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Plasma Malondialdehyde Level, Serum High Sensitivity C-reactive Protein Level and Cognitive Ability in Elderly People

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Oxidative stress and low grade inflammation are associated with cognitive impairment in aging. This study aimed to determine plasma malondialdehyde (MDA) level and serum high-sensitivity C-reactive protein (hs-CRP) level in three groups of elderly people with normal cognitive ability, with minor cognitive impairment and with dementia, to compare both parameters among three studied groups, and to find out the relationship between each of these two parameters and cognitive ability in all elderly people. This study was conducted in elderly people with normal cognitive ability (n=24), elderly people with minor cognitive impairment (n=22) and elderly people with dementia (n=24). Cognitive ability was assessed by Mini Mental State Examination (MMSE) and the cut-off score for dementia was MMSE ≤23, that of minor cognitive impairment was MMSE 24-27, and that of normal cognitive ability was MMSE ≥28. Plasma MDA and serum hs-CRP were determined by spectrophotometric method using thiobarbituric acid and enzyme linked immunosorbent assay, respectively. Plasma MDA level of the elderly people with dementia (2.64±0.66 μmol/l) was significantly higher than that of elderly people with normal cognitive ability (1.26±1.03 μmol/l), ($p<0.001$) and those with minor cognitive impairment (1.86±1.10 μmol/l), ($p<0.05$). There was a significant negative correlation between plasma MDA level and MMSE score (Pearson's $r=-0.469$, $p<0.001$, $n=70$) in all elderly people. The median and interquartile range of serum hs-CRP level of the elderly people with normal cognitive ability, those with minor cognitive impairment and those with dementia were 2.03 (0.89-4.06), 2.95 (0.95-4.34), and 4 (1.45-8.65) mg/l, respectively. There was no significant difference in serum hs-CRP levels among three studied groups. A significant negative correlation was seen between serum hs-CRP level and MMSE score (Spearman's $\rho=-0.247$, $p<0.05$, $n=70$) in all elderly people. The present findings indicated that oxidative stress might be involved in pathogenesis of cognitive impairment but the role of low-grade inflammation in cognitive impairment was still equivocal.

Key words: Oxidative stress, Low grade inflammation, Cognitive ability

INTRODUCTION

At present, the average life expectancy of human is increasing. The number of older persons was 841 million worldwide in 2013 and it will be almost triple by 2050.[1] Although the higher life expectancy reflects a positive development, it also brings new challenges. In particular, age-related diseases such as cognitive impairment and dementia, have become more and more prevalent for both the individual and for society.[2] Cognitive impairment in the elderly exists many forms, ranging from subtle impairments through mild cognitive impairment (MCI) to dementia and Alzheimer's disease (AD).[2] According to the free radical theory, aging can be considered as a progressive, inevitable process partially related to the accumulation of oxidative damage into biomolecules (nucleic acids,

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lipids, proteins or carbohydrates).[3] In the aging brain and in the case of several neurodegenerative diseases, there is a decline in the normal anti-oxidant defense mechanisms, which increases the risk of oxidative damage to the brain.[4]

Some studies reported that oxidative damage to biomolecules occurs early in the pathogenesis of AD and precedes pronounced neuropathological alterations.[5-7] Most of the studies described an increased level of the peripheral MDA in AD patients [8-10] as well as in red blood cells of both MCI and early stage AD patients (mild AD).[7] However, in some studies, it was shown that there was no difference in peripheral level of MDA between AD patients and healthy control subjects.[11,12] Therefore, the association between oxidative stress and cognitive function is still controversial. It was suggested that an important mechanism for age-related cognitive impairment-chronic low-grade inflammation may play a role in age-related cognitive impairment.[13] High sensitivity C-reactive protein (hs-CRP) can reflect the presence of inflammation and can be induced by cytokines.[14] Thus, Arai, *et a*l. in 2006 suggested that hs-CRP can be used as a candidate biomarker for screening patients with cognitive impairment.[15] Some studies described that increased serum CRP levels are associated with concurrent cognitive impairment[16] and with poorer cognitive performance at baseline in elderly.[17]

However, some studies showed that there was no association between serum CRP and baseline cognitive performance.[18, 19] Thus, this issue of possible role of serum CRP in age-related cognitive impairment may be still needed to clarify.

MATERIALS AND METHODS

A cross-sectional, comparative study was carried out from April 2015 to February 2016. Elderly people of age 65 years and above were recruited from Day Care Centre for the Aged, Department of Social Welfare in Mayangone Township and Home for the Aged Poor (Kandawkalay), Mingalar Taungnyunt Township, Yangon. Seventy elderly people were selected according to inclusion and exclusion criteria. History taking and physical examination including anthropometric measurements were done according to proforma.

After the 10-hour fasting (from 8:00 pm to 6:00 am), about 5 ml of fasting blood sample were taken: 2 ml of blood sample were collected into anticoagulant (EDTA) containing tube for plasma MDA assay and another 3 ml of blood were collected in plain tube for serum hs-CRP assay. Blood samples were centrifuged at 3000 rpm for 15 minutes. Plasma MDA level was determined at the day of sample collection. The serum was kept in a separated tube which was stored at -20°C until analysis for hs-CRP. Plasma MDA level was determined by spectrophotometric method using thiobarbituric acid.[20] Serum hs-CRP level was determined by enzyme linked immunosorbent assay according to manufacturer's instruction from DRG instruments, Germany.

Cognitive ability was assessed by Mini Mental State Examination (MMSE) and the cut-off score for dementia was MMSE$\leq$23, that for minor cognitive impairment was MMSE 24-27, and that for normal cognitive ability was MMSE $\geq$ 28. After asking the questions individually, scoring was done according to the instructions given consistency and cognitive ability score was recorded with code number.

Statistical analysis

Data were presented as mean±SD. Data analysis was done by using the Statistical Package for Social Sciences software (SPSS) version 16. The difference between data of the elderly people with normal cognitive ability, those with minor cognitive impairment and dementia was assessed by one way ANOVA test. Pearson's and Spearman's correlation coefficients were computed to explore strength and significance of the relationships among

variables. The statistical significance level was set at $p<0.05$.

Ethical consideration

Ethical consideration was done according to the guideline of Board of Studies (Physiology), University of Medicine 1, Yangon. The elderly people were explained first, and written informed consent was obtained.

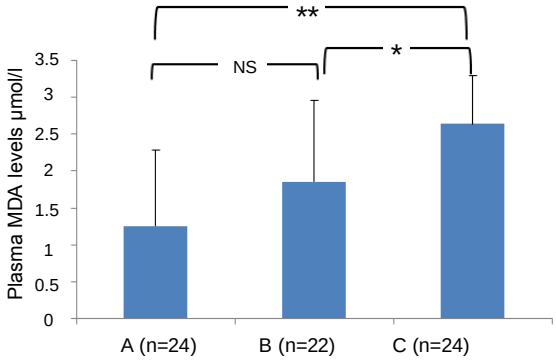
RESULTS

Baseline characteristics of the three different groups of elderly people are shown in Table 1.

Table 1. Baseline characteristics of three different groups of the elderly people

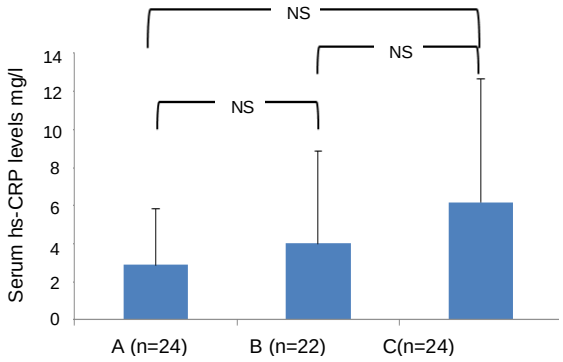
	Elderly people with			P value
	Normal Cognitive Ability (n=24)	Minor cognitive impairment (n=22)	Dementia (n=24)	
Age (years)	76.7±5.10	77.7±4.17	80.1± .29	0.049
Weight (kg)	51.59±8.59	52.68 ±1.34	46.88± 3.04	NS
Height (cm)	158.6±7.54	158.1±7.33	150.4±9.40	0.001
BMI (kg/m^2)	20.51±3.06	21.12±4.66	20.69±5.10	NS
Heart rate (beats/min)	79.8±4.44	81.5±5.32	80.9±4.64	NS
Resting SBP (mmHg)	123.5±8.07	131.7±11.97	126.3 13.99	NS
Resting DBP (mmHg)	77.3±5.43	78.2±5.55	76.9 .75	NS

Data are presented as mean±SD.
NS=No significant difference



A= Elderly people with normal cognitive ability
B= Elderly people with minor cognitive impairment
C= Elderly people with dementia
**=Highly significant difference ($p<0.001$)
*=Significant difference ($p<0.05$)
NS=No significant difference

Fig. 1. Comparison of plasma malondialdehyde levels among three different groups of the elderly people



A=Elderly people with normal cognitive ability
B=Elderly people with Minor cognitive impairment
C=Elderly people with Dementia
NS=No significant difference

Fig. 2. Comparison of serum high sensitivity C-reactive protein levels among three different groups of the elderly people

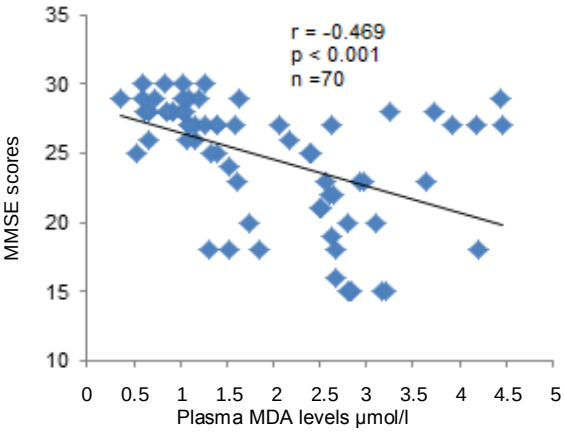


Fig. 3. Correlation between plasma MDA levels and Cognitive ability (MMSE scores) of all elderly people

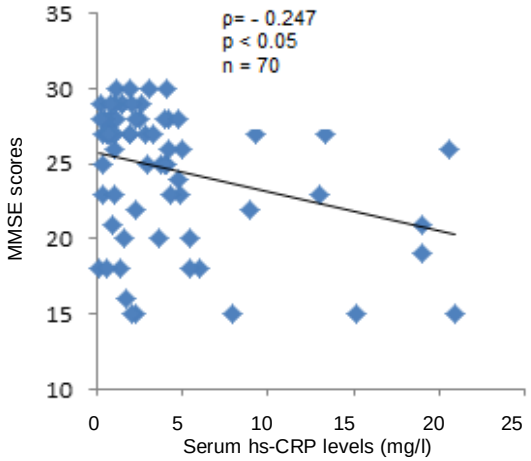


Fig. 4. Correlation between serum hs-CRP levels and Cognitive ability (MMSE scores) of all elderly people

Plasma MDA level of the elderly people with dementia (2.64±0.66 µmol/l) was significantly higher than that of the elderly people with normal cognitive ability (1.26± 1.03 µmol/l), ($p<0.001$) and those with minor cognitive impairment (1.86±1.10 µmol/l), ($p<0.05$) (Fig. 1). There was no significant difference in serum hs-CRP level between three studied groups (Fig. 2).

There was a significant negative correlation between plasma MDA level and MMSE score ($r=-0.469$, $p<0.001$, $n=70$) in all elderly people (Fig. 3). A significant negative correlation between serum hs-CRP level and MMSE score ($p=-0.247$, $p<0.05$, $n=70$) in all elderly people (Fig. 4).

DISCUSSION

In the present study, mean plasma MDA level of the elderly people with normal cognitive ability was 1.26±1.03 µmol/l, those with minor cognitive impairment was 1.86±1.10 µmol/l and those with dementia was 2.64±0.66µmol/l. The normal reference interval of plasma MDA level (20-79 years) is 0.34-1.37 µmol/l.[21] Plasma MDA levels of the elderly people with normal cognitive ability were within normal reference range, whereas that of the other two groups were found to be higher than this reference range. In a study[22] in 2005, plasma MDA level of thirty eight apparently healthy elderly people (63-75 years) was 1.96±0.66 µmol/l before antioxidants supplementation.[22] The plasma MDA level of elderly people with normal cognitive ability in the present study was slightly lower than the other study.[22]

In the present study, plasma MDA levels of the elderly people with dementia were significantly higher than that of the elderly people with normal cognitive ability and minor cognitive impairment. The findings of the present study agreed with Torres, *et al.* in 2011 which demonstrated that average MDA level AD patients was higher than that of MCI patients and healthy aged controls.[23] The present finding was also consistent with that of Greilberger, *et al.* in 2008 and Padurariu, *et al.* in 2010.[11, 24] Greilberger, *et al.* in 2008 reported a significance difference ($p<0.05$) in plasma MDA level of control group ($n=15$; 1.15±0.32 µmol/l) and the neurodegenerative group ($n=16$; 2.62±1.27 µmol/l) in elderly subjects.[11] Padurariu, *et al.* in 2010 also found that a significant increase of the MDA level ($p<0.0005$) was found in the serum of MCI ($n=15$) and AD ($n=15$) patients compared to the age-matched control group ($n=15$).[24]

Many studies showed an association between pathogenesis of AD or neurodegenerative disorders with oxidative stress, which might be responsible for the resulting dysfunction and death of neuronal cells. Thus, the present study also gave supportive evidence that plasma MDA level increases in cognitive impairment such as AD or dementia subjects. There was also a significant negative correlation between plasma MDA level and MMSE score in all elderly. It indicated that low cognitive performance was related to elevated plasma MDA level in aging. The oxidative stress theory of aging postulated that a progressive and irreversible accumulation of reactive oxygen species (ROS) impacts on the senescence process leading to impaired physiology function and increasing incidence of age related pathologies.

Thus, the observed findings of the present study indicated that an oxidative stress might be involved in cognitive impairment in the elderly people. Many studies had been demonstrated that a positive correlation between decreased antioxidant defense and increased lipid peroxidation in MCI and AD patients.[7, 24] It indicated that the progression of AD or dementia may be related to an inadequate capability of the antioxidant defense system to counterbalance the oxidative attack. One of the limitations of the present study was that antioxidant defense parameters (i.e. blood antioxidant enzymes or vitamin levels) related to aging were not determined and assessed in the present study. Thus, it is

recommended that antioxidant enzymes or vitamins should be determined to get insight into oxidative stress and antioxidant imbalance in cognitive impairment in future studies.

In the present study, the median and inter-quartile range of serum hs-CRP levels of the elderly people with normal cognitive ability, those with minor cognitive impairment and those with dementia were 2.03(0.89-4.06 mg/l), 2.95(0.95-4.34 mg/l) and 4(1.45-8.65 mg/l), respectively. There was no statistically significant difference in serum hs-CRP level between the three studied groups.

The present study was in consistent with the findings of Kim, *et al*. in 2015 in which there was no significant difference in serum CRP levels between AD, MCI and control groups.[14] Contrary to the present findings, Dimopoulos, *et al.* in 2006 reported significantly higher serum concentration of adhesion molecules and hs-CRP in patients with dementia (n=37) compared to controls (n=33).[25] Although the present study did not find significant difference in serum hs-CRP among three studied groups, the involvement of low-grade inflammation in the pathogenesis of cognitive impairment could not be excluded. Cross-sectional study design and smaller sample size could be the possible confounding factors for this issue. Since the distribution of hs-CRP was highly skewed, and it is possible that sample size of the present study was not sufficient to detect any significant difference across MMSE score groups.

Thus, longitudinal study with larger sample size would be suggested to determine causal inference and increased hs-CRP with risk of cognitive decline. Another possibility is that the present data showed wide variation in serum hs-CRP level. Selection error may in part explain these results. In the present study, a major effort was made to avoid inclusion of participants with major inflammatory conditions. However, the participants in the present study included hypertensive elderly people except those with malignant hypertension. Previous studies suggested that hypertension may lead to multiple inflammatory stimuli at the vessel wall which in turn promote the production of a number of proinflammatory cytokines such as tumor necrosis factor-α(TNF-α), interleukin-6 (IL-6) and CRP as a defense against injurious factors.[26]

In the present study, there was no correlation between serum hs-CRP level and MMSE score in all three studied groups. However, a significant negative correlation was seen between serum hs-CRP level and MMSE score in all elderly people (Spearman's p=-0.247). There was limited evidence of cross-sectional study to find correlation between hs-CRP and cognitive function in dementia or AD. However, some studies showed increased concentrations of serum hs-CRP has been associated with increased risk of vascular dementia[27] and Alzheimer's disease at follow up.[28] Weuve, *et al.* [29] in 2006 showed there was no evidence of a link between hs-CRP and decrements in cognitive function in older women.

Conclusion

Based on the results of comparison correlation studies, the present study stress might be involved in pathogenesis of cognitive impairment but it could not provide definite evidence that relatively high levels of serum hs-CRP has a negative impact on cognitive impairment.

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