties even after adjustment of factors that are known to cause cognitive defect such as HTN and DM. The results of this study are promising towards the role of drugs treating albuminuria, particularly ACEI and ARB, in improvement of neurocognitive disorder in CKD patients (Barzilay et al., 2008). Studies examined the direct relationship between neurocognitive defect of CKD patients and RAA drugs are lacking.

1.5 RAS gene polymorphism in CKD:

Genes of the RAS have been extensively studied and numerous functional polymorphisms have been identified. Multiple association studies have been performed in cardiovascular diseases, DM, kidney diseases, cancer, central nervous system disorders, and inflammatory conditions. The most commonly studied RAS gene polymorphisms were insertion/deletion (I/D) (NCBI ID rs1799752) of the ACE gene, A1166C (NCBI SNP ID: rs5186) of the AT₁R gene, C3123A (NCBI SNP ID: rs11091046) of AT₂R gene, and two variants of AGT gene; M268T (NCBI SNP ID: rs699) and T207M (NCBI SNP ID: rs4762) (previously described as M235T and T174M, respectively)

A quick review of the involvement of these RAS genes polymorphisms in CKD and neurocognitive defects will be given in this section.

1.5.1 ACE I/D polymorphism

The ACE gene is located on the long arm of chromosome 17 (17q23). The best known polymorphism of the ACE gene is characterized by a presence (insertion, I) or absence (deletion, D) of a 287-base-pair DNA fragment of a repeated *Alu* sequence at intron 16 (rs1799752) (Rieder et al., 1999). A meta-analysis of 145 studies revealed that the D allele overall prevalence is 54%, with race being a major determinant (Saab *et al.*, 2007a; Staessen *et al.*, 1997a). A prevalence of 56% of D allele in Caucasians, 60% in blacks, and 39% in Asians have been reported (Staessen et al., 1997a). In Arabs, a range of 60% of D allele has been reported in Syrians, 66% in Jordanians, and 67% in Egyptians (Salem *et al.*, 2009). Regarding the Saudi population; the target of this study, a prevalence of 69% of D allele has been reported in a study conducted in the central of Saudi Arabia (Dzimiri et al., 2000) with 78% in another study conducted in the southern area of Saudi Arabia (El-Hazmi *et al.*, 2003). The Emiratis population have been studied also and the D allele prevalence was 61%, while in Omanis it was 71% (Bayoumi et al., 2006). Different prevalences have been reported for the D allele in the Lebanese population ranging from 62% (Sabbagh et al., 2007), to 73% (Saab et al., 2007a). The ACE I/D polymorphism accounts for nearly 47% of the total variation in plasma ACE concentration such that subjects with two D allele has 50% higher plasma ACE level than subjects with two I allele (Rigat et al., 1990). This variation in plasma level of ACE has an important influence on the pathophysiology of mainly cardiovascular diseases as well as kidney disease (Abbud *et al.*, 1998; Hadjadj *et al.*, 2007).

Association studies between ACE polymorphisms and disease are many (Gard, 2010) and the most extensively studied is its association with cardiovascular diseases. An in depth investigation of cardiovascular disease studies was beyond the scope of this review, but in general carriers of the D allele have a positive association with cardiovascular diseases especially with hypertension (Higaki et al., 2000) and cardiovascular risk factors such as hyperlipidemia, DM, and obesity (Uemura et al., 2000).

1.5.1.1 Implication of ACE polymorphism in kidney disease

More than 500 articles on the association of ACE polymorphism and kidney disease has been published since 1989 with inconsistent findings. Around 46% of D allele frequency has been reported in ESRD & diabetic nephropathy (DN) Asian (including Chinese and Indian) and Caucasian patients (Lovati *et al.*, 2001; Prasad *et al.*, 2006; Tripathi *et al.*, 2006; Wu *et al.*, 2000)

It has also been reported that the D allele was associated with rapid deterioration in renal function in patients with IgA nephropathy as well as DN (Schmidt *et al.*, 1997), also in Asian Indian DN (Ahluwalia *et al.*, 2009), in Tunisian DN (Ezzidi *et al.*, 2009), and in Chinese type II DM DD genotype carriers (Wang *et al.*, 2005). The risk of renal disease in patients with IgA nephropathy was doubled in Asians carrying the DD genotype (Yong *et al.*, 2006). However, the risk of progression to ESRD was increased by 75% in Asians carrying DD genotype and 90% in DD genotype Caucasians. Iranian patients with Type II DM showed an increased albuminuria in D allele carriers (Nikzamir *et al.*, 2009). DD genotype was also more prevalent in female Mexican DN patients with albuminuria (Palomo-Pinon *et al.*, 2009). Faster decline of renal function has also been reported in non-diabetic renal disease DD genotype carriers, but only when proteinuria was less than 3.5 g/day (Samuelsson *et al.*, 2000).

A meta-analysis including 47 studies with 8663 subjects with DN and 6064 diabetic subjects that were considered as a control showed that II genotype carriers had a 22% lower risk of nephropathy than D allele carriers (Ng *et al.*, 2005). However, a subgroup analysis showed that type II diabetic Asians carrying the II genotype had a 35% reduced risk of nephropathy than type II diabetic Caucasians. A case-control study performed on Caucasians showed that French subjects carrying the D allele had 36% higher risk of diabetic nephropathy in comparison to Danish and Finnish subjects (Hadjadj *et al.*, 2007).

Conversely, a negative association between the risk of renal disease and ACE I/D polymorphism has been reported in 100 south Indian CKD patients (Anbazhagan *et al.*, 2009), and in 5320 French Type II diabetic patients (Hadjadj *et al.*, 2008). In addition, patients with autosomal dominant polycystic kidney disease showed no negative effect

of DD genotype (Pereira et al., 2006). Although the data were inconsistent, most studies supported the association between the D allele of ACE gene and both prevalence and progression of CKD.

1.5.1.2 Implication of ACE polymorphism and RAA therapy in renal disease

The antiproteinuric effect of RAA drugs has proven their superiority over other antihypertensives in controlling blood pressure and protecting kidney function from progressive decline. Carriers of the D allele of ACE gene have higher plasma and tissue ACE levels which may affect the efficacy of treatments with ACE inhibitors. Studies have shown that Asians with the DD genotype treated with ACE inhibitor progressed faster to ESRD (Moriyama *et al.*, 1995; Yoshida *et al.*, 1995). However, failure to reproduce a similar finding in Asians with diabetic nephropathy had been reported (So et al., 2006). The results again were inconsistent in Caucasians. In some studies, patients with DD genotype treated with an ACE inhibitor showed a faster progression of kidney disease and higher residual proteinuria than ID and II carriers (Jacobsen *et al.*, 2003; Nakayama *et al.*, 2009; Parving *et al.*, 1996; van Essen *et al.*, 1996). However, other studies did not show any differences (Bjorck *et al.*, 1997; Burg *et al.*, 1997; Penno *et al.*, 1998; van der Kleij *et al.*, 1997). Surprisingly, two studies showed a favorable effect of ACE inhibitor on renal function in DD genotype carrier (Perna *et al.*, 2000; Vegter *et al.*, 2009). Many reasons can explain the discrepancies among the previous studies which include, but are not limited to, the heterogeneity of study design, environmental factors such as salt intake, and patient characteristics. With respect to ARB therapy, there was no difference in response to ARB therapy among the three genotypes of ACE gene in patients with proteinuria (Andersen *et al.*, 2003; Andersen *et al.*, 2002; Haneda *et al.*, 2004; Park *et al.*, 2006; Parving *et al.*, 2008; Redon *et al.*, 2005).

Controlled trials with large sample size are needed to confirm the implication of ACE I/D genotype in kidney disease and hence the response to RAA therapy and the association of such response to ACE polymorphism.

1.5.1.3 Implication of ACE polymorphism in mood and cognition

RAS gene polymorphism association with neuropsychological defects has been investigated in very few studies and none correlated them to RAA therapy.

Amouyel et al reported 15 years ago the genetic association of the D allele of the ACE ID polymorphism with cognitive impairment in elderly French subjects, as evidenced by a MMSE test score of less than 10 out of 30 and/or with a diagnosis of dementia (Amouyel et al., 1996), the same group later documented the same finding in 1168 elderly subjects with more cognitive decline at 4-year follow-up in DD homozygotes carriers (Richard et al., 2000). However, a negative association between ACE gene polymorphism and normal cognitive aging has been reported in healthy British elderly subjects (Visscher et al., 2003), in vascular dementia patients (Kim *et al.*, 2006; Pandey *et al.*, 2009), and in AD patients (Scacchi et al., 1998) this was supported by recently published meta-analysis (Liu et al., 2009). Using detailed neuropsychological tests measuring different aspects of neurocognitive function including memory, attention, recall, and motor speed in elderly African-Caribbean subjects, no association was found between ACE gene polymorphisms and a decline in cognitive function. However, carriers of the DD genotype showed a strong association between age and cognitive decline (Stewart et al., 2004). In patients with traumatic brain injury, D allele carriers performed worse than I allele carriers in tests involving attention and processing speed (Ariza et al., 2006).

Conversely, a meta-analysis of 39 studies included 6037 patients with AD and 12099 control subjects showed a reduced risk of AD in homozygotic carriers of the D allele of the ACE I/D polymorphism (Lehmann et al., 2005). A similar finding has been reported only in subjects aged 73 years and above having late-onset AD in comparison to control subjects (Helbecque et al., 2009), and in dementia patients (Richard et al., 2001). A suggestion of the beneficial effect of ACE inhibition has been implicated in decreased AD development (Kehoe *et al.*, 2007).

The association of RAS genes with depression has been also investigated. A negative association between ACE gene polymorphisms and depression has been reported (Hong *et al.*, 2002; Meira-Lima *et al.*, 2000; Pauls *et al.*, 2000; Saab *et al.*, 2007b; Segman *et al.*, 2002; Stewart *et al.*, 2009). Studies documenting the positive impact of ACE I/D

polymorphism on mood disorder were carried out by same group of researchers published in 2002, 2004, and 2005. They showed that D allele carriers have more than 5-fold increase in risk of major depression (Bondy et al., 2002), while DD and ID carriers showed rapid improvements in depressive symptoms after treatment with antidepressants (Baghai *et al.*, 2004; Bondy *et al.*, 2005).

There are no published association studies between ACE I/D polymorphism and QOL or anxiety.

In summary, the D allele has been associated with increased CKD prevalence and progression, it is also associated with higher plasma level of ACE and so higher Ang II level. It may have more obvious implications for neurocognitive defects and depression than I allele. Therefore, it may be considered a risk factor for dementia and AD.

1.5.2 Angiotensin II type 1 receptor (AT₁R) gene A1166C polymorphism

This gene is located on chromosomes 3q21-3q25 and consists of five exons and four introns. The AT₁R A1166C polymorphism (rs5186) is located at the 5' end of the 3' untranslated region of the gene (Bonnardeaux et al., 1994). In this polymorphism of the AT₁R gene, the nucleotide Adenine changes to Cytosine at position 1166 of this gene. Response to Ang II was found not to be affected by this mutation (Hilgers et al., 1999).

The overall frequency of the C allele in the population is about 25% and is higher in Caucasians than Asians (Staessen et al., 1997a). The low percentage of the C allele in Asians has been supported by recent studies on Japanese finding a prevalence of 10% (Nishikino et al., 2006), with the Korean population at 13% (Kim et al., 2006), but a surprisingly higher percentage reported in Chinese 30% (Wu et al., 2000). In the Lebanese population, one study has been identified that also reported a low percentage of C allele 24% (Saab et al., 2007b). With respect to cardiovascular and kidney disease, the C allele was found to be increased significantly in hypertensive patients (Bonnardeaux et al., 1994).

Conversely, Losito *et al* reported a similar distribution of AT₁R gene polymorphism in both dialysis patients and control in an Italian population (Losito et al., 2002). A higher frequency of the C allele has been reported in Caucasian ESRD patients of Polish origin

compared to healthy controls (Buraczynska et al., 2006) and in Asians of Japanese origin (Tomino et al., 1999). Black hypertensive Americans also were found to have a higher frequency of C allele (Hsu et al., 2006). It has been shown also that the C allele is associated with a higher risk of renal dysfunction in Type II DM (Lin et al., 2009) and faster progression of renal disease (Buraczynska et al., 2002). After 10 years of Type II DM, CC genotype was found to be associated with albuminuria especially in male patients (Fradin et al., 2002). However, a negative association between AT₁R polymorphism and IgA nephropathy was found in Chinese (Woo et al., 2004), focal-segmental glomerulosclerosis (Luther et al., 2003), and in Japanese polycystic kidney disease (Konoshita et al., 2001). Evaluation of the association of several polymorphisms of RAS genes that included ACE I/D, AGT M268T, and AT₁R A1166C revealed an accelerated decline of kidney function in Caucasian Type I DM patients carrying D, M, and A alleles of ACE, AGT, and AT₁R, respectively and treated with ACE inhibitors (Jacobsen et al., 2003). However, a minor importance of C allele of AT₁R gene in Asian subjects with Type II DM has been reported (Osawa et al., 2007).

With respect to neuropsychological disorders, a Korean study showed that the AT₁R polymorphism did not contribute to genetic susceptibility to vascular dementia patients (Kim et al., 2006). In Lebanese depressed patients, the CC genotype of AT₁R A1166C polymorphism was significantly associated with depression (Saab et al., 2007b).

In summary, evidence of the involvement of AT₁R polymorphism in kidney and neuropsychological disorders does exist, but certain factors may influence this association particularly ethnicity. Furthermore, response to drugs acting on RAA system may be affected by the previously mentioned RAS polymorphisms.

1.5.3 Angiotensin II type 2 receptor (AT₂R) gene C3123A polymorphism

The human AT₂R gene is located on chromosome X (Xq22), so males and females have been studied separately since it is X-linked gene (Koike et al., 1994). It consists of three exons and two introns. In this polymorphism of the AT₂R gene, the nucleotide Cytosine changes to Adenine at position 3123 of this gene. AT₂R C3123A (rs11091046) was identified in humans in 1997 (Katsuya et al., 1997). In healthy Japanese the C allele prevalence was 68% in males and 73% in females. This polymorphism also studied in

depressed Lebanese population in comparison to healthy control (Saab et al., 2007b). The results showed that the C allele accounts for 55% in both healthy and depressed subjects and no association was the final result. In conclusion, few studies have been identified and of those no association between AT₂R polymorphism and cardiovascular or kidney disease has been reported (Akman *et al.*, 2009; Konoshita *et al.*, 2009) nor in depressed patients. One study has reported a significant association between A allele of AT₂R polymorphism and hypertension in only hypertensive males younger than 60 years (Katsuya et al., 1999).

1.5.4 Angiotensinogen gene (AGT) M268T polymorphism

This gene is located on chromosome 1 q42-43. It consists of five exons and four introns (Gaillard et al., 1989). The most clinically significant mutations of this gene is M268T (rs699) that is located in exon 2 of the gene at nucleotide 704 (Jeunemaitre et al., 1992). In this mutation the amino acid methionine changes to threonine at position 268 when thymine (T) substituted by cytosine (C) at position 702 on exon 2. The T allele is more common in blacks (77%) and Asians (78%) than whites (42%) (Wang *et al.*, 2000). Ang II plasma level was not affected by this polymorphism (Hopkins et al., 1996). However, T 268 homozygotes have plasma angiotensinogen levels that are 10% to 20% higher than M268 homozygotes and possibly higher RAS activity (Jeunemaitre et al., 1992). This gene has been found to be strongly associated with essential hypertension (Caulfield et al., 1994).

Evidence related to the association of M268T polymorphism to cardiovascular or kidney disease is weak (Lin *et al.*, 2009; Sethi *et al.*, 2003; Xu *et al.*, 2007). However, significant association of TT genotype of AGT M268T with malignant hypertension has been reported (van den Born et al., 2007). Rapid progression to ESRD has been reported in Caucasian diabetic patients with TT genotype (Lovati et al., 2001) and a significant contribution of the TT genotype to diabetic nephropathy in Chinese (Wu et al., 2000), an Arab population of Tunisian origin (Mehri et al.), and in Asian Indians with Type II DM (Prasad et al., 2006) has been reported. Conversely, the frequency of the M allele was higher in patients with vascular dementia of Korean origin (Kim et al., 2006), while no association was reported in depressed Lebanese (Saab et al., 2007b).

1.5.5 Angiotensinogen gene (AGT) T207M polymorphism

The T207M (rs4762) mutation also exists in exon two of the AGT gene where Methionine displaces Threonine at position 207. This genotype draws little attention in the literature and few studies have been identified. In a group of healthy Caucasians of Italian origin the genotypes distribution was 84.3% of TT, 14.8% of TM, and 0.59% of MM (Losito et al., 2002). In this study the author did not find any significant differences in genotype distribution between dialysis patients and healthy control. The same findings have been reported in Chinese diabetic nephropathy patients compared to healthy control (Wu et al., 2000). Prasad *et al* also reported similar distribution of AGT T207M genotypes in Asian Type II DM with or without nephropathy (Prasad et al., 2006). No reports have been identified discussing the association between this polymorphism and any neuropsychological disorders nor any cardiovascular diseases.

1.6 Conclusion:

There is a high prevalence of neurocognitive impairments in CKD patients and it is most probably multifactorial. Drugs acting on RAS might have a role in improving such defects in cognition. The involvement of RAS gene polymorphisms in both CKD progression and neurocognitive improvement have been an important issue. Inconsistent results have been reported between neurocognitive defects and RAS gene polymorphisms. However, evidence exists regarding the association of neurocognitive defects with both D and C alleles of ACE I/D and AT₁R A1166C polymorphisms, respectively. Targeting the understanding of RAS gene polymorphisms modification of RAA's effect on neurocognitive functions and management of this defect in cognition should be the focus of future research.

At present, there is no study that examines the direct relationship between neurocognitive defect of CKD patients and drugs acting on RAS as well as RAS gene polymorphisms. In addition, there are limited studies and inconclusive findings regarding the contribution of RAS gene polymorphisms in neuropsychological function in CKD patients. More investigation and explanation is warranted.

1.7 Aims

- To study cognition, mood, anxiety, and QOL in Saudi CKD patients. This will be achieved by studying neurocognitive abilities of CKD patients using a number of neurocognitive tools that measure different domains of cognition. This will be conducted since the Saudi CKD patients have been not tested in depth previously for neurocognitive defect to find out if they differ from other healthy controls.
- To compare the effects of different antihypertensive medications (ACEIs and/or ARBs versus other antihypertensives) on cognition in Saudi CKD patients. This will be achieved by comparing the neurocognitive abilities of patients using RAA drugs versus those using other types of antihypertensive. This will be investigated to explore the neurocognitive-improvement effect of antihypertensive drugs acting on RAS such as ACEIs and ARBs.
- To study mood and cognition of colon cancer patients in remission. This is going to be achieved by applying the same neurocognitive tools to assess cognition of colon cancer patients in remission. The aim is to rule out the effect of chronic disease on neurocognitive defect of CKD patients. Several groups of chronically ill patients were considered for this phase of the study, for example chronic inflammatory disorders, chronic respiratory and cardiovascular disorders. Colon cancer patients in remission were selected because they have a potentially fatal illness which is currently in remission. They are currently symptom- and drug-free, but require regular, frequent hospital checks. These patients have a constant fear of their illness and have a regular hospitalization: they are therefore similar, in some aspects, to CKD patients, but without the confounding variables of symptoms and medication such as cardiovascular disease and medication that affect cognition.
- To study cognition, mood, anxiety, and QOL in Saudi HD patients. This will be achieved by studying neurocognitive properties of HD patients using the previously mentioned neurocognitive tools. This is will be carried out since such a population has not been tested previously for neurocognitive defects to find out if they differ from other populations.

- To compare the effects of different antihypertensive medications (ACEIs and/or ARBs versus other antihypertensives) on cognition in Saudi HD patients. This will be achieved by comparing the neurocognitive properties of patients using RAA drugs versus those taking other type of antihypertensive. This will be investigated to investigate the neurocognitive-improvement effect of antihypertensive drugs acting on RAS such as ACEIs and ARBs.

- To investigate the role of RAS in the etiology of subjective cognitive complaints. This will be achieved by correlating between RAS status (gene polymorphisms and RAS-related medications) and neuropsychological dysfunction in HD patients. This will explore whether there is a neurocognitive-improvement effect of drugs acting on RAS.

- To compare RAS polymorphism distribution of Saudi dialysis patients with published RAS polymorphism of Saudi healthy controls. This will give insight into the role of the polymorphisms in the aetiology and prognosis of end-stage renal failure and the association of the polymorphism and neuropsychological consequences.

CHAPTER 2. METHODOLOGY

2.1 Recruitment and study design

2.1.1 Participants

Patients with stable chronic kidney disease (CKD) attending an outpatient nephrology clinic at King Khaled University Hospital (KKUH) as well as healthy controls from the same source were recruited for the first part of the study.

The goal was to recruit 60 CKD patients and 30 healthy volunteers based on sample-size calculations necessary to obtain a 33.0% one-way effect size (obtained from a previous study on healthy volunteers (Mechaeil et al., 2011)) and a probability of 0.05 and a test power of 80% (i.e. 80% chance of detecting an effect which is the recommended power in statistical analysis). The patients were divided into two groups either using renin-angiotensin antagonist drugs (RAA group) as an antihypertensive or using other type of antihypertensives (non-RAA group).

For the second part of the thesis, a group of stable colon cancer patients in remission was chosen as a control group to rule out the effect of chronic disease on neurocognitive defect.

The last part of the thesis include all stable dialysis patients undergoing hemodialysis at Prince Samlan Dialysis Centre (PSDC) and King Abdulaziz Medical City (KAMC) dialysis center were eligible for neurocognitive testing and DNA extraction and renin-angiotensin system (RAS) genotype distribution. A group of Saudi healthy blood donors visiting the Blood Donor Clinic of the King Faisal Specialist Hospital and Research Centre (KFSHRC) were recruited to determine RAS genotype distribution in the general healthy population. The total cohorts were four patient groups from three hospitals and 2 control groups from two hospitals.

2.1.2. Inclusion and exclusion criteria

Eligible patients were those who were adult (18-60 years old), ambulant, and literate having clinically stable CKD or on hemodialysis (HD) as documented based on the charging nephrologists and patient history. Patients were excluded if they were smokers, had psychiatric illness, cerebrovascular disease, visual or hearing impairment, abnormal serum calcium or sodium, abnormal thyroid function tests, uncontrolled high blood pressure (BP>170/110), hemoglobin (Hgb) level of < 7gms/dl, or using glucocorticoids, lipid-soluble beta blockers or any other medications that are known to affect neurocognitive functioning. Age-, sex-, and education-matched healthy volunteers as well as colon cancer patients in remission were also recruited as a comparative group.

2.1.3 Design

In this observational study all participants underwent neurocognitive and psychological testing. Dialysis patients underwent also a 10-ml venous blood sampling for DNA extraction and RAS genotyping. Participants were allocated into one of the two drug groups according to their antihypertensive treatment (RAA and non-RAA) without interference from the main researcher. The main dependent measures were either the total score of self-answered questionnaire, or accuracy depending on task parameters.

2.1.4. Procedure

The eligibility of patients was identified by checking the patient's chart for inclusion and exclusion criteria before administering neuropsychological test. Eligible patients were notified about the procedure of the tests and asked to sign informed consent form (Appendix 1.1 and 1.2) after explanation of the aim of study by the researcher. Each patient then completed the tests in a separate room and the entire session lasted approximately 45 minutes that was administered in the same order to all participants. Thereafter a blood sample was obtained from each participant by the nurse in charge of the patient. Blood samples were transferred by the main researcher in a secure cold container to KFSHRC pharmacogenetic lab for DNA extraction and RAS genes amplification. Each eligible patient was given a code to be known with when antihypertensive therapy type identified. This minimized the bias during interpretation

of neuropsychological tests. An approved statement was obtained from KKHU, PSDC, and KAMC research committee.

2.1.5. Chart review

Each patient was given a code so their medical data were unrevealed by the main researcher. Demographic characteristics (age, gender, education level) and medical data such as cause of CKD, other disease, dialysis and disease duration, Hgb level, sodium, potassium, calcium, phosphorous, thyroid and parathyroid function test, BP, and medication list were obtained from the medical chart and recorded in a specific data collection sheet (an example sheet is attached in Appendix 2.1 and 2.2). Laboratory data obtained were recent and coincided with cognitive function test time.

2.2. Neuropsychological Method

All subjects underwent a battery of 5 neuropsychological tests that assess memory, attention, and executive function. Quality of life (QOL), anxiety, and depression assessment were also performed (see Appendix 3). Neuropsychological tests were carried out in the same order for all patients. The choice of neuropsychological test was made after a consultation of a neuropsychologist and guided by a review of existing literature of this area. Memory, attention, and executive function were represented in this study. The neuropsychological tests used were: Rey-Osterrieth complex figure test (RCF), the Rey auditory-verbal learning test RAVLT), digit symbol substitution task (DSST), mental fluency task, and letter cancellation task (see Appendix 3 for entire battery) (Lezak et al., 2004). These particular neuropsychological tests were selected because they are quite common and administered in an acceptable time frame, and more lengthy tests were avoided to eliminate the effect of stress on patient's performance.

Although the available neuropsychological tests were in English and the tested subjects were Saudi of Arabic origin, a translation had been made to only the fifteen words in RAVLT. English letters and digits in letter cancellation and digit substitution tasks, respectively have not been translated since all subjects were literate and cognisant with English letters and digits. Illiterate subjects were excluded from this study since all neuropsychological tasks necessitate literacy. Measures of accuracy score were

provided as the main dependent measure. All tests require paper and pencil and were printed on A₄ paper.

2.2.1. Cognitive measures

2.2.1.1. Memory

Rey-Osterrieth complex figure (RCF)

The Rey-Osterrieth complex figure test assesses visuospatial organization and visuospatial memory (Fischer, 2004). This test was developed by Rey (1941) and elaborated by Osterrieth (1944). The subject is asked to copy the Rey complex figure that consists of geometric shapes and lines, then reproduce the figure at 3 and 30 minutes later. Scoring was based on a standard scoring system developed for the Rey Complex Figure (Appendix 4). The figure consists of 18 items, each has a score of 2 and the total score is 36, so the score is given to the total corrected items out of 36. It has been reported that education and male gender are associated with better performance among healthy subjects (Lezak et al., 2004).

Rey Auditory-Verbal Learning test (RAVLT)

The Rey Auditory-Verbal Learning Test assesses immediate working and short-term memory and learning skills (Lezak et al., 2004). The original version was developed by Andre Rey (1964). The test later was altered and adapted by Taylor (1959) and Lezak (1983) to be used with English-speaking subjects. In this project the words were translated to Arabic by the main investigator under specialized clinical neuropsychologist supervision. In the RAVLT, 15 translated Arabic nouns are read aloud one at a time for 5-consecutive trials, each trial is followed by a free recall test, then an interference list of another 15 words is presented and followed by a free recall test of that list. Immediately after this, delayed recall of the first list is tested and then after 20 minutes the examinee is again required to recall words from the first list, and finally recognition of the target words presented with distracters. The score was the number of remembered words in the first trial (maximum score 15) and in the five trials (maximum score 75), the number of missed words in the immediate and delayed recall

trials, and the number of recognized words. It has been reported that female performed better than male in recall trials but not on the recognition task (Lezak et al., 2004).

2.2.1.2. Attention

Digit-symbol-substitution-test (DSST)

The digit-symbol test assesses sustained attention, visual searching, visual sequencing, and new-learning abilities of the patients (Lezak et al., 2004). This test was developed by Wechsler (1944). In this test a coding key is provided consisting of 9 abstract symbols, each paired with a number and the examinee is required to scan the key and write down the symbol corresponding to each number, as rapidly as possible. The score was the number of symbols copied correctly in 120 seconds. Some reports indicated women outperform men but only in United States and Canada, not in France (Lezak et al., 2004).

Letter cancellation task

The letter cancellation test was developed by Diller, Ben Yishay et al. (1974) and assesses attention (Lezak et al., 2004). The subject is asked to cancel the letter C and E by marking with ink as quickly as possible from six 52-letter rows in which the target letters are randomly interspersed approximately 18 times in each row. The score was the number of correctly cancelled Cs and Es per second. Translation to Arabic letters was not made since all participants were educated and aware of all English letters. the performance of this test was not affected by age, education, and gender (Lezak et al., 2004).

2.2.1.3. Executive function

Mental fluency

In the semantic verbal fluency test subjects are asked to name as many animals as possible within 60 seconds to assess executive function, working and semantic memory (Lezak et al., 2004). This test has a long history of use in neuropsychology, dated from the work of Thurstone (Thurstone, 1938). The score was the number of names generated

in 60 seconds. Some reports indicated that female especially educated performed better on letter fluency while animal fluency may not be affected by gender (Lezak et al., 2004).

Executive function is a collection of processes that are responsible for guiding, directing, and managing cognitive, emotional, and behavioral functions particularly during active, novel problem solving. Poor executive function indicates poor working memory, difficulty generating and implementing strategies and difficulty correcting errors and carelessness (Lezak et al., 2004).

2.2.2. Quality of Life (QOL)

The 36-item Short-Form Health Survey SF-36 (Ware *et al.*, 1992) assesses quality of life in patients with chronic disease. It is the most widely used generic QOL instrument and was designed by the medical outcomes study, the Arabic version has been validated in Arab population in 2003, and applied in this study (Sabbah et al., 2003). It consists of nine subscales: physical functioning (PF), role limitation due to physical health (RP), role limitation due to emotional problems (RE), vitality (VT), mental health (MH), social functioning (SF), bodily pain (BP), and general health (GH). In addition, one single item determines perceived differences in state of health over the past year called health change (HT). Each scale is composed of a number of items and each has a total score of 100 with averaging items to form a scale. All scales scores are totaled to give the final result of SF-36.

Two SF-36 subscales were also used as a summary of the previous items. The Physical Component Summary (PCS) and the Mental Component Summary (MCS) scales. PCS derived from four domains: PF, RP, BP, and GH while MCS derived from another four domains: VT, SF, MH, and RE.

2.2.3. Anxiety and depression

The Hospital Anxiety and Depression Scale (HADS) (Zigmond *et al.*, 1983) assesses depression and anxiety and was used to examine the possible influence of depression and anxiety symptoms on neurocognitive performance. It is a self-assessment scale composed of 14 items, 7 for anxiety and 7 for depression, each item rated on a four-point scale from 0 to 3. Subscales scores therefore range from 0 (no distress) to 21

(maximum distress). Score cut-off points classify patient's anxiety and depression levels as within normal range (0-7), borderline (8-10), or clinical (11-21). Caseness level starts from 11 and above and indicate symptom severity (Mystakidou *et al.*, 2005; Zigmond *et al.*, 1983). The Arabic version has been validated in Saudi population and was applied in this study (el-Rufaie *et al.*, 1987). Table 2.1 below outlines tests and tests-properties used.

Cognitive domain	Task	Psychometric parameters
Memory	Rey Auditory-Verbal Test (RAVLT)	- Learning - Short-term/ working memory
	Rey-Osterrieth Complex figure (RCF)	- Visuo-spatial organization - Visuo-spatial memory
Attention	Letter cancellation	- Sustained attention - Psychomotor speed
	Digit symbol substitution test (DSST)	- Sustained attention - Visual searching - Visual sequencing
Executive function	Semantic verbal fluency	- mental flexibility
Quality of life	The 36-item Short-Form Health Survey (SF-36 QOL)	- Mental and Physical properties
Anxiety and Depression	Hospital Anxiety and Depression Scale ((HADS)	- Anxiety and Depression

Table 2.1 Summary of cognitive domains, the task used to measure them and the psychometric parameters assessed.

2.3 Biochemical methods

2.3.1. Sample collection and DNA extraction:

A 10 ml venous blood sample was obtained in EDTA-tubes from dialysis patients. Immediate DNA extraction was performed or samples were stored at 4°C for later DNA extraction (maximum 2 days).

DNA was extracted using PURGENE Blood Core Kit C (QIAGEN Sciences, USA). Red blood cells (RBC) were lysed by adding 3 ml RBC Lysis Solution to 10 ml of whole blood in a 50 ml tube labeled with patient' ID which is inverted to mix and incubated for 10 minutes at room temperature. The sample then centrifuged (Centrifuge 5810 R Eppendorf) at 1006 g (3000 rpm) for 10 minutes at 18°C. The supernatant was removed and the white pellet was kept with about 100 µl of the residual liquid. The tube containing the white pellet was vortexed (Vortex-genie 2) for 20 seconds to resuspend the cells in the residual liquid which facilitates cell lysis later on. Cell Lysis Solution 10 ml was added and pipetted up and down to lyse the cells. The sample was incubated at room temperature for 2-3 day.

Protein Precipitation Solution 3.33 ml was added to the cell lysate, then mixed thoroughly by vortexing at high speed for 20 seconds and centrifuged at 1006 g (3000 rpm) at 18°C for 10 minutes.

The supernatant containing the DNA was poured into a clean 50 ml labeled-tube and 10 ml of Isopropanol was added. The sample was mixed gently by inverting 50 times until the white threads of DNA formed a visible clump. The sample was centrifuged at 1006 g (3000 rpm) at 18°C for 3-5 minutes where DNA was visible as a small white pellet. The supernatant was discarded and tube drained briefly on clean absorbent paper, then 3 ml of 70% Ethanol was added to the pellet and the tube inverted several times to wash the DNA pellet. The solution was centrifuged at 1006 g (3000 rpm) for 1 minute at 18°C and the ethanol was carefully poured off. The tube was inverted and drained on a clean absorbent paper and allowed to air dry for 10-15 minutes.

A 250 μ l sample of DNA hydration solution was added for a small pellet, and 350-400 μ l for a big pellet. The sample was incubated at 65°C for 1 hour in a water bath (Isotemp 220, Fisher Scientific) then left overnight at room temperature. DNA was stored at 4°C until Polymerase Chain Reaction (PCR) was performed.

Quantification and purity of the DNA was determined by spectrophotometry. A total volume of 1.5 μ L of DNA solution was loaded into a Nano Drop® ND-1000, (Thermo Scientific, Wilmington, USA) spectrophotometer pedestal and checked for DNA concentration and purity. The concentration of DNA in the sample was displayed based upon the optical density (OD) reading at 260 nm. The ratio between the reading at 260 nm and 280 nm (OD 260/ OD 280) provides an estimate of the purity of DNA. Pure preparation of DNA has OD 260/ OD 280 value of 1.8. A typical spectrophotometer report sample for the DNA reading is included in table 2.2 below.

The stability of DNA in working samples was determined by measuring DNA concentration at the point of isolation and then repeating after one month. A stock solution of the sample was kept frozen at -80°C. The storage period ranges from one to four weeks. Working DNA samples were stored at 4°C and genotyped within 4 weeks.

Sample	Concentration (ng/μL)	Ratio OD 260nm/ OD 280nm
1	396	1.86
2	286	1.88
3	464	1.86
4	710	1.88
5	527	1.85
6	294	1.89
7	485	1.87
8	822	1.86
9	728	1.88
10	615	1.89
11	300	1.88
12	290	1.85
13	305	1.87
14	391	1.86
15	272	1.87

Table 2.2 DNA concentration and purity measurement in a fresh sample. A ratio of 1.80 denotes pure DNA.

2.3.2 Polymerase chain reaction (PCR) Based-Assays

2.3.2.1 PCR-Reagents Used for DNA Amplification

Gene specific predesigned sequences of sense and antisense oligonucleotides were purchased from Metabion International AG, Germany. The primers were thawed, centrifuged for 20 seconds then diluted in 1X Tris-EDTA (TE) buffer to prepare 5 μ M of each primer. TE buffer was freshly prepared by adding 49.5 ml water to 0.5 ml 100X TE solution. PCR stock reagents purchased from QIAGEN[®], Germany were 10X PCR buffer solution [Tris-Cl, KCl, (NH₄)₂SO, 15mM MgCl₂; pH 8.7 (20°C)], 10 mM dNTPs, and 5U/ μ L Hot Star Taq[™] polymerase. Stock solutions were stored at -20°C. Shelf stored nuclease free water, purchased from Baxter Health Care Corporation, USA and DNA solution prepared as mentioned above were also utilized.

2.3.2.2 PCR Gene Polymorphism Genotyping

2.3.2.2.1 ACE (I/D) (rs1799752) Gene Polymorphism Genotyping

The presence of the insertion and deletion allele in intron 16 of the ACE gene was detected using the PCR method of Ryu et al with some modification (Ryu et al., 2002). The sequence of sense oligonucleotide primer was 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and the antisense primer 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'. Polymerase chain reaction was performed in a final volume of 50 μ L containing 10X PCR buffer, 10 mM dNTP, 5 U/ μ L Hot Star Taq[™] DNA polymerase, 25 mM MgCl₂, 5 μ M of each primer, and 4 μ L of DNA solution.

Amplification was performed by using a DNA thermal cycler (PTC-200 Peltier Thermal Cycler, USA). Samples were denatured for 15 minutes at 95°C and then subjected to 35 cycles of 30 seconds at 94°C, 30 seconds annealing at 59°C, and 45 seconds extension at 72°C. An additional 9 min extension time was carried at 72°C.

PCR products were separated, sized by electrophoresis on a 2% agarose gel, stained with ethidium bromide and viewed under UV illumination (the detailed procedure can

be found in section 2.3.4). The insertion allele manifested as a 490-bp band, and the deletion allele was visualized as a 190-bp band. Preferential amplification of the D allele occurs in ID heterozygotes, leading to misclassification of ID as DD in 4% to 5% of cases (Lindpaintner et al, 1995). Therefore, all DD samples were subjected to a second, separate PCR amplification with a primer pair which recognizes the insertion-specific sequence 5'-TGG GAC CAC AGC GCC CGC CAC TAC -3' ; 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3', under identical PCR conditions except for an annealing temperature of 63.5°C for 36 cycles. The reaction yields a 335-bp amplicon only in the presence of an I allele, and no product in samples homozygotes for DD. This procedure correctly identified the 4 to 5 percent of samples with the ID genotype that are misclassified as DD with the insertion-spanning-primers.

All samples were genotyped in King Faisal Specialist Hospital and Research Center (KFSHRC). The genotype of some ambiguous samples was repeated twice to confirm the results. The genotype results were scored by two independent investigators (the author and one of the molecular biology lab technical staff) without knowledge of the sample status of each study individual.

Genotyping was performed after completion of DNA separation process for the total sample size. For 96 samples, a PCR mix of reagents, named PCR master mix, was prepared. The reagent's concentrations in the master mix are based on those needed for 96 samples. An illustration of the needed volume of each reagent per sample reaction, followed by the required volume for the PCR mix is shown in table 2.3 below.

All PCR tubes and pipette tips were nuclease free. The work was developed on ice and under sterile conditions in a hood.

During PCR sample reaction preparation, the required DNA volume (4 μ L in this case) was added to PCR labeled plate, capped and put in the ice. Then PCR master mix was prepared by adding at first water, then the other reagents. Buffer, dNTPs, primers were non-vigorously vortexed prior to use. Hot Star TaqTM was kept at -20°C till the end and then added to the mix. When the latter was ready, the required volume was added to each DNA sample in the PCR plate (in this case 46 μ L of PCR mix was added to 4 μ L

of DNA volume). DNA with other reagents was amplified in the thermal cycler that has the PCR conditions as established in a stored program.

Reagent	Stock Solution	Working Solution	Conc/Sample	Volume/Sample	Volume/PCR Mix for 96 samples
PCR Buffer	10 X	10 X	1X	5 μ L	480 μ L
dNTP	10 mM	10 mM	0.2 mM	1 μ L	96 μ L
Primers Each	100 μ M	5 μ M	0.4 μ M	4 μ L of each	384 μ L
MgCl ₂ *	25 mM	25 mM	1 mM	2 μ L	192 μ L
Hot Star Taq™	5 U/ μ L	5 U/ μ L	0.06 U/ μ L	0.6 μ L	57.6 μ L
DNA	250-900 ng/ μ L	50 ng/ μ L	4 ng/ μ L	4 μ L	xxxxxxx
Water				29.4 μ L	2822.4 μ L
Total reaction volume				50 μ L	46 μ L for each sample reaction

Table 2.3 PCR mixture calculations for ACE (I/D) rs1799752, AGT (M268T) rs699, (T207M) rs4762, AT₁R (A1166C) rs5186 and AT₂R (C3123A) rs11091046 genes.

* MgCl₂ for ACE I/D amplification only.

2.3.2.2.2 AGT (M268T) (rs699) Gene Polymorphism Genotyping

AGT typing was assessed by restriction fragment length polymorphism (RFLP). Sense and antisense sequences of the primer were 5'-CAC GCT CTC TGG ACT TCA CA-3' and 5'-CAG GGT GCT GTC CAC ACT GGA CCC C-3', respectively (Ryu et al., 2002). PCR was performed in a final volume of 50 μ L, which contained 4 μ L of each 5 μ M primer, 1 μ L of 10 mM dNTP, 5 μ L of 10X PCR buffer, 0.6 μ L DNA polymerase, and 4 μ L of 50ng/ μ L DNA solution. Amplification was performed in a PTC-200 Peltier Thermal Cycler, USA. Samples were denatured for 10 minutes at 94°C, followed by 35 cycles of the following steps each: 15 seconds at 94°C, 45 seconds at 60°C, and 45 seconds at 72°C. The PCR product was electrophoresed in 2% agarose gel and visualized directly by ethidium bromide staining. The presence of 164 base-pair (bp) bands indicates the AGT M268T DNA amplification. Amplified DNA was cleaved with the restriction fragment Tth111 enzyme to differentiate between the homozygous MM, TT, and the heterozygous MT (the detailed procedure can be found in section 2.3.3). Bands of the final restricted product was again electrophoresed in 2% agarose gel and visualized by ethidium bromide. The homozygous M268T amplified DNA was incubated with the Tth111 and cleaved to yield a 141 bp and 24bp fragment. In the absence of the M268T variant, the 164 bp amplification product remained intact. Both 164 bp, 140 bp, and 24 bp fragments were apparent for heterozygote. Since the gel image of this polymorphism was not very clear, the result was double checked by sequencing using the MegaBACE DNA analysis system (Amersham Biosciences, Piscataway, NJ, USA) and figure 2.6 shows the results that were the same as the gel picture.

2.3.2.2.3 AGT (T207M) (rs4762) Gene Polymorphism Genotyping

AGT typing was assessed by RFLP. Sense and antisense sequences of the primer were 5'-GAT GCG CAC AAG GTC CTG-3' and 5'-CAG GGT GCT GTC CAC ACT GGC TCG C-3', respectively (Losito et al., 2002). PCR was performed in a final volume of 50 μ L, which contained 4 μ L of each 5 μ M primer, 1 μ L of 10 mM dNTP, 5 μ L of 10X PCR buffer, 0.6 μ L DNA Hot Star TaqTM polymerase, and 4 μ L of 50ng/ μ L DNA solution. Amplification was performed in a PTC-200 Peltier Thermal Cycler, USA. Samples were denatured for 10 minutes at 94°C, followed by 35 cycles of the following steps each: 1 minutes at 94°C, 1 minutes at 61°C, and 1 minutes at 72°C, final extension was at 72°C for 10 minutes. The PCR product was electrophoresed in 2%

agarose gel and visualized directly by ethidium bromide staining. The presence of 100 base-pair (bp) bands indicates the AGT T174M DNA amplification. Amplified DNA was cleaved with the restriction fragment NcoI enzyme to differentiate between the homozygous TT, MM, and the heterozygous TM (The detailed procedure can be found in section 2.3.3.). The final restricted product was again electrophoresed in 2% agarose gel and visualized by ethidium bromide. The homozygous T174M amplified DNA was incubated with the NcoI and cleaved to yield a 64 bp and 33 bp fragment. In the absence of the T174M variant, the 100 bp amplification product remained intact. Both 100 bp, 64 bp, and 33bp fragments were apparent for heterozygotes.

2.3.2.2.4 AT₁ (A1166C) (rs5186) Gene Polymorphism Genotyping

PCR was performed to amplify a fragment encompassing the A→C polymorphic site at 1166 nucleotide position in the 3' untranslated region of the AT₁ gene. The predesigned primer's sequences were as follows: sense, 5'-ATA ATG TAA GCT CAT CCA CC-3'; antisense, 5'-GAG ATT GCA TTT CTG TCA AGT-3' (Losito et al., 2002). The reaction volume was 50 µL consisting of 4 µL of 50ng/ µL DNA, 4µL of each 5 µM primer, 1 µL of 10 mM dNTP, 5 µL of 10X PCR buffer, and 0.6 µL DNA Hot Star TaqTM polymerase. Amplification was carried out using a PTC-200 Peltier Thermal Cycler, USA. It underwent an initial denaturation at 94°C for 10 minutes followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, final extension was at 72°C for 10 min. the PCR products were electrophoresed to confirm the exact amplification, and then were digested with enzyme Dde I for 1 hour at 37°C. the digested products were visualized on 2% agarose gel by ethidium bromide staining. A 367 bp bands indicate AT₁ A1166C DNA amplification, while cleavage to 224 bp and 143 bp indicate the presence of A1166C homozygous. Both 367 bp, 224 bp, and 143 bp fragments were apparent for heterozygotes.

2.3.2.2.5. AT₂ (C3123A) (rs11091046) Gene Polymorphism Genotyping

The reported C to A mutation at the 3123 nucleotide position in the 3' untranslated region of AT₂ receptor gene was amplified using the following primers' sequences: sense, 5'-GGATTC AGA TTT CTC TTT GAA-3'; antisense, 5'-GCA TAG GAG TAT GAT TTA ATC-3' (Katsuya et al., 1997). Each PCR reaction was performed in 50 µL

containing 1 μL of 10 mM dNTP, 5 μL of 10X PCR puffer, 4 μL of each oligonucleotide primer 5 μM , 0.6 μL of Hot Star TaqTM DNA polymerase, and 4 μL of 50 ng/ μL genomic DNA. Amplification was carried out for 30 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, and extension at 72°C for 1 min using PTC-200 Peltier Thermal Cycler, USA. After PCR reaction and confirming DNA amplification by electrophoresis, 10 μL of PCR product was digested with 10 units of Alu I at 37°C for 1 hour, after which a deactivation process is carried out for 15 min at 70°C. electrophoresis using 2% agarose gel with ethidium bromide staining was performed to differentiate between the homozygous C and A allele that has a 321 bp fragments and 214- and 107 bp fragments, respectively. The presence of all 321 bp, 214bp, and 107 bp indicate heterozygosity.

2.3.3. Restriction Fragment Length Polymorphism Gene Genotyping (RFLP)

2.3.3.1. Reagents Used for RFLP

Restriction enzymes including Tth 111, Nco I, Dde I and Alu I were used to cleave AGT M268T, AGT T207M, AT₁R, and AT₂R PCR products, respectively. All enzymes were purchased from NEW ENGLAND BioLabs_{Inc.}, USA. Reagents supplied with the enzymes consist of buffer solutions, i.e., 10X NE Buffer 4TM, 10 X NE Buffer 3TM, 10 X NE Buffer 3TM, 10 X NE Buffer 2TM to be used with Tth 111, Nco I, Dde I and Alu I, respectively.

2.3.3.2. RFLP Procedures

To cleave the DNA (PCR product) in a particular site of the amplicon, a restriction enzyme with the buffer is required for a specific incubation period of time. The calculation of the enzyme buffer, and DNA concentrations, and the incubation period were based on manufacturer instructions. As a general rule, 1 μg of DNA required 1 U of the enzyme for complete digestion after 1 hour at 37°C and 1 μL of DNA contains 0.5 μg . The applied calculation was as follows: The enzyme Tth 111 4000 units/ μL was used to cleave AGT M268T gene. The recognition sequence for Tth 111 is GACN/NNGTC. A 10 μL of the PCR product with 2 μL enzyme and 1.2 μL buffer was incubated at 65°C for 4 hours.

Nco I enzyme (10 units/ μ L) was used also to cleave AGT T207M gene and the recognition sequence for this enzyme is C/CATGG. 10 μ L of the PCR product with 0.6 μ L of the enzyme plus 1.1 μ L buffer was incubated for 1 hour at 37°C. Deactivation process was carried out thereafter for 20 min at 65°C.

Dde I with puffer 3 and Alu I enzymes with buffer 2 (10 units/ μ L) were required to cleave the sequence of the AT₁ and AT₂ genes, respectively. The recognition sequence for Dde I is C/TNAG, and that of Alu I is AG/CT. The incubation for both reactions was at 37°C for one hour. Deactivation process was carried out thereafter for 20 min at 65°C.

2.3.4 Gel Electrophoresis and PCR Product Identification

2.3.4.1 Gel Electrophoresis

2.3.4.1.1 Reagents Used for Gel Electrophoresis

Agarose and ethidium bromide 10mg/ml were purchased from SIGMA^R, Germany, while UltraPureTM 10X TAE Buffer was purchased from InvitrogenTM, USA.

Tris Acitate EDTA (TAE) 1X stock was prepared for the electrophoresis. To prepare a 1 X TAE, 100 ml of 10X TAE buffer was added to 900 ml of water.

For the sample loading process a loading dye (as 6X loading buffer purchased from SIGMA^R, Germany) was utilized. DNA ladder or molecular weight marker is necessary. Thus, 100 and 50 base pair DNA ladder (purchased from Roche, USA) were also needed.

2.3.4.1.2. Agarose Gel Electrophoresis preparation

A 2% agarose concentration was used to separate DNA fragments. To prepare 2% agarose, two grams of agarose powder were weighed, and added to 100 mL of TAE 10%. The solution was microwaved to boiling while stirring, then 1 μ L of ethidium bromide was added. The hot gel solution was poured in the gel electrophoresis bed and left until solidify. Before pouring the hot gel solution, the gel bed upper and lower edges were taped with a scotch tape, and two well-former templates (combs) were placed in the first and middle set of notches of the gel bed. Before DNA loading, the gel was immersed in a buffer solution, TAE 1X.

2.3.4.1.3. DNA Loading

Based on the well-former templates (combs), the amount of PCR product was calculated not to exceed the well size and then loaded into the well in the agarose gel. One sample-well was used for each DNA product. Typically a maximum total of 10 μL of DNA product mixed with loading buffer dye; i.e., 2 μL of diluted loading dye added to 8 μL DNA was loaded in a 5 mm wide \times 3 mm deep well. For precise determination, a smaller practical amount of sample DNA was loaded to give a single fragment that was readily visible and formed a sharp, accurately sizeable band.

2.3.4.1.4. Gel Running

After the samples were loaded, the cover was carefully snapped down onto the electrode terminals. During electrophoresis, the DNA samples migrate through the agarose gel toward the positive electrode. Thus, before loading the samples the gel was properly oriented in the apparatus chamber. The approximate time for electrophoresis varied between 30 and 40 minutes. The usual current/voltage was set at 95 mA/120V.

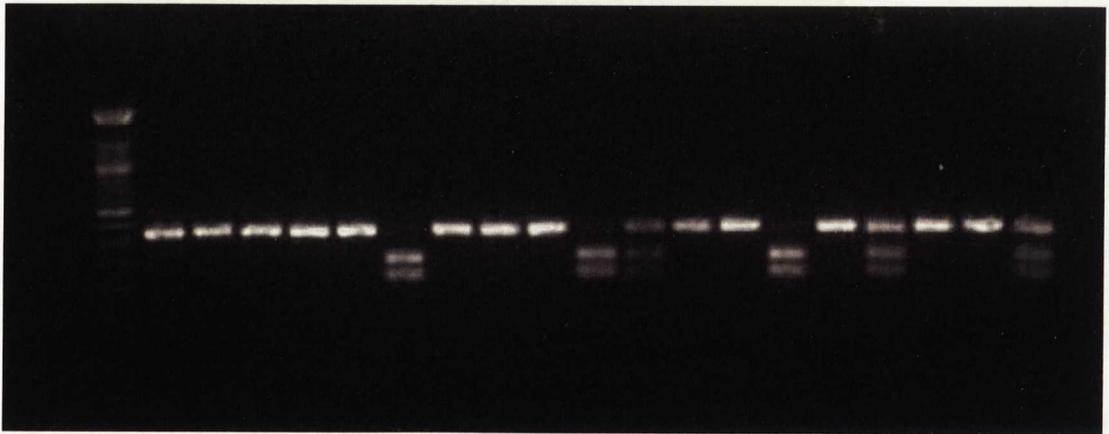
2.3.4.2 PCR Product Identification

2.3.4.2.1 DNA Band Visualization

After running the electrophoresis gel, the gel was transferred from the gel bed to the UV transilluminator Gel DocTM XR (BIO-RAD Laboratorie, Inc., USA). Under UV light, segments of DNA were visualized.

2.3.4.2.2 DNA Band Documentation

For PCR product identification, gel images were printed using Mitsubishi P93D gel documentation printer (Mitsubishi electric, Malaysia). An example for each gene is illustrated in figures 2.1, 2.2, 2.3, 2.4, and 2.5. DNA segment length results were recorded from the whole picture. Actual results yielded specific bands of varying intensities compared to the DNA marker.



1 2 AA AA AA AA CC AA AA AA CC AC AA AA CC AA AC AA AA AC

Figure 2.1 Sample of AT₁R A1166C (rs5186) polymorphism after digestion with Dde I enzyme. Lane 1: 100 bp DNA ladder, lane 2: PCR product before restriction, AA: represent undigested homozygous restricted amplicon product of 367 bp, CC: represent complete digested homozygous restricted amplicon product of 224 and 143 bp, AC: represent partially digested heterozygous restricted amplicon product of 367, 224, and 143 bp.

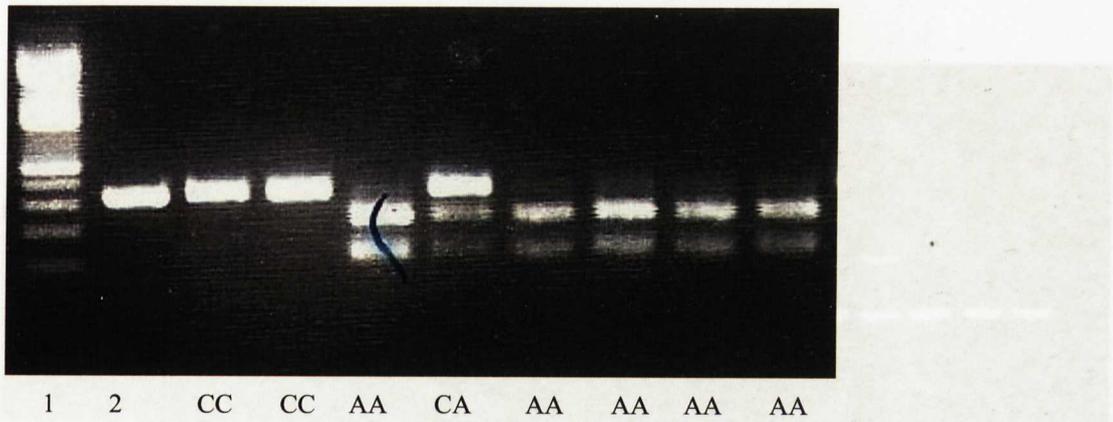
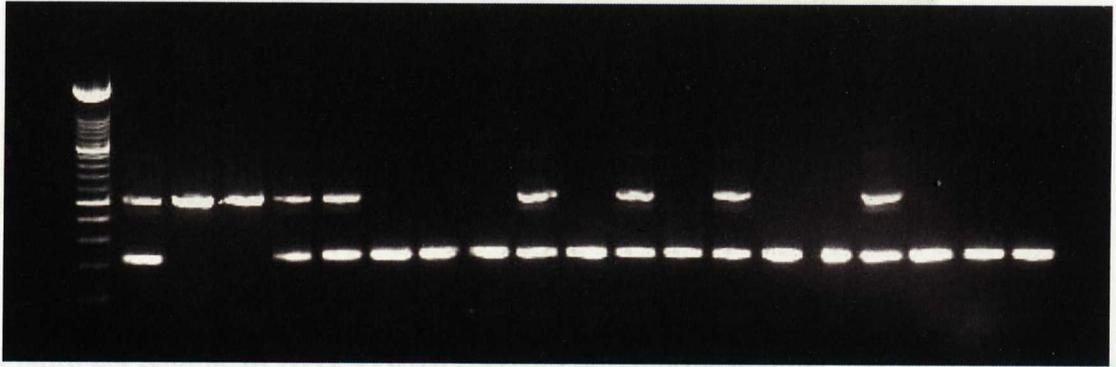
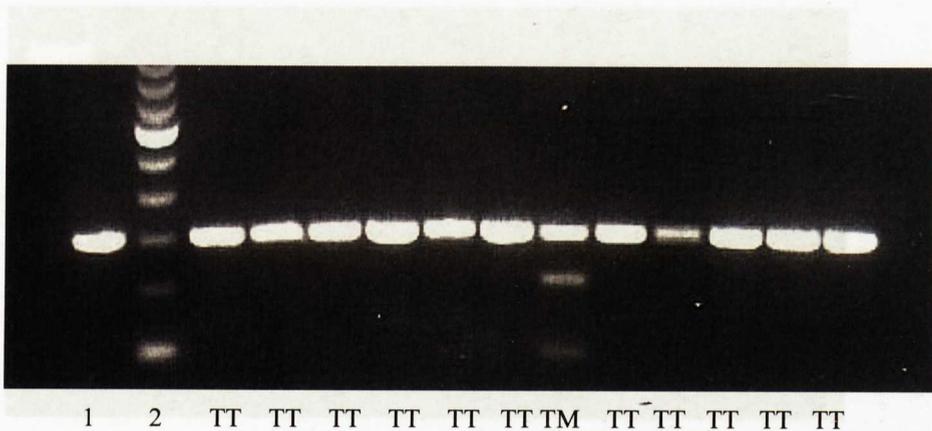


Figure 2.2 Sample of AT₂R C3123A (rs11091046) polymorphism after digestion with AluIenzyme. Lane 1: 100 bp DNA ladder, lane 2: PCR product before restriction, CC: represent undigested homozygous restricted amplicon product of 321 bp, AA: represent complete digested homozygous restricted amplicon product of 214 and 107 bp, CA: represent partially digested heterozygous restricted amplicon product of 321, 214, and 107 bp.



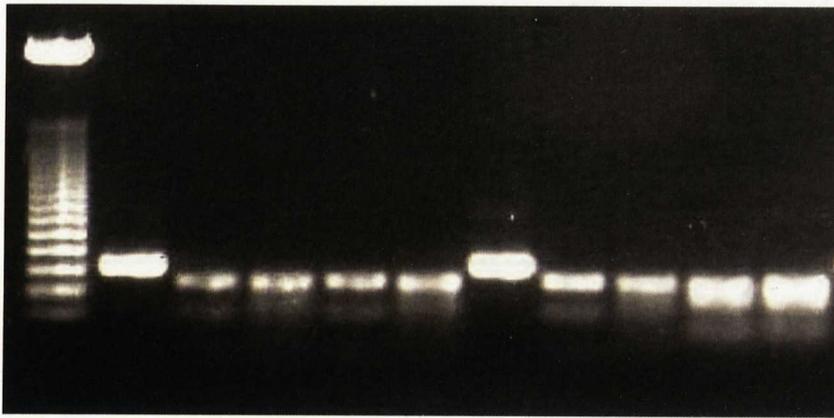
1 ID II II ID ID DD DD DD ID DD ID DD ID DD DD ID DD DD DD
 undigested homozygous restricted amplicon product of 303 bp. TM: represent partially digested heterozygous restricted amplicon product of 303, 211, and 91 bp. There is no

Figure 2.3 PCR sample of ACE I/D (rs1799752) polymorphism.
 Lane 1: 100 bp DNA ladder, ID: represent heterozygous product of 490 and 190 bp, II:
 represent homozygous product of 490, DD: represent homozygous product of 190 bp.



1 2 TT TT TT TT TT TT TM TT TT TT TT TT

Figure 2.4 Sample of AGT T207M (rs4762) polymorphism after digestion with enzyme NcoI. Lane 1: PCR product before restriction, lane 2: 100 bp DNA ladder, TT: represent undigested homozygous restricted amplicon product of 303 bp, TM: represent partially digested heterozygous restricted amplicon product of 303, 211, and 91 bp. There is no patient carrying MM homozygous.



1 2 TT MT TT MT 3 TT MT MM MM

Figure 2.5 Sample of AGT M268T (rs699) polymorphism after digestion with enzyme Tth111. Lane 1: 50 bp DNA ladder, lane 2 and 3: PCR product before restriction, TT: represent digested homozygous restricted amplicon product of 141 and 24 bp, MT: represent partially digested heterozygous restricted amplicon product of 165bp, 141, and 24 bp, MM: represent completely digested homozygous restricted amplicon product of 165bp.



Figure 2.6 Determination of PCR sequencing of AGT M268T

The wavelength next to the black line indicate the specific site of polymorphism through A, B, and C.

- A: show homozygous CC (C in blue)
- B: show heterozygous TC (Y in black)
- C: show homozygous TT (T in red)

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2.5 Statistical methods

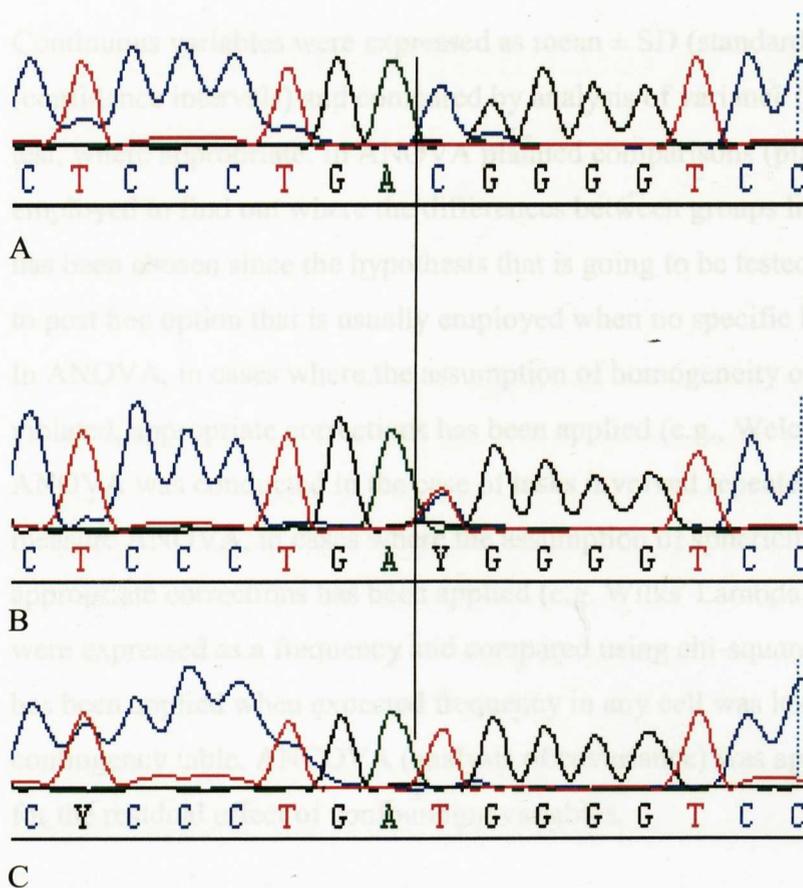


Figure 2.6 Determination of PCR sequencing of AGT M268T
The wavelength next to the black line indicate the specific site of polymorphism through A, B, and C

A: show homozygous CC (C in blue)

B: show heterozygous TC (Y in black)

C: show homozygous TT (T in red)

N.B. this page is "Original in Color"

2.5 Statistical methods

Continuous variables were expressed as mean \pm SD (standard deviation) or CI (confidence intervals) and compared by analysis of variance (ANOVA) or Student *t*-test, where appropriate. In ANOVA planned comparisons (planned contrasts) has been employed to find out where the differences between groups lie. Planned contrasts option has been chosen since the hypothesis that is going to be tested is known in comparison to post hoc option that is usually employed when no specific hypothesis has been tested. In ANOVA, in cases where the assumption of homogeneity of variance has been violated, appropriate corrections has been applied (e.g., Welch F). Repeated measure ANOVA was conducted in the case of tasks involved repeated measure. In repeated measure ANOVA, in cases where the assumption of sphericity has been violated, appropriate corrections has been applied (e.g. Wilks' Lambda F). Categorical variables were expressed as a frequency and compared using chi-square and Fisher's exact test has been applied when expected frequency in any cell was less than 5 in a 2×2 contingency table. ANCOVA (analysis of covariance) was applied in chapter 3 to adjust for the residual effect of confounding variables.

An adjusted linear regression model (method: Stepwise) was employed in chapter 5 where each neurocognitive test was the outcome (dependent) variable and all other confounders (predictors) were independent variables (including age, gender, education, diabetes, smoking, dialysis duration, RAS genotypes, and antihypertensive groups that encompass RAA and non-RAA group). RAS genotypes are highly inter-correlated so their inclusion in the regression model was performed separately to overcome severe multicollinearity.

All significant categorical predictors in each outcome analysis were represented as boxplots to clearly demonstrate the results of regression. In the boxplot, the lowest score (the bottom horizontal line on each plot), and the highest (the top horizontal line of each plot) were represented. The box (the tented area) shows the middle 50% of scores. The middle horizontal thick line of the tinted box represents the value of the median. The circles and asterisks that lie above or below the box represent outliers.

Two-sided *p*-value less than 0.05 were considered to be statistically significant.

Analysis was conducted using standard software (SPSS for window version 18.0).

CHAPTER 3. NEUROPSYCHOLOGY OF SAUDI CKD PATIENTS AND IMPACT OF DRUG THERAPY.

3.1 Introduction

CKD is a progressive problem among patients suffering diabetes mellitus (DM). In Saudi Arabia, the country where this study was undertaken the prevalence of DM is progressively increasing mainly due to increased prevalence of obesity and glomerulointerstitial (glomerular & interstitial inflammation) diseases. The reported prevalence of chronic renal failure is 80-120 per million population (pmp) in Saudi Arabia vs. 283 pmp in Europe, 975 pmp in United States (Shaheen *et al.*, 2005).

The aetiology of CKD is multifactorial however DM is considered one of the most common causes of renal impairment. In Saudi Arabia, epidemiological studies documenting the incidence of DM reported an exponential rise in DM yielding different figures. It has been shown that 23.7% of the Saudi population had DM (Al-Nozha *et al.*, 2004), and diabetes was responsible about 30-45% of patients requiring dialysis (Mitwalli *et al.*, 1997). In patients with type 1 diabetes, the clinical course of nephropathy is relatively well defined . Nephropathy usually becomes clinically evident after 10-15 years of diabetes (Molitch *et al.*, 2004). Fifty percent of patients with type 1 diabetes and overt nephropathy (> 300mg/day) will progress to ESRD within 10 years, and 75% within 20 years where the percentage of CKD patients will approach those in Europe or United States.

Renal impairment is associated with an increased risk of neurocognitive defect (Kurella *et al.*, 2005a; Kurella *et al.*, 2004). The etiology is multifactorial including but not limited to uraemia, anaemia, hypertension, and diabetes mellitus. Kurella *et al* published a number of studies documenting the neurocognitive dysfunction of adult, elderly, and postmenopausal renally-impaired individuals. All these studies used different tools for measuring neurocognitive properties and provide evidence of high risk of neurocognitive defect in CKD individuals than the general population and it was more

obvious in advanced CKD stages (Kurella *et al.*, 2005a; Kurella *et al.*, 2004; Kurella *et al.*, 2005b; Kurella Tamura *et al.*, 2008).

Similar results were obtained especially in concentration and attention after applying computerized neurocognitive tests on adults with moderate CKD aged between 20-59 years (Hailpern *et al.*, 2007). The same finding was also documented when examining the neurocognitive dysfunction in early stages of CKD using a sensitive electrophysiological test (Madan *et al.*, 2007).

Drugs acting on RAS have a role in the management of HTN of CKD patients as well as being able to improve neurocognitive properties in animal studies. It is well documented that RAS has an effect on neuropsychological abilities (Gard, 2002). Drugs acting on the RAS such as ACEIs and ARBs in healthy volunteers proved such an effect especially in attention and short-term memory tests (Currie *et al.*, 1990; Frcka *et al.*, 1988; Olajide *et al.*, 1985). Recently, losartan has been shown to have cognition-improving properties after acute administration in young healthy volunteers (Mechaeil *et al.*, 2011).

In addition, studies of ACEIs and ARBs in hypertensive patients have proved beneficial with respect to mood and cognition (Braszko *et al.*, 2003; Fogari *et al.*, 2003; Muldoon *et al.*, 2002). Recently, ARBs have been associated with a decreased risk of dementia more than ACEI and other antihypertensive drugs (Li *et al.*, 2010).

Patients treated with brain-penetrating ACEIs (perindopril or captopril) and ARB (losartan) demonstrated a lower rate of cognitive decline (Ohruai *et al.*, 2004; Sink *et al.*, 2009; Tedesco *et al.*, 2002).

Cerebral microvascular abnormalities have been implicated as one of the etiologies of vascular dementia (Baker *et al.*, 2007; Wong *et al.*, 2002). In an attempt to implicate the responsibilities of microvascular abnormalities, as evidenced by albuminuria, for neurocognitive defects, Barzilay *et al.* tested urinary albumin and cognitive properties of 2,389 elderly subjects from Cardiovascular Health Cognition Study (CHCS). There were trends for increasing the association between albuminuria and decline in neurocognitive properties even after adjustment of factors that are known to cause cognitive defect such as HTN and DM. The results of this study are promising towards the potential role of drugs treating albuminuria, particularly ACEIs and ARBs, to

improve neurocognitive disorder in CKD patients (Barzilay et al., 2008). Recently, the same author reported an association between microvascular abnormalities in both kidney as manifested by albuminuria and brain as manifested by neurocognitive decline in patients with vascular disease or DM. The cognitive decline was less obvious in patients treated with RAA drugs over placebo (Barzilay et al., 2011).

Although the studies on neurocognitive effect of ACEIs and ARBs are limited, there is good evidence of such an effect which necessitates further investigation using more controlled designs. At present, there is no study that has examined the direct relationship between neurocognitive defect of CKD patients and drugs acting on RAS and studies which examine emotional wellbeing & neurocognitive dysfunction in Saudi CKD patients are lacking.

The purpose of this study is to examine the potential beneficial effect of ACEs and ARBs on neurocognitive properties of CKD patients in comparison to those using other classes of antihypertensives.

3.1.1 The test battery

A battery of neurocognitive tests was selected. Memory, attention, and executive function were represented in this study. The domain of memory and attention was more heavily represented since they are more extensively studied in the literature compared to the domain of executive function. A quality of life measure was also employed in this study to assess both mental and physical health status. Mood and anxiety were assessed also. All of the previously mentioned tests help to assess the neuropsychological functions of patients either directly using neurocognitive specific test domain or indirectly using a measure of QOL.

3.2 Methodology

3.2.1 Participants

Participants (18- 60 yr) were recruited from the nephrology clinic at King Khaled University Hospital (KKUH), 34 patients were on RAA drugs (RAA group) and 26

patients were on other classes of antihypertensives (non-RAA group). Age and education-matched healthy volunteers (36 subjects) were recruited from the same source. Exclusion criteria included smoking, illiteracy, psychiatric or cerebrovascular disease, sensory impairment, concomitant drug therapy that might affect mood and cognition (e.g. steroids or antidepressants), abnormal electrolytes, anaemia or uncontrolled blood pressure.

3.2.2 Measures

Cognition were assessed using the Rey Auditory-Verbal Test (learning & memory); the Rey-Osterrieth complex figure (RCF, visuo-spatial organization and visuo-spatial memory); semantic verbal fluency (executive function); letter cancellation (attention); digit-symbol (sustained attention, visual searching, visual sequencing). The 36-item Short-Form Health Survey (quality of life, QOL) and the Hospital Anxiety and Depression Scale were also used to assess QOL, anxiety, and depression, respectively.

3.2.3 Chart review

See chapter two, section 2.1.5

3.2.4. Statistical methods

See Chapter 2, section 2.5.

3.3 Results

3.3.1 Demographics and medical condition

A total of 60 CKD patients were recruited from the outpatient clinic between February 1, 2007, and March 30, 2008 who met the inclusion criteria (34 patients in RAA and 26 in non-RAA group). During the same period a total of 36 healthy volunteers were also recruited as a comparative group. Demographic characteristics for study groups are summarized in Table 3.1 below.

Detailed drug list is shown in Appendix 5

	RAA (n = 34)	non-RAA (n = 26)	Healthy (n = 36)	P
Age (yrs)	43.4(10.02)	41.7 (11.5)	33.7 (9.9)	< .001*
Male N (%)	28 (82.3)	16 (61.5)	20 (55.6)	.03**
Education (yrs)	12.1 (3.6)	10.8 (4.0)	12.1 (3.0)	.258*

Table 3.1 Mean (\pm SD) or number (%) of Sociodemographic characteristics of chronic kidney disease patients (RAA and non-RAA) and healthy control

* P values are for F statistic (one-way ANOVA test).

** P values are for chi-square test.

While patients in both the RAA and non-RAA have almost similar mean age (43.4 ± 10 vs. 41.7 ± 11.5), the healthy volunteers were younger (33.7 ± 9.9) ($F(2, 93) = 8.49, p < .001$). The gender ratio was different among the groups, as more subjects in RAA group were male ($\chi^2(2) = 6.072, p = .03$). The education pattern was the same among all groups ($p = .258$).

A comparison was also conducted between the clinical characteristics of CKD patients (RAA and non-RAA groups) in terms of variables that may affect neurocognitive properties including Hgb level, HTN, DM, other disease, dialysis and disease duration

as illustrated in Table 3.2 below. None of the above variables were significantly different except for disease duration.

	RAA	non-RAA	P
HTN N (%)	21 (65.6)	17 (73.9)	.512*
DM N (%)	7 (21.9)	6 (26.1)	.717*
Other disease			
N (%)	1 (3.1)	2 (8.7)	.370*
Hemoglobin			
> 11 g/dl, N (%)	26 (81.3)	14 (60.9)	.094**
< 11 g/dl, N (%)	6 (18.8)	9 (39.1)	.092**
Disease duration			
Mean (SD) (yrs)	3.98 (2.6)	5.0 (5.2)	.02**
Dialysis duration			
Mean (SD) (yrs)	2.9 (2.0)	4.5 (6.3)	.13**

Table 3.2 Mean (\pm SD) or number (%) of clinical characteristics of chronic kidney disease patients (RAA and non-RAA)

* P values are for chi-square statistic.

** P values are for student t-test.

Although statistical analysis was not performed on etiology of CKD and CKD stages of the two groups as shown in Table 3.3 below, a substantial number of patients (85%) in advanced CKD stage were in non-RAA group, while the etiology was nearly identical.

Causes	RAA N = 34	non-RAA N = 26
Hypertension N(%)	6	4
Diabetes	3	8
Glomerular disease	8	3
Interstitial disease	1	1
Obstructive uropathy	0	2
IgA nephropathy	3	0
Polycystic kidney disease	2	1
Renal stone	1	1
Unknown	10	6
CKD stage		
Stage III	18	0
Stage IV	8	4
Stage V	3	9
Stage V on HD	5	13

Table 3.3. Number (%) of aetiologies and stages of Chronic Kidney Disease

3.3.2 Neuropsychological function

3.3.2.1 Unadjusted mean cognitive scores

3.3.2.1.1 Memory

Rey Auditory-Verbal Learning Test

Three participants, one from each group refused to perform the recognition task.

Mean learning (total 5 trials), immediate, and delayed recall as well as recognition task for RAA, non-RAA, and healthy groups are presented in Figure 3.1 below

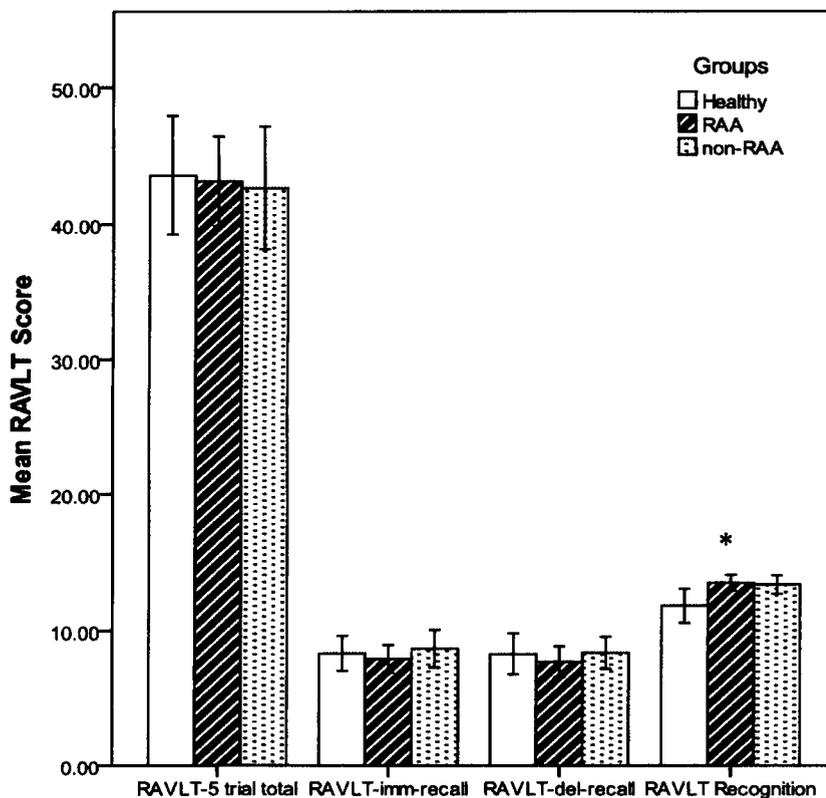


Figure 3.1 Mean \pm 95% CI of RAVLT (5 trial recall, immediate recall, delayed recall, and recognition task) in healthy control (n= 36), RAA (n= 34), and non-RAA (n= 26). * represents significant difference from control group (p<.05).

Four one-way ANOVA with planned contrast were conducted to verify the presence of differences in verbal learning and verbal memory between the three groups. All groups had a similar performance in verbal learning (total five trial recall), immediate and delayed verbal memory. However there was a significant difference in the recognition

task ($F(2, 92) = 8.87, p < .001$). Planned contrast revealed that the significant difference was between healthy control and either RAA or non-RAA where healthy people recognize around 3 words less than either RAA or non-RAA ($t(92) = -4.2, p < .001$).

Since RAVLT and RCF involved repeated measure tasks, repeated measure ANOVA was conducted on RAVLT recall trials and recognition task and on RCF tasks to decrease the chance of type I error as shown in figure 3.2 and 3.4 below.

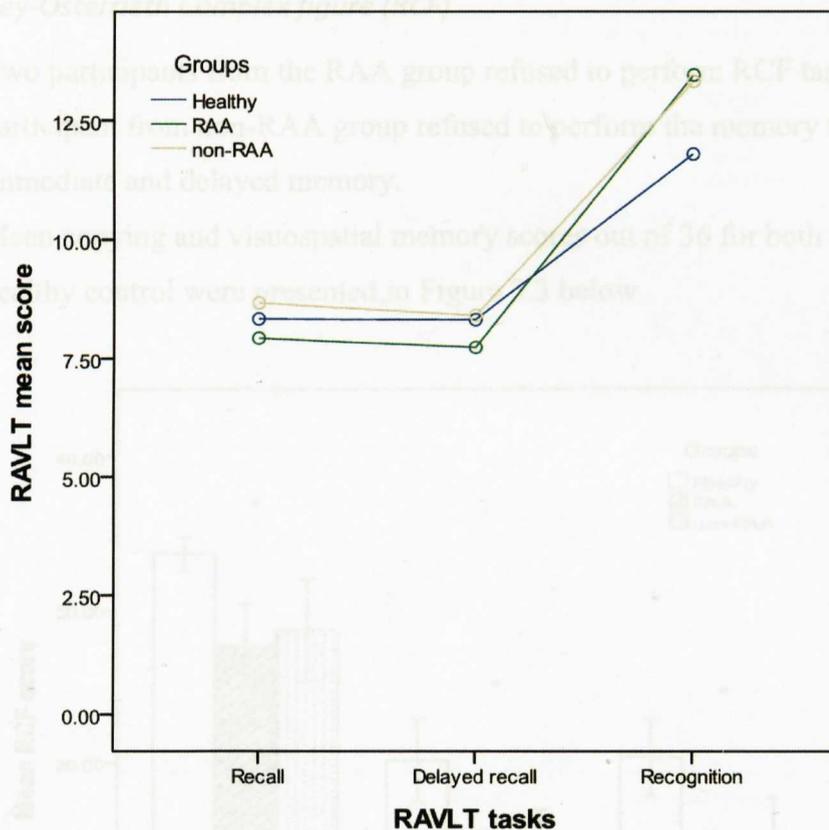


Figure 3.2 Estimated marginal mean plot of immediate recall, delayed recall, and recognition score of RAVLT task in healthy volunteers (n=36), RAA (n=34) and non-RAA group (n=26).

A 3×3 (group \times time) repeated measure ANOVA showed that there was a significant effect of time on RAVLT performance ($F(2,92)= 141, p= .001$), where planned contrast revealed that the difference was between delayed recall and recognition task within each group ($F(2,92)= 269.62, p= .001$).

There was a significant interaction between the groups and tasks of the test i.e. the average change over the tasks is different between the groups ($F(2,90)= 2.76, p= .03$).

Planned contrast analysis revealed that the group task interaction is significant between delayed recall and recognition task ($F(2,90)= 39.41, p= .005$) i.e. no significant differences between healthy controls, RAA and non-RAA groups in RAVLT memory tasks, however, significant differences still existed in recognition task which confirm the previous finding of one-way ANOVA analysis.

Rey-Osterrieth Complex figure (RCF)

Two participants from the RAA group refused to perform RCF task, another one participant from non-RAA group refused to perform the memory tasks including immediate and delayed memory.

Mean copying and visuospatial memory scores out of 36 for both drug groups as well as healthy control were presented in Figure 3.3 below.

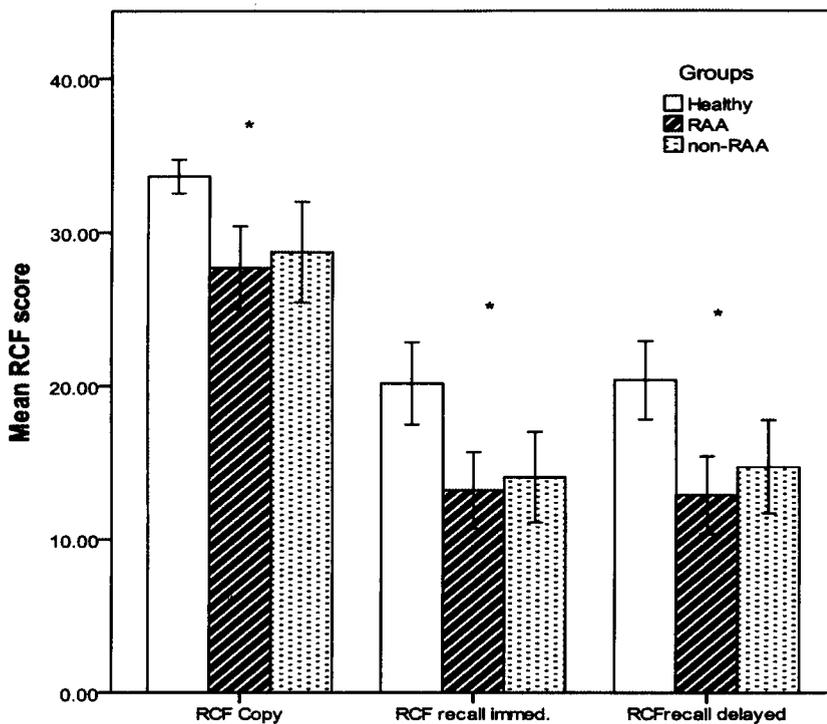


Figure 3.3 Mean \pm 95% CI copying, immediate and delayed visuospatial memory scores on RCF task in healthy control ($n= 36$), RAA ($n= 32$), non-RAA ($n= 26$). * represents significant difference from control group ($p<.05$).

Three one-way ANOVA with planned contrast revealed that both RAA and non-RAA groups were significantly worse in visuospatial organization ($F(2, 93) = 10.38, p < .001$), and visuospatial immediate memory ($F(2, 92) = 10.14, p < .001$), and delayed memory ($F(2, 92) = 10.13, p < .001$). Planned contrast results document the low performance of RAA and non-RAA compared to healthy control; RCF copy ($t(93) = 4.56, p < .001$), immediate recall ($t(92) = 4.48, p < .001$), delayed recall ($t(92) = 4.29, p < .001$).

Digit-symbol-substitution-test (DSST)

Figure 3.4 below represents mean substitution score for RAA, non-RAA, and healthy control groups.

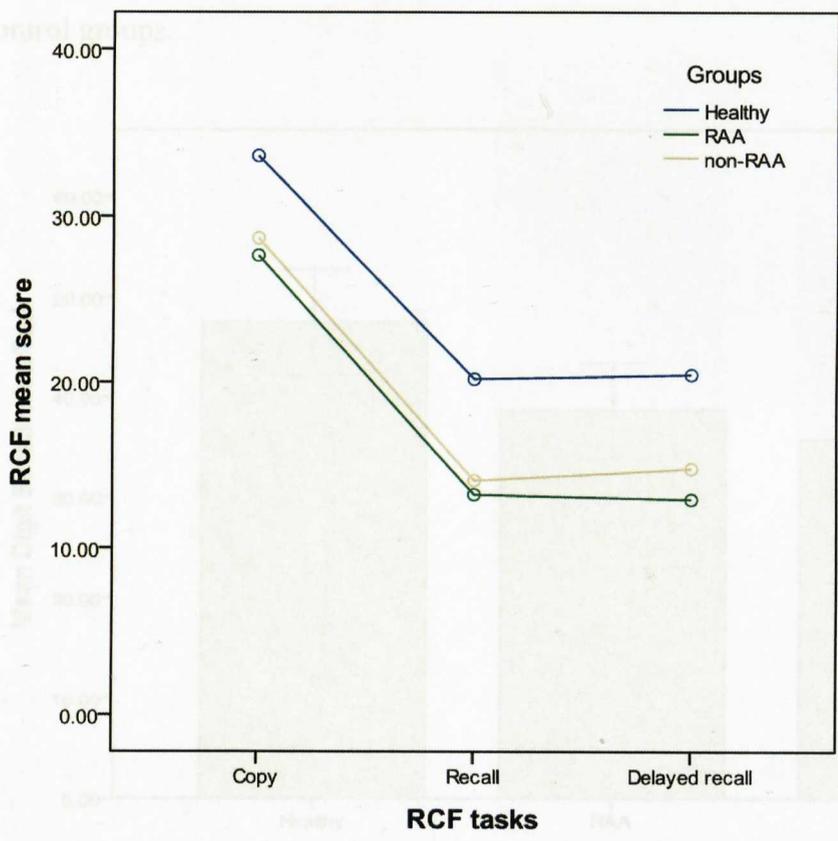


Figure 3.4 Estimated marginal mean plot of Copy, immediate recall, and delayed recall scores of RCF task in healthy control (n=36), RAA group (n=32) and non-RAA group (n=26).

A 3×3 (group \times time) repeated measure ANOVA was conducted on RCF. There was no significant group \times task interaction ($p > .05$). This result does not document the previous result of RCF task when ANOVA has been applied, i.e. no significant differences between the three groups in the three task performance. However, there was

a significant difference in task performance ($F(2,93) = 224.74, p = .001$). Planned contrast revealed that the significant difference was between copy task and both immediate and delayed recall ($F(2,93) = 425.19, p = .001$). This means that the three groups performed similarly on RCF tasks but there was a significant difference between copy task and both immediate and delayed memory tasks.

3.3.2.1.2 Attention

Digit-symbol-substitution-test (DSST)

Figure 3.5 below represents mean substitution score for RAA, non-RAA, and healthy control groups.

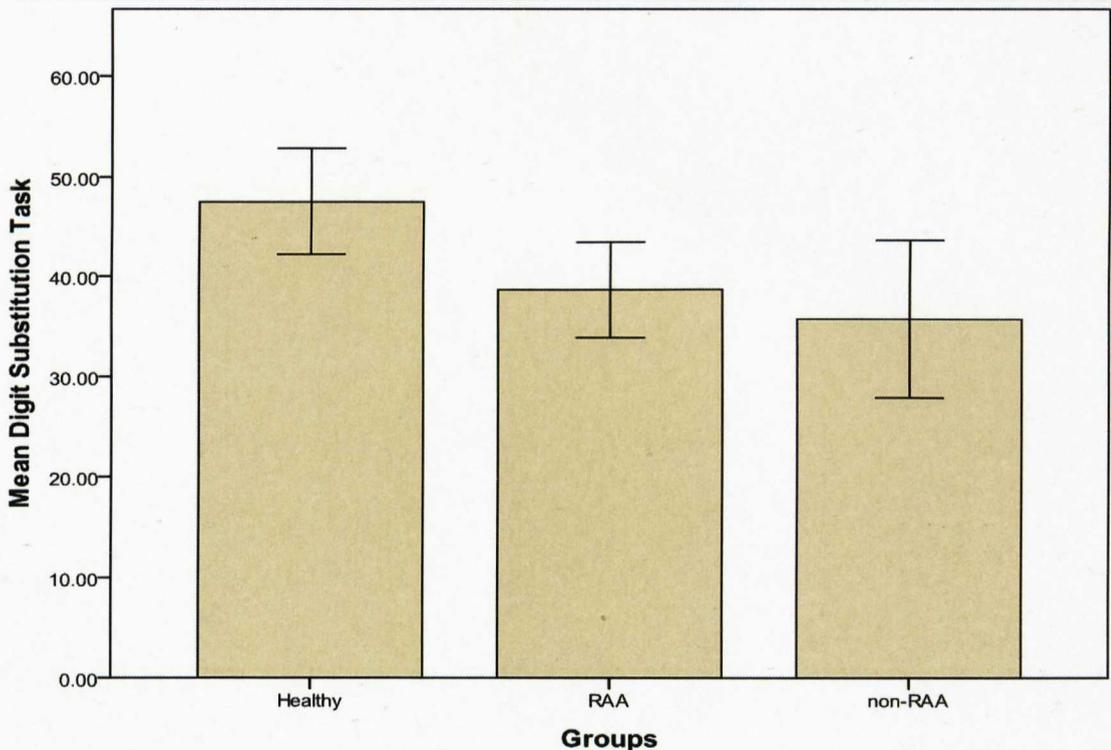


Figure 3.5 Mean \pm 95% CI of digit-symbol-substitution scores in healthy control ($n=36$), RAA ($n=34$), and non-RAA ($n=26$).

Healthy control substituted around 9 and 12 symbols more than the RAA and non-RAA group, respectively ($F(2, 95) = 4.76, p < .05$).

Planned contrast revealed that there was no significant difference between RAA and non-RAA. However, a significant difference was revealed between healthy controls and both RAA and non-RAA groups ($t(95) = 3.07, p < .005$).

Letter cancellation

Figure 3.6 below indicates mean letter cancellation per second for RAA, non-RAA, and healthy control groups.

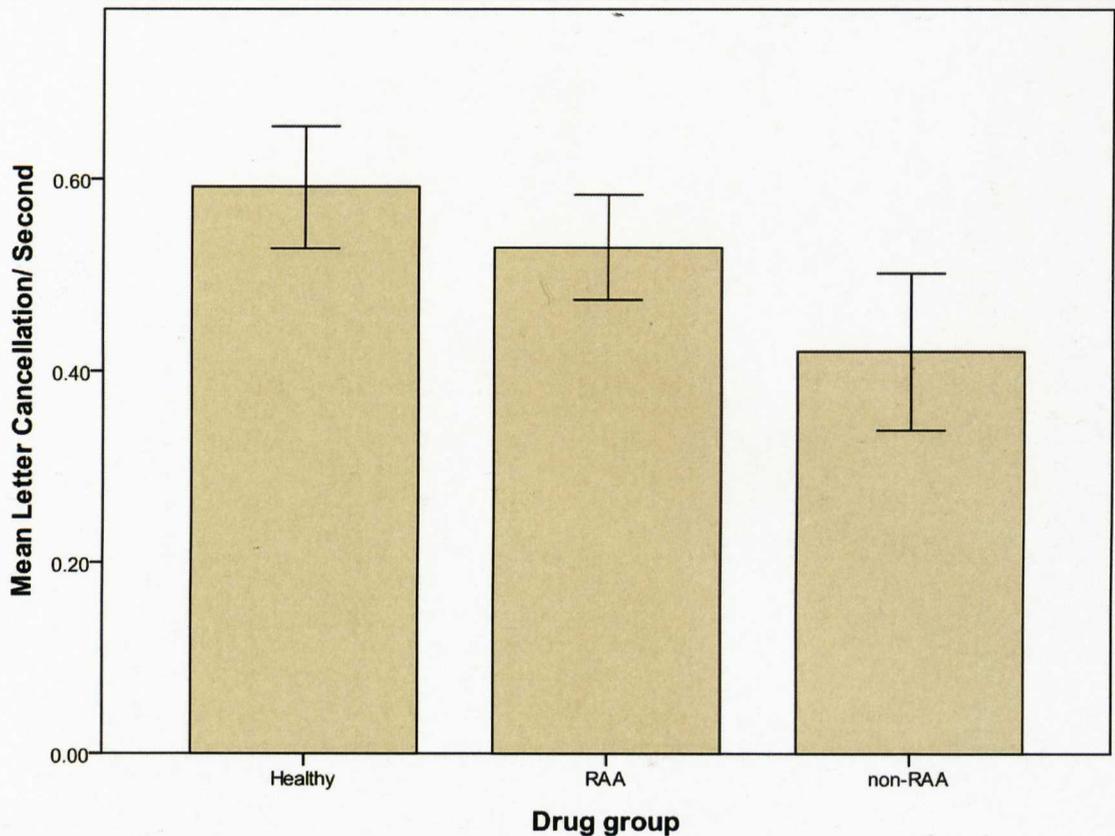


Figure 3.6 Mean \pm 95% CI of letter cancellation scores in healthy control ($n=36$), RAA ($n= 34$), and non-RAA ($n= 26$).

Healthy control cancelled 0.06 and 0.17 letter per second more than RAA and non-RAA groups, respectively, on the letter cancellation task, respectively. One-way ANOVA revealed that participants in the different drug group were statistically significantly different ($F(2, 95) = 6.19, p < .005$). Planned contrast results indicated that RAA group significantly performed better than non-RAA ($t(95) = -2.18, p < .05$), but almost similar to healthy control.

3.3.2.1.3. Executive function

Mental fluency

Figure 3.7 below represents mean words generated in one minute for healthy control as well as RAA and non-RAA CKD patients.

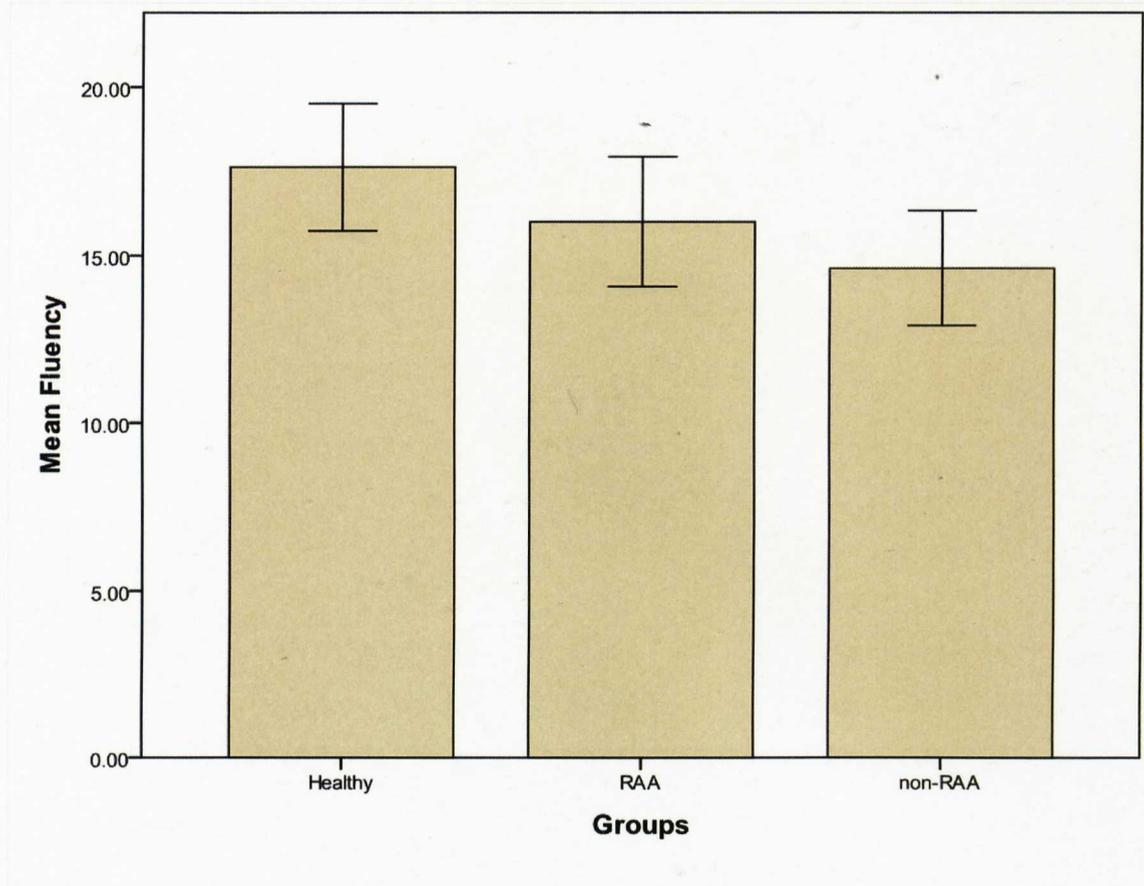


Figure 3.7 Mean \pm 95% CI of mental fluency (animal names generated in one minute) scores in healthy volunteers ($n=36$) as well as RAA ($n= 34$) and non-RAA ($n= 26$).

Executive function result indicates that the non-RAA group also had a worse score on executive function as evidenced by verbal fluency task where healthy controls named 1.6 and 3 names more than RAA and non-RAA groups, respectively ($F(2,93) = 2.53$, $p = .085$). Planned contrast revealed that the non-RAA group is significantly different from healthy control ($t(95) = 2.09$, $p < .05$).

3.3.2.1.2 QOL

Mean QOL, physical component summary (PCS), and mental component summary (MCS) for healthy control as well as RAA and non-RAA CKD patients were presented below as error-bar in figures 3.8 and 3.9, respectively.

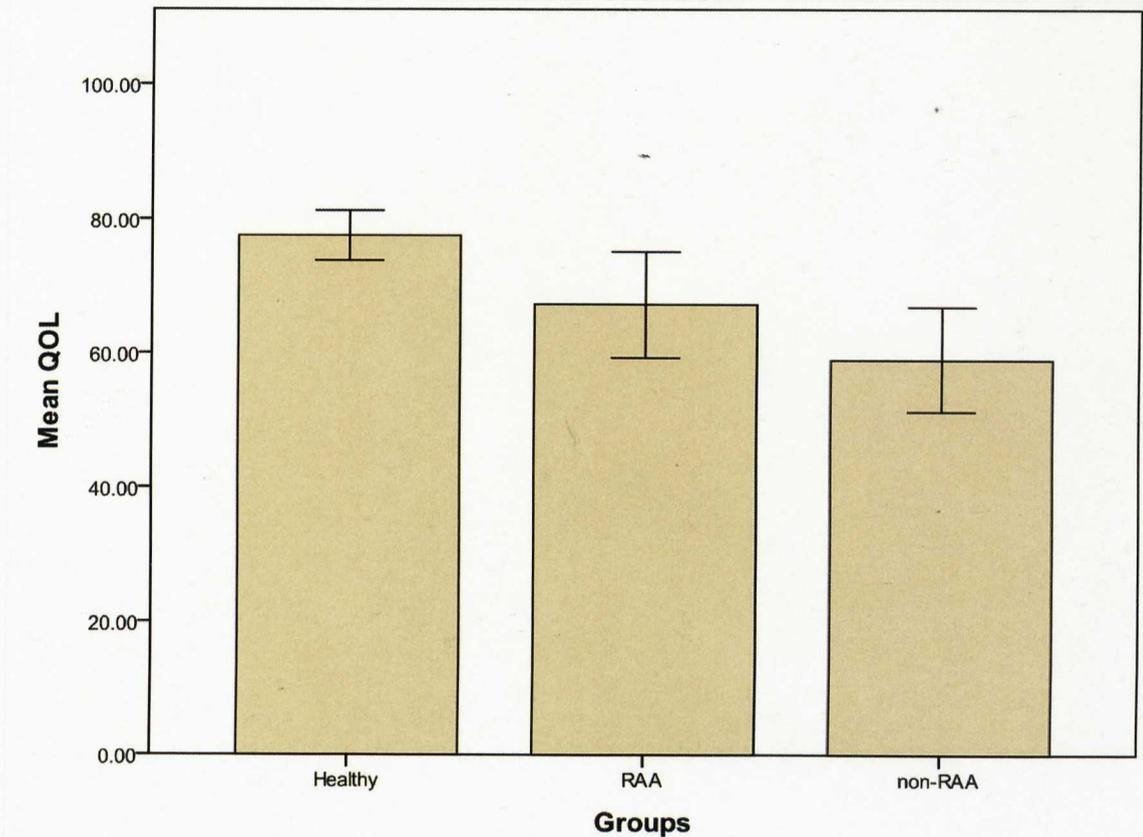


Figure 3.8 Mean \pm 95% CI of QOL scores in healthy volunteers ($n=36$) as well as RAA ($n= 34$) and non-RAA ($n= 26$).

One-way ANOVA revealed that the three drug groups were significantly different ($F(2, 95) = 8.23, p < .001$). Planned contrast revealed that both RAA and non-RAA were significantly different from healthy control ($t(95) = 3.8, p < .001$).

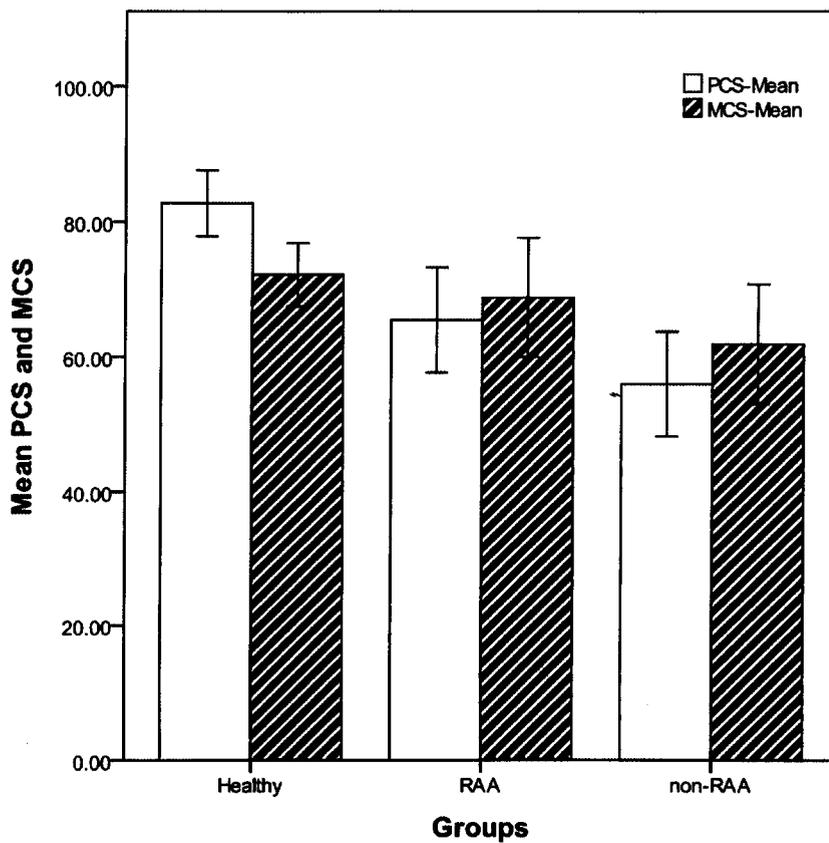


Figure 3.9 Mean \pm 95% CI of PCS and MCS in healthy volunteers (n=36), RAA (n=34), and non-RAA (n=26).

Significant differences among all the three groups were obvious only in physical component summary ($F(2, 95) = 16.83, p < .001$). Planned contrast revealed that both RAA and non-RAA groups were significantly different from healthy control ($t(95) = 5.66, p < .001$), with no differences between RAA and non-RAA.

3.3.2.1.3 Hospital Anxiety and Depression Scale (HADS)

Mean scores of HADS test are indicated as error bars below in figure 3.10

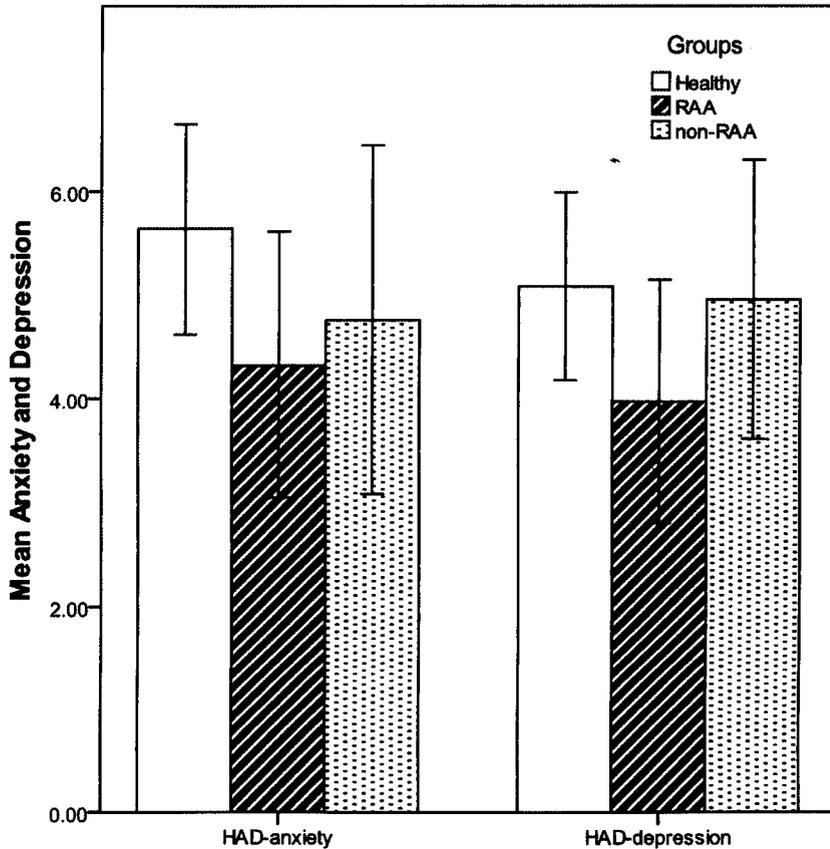


Figure 3.10 mean \pm 95% CI of Anxiety & depression scores in healthy volunteers (n=36) as well as RAA (n= 34), and non-RAA (n= 26).

None of the three groups reached the level of caseness (see section 2.2.3) for anxiety and depression that are non-significantly different.

3.3.2.2 Adjusted mean cognitive scores

To evaluate the independent association between kidney failure and cognitive impairment in different drug groups, ANCOVA (analysis of covariance) was used to adjust for the residual effect of age, sex, education, disease duration, dialysis duration, and comorbidities such as HTN, DM, other disease, Hgb level, CKD stage, and CKD etiology. Among all groups, the significant difference in visuospatial memory (delayed only), attention, and QOL had turned to a non significant difference, while the difference remains significant in visuospatial organization and memory (immediate only) as before covariables adjustment. However, a new significant difference was identified in executive function after controlling for the previously mentioned variables ($F(2,95) = 4.01, p < .05$).

Planned contrast revealed that CKD patients in the RAA group had significantly better performance on executive function ($t(95) = -2.665, p < .05$) compared to CKD patients in the non-RAA group. However, all other planned contrast differences between RAA and non-RAA groups in all remaining adjusted tests were not significant ($p > .05$).

3.4 Conclusion

Table 3.4 below summarizes the significant differences between healthy controls, RAA and non-RAA CKD patients before and after covariables adjustment.

The results indicate that CKD patients have impaired cognition compared to healthy controls. This impairment could reflect differences in age, education, health status (e.g. uraemia) or some other factor. Healthy controls were significantly younger than CKD patients, however the age difference (30 years vs. 40 years) is unlikely to account for difference in cognition; there was no difference in education between the groups. All patients were within therapeutic ranges for Hgb, BUN, electrolytes, thyroid function test and PTH level, hence these parameters, again, cannot account for the cognitive impairment. Differences in cognition may however be correlated with changes in RAS activity.

ANCOVA demonstrated that adjustment for the confounding effect of covariables still identified differences in cognition (executive function) between patients receiving RAA and non-RAA drugs.

Task	Result summary before covariables adjustment	Results summary after covariables adjustment
RAVLT	<p>Five trial learning: No Sig differences.</p> <p>Immediate recall: No sig differences</p> <p>Delayed recall: No Sig differences.</p> <p>Recognition: Sig differences between groups ($p < 0.001$), where both RAA and non-RAA groups performed significantly better than healthy control.</p>	<p>Five trial learning: No Sig differences.</p> <p>Immediate recall: No sig differences</p> <p>Delayed recall: No Sig differences.</p> <p>Recognition: Sig differences between groups ($p < 0.001$), where both RAA and non-RAA groups performed significantly better than healthy control.</p>
RCF	<p>Visuospatial organization: Sig differences between groups ($P < .001$), with both RAA and non-RAA performed significantly lower than healthy control ($P < .001$).</p> <p>Visuospatial immediate recall: Sig differences between groups ($p < 0.001$), with healthy control performing better than RAA and non-RAA groups ($P < .001$)</p> <p>Visuospatial delayed recall: Sig differences between groups ($p < 0.001$), with healthy control performing better than RAA and non-RAA groups ($P < .001$)</p>	<p>Visuospatial organization: Sig differences between groups ($P < .001$), with both RAA and non-RAA performed significantly lower than healthy control ($P < .001$).</p> <p>Visuospatial immediate recall: Sig differences between groups ($p < 0.001$), with healthy control performing better than RAA and non-RAA groups ($P < .001$)</p> <p>Visuospatial delayed recall: No sig differences between the groups.</p>

Table 3.4 Summary of neurocognitive tests comparison results, QOL, and HADS of RAA, non-RAA CKD patients and healthy control before and after controlling for covariables (age, sex, education, hypertension, diabetes, other disease, hemoglobin level, etiology of CKD, dialysis and disease duration, and CKD stage).

Task	Result summary before covariables adjustment	Results summary after covariables adjustment
DSST	Sig differences between groups ($p < .05$), with healthy control performing better than RAA and non-RAA groups ($P < .005$)	No Sig differences.
Letter cancellation	Sig differences between groups ($p < .005$), with RAA group performed better than non-RAA groups ($P < .05$), but similar to healthy control.	No Sig differences.
Mental fluency	No Sig differences among the three groups. However planned contrast revealed that RAA group performed sig better than non-RAA ($p < .05$)	Sig differences among the three groups, with RAA group performed sig better than non-RAA ($p < .05$)
QOL SF-36 (total)	Sig differences between groups ($p < .005$), with healthy control performing better than RAA and non-RAA groups ($P < .005$)	No Sig differences.
PCS	Sig differences between groups ($p < .005$), with healthy control performing better than RAA and non-RAA groups ($P < .005$)	No Sig differences.
MCS	No Sig differences.	No Sig differences.
HAD-Anxiety	No Sig differences.	No Sig differences.
HAD-Depression	No Sig differences.	No Sig differences.

Table 3.4 Summary of neurocognitive tests comparison results, QOL, and HADS of RAA, non-RAA CKD patients and healthy control before and after controlling for covariables (age, sex, education, hypertension, diabetes, other disease, hemoglobin level, etiology of CKD, dialysis and disease duration, and CKD stage).

CHAPTER 4. NEUROPSYCHOLOGY OF SAUDI COLON CANCER PATIENTS

4.1 Forward

Chapter 3 indicates that CKD patients have impaired cognition compared to healthy controls. One possible cause of the impairment is the anxiety, fear, prognosis of chronic illness. This chapter explores cognition in a group of chronically ill patients with intact RAS and without cardiovascular disease and medication that might affect cognition. Abnormal RAS was the hypothesised cause of neurocognitive defect of renally impaired patients, and by choosing a group of patients without any defect in RAS will control for such variable as the case in colon cancer patients in remission.

4.2 Aim of study

The previous study was unable to exclude the contribution of chronic disease and repeated interaction with healthcare professionals on the neurocognitive defects that were observed when CKD patients were tested using multiple domains of cognition. This study was conducted to test colon cancer patients after completion of treatment as an example of patients suffering chronic disease but with intact RAS to assess whether such patients suffer neurocognitive defect, and if so is this neurocognitive defect similar to that observed in CKD patients.

The aim is to rule out the effect of chronic disease on neurocognitive defect of CKD patients. Several groups of chronically ill patients were considered for this phase of the study, for example chronic inflammatory disorders, chronic respiratory and cardiovascular disorders. Colon cancer patients in remission were selected because they have a potentially fatal illness which is currently in remission. They are currently symptom- and drug-free, but require regular, frequent hospital checks. These patients have a constant fear of their illness and have a regular hospitalization: they are therefore similar, in some aspects, to chronic renal failure patients, but without the confounding variables of symptoms, medication, cardiovascular abnormality, and with intact RAS.

Comparison of data from colon cancer patients with data collected previously from healthy subjects was undertaken.

4.2 Introduction

Colorectal cancer is a common disease and its prevalence is second to that of breast cancer worldwide; it affects men and women almost equally (Parkin et al., 2005). The highest incidence rate is in North America, Australia, Western Europe, and Japan. In Saudi Arabia the disease is ranked second after breast cancer and accounts for 8.5% of all tumors (Ibrahim et al., 2008). It has been suggested that the incidence of colorectal cancer in Saudi Arabia will increase fourfold by the year 2030 (Ibrahim et al., 2008). This may be due to high consumption of animal fat especially red meat and inadequate nationwide screening program. However, studies in other countries have reported improvements of early detection of the disease as well as increased survival rates (Verdecchia et al., 2007). Colon cancer is treated by resection with or without adjuvant chemotherapy. Patients are usually followed up consequently to detect recurrence or new disease in the colon. Recurrence rates depend on the stage of colorectal cancer and it is higher during the first 3 years post-surgical resection (Kobayashi et al., 2007). Low recurrence rates (3.7%) has been reported in early stages of colorectal cancer (Kobayashi et al., 2007).

Evaluation of neurocognitive properties as well as QOL of such patients as a consequence of both malignancy and/or exposure to treatments including chemotherapy and surgery is important. QOL studies have received more attention than neurocognitive studies. There is no single study that evaluates the neurocognitive abilities of colon cancer patients after treatment is completed using specific neurocognitive tests that assess different domains such as memory, attention, and executive function. Most of the QOL studies have shown a comparable health-related QOL to the normal population especially in long-term survivors of colorectal cancer (Gall *et al.*, 2007; Kopp *et al.*, 2004; Ramsey *et al.*, 2002; Schneider *et al.*, 2007; Wilson *et al.*, 2006). However, defects in specific aspects of QOL such as emotional and social functioning have been reported (Arndt et al., 2006). On the contrary, depressive symptoms have been reported in colorectal cancer patients (Ramsey et al., 2002). Anxiety and depression were assessed in long-term survivors of cancer including colorectal cancer in Australia and a

comparable level of anxiety and depression to those of general population was shown using the Hospital Anxiety and Depression Scale (HADS) (Boyes et al., 2009).

The aim of this study was to assess a group of Saudi colon cancer patients 6 months after treatment was completed (in remission) to exclude the effect of chronic disease on neuropsychological abilities of such patients. The assessment included different domains of neurocognitive properties, QOL, anxiety, and depression.

4.3 Methodology

4.3.1. Participant

The eligibility of potential volunteers was confirmed by checking the patient's chart for inclusion and exclusion criteria before administering neuropsychological tests. Patients were coded before administering neuropsychological tests so the patient's medical data was not revealed to the main investigator (who carried out the neuropsychological tests). Demographic characteristics (age, gender, education level) and medical history were obtained from the medical records which were recent and coincided with cognitive function test time.

Participants (18- 60 yr) were recruited from the oncology clinic at King Khaled University Hospital (KKUH). Exclusion criteria included smoking, psychiatric or cerebrovascular disease, sensory impairment, abnormal electrolytes, anemia or uncontrolled blood pressure. Eligible patients were notified about the procedure of the tests and asked to sign informed consent form (see Appendix 1.1). Ethical approval for the study was granted by KKUH and College of Medicine Research Ethics Committee.

4.3.2. Measures

Cognition was assessed using the Rey Auditory-Verbal Test RAVLT (learning & memory); the Rey-Osterrieth complex figure (RCF, visuo-spatial organization and visuo-spatial memory); semantic verbal fluency (executive function); letter cancellation (attention); digit-symbol DSST (sustained attention, visual searching, visual sequencing). The 36-item Short-Form Health Survey quality of life (QOL) and the Hospital Anxiety and Depression Scale were also used to assess QOL, anxiety, and depression, respectively.

4.3.3 Statistical methods

See Chapter 2, section 2.5.

4.4 Results

4.4.1 Demographics and medical condition

Data for the healthy control group were presented in Chapter three.

A total of 32 Colon cancer patients in remission were recruited, their mean age was 44.8 years, 23 of them were males (71.9%), while their mean years of education was 13.1 ± 4.06 years. The mean period since surgery was 2.1 ± 1.4 year, while the mean years since chemotherapy completion was 1.2 ± 1.3 , three patients had DM and were receiving oral hypoglycemic medications. Otherwise they did not have any other complaint and/or on any other types of therapy.

Upon comparing colon cancer patients with healthy controls, colon cancer patients in remission were significantly older ($t(66) = -4.2, P < 0.05$).

4.4.2 Neurocognitive function

4.4.2.1 Memory

Rey Auditory-Verbal Learning Test

Mean learning (trial 1 to 5, a total of 45 words), immediate (out of 15 words), and delayed recall (out of 15 words) as well as recognition task (out of 15 words) are presented in Figure 4.1 below

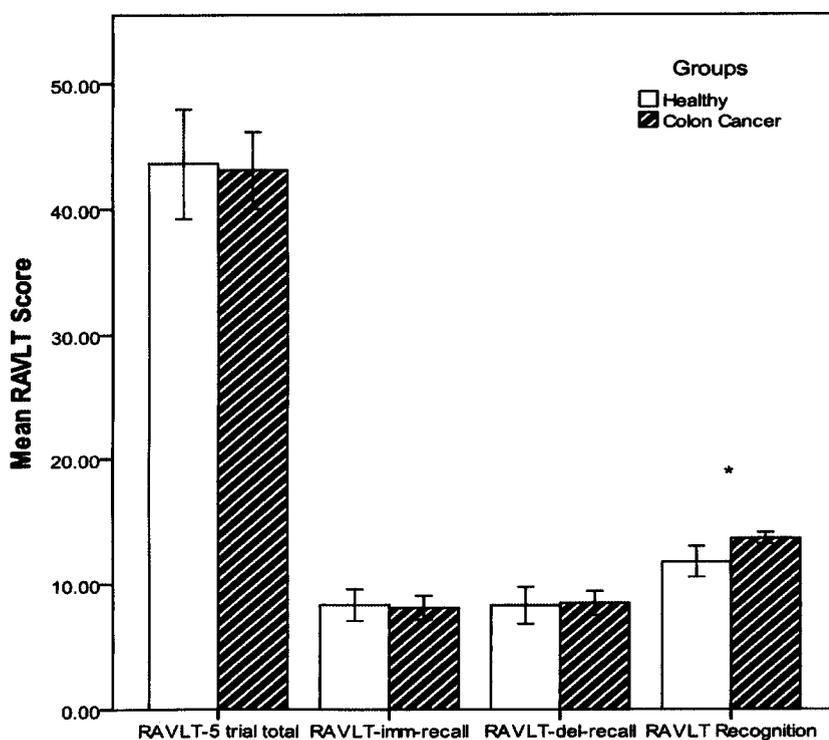


Figure 4.1. Mean \pm 95% CI of RAVLT scores (trial 1 to 5 (RAVLT1-5), immediate (RAVLT imm), and delayed (RAVLT del) recall, as well as recognition task (RAVLT recog) in healthy volunteers (n=36) and colon cancer patients in remission (n=32). * represents significant difference from control group ($p < .05$).

In comparison to healthy control, colon cancer patients in remission had significantly better RAVLT recognition task ($t(67) = 3.922, p < 0.01$). On the other hand no other differences between groups on learning (trial 1 to 5), immediate, and delayed recall.

Since RAVLT and RCF involved repeated measure tasks, repeated measure ANOVA was conducted on RAVLT recall trials and recognition task and on RCF tasks to decrease the chance of type I error as shown in figure 4.2 and 4.4 below.

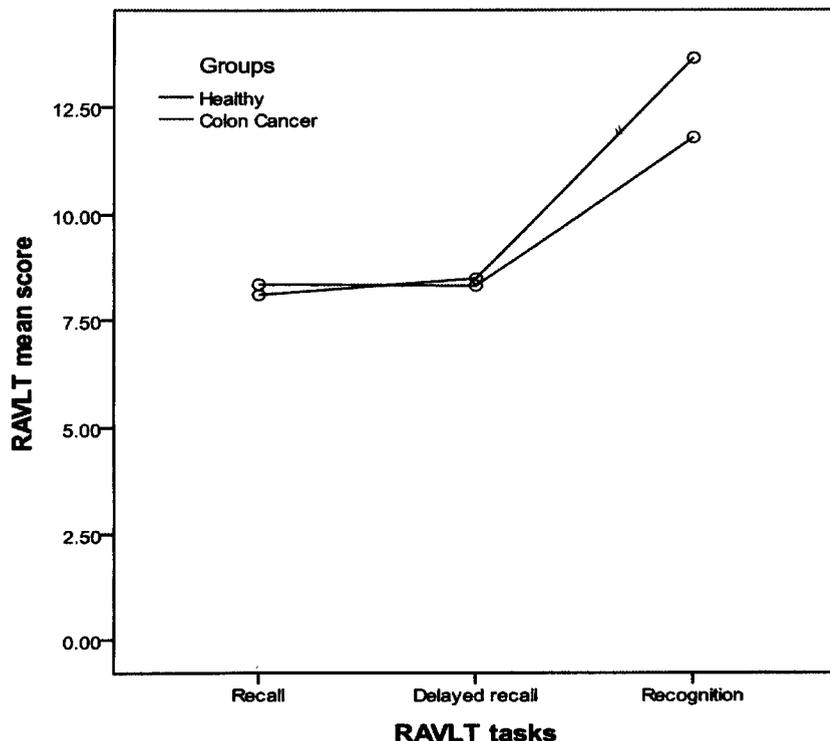


Figure 4.2 Estimated marginal mean plot of immediate recall, delayed recall, and recognition score of RAVLT task in healthy volunteers (n=36) and colon cancer patients(n=32).

A 2×3 (group \times time) repeated measure ANOVA was conducted on RAVLT. The results showed that there was a significant effect of time on RAVLT task performance ($F(2,67)= 78.60, p= .001$), where planned contrast revealed that the difference was between delayed recall and recognition task within each group ($F(2,67)= 138.92, p= .001$). There was also a significant interaction between the groups and tasks of the test i.e. the average change over the tasks is significantly different between the groups ($F(2,67)= 4.19, p= .02$). Planned contrast analysis revealed that the group task interaction is significant between delayed recall and recognition task ($F(2,67)= 5.24, p= .03$) i.e. no significant differences between healthy control and colon cancer patients except in recognition task which confirm the previous finding of one-way ANOVA analysis.

Since RAVLT and RCF involved repeated measure tasks, repeated measure ANOVA was conducted on RAVLT recall trials and recognition task and on RCF tasks to decrease the chance of type I error as shown in figure 4.2 and 4.4 below.

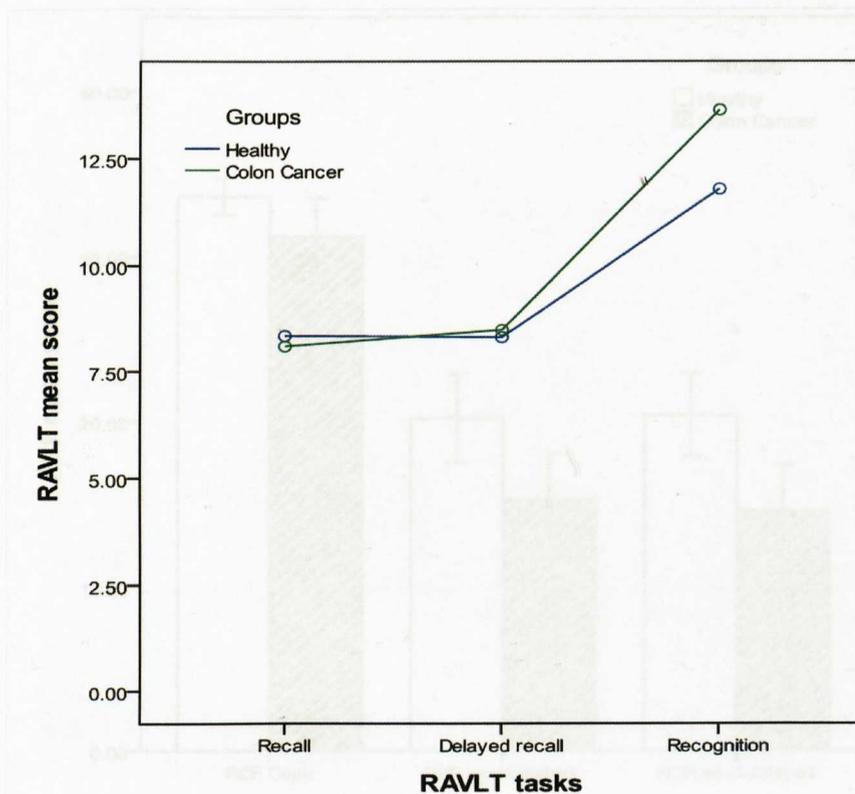


Figure 4.2 Estimated marginal mean plot of immediate recall, delayed recall, and recognition score of RAVLT task in healthy volunteers (n=36) and colon cancer patients(n=32).

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Rey-Osterrieth Complex figure

Figure 4.3 below represent mean copying and visuo-spatial memory scores out of a total of 36.

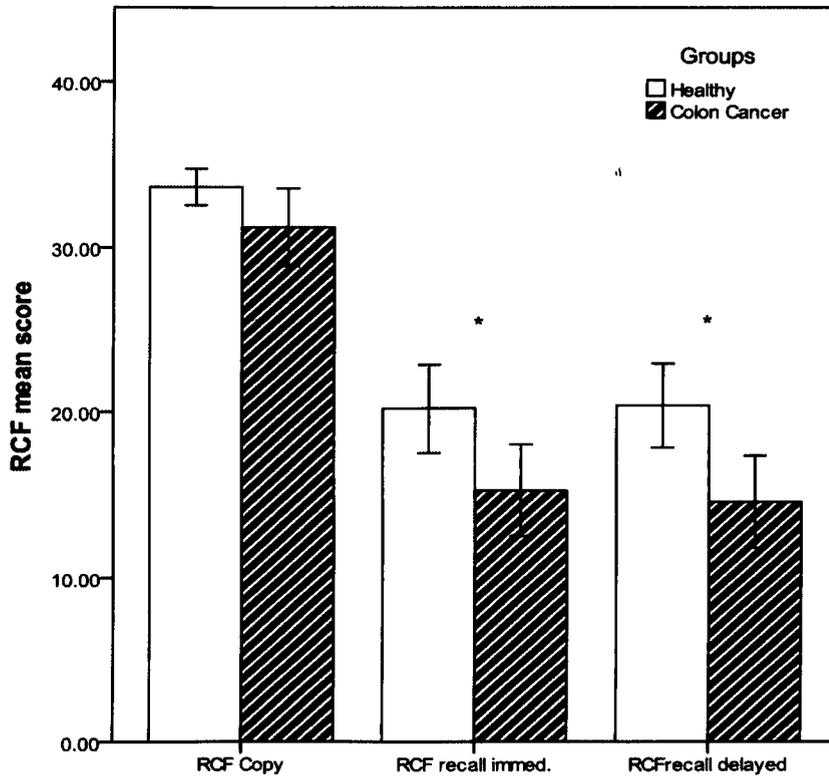


Figure 4.3 Mean \pm 95% CI of RCF scores of copy, immediate and delayed recall tasks in healthy volunteers (n=36) and colon cancer patients in remission (n=32). * represents significant difference from control group ($p < .05$).

In comparison to healthy control, healthy controls had significantly better RCF recall task ($t(67) = 2.61, p < 0.01$) and delayed recall task ($t(67) = 3.16, p = .002$) than colon cancer patients. However, there were no significant differences on copy task.

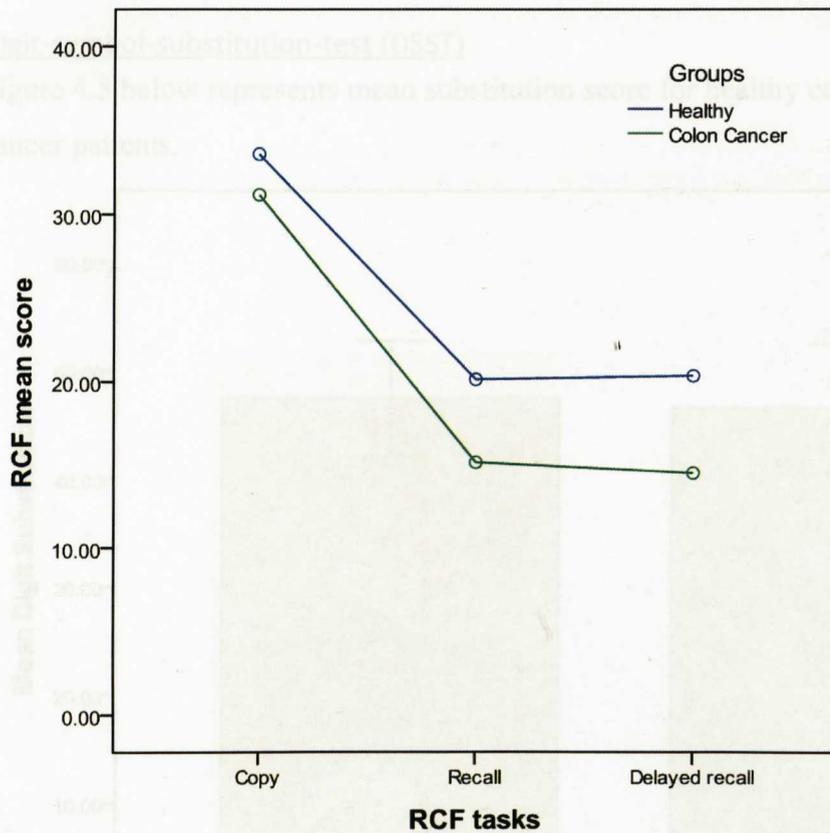


Figure 4.4. Estimated marginal mean plot of copying, immediate and delayed score of RCF task in healthy volunteers (n=36) and colon cancer patients in remission (n=32).

A 2×3 (group \times time) repeated measure ANOVA was conducted on RCF. There was a borderline significant group \times task interaction ($F(2,67) = 3.195, p = .048$), where performance declined sharply in colon cancer patients relative to healthy control performance. However planned contrast failed to represent any significant differences. There was also a significant difference in task performance ($F(2,67) = 178.38, p = .001$). Planned contrast revealed that the significant difference was between copy task and both immediate and delayed recall ($F(2,67) = 317.77, p = .001$). This means that the two groups performed similar on RCF tasks but there was a significant difference between copy task and both immediate and delayed memory tasks among the two groups. This result confirmed the previous finding of t-test comparison.

4.4.2.2 Attention

Digit-symbol-substitution-test (DSST)

Figure 4.5 below represents mean substitution score for healthy control and colon cancer patients.

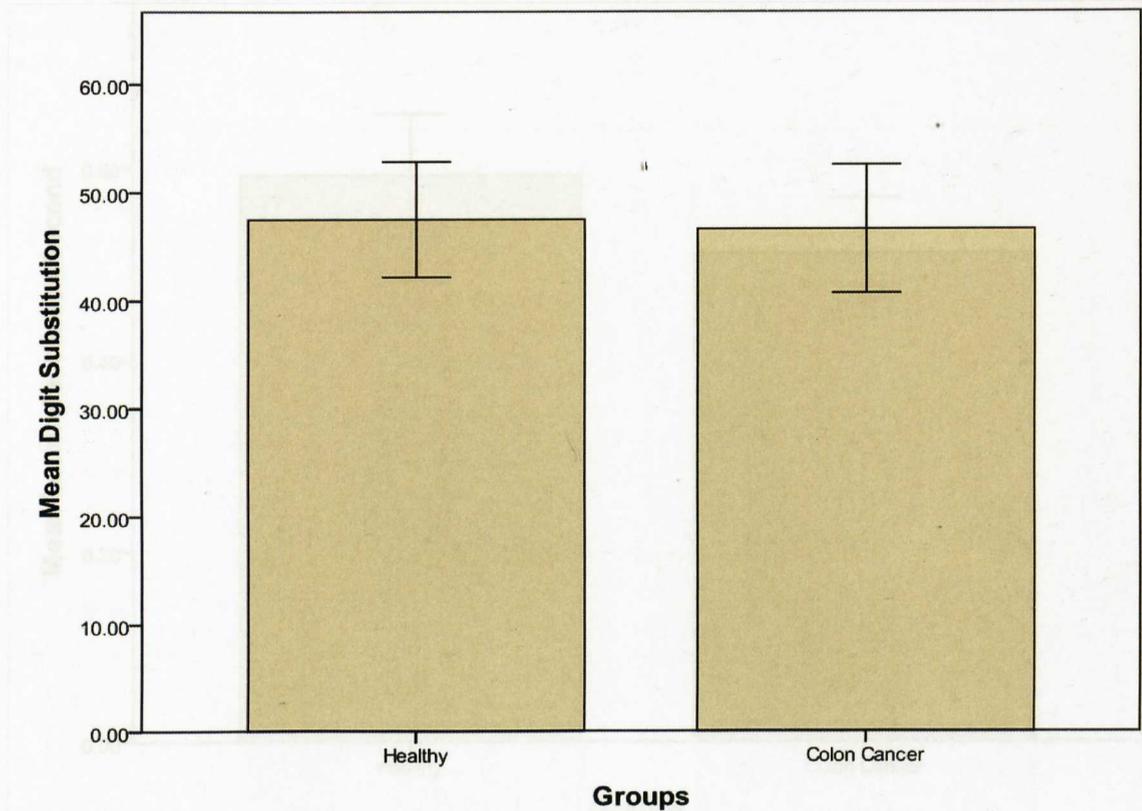


Figure 4.5 Mean \pm 95% CI of Digit-symbol-substitution scores in healthy volunteers (n=36) and colon cancer patients in remission (n=32).

Both healthy control and colon cancer patients were matched for substitution task ($p > 0.05$).

Letter cancellation

Figure 4.6 below indicates mean letter cancellation per second for healthy control and colon cancer patients.



Figure 4.6 Mean \pm 95% CI of letter cancellation scores in healthy volunteers (n=36) and colon cancer patients in remission (n=32).

Results indicate that colon cancer patients in remission were similar to healthy controls in the letter cancellation task.

4.4.2.3 Executive function

Mental fluency

Figure 4.7 below represent mean number of words generated in one minute for healthy control and colon cancer subjects.

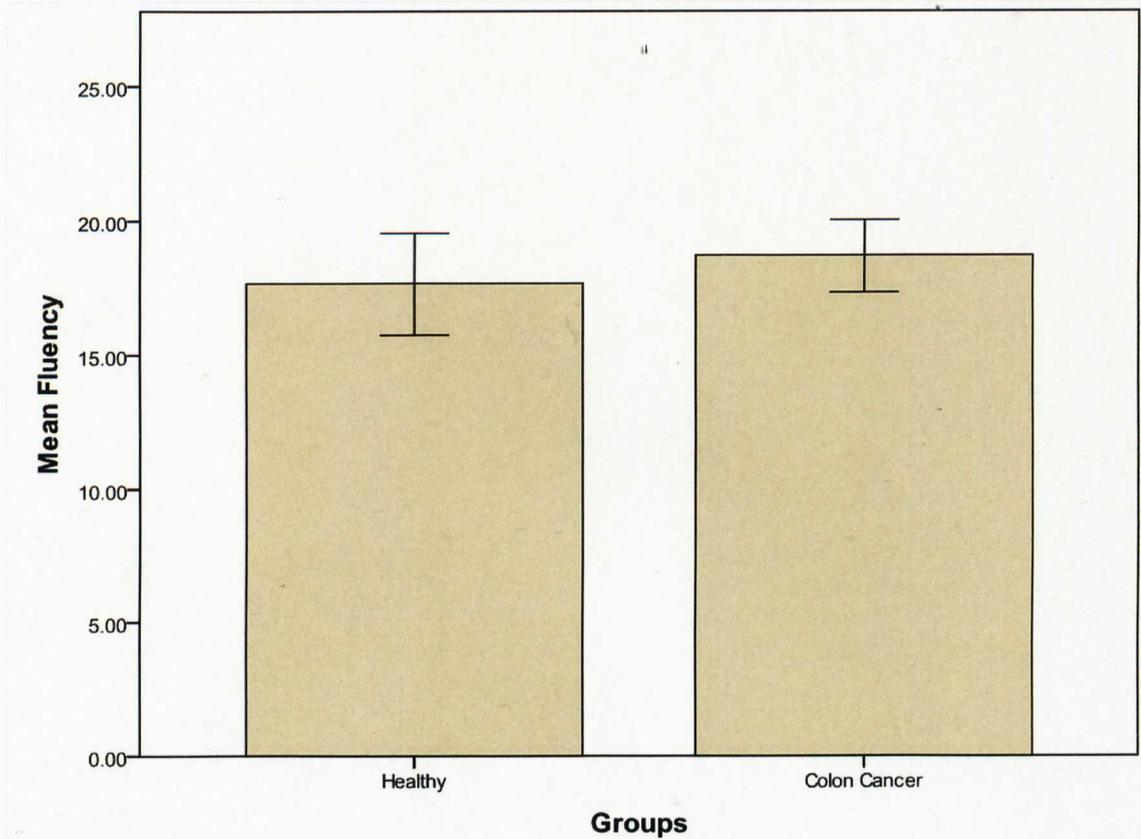


Figure 4.7 Mean \pm 95% CI of mental fluency (animal names generated in one minute) scores in healthy volunteers (n=36) and colon cancer patients in remission (n=32).

Mental fluency results indicate that colon cancer patients in remission were similar to healthy controls.

4.4.3 QOL

Figure 4.8 below represents mean QOL for healthy control and colon cancer patients.

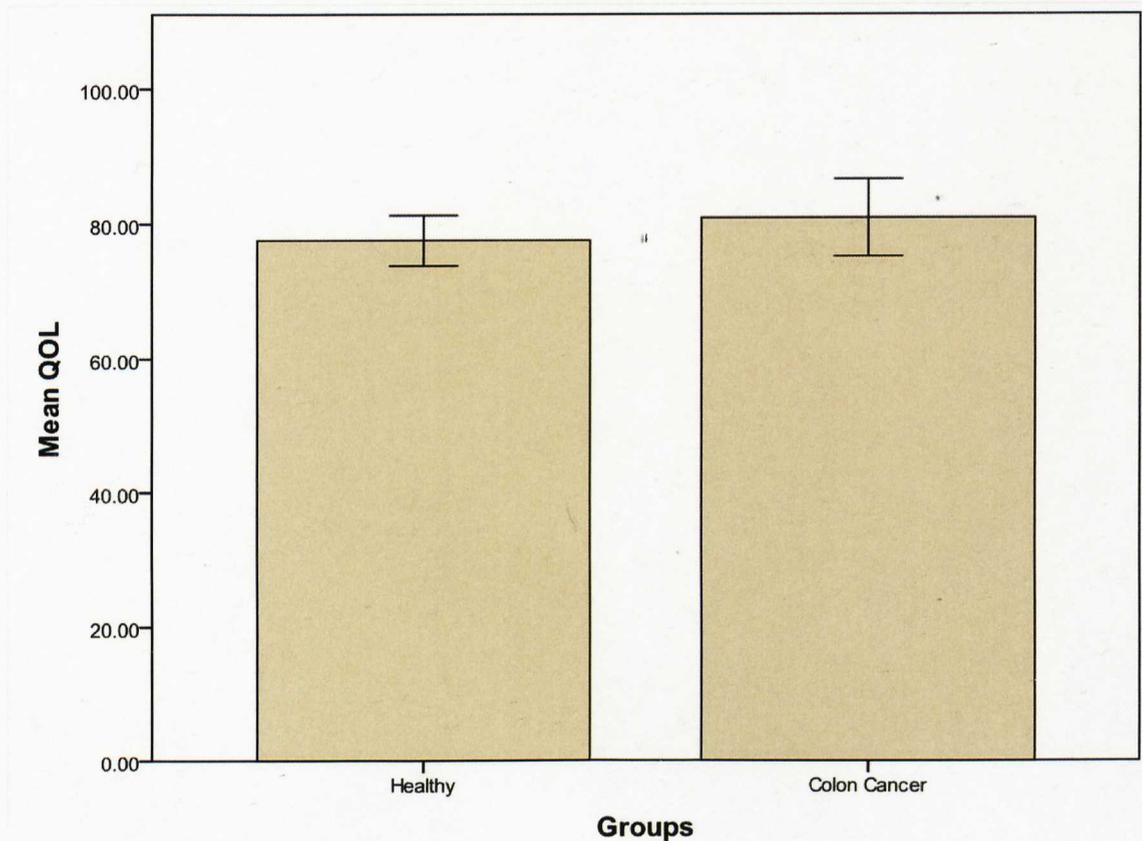


Figure 4.8 Mean \pm 95% CI of Quality of Life scores in healthy volunteers (n=36) and colon cancer patients in remission (n=32).

There were no significant differences in QOL between the healthy controls and the colon cancer patients.

Figure 4.9 below represents PCS and MCS for healthy control and colon cancer patients.

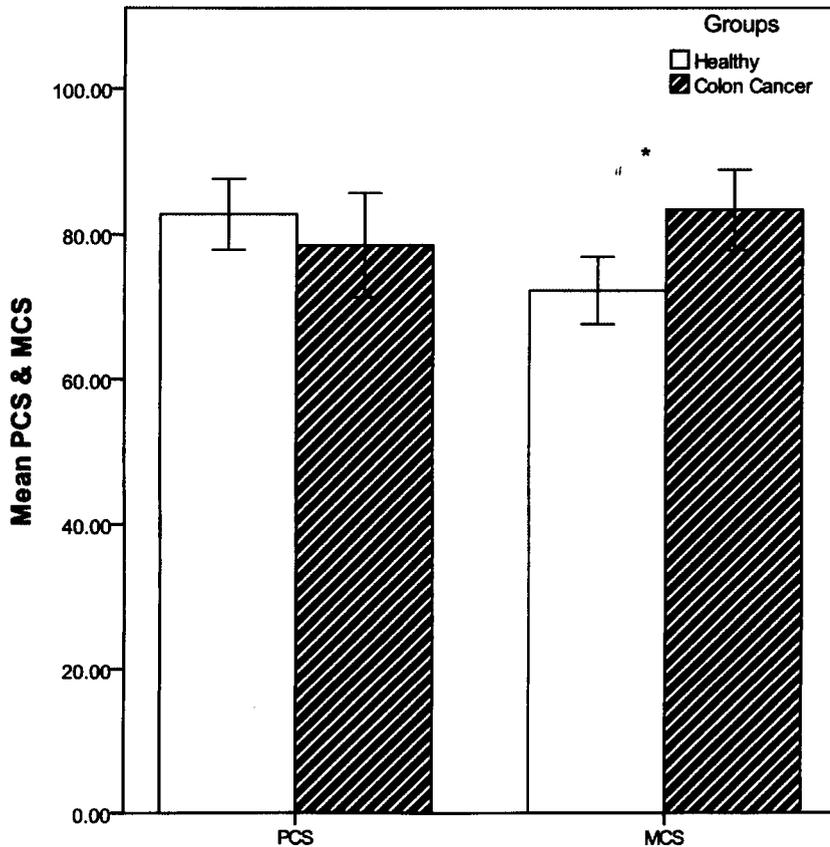


Figure 4.9 Mean \pm 95% CI of PCS and MCS in healthy volunteers (n=36) and colon cancer patients in remission (n=32). * represents significant difference from control group ($p < .05$).

Colon cancer patients had significantly better MCS score ($t(66) = - 3.1, p < 0.01$) than healthy control. There were no significant differences in scores on majority of the subscales of the SF-36 QOL scale between healthy controls (n= 36) and colon cancer patients in remission (n=32). Scores for the vitality ($t(66) = - 2.2, p < 0.05$), mental health ($t(66) = - 4.2, p < 0.01$) and reported health transition ($t(66) = - 2, p = 0.05$) scales, however, were significantly higher in the cancer patients ($p < 0.05$), whilst the scores for role physical ($t(66) = 2.1, p < 0.05$), were significantly lower ($p < 0.05$), see table 4.1 below

SF-36 Scales	Healthy (36)	Colon Cancer (32)
Physical Functioning (PF)	86.1 ± 15.2	82.7 ± 22.5
Role-Physical (RP)	87.5 ± 25.0	71.5 ± 37.2 *
Bodily Pain (BP)	85.8 ± 17.1	85.4 ± 20.3
General Health (GH)	71.7 ± 15.5	74.4 ± 19.6
Vitality (VT)	63.8 ± 13.6	73.0 ± 20.8 *
Social Functioning (SF)	79.5 ± 21.8	85.9 ± 17.3
Role-Emotional (RE)	75.9 ± 33.4	89.6 ± 27.4
Mental Health (MH)	69.7 ± 15.1	84.9 ± 14.7 *
Reported Health Transition (HT)	61.1 ± 21.1	72.7 ± 26.5 *

Table 4.1. Comparison of QOL subscale scores (mean ± SD) between healthy volunteers (n= 36) and colon cancer patients in remission (n= 32), * indicates significant difference from control group p<0.05.

4.4.4 Hospital Anxiety and Depression Scale (HADS)

In comparison to healthy controls, colon cancer patients in remission has significantly less anxiety ($t(1,66) = 4.634, p < 0.01$) and less depression ($t(1,66) = 4.173, p < 0.01$). Mean scores of HADS tests are indicated in figure 4.10 and 4.11

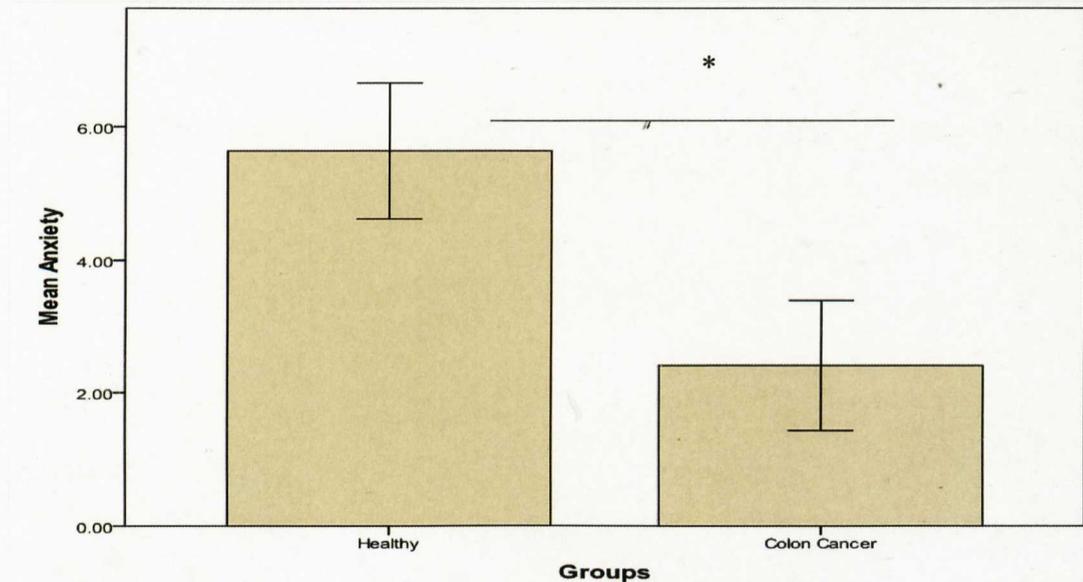


Figure 4.10 Mean \pm 95% CI of HAD-Anxiety scores (mean \pm SD) in healthy volunteers (n=36) and colon cancer patients in remission (n=32). * represents significant difference from control group ($p < .05$).

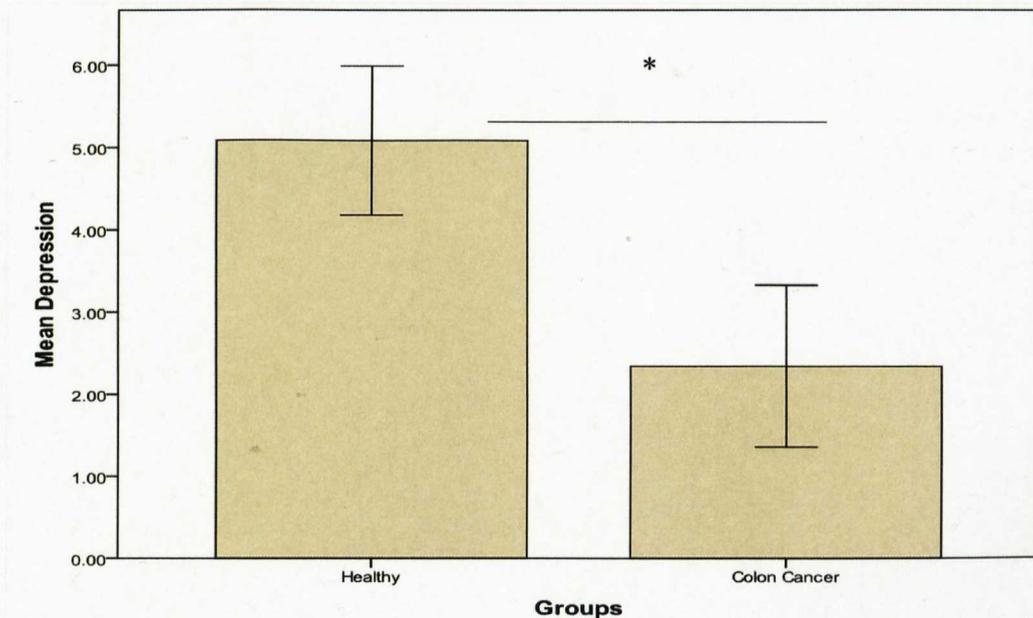


Figure 4.11 Mean \pm 95% CI of HAD-Depression scores in healthy volunteers (n=36) and colon cancer patients in remission (n=32). * represents significant difference from control group ($P < .05$).

4.5 Conclusion

Table 4.2 below summarizes the significant differences between colon cancer patients and healthy controls.

In this study, colon cancer patients in remission were selected to exclude the effect of chronic disease on neurocognitive properties that have been observed in the CKD study. Colon cancer patients in remission have potentially fatal illness which is currently in remission. They are currently symptom- and drug-free, but require regular, frequent hospital checks. These patients have a constant fear of their illness recurrence and have regular hospitalization: they are therefore similar, in some aspects, to chronic renal failure patients, but without the confounding variables of symptoms and medication.

Colon cancer patients in remission had no impairments in cognition with some decrease in physical components of quality of life. These results suggest that the cognitive impairment in CKD patients is not due to the presence of chronic disease nor interaction with health services.

The results of this study documented the absence of the bad effect of any chronic disease on mood and cognition at least in the tested population. In addition it supported the suggestion that the finding of the CKD study is not due to the disease itself and other factors should be considered. One of these factors is the contribution of RAS in mood and cognition. This contribution was investigated in the third study where dialysis patients were tested for RAS genotypes and its association to mood and cognition as well as RAA drugs.

Task	Result summary
QOL SF-36 (total)	No Sig differences.
PCS	No Sig differences.
MCS	Sig differences between groups ($p < 0.01$), with colon cancer patients performing better.
HAD-Anxiety	Sig differences between groups ($p < 0.01$), with colon cancer patients having less anxiety.
HAD-Depression	Sig differences between groups ($p < 0.01$), with colon cancer patients having less depression.
RAVLT	Five trial learning: No Sig differences. Delayed recall: No Sig differences. Recognition: Sig differences between groups ($p < 0.01$), with colon cancer patients performing better.
RCF	Visuospatial organization: No Sig differences. Visuospatial immediate recall: Sig differences between groups ($p < 0.05$), with healthy control performing better. Visuospatial delayed recall: Sig differences between groups ($p < 0.01$), with healthy control performing better.
DSST	No Sig differences.
Letter cancellation	No Sig differences.
Mental fluency	No Sig differences.

Table 4.2. Summary of QOL, HADS, and neurocognitive tests results of colon cancer study.

CHAPTER 5. GENOTYPING OF SAUDI DIALYSIS PATIENTS AND THEIR NEUROPSYCHOLOGICAL ABILITIES

5.1 Aim of study

As discussed previously, the main aim of this thesis is to explore neurocognitive defects in chronic kidney disease (CKD) patients and to highlight the possible role of renin-angiotensin system (RAS) in any defect. The literature review of this thesis documents the favourable impact of drugs acting on RAS on memory and cognition. Furthermore, good memory and cognition in CKD patients has been shown in the first study of this thesis. This study was designed to ascertain whether the neuropsychological abilities of dialysis patients and their responses to drug therapy were influenced by gene polymorphisms. This was achieved by measuring RAS gene polymorphisms of dialysis patients and correlating them to RAS inhibition and neuropsychological performance of tested patients. Dialysis patients were chosen in this study since they are in the same stage of kidney impairment but biochemically controlled. The RAS component polymorphisms that were studied in this section are angiotensin converting enzyme insertion/deletion (ACE I/D) rs1799752, Angiotensinogen (AGT M268T) rs699 and (AGT T207M) rs4762, angiotensin type 1 receptor (AT₁R A1166C) rs5186, and angiotensin type 2 receptor (AT₂R C3123A) rs11091046.

5.2 Introduction

The finding of the CKD study (chapter 3) where CKD patients treated with renin-angiotensin-antagonist (RAA) drugs had better neuropsychological abilities than CKD patients treated with non-RAA needs to be supported and correlated to RAS activity. Evidence exists to involve the RAS gene polymorphisms in mood and cognition. Thus RAS gene polymorphisms were explored in healthy control and kidney disease Arabs as well as their association with neurocognitive defects.

5.2.1 Acknowledging RAS polymorphism in health and CKD

RAS polymorphisms have been implicated in many diseases where cardiovascular diseases were the most extensively studied along with kidney disease. Lately, the contribution of RAS polymorphisms to other diseases has been indicated for example in

anxiety, depression, and disorders of memory and cognition. Activation of RAS potentiates the prevalence of hypertension (HTN) in CKD which is considered a major contributor in the progression of renal failure. Therefore, a genetic predisposition to overactivation of RAS may predispose and/or promote kidney function loss. Moreover, disorders of memory and cognition have been identified in patients with kidney disease and again RAS polymorphisms have been associated with defects in mood and cognition. RAS genes are highlighted in genetic studies of CKD as well as memory and cognition, of these are the polymorphisms of ACE, AGT, AT₁R, and AT₂R genes that have been considered to be implicated in chronic renal disease and are of particular interest.

As discussed in the introductory chapter of this thesis, there is strong evidence from the literature indicating that dialysis patients suffer neurocognitive defects despite efficient hemodialysis where urea clearance satisfies its target value that is equal to 1.2-1.4 (Kurella *et al.*, 2004; Murray *et al.*, 2006). This necessitates looking for factors that impair neurocognitive function of such patients despite adequate dialysis. RAS genotype association with defects in memory and cognition is investigated in this study.

Inconsistent data has been reported from RAS genotyping studies in CKD patients in different populations. Previous studies reported a similar frequency of D allele (around 45%) of the ACE gene in most of the CKD population, including Caucasian, Indian, and Chinese as detailed in the introduction. This prevalence was quite different among healthy populations where it was highest in healthy Caucasian (around 60%) and lowest in healthy Korean, Japanese, and Chinese (around 20%). Moreover, rapid progression toward end-stage renal disease (ESRD) has been associated with the DD genotype of the ACE gene especially in patients carrying the MM genotype of the AGT M268T gene (Lovati *et al.*, 2001). This may suggest the association between ACE I/D polymorphism and CKD.

The prevalence of the A allele of AT₁R A1166C was around 90% in Chinese and Indian diabetic nephropathy patients. A prevalence of C allele between 10-30% has been reported in healthy Japanese, Korean, Arabs, and Chinese healthy subjects. This may suggest a weak association of this polymorphisms with CKD. However, a higher

frequency of C allele has been reported in Caucasian ESRD patients. Data on the prevalence of AT₂R C3123A in CKD patients is lacking.

The prevalence of the M variant (T allele) of the AGT M268T polymorphism in CKD was around 57% in Caucasian, 15% in Chinese, and 37% in Indian population. This difference in prevalence among different populations may suggest the strong effect of ethnic origin on the disease. In healthy individuals, the prevalence of the M variant of the AGT M268T was 60% in Caucasian and 21% in Chinese.

A high prevalence of T variant (C allele) of AGT T207M polymorphism has been reported in Chinese, Indian, and Caucasian diabetic nephropathy patients that was 87% and non-significantly different from its prevalence in healthy subjects of the same population. All these reports suggest a possible association of ACE I/D polymorphism with CKD while the other components of RAS gene lack such association.

5.2.2 Acknowledging RAS polymorphism in neuropsychological disorders

Only a handful of reports exist addressing the RAS polymorphism association with neuropsychological defects but none have correlated them to neurocognitive defects in dialysis patients.

The association of D allele or DD genotype of ACE I/D polymorphism with cognitive impairments in elderly subjects has been documented a long time ago as detailed in the introductory chapter of this thesis. Again these studies gave inconsistent findings regarding the association of D allele with neurocognitive defects in elderly subjects with either normal cognitive aging or dementia. Some of these studies prove a positive association between D allele and neurocognitive impairment especially those published before 2001 (Amouyel *et al.*, 1996; Bartres-Faz *et al.*, 2000; Richard *et al.*, 2000). More recent reports deny such an association in both dementia and normal cognitive aging (Kim *et al.*, 2006; Stewart *et al.*, 2004; Visscher *et al.*, 2003). Furthermore, a reduced risk of Alzheimer disease (Helbecque *et al.*, 2009; Lehmann *et al.*, 2005), and dementia (Richard *et al.*, 2001) has been reported in D allele carriers.

Genes of the RAS other than the ACE gene, for example AT₁R, AT₂R, and AGT have not been extensively studied in neurocognitively impaired patients. AT₁R gene polymorphisms did not contribute to genetic susceptibility to vascular dementia of Korean subjects, while the M variant of AGT T268M polymorphism was more common in vascular dementia patients (Kim et al., 2006). However, a recent report addresses the contribution of the TT polymorphism of the AGT M268T gene in a protective effect of ACEIs against executive function decline in elderly subjects (Hajjar et al., 2010).

It is very difficult to draw a conclusion regarding the association of RAS polymorphisms with neurocognitive defects due to the high discrepancy in published data.

5.2.3 Acknowledging RAS polymorphism in depression

The association of RAS genes with depression has been also investigated. Negative association between ACE gene polymorphisms and depression have been reported (Hong et al., 2002; Meira-Lima et al., 2000; Pauls et al., 2000; Saab et al., 2007b; Segman et al., 2002). Conversely, the M variant of the AGT M268T polymorphism was more frequent in the depressed population (Meira-Lima et al., 2000). Female depressed patients carrying the D allele of the ACE I/D polymorphism showed more rapid response to antidepressant treatment than carriers of the I allele (Baghai et al., 2004). Good improvement has also been achieved in depressed patients carrying the haplotype combination of CC genotype of AT₁R A1166C polymorphism and the DD/ID genotypes of the ACE I/D polymorphism (Bondy et al., 2005).

5.2.4 Experiment outline and predictions

There was a clear contribution of RAS polymorphism in CKD (Tyrrell et al., 2005). The data for the contribution of RAS polymorphisms to neuropsychological defects were inconsistent. Implication of the cognitive enhancing effect of drugs acting on RAS in CKD patients needs to be proved irrespective of the improvements in kidney function. This experiment was designed to overcome such a deficit. This was achieved by investigating the relationship between the effectiveness of RAA drugs in improving memory and cognition and polymorphisms of RAS genes in clinically stable dialysis patients. The association of allele frequency and/or genotype frequencies of RAS genes

with neurocognitive parameters in Saudi dialysis patients was evaluated to understand the genetic etiology of neurocognitive defects. Few studies have been published in relation to RAS gene polymorphisms in Saudi subjects, and this is the first report of RAS gene polymorphisms in relation to neurocognitive parameters in Saudi dialysis patients.

The expectation based on the previous reports was to find positive impact of RAA drugs on neurocognitive abilities of dialysis patients that was related to RAS polymorphisms in such patients. Polymorphism associated with increased RAS activity such as The D allele of ACE gene were predicted to worsen neurocognitive function that might be antagonized by RAA drugs. Furthermore, polymorphisms that were known to decrease the RAS activity might have a good impact on neurocognitive function especially with RAA drugs.

5.3 Methodology

5.3.1 Recruitment and study design

A total of fifty-three Saudi dialysis patients were recruited in this study. Among the subjects, 13 (24.5%) were using RAA drugs, 40 (75.5%) were on other types of antihypertensive. Patients were recruited from two dialysis centres at Riyadh, the capital city of Saudi Arabia. Prince Salman dialysis centre (PSDC) accommodates around 210 hemodialysis patients, of those only 27 were eligible for this study. The dialysis centre at King Abdulaziz Medical City (KAMCDC) also accommodates around 230 hemodialysis patients and only 26 were eligible for this study. Table 5.1 below shows the characteristics of ineligible patients at both centres.

	PSDC 210 patients N (%)	KAMCDC 230 patients N (%)
Total Male/female	124/86 (59.05/ 40.95)	126/104 (54.8/ 45.2)
Eligible Male/female	19/8 (9.05/ 3.8)	15/11 (6.5/ 4.8)
Older than 60 and illiterate	62/48 (29.5/ 22.9)	65/49 (28.3/ 21.3)
Younger than 60 and illiterate	25/15 (11.9/ 7.1)	29/20 (12.6/ 8.7)
Non-Saudi	0/1 (0/ 0.5)	2/3 (0.87/ 1.3)
Visual/auditory problem	6/6 (2.9/ 2.9)	7/8 (3/ 3.5)
CVA	1/2 (0.5/ 0.95)	2/2 (0.87/ 0.87)
SLE on CS	0/2 (0/ 0.95)	0/2 (0/ 0.87)
Epilepsy on antiepileptic	2/1 (0.95/ 0.5)	2/1 (0.87/ 0.4)
On antidepressant	3/2 (1.4/ 0.95)	2/4 (0.87/ 1.7)
Eligible but refused	6/1 (2.9/ .5)	2/4 (0.87/ 1.7)

Table 5.1 Dialysis patients from Prince Salman (PSDC) and King Abdulaziz Medical City (KAMCDC) dialysis centers who were ineligible for inclusion into the current study.

Reason for ineligibility split between Male and female.

CVA: cerebro-vascular accident, SLE: systemic lupus erythematosus, CS: corticosteroids

A total of 42 Saudi healthy male blood donors visiting the Blood Donor Clinic of King Faisal Specialist Hospital and Research Center (Riyadh, Saudi Arabia) were recruited to determine the RAS genotypes distribution among Saudi healthy controls.

All patients were instructed to complete the questionnaire and neurocognitive tests during hemodialysis session under the supervision of the main researcher.

5.3.2 Inclusion & Exclusion criteria

Eligible patients were those who were adult (18-60 years old), ambulant, and literate having clinically stable ESRD and on hemodialysis as documented by the charging nephrologists and patient history. Patients were excluded if they had psychiatric illness, cerebrovascular disease, visual or hearing impairment, abnormal serum calcium or sodium, abnormal thyroid function tests, uncontrolled high blood pressure (BP>170/110), hemoglobin level of < 11g/dl, and using glucocorticoids, antidepressant, or lipid-soluble beta blockers or any other medications that are known to affect neurocognitive functioning.

5.3.3 Design

In this observational study, dialysis patients underwent neurocognitive and psychological testing. Dialysis patients underwent also a 10-ml venous blood sampling for DNA extraction and RAS genotyping. Participants were allocated into one of the two drug groups according to their antihypertensive treatment (RAA and non-RAA) without interference of the main researcher. The main dependent measures were either total score of self-answered questionnaire, or accuracy depending on task parameters.

5.3.4 Procedure

An approved ethics statement was obtained from PSDC, and KAMC research committees. Eligible patients were notified about the procedure of the tests and asked to sign informed consent form (Appendix 1.2) after explanation of the aim of study by the researcher.

Each patient then completed the tests in a separate room and the entire session lasted approximately 45 minutes. Tests were administered in the same order to all eligible patients. Thereafter a blood sample was obtained from each participant by the nurse in charge of the patient. Blood samples were transferred by the main researcher (Norah Abanmy) in a secure cold container to the KFSHRC pharmacogenetic lab for DNA extraction and RAS genes amplification. Each eligible patient was given a code for identification when antihypertensive therapy type was identified. This minimized the bias during interpretation of neuropsychological tests.

5.3.5 Chart review

See Chapter 2, section 2.1.5.

5.3.6 Measurement of cognitive function

Chapter 2, section 2.2 provides details of neurocognitive tests, quality of life (QOL), and hospital anxiety and depression scale (HADS) tests.

5.3.7 Genotyping

Chapter 2, section 2.3 provides details of biochemical and genotyping methods.

5.3.8 Covariates

Several covariates were examined in the analysis. Demographic variables included age, gender, and education. Behavioral variables included current cigarette smoking.

Comorbid conditions included diabetes and antihypertensive therapy as well as dialysis duration. Biochemical data (sodium, potassium, phosphate, calcium, hemoglobin, and dialysis adequacy as measured by KtV) were not included in the covariates since all of them were in the therapeutic range and the target population were stabilized dialysis patients.

5.3.9 Statistical methods

See Chapter 2, section 2.5.

5.4 Results

5.4.1 Baseline characteristics

Among the dialysis patients, male gender was predominant (64.2%); otherwise, there were no significant differences in age, education level, diseases and hemodialysis duration, proportion of diabetic patients, urea clearance (Kt/V) as a measure of dialysis adequacy, target haemoglobin, and electrolytes levels between RAA group and Non-RAA as represented below in table 5.2.

Parameters	RAA	non-RAA	P
Gender (male/female)	11/2	23/17	< 0.05
Age (years)	35.38 (10.33)	36.93 (11.19)	> 0.05
Education (years)	11 (3.39)	11.69 (3.34)	> 0.05
Disease duration (years)	6.73 (7.21)	7.61 (6.05)	> 0.05
Years on dialysis	4.21(4.42)	6.38 (5.46)	> 0.05
HTN yes/no	13/0	40/0	> 0.05
DM yes/no	4/9	8/32	> 0.05
Other disease yes/no	0/13	1/39	> 0.05
Smoking yes/no	1/12	5/35	> 0.05
Kt/V	1.47 (0.19)	1.53 (0.31)	> 0.05
Hgb g/dl (equal to/or above 11)	11/2	33/7	> 0.05
Na mEq/L	136.62 (2.22)	136.50 (2.12)	> 0.05
K mEq/L	5.05 (0.73)	4.87 (0.81)	> 0.05
Ca mg/dl	2.19 (0.22)	2.29 (0.26)	> 0.05
Po3 mg/dl	1.54 (0.47)	1.65 (0.51)	> 0.05
Ca×Po3 product	3.38 (1.11)	3.76 (1.30)	> 0.05
PTH pg/ml	45.55 (31.47)	60.57 (68.89)	> 0.05

Table 5.2 Mean ± SD of baseline characteristics and comparison of Renin-Angiotensin Antagonist group (RAA) (13 patients) and non-Renin-Angiotensin Antagonist group (non-RAA) (40 patients) groups. P represents statistical significance of observed differences.

Na: sodium, K: potassium, Ca: calcium, Po3: phosphate, PTH: parathyroid hormone level.

Most of the biochemical variables among the two dialysis group (RAA and non-RAA) were within therapeutic range which indicates that all patients were clinically stable. A detailed drug list is shown in Appendix 6.

The most common aetiology of chronic kidney disease in dialysis patients was hypertension in 20 patients (37.7%) and diabetes in 11 patients (20.8%). Other aetiologies are shown in table 5.3 below.

Aetiology	RAA	non-RAA	P
HTN	2 (15.4)	18 (45.0)	> 0.05
DM	4 (30.8)	7 (17.5)	> 0.05
Glomerular disease	1 (7.7)	3 (7.5)	> 0.05
Interstitial disease	1 (7.7)	2 (5.0)	> 0.05
Obstructive disease	0 (0)	2 (5.0)	> 0.05
IGA nephropathy	0 (0)	3 (7.5)	> 0.05
Polycystic kidney disease	2 (15.4)	2 (5.0)	> 0.05
Renal stone	3 (23.1)	1 (2.5)	> 0.05

Table 5.3 Aetiology of CKD in dialysis patients N(%) . P represents statistical significant of observed differences.

HTN: hypertension, DM: diabetes mellitus, IGA: immune-globulin A.

5.4.2 Neuropsychological tests comparison between dialysis patients and healthy control

Data on healthy control from the CKD study (chapter 3) were used as a comparative group to dialysis patients.

5.4.2.1 Neurocognitive tests comparison

The dialysis patients were similar to normal healthy controls in most of the neurocognitive tests except in the Rey-auditory-verbal learning test (RAVLT) where dialysis patients performed significantly better in learning ($t(87) = -2.99, p = .004$), immediate memory ($t(87) = -2.21, p = .03$), and recognition task ($t(87) = -4.31, p = .001$) than healthy controls as shown in figure 5.1 below.

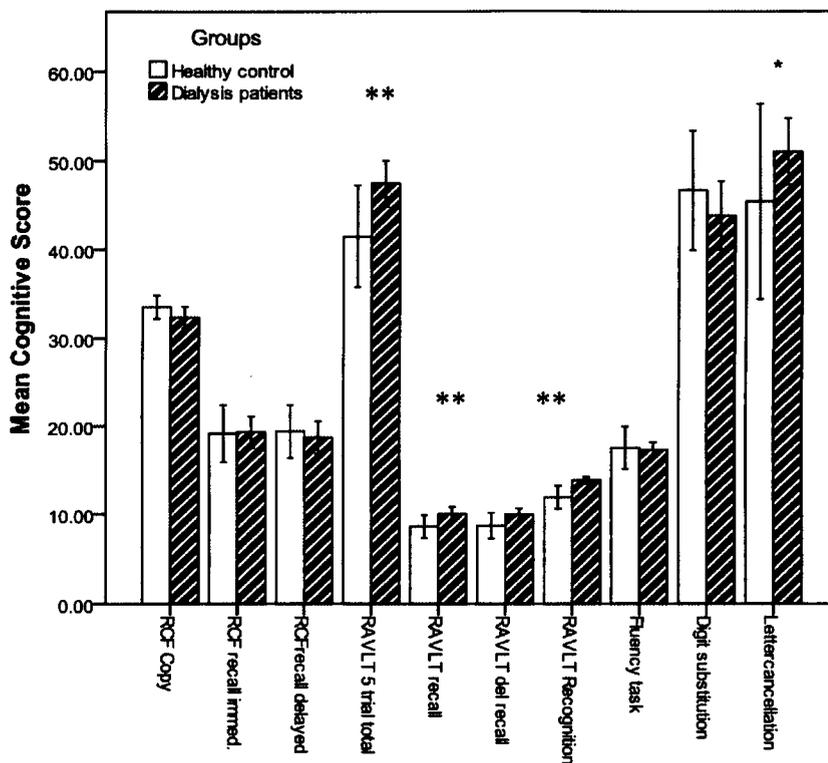


Figure 5.1 Mean \pm 95% CI of neurocognitive tests scores for dialysis patients and healthy control . * multiplied by a factor of 100 to clearly illustrate the results.

RCF: Rey complex figure, RCF recall immediate: RCF recall delayed, RAVLT: Rey-auditory-verbal learning test. ** represents significant difference from control group ($p < .05$).

Since a repeated measure has been undertaken in both RAVLT and RCF tasks, a general linear model with repeated measure analysis was undertaken to try to reduce the

likelihood of type I errors and to replicate the previous finding of a simple t-test comparison as shown below in figures 5.2 and 5.3.

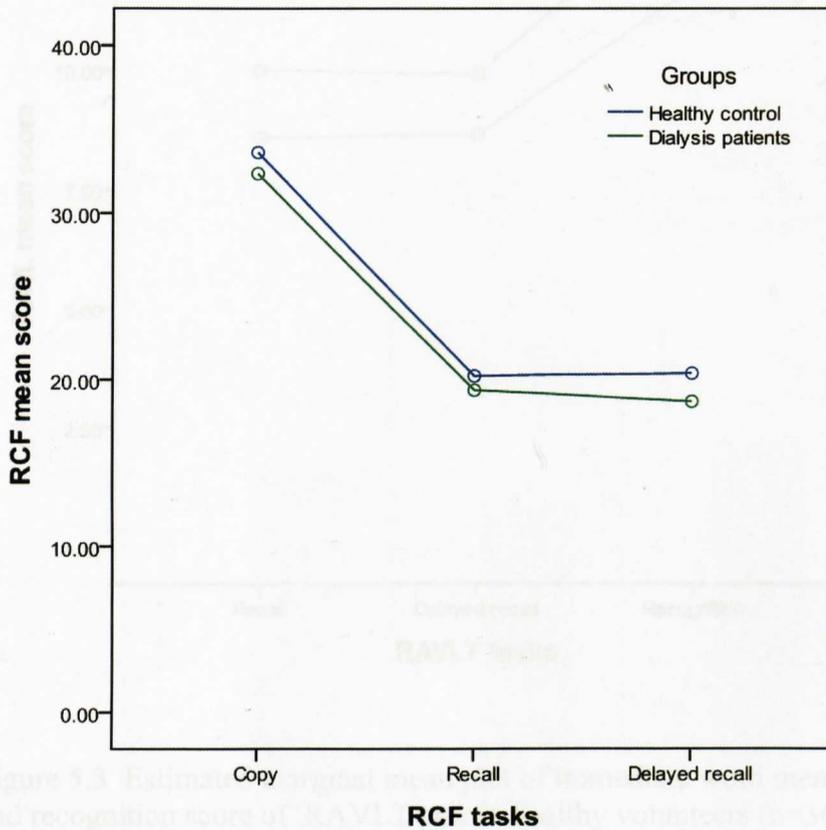


Figure 5.2 Estimated marginal mean plot of Copy, immediate recall and delayed recall scores of RCF task in healthy volunteers (n=36) and dialysis patients (n=53).

A 2×3 (group \times time) repeated measure ANOVA was conducted on RCF. There was a significant main effect of time within each group ($F(2,88) = 276.13, p = .001$). Planned contrast revealed that the significant difference was between copy task and either recall or delayed recall task ($F(2,88) = 452.68, p = .001$).

There was no significant group \times time interaction ($p > .05$). This result documents the previous result of RCF task when simple t-test comparison has been undertaken, i.e. no significant differences between dialysis patients and healthy controls in RCF tasks.

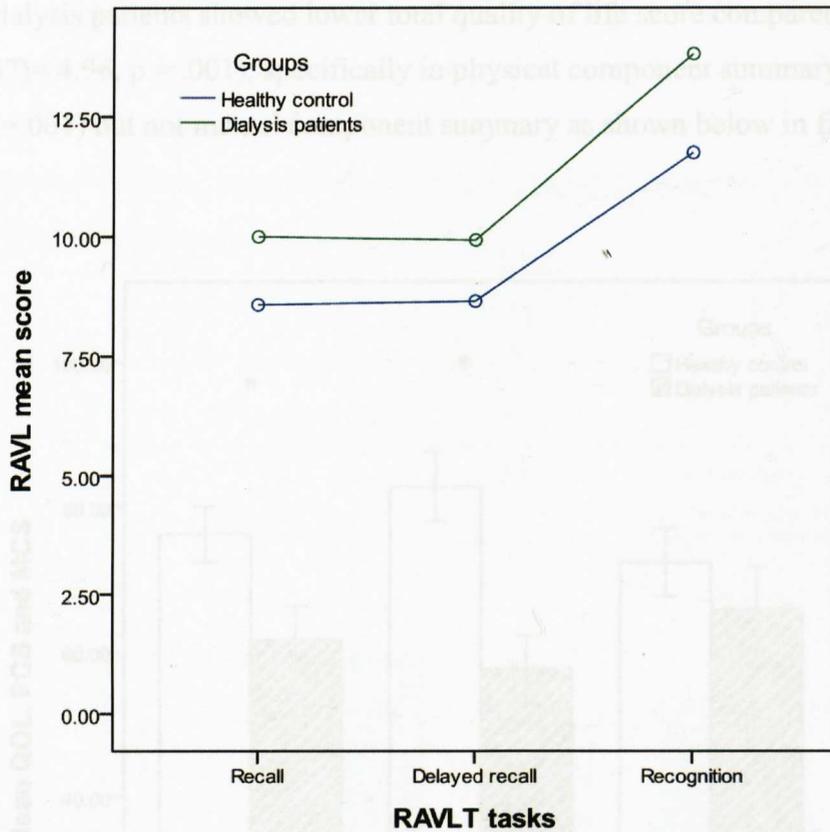


Figure 5.3 Estimated marginal mean plot of immediate word memory, delayed recall, and recognition score of RAVLT task in healthy volunteers (n=36) and dialysis patients (n=53).

A 2×3 (group \times time) repeated measure ANOVA was conducted on RAVLT. There was a significant main effect of time within each group ($F(2,88) = 2.88, p > .05$).

Planned contrast revealed that the significant difference was between recognition task and either immediate or delayed recall ($F(2,88) = 151.38, p = .001$).

There was no significant group \times time interaction ($p > .05$). This result does not confirm the previous finding of simple t-test comparison, i.e. no significant differences between dialysis patients and healthy controls in RAVLT memory and recognition tasks.

5.4.2.2 Quality of life:

Dialysis patients showed lower total quality of life score compared to healthy controls ($t(87) = 4.96, p = .001$), specifically in physical component summary (PCS) ($t(87) = 7.32, p = .001$) but not mental component summary as shown below in figure 5.4.

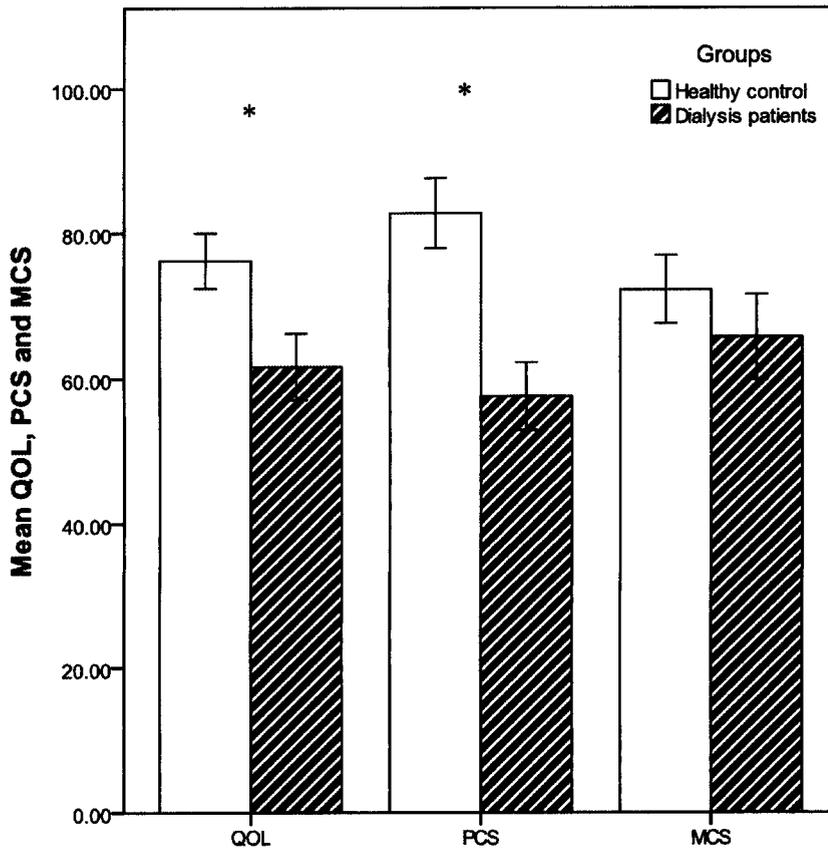


Figure 5.4 Mean \pm 95% CI of QOL, PCS, and MCS scores for dialysis patients and healthy controls. * represents significant difference from control group ($p < .05$). QOL: quality of life, PCS: physical component summary, MCS: mental component summary.

5.4.2.3 HAD-anxiety and depression

The two groups scored similarly on both anxiety and depression scales. Moreover, none of the scores reach level of casesness (see section 2.2.3) which is usually equal to or above 11 (Zigmond *et al.*, 1983; Mystakidou *et al.*, 2005).

Figure 5.5 below represents mean anxiety and depression score for both dialysis patients and healthy controls.

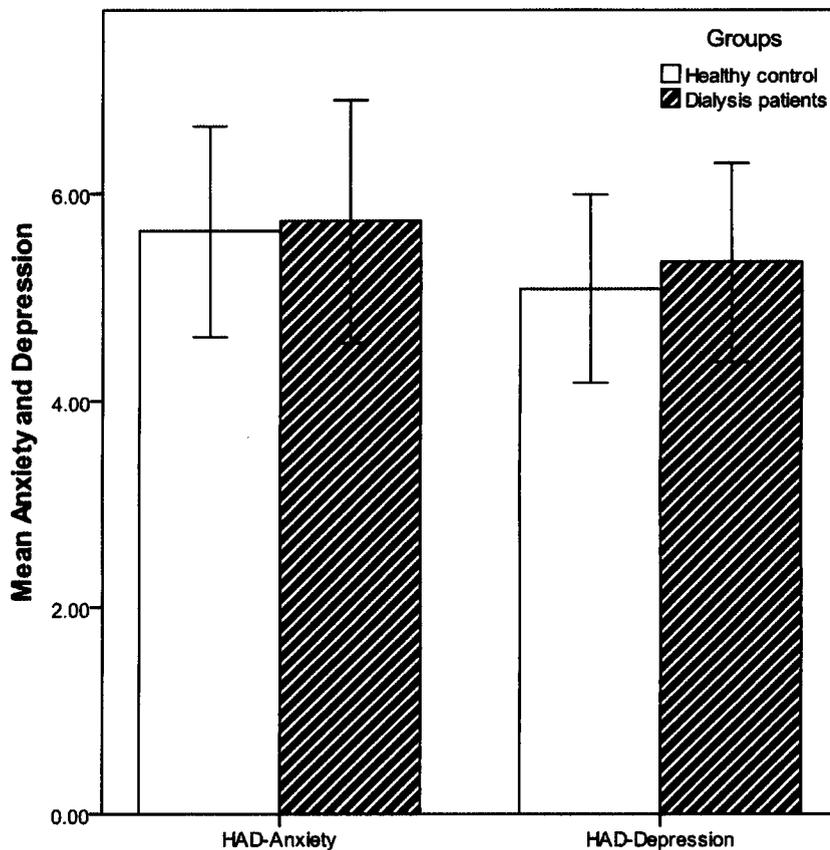


Figure 5.5 Mean \pm 95% CI of anxiety and depression scores of HAD Scale of dialysis patients and healthy controls.

5.4.3 Neuropsychological tests comparison between RAA group and non-RAA group of dialysis patients

5.4.3.1 Neurocognitive tests comparison

The RAA group showed similar performance to the non-RAA group in most of the neurocognitive tests except in RAVLT as shown in figure 5.6 below. The results of RAVLT showed significantly better learning skills in the non-RAA group compared to the RAA group ($t(51) = -2.1, p = 0.045$). However, immediate & short-term memory were similar among the two groups.

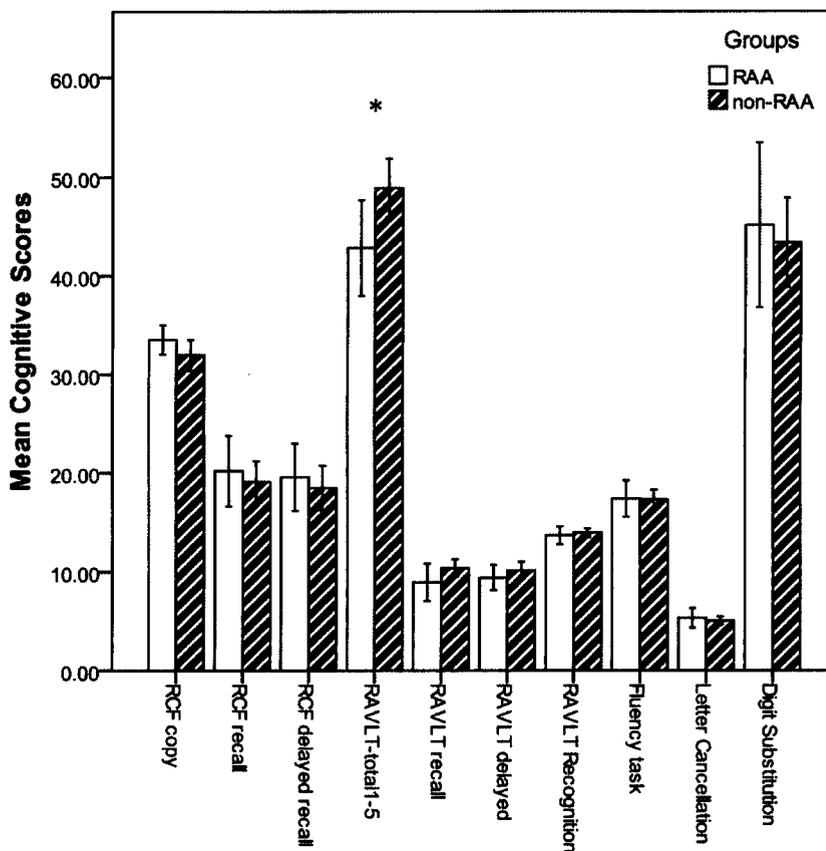


Figure 5.6 Mean \pm 95% CI of neurocognitive tests scores for RAA and non-RAA dialysis patients. * represents significant difference ($p < .05$).

Since a repeated measure has been undertaken in both RAVLT and RCF tasks, a general linear model with repeated measure analysis was undertaken to reduce the likelihood of type I error and to replicate the previous finding of simple t-test comparison as shown below in figures 5.7 and 5.8.

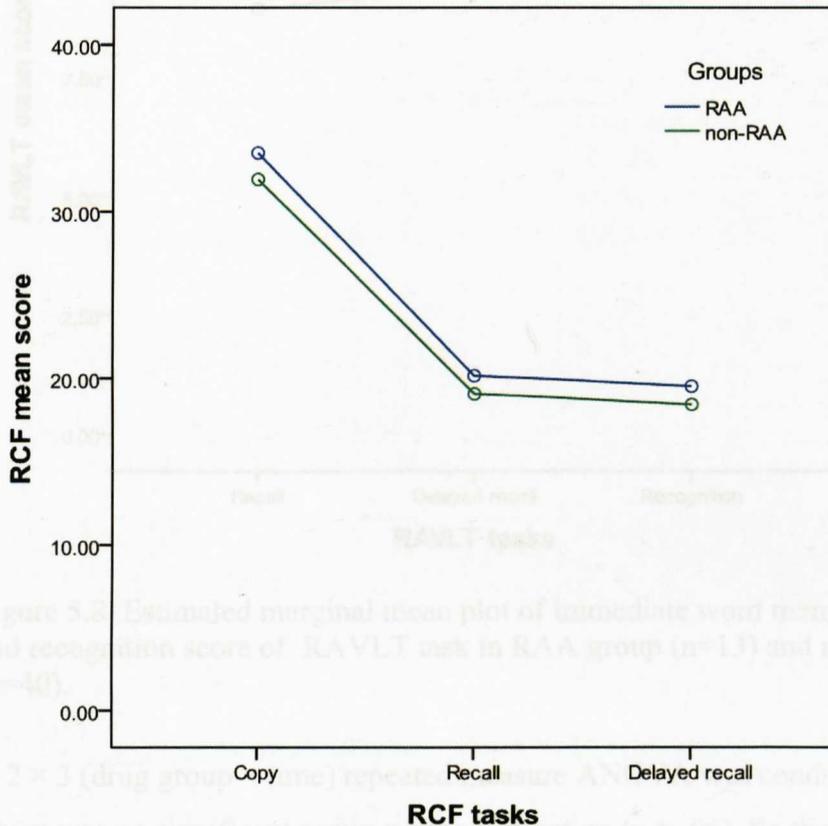


Figure 5.7 Estimated marginal mean plot of copy, immediate recall, and delayed recall scores of RCF task in RAA group (n=13) and non-RAA group (n=40).

A 2×3 (group \times time) repeated measure ANOVA was conducted on RCF. There was no significant group \times time interaction ($p > .05$). This result documents the previous result of RCF task when simple t-test comparison has been undertaken, i.e. no significant differences between RAA and non-RAA groups. However, there was a significant main effect of time on task performance ($F(2,52) = 208.04, p = .001$). Planned contrast revealed that the significant difference was between copy task and immediate both immediate and delayed recall ($F(2,52) = 317.69, p = .001$)

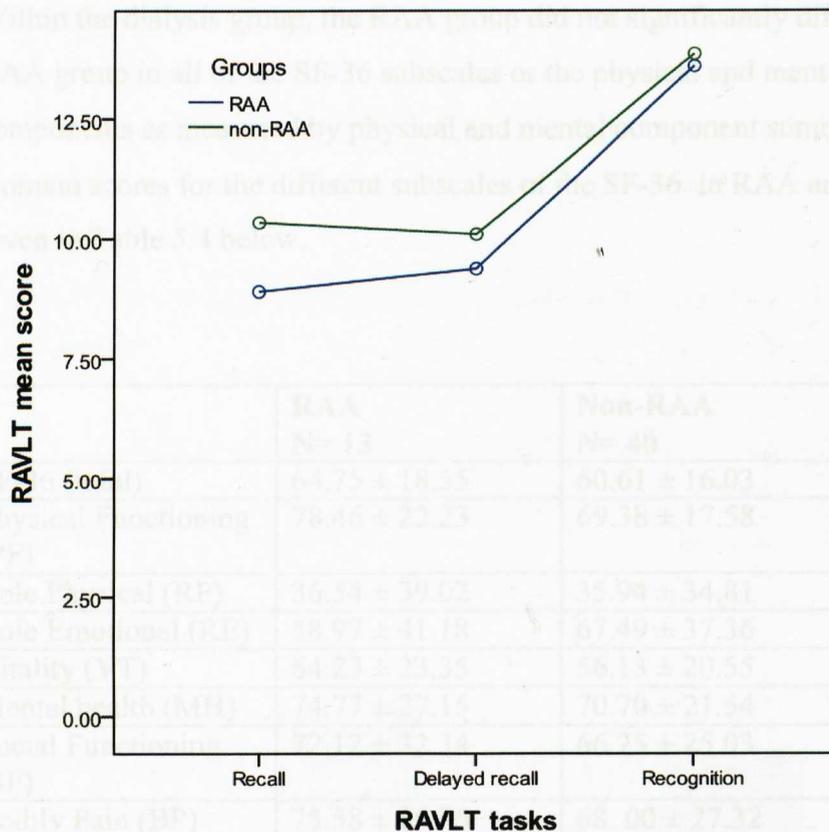


Figure 5.8 Estimated marginal mean plot of immediate word memory, delayed recall, and recognition score of RAVLT task in RAA group (n=13) and non-RAA group (n=40).

A 2 × 3 (drug group × time) repeated measure ANOVA was conducted on RAVLT. There was no significant group × time interaction ($p > .05$). So the two groups performed similarly. However, there was a significant effect of time on task performance ($F(2,52) = 111.89, p = .001$). Planned contrast revealed that the significant difference was between delayed recall and recognition task in both groups ($F(2,52) = 174.98, p = .001$). This result confirms the previous finding of the simple t-test comparison.

5.4.3.2 Quality of life:

Within the dialysis group, the RAA group did not significantly differ from the non-RAA group in all of the SF-36 subscales or the physical and mental well-being components as measured by physical and mental component summary, respectively.

Domain scores for the different subscales of the SF-36 in RAA and non-RAA group are given in Table 5.4 below.

	RAA N= 13	Non-RAA N= 40	P
SF-36 (total)	64.75 ± 18.55	60.61 ± 16.03	> 0.05
Physical Functioning (PF)	78.46 ± 22.23	69.38 ± 17.58	> 0.05
Role Physical (RP)	36.54 ± 39.02	35.94 ± 34.81	> 0.05
Role Emotional (RE)	58.97 ± 41.18	67.49 ± 37.36	> 0.05
Vitality (VT)	64.23 ± 23.35	56.13 ± 20.55	> 0.05
Mental health (MH)	74.77 ± 27.15	70.70 ± 21.54	> 0.05
Social Functioning (SF)	72.12 ± 32.34	66.25 ± 25.03	> 0.05
Bodily Pain (BP)	75.58 ± 16.96	68.00 ± 27.32	> 0.05
General Health (GH)	57.31 ± 13.33	51.00 ± 17.03	> 0.05
Reported Health Perception (HP)	57.69 ± 32.89	65.63 ± 29.79	> 0.05
Physical component score (PCS)	61.97 ± 19.1	56.08 ± 16.2	> 0.05
Mental component score (MCS)	67.52 ± 25.53	65.14 ± 19.91	> 0.05

Table 5.4 Mean ± SD of quality of life subscales of both RAA and non-RAA group. P represents statistical significant of observed differences.

5.4.3.3 HAD-anxiety and depression

None of the scores of either anxiety or depression reached level of casesness (see section 2.2.3) which is usually equal or above 11 (Zigmond *et al.*, 1983; Mystakidou *et al.*, 2005). Anxiety scores (4.85 vs. 6.03) and depression scores (4.69 vs. 5.55) for RAA and non-RAA, respectively. Both RAA and non-RAA were scored similar on anxiety and depression.

Figure 5.9 below represents mean anxiety and depression score for both RAA and non-RAA group.

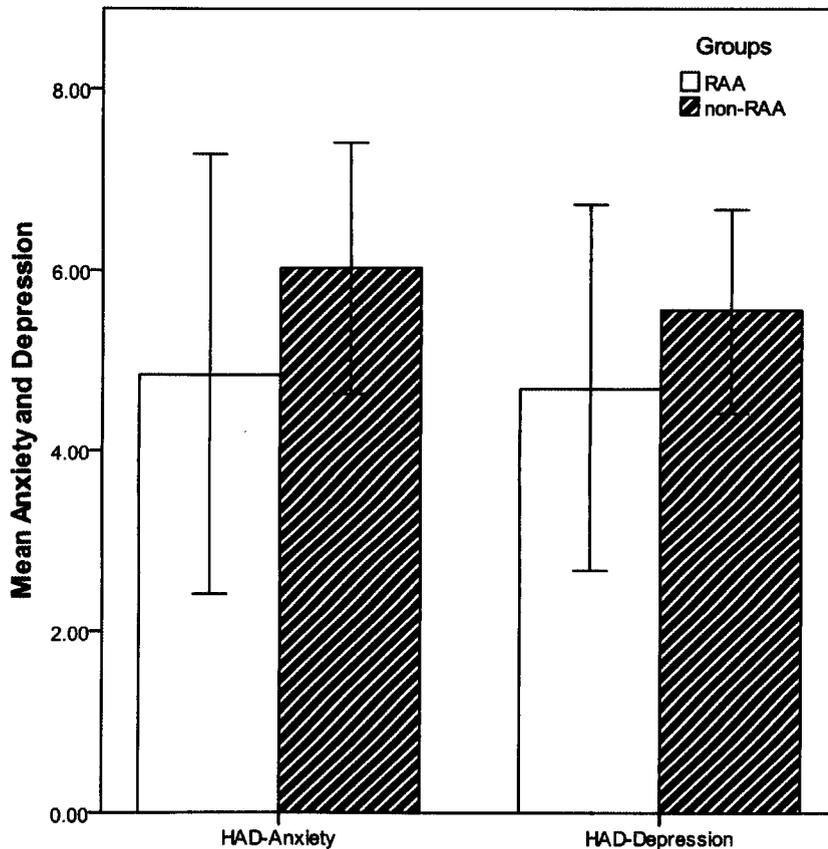


Figure 5.9 Mean \pm 95% CI of anxiety & depression scores for both RAA and non-RAA groups.

5.4.4 Genotyping

5.4.4.1 Hardy-Weinberg equilibrium

Any population genotype should follow the rule of Hardy-Weinberg to make proper correlation between polymorphism and any disorder. The genotype distribution of control population should follow the equation $p^2 + 2pq + q^2 = 1$, where p is the frequency of the A allele, q is the frequency of the a allele, and $2pq$ is the frequency of Aa heterozygotes (Stark, 1976). Adherence to Hardy-Weinberg indicates that the population is not subject to specific distorting influences, such as non-random mating. The genotype distribution in the tested population is compared with the genotype distribution of the control population. A correlation of any genotype with certain disorder will be made if an excess of such genotype in the tested population is attained. Since AT₂R is an X-linked gene and males cannot have the heterozygote genotypes, Hardy-Weinberg equilibrium satisfaction was not calculated for male gender. Regarding this study an agreement with Hardy-Weinberg equilibrium was attained in all tested groups (healthy control and dialysis patients) with all tested genes: ACE I/D, AT₁R A1166C, AT₂R C3123A, AGT M268T, and AGT T207M except AT₁R and AT₂R genotypes in dialysis patients as shown below in table 5.5 and 5.6.

	Observed Genotype N (%)	Observed Allele Frequency	Expected Genotype N (%)	Chi-square	P	DF
ACE I/D						
DD	21 (50)	I: 0.27	22 (0.53)	0.79	> 0.05	1
II	2 (4.8)	D: 0.73	3 (0.07)			
ID	19 (45.2)		17 (0.40)			
All	42 (100)		42 (100)			
AT ₁ R (A1166C)						
AA	32 (76.2)	A: 0.86	30 (0.73)	2.07	> 0.05	1
CC	2 (4.8)	C: 0.14	1 (0.02)			
AC	8 (19)		11 (0.25)			
All	42 (100)		42 (100)			
AT ₂ R (C3123A)						
Female/Male	0/21 (50)	C: 0.5	11 (0.26)	-	-	-
CC/C-	0/21 (50)	A: 0.5	10 (0.24)			
AA/A-	0/0		21 (0.5)			
CA	42 (100)		42 (100)			
All						
AGT (T207M)						
TT	35 (83.3)	T: 0.92	35 (0.84)	0.35	> 0.05	1
MM	7 (16.7)	M: .08	7 (0.15)			
TM	0		0 (0.007)			
All	42(100)		42 (100)			
AGT (M268T)						
MM	5 (11.9)	M: 0.37	6 (0.14)	0.23	> 0.05	1
TT	16 (40)	T: 0.63	16 (0.39)			
MT	21 (50)		20 (0.47)			
All	42 (100)		42 (100)			

Table 5.5 ACE (I/D), AT₁R (A1166C), AT₂R (C3123A), AGT (T207M), and AGT (M268T) genotype distribution and allele frequency in Saudi normal population compared to Hardy-Weinberg Equilibrium.

A p value of < 0.05 is considered statistically significant.

DF: degrees of freedom.

	Observed Genotype N (%)	Observed Allele Frequency	Expected Genotype N (%)	Chi-square	P	DF
ACE I/D						
DD	28 (52.8)	I: 0.28	27 (51)	0.26	> 0.05	1
II	5 (9.4)	D: 0.72	4 (8)			
ID	20 (37.7)		22 (41)			
All	53 (100)		53 (100)			
AT ₁ R (A1166C)						
AA	42 (79.2)	A: 0.87	40 (75)	6.19	< 0.05	1
CC	3 (5.7)	C: 0.13	1 (2)			
AC	8 (15.1)		12 (23)			
All	53 (100)		53 (100)			
AT ₂ R (C3123A)						
Female/Male	8/14	C: 0.44	11 (20)	9.78	< 0.05	1
CC/C-	(41.5)	A: 0.56	16 (30)			
AA/A-	9/19		26 (50)			
CA	(52.8)		53 (100)			
All	3/0 (5.7)		53 (100)			
AGT (T207M)						
TT	45 (84.9)	T: 0.92	45 (85)	0.35	> 0.05	1
TM	8 (15.1)	M: .08	7 (13)			
MM	0		1 (2)			
All	53 (100)		53 (100)			
AGT (M268T)						
MM	3 (5.7)	M: 0.32	6 (11)	2.39	> 0.05	1
TT	22 (41.5)	T: 0.68	24 (45)			
MT	28 (52.8)		23 (44)			
All	53 (100)		53 (100)			

Table 5.6 ACE (I/D), AT₁R (A1166C), AT₂R (C3123A), AGT (T207M), and AGT (M268T) genotype distribution and allele frequency in Saudi dialysis patients compared to Hardy-Weinberg Equilibrium.

A P value of < 0.05 is considered statistically significant.

DF: degrees of freedom

5.4.4.2 RAS gene polymorphisms distribution

RAS genotypes frequencies of AT₁R A1166C, AT₂R C3123A, ACE I/D, AGT M268T, and AGT T207M polymorphisms are illustrated in table 5.7 below for the RAA and non-RAA groups as well as the dialysis group and healthy controls. There were no significant differences in genotypes frequencies between patients and control or between RAA and non-RAA.

	Control (42) N (%)	Patients (53) N (%)	P	RAA (13) N (%)	Non-RAA (40) N (%)	P
ACE I/D						
DD	21 (50)	28 (52.8)	> 0.05	9 (69.2)	19 (47.5)	> 0.05
II	2 (4.8)	5 (9.4)		1 (7.7)	4 (10)	
ID	19 (45.2)	20 (37.7)		3 (23.1)	17 (42.5)	
AT ₁ R (A1166C)						
AA	32 (76.2)	42 (79.2)	> 0.05	9 (69.2)	33 (82.5)	> 0.05
AA	2 (4.8)	3 (5.7)		1 (7.7)	2 (5)	
CC	8 (19)	8 (15.1)		3 (23.1)	5 (12.5)	
AC						
AT ₂ R (C3123A) Female/Male						
CC/C-	0/21 (50)	8/14(41.5)	> 0.05	1/4 (38.5)	7/10 (42.5)	> 0.05
AA/A-	0/21 (50)	9/19(52.8)		1/6 (53.8)	8/13 (52.5)	
CA	0/0	3/0 (5.7)		1/0 (7.7)	2/0 (5)	
AGT (T207M)						
TT	35 (83.3)	45 (84.9)	> 0.05	11 (84.6)	34 (85)	> 0.05
TM	7 (16.7)	8 (15.1)		2 (15.4)	6 (15)	
MM	0	0		0	0	
AGT (M268T)						
MM	5 (11.9)	3 (5.7)	> 0.05	1 (7.7)	2 (5)	> 0.05
TT	16 (40)	22 (41.5)		4 (30.8)	18 (50)	
MT	21 (50)	28 (52.8)		8 (61.5)	20 (45)	

Table 5.7 Distribution of RAS genotypes among dialysis patients, controls, RAA, and non-RAA groups.

P represents statistical significant of observed differences from control group.

RAS allele frequencies of AT₁R A1166C, AT₂R C3123A, ACE I/D, AGT M268T, and AGT T207M polymorphisms are illustrated in table 5.8 below for the RAA and non-RAA groups as well as the dialysis group and healthy controls. There were no significant differences in allelic frequencies between patients and control or between RAA and non-RAA.

	Controls	Patients	P	RAA	non-RAA	P
ACE						
D	61 (72.6)	76 (71.7)	> 0.05	21 (80.8)	55 (68.8)	> 0.05
I	23 (27.4)	30 (28.3)		5 (19.2)	25 (31.3)	
AT ₁ R						
A	72 (85.7)	92 (86.8)	> 0.05	21 (80.8)	71 (88.8)	> 0.05
C	12 (14.3)	14 (13.2)		5 (19.2)	9 (11.3)	
AT ₂ R						
C	42 (50)	47 (44.3)	> 0.05	11 (42.3)	36 (45)	> 0.05
A	42 (50)	59 (55.7)		15 (57.7)	44 (55)	
AGT (T207M)						
T	77 (91.7)	98 (92.5)	> 0.05	24 (92.3)	74 (92.5)	> 0.05
M	7 (8.3)	8 (7.5)		2 (7.7)	6 (7.5)	
AGT (M268T)						
M	31 (36.9)	34 (32.1)	> 0.05	10 (38.5)	24 (30)	> 0.05
T	53 (63.1)	72 (67.9)		16 (61.5)	56 (70)	

Table 5.8 Distribution of RAS alleles (%) among healthy controls, dialysis patients, RAA, and non-RAA groups.

P represents statistical significant of observed differences.

5.4.5 Linear regressions

A series of linear regression analyses was conducted to evaluate the unique contribution of genetic polymorphism, demographic, behavioural variables (age, gender, level of education, smoking), and clinical variables (antihypertensive therapy, diabetes, dialysis duration) to neuropsychological test scores (cognition, depression, anxiety) and QOL including mental component summary (MCS) and physical component summary (PCS). In the first part of linear regression analysis, RAS genotypes were included in the analysis along with other variables. A second linear regression analysis was conducted on RAS alleles and other variables. This means that the linear regression analysis was carried out twice.

5.4.5.1 Linear regression analysis of neuropsychological tests, QOL, anxiety and depression with RAS genotypes

5.4.5.1.1 Memory

Rey Auditory-Verbal Learning Test (RAVLT)

When linear regression was applied to RAVLT, both learning and memory task, diabetes mellitus (DM), gender, age, drug group, AT₂R genotype, AGT T207M and AGT M268T genotype were found to make significant contribution to the model of learning and of memory tasks as shown in tables 5.9, 5.10, 5.11, 5.12 below

1. Learning

	B	SE B	Beta	P
Step 1				
Constant	49.49	1.36		
DM	- 9.24	2.86	- .412	.002
Step 2				
Constant	42.19	3.66		
DM	-8.39	2.79	- .374	.004
Gender	5.16	2.41	.267	.037
Step 3				
Constant	48.34	4.53		
DM	- 9.10	2.71	- .404	.002
Gender	5.81	2.35	.300	.017
AT ₂ R	- 4.20	1.94	- .263	.035

Table 5.9 RAVLT report of multiple regression. Note $R^2 = .170$ for step 1; $\Delta R^2 = .07$ for step 2. $\Delta R^2 = .07$ for step 3. P represents statistical significant of observed differences.

- The B value indicates the relationship between RAVLT and each predictor. A positive value indicates a positive relationship between the predictor and the outcome whereas a negative coefficient represents a negative relationship.

- The SE B is the standard error of B value.

- The standardized beta value represents the number of standard deviations that the outcome (RAVLT) will change as a result of one standard deviation change in the predictor (DM and gender).
- R^2 represents a measure of how much the variability in the outcome (RAVLT) is accounted for by the predictor (DM in step 1). ΔR^2 represents a measure of how much the variability in the outcome (RAVLT) is accounted for by the second predictor (gender in step 2) and the third predictor (AT₂R in step 3).

The boxplots of observed RAVLT learning tasks for DM, gender and AT₂R are illustrated below in figures 5.10, 5.11, 5.12, respectively. The results indicate that patients without DM had significantly better learning. It also indicates that female patients performed better on the learning task and the patients carrying the CA genotype performed better on learning task.

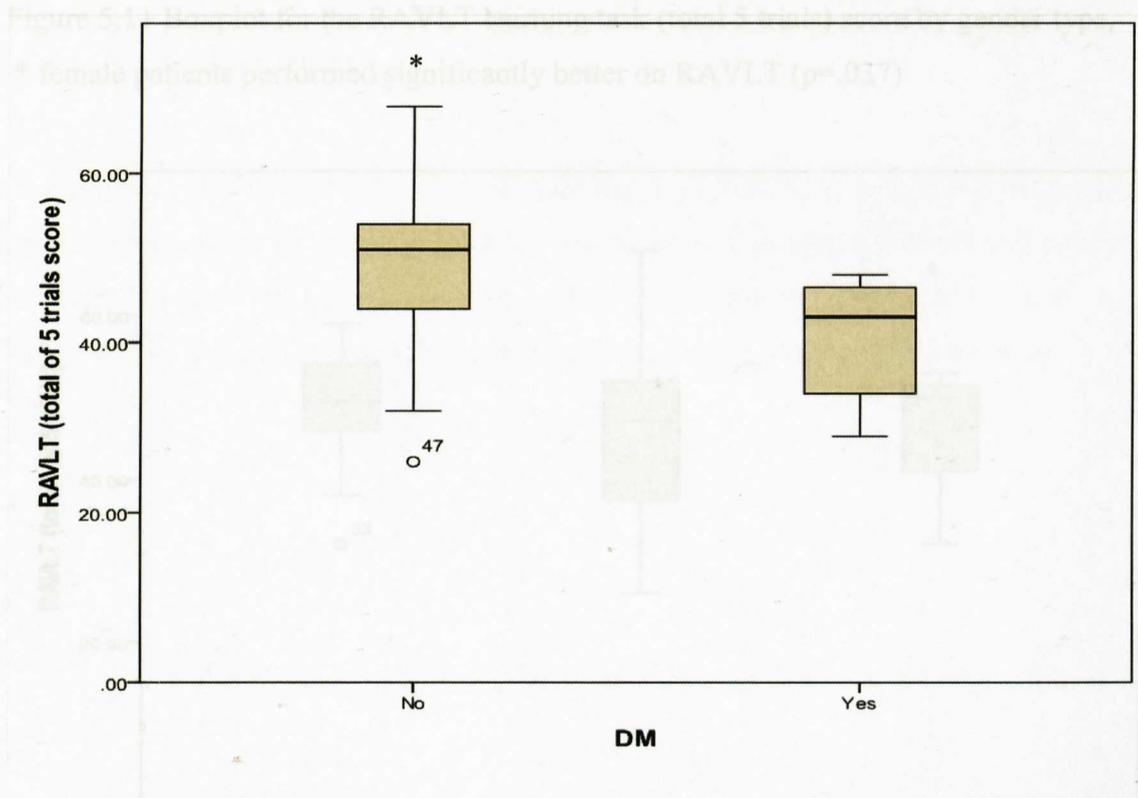


Figure 5.10 Boxplot for the RAVLT learning(total of 5 trials) task score by DM group. * patients without DM performed significantly better on RAVLT ($p=.002$)

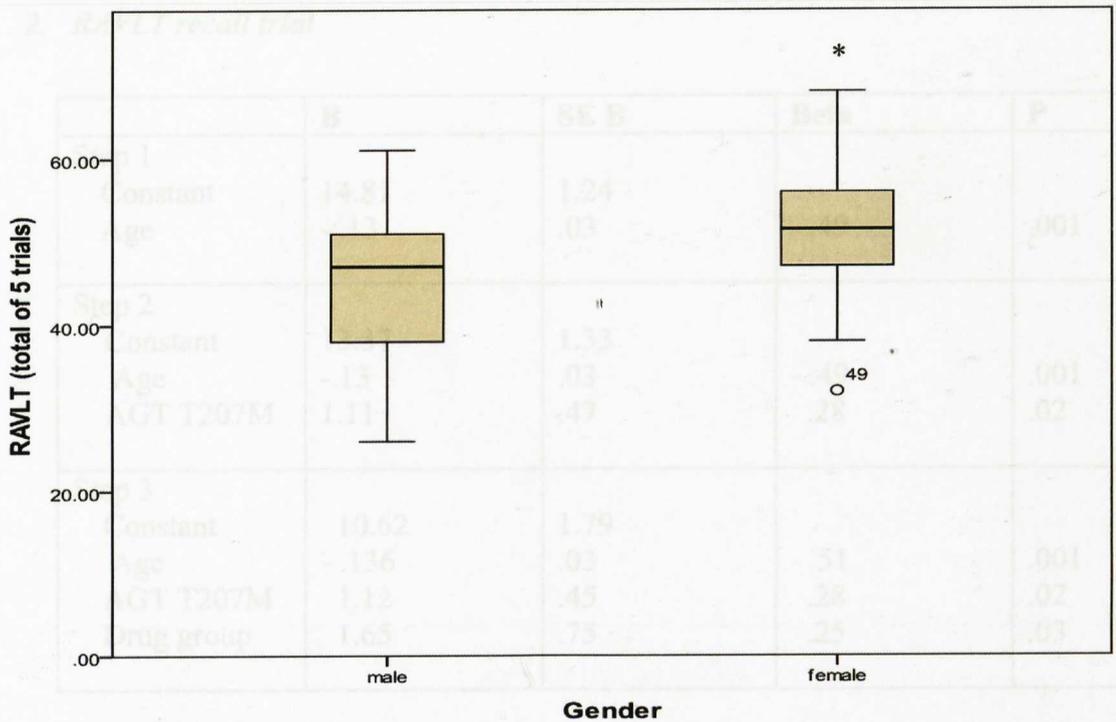


Figure 5.11 Boxplot for the RAVLT learning task (total 5 trials) score by gender type.

* female patients performed significantly better on RAVLT ($p=.037$)

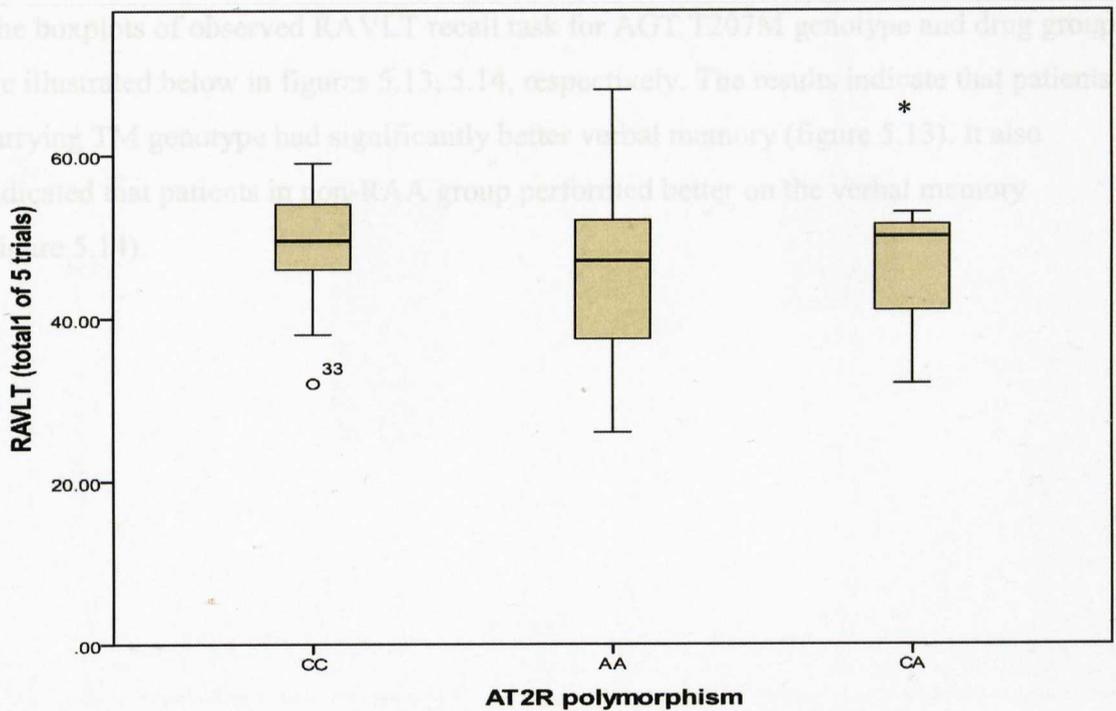


Figure 5.12 Boxplot for the RAVLT learning task (total 5 trials) score by AT₂R genotypes. * patients with CA genotype performed significantly better on RAVLT.

2. RAVLT recall trial

	B	SE B	Beta	P
Step 1				
Constant	14.81	1.24		
Age	-.13	.03	-.49	.001
Step 2		"		
Constant	13.37	1.33		
Age	-.13	.03	-.49	.001
AGT T207M	1.11	.47	.28	.02
Step 3				
Constant	10.62	1.79		
Age	-.136	.03	-.51	.001
AGT T207M	1.12	.45	.28	.02
Drug group	1.65	.75	.25	.03

Table 5.10 RAVLT recall report of multiple regression. Note $R^2 = .24$ for step 1; $\Delta R^2 = .08$ for step 2. $\Delta R^2 = .06$ for step 3. P represents statistical significant of observed differences

The boxplots of observed RAVLT recall task for AGT T207M genotype and drug group are illustrated below in figures 5.13, 5.14, respectively. The results indicate that patients carrying TM genotype had significantly better verbal memory (figure 5.13). It also indicated that patients in non-RAA group performed better on the verbal memory (figure 5.14).

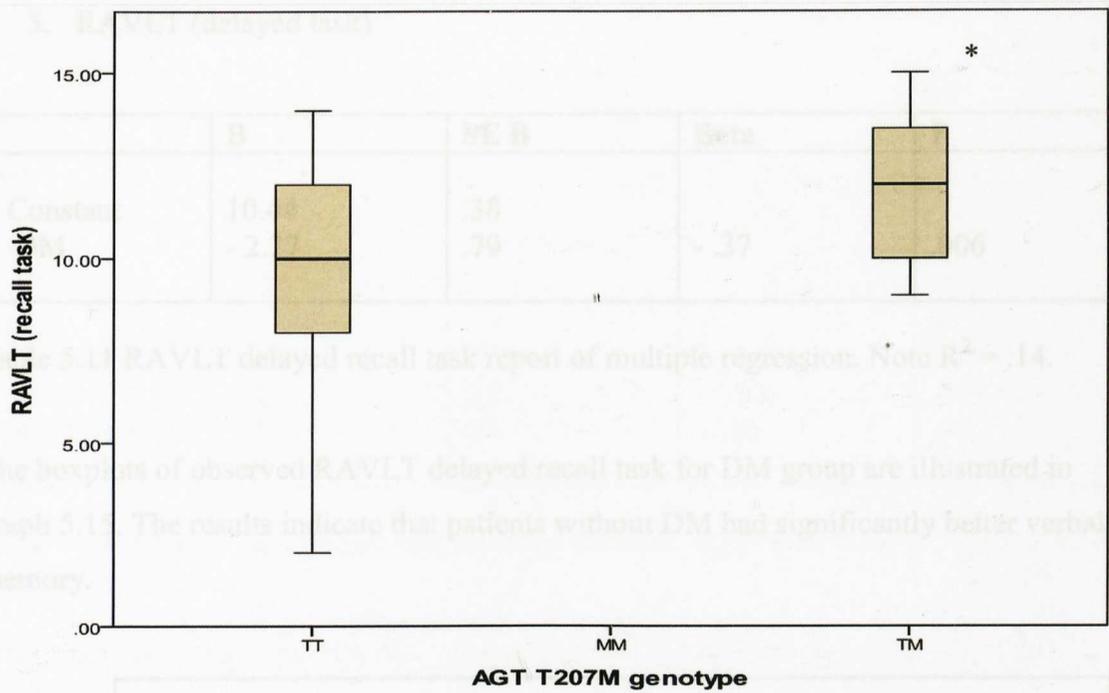


Figure 5.13 Boxplot for the RAVLT recall task score by AGT T207M genotypes. * patients with TM genotype performed significantly better on RAVLT recall trial.

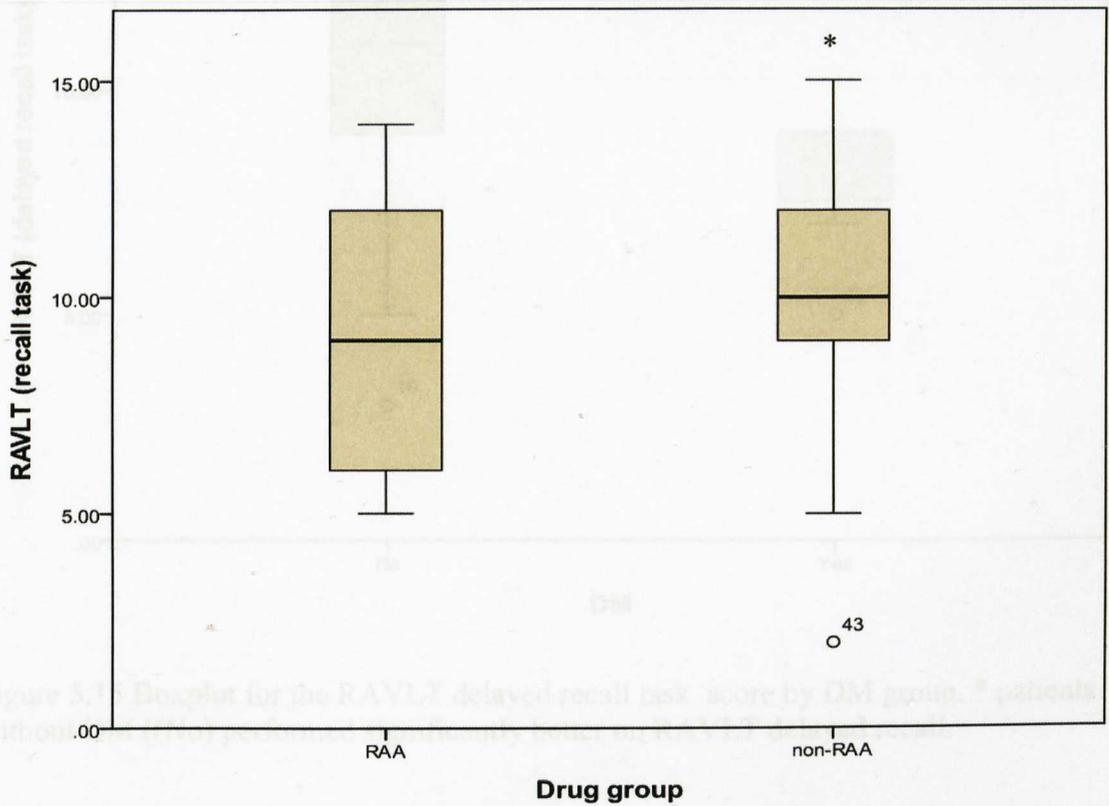


Figure 5.14 Boxplot for the RAVLT recall task score by drug group. * patients in non-RAA group performed significantly better on RAVLT recall trial.

3. RAVLT (delayed task)

	B	SE B	Beta	P
Constant	10.44	.38		
DM	- 2.27	.79	- .37	.006

Table 5.11 RAVLT delayed recall task report of multiple regression. Note $R^2 = .14$.

The boxplots of observed RAVLT delayed recall task for DM group are illustrated in graph 5.15. The results indicate that patients without DM had significantly better verbal memory.

The boxplots of observed RAVLT recognition task for DM group and AGT M268T

genotype are illustrated in figure 5.16 and 5.17, respectively. The results indicate that patients without DM had significantly better recognition. It also indicates that patients carrying MM genotype of AGT M268T had significantly better recognition.

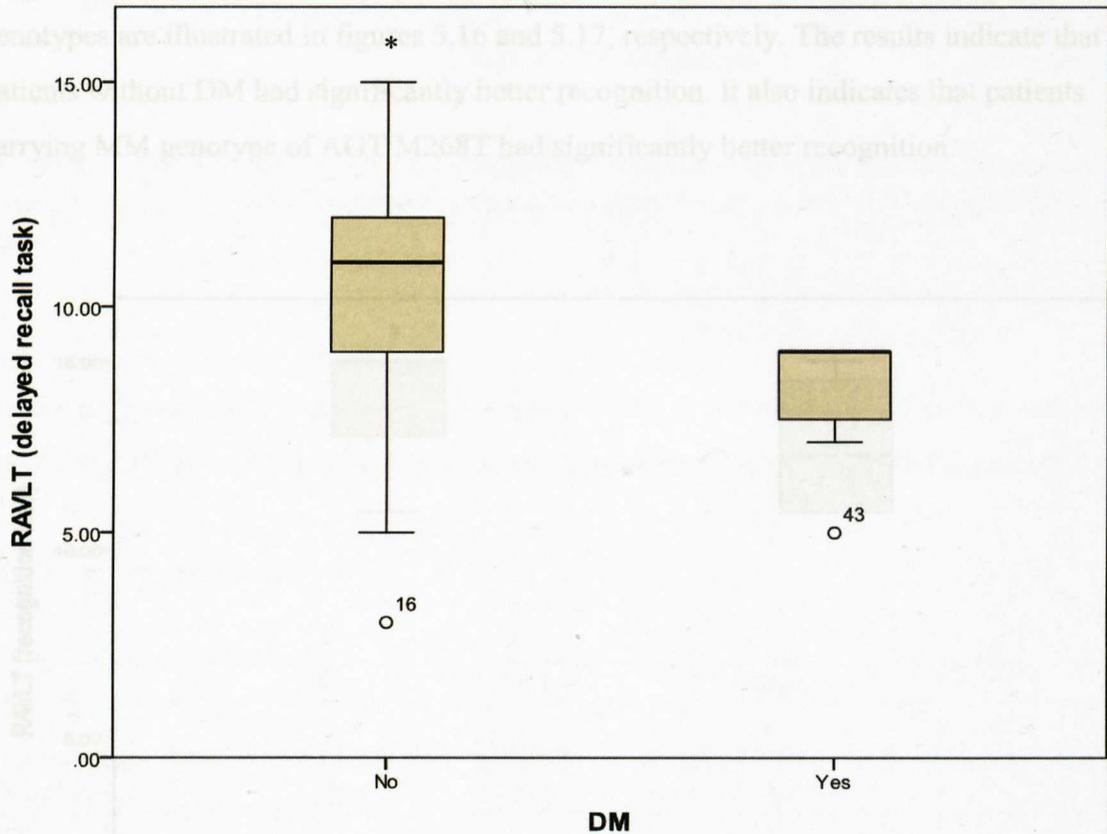


Figure 5.15 Boxplot for the RAVLT delayed recall task score by DM group. * patients without DM ((No) performed significantly better on RAVLT delayed recall.

Figure 5.16 Boxplot for the RAVLT recognition task score by DM group. * patients without DM ((No) performed significantly better on RAVLT recognition task.

4. Recognition task

	B	SE B	Beta	P
Step 1				
Constant	14.09	.20		
DM	- 1.26	.42	- .39	.004
Step 2				
Constant	15.27	.59		
DM	-1.29	.41	- .39	.003
AGT M268T	-.51	.24	- .26	.04

Table 5.12 RAVLT recognition task report of multiple regression. Note $R^2 = .15$; $\Delta R^2 = .07$ for step 2.

The boxplots of observed RAVLT recognition task for DM group and AGT M268T genotypes are illustrated in figures 5.16 and 5.17, respectively. The results indicate that patients without DM had significantly better recognition. It also indicates that patients carrying MM genotype of AGT M268T had significantly better recognition.

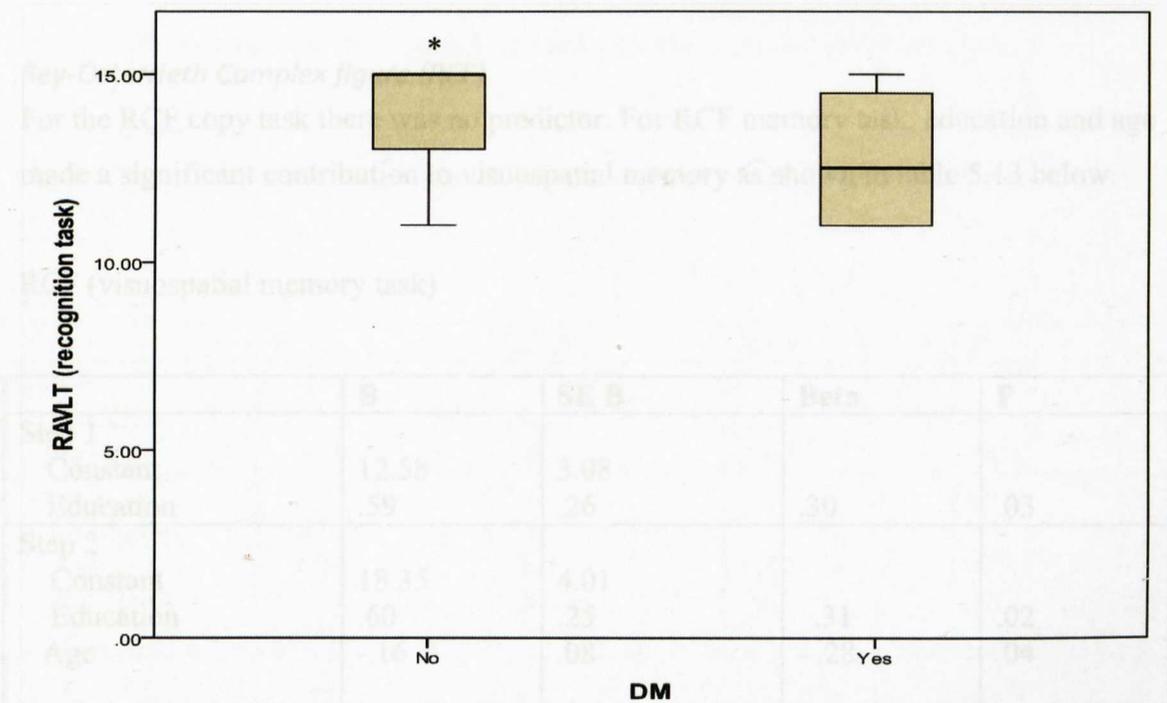


Figure 5.16 Boxplot for the RAVLT recognition task score by DM group. * patients without DM (No) performed significantly better on RAVLT recognition task.

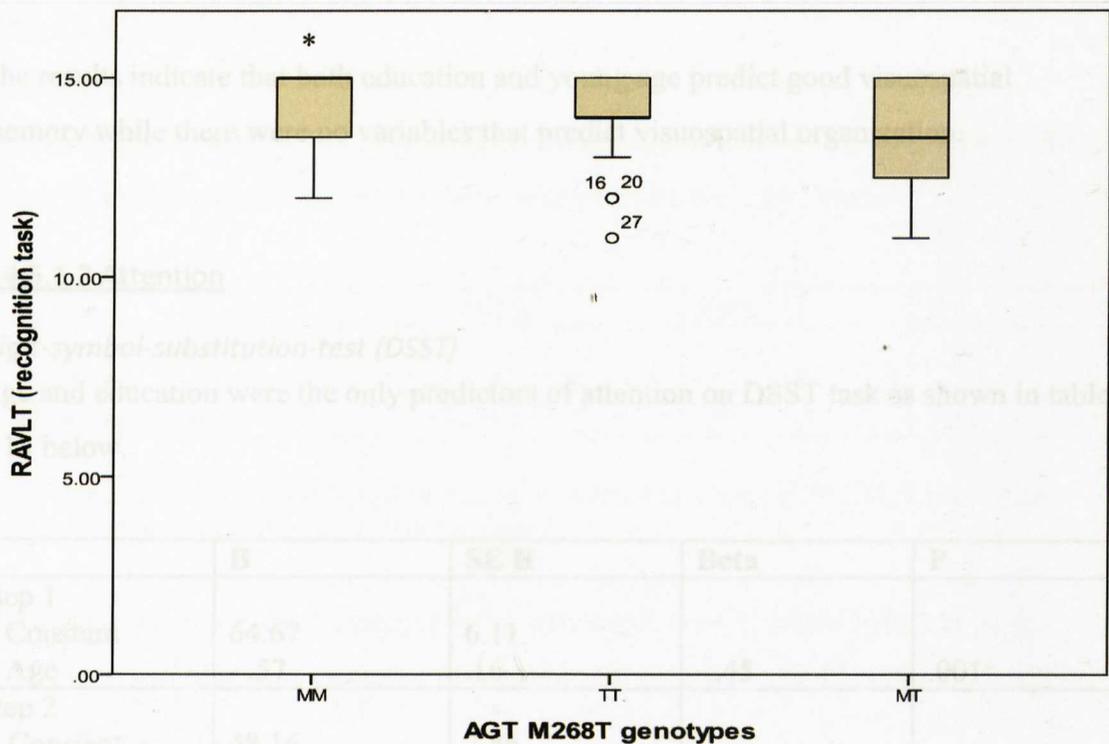


Figure 5.17 Boxplot for the RAVLT recognition task score by AGT M268T genotypes. * patients with MM genotype performed significantly better on RAVLT recognition task.

Rey-Osterrieth Complex figure (RCF)

For the RCF copy task there was no predictor. For RCF memory task, education and age made a significant contribution to visuospatial memory as shown in table 5.13 below.

RCF (visuospatial memory task)

	B	SE B	Beta	P
Step 1				
Constant	12.58	3.08		
Education	.59	.26	.30	.03
Step 2				
Constant	18.35	4.01		
Education	.60	.25	.31	.02
Age	-.16	.08	-.28	.04

Table 5.13 RCF copy report of multiple regression. Note $R^2 = .09$ for step 1; $\Delta R^2 = .08$ for step 2. P represents statistical significant of observed differences.

The results indicate that both education and young age predict good visuospatial memory while there were no variables that predict visuospatial organization.

5.4.5.1.2 Attention

Digit-symbol-substitution-test (DSST)

Age and education were the only predictors of attention on DSST task as shown in table 5.14 below.

	B	SE B	Beta	P
Step 1				
Constant	64.67	6.11		
Age	-.57	.16	-.45	.001
Step 2				
Constant	48.16	7.86		
Age	-.58	.15	-.46	.001
Education	1.48	.49	.35	.004

Table 5.14 DSST report of multiple regression. Note $R^2 = .20$ for step 1; $\Delta R^2 = .12$ for step 2. P represents statistical significant of observed differences.

The results indicate that both young age and education predict good attention.

Letter cancellation

Again age and education significantly predicted good attention as shown below in table 5.15.

	B	SE B	Beta	P
Step 1				
Constant	.72	.06		
Age	-.006	.002	-.47	.001
Step 2				
Constant	.59	.08		
Age	-.006	.001	-.47	.001
Education	.01	.005	.30	.01

Table 5.15 Letter cancellation report of multiple regression. Note $R^2 = .22$ for step 1; $\Delta R^2 = .09$ for step 2. P represents statistical significant of observed differences.

5.4.5.1.3 Executive function

Mental fluency

Results indicated that the AT₂R genotypes made a significant contribution to executive function, working and semantic memory as shown in table 5.16 below.

	B	SE B	Beta	P
Step 1				
Constant	14.75	1.2		
AT ₂ R genotypes	1.52	.69	.29	.032

Table 5.16 Mental fluency report of multiple regression. Note $R^2 = .09$. P represents statistical significant of observed differences.

The boxplot of observed fluency for AT₂R genotypes are illustrated below in figure 5.18. The result indicates that patients carrying AA genotype significantly predict good executive function.

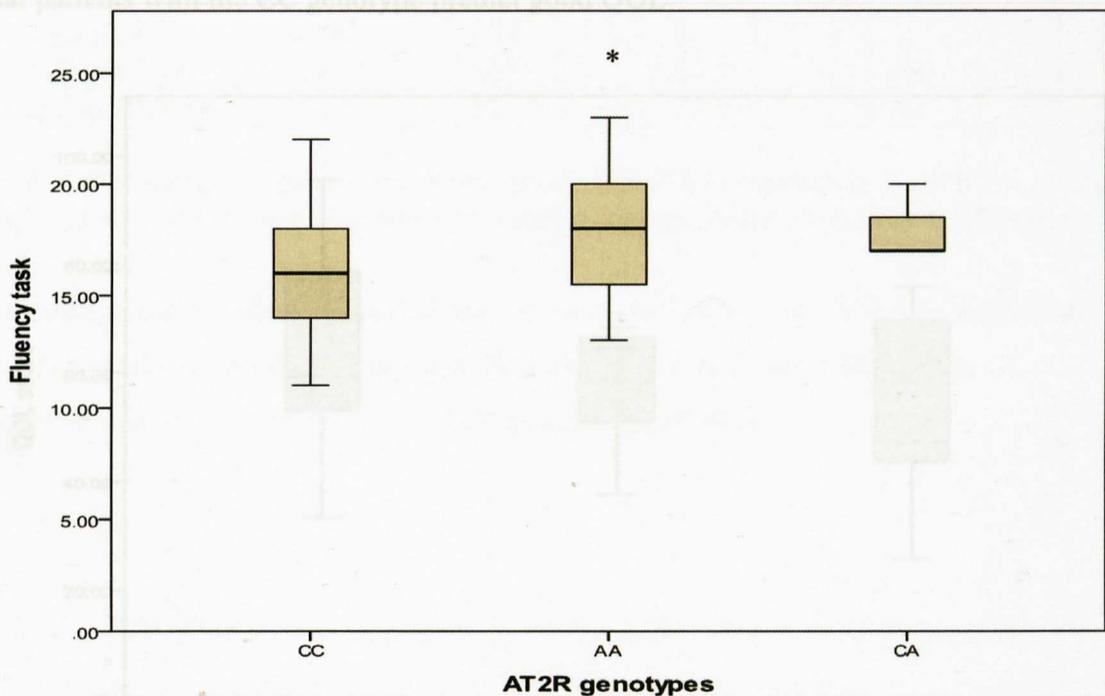


Figure 5.18 Boxplot for the fluency task score by AT₂R genotypes. * patients with AA genotype performed significantly better on mental fluency (executive function).

5.4.5.1.4 Quality of Life

Both dialysis duration and AT₁R genotypes were predictors of QOL as shown in table 5.17 below

	B	SE B	Beta	P
Step 1				
Constant	66.78	3.32		
Dialysis duration	-.88	.42	-.28	.04
Step 2				
Constant	77.96	5.57		
Dialysis duration	-1.11	.42	-.35	.01
AT ₁ R genotypes	-7.25	2.97	-.32	.02

Table 5.17 QOL report of multiple regression. Note $R^2 = .08$ for step 1; $\Delta R^2 = .09$ for step 2. P represents statistical significant of observed differences.

The results indicate that shorter dialysis duration predict good QOL. A boxplot of observed QOL for AT₁R genotypes is illustrated below in figure 5.19 which indicates that patients with the CC genotype predict good QOL.

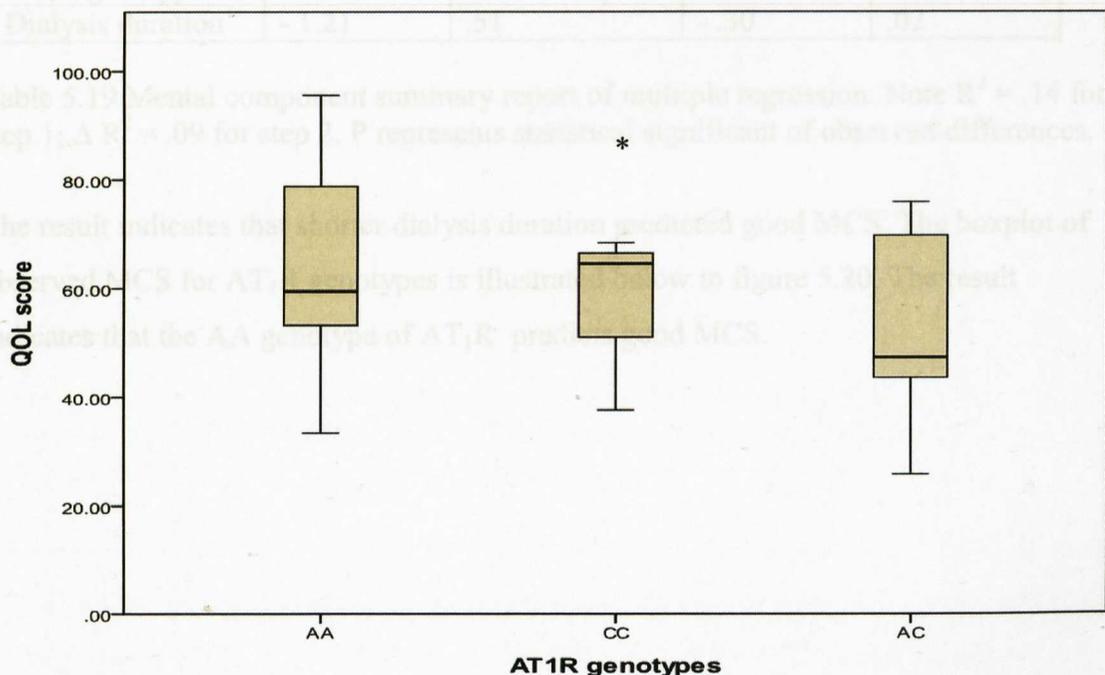


Figure 5.19 Boxplot for the QOL score by AT₁R genotypes. * patients with CC genotype have significantly better QOL.

When physical and mental component summary of QOL were analyzed separately, linear regression results indicated that dialysis duration significantly predicted physical component summary as shown in table 5.18 below. However AT₁R genotypes and dialysis duration predict mental component summary as shown in table 5.19 below.

	B	SE B	Beta	P
Step 1				
Constant	63.06	3.37		
Dialysis duration	-.95	.43	-.29	.03

Table 5.18 Physical component summary report of multiple regression. Note $R^2 = .09$. P represents statistical significant of observed differences.

	B	SE B	Beta	P
Step 1				
Constant	80.26	5.77		
AT ₁ R genotype	- 10.70	3.74	-.37	.006
Step 2				
Constant	90.00	6.90		
AT ₁ R genotype	- 12.65	3.68	-.44	.001
Dialysis duration	- 1.21	.51	-.30	.02

Table 5.19 Mental component summary report of multiple regression. Note $R^2 = .14$ for step 1; $\Delta R^2 = .09$ for step 2. P represents statistical significant of observed differences.

The result indicates that shorter dialysis duration predicted good MCS. The boxplot of observed MCS for AT₁R genotypes is illustrated below in figure 5.20. The result indicates that the AA genotype of AT₁R predicts good MCS.

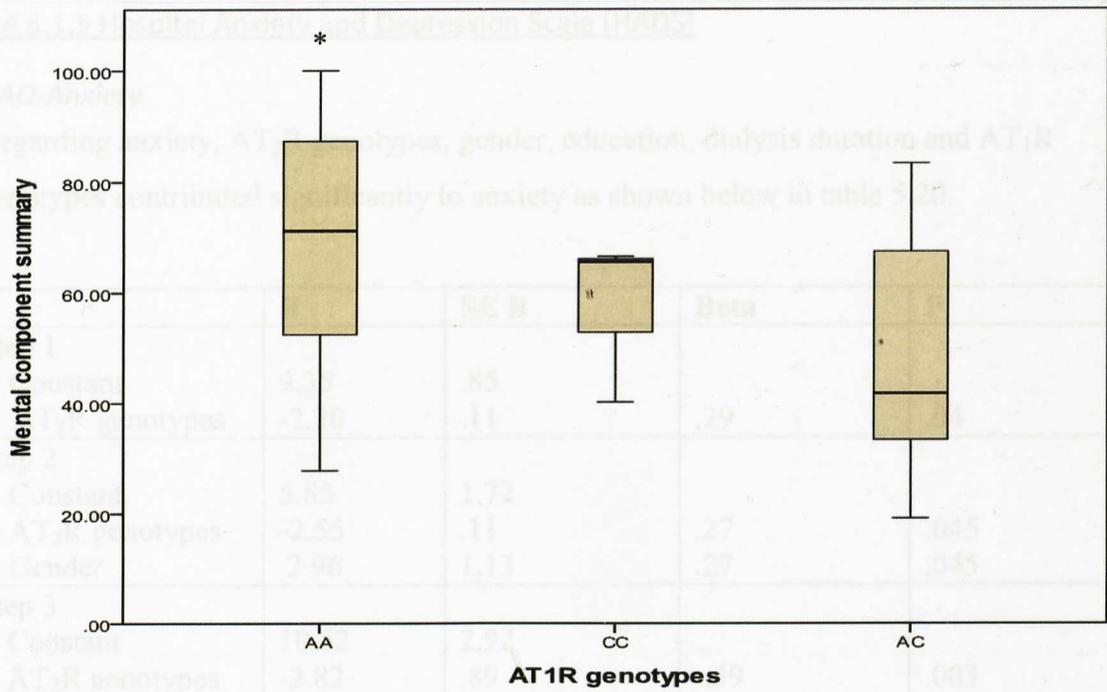


Figure 5.20 Boxplot for the MCS score by AT₁R genotypes. * patients with AA genotype have significantly better MCS.

	Beta	SE B	Beta	P
Step 1				
Constant	9.3	1.85		
AT ₁ R genotypes	-2.0	.11	.29	
Step 2				
Constant	5.85	1.72		
AT ₁ R genotypes	-2.55	.11	.27	.045
Gender	2.96	1.13	.27	.045
Step 3				
Constant	2.5	1.7		
AT ₁ R genotypes	-2.82	.85	.003	
Gender	2.75	1.07	.01	
Education	-.34	.16	.04	
Step 4				
Constant	5.92	3.08		
AT ₁ R genotypes	-2.76	.87	.002	
Gender	2.59	1.04	.02	
Education	-.32	.15	.045	
Dialysis duration	.19	.1	.048	
Step 5				
Constant	5.92	3.08		
AT ₁ R genotypes	-2.17	.86	.02	
Gender	2.46	.99	.02	
Education	-.35	.15	.03	
Dialysis duration	.25	.09	.01	
AT ₁ R genotypes	1.70	.7	.02	

Table 5.20 Anxiety report of multiple regression. Note $R^2 = .094$ for step 1; $R^2 = .11$ for step 2; $R^2 = .07$ for step 3; $R^2 = .06$ for step 4; $R^2 = .08$ for step 5. P represents statistical significant of observed differences.

A boxplot of observed anxiety for AT₂R genotypes, gender and AT₁R genotypes is illustrated below in figures 5.21, 5.22, 5.23, respectively. The results indicate that male gender and carrying the AA genotype of AT₂R and AT₁R predict lower anxiety.

5.4.5.1.5 Hospital Anxiety and Depression Scale (HADS)

HAD-Anxiety

Regarding anxiety, AT₂R genotypes, gender, education, dialysis duration and AT₁R genotypes contributed significantly to anxiety as shown below in table 5.20.

	B	SE B	Beta	P
Step 1				
Constant	9.35	.85		
AT ₂ R genotypes	-2.20	.11	.29	.04
Step 2				
Constant	5.85	1.72		
AT ₂ R genotypes	-2.55	.11	.27	.045
Gender	2.96	1.13	.27	.045
Step 3				
Constant	10.52	2.92		
AT ₂ R genotypes	-2.82	.89	-.39	.003
Gender	2.75	1.07	.32	.01
Education	-.34	.16	-.27	.04
Step 4				
Constant	9.19	2.91		
AT ₂ R genotypes	-2.76	.87	-.38	.002
Gender	2.59	1.04	.30	.02
Education	-.32	.15	-.25	.045
Dialysis duration	.19	.1	.24	.048
Step 5				
Constant	5.92	3.08		
AT ₂ R genotypes	-2.17	.86	-.30	.02
Gender	2.46	.99	.28	.02
Education	-.33	.15	-.26	.03
Dialysis duration	.25	.09	.31	.01
AT ₁ R genotypes	1.70	.7	.29	.02

Table 5.20 Anxiety report of multiple regression. Note $R^2 = .094$ for step 1; $\Delta R^2 = .11$ for step 2; $\Delta R^2 = .07$ for step 3; $\Delta R^2 = .06$ for step 4; $\Delta R^2 = .08$ for step 5. P represents statistical significant of observed differences.

A boxplot of observed anxiety for AT₂R genotypes, gender and AT₁R genotypes is illustrated below in figures 5.21, 5.22, 5.23, respectively. The results indicate that male gender and carrying the AA genotype of AT₂R and AT₁R predict lower anxiety.

Figure 5.22 Boxplot for the HAD-Anxiety score by gender type. * male patients have significantly lower anxiety ($p < .01$).

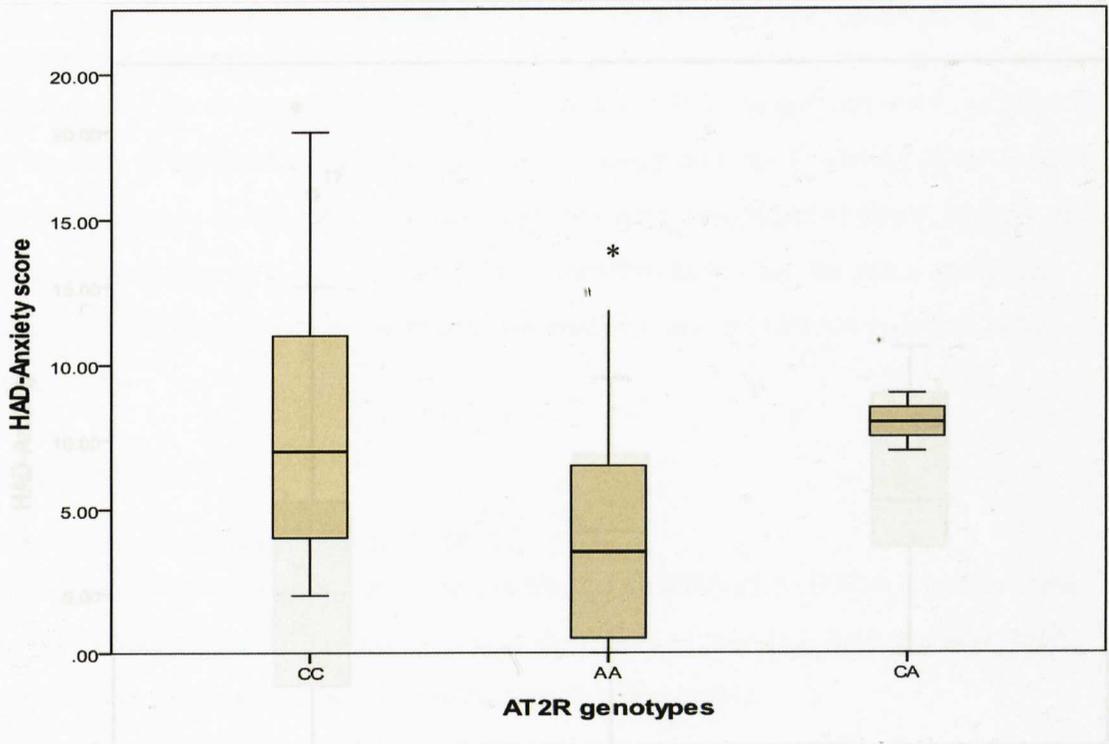


Figure 5.21 Boxplot for the HAD-Anxiety score by AT₂R genotypes. * patients with AA genotype have significantly lower anxiety (p= .04).

Figure 5.23 Boxplot for the HAD-Anxiety score by AT₁R genotypes. * patients with AA genotype have significantly lower anxiety (p= .02).

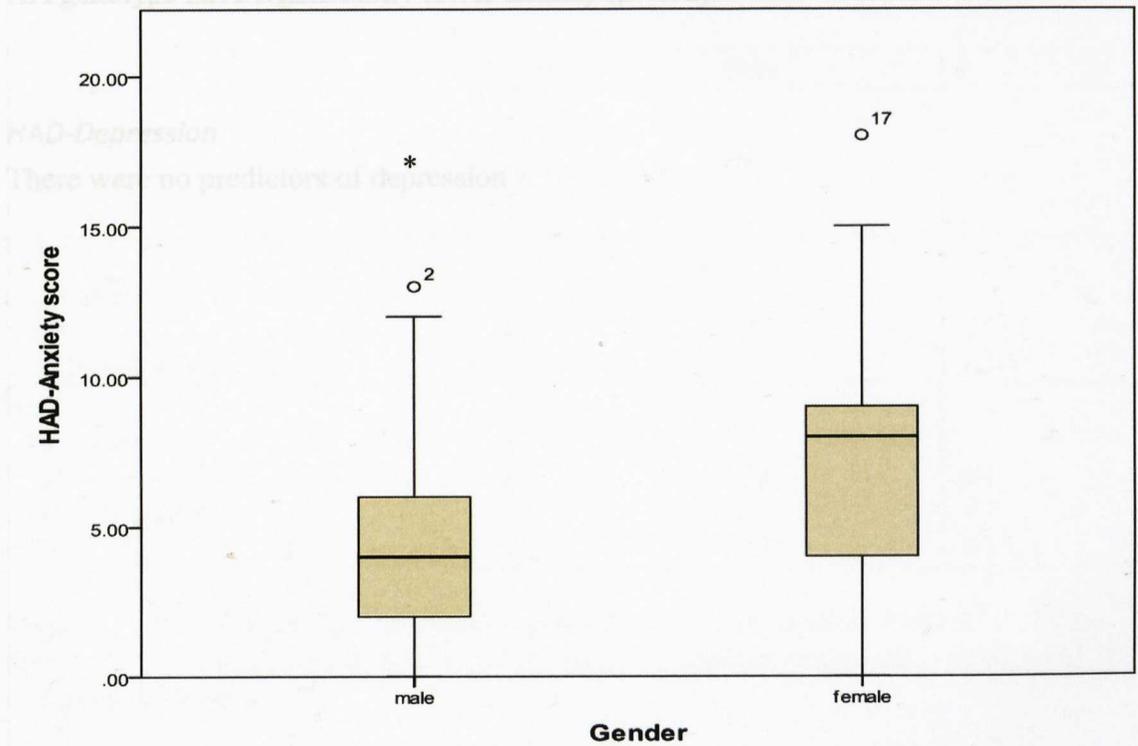


Figure 5.22 Boxplot for the HAD-Anxiety score by gender type. * male patients have significantly lower anxiety (p= .045).

5.4.5.2 Linear regression analysis of neuropsychological tests, QOL, anxiety and depression

In this part of linear regression analysis, the alleles of RAS genotypes were included in the analysis of predicted variables of neuropsychological tests. Doubling of the sample size is mandatory in this case since each patient carries two types of allele. Results of predicted variables (e.g. age, gender, DM, ... etc) that have been the same as the one reported in the first part of linear regression analysis will not be repeated here in this section.

5.4.5.2.1 Memory

Rey-Auditory-Verbal Learning Test (RAVLT)

When linear regression analysis was applied to learning task of RAVLT; DM as a gender were found to make a significant contribution to the total of learning. Age and DM were also found to make a significant contribution of memory trial.

DM was found to make a significant contribution of memory. DM, ACE ID alleles, and age were making a significant contribution of recognition task as shown in

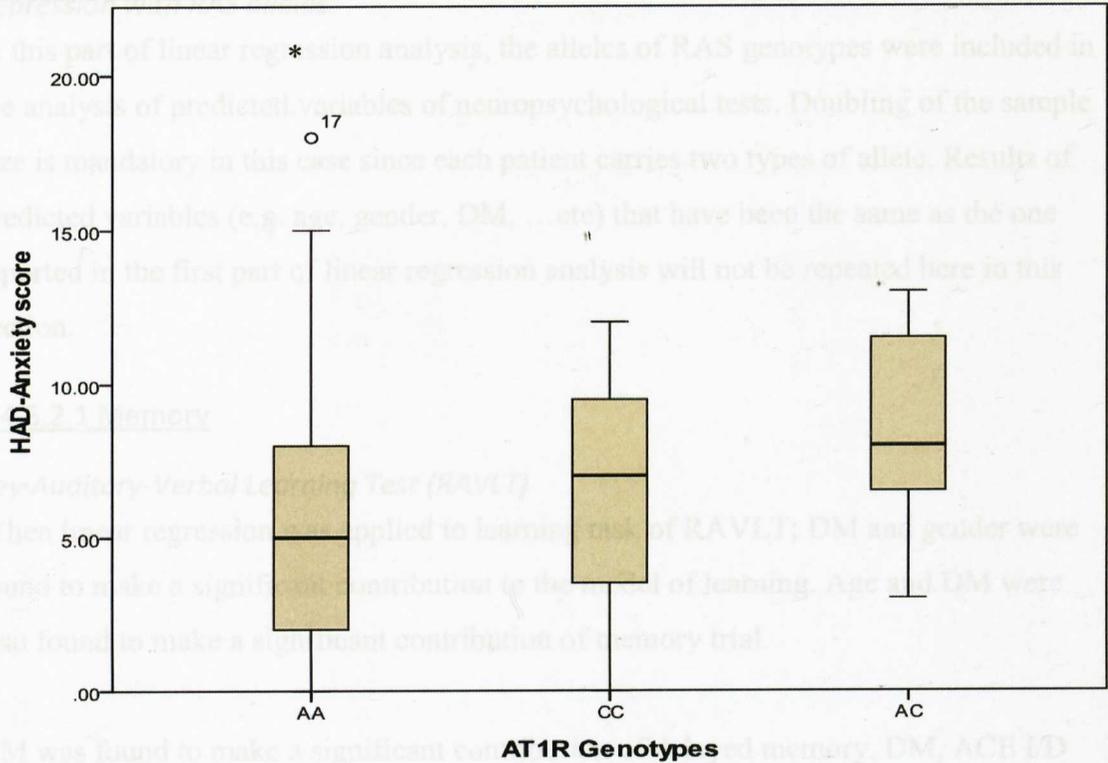


Figure 5.23 Boxplot for the HAD-Anxiety score by AT₁R genotypes. * patients with AA genotype have significantly lower anxiety (p= .02).

	B	SE B	Beta	P
HAD-Depression				
There were no predictors of depression				
Step 2				
Constant	12.64	.66		
DM	-.39	.41	-.42	.001
ACE ID allele	1.36	.59	.29	.03
Step 3				
Constant	13.77	.84		
DM	-.97	.45	-.29	.04
ACE ID allele	1.44	.57	.31	.02
Age	-.04	.02	-.28	.044

Table 5.21 RAVLT (recognition task) report of multiple regression. Note R² = .15 for step 1; ΔR² = .08 for step 2; ΔR² = .06 for step 3. P represents statistical significant of observed differences.

5.4.5.2 Linear regression analysis of neuropsychological tests, QOL, anxiety and depression with RAS alleles

In this part of linear regression analysis, the alleles of RAS genotypes were included in the analysis of predicted variables of neuropsychological tests. Doubling of the sample size is mandatory in this case since each patient carries two types of allele. Results of predicted variables (e.g. age, gender, DM, ...etc) that have been the same as the one reported in the first part of linear regression analysis will not be repeated here in this section.

5.4.5.2.1 Memory

Rey Auditory-Verbal Learning Test (RAVLT)

When linear regression was applied to learning task of RAVLT; DM and gender were found to make a significant contribution to the model of learning. Age and DM were also found to make a significant contribution of memory trial.

DM was found to make a significant contribution of delayed memory. DM, ACE I/D alleles, and age were making a significant contribution of recognition task as shown in table 5.21 below.

	B	SE B	Beta	P
Step 1				
Constant	14.09	.20		
DM	- 1.26	.42	- .39	.004
Step 2				
Constant	12.64	.66		
DM	-1.39	.41	- .42	.001
ACE I/D allele	1.36	.59	.29	.03
Step 3				
Constant	13.77	.84		
DM	-.97	.45	-.29	.04
ACE I/D alleles	1.44	.57	.31	.02
Age	-.04	.02	-.28	.044

Table 5.21 RAVLT (recognition task) report of multiple regression. Note $R^2 = .15$ for step 1; $\Delta R^2 = .08$ for step 2; $\Delta R^2 = .06$ for step 3. P represents statistical significant of observed differences.

The boxplot of observed RAVLT recognition task for ACE I/D alleles is illustrated below in figure 5.24. The results indicate that patients carrying I allele significantly predict good recognition task.

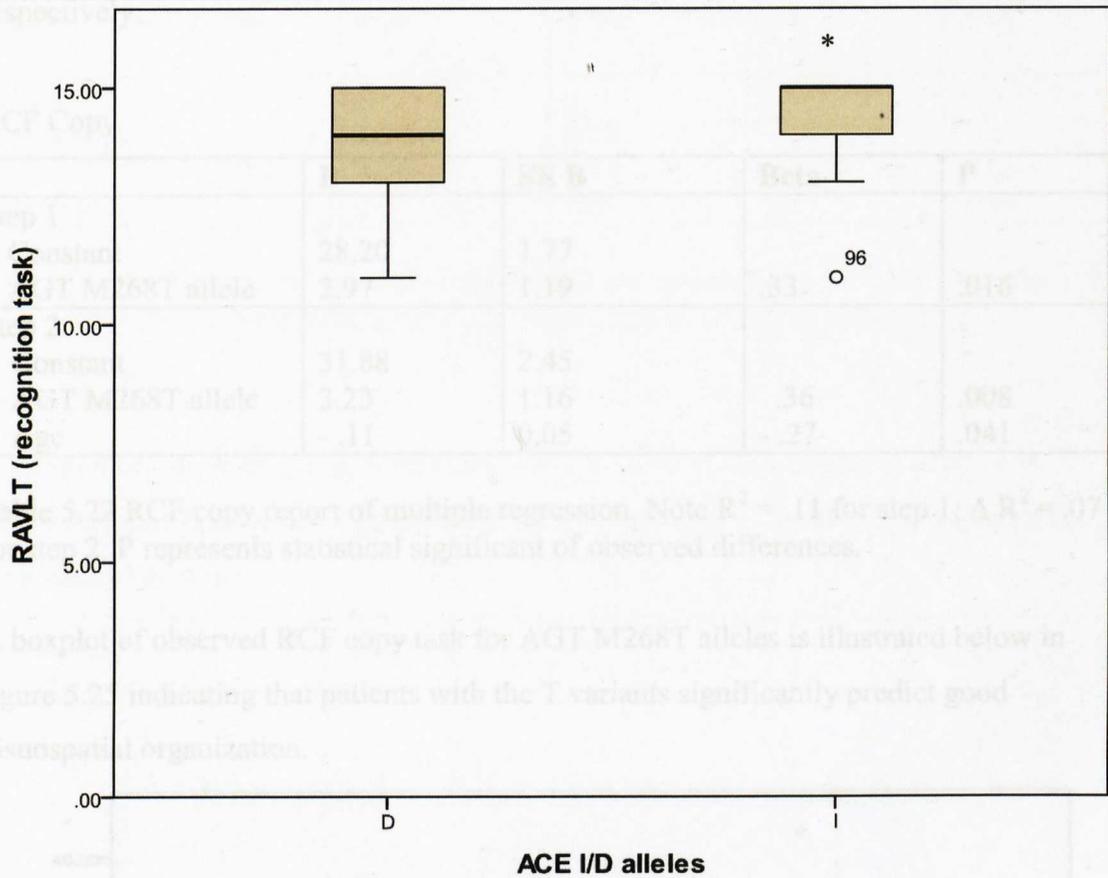


Figure 5.24 Boxplot for the RAVLT recognition task by ACE I/D alleles. * patients carrying I alleles have significantly better recognition task ($p = .02$).

Rey-Osterrieth Complex figure (RCF)

For the RCF copy task, AGT M268T alleles and age made a significant contribution to visuospatial organization. While education and AGT M268T allele made a significant contribution to visuospatial memory as shown in table 5.22 and 5.23 below, respectively.

RCF Copy

	B	SE B	Beta	P
Step 1				
Constant	28.20	1.77		
AGT M268T allele	2.97	1.19	.33	.016
Step 2				
Constant	31.88	2.45		
AGT M268T allele	3.23	1.16	.36	.008
Age	-.11	0.05	-.27	.041

Table 5.22 RCF copy report of multiple regression. Note $R^2 = .11$ for step 1; $\Delta R^2 = .07$ for step 2. P represents statistical significant of observed differences.

A boxplot of observed RCF copy task for AGT M268T alleles is illustrated below in figure 5.25 indicating that patients with the T variants significantly predict good visuospatial organization.

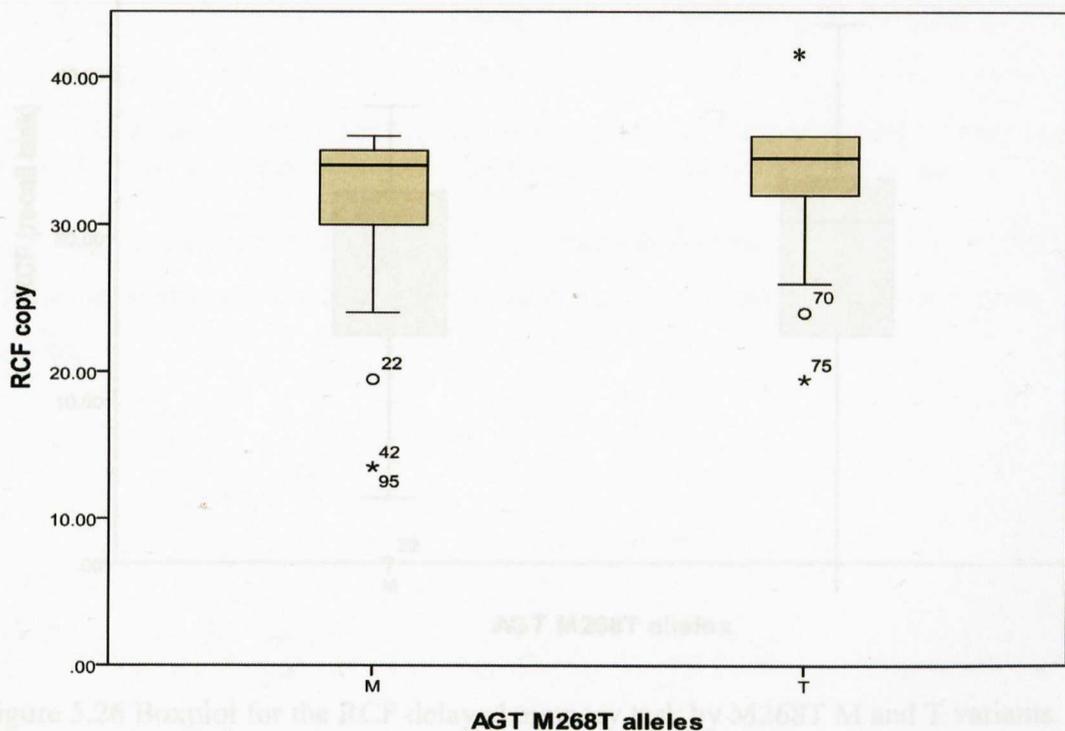


Figure 5.25 Boxplot for the RCF copy task by M268T M and T variants. * patients carrying T variants significantly predict good visuospatial organization ($p = .016$).

RCF recall

	B	SE B	Beta	P
Step 1				
Constant	12.31	2.66		
AGT M268T alleles	4.59	1.80	.34	.014
Step 2				
Constant	5.90	3.90		
AGT M 268T alleles	4.54	1.74	.33	.012
Education	.56	.26	.28	.034

Table 5.23 RCF memory task report of multiple regression. Note $R^2 = .113$ for step 1; $\Delta R^2 = .08$ for step 2. P represents statistical significant of observed differences.

A boxplot of the observed RCF memory task for AGT M268T alleles is illustrated below in figure 5.26 which indicates that patients with the T variant perform better on visuospatial memory.

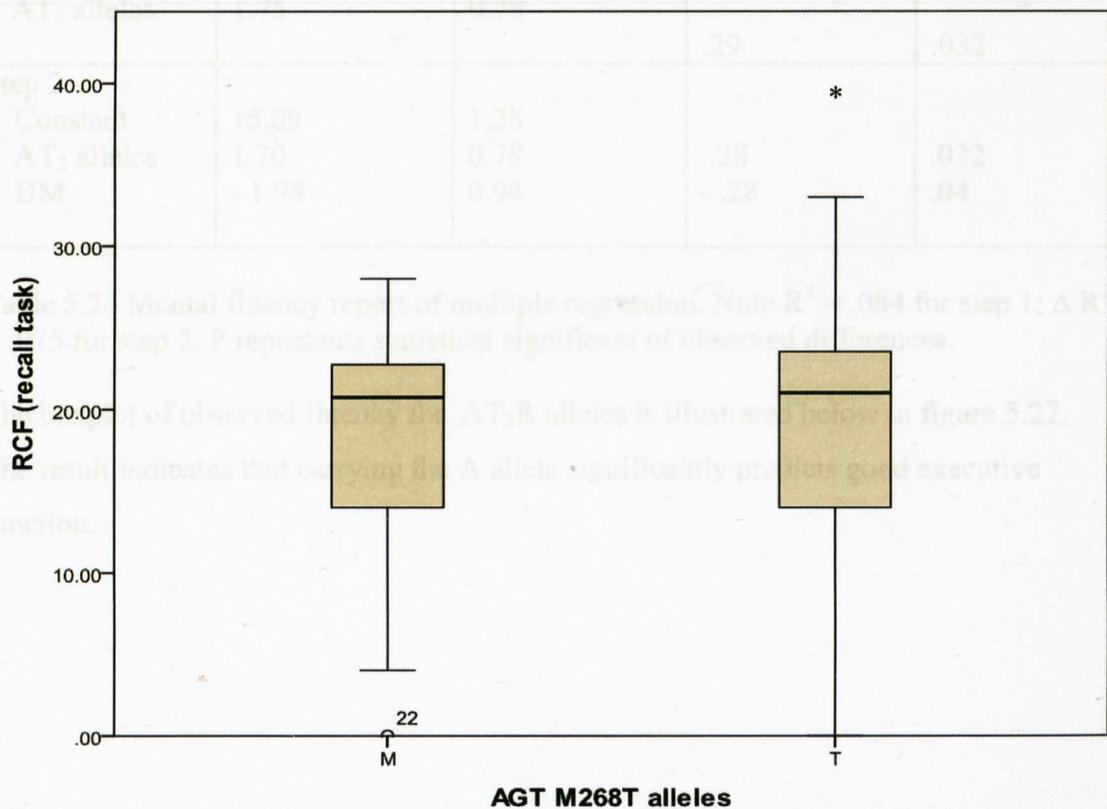


Figure 5.26 Boxplot for the RCF delayed memory task by M268T M and T variants.
* patients carrying T variants significantly predict good visuospatial memory (p= .014).

5.4.5.2.2 Attention

Digit-symbol-substitution-test (DSST)

No alleles were found to be a predictor of attention

Letter cancellation

No alleles were found to be a predictor of attention

5.4.5.2.3 Executive function

Mental fluency

Results indicated that the AT₂R alleles and DM made a significant contribution to executive function, working and semantic memory as shown in table 5.24 below.

	B	SE B	Beta	P
Step 1				
Constant	14.57	1.30		
AT ₂ alleles	1.75	0.78		
			.29	.032
Step 2				
Constant	15.09	1.28		
AT ₂ alleles	1.70	0.78	.28	.032
DM	- 1.98	0.94	- .28	.04

Table 5.24 Mental fluency report of multiple regression. Note $R^2 = .084$ for step 1; $\Delta R^2 = .075$ for step 2. P represents statistical significant of observed differences.

The boxplot of observed fluency for AT₂R alleles is illustrated below in figure 5.27.

The result indicates that carrying the A allele significantly predicts good executive function.

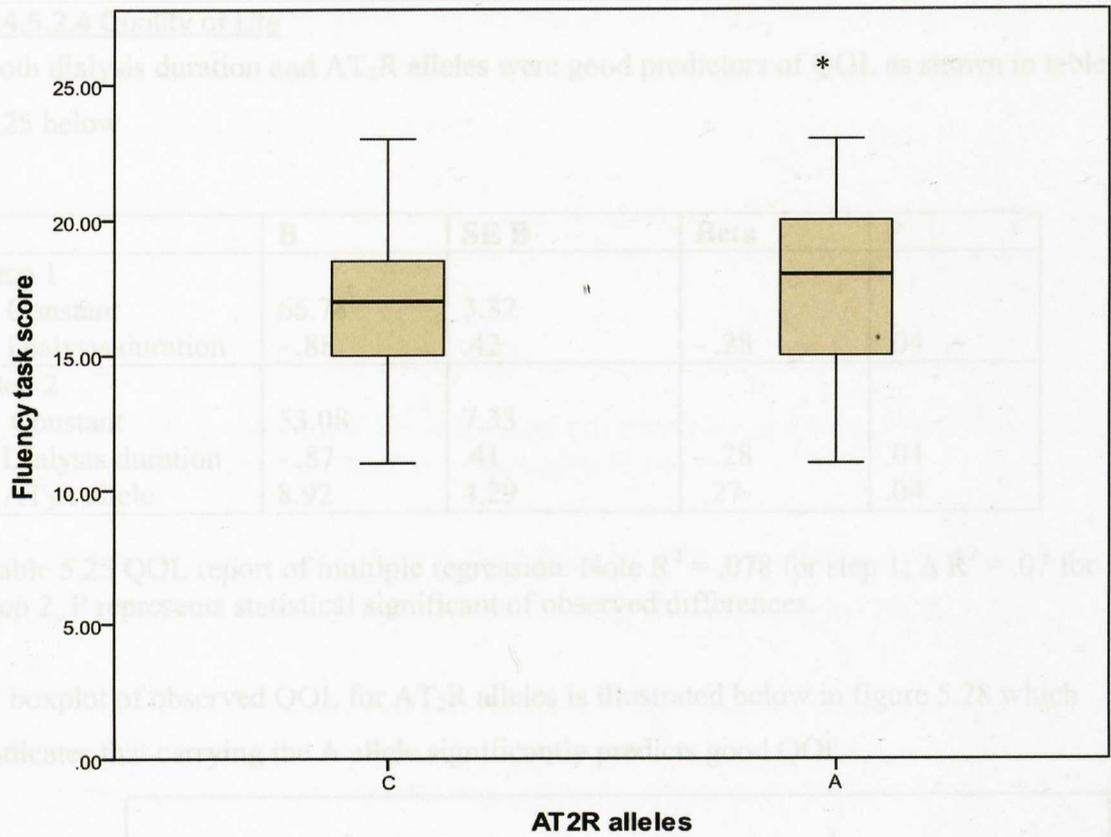


Figure 5.27 Boxplot for the fluency task score by AT₂R C and A alleles. * patients carrying A alleles significantly predict good executive function (p= .032).

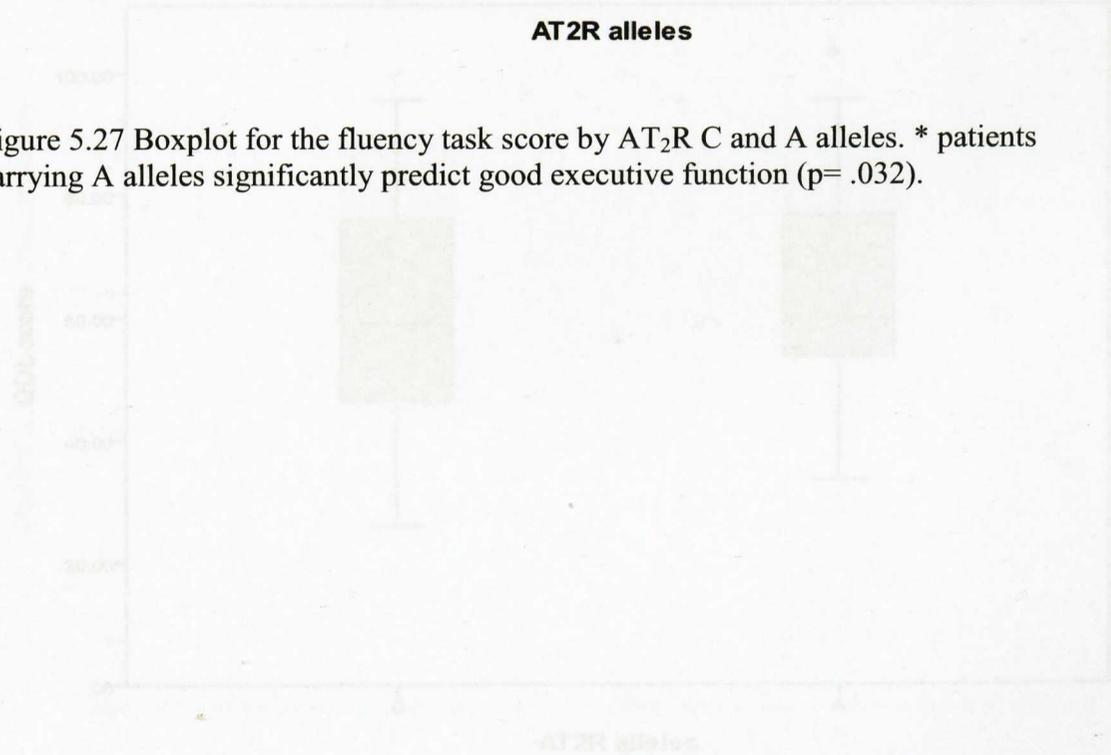


Figure 5.28 Boxplot for the QOL score by AT₂R C and A alleles. * patients carrying A alleles significantly predict good QOL (p= .04).

5.4.5.2.4 Quality of Life

Both dialysis duration and AT₂R alleles were good predictors of QOL as shown in table 5.25 below

	B	SE B	Beta	P
Step 1				
Constant	66.78	3.32		
Dialysis duration	-.88	.42	-.28	.04
Step 2				
Constant	53.08	7.33		
Dialysis duration	-.87	.41	-.28	.04
AT ₂ R allele	8.92	4.29	.27	.04

Table 5.25 QOL report of multiple regression. Note $R^2 = .078$ for step 1; $\Delta R^2 = .07$ for step 2. P represents statistical significant of observed differences.

A boxplot of observed QOL for AT₂R alleles is illustrated below in figure 5.28 which indicates that carrying the A allele significantly predicts good QOL.

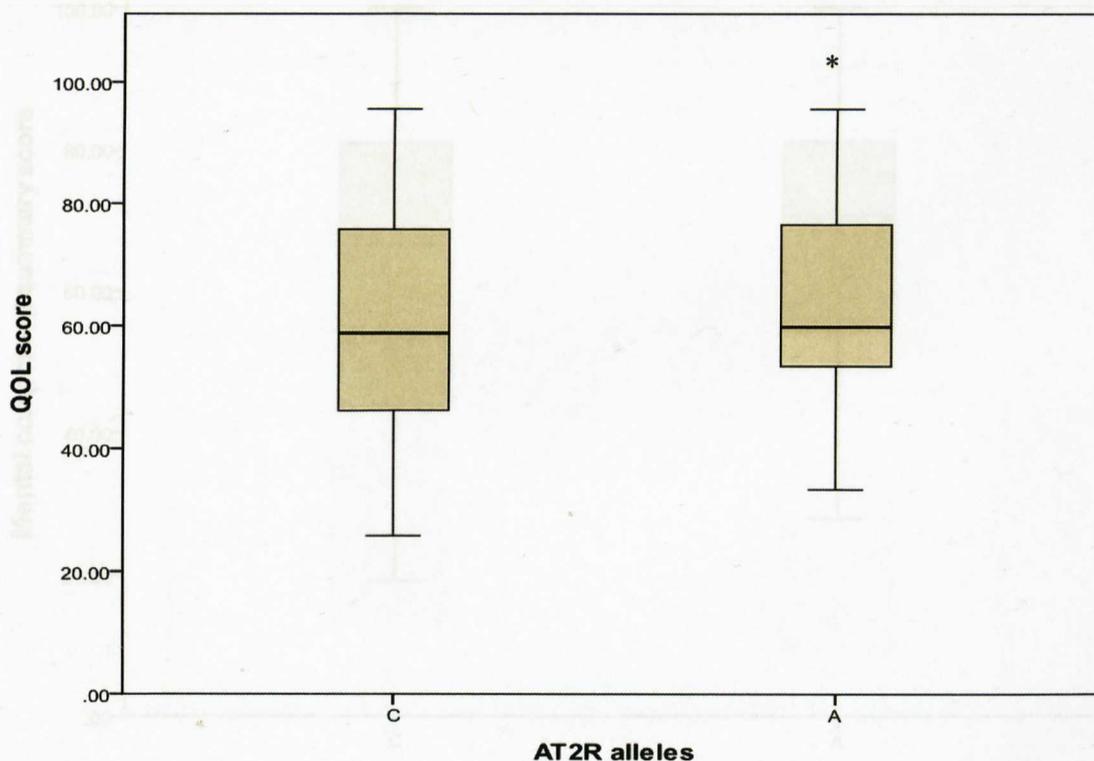


Figure 5.28 Boxplot for the QOL score by AT₂R C and A alleles. * patients carrying A alleles significantly predict good QOL (p= .04).

When physical and mental summary components were analyzed separately, linear regression results indicated that the AT₂R allele predicts good mental component summary as shown in table 5.26 below.

	B	SE B	Beta	P
Step 1				
Constant	44.87	8.95		
AT ₂ R allele	13.65	5.57	.33	.02

Table 5.26 Mental component summary report of multiple regression. Note $R^2 = .105$. P represents statistical significant of observed differences.

The boxplots of observed MCS for AT₂R alleles are illustrated below in figure 5.29.

The results indicate that carrying the A allele of AT₂R predicts good MCS.

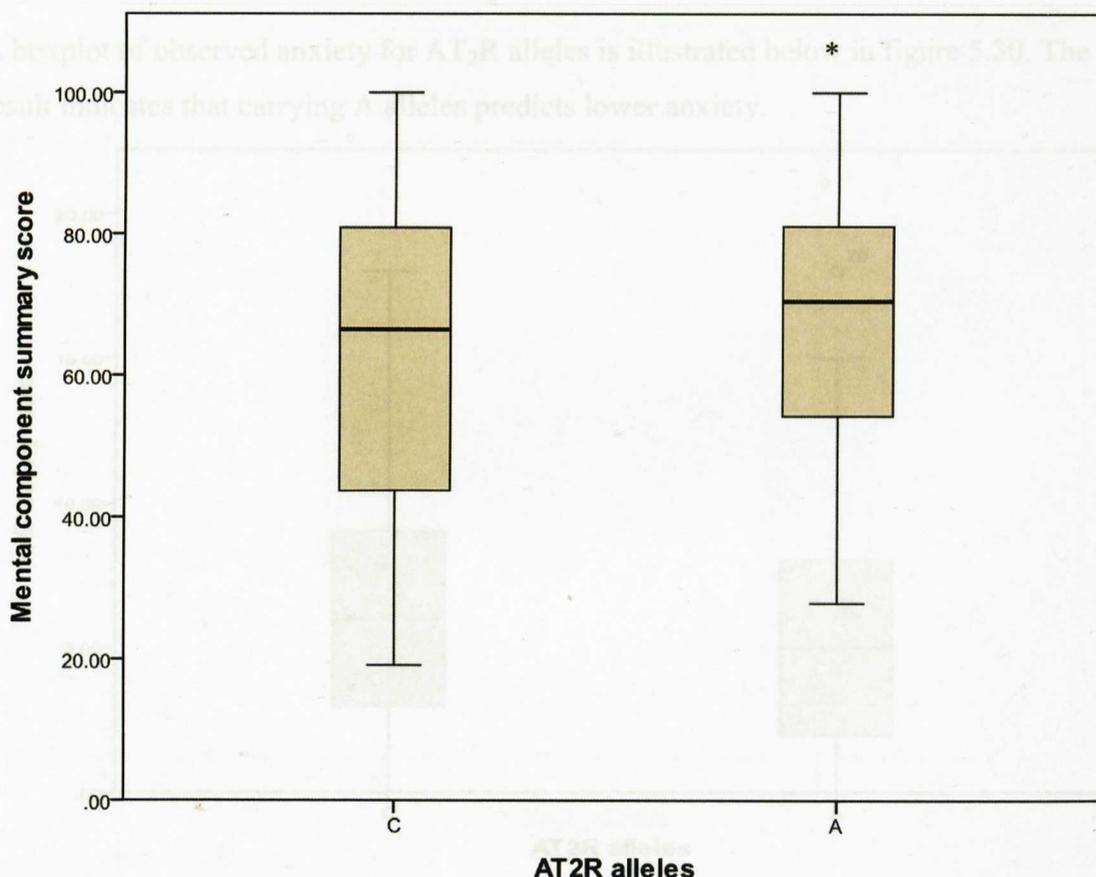


Figure 5.29 Boxplot for the MCS score by AT₂R C and A alleles. * patients carrying A alleles significantly predict good MCS (p= .02).

5.4.5.2.5 Hospital Anxiety and Depression Scale (HADS)

Anxiety

Regarding anxiety, AT₂R alleles and dialysis duration contributed significantly to anxiety as shown below in table 5.27.

	B	SE B	Beta	P
Step 1				
Constant	11.94	1.67		
AT ₂ R allele	- 4.06	1.04	- .48	.001
Step 2				
Constant	10.55	1.69		
AT ₂ R alleles	- 4.02	.99	- .48	.001
Dialysis duration	.23	.095	.28	.02

Table 5.27 Anxiety report of multiple regression. Note $R^2 = .23$ for step 1; $\Delta R^2 = .08$ for step 2. P represents statistical significant of observed differences.

A boxplot of observed anxiety for AT₂R alleles is illustrated below in figure 5.30. The result indicates that carrying A alleles predicts lower anxiety.

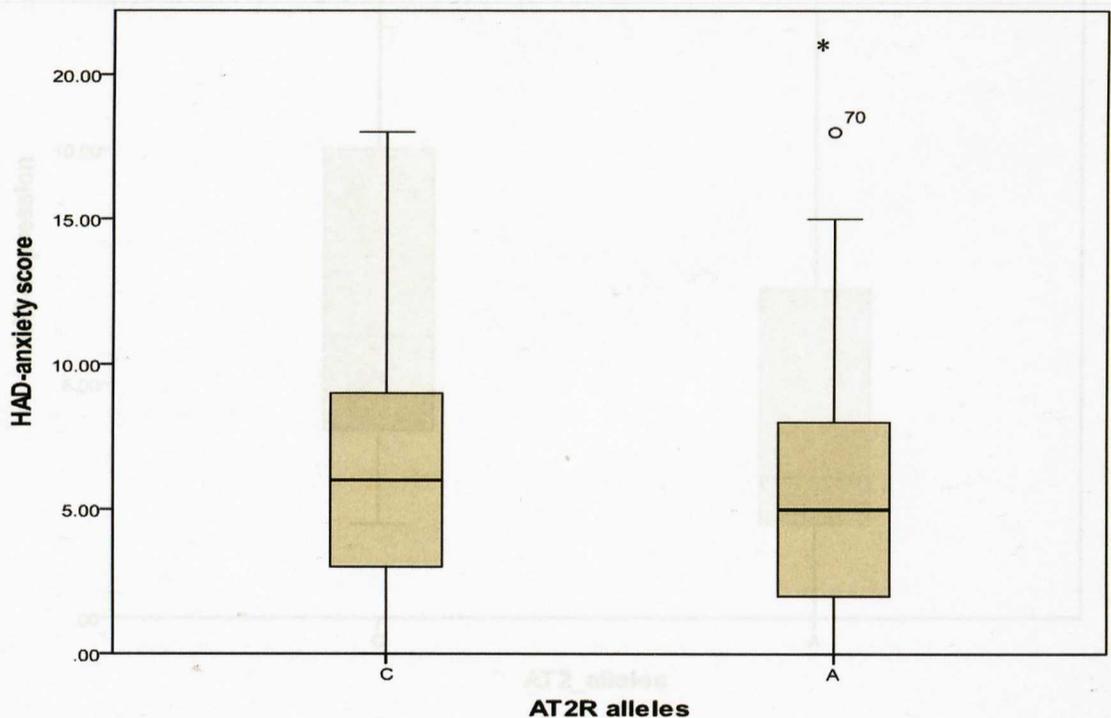


Figure 5.30 Boxplot for anxiety score by AT₂R C and A alleles group. * patients carrying A alleles significantly predict lower anxiety (p= .001).

5.5 Conclusion

Depression

AT₂R alleles were the only predictors of Depression as shown below in table 5.28

	B	SE B	Beta	P
Step 1				
Constant	9.45	1.43		
AT ₂ R allele	- 2.69	.89	- .39	.004

Table 5.28 Depression report of multiple regression. Note $R^2 = .152$. P represents statistical significant of observed differences.

A boxplot of observed depression for AT₂R alleles is illustrated below in figure 5.31.

The result indicates that carrying A alleles predicts lower depression.

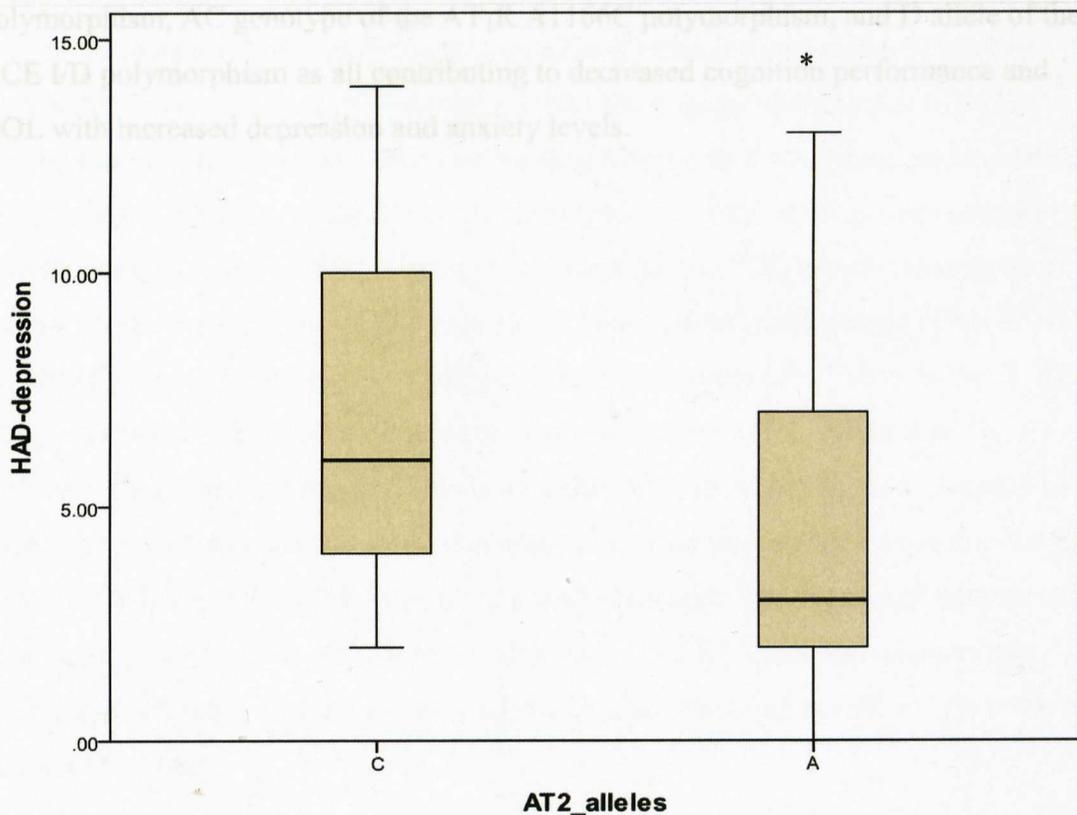


Figure 5.31 Boxplot for the depression score of AT₂R alleles A and C carriers.
* patients carrying A alleles significantly predict lower depression (p= .004).

5.5 Conclusion

Dialysis patients were similar to healthy controls in term of cognition. This differs from results of previously published studies (as discussed in detail in chapter 1), and from the previous study in CKD patients (see chapter 3). Dialysis patients have more severe kidney disease than those studied in chapter 3, but more stable uraemia.

There were no significant differences in cognition between those patients receiving RAA treatment and those receiving non-RAA.

Linear regression identified DM, age, gender, dialysis duration and drug group contributed significantly to mood, cognition, QOL, and anxiety. In addition, MT genotype and M variant of the AGT M268T polymorphism, TT genotype of the AGT T207M, CC and CA genotype (in case of executive function and anxiety, respectively). Moreover, AA genotype (in case of verbal learning) and C allele of the AT₂R C3123A polymorphism, AC genotype of the AT₁R A1166C polymorphism, and D allele of the ACE I/D polymorphism as all contributing to decreased cognition performance and QOL with increased depression and anxiety levels.

CHAPTER 6. ANALYSIS OF A SUB-GROUP OF CKD PATIENTS THAT WERE NON-DIALYSIS PATIENTS FROM CKD STUDY IN CHAPTER THREE

6.1 Aim and expectation

In chapter 3, when the decision was made to recruit CKD patients from the nephrology clinic at KKHU, inclusion of all CKD patients was made irrespective of CKD stage. A substantial number of dialysis patients were included. Thereafter a decision was made to recruit only stable dialysis patients (chapter 5) to decrease confounding variables as much as possible. The other important reason for recruiting dialysis patients in chapter 5 was the easiness of the accessibility of venous blood to be used in genotyping analysis.

Analysis of neuropsychological abilities of dialysis patients in chapter 5 revealed that dialysis patients had good neuropsychological properties in comparison to healthy control. Moreover, dialysis patients treated with RAA drugs were similar to those treated with non-RAA except in verbal learning where non-RAA group perform better, this conflicted with the results of the earlier chapter. Since there are a large number of dialysis patients in the non-RAA group (13 patients) in the CKD study (chapter 3) in comparison to the RAA group (5 patients), exclusion of the confounding effect of being a dialysis patient on the neuropsychological results is mandatory. The variable; "CKD stage" was taken into consideration when results of neuropsychological tests were analyzed. However, in the light of the results from chapter 5, further consideration of the earlier results was prompted. At this point a decision was made to reanalyze the data from the CKD study (chapter 3), removing the independent association of dialysis with good neuropsychological performance. Data from 29 CKD (non-dialysis) patients receiving RAA drugs and the 13 non-dialysis patients receiving non-RAA drugs were therefore revisited.

6.2 Results

There were 13 non-hemodialysis patients in the non-RAA group and 29 patients in the RAA group. ANOVA results indicate that there is no significant difference in education level among the three groups. ANOVA also indicates that there is a significant difference in age between the groups ($F(2,77)= 7.97, p<.05$). However, planned contrast revealed that the significant difference is only between healthy control and RAA group ($t(77)= -3.98, p<.05$). Gender analysis revealed that there was no significant difference in male and female ratio among the three groups.

QOL, PCS, MCS, anxiety and depression are shown below in figure 6.2 and compared with healthy control (36 patients) using ANOVA test.

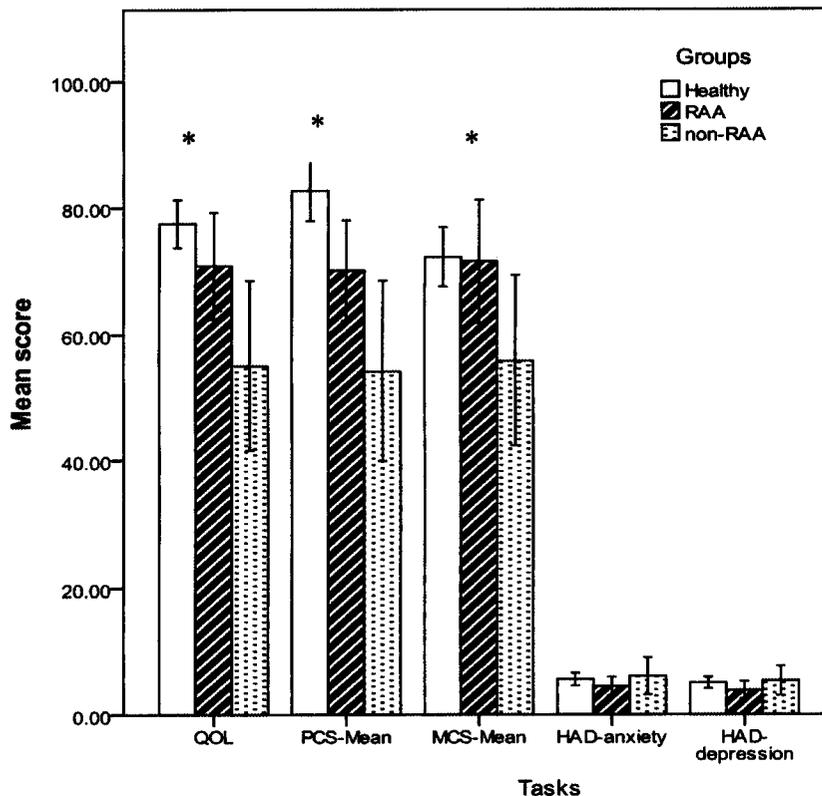


Figure 6.1 Error bar graph show 95% CI of mean QOL, MCS, PCS, anxiety and depression healthy control (36), RAA (29) and non-RAA (13) group (a subgroup of CKD study in chapter 3). * represents significant difference from control group ($p<.05$).

The results of the comparison using ANOVA test revealed that the three groups were significantly different in QOL ($F(2,77) = 8.14, p < .05$), PCS ($F(2,77) = 12.33, p < .05$), and MCS ($F(2,77) = 3.9, p < .05$).

Neuropsychological tests are shown below in figure 6.3 and compared with healthy control (36 patients) using ANOVA test.

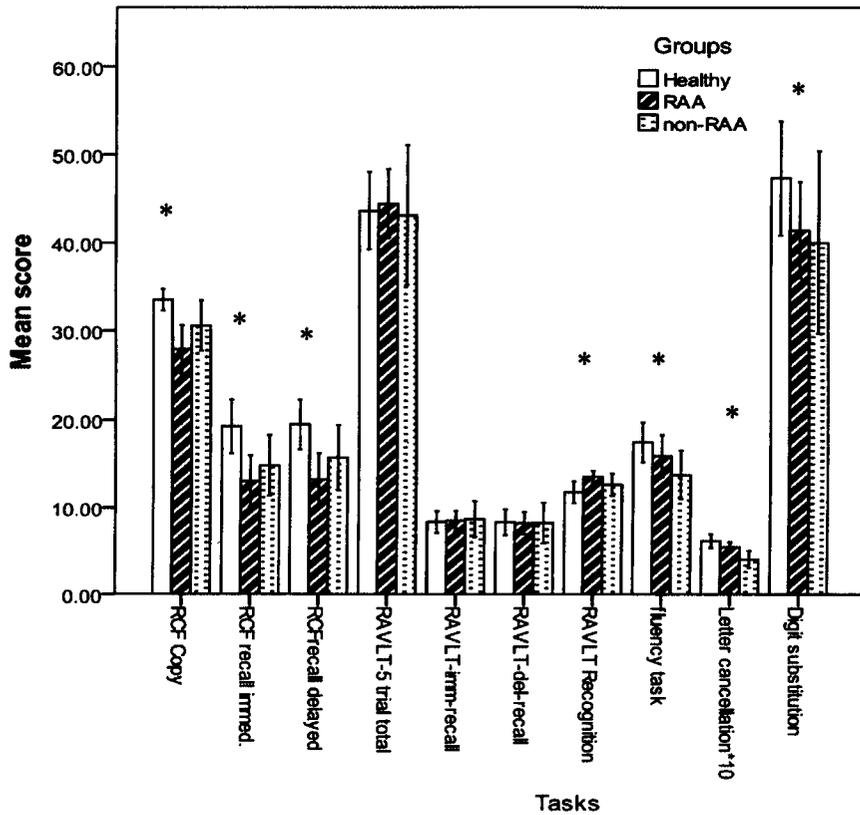


Figure 6.2 Error bar graph show 95% CI of mean RCF copy and memory tasks, RAVLT learning and memory tasks, fluency, cancellation and substitution tasks of healthy control (36), RAA (29) and non-RAA (13) group (a subgroup of CKD study in chapter 3). * represents significant difference from control group ($p < .05$). Results of letter cancellation multiplied by 10 to clearly demonstrate the results on the graph.

The results of the comparison using ANOVA test revealed that the three groups were significantly different in RCF copy ($F(2,77)=12.56, p<.05$), RCF immediate recall ($F(2,77)=9.79, p<.05$), RCF delayed recall ($F(2,77)=9.73, p<.05$), RAVLT recognition task ($F(2,77)=6.19, p<.05$), Fluency ($F(2,77)=3.30, p<.05$), Letter cancellation ($F(2,77)=6.35, p<.05$), and digit substitution ($F(2,77)=3.72, p<.05$).

Planned contrasts between healthy, RAA and non-RAA groups revealed that the significant differences indicate that CKD disease patients, compared to healthy controls, have defects in physical component summary of QOL, visuospatial organization, visuospatial memory, recognition and attention abilities as indicated below:

1. Healthy control versus RAA group in the following parameters:

- Age ($t(77)=-3.98, p<.05$)
- PCS ($t(77)=2.99, p<.05$)
- RCF copy ($t(77)=4.46, p<.05$)
- RCF recall ($t(77)=4.15, p<.05$)
- RCF delayed recall ($t(77)=4.36, p<.05$)
- RAVLT recognition ($t(77)=-3.53, p<.05$)
- Digit substitution ($t(77)=2.12, p<.05$)

2. Healthy control versus non-RAA group in the following parameters:

- QOL ($t(77)=3.79, p<.05$)
- PCS ($t(77)=4.31, p<.05$)
- MCS ($t(77)=2.67, p<.05$)
- RCF copy ($t(77)=3.20, p<.05$)
- RCF recall ($t(77)=2.84, p<.05$)
- RCF delayed recall ($t(77)=2.02, p<.05$)
- Fluency ($t(77)=2.48, p<.05$)
- Letter cancellation ($t(77)=3.56, p<.05$)
- Digit substitution ($t(77)=2.33, p<.05$)

However, planned contrast revealed that CKD patients in the RAA group performed significantly better in QOL, MCS and letter cancellation tasks than CKD patients in the non-RAA group as indicated below.

- QOL ($t(77) = 2.27, p < .05$)
- MCS ($t(77) = 2.48, p < .05$)
- Letter Cancellation ($t(77) = 2.76, p < .05$)

Analysis adjusted for covariables that include age, gender, education, disease duration, HTN, DM, Hgb level were performed. Among all groups, the significant differences remained the same as before covariable adjustment in the cases of QOL ($F(2,67) = 4.05, p < .05$) and letter cancellation as a measure of attention ($F(2,67) = 5.27, p < .05$). In addition to new significant difference in PCS ($F(2,67) = 4.46, p < .05$). Planned contrast revealed that CKD patients in the RAA group had significantly higher QOL, PCS, and attention score compared to CKD patients in non-RAA group ($p < .05$).

6.3 Conclusion

A subgroup analysis of CKD patients (chapter 3) revealed that the non-hemodialysis CKD patients in the RAA group had better scores in QOL and PCS in addition to better attention as measured by letter cancellation task than patients receiving non-RAS drugs. In conclusion results of CKD patients in chapter three which showed good executive function of RAA group compared to the non-RAA group is a real finding and not affected by the large number of dialysis patients in the non-RAA group.

CHAPTER 7. GENERAL DISCUSSION

7.1 Summary

The main findings of this thesis are:

- Saudi CKD patients suffer neurocognitive defects particularly in executive function, visuospatial organization and memory in addition to low QOL. When RAA drugs were used as antihypertensive agents in these patients, better neurocognitive function (executive function) was observed in comparison to patients receiving non-RAA antihypertensives. This is a novel finding.
- Saudi colon cancer patients in remission exhibited good neurocognitive function except in visuospatial memory where healthy controls exhibited marginally significantly higher scores. These results suggest that chronic illness itself does not lead to impaired cognition. This is a novel finding.
- Saudi dialysis patients had good neurocognitive properties in comparison to healthy controls. This is a novel finding.
- Dialysis patients treated with RAA drugs as an antihypertensive had good neurocognitive function except in verbal learning where significant better performance has been reported in dialysis patients treated with non-RAA drugs as an antihypertensive. This is a novel finding.
- Saudi dialysis patients possessed RAS genotypes similar to healthy controls, suggesting a lack of association between RAS genotype and stage V kidney disease. This is a novel finding.
- Better cognition, QOL with less anxiety and depression were found to be associated with certain RAS-associated gene polymorphisms, notably of AGT M268T and AT₂R C3123A. This is a novel finding.

7.2 Discussion

Human beings suffer a lot from chronic diseases and the increased burden of such conditions makes them behave differently to normal healthy subjects. One of the most important properties of human beings that can be easily affected due to disease is neuropsychological abilities such as cognition, memory, and concentration. Although neuropsychological defects are documented in CKD patients, little is known about the causative factors (Murray *et al.*, 2006). One study reported as much as 30% prevalence of mild cognitive defect among ESRD patients (Sehgal *et al.*, 1997).

The current study demonstrates a defect in neuropsychological behaviours of CKD patients particularly in executive function, visuospatial organization, and visuospatial memory compared to healthy controls who were younger but were of similar educational pattern. Neurocognitive defects of CKD patients reported in this study were in accordance with the previously published studies as Kurella *et al.* and Murray *et al.* who reported an obvious defect in executive function; attention was also impaired in some studies (Hailpern *et al.*, 2007; Kurella *et al.*, 2004; Kurella *et al.*, 2005b; Murray *et al.*, 2006; Pliskin *et al.*, 1996; Williams *et al.*, 2004). Defects in immediate and delayed verbal memory have also been documented (Kurella *et al.*, 2005b; Lee *et al.*, 2004; Williams *et al.*, 2004). The defect in executive function may affect a patient's ability to follow medical advice especially in elderly patients (Royall *et al.*, 2005).

Disturbance of visuospatial organization and memory have not been documented before as a defect in CKD patients, however the ethnic origin of this sample; Arabs of medium to low educational level and not familiar with such types of written tests, might explain this defect. Although cognitive decline has been documented in the previous studies and also in this study, it is unclear whether this defect is due to chronic disease or any other factors that were suggested in previous studies but not documented. Such factors were anaemia, DM, HTN, age, education level (see chapter one for more details). Cognitive dysfunction after adjustment of such factors however, remains significant (Kurella *et al.*, 2005b). Additional risk factors (e.g. hyperparathyroidism, oxidative stress) for neurocognitive defect in CKD patients has been studied insufficiently (detailed in chapter one).

Patients in this study had stable CKD and were either in moderate (stage III), severe (stage IV), or in ESRD (stage V) (Young, 1995). The glomerular filtration rate (GFR) declines to less than 60 ml/min/1.73 m² in stage III with the appearance of the first clinical sign of HTN and mild anaemia. In stage IV severe decline in renal function occurs where GFR declines to less than 30 ml/min/1.73 m² with the appearance of all complications of renal disease. Once kidney function reaches stage V where GFR becomes less than 10 ml/min/1.73 m², dialysis or kidney transplantation becomes necessary to sustain life and relieve uraemic symptoms. Patients were carefully selected so that those with unstable conditions such as refractory anaemia, severe HTN, coronary or cerebrovascular disease were excluded to overcome as much as possible the effect of such conditions on the results. However, multiple risk factors still exist and were taken into consideration when analyzing the results.

It would be feasible to speculate that CKD may be associated with abnormal RAS function, and in the knowledge that the RAS has been associated with cognition it can be hypothesized that a disordered RAS may underlie the cognitive deficits observed in CKD patients.

Patients treated with RAA drugs experienced an improvement in neuropsychological properties over the non-RAA group particularly in verbal learning, executive function and attention. Accounting for existing risk factors made the improvements in neurocognitive defects obvious in executive function. This might suggest either a beneficial effect of RAA drugs over other antihypertensives on neuropsychological properties of CKD patients, the better medical condition of the RAA group, or some other beneficial feature of the chronic illness that such patients have. Attention, immediate, and delayed memory-enhancing effects have been shown in studies investigating the beneficial effects of ACEIs and ARBs on neurocognition in hypertensive patients (Braszko *et al.*, 2003; Currie *et al.*, 1990; Fogari *et al.*, 2003). Furthermore the RAA group had less DM and HTN but not statistically significant, shorter disease duration, and a lower percentage of patients in end-stage renal disease (23.5% in the RAA group vs. 84.6% in the non-RAA group).

In addition, increased anxiety, depression, and decreased QOL might be associated with lower neuropsychological performance (Kizilbash *et al.*, 2002; Mikkelsen *et al.*, 2009;

Wilson *et al.*, 2002; Wilson *et al.*, 2004), however; the non-significant difference in QOL, anxiety, and depression between the two groups ruled out such confounding factors on neurocognitive defect. Patients characterized their QOL, anxiety, and depression from their subjective perspective and they gave high scores for items that seemed impaired; the patient's capacity to judge their QOL, anxiety, and depression may have been impaired either due to their neurocognitive defect or some other factors such as religious belief, which may let them score such items highly.

Any neurocognitive decline induced by chronic illness was investigated in chapter four where colon cancer patients in remission were chosen. Results indicated that patients suffering chronic disease had good neurocognitive properties and thus the assumption of the deleterious impact of chronic disease on neurocognitive properties was unlikely. A direct role of RAA drugs on neurocognitive functioning of CKD patients cannot be proven in this study due to the lack of randomization and the presence of multiple confounders (e.g. DM, polypharmacy, and anaemia), however, the results suggest a direct effect of RAS drugs on neurocognitive function which warrants further investigation.

One of the theoretical assumptions of the beneficial effect of ACEIs and ARBs is based on the suggestion that elevated ACE activity enhances the formation of Ang II which increases the inhibitory effect of angiotensin on acetylcholine release so that acetylcholine concentration is increased in the neurons by using ACEIs or ARBs and may be beneficial in improving cognition as evidenced in patients with Alzheimer's disease (Barnes *et al.*, 1991; Hanes *et al.*, 2007; Kehoe *et al.*, 2007). In addition, an involvement of Ang IV, a derivative of Ang II peptide, in neurocognitive behavior has been proposed, based on the finding that Ang II intracellular degradation to Ang IV is necessary to elicit its cognitive enhancing effects in rats (Braszko *et al.*, 2006). The CKD study is observational and was the first in examining neuropsychological properties of Saudi CKD patients, and in examining the impact of RAA drugs on neuropsychological properties of CKD patients.

It is documented that Ang II and Ang IV plasma concentrations in CKD and dialysis patients are similar to healthy control (Shibasaki *et al.*, 1999). When AT₁R antagonist (candesartan) treatment was used for 7 days in CKD and dialysis patients, an increase in

Ang II and Ang IV level to 5.5 and 4.1 fold, respectively was reported (Shibasaki et al., 1999). When plasma concentrations were measured 28 and 56 days later, the Ang II level had declined significantly while Ang IV level were not significantly changed. The author suggested that the sustained increase in Ang IV level might be due to the continuous production of renin due to AT₁R antagonism that may possibly increase Ang I production which could then be converted to Ang IV. Enhanced AT₁R expression in leukocytes of CKD patients that is not related to RAA therapy has also been reported (Chon et al., 2011). All of this might indicate a high activity of Ang II or Ang IV in renal impaired patients which may explain the neuropsychological properties of CKD patients. Such increased activity may be being resolved by use of the RAA antihypertensives. Increased Ang II may cause impairment, increased Ang IV may cause improvement.

This study has some limitations. The large number of variables and the relatively small sample size. The Cross-sectional design of the study cannot provide evidence of a causal relationship between RAS drugs and cognitive improvement and a longitudinal analysis would be better to provide evidence of a causal relationship by studying the neurocognitive changes since diagnosis of CKD, before and after treating with drugs acting on RAS. A global test of neurocognitive defect, was not used, such as Mini-Mental State examination (3MS) (Teng *et al.*, 1987) would also have been helpful in assessing general cognitive status of patients. The main reason behind not using 3MS is its generalizability in assessing different domains of memory and cognition and the low acceptability of this test by the tested group. The low participation rate of females may make these results not generalizable to female CKD patients. In addition to the lack of information about non-participants who were either non-eligible because they were elderly and illiterate or having obvious risk factor such as cerebrovascular disease and those are the majority. A small number of patients refused and claimed they were too busy or not willing to participate. However, most of the previously mentioned limitations were overcome in the third study carried out on stable dialysis patients. One of the most important strengths of the CKD study, however, was the use of a healthy control group of similar education and ethnic origin for comparison rather than comparison to published norms which are lacking in this case. It is also a prospective study using multiple tools to test multiple domains with validated neuropsychological tests.

Knowing the causative factors related to neurocognitive defect could improve memory problems and poor concentration and could help improve patients' ability to follow medical staff instructions and improve medication compliance. The number of CKD and hemodialysis patients is rapidly growing in Saudi population, therefore, detection, monitoring, and management of cognitive impairment in such a population is needed. Diagnosis of cognitive impairment should be included in the medical record of CKD patients.

Within the volunteers for the CKD study, it was found that a larger proportion of the non-RAA patients were on hemodialysis, it is very difficult at this point to explain the large number of dialysis patients within this group. One explanation might be because they were not treated with RAA drugs in the early stages of their disease. RAA drugs are known to halt the progression of CKD to end-stage renal disease where dialysis or kidney transplantation is mandatory to sustain patient's life. The lack of drug therapy data from the diagnosis of CKD to the point of checking the neuropsychological properties of CKD patients is considered to be a limitation of CKD study.

The re-analysis of the results indicate that the non-dialysis CKD patients in the RAA group had better QOL and PCS as well as good attention in comparison to the non-RAA group. This finding excludes the effect of the high number of dialysis patients in the non-RAA group and confirms the beneficial effect of RAA drugs on executive function that were documented in the CKD study (chapter 3).

It is suggested above that the neurocognitive impairment of the CKD patients may have been independent of any renal impairment and may be, in fact, a result of the chronic illness itself. The study in colon cancer patients revealed no evidence of impaired cognition in this group, thus suggested that the impairment in CKD patients was a specific feature of their disease. The colon cancer patients had previously been informed of their chronic condition and had received surgical treatment approximately 2 years previously together with chemotherapy; there had been repeated interaction with healthcare professionals, with repeated hospital appointments and stays. They were all currently in remission but were aware of the risk of reoccurrence. The 5-year survival rate is approximately 90% for patients with localized disease and around 66% in those

with regional disease determined at diagnosis (Jemal et al., 2007). The incidence of disease recurrence is 25% in the absence of regional node involvement. Therefore more advanced disease indicates involvement of lymph node which worsens the prognosis (Bilchik et al., 2007).

In comparison to the healthy control group they were significantly older, but there was no difference in the degree of education. The psychological and neurocognitive assessments indicated that the cancer patients had significantly lower depression and anxiety scores with better pattern of recognition and mental component summary than healthy controls. Healthy controls, however, had significantly higher scores on health transition and visuospatial recall. These findings suggest an overall good neurocognitive and psychological functions of colon cancer patients in remission. This study tested the neurocognitive properties of colon cancer patients in remission to exclude the effect of chronic disease on neurocognitive properties. The results for memory, attention and executive functions were comparable to healthy control and in some domains were even superior in colon cancer patients. The only exception was in visuospatial memory as measured by RCF immediate and delayed recall. The superiority of visuospatial memory as measured by RCF in healthy control in comparison to colon cancer patients may be explained by the young age (mean age 33.7 years) of healthy control with respect to colon cancer patients who were older (mean age 44.8 years).

In this study visuospatial memory was assessed by a task involving copying geometric shapes and lines which was considered by the majority of subjects as a drawing trial that they may not be used to and was difficult for them to perform precisely. Furthermore, colon cancer patients were more than 10 years older than the healthy controls which might explain the lower score that they attained in RCF recall trials in comparison to healthy control. The mean performance of young colon cancer patients (< 40 years) in visuospatial memory was much higher than the mean of older colon cancer patients (\geq 40 years). The young colon cancer patient's mean was 18 which was approaching the mean performance of healthy control that was 20. This documents the effect of age on colon cancer patients' performance, furthermore the negative effect of age on RCF recall has been documented in the recent literature (Gallagher *et al.*, 2007; Pena-Casanova *et al.*, 2009). However, such 11 years difference in this age range (30-40 years) might not be considerably important.

The good QOL measures of colon cancer patients especially MCS is in line with neurocognitive measures as well as anxiety and depression. However, depressive symptoms have been reported in colon cancer patients in remission in two recent systematic reviews that covers studies from US, Japan, Italy, and Sweden (Harrington *et al.*, 2010; Jansen *et al.*, 2010). This might be explained by the fear of recurrence and further spread of cancer that many colon cancer survivors reported (Phipps *et al.*, 2008). These results exclude the bad effect of chronic disease on mood and cognition as well as QOL at least in the target population (Saudi colon cancer patients). The majority of QOL studies have focused on women with breast cancer. Furthermore, the QOL for colon cancer patients in remission has received little attention compared with rectal cancer patients. Most of these studies documented overall good QOL of colon cancer survivors that was less burden than rectal cancer but equivalent or worse than breast cancer survivors (Di Fabio *et al.*, 2008; Mosher *et al.*, 2009). The explanation behind the difference in the findings of this study compared to the previous studies might be the population type. The Saudi population seemed to accept minor complaints after a cancer cure and appeared to have adjusted well to the disease experience, in addition their strong belief in God hindered them reporting such minor complaints and feeling that life after cancer is not doom and gloom. Muslims in general tend to use word "Insha'allah" which means God willing when answering QOL questions and so they tend to choose the best answer although not the exact answer that explain their illness in this study which is also reported by other studies (Naser, 2011). Non-specificity of the QOL instrument for colon cancer patients in remission can be another explanation. The SF-36 QOL instrument was used in this study although it might be non-specific for cancer patients but because of the consistency of measures employed over all the studies in this thesis its use was mandatory. In addition, the cross-sectional design of this study and the previously published studies makes the measurements of changes in QOL over time impossible.

Hemodialysis patients represent a population of stable, chronic kidney disease patients. The dialysis patients included were selected randomly from two dialysis centers after checking that they were in accordance with inclusion and exclusion criteria. Including the two dialysis centers gives a representative sample of the dialysis patients in Saudi Arabia. The controls also represent healthy subjects in the same region.

The studied dialysis patients were stable and did not differ in clinical variables such as electrolyte level, anaemia profile, and dialysis adequacy. Such variables may affect neurocognitive properties. However other variables of dialysis patients could not be controlled such as demographics (age, gender, and education level). Dialysis patients were also chosen for RAS genotyping analysis due to the easy access of venous blood samples during dialysis session. RAS genotyping was not possible for CKD patients recruited in the first part of this thesis since they do not have venous access for blood sampling and ethics approval for collection of an additional venous blood sample was not sought.

The aim of the study was to find any association between RAS polymorphism and neurocognitive defects that might explain the mechanism of neurocognitive defect in dialysis patients.

This is the first study to investigate the association between four RAS genes and neurocognitive defect in Saudi dialysis patients. Furthermore, neurocognitive assessment of Saudi dialysis patients have never been undertaken before.

The results of this study showed that:

- Dialysis patients had significantly better learning as determined by the RAVLT task than healthy control. There were no differences on any other neuropsychological task.
- The total quality of life score for dialysis patients was lower than that for healthy controls specifically in physical component summary but not mental component summary.
- Anxiety and depression test scores for dialysis patients were similar to that for normal healthy controls.
- There were no significant differences in RAS gene polymorphism distribution between dialysis patients and normal, healthy controls.
- Female gender was associated with better learning and increased anxiety amongst dialysis patients.
- Increased age was associated with impaired visuospatial memory, impaired verbal memory and impaired attention amongst dialysis patients.

- Education was associated with better visuospatial memory, better attention and lower anxiety in dialysis patients.
- Diabetes mellitus was associated with impaired learning, impaired verbal memory, impaired recognition and impaired executive function in dialysis patients.
- Increased dialysis duration was associated with lower quality of life, lower MCS, lower PCS and higher anxiety in dialysis patients.
- Patients receiving non-RAA-based medications had significantly better learning, as determined by the RAVLT task, than those patients receiving RAA-based antihypertensives. There were no differences on any other neuropsychological task.
- Non-RAA group-based therapy was associated with better verbal memory in dialysis patients.
- The AGT M268T MM genotype was associated with better recognition while T allele was associated with better visuospatial organization and memory in dialysis patients.
- The AGT T207M TM genotype was associated with better verbal memory in dialysis patients (dialysis patients in this study have only TM and TT genotypes).
- The ACE I/D I allele was associated with better recognition.
- The AT₁ CC genotype was associated with better QOL, while AA genotype was associated with better MCS and lower anxiety in dialysis patients.
- The AT₂ CA genotype was associated with better verbal learning but higher anxiety while AA genotype was associated with better executive function. The A allele was associated with better executive function, QOL, MCS, less anxiety and depression in dialysis patients.

These are all novel findings.

This is the first study of neuropsychological characteristics of Saudi dialysis patients. The CKD (chapter 3) study had identified impaired cognitive function in CKD patients and a sparing effect of RAA drugs on one aspect of neurocognitive function. However, the dialysis study (chapter 5) showed that dialysis patients did not exhibit impaired cognition, thus there was no opportunity for a 'sparing' effect of any drug treatment.

The observation from the Saudi CKD study was not reproduced in Saudi dialysis patients. Dialysis patients were not impaired in comparison to healthy control. The normal neurocognitive properties of Saudi dialysis patients were not in accordance with the previously reported neurocognitive defects in dialysis patients that mainly tapped domains of verbal memory, concentration, and executive function. Many explanations can be given.

Most of the previously studied dialysis patients were either older than 60 y.o with some risk factors of neurocognitive defects such as inadequate dialysis, anaemia, and stroke. The cohort of this study were closely monitored and on regular renal replacement therapy, therefore they were neurocognitively intact and drugs acting on RAS did not produce any neurocognitive effect. However, hemodialysis patients were found to perform better in neurocognitive tests 2 hours post-dialysis than 2 hours pre-dialysis (Tilki et al., 2004). The RAA drugs appear to improve impaired cognition, but do not enhance 'normal' cognition. This may reflect control of uraemia and accumulation of uremic toxins that are known to cause deterioration of neurocognitive symptoms. RAA and non-RAA groups did not show any significant differences in neurocognitive testing except in learning skills where the non-RAA group showed significantly better learning skills. More than one explanation can be given for such observation.

The low participation of females in the RAA group as well as the small sample size of RAA group might be a cause for superiority of non-RAA group in RAVLT performance. Women tend to perform better on most of the neurocognitive tests especially verbal tasks (Schaie, 1994). Recruiting more female patients from other dialysis centres in the same region was difficult due to the restriction of time and the time-consuming approval process for the medical protocols.

The renal replacement therapy that is the only treatment choice for ESRD patients to sustain their life makes their medical condition more stable than CKD patients and as a result less neurocognitively impaired. This may raise another issue of debate whether the good neurocognitive function of dialysis patients related to management of uraemia only or the use of RAA drugs.

This study dealt with chronic disease with many complications and variables that makes the definite conclusion regarding the sparing effect of RAA drugs on neurocognitive defects difficult. Looking for the involvement of RAS polymorphism in modulating neurocognitive response to RAA drugs will be explored in the following sections.

With respect to QOL, dialysis patients showed lower total quality of life and this decline in QOL was significantly clear in physical component summary of QOL measure. This is expected from such kind of patients with multiple hospital admissions (three times per week) for dialysis sessions that usually extend for four or five hours. In addition to the restraining effect that such a procedure will have on dialysis patients that will render them partially physically impaired. None of the dialysis patients nor the healthy control reached the level of caseness in either anxiety or depression which is usually equal or above 11 (Zigmond *et al.*, 1983; Mystakidou *et al.*, 2005). So neither dialysis patients nor healthy control fulfilled diagnostic criteria for anxiety or depression. However, poor QOL and depression have been reported in previously published studies in dialysis patients.

Several factors have been implicated in the neurocognitive defects in dialysis patients. Of these are mainly demographics such as age, gender and education level. DM and dialysis duration were also involved. All of these co-variables were documented in the literature to affect the neurocognitive outcome and may interact with RAS genotypes in a way that may mask or hide the effect of RAS polymorphisms on memory and cognition. When age, gender, education level, and DM were controlled in previous studies in CKD patients and dialysis patients, the neurocognitive defect of such patients was documented (see chapter 1 for more details). The current study has found DM to be associated with impaired learning, verbal memory, recognition and executive function, hence there could be an association between DM, ACE polymorphism, progression to ESRD and impaired cognition. The fact that might document such relationship is the association of D allele of ACE I/D polymorphism with DN and its progression to advanced disease. In this study the D allele was associated with lower recognition and this suggests the association between DM, ACE polymorphism, impaired cognition and progression to ESRD.

Female gender in this study has proven superiority in learning task which is in accordance with the published facts. Females also exhibit higher anxiety as is expected. Visuospatial memory and attention was affected by both age and education level. The RCF task involved a drawing trial of certain geometric shapes and performance usually declined in the elderly and patients with low education level. The attention task was not found to be affected by age nor education (Lezak et al., 2004). However, such finding may be pertinent to Saudi population that have been not tested before. The disease burden and the stressful condition of dialysis procedure will affect QOL and increased anxiety level that is documented by the significant association between dialysis duration and both QOL and anxiety. However, dialysis patients in this study were not anxious and their scores of anxiety did not reach the level of caseness.

The genotype association study attempted to relate neurocognitive performance to RAS genotype amongst Saudi dialysis patients. Before commenting on the associations, the comparison of genotype prevalence observed with those reported for other populations will be discussed.

It is documented that the most studied ACE gene polymorphism, the 278- base pair insertion (allele I) or deletion (allele D) variant in intron 16 on chromosome 17q23, is associated with serum and tissue ACE levels (Rigat et al., 1990). This is manifested by subjects with D alleles having elevated plasma ACE level as well as activity. Around 20% of the variation in serum ACE concentration and 16-24% of the variation of ACE activity is attributed to genetic factors (Danilov et al., 1996). However, other factors can influence ACE activity such as salt intake, cardiovascular disorders, diabetic nephropathy, as well as ACE inhibitor therapy (Rudnicki *et al.*, 2003).

ACE genotype frequencies in this study did not significantly differ between dialysis patients and control subjects. The distribution also fulfilled the Hardy-Weinberg equilibrium. However, several studies on Arabic populations with different findings have been published. Focusing on ACE gene distribution in healthy Arabic population revealed varying percentage of DD genotype that ranges from 32.9% in Syrian population to 58.7% in Saudi population. Our population DD genotype was within the reported range of Arabs population and it is at 50%.

With respect to D allele, a range from 0.60 in Syrian population to 0.78 in Saudi population was reported and our finding (0.72) is within this range. There were only two studies on the Saudi population addressing the ACE I/D distribution (Dzimiri *et al.*, 2000; El-Hazmi *et al.*, 2003). The results of the Saudi population genotyping studies coincide with the geographic distribution trend of II genotype reported by Saab *et al.* (Saab *et al.*, 2007a). Dzimiri *et al.* reported a prevalence of 7.3% of II genotype while El-Hazmi *et al.* reported only 1.3% in southern area of Arabian peninsula. The current study covers a similar region to Dzimiri *et al.* study with finding of II genotype prevalence (5%) quite similar to them.

This is the first study investigating RAS genotype distribution in Saudi dialysis patients. The DD genotype frequency (52.8%) is higher than that reported in Indian ESRD (29%) (Tripathi *et al.*, 2006), Indian DN patients (28%) (Prasad *et al.*, 2006), Chinese DN patients (25%) (Wu *et al.*, 2000), and Swiss-German ESRD patients (15.8%) (Lovati *et al.*, 2001).

Data on Arab dialysis or ESRD patients is lacking. Since the DD genotype distribution accounted for 50% in healthy Saudi and 52.8% in Saudi dialysis patients, the ethnic origin of Saudi healthy and dialysis patients might be the only explanation for this difference, but still other variables cannot be excluded since data on Saudi ESRD from other studies are lacking. The D allele frequency of ACE gene was also higher (0.73) in Saudi dialysis patients than Indian ESRD (0.46) (Tripathi *et al.*, 2006), Indian DN (0.47) (Prasad *et al.*, 2006), Chinese DN (0.47) (Wu *et al.*, 2000), and Caucasian ESRD (0.46) (Lovati *et al.*, 2001) patients which strengthens the ethnic origin as a cause of this difference in ACE genotype distribution.

Most of the physiological effects of Ang II were modulated by AT₁R which mainly includes renal, hepatic and neuronal effects. The result of genotype prevalence of AT₁R A1166C polymorphism in this study is quite similar to the study by Saab *et al.* which was performed in the Lebanese population. The C allele prevalence in this study (24%) is similar to that reported by Saab *et al.* and it has been documented that the prevalence of the C allele was significantly higher in Caucasians than in Asian populations. Hardy-Weinberg equilibrium was confirmed for this polymorphism only in Saudi healthy population but not in dialysis patients. It is also worth noting that most of the clinical

studies addressing the prevalence of AT₁R polymorphism in kidney disease found that C allele is higher and associated with faster progression of renal disease. However, limited studies reported no association (see details in chapter 1).

The AT₂R C3123A polymorphism is located on chromosome X, so males and females have been studied separately. The C allele prevalence in Saudi healthy control (50%) in this study is almost similar to that documented in Lebanese population (55%). However a lower prevalence of the C allele was reported in Saudi dialysis patients (44%) in this study. Such a difference in dialysis patients may favor the relative contribution of A allele to disease state which was documented in hypertensive young male patients (Katsuya *et al.*, 1999). Making a conclusive statement is difficult due to the lack of enough genotyping studies of this genotype. Testing for Hardy-Weinberg equilibrium was not confirmed in either healthy controls (no females) and dialysis patients.

It is well known that the TT genotype of the AGT M268T polymorphism is associated with increased circulating AGT where it is associated with 10-20% higher level of angiotensinogen than MM genotype (Jeunemaitre *et al.*, 1992). This association may predispose such patients with TT genotype to multiple diseases and probably more neurocognitive defect. Angiotensinogen is not rate-limiting step in RAS activity. This means that higher level of angiotensinogen will not lead to increased level of angiotensin II that might be responsible about impaired cognition, although a decrease in angiotensinogen may result in a decrease in angiotensin II.

The prevalence of M variant in the Lebanese population is 46% (Saab *et al.*, 2007b), in Caucasian 60% (Lovati *et al.*, 2001), and around 18% in Asian population (Japanese, Korean, and Chinese) (Kim *et al.*, 2006; Nishikino *et al.*, 2006; Wu *et al.*, 2000). The T variant is less common in whites than in blacks and Asians (Wang *et al.*, 2000). TT genotype of this polymorphism was confirmed to be associated with DN in Asians, Caucasians, and Arabs. This study confirmed a similar prevalence where the T variant accounts for 63% and 68% in Saudi healthy control and dialysis patients, respectively. The result appeared to favor the T variant association with ESRD although the difference was non-significant. The genotype distribution is in agreement with Hardy-Weinberg equilibrium.

Little is known regarding the association of AGT T207M polymorphism with disease state. However, similar genotype distribution of this gene was reported in dialysis patients, DN patients, and healthy control of Asians and Caucasians origin (Losito *et al.*, 2002; Prasad *et al.*, 2006; Wu *et al.*, 2000). This study confirmed the same finding. This genotype distribution also fulfilled the Hardy-Weinberg equilibrium.

The basic hypothesis of this thesis was that polymorphisms associated with increased angiotensin activity might be associated with poorer neurocognitive function. Part of the study was looking for the involvement of ACE I/D genotype in modulating neurocognitive abilities of dialysis patients as a consequence to the involvement of D alleles in activity and plasma level of ACE. The hypothesis that this study is testing is whether the D allele carriers that have higher plasma level and activity of ACE will be more neurocognitively impaired. In addition, will this impairment be opposed by RAA drugs that block the action of any increased Ang II that may accumulate due to high ACE level?

Previous reports have documented the higher risk of diabetic nephropathy and progression to end-stage renal disease in Asian carriers of DD polymorphism of ACE I/D genotype (Ng *et al.*, 2005), as well as hypertension and left ventricular hypertrophy (Staessen *et al.*, 1997b). The current study has found that the I allele of the ACE I/D is associated with better recognition. It also has found DM to be associated with impaired learning, verbal memory, recognition and executive function, hence there could be an association between DM, ACE polymorphism, progression to ESRD and impaired cognition.

RAS gene polymorphism was also implicated as a cause of neurocognitive defect in many studies. Again no such relationship between RAS genotypes and neurocognitive defects in Saudi or Arab population has been studied before, so exploring such a relationship will be done on variety of ethnic groups published in the literature rather than to Saudi or Arabs.

All studied populations were either elderly with neurocognitive defect or with vascular dementia (VD) or Alzheimer's disease (AD). A problem in comparison may arise because the studied Saudi population is much younger (mean age 35 years). This is

going to make the comparison between the underlying studied Saudi population and other published studied population that were older with diagnosed neurocognitive defects unfair.

The DD genotype of ACE gene was only 20% in a group of elderly African-Caribbean with cognitive decline (Stewart et al., 2004), about 27% in Caucasian with late-onset AD (Helbecque et al., 2009), 16% in Korean VD (Kim et al., 2006), and surprisingly about 38% in Caucasian elderly with cognitive impairment (Amouyel et al., 1996). DD genotype had the lowest cognitive score in a group of highly performing elderly subjects when the MMSE was used to assess subject's cognitive function (Richard et al., 2000).

There were no significant differences in allelic distribution of any ACE I/D polymorphism between RAA and non-RAA groups or between the dialysis patients and the control group. In this study, regression analysis was performed twice. The first analysis involved the different genotypes of the previously mentioned RAS genes. The second regression analysis involved alleles of the different RAS gene. A very important aspect of alleles analysis is that each patient carries two alleles and so doubling of the sample size is mandatory in regression analysis. Doubling the sample size in case-control studies is appropriate when the Hardy-Weinberg equilibrium is satisfied (Sasieni, 1997).

Regression analysis documented the association of the I allele of the ACE I/D polymorphism with better recognition in dialysis patients. It is worth noting that most dialysis patients perform well on neurocognitive tests in comparison to both CKD and healthy subjects tested in the first study of this thesis. The implication of D allele-induced cognitive function decline in dialysis patients replicated in this study in only recognition properties. Most of the previous studies documented the association of D allele only with demented or depressed patients. However, dialysis patients in this study are not demented or depressed which might explain the absence of such association.

Studies documenting the positive impact of ACE I/D polymorphism in mood disorder were carried out by same group of researchers published in 2002, 2004, and 2005. They showed that D allele carriers have more than 5-fold increase in risk of major depression (Bondy et al., 2002), while DD and ID carriers showed rapid improvements in

depressive symptoms after treatment with antidepressants (Baghai *et al.*, 2004; Bondy *et al.*, 2005). On the other hand, no association was found between ACE I/D polymorphism and depression in patients suffering bipolar depression nor antidepressant response.

With respect to AT₁R A1166C polymorphism, the CC genotype was significantly associated with better QOL. However, better mental component summary of QOL measure and lower anxiety were associated with the AA genotype. Mental component summary of QOL measure does involve a measure of neuropsychology. The AT₁R polymorphism makes a significant contribution to QOL, mental component summary and anxiety. However, it is very difficult to correlate between the difference in genotype association in this gene. At this point it is not clear which genotype is associated with good neuropsychological properties especially since allele association was not found in this study and further work is necessary to confirm such a finding. However, C allele was reported to be associated with increased sensitivity to Ang II (Spiering *et al.*, 2000) and might be responsible for neurocognitive defect rather than the A allele. A lack of association between the AT₁R polymorphism and neurocognitive defect has been seen in vascular dementia patients (Kim *et al.*, 2006). It is documented that angiotensin II plasma concentrations in CKD and dialysis patients are similar to healthy control. After a 7-day treatment with AT₁R antagonist (candesartan), Ang II was increased 5.5 fold relative to the level before candesartan treatment. However, this level declines significantly when measured 28 and 56 days later. This might document the absence of the impact of the AT₁R polymorphism on memory and cognition (Shibasaki *et al.*, 1999). This finding indicates that angiotensin II level is not altered in kidney disease whatever the type of the polymorphism. As a consequence of this, RAA drugs that either antagonize the AT₁R receptor or inhibit ACE will not affect plasma angiotensin II level in kidney disease patients and so will have no effect on mood and cognition. However, enhanced AT₁R expression in leukocytes of CKD patients that is not related to RAA therapy has been reported (Chon *et al.*, 2011). This finding might indicate the high activity of angiotensin II in renal impaired patients which makes the interpretation of the association of AT₁R polymorphism and neuropsychological properties of dialysis patients difficult.

Linear regression results indicate that the AT₂R C3123A polymorphism makes a significant contribution to a executive function, verbal learning, QOL, MCS, anxiety and depression. The results indicate that the CA genotype was associated with better verbal learning but higher anxiety. However, the small sample size of female patients carrying CA genotype (3 females) warrant cautious interpretation of such result. The A allele predicted better executive function, good QOL and MCS. Exploring the literature shows that no similar results have been published before. It is worth noting that the A allele was also significantly associated with lower anxiety and depression. However, no association has been reported in depressed population of Lebanese origin. This finding although first reported in the current study can be the bases for future studies that might help to explain such association.

The AGT M268T polymorphism contributed significantly to recognition, visuospatial memory and organization. The MM genotype significantly predicted good recognition while the T variant significantly predicted better visuospatial organization and visuospatial memory. This may suggest a possible role of this polymorphism in memory and cognition. Recently, it has been reported that the protective effect of ACEIs against decline of executive function was evident only in TT homozygotes carriers (Hajjar et al., 2010). However, the M variant of AGT M268T polymorphism was more common in vascular dementia patients (Kim et al., 2006). The contribution of the T variant with memory and orientation is supported by the association of increased plasma angiotensinogen in T variant carriers. However Ang II concentration was not affected. Although some reports documented the association of this polymorphism to depression (Meira-Lima et al., 2000), this study was unable to reproduce same finding.

The TM genotype of AGT T207M polymorphism predicted better verbal memory in dialysis patients. Exploring the literature discussing the association of this polymorphism identified with no such studies. However exploring its distribution in Saudi dialysis patients and healthy control as well as its association with memory may provide useful knowledge.

The RAA drugs did not show any neurocognitive-improving effects in dialysis patients in comparison to dialysis patients treated with other antihypertensives. However, non-RAA-based therapy predicted good verbal learning. High cognitive scores have been

reported in CKD patients treated with RAA drugs, reported in CKD study in chapter 3. This might suggest that the RAA drugs treat or correct the underlying cause of the impairment in CKD patients but do not have a non-specific cognition enhancing effect or in another way do not improve the 'normal cognition' that dialysis patients have.

Regarding anxiety and depression, dialysis patients did not reach level of casesness and there is no place for anti-anxiety or antidepressant effects of RAA drugs. However, evidence of a link between antidepressant activity and reduced angiotensin function has been identified, for example both losartan and captopril showed antidepressant like effects (Gard et al., 1999). Although PCS score of QOL was lower in dialysis patients compared to healthy control, the involvement of RAA drugs on this defect was not proven in dialysis study.

It should be understood that the regression technique employed in this work is designed to analyse the data arising from observational studies not randomized controlled experiments. Therefore, one should be able to include in the model the relevant risk factors that may affect the outcome and possible confounders that can reduce the bias in estimating the regression coefficient of the main risk factors. Quite often, the researcher can not include all possible confounders and to minimize the bias one should reasonably increase the sample size to reduce the residual error. This might be costly and time-consuming, however, it is possible to get a good idea about the likely size of uncertainty in the regression model by either employing a non-parametric regression technique or the use of bootstrap technology to find an empirical estimate of the measures of uncertainty which is beyond the scope of this thesis.

7.3 Conclusion

There are many reasons for defects in the neurocognitive properties of CKD patients. This kind of patient suffers chronic disease that usually progresses overtime especially those patients that are not strictly compliant to their medication or physician instructions. As a result of this kind of progressive disease, a fear of progression is also an important factor in disturbing a patient's neurocognitive properties. The multiple biochemical disorders that CKD patients usually suffer from especially anaemia, hyperphosphatemia, and high blood pressure also contribute to such neurocognitive defects. However all these factors have been controlled in the CKD study as much as possible and a finding of neurocognitive defects have been proven. RAA drugs demonstrated a sparing effect on a neurocognitive defect of CKD patients. This might suggest a role of the RAS in improvements of neurocognitive defects of CKD patients irrespective of the control of uraemia or any other kidney failure abnormality. RAA drugs have cognition-enhancing effects in CKD patients which is indicative of the involvement of RAS in neurocognitive changes in CKD patients. Absence of neurocognitive defects in colon cancer patients helped to exclude the deleterious effect of chronic disease as well as worry and fear of cancer recurrence on neurocognitive properties of colon cancer patients in remission and so exclude chronic disease, fear, and worry as causative factors of neurocognitive defects of CKD patients.

It is well known that RAA drugs prove beneficial in improving microalbumiurea (irrespective of their antihypertensive action) in renally-impaired patients and so delay progression of CKD to ESRD where dialysis or kidney transplantation is mandatory to sustain patient's life. Evidence exists from the association of the D allele of the ACE I/D polymorphism with microalbumiurea in DM and also with progression of kidney failure. This evidence might explain some abnormality of RAS in CKD which will have a deleterious effect on neurocognitive function and so RAA drugs will improve such defects.

While significant cognitive benefit of drugs acting on RAS have been documented in CKD patients, a lack of such benefit have been documented in dialysis patients. The proposed explanation of this finding is that dialysis patients chosen for this study were biochemically stable. They have most of their biochemical data within therapeutic range, closely monitored and on renal replacement therapy (hemodialysis) which all

rendered them, at least during the examination session, stable and exhibiting normal neurocognitive properties and there is no space for RAA drugs to improve their neurocognitive properties. However, previous studies have confirmed neurocognitive defect in dialysis patients that is related mainly to dialysis adequacy and history of stroke. In this study dialysis adequacy was maintained and stroke was considered an exclusion criteria. The explanation of discrepancy in the finding of neurocognitive function of dialysis patients in this study and other published studies can only be explained by the stabilized medical condition of such patients chosen for this study.

The main aim of the dialysis study was to look for the reason behind the neurocognitive defect in kidney disease patients and if it is associated to RAS gene polymorphisms. The RAS gene polymorphism's modification of the effect of RAA drugs on neurocognitive behavior was investigated. There were no neurocognitive defects in dialysis patients and as a consequence RAS gene polymorphisms did not show any modification of RAA drug effects on neurocognitive behavior of dialysis patients. Dealing with stable dialysed patients, non-depressed, non-anxious, with reasonable high score of QOL and neurocognitive properties and without severe neurocognitive risk factors such as stroke or CVA might be the suitable explanation for the absence of such association. However, a significant association of the RAS gene polymorphisms with better cognition, mood, and QOL has been demonstrated in dialysis study which makes the hypothesis of the involvement of RAS in neurocognitive behavior improvements still tenable.

It is well known that the TT genotype of the AGT M268T polymorphism was associated with 10-20% higher level of angiotensinogen (the precursor of angiotensin I and II) which may predispose individuals carrying this genotype to multiple disease and probably more neurocognitive defect. On the other hand, the association of the T variant of AGT M268T with better visuospatial organization documented in this study might explain the beneficial effect that RAA drugs exert on such T variant carriers (i.e. T variant exhibit some neurocognitive defect due to high angiotensinogen which is a precursor of Ang II, but RAA drugs abolish such bad effects that are only present in T variant carriers and so the T variant was associated with better neurocognitive function). It is also worth noting that the D allele of ACE I/D polymorphisms was associated with

impaired recognition and the D allele is known to be associated with elevated plasma level of ACE and neurocognitive properties of dialysis patients.

A very important aspect should be considered; the ACE and AGT activity were not the rate-limiting step in Ang II activity while renin is the rate limiting step. This means that only when ACE and AGT activity decrease they will decrease Ang II synthesis. As a consequence to this any polymorphism that affects the activity RAS will affect the activity of Ang II might be responsible for impaired cognition. Of these are T variant of AGT that increase AGT activity, A allele of AT₂R that increase RAS activity and C allele of AT₁R that increase RAS activity. All of the previous polymorphisms have been reported to be implicated in neurocognitive properties of dialysis patients. Finally, the increased plasma level of Ang IV that is observed after AT₁R antagonist treatment might explain the sparing effect of RAS on neurocognitive abilities of renally impaired patients.

More investigation is needed to document the mechanism of RAA drugs' neurocognitive improvements. This will necessitate using more controlled design and a large number of participants of equal male to female ratio.

In summary, CKD patients have impaired cognition which is not simply due to disease 'burden'. Dialysis patients are not impaired suggesting that kidney failure itself is not the cause of the impairment, but some consequence of the failure is. RAA drugs have better neuropsychological outcome in CKD than non-RAA drugs, suggesting either a direct effect via RAS, or better control of consequences of ESRD. Importantly, five gene polymorphisms of the RAS have been associated with neurocognition in ESRD patients. This association could reflect a positive role of RAS gene polymorphisms in modification of the RAA drugs' effect on neurocognitive function. It may suggest also that genotyping of ESRD patients may provide more information concerning selection of drug therapy for optimum neuropsychological outcome.

Future work would include recruiting a larger sample size and investigating neurocognitive properties progression during the course of the CKD disease. This is done in order to watch for the possible development of dementia in CKD patients especially patients treated with antihypertensives other than RAA drugs.

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APPENDICES

Appendix 1. Pre-testing material

- 1.1 Consent form and participant information form - study 1 and 2
- 1.2 Consent form – study 3
- 1.3 Open letter - study 3

Appendix 1.1. Consent form and participant information form – study 1 and 2

موافقة خطية

CONSENT FORM



**College of Medicine
King Saud University
Research Ethics Committee**

CONSENT FORM	نموذج إقرار
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دور الفشل الكلوي في التأثير على المعرفة و الإدراك

الباحث الرئيسي: نوره أبانمي
الباحث المشارك: د. عبد الكريم السويدة

The effect of chronic renal failure on cognitive function

Principle Investigator: Norah Abanmy

Co-Investigator: Dr. Abdulkareem Al Suwaida

You are being asked to participate voluntarily in a Research Study. If you decide to take part in this study, please sign this consent form and return it.

This consent form, a copy of which has been given to you, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

STUDY PURPOSE:

1. To develop a suitable test battery to assess cognition in Arabic speaking subjects.

نرجو منك المشاركة في هذه الدراسة البحثية وعند موافقتك بذلك نرجو منك التوقيع على هذه الورقة وإرجاعها إلينا.

هذا الإقرار، والذي سيكون لديك منه نسخة، سيعطيك فكره عن ماهية الدراسة وكيفية مشاركتك فيها. لك مطلق الحرية في السؤال عن أي شيء غير واضح في هذا النموذج أو حتى معلومات لم تذكر تود الإستفسار عنها. لديك الوقت الكافي لقراءة النموذج و فهمه.

الغرض من الدراسة:

1. تطوير استخدام اختبارات تقييم المعرفة و الإدراك للمرضى متحدثي اللغة العربية حيث انه لا توجد دراسات

2. To study cognition in patients with chronic renal failure in comparison to colon carcinoma patients in remission and healthy volunteers.
3. To compare the effects of different medications on cognition in patients with chronic renal failure

منشورة من هذا النوع .
 2. دراسة الحالة النفسية و العصبية
 لمرضى الفشل الكلوي دون تدخل طبي
 ومقارنتهم بمتطوعين أصحاء وبمرضى
 سرطان القولون المتعافين حيث تعمل
 لديهم وضائف الكلى بشكل جيد.
 3. مقارنة تأثير الأدوية المختلفة على المعرفة و
 الإدراك في مرضى الفشل الكلوي.

STUDY PLAN:

60 patient (30 using ACEI or ARB and 30 using other antihypertensive drugs) from inpatient and outpatient adult renal failure patients in King Khaled University Hospital (KKUH) are going to be compared against patients with colon cancer in remission and healthy volunteers to look for any cognitive differences among them.

The cognitive tests being used are going to be carried out and translated to Arabic by the investigators under clinical psychologist supervision for 30-45 min. A relationship between the improvement in cognition and different medications is also going to be investigated.

- Detailed explanation of the tests will be provided to patient practically by the researcher.

الهدف من الدراسة:
 سوف يخضع 60 (30 يتعالجون بأدوية علاج ارتفاع الضغط من فئة الأدوية التي تعمل على الأنجيوتنسين و 30 يتعالجون بأدوية علاج الضغط الأخرى) من مرضى الفشل الكلوي البالغين في مستشفى الملك خالد الجامعي المنومين و مراجعي العيادات الخارجية لإختبارات تقييم المعرفة و الإدراك من قبل متدرب جيد تحت اشراف طبيب متخصص ولمدة 30-45 دقيقة. كما سيخضع ايضا كذلك 30 من مرضى سرطان القولون المتعافين وكذلك 30 من متطوعين اصحاء لنفس الإختبارات. هذه الإختبارات طورت لتناسب المجتمع المحلي و ترجمت الى العربية تحت اشراف طبيب متخصص. كما سيكون هناك اجراء علاقة بين الأدوية المختلفة التي يستخدمها المرضى و التحسن في المعرفة و الإدراك.
 - سيقوم الباحث بشرح تفصيلي للإختبارات بشكل عملي عند لقاء المريض.

N.B. ACEI: angiotensin converting enzyme inhibitors, ARB: angiotensin receptor blockers.

BENEFITS:

The result of this study may not benefit you directly, but in the future patients might benefit from the knowledge acquired.

الاستفادة المرجوة من الدراسة:
 إن الاستفادة من هذه الدراسة قد لا تعود عليك مباشرة ولكن قد تكون لنتائج هذا البحث تأثيرات على معالجة المرضى الآخرين.

SIDE EFFECT: There are no side effects. Your participation in this study has any further risks or discomfort to you.

الآثار الجانبية: لا توجد هناك أي أضرار جانبية من هذه الدراسة ومشاركتك لا تسبب أي إزعاج أو مخاطر مستقبلاً

REFUSAL:

- I do have the right to withdraw from this

عدم الرغبة في المشاركة:
 - إنني أعلم بأنه لي الحق في الانسحاب في أي

study at any time, by telling my doctor. My decision to withdraw, or to decide not to participate, will in no way affect my ongoing treatment, to my relationship with my doctor and there will be no penalty or loss of benefits.

CONFIDENTIALITY:

Your participation in this study will be kept confidential. The results of this research may be published, however, your identity will never be revealed.

All documents will be identified only by code number and kept in a locked filing cabinet. Subjects will not be identified by name in any reports of the completed study.

APPROVAL:

I fully understand the information and the consent form.

Your participation in this study is entirely voluntary and you may refuse to participate or withdraw from the study at any time without jeopardy to your privileges and access to health care.

Detailed explanation of tests has been provided by the researcher.

Your signature below indicates that you have received a copy of this consent form for your own records.

Your signature indicates that you consent to participate in this study.

I sign freely and voluntarily. A copy has been given to me.

Investigator: Norah Abanmy

Signature:

Date:

Patient Name:

Signature:

Date:

Witness Name:

Signature:

Date:

If you have any further concerns or questions, you can contact Dr. Norah Abanmy Tel # 4919282.

وقت من هذه الدراسة وذلك بإخطار طبيبي المعالج بأني قررت الانسحاب أو قررت عدم المشاركة وعليه فإنك لن تتعرض لأي جزء أو فقدان للمزايا ولن يؤثر ذلك على علاجك أو علاقتك بالطبيب.

سرية المعلومات:

إن مشاركتك في هذه الدراسة ستكون في غاية السرية. قد يتم نشر نتائج هذا البحث لأغراض أكاديمية ولكن لن يتم الكشف عن هويتك في أي حال من الأحوال. سيتم التعرف على و حفظ هذه الإختبارات بأرقام سرية في أدراج آمنه و مقفله بدون اشاره الى إسم المريض.

الموافقة بالمشاركة:

استوعبت المعلومات في هذا النموذج, لذا أوافق طوعا بالمشاركة في هذه الدراسة.

لك الحق في الإنسحاب في أي وقت دون أن تتعرض لأي جزء أو فقدان للمزايا ولن يؤثر ذلك على علاجك أو علاقتك بالطبيب.

لقد تم شرح هذا النموذج للمتبرع بواسطة أحد الباحثين قبل طلب التوقيع منه.

إن توقيعك يثبت أنك حصلت على نسخة من هذا الإقرار وأنك وافقت بمحض إرادتك على المشاركة في هذه الدراسة.

أوقع أنا بمحض إرادتي وحرיתי وقد تم إعطائي نسخة من الإقرار.

أسم الباحث: نوره أبانمي

التوقيع:

التاريخ:

أسم المريض:

التوقيع:

التاريخ:

أسم الشاهد:

التوقيع:

التاريخ:

عند الرغبة في أي استفسار عن هذه الدراسة يمكن أن تتصل بالباحثة: نوره أبانمي على تليفون رقم 4919282.

Appendix 1.2. Consent form - study 3



INFORMED CONSENT (CLINICAL STUDY)

مذكرة موافقة (دراسة سريرية)

TITLE	
The role of angiotensin in mood and cognition in end-satge renal disease	
PRINCIPAL INVESTIGATOR / DOCTOR	
Norah Abanmy in collaboration with Dr. Hejaili	

عنوان الدراسة	
دور هرمون الأنجيوتنسين في التأثير على التفكير والإدراك في مرضى الفشل الكلوي	
الطبيب الباحث	
نوره ابانمي بالمشاركة مع د. فايز الحجيلي	

Having discussed this research project with

Dr.	Norah Abanmy
------------	--------------

And reviewed the OPEN LETTER, which is attached, I agree, voluntarily to the participation in this study:

بعد مناقشة بحث هذه الدراسة مع :

الطبيب	نوره ابانمي
---------------	-------------

ومراجعة المعلومات المفصلة عن الدراسة المرفقة فأذني أوافق طوعاً على المشاركة في هذه الدراسة .

Patient's name	
Relationship	

اسم المريض	
العلاقة بالمريض	

1. I understand that I will be participating in a study, which may, or may not benefit me directly, but will provide new knowledge, which could benefit other patients with similar conditions to mine in the future.
2. I also understand that I do have the right to withdraw from this study at any time, by telling my doctor. My decision to withdraw, or to decide not to participate, will in no way affect my ongoing treatment, to my relationship with my doctor.
3. I give permission for the doctor to read my medical records, and to publish or report the findings of this study at scientific meetings in the future, knowing that my identity will not be revealed. The doctor will explain the results of this study at the end.

1- أنا أعلم بأنني سوف أشترك في هذه الدراسة ومن المحتمل أن تكون ذات فائدة بطريقة مباشرة أو غير مباشرة ولكنها سوف توفر معلومات يمكن أن تفيد مرضى آخرين يمثل حالتني في المستقبل.

2- بالإضافة إلى ذلك فأذني أعلم بأنه لي الحق في الانسحاب في أي وقت من هذه الدراسة وذلك بإخطار طبيبي المعالج فأذني قررت الانسحاب أو قررت عدم المشاركة ولن يؤثر ذلك على علاجي أو علاقتي بالطبيب

3- أذني قد فوضت الطبيب بمراجعة ملفي الطبي ونشر أو تقديم نتائج الدراسة في المؤتمرات الطبية في المستقبل مع عدم ذكر اسمي. وفي نهاية الدراسة سيشرح لي الطبيب نتائجها.

Signature	
Witness	
Investigator/Doctor	

توقيع المريض	
شاهد	
الباحث / الطبيب	

Appendix1.3. Open letter - study 3

OPEN LETTER TO STUDY PARTICIPANTS رسالة توضيحية للمشاركين في الدراسة

TITLE: The Role of Angiotensin in Mood and Cognition in End-Stage Renal Disease

عنوان
لدراسة

دور هرمون الأنجيوتنسين في التأثير على المزاج والإدراك والذاكرة في مرضى الفشل الكلوي

PRINCIPAL DOCTOR/ INVESTIGATOR: Norah Abanmy

الطبيب الباحث : نوره ابانمي

TELEPHONE #:

الهاتف : 0505467885

◆ مشكلة البحث :-
يعاني مرضى الفشل الكلوي من تغيرات في المزاج والتفكير والإدراك، لذا نود ان نقوم بدراسة هذه التغيرات في مرضى الفشل الكلوي. بالإضافة الى التوصل الى ايجاد السبب خلف هذه الأعراض حيث أثبتت بعض الدراسات ان هرمون الأنجيوتنسين المفرز من الكلى له دور في هذه الأعراض.

◆ أهمية البحث :-
دراسة السبب خلف التغيرات في المعرفة و الإدراك و ايجاد حل لتحسينها.

◆ أهداف البحث :-
1. دراسة التغيرات التي تطرأ على المزاج و التفكير والإدراك لمرضى الفشل الكلوي دون تدخل طبي.
3. مقارنة تأثير الأدوية المختلفة على التفكير و الإدراك في مرضى الفشل الكلوي.

◆ منهجية البحث :-
سوف يخضع مرضى الفشل الكلوي لإختبارات تقييم التفكير و الإدراك من قبل الباحث الرئيسي لمدة لا تتجاوز النصف ساعه بالإضافة الى سحب مقدار 10 مل من الدم لتحليلها بهدف فحص التغيرات الطارئة على جينات هرمون الأنجيوتنسين. كما سيكون هناك اجراء علاقة بين الأدوية المختلفة التي يستخدمها المرضى و الأنجيوتنسين و التحسن في التفكير و الإدراك.

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Appendix 2. Data collection Sheets

2.1 Demographics and medical condition

2.2 Biochemical variables

2.1 Demographics and medical condition

Prince Salman

Data collection sheet:

Disease since:	Pt. ID	sex	age	Educa- tion	ACEI/d ate	ARBs/date	B- Block/date	Ca Chan. Block/date	Diuretic/ date	Initial diagnosis	HTN	DM	Other disease	Duration of dialysis
3 yr	S355	♂	27	6	-	-	-	Amlor	-	unknown	-	*	-	3 yr
2.18 yr	S224	♂	26	6	-	-	-	Amlor	-	=	-	x	-	2.3
one yr	S487	♂	25	12	-	Isosorbide	-	-	-	↑BP	-	✓	-	5
5 yr	S006	♀	42	9	-	-	-	Amlor	-	unknown	-	✓	-	2
2 yr	S464	♂	29	14	fenelapril	-	Atenolol	Amlor	-	HTN	-	✓	-	6
3 yrs	S74	♂	56	16	enalapril	-	-	amlor	-	unknown	-	✓	-	4
4 yr	S062	♂	27	12	-	-	-	Amlor	-	=	✓	✓	-	2

2.2 Biochemical variables

Prince Siliem

Patient ID#	Hgb	BP	Na	K	Ca ²⁺	PTH	TSH	Steroids	Hx. Of stroke	KtV
S355	10.3	132/90	140	4.2	1.81	1.92	24.2	N	—	1.36
S224	10.2	152/90	140	3.3	2.27	1.38	64.76	N	—	1.43
S487	13.1	132/90	139	4.4	2.29	1.22	39.63	N	—	1.41
S06	13.3	142/90	135	3.1	2.4	1.21	83.83	N	—	1.37
S464	11.2	141/90	134	3.7	2.26	1.14	23.48	N	—	1.38
S474	10.1	140/80	135	4.2	2.18	1.36	91.73	N	—	1.4
S062	13	132/80	140	4.6	2.4	2.32	103.8	N	—	1.39
S273	11.3	132/90	137	4.6	2.14	1.96	44.95	N	—	1.4
S235	11.2	122/90	138	4.5	2.11	1.25	14.29	N	—	1.41
S450	10	122/80	138	3.7	2.06	1.40	42.62	N	—	1.4
S415	11.5	130/80	140	4.9	2.49	1.87	99.76	N	—	1.31
S432	12.2	135/90	136	3.8	2.77	2.05	23.05	N	—	1.32

Ca x PTH < 70 no sent calcimim therapy

Appendix 3. TEST BATTERY

- 3.1. Rey auditory-verbal learning test (RAVLT)
- 3.2. Rey-Osterrieth complex figure (ROF)
- 3.3. Digit-symbol-substitution test (DSST)
- 3.4. Letter cancellation
- 3.5. Mental Fluency
- 3.6. SF-36 Quality of Life (SF-36 QOL)
- 3.7. Hospital anxiety and depression scale (HADS)

Appendix 3.1. Rey auditory-verbal learning test

7 بعد 20 دقيقه من آخر محاولة	6 (بدون اعاده)	ب	5	4	3	2	1
أنف	أنف	قارب	أنف	أنف	أنف	أنف	أنف
دجاجه	دجاجه	خروف	دجاجه	دجاجه	دجاجه	دجاجه	دجاجه
لون	لون	بندقية	لون	لون	لون	لون	لون
شقه	شقه	قلم رصاص	شقه	شقه	شقه	شقه	شقه
نهر	نهر	مسجد	نهر	نهر	نهر	نهر	نهر
ستاره	ستاره	سمكه	ستاره	ستاره	ستاره	ستاره	ستاره
طبله	طبله	مكتب	طبله	طبله	طبله	طبله	طبله
جرس	جرس	حارس	جرس	جرس	جرس	جرس	جرس
قهوه	قهوه	طائر	قهوه	قهوه	قهوه	قهوه	قهوه
مدرسه	مدرسه	حذاء	مدرسه	مدرسه	مدرسه	مدرسه	مدرسه
أهل	أهل	موقد	أهل	أهل	أهل	أهل	أهل
قمر	قمر	جبل	قمر	قمر	قمر	قمر	قمر
حديقه	حديقه	نظارات	حديقه	حديقه	حديقه	حديقه	حديقه
طاقية	طاقية	منشفه	طاقية	طاقية	طاقية	طاقية	طاقية
مزارع	مزارع	سحاب	مزارع	مزارع	مزارع	مزارع	مزارع

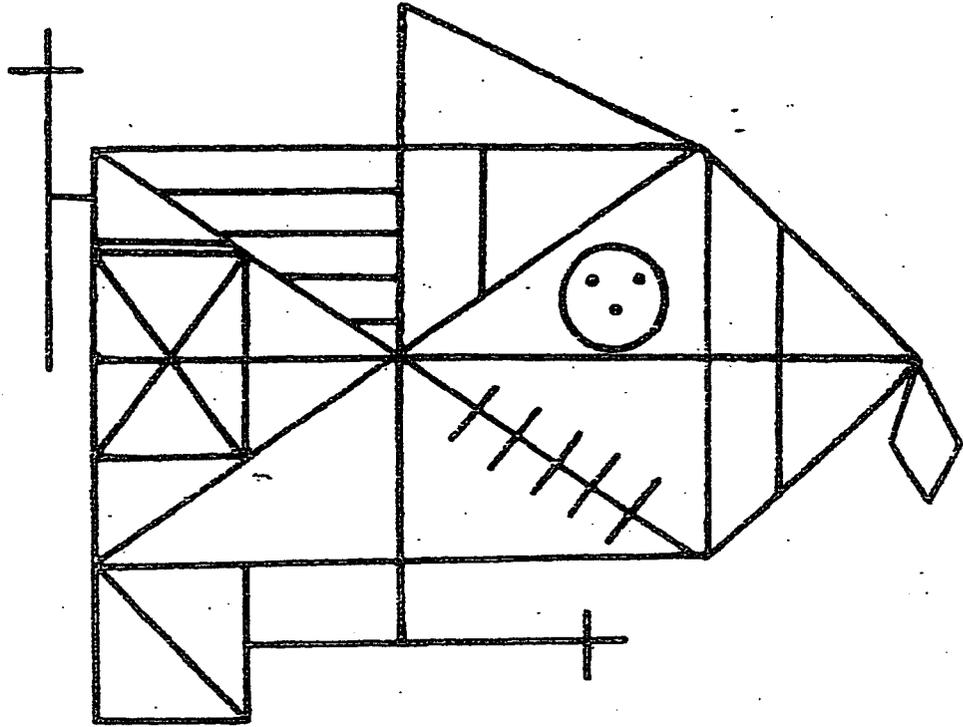
Recognition List of RAVLT

نظارات	قارب	منشفه	بيت	جرس
شراب	حر	ستاره	سمكه	شباك
حذاء	أهل	زهرة	قمر	طاقیه
مدرس	ماء	لون	شجره	حضیره
موقد	مزارع	مكتب	بالون	حارس
شبكة	ورده	بندقیه	طائر	أنف
طفل	سحاب	قلم ألوان	جبل	طقس
طبلة	شقه	مسجد	قهوه	مدرسه
حلوى	غريب	دجاجه	فأر	يد
مصباح	حديقه	نافوره	نهر	قلم رصاص

Appendix 3.2. Rey-Osterrieth complex figure

- الرجاء نسخ الشكل في المكان الفارغ اسفل الشكل.

- Please copy the figure below in the space provided.



Appendix 3.3. Digit-symbol-substitution test

Digit	1	2	3	4	5	6	7	8	9
Symbol	—	⊥	⊏	⊥	○	⊔	∧	×	=

2	1	3	7	2	4	4	8	2	1	3	2	1	4	2	3	5	2	3	1	4	5	6	3	1	4
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

9	2	8	1	7	9	4	6	8	5	9	7	1	8	5	2	9	4	8	6	3	7	9	8	6
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Appendix 3.4. Letter cancellation test

- الرجاء شطب الحرفين C و E المتكررين في الصفحة التالية, حاول تكون سريع ودقيق في الاختيار.

- Please try to cancel each C and E in the list below, try to be quick and accurate as you can.

BEIFHEFEGICHEICBDACHFBEDACDAFCIHCFEBAFEACFCHBDCFCHE

CAHEFACDCFEHBFCADEHAIEGDECHBCAGGCIERICDBCCFDEBA

EBCAFCBEHFAEFECCHGDEHBAEGDACHEBAEEDCCDAFCBIFEADCCBEACG

CDCACHEFBCAFEABFCHDEFCCACBEDCFAHHEFDICHBIEBCAHCDEFB

ACBCCBIEHACAFICABECFBFEFAEBCCGFACDBEBCHFEADHCAIEFEC

EDHBCADCEADFEBEICACGEDACHGEDCABAEFBCHDACCCBEHCDFEHAIE

Appendix 3.5 Mental fluency

- في هذا الاختبار حاول اعطاء اسماء حيوانات باكثر عدد ممكن خلال دقيقه واحده.

- for this task try to give animal names as much as you can in one minute.

استبيان صحى Appendix 3.6. SF-36 Quality of life

- الجنس ذكر
 انثى
- العمر _____ سنة
- المؤهل العلمي:
- ابتدائي
 - اعدادي
 - ثانوي
 - بكالوريوس
 - ماجستير
 - دكتوراه

من فضلك، أجب على كل الأسئلة الموجودة في هذا الاستبيان. في حالة عدم وضوح أي سؤال، أرجو اختيار أقرب اجابة لمفهومك للسؤال.

١- بصورة عامة، كيف ترى حالتك الصحية؟

(اختر اجابة واحدة وضع علامة ✓ أمام الاجابة المناسبة)

- ممتازة
- جيد جدا
- جيدة
- لا بأس بها
- سيئة

٢- مقارنة بعام مضى، كيف تقيم حالتك الصحية الآن بصورة عامة؟

(اختر اجابة واحدة وضع علامة ✓ أمام الاجابة المناسبة)

- أفضل بكثير مما كانت عليه قبل عام
- أفضل نوعا ما من العام الماضي
- تقريبا على ما هي عليه
- أسوأ نوعا ما من العام الماضي
- أسوأ بكثير مما كانت عليه قبل عام

(اختر اجابة واحدة وضع علامة ✓ تحت الاجابة المناسبة)

٣- تتعلق البنود التالية بأنشطة يمكن أن تقوم بها خلال يومك العادي،
في الوقت العالي، الى اي مدى تقيدك حالتك الصحية:

لا تقيدني اطلاقا	نعم تقيدني قليلا	نعم تقيدني كثيرا	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(أ) من ممارسة الأنشطة الشاقة مثل: الجري، حمل الأشياء الثقيلة او مزاولة الأنشطة الرياضية المجهدة جدا؟
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(ب) من ممارسة الأنشطة متوسطة الجهد، كتحريك الطاولة او التنظيف باستخدام المكنسة الكهربائية او تنظيف حديقة المنزل والعناية بها ؟
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(ج) من حمل المشتريات من البقالة او السوق المركزي (السوبرماركت)؟
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(د) من صعود الدرج لعدة ادوار؟
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(هـ) من صعود الدرج لدور واحد فقط؟
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(و) من الانحناء او الركوع او السجود ؟
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(ز) من المشي لأكثر من كيلومتر ونصف؟
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(ح) من المشي مسافة نصف كيلومترا؟
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(ط) من المشي مسافة مئة متر؟
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	(ي) من الاستحمام او ارتداء الملابس بنفسك؟

الصحة الجسمية

(اختر اجابة واحدة وضع علامة ✓ تحت الاجابة المناسبة)

٤- تتعلق البنود التالية (أ ، ب ، ج ، د) بالمشاكل التي يمكن ان تواجهك خلال تديتك لعملك او للأنشطة اليومية المعتادة نتيجة لحالتك الصحية الجسمية. خلال الأسابيع الأربعة الماضية، هل تسببت حالتك الصحية الجسمية في:

لا	نعم	
<input type="checkbox"/>	<input type="checkbox"/>	(أ) التقليل من الوقت الذي تقضيه في العمل او اي أنشطة أخرى؟
<input type="checkbox"/>	<input type="checkbox"/>	(ب) التقليل مما تود انجازه من العمل أو أي أنشطة أخرى؟
<input type="checkbox"/>	<input type="checkbox"/>	(ج) تقييدك في أداء نوع معين من الأعمال أو أي أنشطة أخرى؟
<input type="checkbox"/>	<input type="checkbox"/>	(د) أن تجد صعوبة في تذية العمل أو أي أنشطة أخرى؟ (على سبيل المثال، احتجت الى جهد اضافي لتذيتها)

الصحة النفسية

(اختر اجابة واحدة وضع علامة ✓ تحت الاجابة المناسبة)

٥- تتعلق البنود التالية (أ ، ب ، ج ، د) بالمشاكل التي يمكن ان تواجهك خلال تديتك لعملك او للأنشطة اليومية المعتادة كنتيجة لحالتك الصحية النفسية. (مثلا الشعور بالاكئاب او القلق) خلال الاسابيع الأربعة الماضية، هل تسببت حالتك الصحية النفسية في:

لا	نعم	
<input type="checkbox"/>	<input type="checkbox"/>	(أ) التقليل من الوقت الذي تقضيه في العمل او اي أنشطة أخرى؟
<input type="checkbox"/>	<input type="checkbox"/>	(ب) التقليل مما تود انجازه من العمل أو أي أنشطة أخرى؟
<input type="checkbox"/>	<input type="checkbox"/>	(ج) عدم انجاز العمل او اي أنشطة أخرى بالحرص المعتاد؟

الصحة الجسمية او النفسية

٦- خلال الاسبوع الاربعة الماضية، الى اي مدى تعارضت صحتك الجسمية او النفسية مع تأديتك لنشاطاتك الاجتماعية المعتادة مع عائلتك او اصدقائك او جيرانك او اي من المناسبات الاجتماعية الأخرى؟

(اختر اجابة واحدة وضع علامة ✓ أمام الاجابة المناسبة)

- لم يكن هناك أي تعارض اطلاقا
- كان هناك تعارض قليل
- كان هناك تعارض متوسط
- كان هناك تعارض كبير
- كان هناك تعارض كبير جدا

شدة الألم

٧- ما شدة الألم الجسدي الذي عانيت منه خلال الاسبوع الاربعة الماضية؟

(اختر اجابة واحدة وضع علامة ✓ أمام الاجابة المناسبة)

- لم يكن هناك أي ألم
- كان هناك ألم خفيف جدا
- كان هناك ألم خفيف
- كان هناك ألم متوسط
- كان هناك ألم شديد
- كان هناك ألم شديد جدا

٨- خلال الاسبوع الاربعة الماضية، الى اي مدى ادى الالم الجسمي الى التعارض مع تأديتك لأعمالك المعتادة (سواء داخل المنزل او خارجه)؟

(اختر اجابة واحدة وضع علامة ✓ أمام الاجابة المناسبة)

- لم يكن هناك أي تعارض
- كان هناك تعارض قليل جدا
- كان هناك تعارض متوسط
- كان هناك تعارض كبير
- كان هناك تعارض كبير جدا

٩- الأسئلة التالية تتعلق بكيفية شعورك وطبيعة سير الأمور معك خلال الأسابيع الأربعة الماضية، الرجاء إعطاء اجابة واحدة لكل سؤال بحيث تكون هذه الاجابة هي الأقرب الى الحالة التي كنت تشعر بها.
خلال الأسابيع الأربعة الماضية، كم من الوقت:

(اختر اجابة واحدة وضع علامة ✓ تحت الاجابة المناسبة)

لم اشعر في أي وقت من الأوقات	في قليل من الأوقات	في بعض الأوقات	في كثير من الأوقات	في معظم الأوقات	في كل الأوقات
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
أ) شعرت بأنك مليء بالحيوية والنشاط؟					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ب) كنت شخصاً عصيباً جداً؟					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ج) شعرت بأنك في حالة اكتئاب الى درجة لم يمكن معها إبخال السرور اليك؟					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
د) شعرت بالهذوء والطمأنينة؟					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
هـ) كانت لديك طاقة كبيرة؟					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
و) شعرت بالاحباط واليأس؟					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ز) شعرت بأنك منهك (استنفذت قواك)؟					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ح) شعرت بأنك شخص سعيد؟					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ط) شعرت بأنك تعبان؟					

١٠- خلال الاسابيع الاربعة الماضية، ما مقدار الوقت الذي تعارضت فيه صحتك الجسمية او مشاكلك النفسية مع نشاطاتك الاجتماعية (مثل زيارة الأصدقاء والأقارب وغير ذلك) ؟

(اختر اجابة واحدة وضع علامة ✓ امام الاجابة المناسبة)

- كان التعارض في كل الاوقات
- كان التعارض في معظم الاوقات
- كان التعارض في بعض الاوقات
- كان التعارض في قليل من الاوقات
- لم يكن هنالك تعارض في أي وقت من الاوقات

١١- ما مدى صحة او خطأ كل من العبارات التالية (أ ، ب ، ج ، د) بالنسبة الي حالتك الصحية؟

(اختر اجابة واحدة وضع علامة ✓ تحت الاجابة المناسبة)

صحيحة بلا شك	صحيحة غالبا	لا اعلم	خطأ غالبا	خطأ بلا شك	
<input type="checkbox"/>	(أ) يبدو أنني أصاب بالمرض أسهل من الآخرين.				
<input type="checkbox"/>	(ب) حالتي الصحية مساوية لأي شخص أعرف.				
<input type="checkbox"/>	(ج) أتوقع أن تسوء حالتي الصحية.				
<input type="checkbox"/>	(د) حالتي الصحية ممتازة.				

***** شكرا لتعاونكم *****

Appendix 3.7. Hospital anxiety and depression scale

- الرجاء اختيار الاجابة المناسبه.
- Please choose the suitable answer

ق- اشعر بحالة توتر و ضيق:

- 3- معظم الوقت
- 2- كثير من الوقت
- 1- احيانا
- 0- ابدا

إ-مازلت استمتع بالأشياء التي كنت استمتع بها من قبل:

- 0 - بنفس الدرجة تماما
- 1 - بدرجة أقل
- 2 - بدرجة قليلة
- 3 - لا أستمتع بأي منها ابدا

ق- ينتابني احساس مخيف وكان شيئا سينا على وشك الحدوث:

- 3- اكيد وبدرجة عالية
- 2- نعم لكن ليس بدرجة عالية
- 1- قليلا لكن لا يقلقني
- 0- أبدا

إ-استطيع ان أضحك و أرى الفكاهة في الموقف:

- 0 - تماما وبنفس القدر من قبل
- 1 - بدرجة أقل
- 2 - بدرجة قليلة ولكن ليس مثل ماكنت في الماضي
- 3 - ابدا

ق- تراودني أفكار مقلقه:

- 3معظم الوقت
- 2-كثير من الوقت
- 1-احيانا وليس كثيرا
- 0-قليل جدا

إ- اشعر بالمرح:

- 3-ابدا
- 2-قليلًا
- 1-أحيانا
- 0-معظم الوقت

ق- استطيع ان أشعر بالهدوء و الإرتياح:

- 0-اكيد
- 1-عادة
- 2-ليس كثيرا
- 3-ابدا

إ-أشعر و كأننى اصبحت بطيء الحركة:

- 3-معظم الوقت
- 2-كثير من الوقت
- 1-أحيانا
- 0-ابدا

ق- أشعر بالوجس:

- 0-ابدا
- 1-أحيانا
- 2-كثيرا
- 3-كثيرا جدا

إلم اعد أهتم بمظهري:

- 3-اكيد
- 2-لا أهتم بذلك كما يجب
- 1-لا أهتم بذلك
- 0-مازلت اهتم بمظهري كما كنت

ق- اشعر باضطراب و عدم القدرة على الاستقرار فى أى موضع:

- 3-بدرجة كبيره جدا
- 2-بدرجة كبيره
- 1-بدرجة قليله
- 0-ابدا

إ-اتوقع الأمور بالتفاؤل:

- 0-كما كنت دائما
- 1-أقل من السابق
- 2-أقل كثيرا
- 3-لا أتطلع الى ذلك نهائيا

ق-ير اودنى احساس بالرعب:

- 3-كثير جدا
- 2-كثيرا
- 1-قليلًا
- 0-ابدا

ا-استطيع الإستمتاع بقراءة كتاب جيد او الإستماع الى الراديو او مشاهدة برنامج بالتلفزيون:

- 0-كثيرا
- 1-احيانا
- 2-قليلًا
- 3-نادرا

Appendix 4. Scoring system for the Rey-Osterrieth complex figure

Units	
1	Cross upper left corner, outside of rectangle
2	Large rectangle
3	Diagonal cross
4	Horizontal midline of 2
5	Vertical midline
6	Small rectangle, within 2 to the left
7	Small segment above 6
8	Four parallel lines within 2, upper left
9	Triangle above 2, upper right
10	Small vertical line within 2, below 9
11	Circle with three dots within 2
12	Five parallel lines within 2 crossing 3, lower right
13	Sides of triangle attached to 2 on right
14	Diamond attached to 13
15	Vertical line within triangle 13 parallel to right vertical of 2
16	Horizontal line within 13, continuing 4 to right
17	Cross attached to 5 below 2
18	Square attached to 2, lower left

Scoring:

Consider each of the 18 units separately. Appraise accuracy of each unit and relative position within the whole of the design. For each unit count as follows:

Correct	Placed properly	2 points
	Placed poorly	1 point
Distorted or incomplete But recognizable	Placed properly	1 point
	Placed poorly	1/2 point
Absent or not recognizable		0 point
Maximum		36 points

Appendix 5. Drug list of CKD patients from Chapter 3

Drug name	RAA group 34 patients	Non-RAA group 26 patients
Lisinopril	4	0
Perindopril	1	0
Enalapril	3	0
Irbesartan	13	0
Losartan	12	0
Valsartan	2	0
Amlodipine	2	22
Atenolol	3	4
Furosemide	4	3
Insulin	7	6
Folic acid	30	24
Cinacalcet	25	18
Calcium carbonate	20	15
Calcitriol	27	21
Omeprazole	15	12
Ranitidine	13	9
Multivitamine	30	22
Erythropoietin	32	24
Polystyrene sulphonate	29	22
Lactulose	12	10
Ferrous sulphate	14	17

Appendix 6. Drug list of dialysis patients from Chapter 5

Drug name	RAA group 13 patients	Non-RAA group 40 patients
Lisinopril	5	0
perindopril	4	0
Enalapril	1	0
Irbesartan	3	0
Losartan	0	0
Valsartan	0	0
Amlodipine	4	30
Atenolol	3	6
Labetalol	0	4
Furosemide	2	5
Insulin	4	8
Folic acid	13	39
Cinacalcet	11	35
Calcium carbonate	13	38
Calcitriol	13	39
Omeprazole	7	15
Ranitidine	8	13
Multivitamine	13	40
Erythropoietin	13	40
Polystyrene sulphonate	11	36
Lactulose	9	25
Ferrous sulphate	12	37