Factors associated with Dementia with special reference to Serum Homocysteine Level: A Case-Control Study

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Abstract—*Prevalence of degenerative dementias and dementias associated with cerebrovascular disease is increasing with the time. Dementia is one of the most significant public health problems. Demographic data, medical history, general biochemical data and serum total homocysteine (tHcy) levels was used in this study to examine the differences between dementia and normal control groups. A cross-sectional study was conducted on 236 individuals who were above the age of 65 years. These participants went through the Mini-Mental State Examination (MMSE), Clinical Dementia Rating (CDR), demographic characteristics, biochemical data and tHcy level. Each of the above mentioned factors was assessed. There were significant differences in the history of hypertension, diabetes mellitus, marital status, alcohol consumption (AC), BMI value, and triglyceride (TG) and serum tHcy levels. The logistic regression analysis showed significant differences in marital status, AC and tHcy. So it can be concluded that elevated serum tHcy, no AC and no partner are associated with the risk of dementia in elders of Southern Taiwan. It needs further researches to identify and reduce the risk of dementia.*

Keywords: Dementia; Homocysteine; partner; alcohol consumption.

I. INTRODUCTION

Dementia is characterized by a progressive deterioration of cognitive skills that leads to a decline in the ability to perform daily activities; Current data from developing countries suggest that age-adjusted dementia prevalence estimates in 65 year olds are high (≥5%) in certain Asian and Latin American countries.[1] The aging population in Taiwan has grown rapidly in the past decades, with an increase in the percentage of the population over age 65 from 6.8% to 12.51% in 1992 and 2015, respectively.[2] Sun's study showed that the prevalence of mild cognitive impairment (MCI) prevalence was 18.76% (95% CI 17.91–19.61), this nationwide epidemiological study showed that MCI affects a considerable percentage of the population of Taiwan aged 65 and above and is more prevalent than dementia in Taiwan.[3] Chang's research revealed that feeding difficulty has a higher prevalence in elderly patients with dementia in Taiwan.[4] Dementia is characterized by significant impairments in multiple cognitive domains, functioning, and behavior; dementia places a tremendous burden on the individuals and society. Chen's research identified that dementia is a hidden health issue due to its underestimation in the elderly population within Taiwan and many other counties.[5]

Homocysteine level is associated with endothelial dysfunction and vascular disease, as well as neuropsychiatric disorders.[6] There is evidence suggesting that increased serum tHcy levels are associated with declining cognitive function and dementia, which is currently believed to play a

significant role in AD etiology.[7] Research has recently found that tHcy concentrations are a direct cause of AD.[8] In fact we have observed that plasma tHcy itself seems to play a minor role in cognitive impairment in patients with dementia or other psychogeriatric diseases.[9]

Early detection and management of dementia are very important. They prevent overuse of costly healthcare resources and allow affected individuals and caregivers time to prepare for future medical, financial, and emotional challenges. The main purpose of this study was to compare the serum folic acid, vitamin B12, tHcy and other indexes in patients with and without dementia. Other secondary purposes were to understand the unique differences in gender, age, body mass index (BMI), education, marital status, lifestyle, medical history and serum biochemical data between the elderly with and without dementia

II. METHODOLOGY

This cross-sectional analytic type of descriptive study was conducted from March 1st, 2011 to January 31st, 2014 in Kaohsiung city of Tainan, Taiwan.

2.1 Subjects and setting:

Subjects enrolled in this study were over 65 years of age, and included volunteers from Tainan、Kaohsiung city and county community dwellers. The community dwellers consisted of the population in Tainan、Kaohsiung city and county of the 10 local community in the main southern Taiwan counties and cities. Each community has about 10~20 participants, the participants were randomly selected and consented to the study by the local community village officials and researchers.

Inclusion of residents into the study was completed using the following procedure:

1. Individuals over 65 years old who had resided permanently in the community for the past 6 months and had intentions of stay, were identified by the Director- General in the village.
2. From this list, a minimum of 10 participants from each community (maximum 20) were selected to participate in the study.

Patients with impaired consciousness, severe difficulty hearing, severe speech and language disorders, and those who were unable to complete the evaluation due to severe dementia were excluded. Informed consent was obtained from the subjects before interview with the research team. All assessment measures were administered in accordance with written protocols constructed from original materials, where they were available. This study was reviewed and approved by the Department of Nursing, Chung Hwa University of Medical and Technology Institutional Review Board (IRB). Each participant gave their informed consent before any procedure was performed, and informed consent was obtained from all patients and/or caregivers concerning the nature and purpose of the procedures.

A total of 236 elderly persons above the age of 65 were selected and each candidate responded to a survey. Diagnosis of dementia and assignment of a specific cause was made by consensus of 2 psychiatrists and 1 neuropsychologist based on baseline and follow-up information. From the 236 participants, those with a clinical diagnosis MMSE, CDR, DSM-IV criteria for dementia and computed tomography (CT) scan of the brain as a standard to diagnose of dementia were grouped as 'Dementia group' and grouped the others as the 'Normal control' in the study. The principal investigator made their diagnosis of dementia based on the DSM-IV criteria.[10]

2.2 Assessment

A comprehensive medical history was put together for each subject. Both the neurological examination and the mental status examination were performed by experienced geriatric psychiatrists. This assessment included a complete evaluation of cognitive symptoms, using input from the patient's family members and caregivers. Brain CT imaging and laboratory assessments (including chemistries, electrolytes, complete blood count, liver tests, thyroid tests, serum B12, serum folate, sedimentation rate, tHcy, urinalysis, and chest X-ray) were performed. Plasma levels of tHcy, vitamin B12, folate, and lipids (total cholesterol, HDL cholesterol, and TG) were mostly obtained at the Taiwan Accrediation Foundation-approved laboratory at Yong Kang Veterans Hospital. Then, the psychiatrists checked the subjects' medical history, physical and neurological examinations, and blood test results, and evaluated them using the DSM-IV, MMSE, CDR and CT scan of the brain. Basic surveys were employed to obtain the subjects' information and lifestyle factors, such as age, educational level, partner, medical history, regular AC, smoking habits if any, intellectual activities, and whether the subjects had long-term use of anti-inflammatory medication, painkillers and vitamins.

Total 236 subjects in this study were recruited from March 1st, 2011 to January 31st, 2014. The psychiatrists and psychologists focused on those with suspected dementia according to medical records, physical and neurological examinations, MMSE, CDR and Hachinski Ischemic Score, and the psychiatrists then confirmed the diagnosis using DSM-IV criteria. The MMSE originally suggested using 23/24 points as the cut-off point,11 categorizing those with less than 23 points as having suspected impaired cognitive functioning and those with 24 points or more as normal. However, in China, researchers found that the MMSE scores were greatly affected by the level of education. Therefore, those who were illiterate, the score of 17/18 would be used as the cut-off point, 20/21 for those with less than six years of education, and 24/25 for those with over six years of education. For a place like Taiwan, in which there is a large population of illiterate elderly, this approach is more suitable.[12] We also used CDR $\geq$ 1 as the cut-off point. We then confirmed the diagnostic criteria from a CT scan of the brain, and performed statistical analysis again using the result. The results of these examinations yielded 131 patients with dementia and 105 normal individuals for this research.

At baseline, participants' blood profiles were obtained after a 12-hour fast. The blood was transported on ice to a hospital laboratory for analysis, for processing within 4 hours of collection, and it was isolated and stored at −30 °C until it was analyzed. Plasma tHcy concentrations were measured by HPLC with postcolumn fluorescence detection. Plasma vitamin B-12 concentrations were measured by using a radioassay (Quantaphase II; BioRad Diagnostics, Hercules, CA). Biochemical markers such as glucose, total cholesterol, HDL cholesterol, and TG were analyzed by a biochemical autoanalyzer (Beckman Coluter, Lx-20, USA) at the Clinical Laboratory Department of YangKang veterans Hospital. Fasting blood samples were obtained between 08:00 and 11:00 am to minimize diurnal variation.

2.3 Statistical Analysis

SPSS version 15.0 (SPSS Inc, Chicago, Ill) statistical software was also uses to perform the statistical analysis. Two sample t-tests were used to test the difference in the age of the participants (or their BMI, educational level, serum TG, cholesterol, Vitamin B12, folic acid) between dementia patients and normal control. We also used tHcy as a categorical variable, using the acknowledged level of 15 μmol/L

as the cut-off point. Chi-square was used to test the relationship of dementia with hypertension (or diabetes, current marital status, AC, smoking and tHcy. Stepwise logistic regression analysis was used for the association between lifestyle factors, cardiac risk factors and dementia. Odds ratios (OR) and their 95% confidence intervals (CI) were computed. Level of significance was set at 'p value' 0.05.

III. RESULTS

Total 236 eligible subjects were recruited from March 1st, 2011 to January 31st, 2014 and participated in this study. Out of these 236 eligible participants 131 (55.51%) had dementia. (Figure 1)

As per diagnosis of Dementia these participants were divided into two groups: those with and those without dementia (mean MMSE of the normal control group: 27.78±2.24; mean MMSE of the dementia patients: 11.53±6.04; P<0.001). (Figure 2)

Figure 1

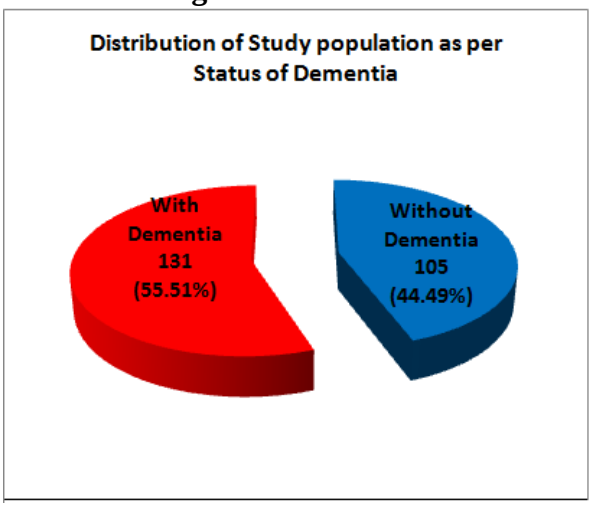
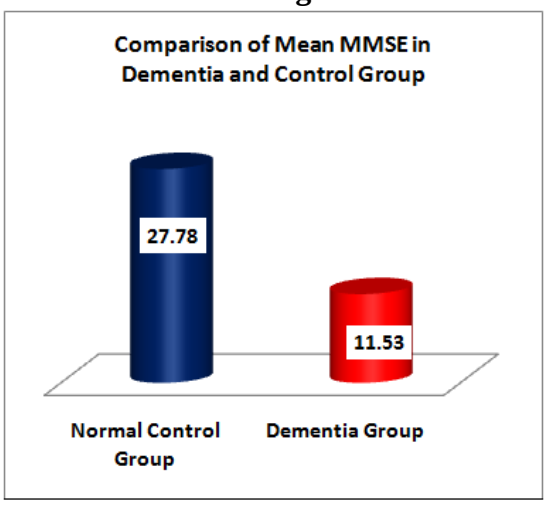


Figure 2



It was observed that although there was no significance difference in age (79.95 ± 3.40 years vs. 80.89 ± 5.39 years; P = 0.322), educational level (6.90 ± 4.23 years vs. 6.58 ± 2.16 years; P = 0.727) but BMI of the dementia patients was significantly lower (24.08 ± 3.26, vs. 22.60±2.99; P<0.001). It was also found that those who lost their spouse were less prone (63.8% v/s 96.9% i.e. p<0.05) to dementia. (Table 1).

Table 1
Comparison of demographic data, medical history, homocysteine and serum biochemical data of dementia and normal control groups

S. No.	Variables	Control Group (N=105)	Dementia Group (N=131)	P value
1	**Age**	79.95 ± 3.40	80.89 ± 5.39	*P = 0.322
2	**Educational level**	6.90± 4.23	6.58± 2.16	*P = 0.727
3	**BMI value**	24.08 ±3.26	22.60 ±2.99	*P <0.001
4	**Lost Spouse Partner (N=194)**	67 (63.8%)	127 (96.9%)	†P <0.001
5	**Triglyceride, mg/dl**	107.15 ± 32.60	91.60 ± 37.03	*P < 0.001
6	**Vitamin B12, pg/dl**	450.93.± 275.38	283.88 ± 116.67	*P < 0.001
7	**Cholesterol, mg/dl**	160.16 ± 35.20	153.11± 31.71	*P = 0.107
8	**Folic acid, ng/dl**	4.94 ± 2.96	3.84 ± 2.60	*P = 0.001
9	**Smoking (N=68)**	33 (31.4%)	35 (26.7%)	†P = 0.427
10	**Alcohol Consumption (N=54)**	43 (41.0%)	11 (8.4%)	†P <0.001
11	**Hypertension (N=74)**	25 (23.8%)	49 (37.4%)	†P = 0.025
12	**Diabetes (N=24)**	6 (5.7%)	18 (13.7%)	†P = 0.043
13	**tHcy Normal (N=77)**	66 (62.9%)	11 (8.4%)	†P <.001

*Unpaired 't' test †Chi-square Test

Participants with dementia had a significantly lower serum TG level (107.15±32.60 vs. 91.60 ± 37.03; P < 0.001), serum Vitamin B12 (450.93 ± 275.38 vs. 283.88 ± 116.67; P < 0.001) and serum cholesterol (160.16 ± 35.20 vs. 153.11± 31.71; P=0.107) than those in the normal control group. But it was found significant in case on TG level and serum Vitamin B12 level only. (Table 1)

It was also revealed from this study that dementia patients having significantly (p<0.001) higher tHcy. It was found that however there were no significant differences between dementia patients and normal controls in their history of smoking but there was significant difference in the history of hypertension (χ^2 = 5.00), and diabetes (χ^2 = 4.11), AC (χ^2 = 35.01). Participants with hypertension and diabetes were more prone to have Dementia. (Table 1)

Taking all the subjects as a single group (n=236), Pearson correlation coefficient was computed to find the relationships among dementia-related risk factors. MMSE correlated significantly with TG, vitamin B12, folic acid, BMI, diabetes, tHcy, AC and partner; partner was correlated with MMSE, TG, vitamin B12, folic acid, BMI, diabetes and tHcy. Because there were multiple associations among these variables, we used the partner, AC and tHcy (with larger chi square value) as factors of the logistic regression model. This study also revealed that there was significant difference in the averages of MMSE, serum TG, vitamin B12 and folic acid; and in the proportions of hypertension, diabetes, AC, and partner between tHcy abnormal and normal controls. (Table 2)

Table 2
Comparison of dementia-related risk factors and Homocystiene (tHcy) level

S. No.	Variables	Normal tHcy level (N=77)	Abnormal tHcy level (N=159)	P value
1	**MMSE**	25.70 ±6.19	15.40 ±8.80	*P <0.001
2	**Lost Spouse Partner (N=194)**	48 (62.3%)	146 (91.8%)	†P <0.001
3	**Triglyceride, mg/dl**	104.49 ± 30.12	95.62 ± 38.14	P =0.023*[a]
4	**Vitamin B12, pg/dl**	469.57 ± 250.33	304.27 ± 179.70	*P < 0.001
5	**Cholesterol, mg/dl**	5.87 ± 3.79	3.58 ± 1.77	*P < 0.001
6	**Alcohol Consumption (N=54)**	30 (39.0%)	24 (15.1%)	†P <0.001
7	**Hypertension (N=74)**	14 (18.2%)	60 (37.7%)	†P = 0.002
8	**Diabetes (N=24)**	2 (2.6%)	22 (13.8%)	†P = 0.007

*Unpaired 't' test †Chi-square Test

This study also revealed Odds ratio for lost partner (odds ratio = 0.051, p < 0.001), AC (odds ratio = 0.099, p < .001) and serum tHcy levels (odds ratio = 12.661, p < .001) showed obvious association with dementia. (Table 3).

Table 3
Multiple Logistic Regression Analysis for Dementia Patients and Normal Control Subjects

Variables	Coefficients	Standard Error	P-value	Odds Ratio	95% CI of odds ratio
Intercept	-0.592	0.372	0.112		
Homocystiene (tHcy) level	2.539	0.426	P <0.001	12.661	5.494 to19.180
Lost Partner/Spouse	-2.938	0.614	P <0.001	0.051	0.015 to 0.169
Alcohol Consumption	-2.312	0.447	P <0.001	0.099	0.041 to 0.238

The area under receiver operation characteristic (AUROC) curve was used to measure the accuracy of above logistic regression model. This showed that the accuracy was 0.877 when we used the logistic regression model of partner, AC and tHcy to predict dementia. (Table 4).

Table 4
Area under ROC curve for different logistic regression models

Logistic regression models	AU ROC	Standard Error	[a] P-value	95% CI of AU ROC
(1) AC+partner+tHcy	.877	.024	**<.001**	(0.830 to 0.924)
(2) AC+partner+tHcy+BMI	.888	.023	**<.001**	(0.844 to 0.933)
(3) AC+partner+tHcy+BMI+religion	.898	.021	**<.001**	(0.857 to 0.939)

[a]the null hypothesis: AUROC=0.5

IV. DISCUSSION

Measurement of tHcy levels has been a recent focus of attention, with epidemiological studies suggesting that elevated levels may be a risk factor for AD and vascular dementia (VaD). Reynolds pointed out that tHcy elevation is associated with endothelial dysfunction and vascular disease [6]. The results of our study showed that serum folic acid and vitamin B12 levels are negatively correlated with plasma tHcy levels in clinically diagnosed dementia patients, and an inverse correlation between MMSE scores and tHcy was observed in these patients. These results were similar to what Inara reported and he pointed out that elevated tHcy is associated with increased dementia risk, and the risk is greater for VaD than for AD.[13] Elevated tHcy, a risk factor for cardiovascular disease, have been shown to be associated with an increased risk for dementia.

Hypertension is the main cause of stroke, but it also increases the risk of dementia. Both cerebral infarction and cerebral hemorrhage could lead to the development of dementia as a complication in stroke patients. The chance of developing VaD is especially high in patients who suffer multiple recurrent strokes. Evidence from prospective cohort studies suggests that individuals who develop high blood pressure during midlife have an increased risk of dementia and cognitive decline.[14] Kivipelto also pointed out that hypertension in middle age is related to higher AD risk, which was similar to our results.

As for marital status, in a previous study of 3,675 subjects above the age of 65 and without dementia, 2,106 were married or cohabiting, 1,287 were widowed, 179 were single, and 105 were divorced or separated. A 5-year follow-up found that the occurrence of dementia was obviously much higher in the group of single subjects than among those who were married or cohabiting.[15] Håkansson et al. also found that people cohabiting with a partner in midlife (mean age, 50.4 years) were less likely than those who were single, separated, or widowed to show cognitive impairment later in life (age 65 to 79 years); these findings are similar to those obtained in this research.[16]

With regard to BMI value, this study showed that the BMI value of the dementia group was obviously lower than that of the normal control group. Perhaps, this was due to the fact that subjects with dementia have decreased self-care ability and they often do not have a regular diet, which leads to nutritional deficiency; thus their BMI values are lower than 24 kg/m^2. Stewart et al. also pointed out that dementia may cause weight loss and lower BMI due to feeding difficulties and other catabolic changes that are not totally understood.[17]

Recent studies have shown that subjects with moderate AC have a lower occurrence rate of AD or dementia when compared with subjects who drink excessively or do not drink. Light to moderate AC appears to have disability prevention benefits only in those men and women in relatively good health.[18] Long-term excessive drinking causes great harm to the body, such as liver cirrhosis, cerebellum

degeneration, trembling hands, peripheral neuropathy, cerebral atrophy, and dementia. On the other hand, many studies have reported that consumption of a small amount of alcohol may reduce the occurrence of cardiovascular disease and ischemic stroke; our study had similar findings.

AD is a degenerative disorder of the brain, but many medical data in recent years had shown that vascular risk factors, such as diabetes, hypertension, hyperlipidemia, and carotid arteriosclerosis, which were previously only recognized for their relationship with stroke, are also risk factors for AD.[19] Given such information, consumption of a small amount of alcohol may lower the occurrence rate of cardiovascular disease and cerebral stroke.[20] Flavonoids are natural anti-oxidants found in alcohol and potentially play a role in protecting subjects that drink moderately from developing AD. This study also showed a higher ratio of moderate AC in the normal control group than in the dementia group (41.0% vs. 8.4%). Whether moderate consumption of alcohol plays a protective role or not requires further verification.

In recent years, it has been found that Hcy also plays an important role in the development of dementia and cognitive impairment without dementia.[21] Homocysteine, an amino acid, is a product of methionine metabolism; therefore, it requires the vitamin B group, such as B12, B6 and folic acid, during the metabolic process. Another study had pointed out that an increase in serum tHcy concentration is related to cognitive degeneration and dementia. There is clear evidence that supports the relationship between serum Hcy level and risk factors for vascular disease.[22] Some cross-sectional or longitudinal studies had reported the associations between elevated tHcy and an increased risk of cognitive impairment.[21] Homocysteine is an important pathological factor not only for VaD but also for AD.[21] This study obtained a similar result, in that the dementia group had a significantly higher ratio in serum tHcy level (normal control group: 37.1%, dementia group: 91.6%, $\chi^2 = 78.64$). Garcia and Zanibbi reported similar results in 2004.[23] They found that increased serum tHcy levels were associated with declining cognitive function and dementia. Our study further carried out stepwise logistic regression analysis of factors that showed significant differences in marital status, AC and serum tHcy level.

One limitation of this study was our use of only one measurement of the tHcy, which could have led to a measurement error and an underestimation of the association between tHcy and cognitive impairment. Another limitation is that the patient's information was based on clinical records or on the presence of a specified risk factor. Furthermore, we did not identify AD or VaD, which could bias the results in some way. Finally, we consider that the next step should be a longitudinal study of the same population.

V. CONCLUSION

Among the items that were examined, three factors: partner, AC and tHcy had significant influence on dementia. All these factors could be improved with changes in everyday life, such as having a better lifestyle (Living with partner or other family members), taking vitamins B-complex daily, improving current living conditions, having more social interaction with others and enjoying an active retirement by participating in community activities.[24] Alcohol consumption should be kept to a moderate amount. One should not drink excessively, and instead, utilize the flavonoids in alcohol for natural protection. In addition, a regular diet and exercise, avoiding high-salt foods, and good control of blood pressure are all ways to delay dementia. We could also use the tHcy as an indicator in the screening assessment and prevention of senile cognitive functioning. Some studies in recent years have shown that the tHcy is related to cognitive functioning. Folic acid, vitamin B12 and vitamin B6 can all reduce the serum tHcy level.[25] In other words, examining these items can provide an indication of cognitive functioning, and is

informative for public health. Evidence shows that an increased tHcy is accompanied by decreased cognitive functioning and increased dementia symptoms.

CONFLICT

None declared till date.

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