Association between Plasma Leptin and Estrogen in Female Patients of Amnestic Mild Cognitive Impairment

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Increasing evidences suggested the association between leptin and cognitive functions. Estrogen is an important factor that regulates the production and metabolism of leptin. However, little is known about the relationship between leptin and estrogen in mild cognitive impairment (MCI). Plasma levels of leptin, total estradiol, and β-amyloid protein (Aβ) were measured in a total of 23 female amnestic MCI (aMCI) patients and 19 female cognitively normal controls. This study showed that female aMCI patients had lower plasma levels of leptin and higher levels of estradiol compared to female normal controls. Leptin and estradiol levels were not correlated with cognitive performances or plasma Aβ levels in either aMCI patients or normal controls. There was a significant negative correlation between leptin and estrogen in female aMCI patients ($r = -0.633, p = 0.002$) but not in female normal controls. The potential mechanisms of this disease-stage-specific association between leptin and estrogen need further investigations.

1. Introduction

Alzheimer’s disease (AD) is the major cause of senile dementia. The deposition of extracellular β amyloid peptide (Aβ) and the presence of neurofibrillary tangles formed by hyperphosphorylated tau protein are the neuropathological hallmarks of AD. It has been well-known that AD pathological changes begin several years before the onset of dementia. Mild cognitive impairment (MCI) is the symptomatic pre-dementia stage on the continuum of cognitive decline [1]. Considering that neurons have been irreversibly damaged in the dementia stage, the MCI stage might be more valuable for early detection and prevention.

Previous studies have shown that plasma leptin levels were significantly lower in MCI patients than those in normal controls [2]. The longitudinal studies also demonstrated that higher leptin levels were associated with decreased risk of cognitive decline [3]. The laboratory studies identified that the leptin signaling pathways were linked to the pathophysiology of AD. In AD hippocampus, leptin signaling was decreased and its localization was shifted to reactive astrocytes instead of neurons [4]. Accumulating in vivo and in vitro studies have suggested that leptin is associated with decreased Aβ production and tau phosphorylation [5].

The hormone leptin is secreted predominantly by adipocytes and involved in regulating appetite, energy balance, and neuroendocrine. Estrogen is one of the important factors that regulate the metabolism, accumulation, and distribution of adipose tissue and exerts major effects on leptin production. In cell models, estrogen enhanced leptin gene expression [6], and in ovariectomized rats, supplement of estrogen increased leptin concentration [7]. Furthermore, estrogen plays important roles in pathogenic mechanisms of AD. Numerous studies have demonstrated the neuroprotective
effects of estrogen. Estrogen can inhibit the formation of Aβ plaques and neurofibrillary tangles [8]. Thus, all these
evidences showed that there is a close relationship between
leptin and estrogen and both of them influence the patho-
physiological changes of AD. However, in MCI stage, the
study of association between leptin and estrogen is still
lacking.

In this study, we selected female amnestic MCI (aMCI)
patients as our study subjects. The longitudinal clinical-
pathological studies have identified that aMCI patients are on
a pathway toward AD, and 75~80% of aMCI patients finally
convert to AD [9]. We investigated the changes of leptin and
estrogen levels in female aMCI patients compared to those
in normal controls and the relationship between leptin and
estrogen in aMCI. We were also interested in the influences
of leptin and estrogen on memory function and plasma Aβ
levels.

2. Methods

2.1. Subjects. The aMCI patients and cognitively normal
controls were recruited from the memory clinic of Xuan Wu
Hospital from January 2012 to March 2014. For all of our
patients, aMCI was a diagnosis made by at least
two experienced neurologists. These neurologists made the
diagnosis strictly according to the published criteria [10, 11]:
cognitive concern by subject, informant, nurse, or physician;
impairment in 1 or more of the 4 cognitive domains; essen-
tially normal functional activities; and absence of dementia.
Subjects with MCI were classified as aMCI if the memory
domain was impaired.

All the controls had normal cognitive functions, assessed
by the Mini-Mental State Examination (MMSE), Montreal
Cognitive Assessment (MoCA), and the Clinical Dementia
Rating (CDR) scale, and none of them had previous history
of neurological or psychiatric disorders.

Subjects with acute illnesses or taking exogenous hor-
mones currently or previously were excluded. All the sub-
jects were postmenopausal in this study. Written informed
consents were obtained from all participants or their relatives
before the study. This study was approved by the Institutional
Review Board of Xuan Wu Hospital.

2.2. Assessments. Both cases and controls were asked to
provide information on age, years of formal education,
height, and weight. The body mass index (BMI) was cal-
culated as weight (kilograms) divided by squared height
(meters). The MMSE and MoCA were used to assess global
cognitive ability [12, 13]. The CDR was used to examine
the different cognitive domains [14]. We used the World Health
Organization-University of California-Los Angeles Auditory
Verbal Learning Test (WHO-UCLA AVLT) to measure verbal
memory [15]. In this test, the subjects were asked to recall a 15-
word list right after the presentation. This kind of immediate
recall was repeated 3 times (maximum score = 45). Thirty
minutes later, the subjects performed the long delay free recall
(maximum score = 15) and long delay recognition (maximum
score = 15).

2.3. Plasma Examinations. Plasma samples were obtained
in the morning following an overnight fast. The samples were
kept at −80°C until being analyzed. Plasma leptin levels were
measured by a commercial enzyme-linked immunosorbent
assay (ELISA) kit (R and D Systems, Inc., Minneapolis,
MN, USA). The lower limit of detection was 7.8 pg/mL.
The interassay precision was 3.5~5.4%, and the intra-assay
precision was 3.0~3.2%. The plasma levels of estradiol
were detected by an electrochemiluminescence immunoas-
say (cobas e 601, Roche Diagnostics GmbH, Mannheim,
Germany) [16]. The detection limit of estradiol was 18.4 pmol/L.
The intra-assay coefficient of variation (CV) at 130 pmol/L
was 3.3%, and interassay CV at 120 pmol/L was 4.7%.
The plasma levels of Aβ42 and Aβ40 were determined by ELISA
kit (Invitrogen, USA) [17]. The intra-assay and interassay CVs
for Aβ42 were 3.1% and 4.0%, respectively. For Aβ40, the in-
tra-assay and interassay CVs were 2.4% and 3.6%, respectively.

2.4. Statistical Analysis. Normality of distribution was exam-
ined using Kolmogorov-Smirnov test. We used independent
samples t-tests to explore the characteristics of our sub-
jects, including age, education, BMI, and cognitive scores.
However, the levels of Aβ42 and Aβ40 were skewed distrib-
ution, so they were analyzed with a nonparametric Mann-
Whitney U test. For comparisons of leptin and estrogen levels
between aMCI group and normal controls, linear regression
models were constructed. The levels of leptin and estrogen
were added to regression models as dependent variables,
respectively; disease status (aMCI or normal control) was
independent variable, and age and BMI were adjusted. We
analyzed the correlations using Pearson or Spearman tests
when involving Aβ42 and Aβ40 levels. For the correlations
between leptin and estradiol and cognitive scores, the partial
correlation analyses were used to control confounded factors,
including age, education, and BMI. A p value <0.05 was
regarded as statistically significant.

3. Results

3.1. Subjects’ Characteristics. A total of 23 female aMCI
patients and 19 female cognitively normal controls were
included. The characteristics of our subjects were presented
in Table 1. Age, education, and BMI were matched between
these two groups. All the aMCI patients had a CDR score of
0.5, and all the controls had a CDR score of 0. The MMSE
and MoCA scores were significantly lower in aMCI patients
than those in normal controls. The aMCI patients performed
significantly worse in memory tests compared to the controls.
There were no group differences in the plasma levels of Aβ42,
Aβ40, or Aβ40/Aβ42.

3.2. Comparisons of Plasma Leptin and Estradiol Levels
between aMCI Patients and Normal Controls. All aMCI
patients had the levels of estradiol higher than the detec-
tion limit. Two normal controls had undetectable plasma
estradiol levels, which were regarded as the lower detection
limit (18.4 pmol/L). The comparisons of plasma leptin and
estradiol levels between groups were shown in Table 2. The
Table 1: The characteristics of our subjects.

|                    | aMCI, n = 23 | Control, n = 19 | p    |
|--------------------|--------------|-----------------|------|
| Age                | 67.2 (6.9)   | 68.1 (7.3)      | 0.707|
| Education (year)   | 9.4 (3.1)    | 11.3 (3.4)      | 0.073|
| BMI                | 23.5 (1.5)   | 24.7 (3.1)      | 0.110|
| Cognitive tests    |              |                 |      |
| MMSE               | 24.6 (2.9)   | 27.7 (1.7)      | <0.001|
| MoCA               | 20.4 (3.2)   | 25.8 (2.5)      | <0.001|
| Immediate verbal recall | 15.1 (3.2) | 24.7 (4.4)      | <0.001|
| Delay free recall  | 3.6 (1.5)    | 8.7 (2.3)       | <0.001|
| Delay recognition  | 6.9 (2.2)    | 12.1 (1.6)      | <0.001|
| * Plasma Aβ42 (pg/mL) | 24.6 (20.8, 27.3) | 22.2 (19.8, 29.7) | 0.695|
| * Plasma Aβ40 (pg/mL) | 108.3 (89.2, 136.7) | 100.7 (90.9, 148.6) | 0.771|
| Plasma Aβ40/Aβ42   | 4.5 (0.6)    | 4.8 (0.9)       | 0.258|

Values are means (SDs).
* Values are medians (interquartile ranges).

Table 2: Comparisons of plasma leptin and estradiol levels between aMCI patients and normal controls.

|                        | aMCI, n = 23 | Control, n = 19 | β   | p  |
|------------------------|--------------|-----------------|-----|----|
| Plasma leptin (mean ± SD, ng/mL) | 2.6 (1.1)    | 6.7 (4.2)       | 1.165| <0.001|
| Unadjusted             |              |                 |     |    |
| Adjusted               |              |                 | 1.339| <0.001|
| Plasma estradiol (mean ± SD, pmol/L) | 35.5 (10.8) | 28.4 (7.1)      | -0.093| 0.029|
| Unadjusted             |              |                 |     |    |
| Adjusted               |              |                 | -0.118| 0.019|

Adjusted for age and BMI.

aMCI patients had significantly lower plasma leptin levels than normal controls; however, their plasma estradiol levels were significantly higher than those in normal controls. After adjusting for age and BMI, the results were still unchanged.

3.3. Correlations between Leptin, Estrogen, Cognitive Functions, and Plasma Aβ Levels. The correlation analyses were conducted in aMCI patients and normal controls, respectively. In normal controls, there was no correlation between leptin and estradiol levels. Neither leptin nor estradiol was correlated with any cognitive tests, and further adjustment for age, education, and BMI did not change the results. Leptin and estradiol levels were not correlated with plasma Aβ levels (including Aβ42, Aβ40, and Aβ40/Aβ42) in normal controls. In aMCI patients, similarly, the levels of leptin and estradiol were not correlated with the scores of cognitive examinations or plasma Aβ levels. However, there was a significantly negative correlation between leptin and estradiol levels in aMCI patients (r = −0.621, p = 0.002). This correlation was also shown in the scatter plot (Figure 1).

4. Discussion

An increasing number of evidences suggested the association between leptin and AD, and numerous laboratory studies have demonstrated the extensive roles that estrogen plays in the pathogenic mechanisms of AD. Interestingly, there is a close relationship between estrogen and leptin; estrogen is one of the important regulating factors in the metabolism of leptin [18, 19]. Some previous researches have studied the links between leptin and estrogen in normal females; however, to our knowledge, our study is the first one that investigated the association between leptin and estrogen in aMCI patients. The results of this study showed that (1) female aMCI patients had lower levels of leptin and higher levels of estradiol compared to female normal controls, (2) there was a significant negative correlation between leptin and estradiol levels in female aMCI patients, but leptin and estradiol levels were not correlated in cognitively normal women, and (3) leptin and estradiol levels were not correlated with the scores of cognitive tests or plasma Aβ levels in either aMCI patients or normal controls.

In previous cross-sectional studies, plasma leptin levels were decreased in MCI patients [2]. In longitudinal studies,
higher leptin levels were associated with a lower risk of AD and cognitive decline [3, 20, 21]. Our result of lower leptin levels in aMCI patients was consistent with these studies. However, some other studies showed that the plasma levels of leptin were not altered in MCI patients and not associated with the risk of cognitive decline [22–24]. Based on the controversial results, one study identified the influences of race and gender, and the result suggested that leptin levels were negatively associated with the scores of cognitive tests in black men but showed an opposite effect in white men; more interesting, for women, there was no relationship between leptin levels and cognitive performances in this previous study [25]. In our study, all subjects were female and no correlation was found between leptin and cognitive performances. Thus, the influences of gender and race should be considered in further studies on leptin and cognition, and our results need confirmation in larger Asian samples.

Although the exact change of leptin in cognitive impairment population was still inconsistent, many laboratory studies had suggested the neuroprotective effects of leptin against AD [26]. Multiple studies demonstrated that leptin reduced Aβ production and downregulated the activity and expression levels of BACE1 [27]. The plasma leptin was strongly negatively associated with Aβ levels in the mouse brain [28]. Thus, in our study, we investigated the correlation between leptin and plasma Aβ levels (including Aβ42, Aβ40, and Aβ40/Aβ42). However, no correlation was found in either aMCI patients or normal controls. Similarly, using CSF, the previous study also showed no correlation between CSF levels of leptin and Aβ42 [4].

The most interesting finding of our study was the higher estradiol levels in aMCI patients and the negative correlation, which was not found in normal controls, between plasma leptin and estradiol levels in aMCI patients. Although estrogen is usually regarded as a neuroprotective factor, the effects of estrogen on AD are still ambiguous in population studies. Some studies reported comparable or even higher levels of estrogen in patients with AD [29, 30]. Consistent with previous studies [31–33], our study did not find the correlation between leptin and estradiol in cognitively normal postmenopausal women. However, in female aMCI patients, the leptin levels were significantly negatively correlated with estradiol. The “healthy cell bias of estrogen action” hypothesis may partially explain these inconsistent results [34]. This hypothesis suggests that estrogen has beneficial effects on neurological function if neurons are intact; however, oppositely, if neurological health has already been compromised, estrogen may exacerbate neurological impairment.

There are some limitations in our study. First, as a preliminary study, our sample size is relatively small. Since there was no previous study that investigated the relationship between leptin and estrogen in aMCI patients, our result needs confirmation in larger samples. For the aim of our study, the study samples were females only and our results are probably not suitable for men, and considering the possible influences of race on the results, our results may be more valuable for Asian population. Furthermore, the potential confounded factors were controlled when we analyzed the data; however, the influences of unknown or unmeasured factors cannot be excluded. Last, the cross-sectional study design precludes the explanation of causal relationship.

5. Conclusions
In conclusion, our study is valuable for further observation and intervention studies on leptin and estrogen in MCI patients. As a preliminary study, our results suggested that there were opposite changes in plasma leptin and estrogen levels in female aMCI patients. Interestingly, the significant negative correlation between leptin and estrogen existed in female aMCI patients but not in female normal controls. Our study suggested that there may be a disease-stage-specific association between leptin and estrogen, and the potential mechanisms deserve further investigations.

Conflict of Interests
The authors have no conflict of interests to declare.

Authors’ Contribution
Yi Xing, Jianghong Liu, and Jingdong Xu contributed equally to this work.

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