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ASSOCIATION BETWEEN STRUCTURAL MEASURES OF SPECIFIC REGIONAL BRAIN VOLUMES MEASURED BY QUANTITATIVE MAGNETIC RESONANCE IMAGING AND NEUROCOGNITIVE PERFORMANCE IN ELDERLY BREAST CANCER SURVIVORS EXPOSED TO CHEMOTHERAPY

by

Haris Hamsakutty

A thesis submitted in partial fulfillment
of the requirements for the Master of
Science degree in Free Radical and Radiation Biology
in the Graduate College of
The University of Iowa

December 2009

Thesis Supervisors: Professor Peg Nopoulos
Professor Susan K. Schultz

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Graduate College
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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

Haris Hamsakutty

has been approved by the Examining Committee
for the thesis requirement for the Master of Science
degree in Free Radical and Radiation Biology at the December 2009
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To My Teachers

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ABSTRACT

Recent advances in early detection and treatment of breast cancer have led to increasing numbers of long term survivors of breast cancer. There is a growing concern about the potential adverse effects of chemotherapy on cognitive functioning.

The current study examines the neuroanatomical correlates of late neurocognitive effects of chemotherapy in elderly breast cancer survivors who have survived more than ten years and were exposed to chemotherapy at the time of their cancer treatment.

The participants in this study are 30 women breast cancer survivors in the age range of 65-81 years. In this cross sectional design, regional brain volumes measured using magnetic resonance imaging were correlated with cognitive test scores using multiple regression analyses. The test scores from Wisconsin Card Sorting Test and Trail Making Test B are used as measures of executive function. The test scores from the Letter Number Sequencing subset of the Wechsler Adult Intelligence Scale (WAIS) are used for measures of working memory.

We found support for the hypothesized association between reduction in performance on specific neuropsychological tests and reduced volumes predominantly in the frontal, temporal and subcortical white matter regions. These results suggest that the frontal, temporal and subcortical white matter region are a neuroanatomical correlate of cognitive impairment seen in our study population.

Future research will be needed to discern whether the structural correlates of cognitive impairment seen in long term cancer survivors is likely to be developed as an imaging marker for cognitive evaluation and rehabilitation.

TABLE OF CONTENTS

LIST OF TABLES viii

LIST OF FIGURES ix

CHAPTER I: BACKGROUND, HYPOTHESIS, AND SIGNIFICANCE 1

1.1 Introduction 1
1.2 Background 2
1.2.1 Breast Cancer 2
1.2.1.1 Breast Cancer Staging 2
1.2.1.2 Breast Cancer Treatment 3
1.2.2 Chemotherapeutic Agents in Context of Current Study 3
1.2.3 Chemotherapy Related Cognitive Dysfunction: An Emerging Target for Research 4
1.2.4 Candidate Mechanisms for Chemotherapy Associated Cognitive Changes 5
1.2.4.1 Direct Neurotoxic Mechanisms of Chemotherapy 5
1.2.4.2 Oxidative Stress Mediated Neurotoxic Mechanism of Chemotherapy 6
1.2.4.3 Genetic Factors Predisposing to Neurotoxicity of Chemotherapy 6
1.2.5 Review of Published Scientific Reports 7
1.3 Significance 11

CHAPTER II: MATERIALS AND METHODS 13

2.1 Experimental Design 13
2.2 Participants 13
2.3 Neurocognitive Testing 14
2.4 Magnetic Resonance Imaging Protocol 15
2.5 Magnetic Resonance Image Analysis 16
2.6 Data Processing and Management 17
2.7 Statistical Analysis 17

CHAPTER III: RESULTS 18

3.1 Participants 18
3.2 Results 18
3.2.1 Multiple Regression Analyses: Effect of Regional Brain Volume on Trail Making Test B 18
3.2.2 Multiple Regression Analyses: Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST - PE) 19
3.2.3 Multiple Regression Analyses: Effect of Regional Brain Volume on Letter Number Sequencing Test 19

CHAPTER IV: DISCUSSION 21

CHAPTER V: FUTURE DIRECTION 26

BIBLIOGRAPHY.....45

LIST OF TABLES

Table 3.1 Descriptive Data: Demographic.....27

Table 3.2 Descriptive Data: Neurocognitive Test Performance 28

Table 3.3 Multiple Regression Analyses: The Effect of Regional Brain Volume on Trail Making Test B.....29

Table 3.4 Multiple Regression Analyses: The Effect of Regional Brain Volume on Trail Making Test B.....30

Table 3.5 Multiple Regression Analyses: The Effect of Regional Brain Volume on Trail Making Test B.....31

Table 3.6 Multiple Regression Analyses: The Effect of Regional Brain Volume on Trail Making Test B.....32

Table 3.7 Multiple Regression Analyses: The Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST–PE) 33

Table 3.8 Multiple Regression Analyses: The Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST–PE) 34

Table 3.9 Multiple Regression Analyses: The Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST–PE) 35

Table 3.10 Multiple Regression Analyses: The Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST–PE) 36

Table 3.11 Multiple Regression Analyses: The Effect of Regional Brain Volume on Letter Number Sequencing Test 37

Table 3.12 Multiple Regression Analyses: The Effect of Regional Brain Volume on Letter Number Sequencing Test 38

Table 3.13 Multiple Regression Analyses: The Effect of Regional Brain Volume on Letter Number Sequencing Test 39

Table 3.14 Multiple Regression Analyses:The Effect of Regional Brain Volume on Letter Number Sequencing Test 40

LIST OF FIGURES

Figure 3.1 Scatter plot of the relationship between ratio of temporal white matter volume with intracranial volume *vs* Trail Making test B performance. 41

Figure 3.2 Scatter plot of the relationship between ratio of sum of temporal white matter volume and temporal grey matter volume with intracranial volume *vs* Trail Making test B performance.....42

Figure 3.3 Scatter plot of the relationship between ratio of sum of frontal white matter volume, temporal white matter volume, and subcortical white matter volume with intracranial volume *vs* Trail Making test B performance 43

Figure 3.4 Scatter plot of the relationship between ratio of sum of frontal white matter volume, temporal grey matter volume, and subcortical white matter volume with intracranial volume *vs* Letter Number Sequencing Test –Raw score.....44

CHAPTER I: BACKGROUND, HYPOTHESIS, AND SIGNIFICANCE

1.1 Introduction

Breast cancer is the most common type of malignancy and the second leading cause of cancer deaths in women in the United States. The treatment of breast cancer includes some combination of surgery, radiation therapy, chemotherapy, hormonal therapy, or biologic therapy. With the development of systemic therapies, the survival rate of breast cancer patients is increasing and hence there is a need to understand the long term toxicities of systemic therapies.

The current study examines the neuroanatomical correlates of late neurocognitive effects of chemotherapy in elderly breast cancer survivors who have survived more than ten years and were exposed to chemotherapy at the time of their cancer treatment. The participants in this study are human subjects in the age range (65-81 years) where cognitive impairment may become more readily apparent.

Based on the previous studies examining cognitive impairment following chemotherapy, the current study looks at lines of evidence that implicate specific effects of chemotherapy on subcortical as well as frontal and temporal brain regions that may result in a distinct pattern of cognitive decline. Neuropsychological assessment was used in combination with magnetic resonance imaging to conduct an analysis of regional brain differences in relation to cognitive outcomes.

We hypothesized that cognitive dysfunction in breast cancer survivors exposed to chemotherapy would be directly related to measures of regional brain structure obtained from magnetic resonance imaging.

The stated hypothesis was investigated by accomplishing the following objectives

1: To evaluate whether measures of frontal, temporal and subcortical brain regions obtained from magnetic resonance imaging scans would correlate with impairment in neuropsychological tests of executive function and working memory.

Brain scans of breast cancer survivors acquired using high resolution magnetic resonance imaging were used to derive the volumetric data for regions of interest. A correlation analysis was then performed between regional brain volumes of interest and neuropsychological test scores. The neuropsychological tests selected for this study are Trail Making Test B, Wisconsin Card Sorting Test – Perseverative Errors and Letter Number Sequencing test.

1.2 Background

1.2.1 Breast Cancer

Breast cancer is the most common type of malignancy and the second leading cause of cancer deaths in women in the United States. Recent advances in early detection and treatment have led to increasing numbers of long term survivors of breast cancer. The estimated number of breast cancer survivors (BCS) alive in US as of January 2005 is 2,477,850 women (SEER Program, NCI 2008).

1.2.1.1 Breast Cancer Staging

Breast cancer is an abnormal and uncontrolled proliferation of epithelial cells lining the ducts or lobules of the breast. An important aspect in determining the prognosis and treatment option of breast cancer is its stage. The stage describes the spread of cancer in the body and staging system is a standardized way to summarize this information. *In situ* or non invasive breast cancer refers to cancer in which cells are confined within the site of origin. On the other hand invasive or infiltrating breast cancers spread beyond the site of origin to invade the surrounding or distant tissue. The TNM staging system classifies cancers based on the size of primary tumor (T), the extend of involvement of regional lymph node (N), and the presence or absence of metastasis (M). Information from TNM staging is used to define the five main stage groups (stages 0, I, II, III, IV) characterized by increasing disease severity.

1.2.1.2 Breast Cancer Treatment

The stage of breast cancer is integral to the treatment planning of breast cancer. The current standard in treatment of breast cancer is surgery, chemotherapy, adjuvant hormonal therapy, biological therapy and radiation therapy or some combination of this depending on the cancer stage. Chemotherapy, hormonal therapy and biological therapies are considered as systemic therapies. With the development of systemic therapies, the survival rate of breast cancer patients is increasing and hence the need to understand the long term toxicities of systemic therapies.

1.2.2 Chemotherapeutic Agents in Context of Current Study

A review of multi-agent chemotherapy from the Early Breast Cancer Trialists' Collaborative group provides a meta-analysis of all randomized trials in the period up to 1990. Of the 47 clinical trials, the majority involved a cyclophosphamide (C), methotrexate (M), 5-fluorouracil (F) combination. Nineteen of the trials examined CMF alone, 11 trials involved CMF of varying durations, 9 studies involved CMF with various adjuvant drugs, 11 studies compared anthracyclines-containing regimens to CMF, and nineteen involved other combinations (Group, 2001). This information is of interest in the setting of the current study of long term survivors of breast cancer, as these agents are most representative of the treatment our sample cohort would have received in 1990-95.

The following is a brief overview of these chemotherapeutic agents and their mechanisms of action:

- Cyclophaphamide (C) is an alkylating agent that acts by causing DNA cross linking and thereby compromising the ability of DNA to replicate leading to cell death.

- Methotrexate (M) is an antimetabolite agent that inhibits the formation of tetrahydrofolic acid. Cell death is induced by the inability to synthesize pyrimidines, acting synergistically with 5-FU.
- 5-fluorouracil (5-FU) is an antimetabolite that acts by inhibiting thymidylate synthetase (TS) enzyme. TS enzyme is involved in the methylation of deoxyuridylic acid to thymidylic acid. 5-FU thereby interferes with DNA synthesis and cellular death ultimately occurs as a result of thymine (pyrimidine) deficiency.
- Doxorubicin (A) is an antitumor antibiotic that acts by intercalating DNA, inhibition of DNA and RNA synthesis, fragmentation of DNA as well as by inhibition of DNA repair. Doxorubicin interference with coenzyme-Q dependent mitochondrial electron transfer reactions and can lead to the formation of free radicals. Mitochondrial DNA was also shown to be a critical target for the anthracycline daunorubicin (Lo, et al., 2005).

1.2.3 Chemotherapy Related Cognitive Dysfunction: An Emerging Target for Research

Cognitive dysfunction has been reported following cancer treatments, with the greatest effect noted among persons receiving systemic treatments (Anderson-Hanley, Sherman, Riggs, Agocha, & Compas, 2003). The possibility that chemotherapy might impair cognition is a growing concern among healthy women with breast cancer, when many of them have normal life expectancies that may span many decades. Previous studies have shown adverse cognitive impairments in breast cancer patients exposed to adjuvant chemotherapy. However there is relatively little information to date regarding the specific brain changes that may reflect the problem. While most patients report minor difficulties with cognitive functioning, a small subset of patients are more markedly

impaired to the extend that it impacts significantly on their quality of life (QOL) and in functions related to the activities of daily living.

1.2.4 Candidate Mechanisms for Chemotherapy Associated Cognitive Changes

The mechanisms through which chemotherapy might cause cognitive impairment are mostly unknown, but they are likely to be multifactorial. Ahles *et al* (2007) conducted a review of potential mechanisms for chemotherapy induced cognitive changes. It is likely that in the background of a genetic variability in neural repair and/or plasticity, chemotherapy agents might cause direct damage or indirectly impair cellular maintenance through an increase in oxidative stress in genetically vulnerable individuals. The genetic variability in the blood brain barrier and blood brain drug transporters might influence the amount of chemotherapeutic agents that enters brain following systemic therapy. Chemotherapy induced hormonal changes; secondary immunologic changes with release of cytokines were other factors speculated (Ahles & Saykin, 2007).

1.2.4.1 Direct Neurotoxic Mechanisms of Chemotherapy

The chemotherapy agents could cause direct damage to the cerebral parenchyma, including the microglia, oligodendrocytes, and axons producing cerebral gray matter atrophy and white matter demyelination. (Saykin, Ahles, & McDonald, 2003). Imaging studies using voxel based morphometry in long term survivors of cancer approximately 10 years after chemotherapy showed reductions in bilateral gray and white matter compared to controls (Saykin, et al., 2003)

Studies integrating the assessment of brain structure with function by combining neuroimaging with neurocognitive assessments have revealed results that support this model. Inagaki *et al* (2007) reported a reduction in gray and white matter volumes that correlated with poorer performance on cognitive testing one year after treatment

compared with controls, however, structural volume reductions were not present three years after treatment.

In small animal studies it was shown that neural progenitor cells and myelin forming oligodendrocytes were vulnerable to increased cell death or decreased cell division following systemic administration of chemotherapeutic agents at a lower dose than required to cause tumor cell death (Dietrich, Han, Yang, Mayer-Proschel, & Noble, 2006).

The neurotoxicity also depends on the chemotherapy agent used. Chemotherapy agents are almost always used in combination rather than as a single agent and so it is difficult to determine the toxic effects of a single agent.

1.2.4.2 Oxidative Stress Mediated Neurotoxic Mechanism of Chemotherapy

Chemotherapy has been associated with increased levels of non protein bound iron (Weijl, et al., 2004), free radicals (Kaya, et al., 2005) and reduced antioxidant capacity (Papageorgiou, et al., 2005), all of which can increase oxidative stress. Oxidative damage resulting from this could lead to nuclear and mitochondrial DNA damage leading to neuron degeneration.

1.2.4.3 Genetic Factors Predisposing to Neurotoxicity of Chemotherapy

Individual genetic differences in blood brain barrier transporters, DNA repair mechanisms, rate of telomere shortening, cytokine regulation, neuronal repair and plasticity, and neurotransmission could all increase the vulnerability to neurotoxicity associated with chemotherapy (Ahles & Saykin, 2007).

Ahles *et al* (2003) evaluated the relationship of apolipoprotein E (APOE) genotype to neuropsychological performance in long term cancer survivors treated with chemotherapy. It was found that survivors with at least one E4 allele scored significantly lower in visual memory and spatial ability domains, with a trend to score lower in executive functioning compared with survivors who did not carry and E4 allele. This gene has also been related to reduced cognitive performance in healthy aging (Small, Rosnick, Fratiglioni, & Backman, 2004) and also in Alzheimers disease (Saunders & Schmader, 1993). Previous studies also have shown that E4 allele hinders the ability of the brain to repair following an insult.

In the context of breast cancer treatment regimen involving methotrexate, a gene involved in folate metabolism, methylene tetrahydrofolate reductase (MTHFR) gene deserves mention. Common polymorphisms of MTHFR gene have shown to be associated with decrease levels of folate in response to methotrexate, a folic acid antimetabolite, leading to accumulation of homocysteine, an excitotoxin (Toffoli & De Mattia, 2008; Ulrich, et al., 2001). Oxidative damage secondary to folate deprivation and homocysteine accumulation may be another potential cause of neurocognitive dysfunction associated with chemotherapy treatments involving methotrexate.

1.2.5 Review of Published Scientific Reports

The majority of studies have investigated the short-term effects on cognitive functioning, assessing participants within one to three years post-chemotherapy treatment. Very few studies have assessed for potential late effects of chemotherapy on cognitive functioning.

Scherwath *et al* (2006) studied breast cancer patients on average five years after treatment. Forty seven patients (23 high dose and 24 standard dose patients) were studied along with 29 early breast cancer patients matched for age, education and time since treatment as controls. Results showed 13% of standard dose, 8% of high dose, and 3% of

early breast cancer patients having global cognitive impairment. Also impairments were reported for the domains of executive functioning and attention.

Ahles *et al* (2002) studied long term survivors of breast cancer and lymphoma who were, on average, approximately ten years after standard dose chemotherapy. Breast cancer (n=35, age 59.1 ± 10.7 years) and lymphoma (n=36, age 55.9 ± 12.1 years) survivors treated with chemotherapy were compared to breast cancer (n=35, age 60.6 ± 10.5 years) and lymphoma (n=22, age 48.7 ± 11.7 years) survivors treated with local therapy (surgery or radiation) only. Results from the study using multivariate analysis of variance, controlling for age and education, demonstrated significantly more impairment in the cognitive domains of verbal memory and psychomotor functioning in survivors exposed to chemotherapy. Data from this study support the hypothesis that systemic chemotherapy can have a negative impact of cognitive functioning and can persist long after treatment.

Hurria *et al* (2006) investigated the cognitive function of 28 older women (age 65 and older at diagnosis) with a prospective, longitudinal design. Neuropsychological assessments were made before receiving chemotherapy and six months after chemotherapy. Comparison group was not included in the study. Fifty percent (n=14) of participants demonstrated no change in cognitive function, 39% (n=11) showed impairment in psychomotor speed, visuo-spatial abilities and visual memory. Results from this study warrant the need for further study with older patients.

Bender *et al* (2006) designed a prospective study incorporating neurocognitive assessments at three time points, a baseline cognitive assessment before chemotherapy, within one week of chemotherapy and further assessment about one year after chemotherapy. Participants were grouped into those who received either chemotherapy (n=19) or chemotherapy and tamoxifen (n=15), and a group who received only local surgical therapy (n=12). Patients treated with chemotherapy and tamoxifen showed a decline on one year follow up on visual memory. Differences between groups when

analyzed showed that differences were more evident at one year follow up. This study suggests that chemotherapy may negatively affect memory over time, particularly when patients are exposed to chemotherapy and tamoxifen. Also the inclusion of a control group with local surgical therapy points that chemotherapy is more likely influencing the outcome.

With recent advances in neuroimaging modalities, there is a considerable interest among researchers to integrate non invasive imaging methods to understand the structural abnormalities, if any, associated with cognitive dysfunction in patients exposed to chemotherapy.

Saykin *et al* (2003) studied structural brain changes associated with chemotherapy five years post treatment in survivors (n=12) of breast cancer (n= 10) and lymphoma (n= 2) in comparison to healthy controls (n=12). The study was done using magnetic resonance imaging. Results from the voxel based morphometric study showed a bilateral reduction of grey matter, and cortical and subcortical white matter in the patients exposed to chemotherapy compared to healthy controls. Given these effects in a mixed age sample, it is anticipated that brain abnormalities examined in patients who are elderly, as proposed, in this study may be more readily detectable but complex in terms of isolating the various contributing factors to degenerative change.

Inagaki *et al* (2007) investigated the regional brain volumes in breast cancer survivors exposed to chemotherapy using voxel based morphometry and neurocognitive outcomes. Participants were grouped into those who received either chemotherapy (n= 51), or no adjuvant therapy (n= 54) and comparison group of healthy controls (n=55). MRI scans of within 1 year (range: 3–15 months) or 3 years (range: 27–39 months) after initial surgery were compared with healthy controls' scans and correlation analysis was done. Patient treated with chemotherapy showed reductions in volumes of grey matter and white matter at one year study, when compared to the healthy controls and no adjuvant therapy group. The regional volume reductions correlated with neurocognitive

performance of attention and visual memory. The analyses also showed that differences were more evident at one year study and no significant volume differences were seen on three year study.

Abraham *et al* (2008) used diffusion tensor imaging to study the white matter integrity in breast cancer patients treated with chemotherapy. Patients who were on average 22 months post treatment (n=10) and had cognitive complaints were compared with age and education matched healthy controls (n=9). The chemotherapy group showed lower fractional anisotropy, a sensitive marker of white matter integrity, in the genu of corpus callosum and this was correlated with reduced graphomotor speed.

Ferguson *et al* (2007) studied a case of monozygotic twins who are discordant for breast cancer and chemotherapy exposure (i.e., one twin contracted breast cancer and underwent chemotherapy, and the other had no breast cancer). Evaluation with standardized, self-report measures of cognitive function, standard neuropsychological tests, and structural and functional magnetic resonance imaging showed that the twin who underwent chemotherapy had substantially more subjective cognitive complaints, more white matter hyperintensities on MRI, and an expanded spatial extent of brain activation during working memory processing than her nonaffected twin. There were no significant differences on tests of memory and executive functions.

In summary, several studies of breast cancer patients exposed to chemotherapy reported cognitive impairment in significant proportion of participants. Recent neuroimaging studies have reported structural and functional abnormalities in brain associated with chemotherapy. But we did not find any study that looked at the late effects of chemotherapy past ten years of exposure.

Clearly there is a need to study the association between structural brain morphology and neurocognitive performance in survivors exposed to chemotherapy as it will provide information on designing future interventions for cognitive evaluation and rehabilitation. The present study is conducted with the purpose of attempting to better

understand the late effects of chemotherapy on cognitive functioning by investigating the association with specific regional brain structure.

1.3 Significance

A substantial body of evidence showed that patients exposed to chemotherapy shows objective evidence of cognitive impairment. Chemotherapeutic agents may be directly or indirectly involved in the development of cognitive impairment. A previous report from preliminary analysis (Yamada *et al*, in press) to this study showed cancer survivors exposed to chemotherapy scored lower on tests of executive function and working memory compared to age matched healthy controls.

In this preliminary analysis (larger study) to the current project, 30 women breast cancer survivors were matched with 30 non-cancer, healthy older women in age, education, and intellect. It was observed that cancer survivors scored significantly worse in executive function as measured by the Trail Making Test B and Wisconsin Card Sorting Task. Cancer survivors also scored lower on tasks of working memory as measured by the Wechsler Adult Intelligence Scale, Digit Span Reverse and Letter Number Sequencing (Yamada *et al*, in press). It was concluded that these findings are likely reflecting dysfunction in frontal-temporal-subcortical brain regions.

These results from this preliminary analysis support the hypothesis that structural magnetic resonance imaging will demonstrate that the structural measures of the frontal, temporal and subcortical brain regions will be correlated with impairment in neuropsychological tests of executive function and memory.

In the current study using structural magnetic resonance imaging we are exploring to detect a meaningful association between the frontal-temporal-subcortical brain regions and the neuropsychological test outcomes. By studying an older patient sample, this study may discern neurodegenerative changes potentially related to chemotherapy that may be too subtle to detect earlier in otherwise healthy women. This study is one of the earliest

of its kind to look at the long-term neurocognitive implications of chemotherapeutic treatments by correlating with structural imaging modalities in a sample population who are more than 65 years of age and more than 10 years post treatment.

If this study detects an association between brain morphology and performance on neuropsychological testing in survivors exposed to chemotherapy, it will provide new insights for future research aimed at improving the quality of life of cancer patients. Also it will contribute to further study the lifestyle mediators of variance in outcome. Data correlating structural brain imaging with cognitive performance could be used to develop a quantitative imaging marker that has implications in cognitive evaluation and rehabilitation.

CHAPTER II: MATERIALS AND METHODS

2.1 Experimental design

The design of this study is based on an approach integrating the volumetric data from magnetic resonance imaging with the neuropsychological test scores to make an assessment for volumetric correlates of cognitive impairment. In this cross sectional design, regional brain volumes measured using magnetic resonance imaging will be correlated using multiple regression analyses with cognitive test scores.

2.2 Participants

The sample consists of breast cancer survivors (hereafter referred to as BCS) who were administered a battery of neuropsychological tests and imaging studies as part of a larger study (IRB # 200603774, R01 CA122934-01). This larger study titled "Elderly Cancer Survivors: Cognitive Outcomes and Markers of Neurodegeneration" investigates the potential contribution of chemotherapy to cognitive impairment in late life. Breast cancer survivor participants were recruited in collaboration with the Iowa Cancer Registry, a statewide registry of cancer patients begun in 1973.

The study specified enrollment criteria that the participants were women over the age of 65 years, at least 50 years of age at the time of cancer diagnosis and treatment, and at least 10 years post-cancer treatment. Participants for this report were diagnosed and treated for early malignant breast cancer Stage II through Stage IIIA without evidence of metastasis and had received a standard multi-agent chemotherapy regimen involving cyclophosphamide, methotrexate and 5-fluorouracil (CMF) or an anthracycline (doxorubicin).

Participants were excluded on the basis of one or more of the following:

- If there had been a recurrence of any kind of cancer in the 10-15 year period since initial diagnosis, excluding basal cell or relatively benign skin lesions.

- If they possessed a central nervous system (CNS) disorder, such as multiple sclerosis, Parkinson's disease, closed head trauma with an extended loss of consciousness, or other CNS lesion.

All breast cancer survivor participants were free of currently active and unstable metabolic, psychiatric, and cardiovascular diseases, including cerebrovascular events and substance abuse.

In the preliminary study, to analyze the neuropsychological outcomes of breast cancer survivors, each BCS participant was demographically matched to a non-cancer comparison participant from an existing database. Participants met inclusion criteria if they were free of neurological and psychiatric illness, as indicated above. All participants signed a written informed consent document approved by the Iowa Institutional Review Board.

2.3 Neurocognitive Testing

Each participant completed a three hour standardized neuropsychological battery designed to evaluate a broad range of cognitive abilities involving attention, global intellect, memory, language, visuospatial skills and executive functioning as part of the larger study. In the larger study the chemotherapy exposed group showed significant impairment in the domains of working memory and executive function compared to the age, education and intellect matched healthy controls.

For our study, we are focusing on the working memory and executive domains and the neuropsychological tests used to detect the differences.

The test scores from Wisconsin Card Sorting Test and Trail Making Test B are used as measures of executive function. The test scores from the letter number sequencing subset of the Wechsler Adult Intelligence Scale (WAIS) are used for measures of working memory.

Wisconsin Card Sorting Test – Perseverative Errors (WCST_PE): This test measures problem solving, mental flexibility and abstract reasoning. The participant must "break the code" by using the examiners corrective feedback during a novel card task (Heaton et al 1993). The higher scores in this test mean a worse performance.

The Trail Making Test (TMT-B): This is a test indicating a measure of conceptual ability and visuomotor tracking. The participant is required to draw lines to connect consecutively numbered and lettered circles as quickly as they can (Spreen and Strauss 1998). The subject is scored for the time taken to complete the task. A higher score in this test is interpreted as a worse performance.

The Letter Number Squencing subtest of WAIS: This test measures auditory verbal working memory. Participant is read strings of digits and letters, and must order what they hear in numerical and alphabetical form, prior to repeating the digits and then letters back to the examiner (Wechsler 1997). The subjects' higher score in this test is interpreted as better performance.

2.4 Magnetic Resonance Imaging Protocol

Subjects recruited into the larger study underwent a high resolution multimodality MR imaging protocol on a Siemens 3T Trio scanner. The study acquired T1, T2, and FLAIR data. T1 weighted scan was acquired in the coronal plane using a 3D MP-RAGE sequence with the following parameters: TE=4ms, TR=2530ms, TI=1100ms, flip angle=10°, FOV=256x256x224mm, Matrix=256x256x224, BW=180Hz/Pixel, NEX=2. The T2 weighted scans was acquired using a coronal 2D FSE sequence: TE=15ms, TR=7060ms, NEX=1, Matrix=256x256, FOV=256x256mm, BW=315 Hz/Pixel, turbo Factor = 9, slice thickness/gap=1.5/0.0mm. The axial FLAIR images were in 2D using the following scan parameters: TE=116ms, TR=9120ms, TI=2500ms, FOV=240x240mm, Matrix=256x256, Slice Thickness/Gap = 1.8/0.0mm, BW=241 Hz/Pixel, Turbo factor = 23, NEX=1.

2.5 Magnetic Resonance Image Analysis

MRI analysis was done using software tool known as BRAINS2 (Brain Research: Analysis of Images, Networks, and Systems). All BRAINS tools are subjected to careful validation and reliability checks. BRAINS software has grown into a complex and powerful group of image analysis tools.

The imaging data was transferred to the image processing lab in the Department of Psychiatry where it was analyzed using a standard image processing pipeline. The first step in the analysis was aligning the T1 weighted scan along the AC-PC line and the interhemispheric fissure. The T2 and FLAIR scans were then co-registered to the resample T1 weighted scan (Woods, Grafton, Holmes, Cherry, & Mazziotta, 1998; Woods, Grafton, Watson, Sicotte, & Mazziotta, 1998). The second step in the pipeline involved tissue classification. This was done by a multispectral discriminant analysis method that uses training classes in order to classify tissue throughout the brain into grey matter, white matter and CSF. Once we have performed the tissue classification, we were then able to regionally quantify tissue volumes by region. For automated labeling of the brain currently two techniques are being used. Gross regional measures of brain tissue composition was obtained using a Talairach based atlas system (Andreasen, et al., 1996). This method provides measures for lobar regions including frontal, temporal, parietal and occipital lobes. The amount of grey matter, white matter and CSF was obtained for each of these regions. Artificial neural network (ANN) was used to perform skull stripping and for the definition of several subcortical (caudate, putamen, and thalamus) (Magnotta, Andreasen, et al., 1999; Spinks, et al., 2002) and cerebellar structures. The main variables of interest for this study include the lobar based measurements of tissue volumes including grey matter, white matter and CSF. Artificial neural networks were used to define the hippocampus and basal ganglia structures (caudate, putamen, and globus pallidus).

2.6 Data Processing and Management

Imaging data were processed and analyzed in cooperation with the University of Iowa's Psychiatric Iowa Neuroimaging Consortium. All psychometric data were double-entered, as were summaries of the imaging and genetic data that are transferred into the master datafile. Data storage is performed on a Windows-Based PC with off-site back-up performed weekly. Imaging data is initially transferred to data tapes which are then downloaded and stored on an Octane hard drive. The images on the hard drive are routinely backed up onto 10GB tapes.

2.7 Statistical Analysis

Using SPSS 17.0 for windows software (SPSS Inc., Chicago), we conducted a regression analysis to determine the effect of regional brain volumes on executive function after controlling for education. Independent measures included frontal, temporal and subcortical grey and white matter volumes. The dependent measures included the quantitative performance scores from the Trail Making Test B, the Wisconsin Card Sorting Test- Perseverative Errors, and the Letter Number Sequencing Task from the Wechsler Adult Intelligence Scale. Years of education were included as a covariate as it may account for significant variance within neuropsychological test performance. As the sample was recruited specifically as an elderly group, age was not used as a covariate. In the regression analysis, regional volumes were used as independent measure for individual frontal, temporal and subcortical grey and white matter individually, as well as the sum of volumes of frontal, temporal and subcortical white matter, which provided a more comprehensive evaluation of white matter disruption. The sums of volumes of frontal, temporal and subcortical gray matter were also used as an independent variable. Grey and white matter volumes were also summed for the individual frontal and temporal lobes to examine total volume in these areas. All measures were corrected for total intracranial volume by creating a ratio variable.

CHAPTER III: RESULTS

3.1 Participants

To be included in the analyses of present study, breast cancer survivor participants had to have completed the selected neuropsychological tests and also have magnetic resonance imaging (MRI) scan as part of the larger project. Of the 30 breast cancer survivors who had neuropsychological test data, only 25 who had successfully completed the MRI scan were included in this study. The final sample of the present study was aged between 65 years and 81years (mean = 72.9; SD = 4.64) and varying levels of education (mean = 13.6; SD = 1.98).

Descriptive Findings: Table 3.1 reports the demographic data for all the participants . The neuropsychological score data is summarised on Table 3.2

3.2 Results

Results of the multiple regression using enter method are reported in Tables 3.3 through 3.11. In each model the effect of specific regional brain volume measured using magnetic resonance imaging will be correlated with a specific cognitive test score using education as a covariate.

3.2.1 Multiple Regression Analyses:
Effect of Regional Brain Volume on Trail Making Test B

The statistical significant correlation was observed for the Trail making test B with volume of temporal white matter region (p= .037) and also for the combined volume of temporal white and gray matter (p= .046). These results are shown on Table 3.4, model 4 and Table 3.4, model 6 respectively.

There was statistically significant (p= .007) correlation of Trail making test B (TMT-B) score and combined volumes of the frontal, temporal and subcortical white matter regions, such that an increased score TMT-B corresponded to lower white matter

volume. In TMT-B, the time taken to complete the task is scored in seconds and a higher score means worse performance. These results are tabulated in Table 3.6**,** model 8**.**

Scatter plots of the relationship between the regional volumes corrected for intracranial volume *vs* Trail Making test B displays a pattern in which the performance of task is worse in subjects with reduced volume in these regions. These patterns for the statistically significant models are shown in Figures 3.1, Figure 3.2, and Figure 3.3 with a negative slope of the linear fit line.

No statistically significant correlation with Trail Making test B was observed for volumes corresponding to frontal white matter (Table 3.3, model 1), frontal gray matter (Table 3.3, model 2), sum of frontal white and gray matter (Table 3.3, model 3), temporal gray matter (Table 3.4, model 5), subcortical white matter (Table 3.5, model 7).

Trail making test B performance reduction was not found to be correlated in a statistically significant manner with the combined volumes of frontal, temporal and subcortical gray matter regions also (Table 3.6, model 9).

3.2.2 Multiple Regression Analyses: Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST - PE)

No statistically significant correlation with WCST - PE was observed for any of the individual or summed volumes evaluated. The results of these analyses are tabulated in Tables 3.7, 3.8, 3.9, 3.10.

3.2.3 Multiple Regression Analyses: Effect of Regional Brain Volume on Letter Number Sequencing Test

There was statistically significant (p=.005) correlation of Letter Number Sequencing test score and combined volumes of the frontal, temporal and subcortical white matter regions, such that a decreased score letter number sequencing test

corresponded to lower white matter volume. In letter number sequencing test, a higher score means better performance. These results are tabulated in Table 3.14**,** model 8.

A scatter plot of the relationship between the independent variables of frontal-temporal-subcortical white matter regional volume corrected for intracranial volume *vs* Letter Number Sequencing test score displays a pattern in which the performance of task is worse in subjects with reduced volume in these regions. This pattern is observed in Figure 3.4 with a positive slope of the linear fit line.

We did not observe any statistical correlation with other individual volumes of frontal, temporal, subcortical white matter regions. Neither individual frontal nor temporal gray matter did show statistical significance. The summed volumes of frontal, temporal and subcortical gray matter also did not reach statistical significance. Also the summed volumes of gray and white matter volumes of frontal regions, temporal regions did not show statistical significance. The results of the analyses are tabulated in Tables 3.11, 3.12, 3.13, 3.14.

CHAPTER IV: DISCUSSION

We found support for the hypothesized association between reduction in performance on specific neuropsychological tests and reduced volumes predominantly in the frontal, temporal and subcortical white matter regions. These results suggest that the frontal, temporal and subcortical white matter region are a neuroanatomical correlate of cognitive impairment seen in our study population. These findings are important in the light of preliminary studies in this group of long term breast cancer survivors, showing cancer survivors to have statistically significant cognitive impairment in domains of working memory and executive function when compared to healthy matched controls. When we then examined the same tests of working memory and executive function in relation to white matter volumes, we observed a pattern of reduced volumes, as described below.

Overall, the significant results were as follows: 1) A regression analysis revealed that the sum of volumes of frontal, temporal and subcortical white matter significantly predicted poorer performance in the neuropsychological test scores of executive function on the Trail Making Test B (TMT-B) and working memory on the Letter Number Sequencing Test. 2) The temporal white matter was the only individual independent predictor within the group that showed significance for TMT-B, a test reflecting executive function. 3) The sum of volumes of temporal white and gray matter demonstrated statistical significance for TMT-B. 4) The sum of volumes of frontal, temporal, subcortical gray matter regions or any of the individual gray matter volumes did not show statistical significance with the tests for TMT-B, or Letter Number Sequencing test. 5) No statistical correlation was also not with any of the individual or combined regional volumes with Wisconsin Card Sorting Test–Perseverative Errors (WCST-PE).

Interestingly, the association did not reach statistical significance when analyses were conducted for the individual volumes of white matter regions, except for the temporal white matter region, although a trend in the expected direction did emerge for individual frontal white and subcortical white matter regions.

This failure to reach statistical significance for the individual white matter volumes may be due to the low variance in the individual volume relative to the sum of frontal, temporal and subcortical volume. This could be addressed by increasing the sample size. Though the sample size in this study is modest, this is a relatively good sample size considering the age group and the long-term survivor status of this sample. Importantly, this group was recruited in a unique manner, as these patients are not necessarily seen in specialty cancer clinics given that they are decade or more past the acute illness. Many no longer consider themselves cancer patients and do not consider it to be a part of their active medical problem list. Consequently these women were recruited by invitation through cancer registry, as described above. Many have retired to warmer climates or were no longer engaged in active medical care, so it was a unique challenge to engage them in a research study as opposed to the typical recruitment procedures that involve directly approaching patients who are actively being seen for cancer treatment or post-treatment follow-up.

In view of the small sample sizes in many cancer survivor projects, meta-analyses are helpful. Anderson-Hanley conducted a meta-analysis (2003) of chemotherapy effects on neuropsychological function across 30 research studies, which included 838 patients, and noted consistent impairments in measures of executive function, verbal memory and motor function, implicating involvement of frontal cortex, medial temporal lobe / hippocampi and subcortical white matter. Our results are in agreement with the current literature and further support the theoretical concept that a linear pattern of progressive and persistent structural abnormality and cognitive impairment could result following exposure to chemotherapy. However, the pooled studies in the Anderson-Hanley meta-

analysis included patients who were treated for a variety of cancers who were an average of 86 weeks (or 1 ½ years) from the time of diagnosis and treatment. Hence our analysis of patients who are more than 10 years past their treatment involves a group where there is also an interaction with aging-related changes in cellular repair such that a linear effect may begin to change in slope at a later phase of life.

In the context of current literature, studies such as the Anderson-Hanley analysis have shown a pattern of cognitive symptoms that could be detected in the immediate time frame after exposure to chemotherapy. Some studies that have looked at the structural brain volumes such as the Inagaki et al study noted above, have suggested that brain volume changes may be detected in the first year after treatment but may not observed after few years. Our results suggest that this group may or may not have had structural abnormalities that were too subtle to detect earlier, but now in the context of approaching later life and the age range where variance begins to occur in neuropsychological functioning, we are able to detect differences associated with the cancer treatment. This observation, if further explored can explain some of the gaps in current literature and assist in an understanding of how earlier life exposures such as chemotherapy may have latent effects on the aging process.

Our results lead us to conclude that long term cancer survivors may be at increased risk for structural abnormalities long after cancer treatments are completed, that may reflect changes in their ability to maintain cellular repair as aging-related processes begin to occur. Future research will be needed to discern whether the structural correlates of cognitive impairment seen in long term cancer survivors is likely to be developed as an imaging marker for cognitive evaluation and rehabilitation.

Several limitations in the study should be noted that limit the confidence and ability to generalize our results. The current study did not have a comparison group of healthy older women who did not have previous cancer treatment. As noted above, we did conduct a preliminary analysis that determined differences were observable in

working memory and executive function between healthy comparison subjects and cancer survivors, but this analysis only reports on how those cognitive tests correlated with brain volumes in cancer patients only. Future work will help in making comparisons not only to healthy subjects, but also to cancer survivors who had localized surgical treatments, but no chemotherapy exposure.

In this study we were unable to access information about the duration and doses of exposure to the treatment agents among our participants. There are studies showing the implication of such considerations and potential contribution of these variables in the outcome we measured.

Diagnostic and therapeutic techniques have significantly improved the accuracy of breast cancer diagnosis and there have been significant advances in genetic testing that help identify patients at great risk for recurrence. These new strategies may help inform the use of chemotherapy in a way that minimizes the exposures as much as possible. Furthermore there are likely to be new developments that help minimize any potentially neurotoxic effects of future treatment recipients. However, the findings from this study could give us future directions to locate the regions of cortical structures that are more vulnerable to degenerative changes, which may be relevant to the millions of patients who have already received chemotherapy that has successfully treated their breast cancer.

Despite these limitations, this study also has several strengths. To our knowledge, this study is the earliest of its kind to examine the relationship between structural abnormalities and cognitive function in long term survivors who are more that ten years post treatment. With cancer survivors with such a long span of time after cancer treatment, we believe that this study represents an important step in exploring the cortical structural correlates of cognitive impairment among survivors who are at increased risk for long term cognitive dysfunction. More importantly, these structural regions could be compared with other neurodegenerative diseases which show similar patterns of cognitive

dysfunction so as to identify a common underlying mechanism in the neurodegenerative pathway.

CHAPTER V: FUTURE DIRECTION

Our study showed that the revealed that the white matter regions of frontal, temporal and subcortical white matter significantly predicted the performance in the neuropsychological test scores of executive function (TMT-B) and working memory (Letter Number Sequencing test). Further efforts are necessary to do a comparison with an age, education matched control sample population. In an effort to delineate the effects of disease factors from treatment factors that could lead to cognitive and/or structural abnormalities, a control population of breast cancer survivors who been treated only with local therapy would be ideal and is presently the goal of the overall study. A comparison with age, education matched healthy controls would also strengthen the data to show whether the effects are not part of the healthy ageing process.

We can further investigate the white matter structural integrity in this population by using diffusion tensor imaging (DTI) and this data could be correlated with the neurocognitive function. DTI is a more sensitive imaging tool to identify the white matter integrity by non invasive magnetic resonance imaging.

Further studies integrating cognitive assessment, structural imaging and genetic assessment of polymorphisms that make individuals more vulnerable to chemotherapeutic agents will help to develop effective treatment strategies and remediation interventions.

Table 3.1 Descriptive Data: Demographic

	(Participants) N = 25		
Variable	**Mean**	**SD**	**Range**
Age (years)	72.9	4.64	66 - 81
Education (years)	13.6	1.98	11 - 20

Mean value, standard deviation, and range for demographic variables of the sample shown.

SD = Standard deviation

Table 3.2 Descriptive Data: Neurocognitive Test Performance

Variable	Mean	SD	Range
Letter Number Sequencing Test - Raw Score	9.2	1.98	5-13
Trail Making Test B (Seconds)	92.88	35.85	51-184
Wisconsin Card Sorting Test - Perseverative Errors	12.12	7.38	4-36

Mean value, standard deviation, and range for neurocognitive test performance variables of the sample shown

SD = standard deviation.

Table 3.3 Multiple Regression Analyses: The Effect of Regional Brain Volume on Trail Making Test B

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	**Std. error**				
Model 1	0.141				0.187		
Education		-5.46	3.128	-0.345	0.095	-0.345	0.119
Frontal white		-556.35	647.036	-0.17	0.399	-0.17	0.029
Model 2	0.181				0.111		
Education		-4.44	3.113	-0.281	0.168	-0.275	0.076
Frontal gray		-841.98	618.88	-0.268	0.187	-0.262	0.069
Model 3	0.233				0.054		
Education		-4.638	2.972	-0.293	0.133	-0.291	0.085
Frontal white + Frontal gray		-904.15	485.51	-0.35	0.076	-0.348	0.122

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

Table 3.4 Multiple Regression Analyses: The Effect of Regional Brain Volume on Trail Making Test B

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	**Std. error**				
Model 4	0.274				**0.029***		
Education		-6.178	2.898	-0.391	0.044	-0.387	0.150
Temporal white		-2606.7	1176.98	-0.406	**0.037***	-0.402	0.162
Model 5	0.14				0.191		
Education		-5.246	3.126	-0.332	0.108	-0.332	0.1102
Temporal gray		-913.56	1093.8	-0.165	0.413	-0.165	0.0272
Model 6	0.262				**0.035***		
Education		-5.755	2.902	-0.364	0.06	-0.363	0.132
Temporal white + Temporal gray		-1630.48	771.14	-0.388	**0.046***	-0.387	0.150

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

Indicates significance at $p < .05$

Table 3.5 Multiple Regression Analyses: The Effect of Regional Brain Volume on Trail Making Test B

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	Std. error				
Model 7	0.184				0.107		
Education		-6.024	3.088	-0.381	0.064	-0.376	0.141
Subcortical white		-3362.1	2418.1	-0.272	0.178	-0.268	0.072

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

Table 3.6 Multiple Regression Analyses: The Effect of Regional Brain Volume on Trail Making Test B

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	**Std. error**				
Model 8	0.368				**0.006***		
Education		-5.521	2.679	-0.349	0.051	-0.349	0.122
Frontal white + Temporal white + Subcortical white		-0.606	0.203	-0.506	**0.007***	-0.506	0.256
Model 9	0.173				0.124		
Education		-4.84	3.09	-.306	0.131	-.304	0.092
Frontal gray + Temporal gray + Subcortical gray		-.487.16	383.22	-.248	0.217	-.246	0.060

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

Table 3.7 Multiple Regression Analyses: The Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST–PE)

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	Std. error				
Model 1	0.087				0.368		
Education		-0.346	0.664	-0.106	0.608	-0.106	0.011
Frontal white		-189.5	137.4	-0.281	0.182	-0.281	0.079
Model 2	0.052				0.556		
Education		-0.151	0.69	-0.046	0.829	-0.045	0.002
Frontal gray		-138.6	137.2	-0.214	0.323	-0.21	0.044
Model 3	0.162				0.142		
Education		-0.138	0.64	-0.042	0.831	-0.042	0.001
Frontal white + Frontal gray		-210.5	104.5	-0.396	0.056	-0.393	0.154

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

Table 3.8 Multiple Regression Analyses: The Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST–PE)

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	Std. error				
Model 4	0.121				0.241		
Education		-0.444	0.657	-0.136	0.506	-0.135	0.018
Temporal white		-449.6	266.8	-0.34	0.106	-0.337	0.114
Model 5	0.031				0.703		
Education		-0.282	0.683	-0.087	0.684	-0.087	0.007
Temporal gray		-174.5	239.1	-0.153	0.473	-0.153	0.023
Model 6	0.121				0.243		
Education		-0.374	0.653	-0.115	0.573	-0.114	0.013
Temporal white + Temporal gray		-291.0	173.445	-0.336	0.108	-0.335	0.112

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

Table 3.9 Multiple Regression Analyses: The Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST–PE)

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	Std. error				
Model 7	0.045				0.605		
Education		-0.399	0.688	-0.122	0.568	-0.121	0.146
Subcortical white		-494.646	539.026	-0.194	0.369	-0.191	0.036

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

Table 3.10 Multiple Regression Analyses: The Effect of Regional Brain Volume on Wisconsin Card Sorting Test – Perseverative Errors (WCST–PE)

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	Std error				
Model 8	0.117				0.255		
Education		-0.322	0.653	-0.099	0.627	-0.099	0.01
Frontal white + temporal white + subcortical white		-0.081	0.049	-0.33	0.114	-0.33	0.109
Model 9	.052						
Education		-.212	.680	-.065	.758	-.065	0.004
Frontal grey + Temporal grey + Subcortical grey		-85.62	84.52	-.212	.322	-.210	0.044

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

Table 3.11 Multiple Regression Analyses: The Effect of Regional Brain Volume on Letter Number Sequencing Test

	R^2	Unstandardized coefficients		β std	p-value	sr	sr^2
		B	Std. error				
Model 1	0.041				0.633		
Education		0.079	0.183	0.091	0.669	0.091	0.008
Frontal white		33.48	37.757	0.185	0.385	0.185	0.034
Model 2	0.029				0.721		
Education		0.042	0.187	0.049	0.823	0.048	0.002
Frontal gray		26.79	37.206	0.154	0.479	0.151	0.023
Model 3	0.079				0.404		
Education		0.041	0.180	0.047	0.820	0.047	0.002
Frontal white + Frontal gray		38.719	29.377	0.272	0.201	0.27	0.073

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

Table 3.12 Multiple Regression Analyses: The Effect of Regional Brain Volume on Letter Number Sequencing Test

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	Std. error				
Model 4	0.096				0.329		
Education		0.106	0.179	0.121	0.559	0.12	0.014
Temporal white		107.191	72.516	0.302	0.154	0.3	0.09
Model 5	0.014				0.855		
Education		0.071	0.185	0.082	0.704	0.082	0.007
Temporal gray		-26.893	64.644	-0.088	0.681	-0.088	0.008
Model 6	0.023				0.777		
Education		0.078	0.184	0.089	0.677	0.089	0.008
Temporal white + Temporal gray		29.682	49.004	0.128	0.551	0.128	0.016

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

Table 3.13 Multiple Regression Analyses: The Effect of Regional Brain Volume on Letter Number Sequencing Test

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	Std. error				
Model 7	0.105				0.295		
Education		0.117	0.179	0.134	0.52	0.132	0.017
Subcortical white		217.8	139.8	0.319	0.134	0.314	0.099

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

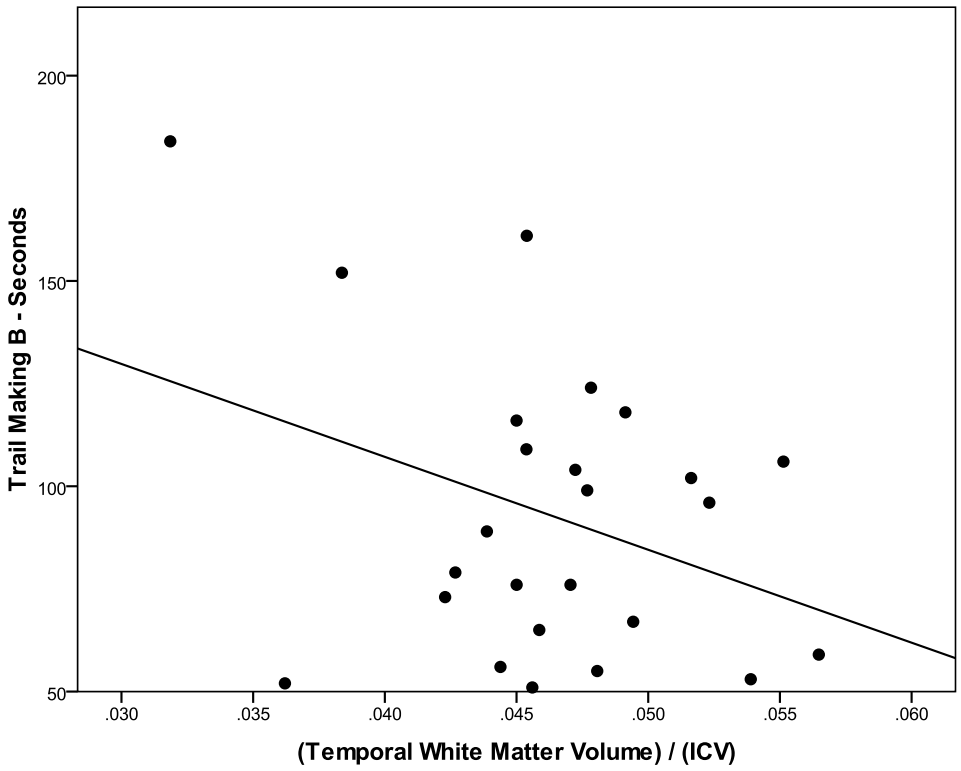
Table 3.14 Multiple Regression Analyses:The Effect of Regional Brain Volume on Letter Number Sequencing Test

	R^2	Unstandardized coefficients		β std	*p*-value	sr	sr^2
		B	Std. error				
Model 8	0.308				**0.017***		
Education		0.083	0.155	0.095	0.597	0.095	0.009
Frontal white + Temporal white + Subcortical white		0.036	0.012	0.549	**0.005***	0.549	0.301
Model 9	0.024				0.769		
Education		.056	.185	.064	.764	.064	.004
Frontal gray + Temporal gray + Subcortical gray		14.32	22.99	.132	.540	.131	.017

β std = Standardized beta coefficient, sr = Part correlation coefficient
sr^2 =Square of part correlation coefficient

* Indicates significance at $p < .05$

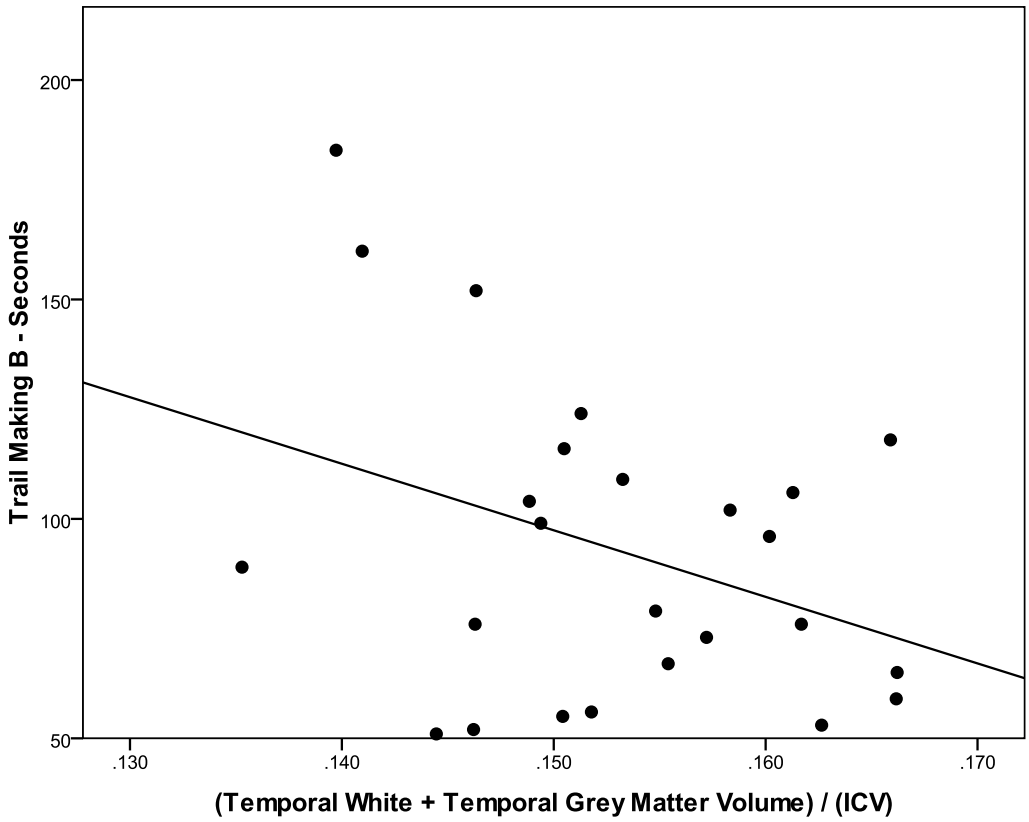
Figure 3.1 Scatter plot of the relationship between ratio of temporal white matter volume with intracranial volume *vs* Trail Making test B performance.



Solid line represents the linear regression fit line for the subjects.

ICV = Intracranial volume

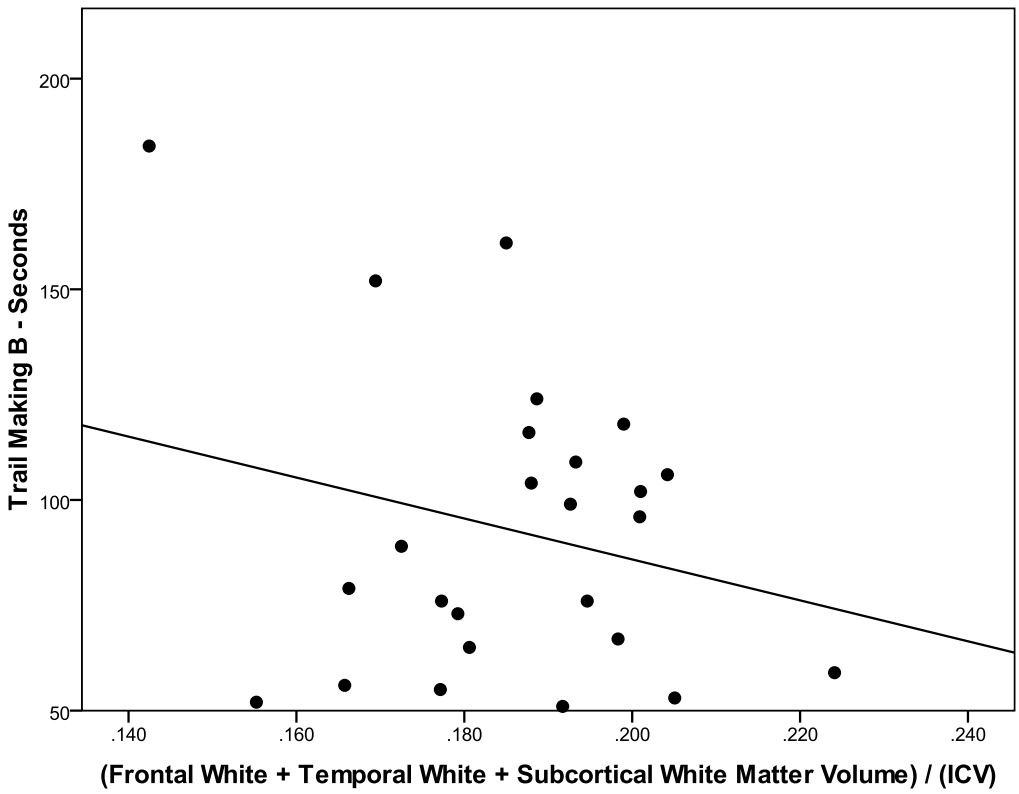
Figure3.2 Scatter plot of the relationship between ratio of sum of temporal white matter volume and temporal grey matter volume with intracranial volume *vs* Trail Making test B performance



Solid line represents the linear regression fit line for the subjects.

ICV = Intracranial volume

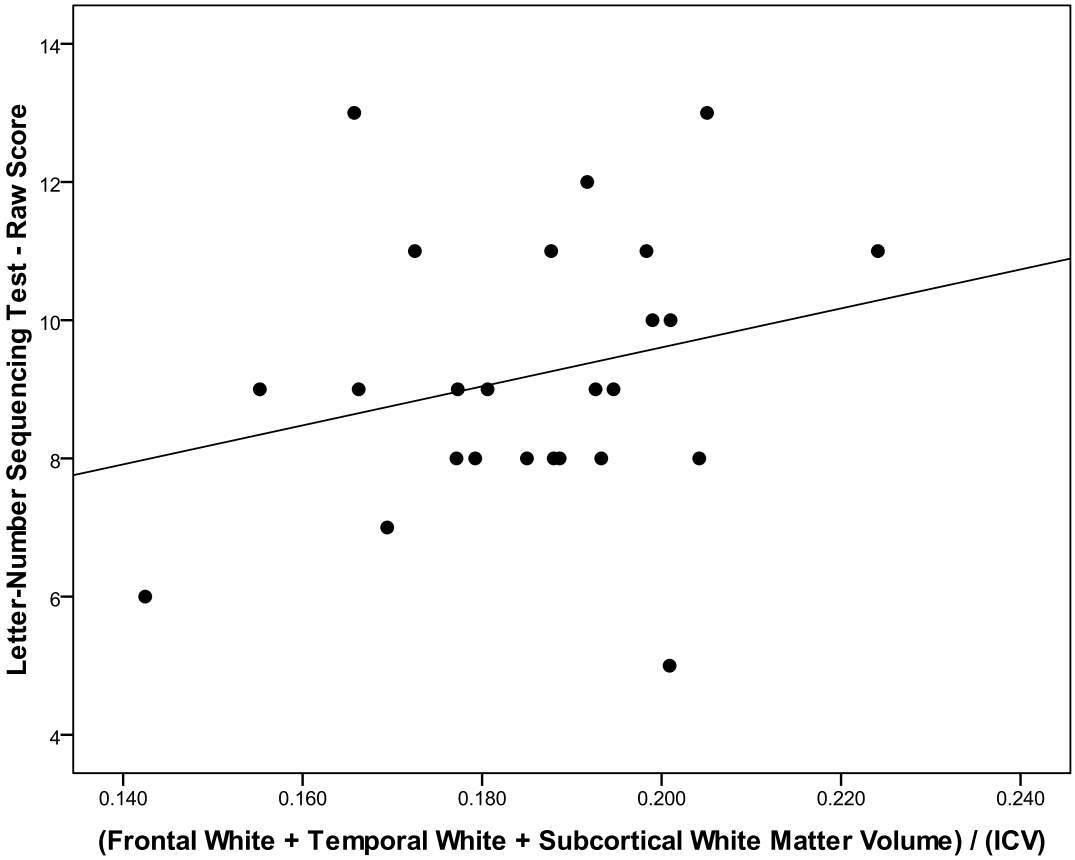
Figure 3.3 Scatter plot of the relationship between ratio of sum of frontal white matter volume, temporal white matter volume, and subcortical white matter volume with intracranial volume *vs* Trail Making test B performance



Solid line represents the linear regression fit line for the subjects.

ICV = Intracranial volume

Figure 3.4 Scatter plot of the relationship between ratio of sum of frontal white matter volume, temporal grey matter volume, and subcortical white matter volume with intracranial volume *vs* Letter Number Sequencing Test –Raw score



Solid line represents the linear regression fit line for subjects.

ICV = Intracranial volume

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