

The expression of insulin-like growth factor-1 in senior patients with diabetes and dementia

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Abstract. This study was conducted to investigate the expression of insulin-like growth factor-1 (IGF-1) in elderly patients with diabetes and dementia and to analyze the expression mechanism. A total of 30 senior patients with diabetes and dementia (group A), 30 senior patients with dementia but no diabetes (group B), 30 senior patients with diabetes but no dementia (group C), and 30 healthy seniors (group D) were continuously selected. The ELISA method was used to test the level of serum IGF-1, β -amyloid peptide (A β) and the phosphorylation of immunohistochemical staining of microtubule associated protein (tau protein). Western blot analysis was utilized to test the level of prion protein (PrP), forkhead transcription factor O (FOXO) subfamily protein, p-PI3K and p-Akt. The levels of IGF-1, A β , tau protein positive rate, PrP, FOXO protein, p-PI3K, and p-Akt in group A were significantly higher than that in group B, which was higher than in groups C and D. The results between groups A and B, but not groups C and D, were statistically significant ($P < 0.05$). IGF-1 was highly expressed in senior patients with diabetes and dementia. Thus, IGF-1 can adjust the expression of PrP and FOXO through p-PI3K/Akt pathway and further impact the formation of A β and tau protein, leading to dementia.

Introduction

Alzheimer's disease (AD) is related to age and is a neurodegenerative disease featuring progressive cognitive disorder

and memory damage. Since diabetes can increase the risk of vascular dementia and AD, some scholars regard AD as another type of diabetes (1). Rasgon and Jarvik (2) found that cognitive disorders or AD incidence rates were 2- to 3-fold higher in patients with type 2 diabetes mellitus (T2DM) than those in control group. Thus, T2DM was found to be a risk factor in cognitive disorders among seniors. The main pathologic features of AD include senile plaques, neurofibrillary tangles, neuro-reduction and insulin neuro-transduction involved in its metabolic pathway (2).

Insulin-like growth factor-1 (IGF-1) is an important neurotrophic factor and its receptor IGF-1R is prevalently expressed in the nervous system (3). IGF-1 can impact the elimination of β -amyloid peptide (A β) and the phosphorylation of microtubule associated protein (tau), which is related to PI3K/Akt and MAPK/ERK1/2 signal pathways (4). Forkhead transcription factor O (FOXO) subfamily protein, an important downstream responsive molecule in IGF-1 signal pathway, is modified and adjusted by the post-translational modification in the PI3K/Akt pathway (5). Most studies conducted are centered on AD animal models while few involve in-clinic observation. Therefore, this study serves as a reference to clinical diagnosis and treatment by examining whether the IGF-1 expression in different groups of seniors is related to diabetes and AD, and the possible action mechanism.

Patients and methods

Subject information. A total of 30 senior patients who were admitted to and diagnosed by our hospital with diabetes and AD (group A), 30 with AD but no diabetes (group B), 30 with diabetes but no AD (group C), and 30 healthy seniors (group D) were continuously selected from June 2013 to January 2016. The selection criteria for the study were: i) Age, ≥ 65 ; and ii) meet WHO's T2DM diagnosis standard and Chicago AD diagnosis standard, including pre-clinical phase and dementia phase. The exclusion criteria included: i) Serious diabetes complications, such as eye-ground retinal hemorrhage, diabetic kidney disease, diabetic foot, and cardiovascular and cerebrovascular diseases, such as ischemic stroke, head injury, history of surgery and cancer; ii) neuropsychiatric disorders, autoimmune diseases, severe anxiety and depression; and iii) non-compliant patient.

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The present study was approved by the ethics committee of Yantai Affiliated Hospital of Binzhou Medical University. Informed consent was obtained from the patients or their families. There were 17 males and 13 females in group A; average age of 70.5 ± 6.3 ; fasting blood sugar averaged 8.2 ± 2.0 mmol/l; and the course of disease averaged 5.6 ± 1.7 years. There were 16 males and 14 females in group B, and the average age was 71.7 ± 6.5 . There were 15 males and 15 females in group C; average age of 71.6 ± 6.8 ; fasting blood sugar averaged 8.5 ± 2.2 mmol/l; and the course of disease averaged 5.8 ± 1.4 years. There were 16 males and 14 females in group D, and the average age was 71.5 ± 6.3 . The difference in gender and age among groups was not statistically significant ($P > 0.05$).

Study methods. We utilized the ELISA method to test the levels of serotonin IGF-1, A β and the phosphorylation of immunohistochemistry staining microtubule associated protein (tau protein). We utilized the western blot method to test the level of prion protein (PrP), FOXO subfamily protein, p-PI3K and p-Akt. ELISA kits were purchased from Sigma (St. Louis, MO, USA) and the procedures in the manual were followed during testing. The main procedures of the immunohistochemical (SP) staining method were producing peripheral mononuclear cells of paraffin through conventional steps, including dewaxing, gradient alcoholic dehydration, 3% H₂O₂ inactivation of peroxidase, antigen retrieval, closure, adding phosphorylated tau protein (pSer202) antibody (1:100; Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China), negative control plus phosphate-buffered saline (PBS), at 4°C overnight, 5 times of 3-min PBS washing, adding biotin-marked antibody (Beijing ZS-Bio Co., Ltd., Beijing, China), 30-min at 37°C incubation, 5 times of 3-min PBS washing, DAB coloration, hematoxylin staining, conventional dehydration, transparency made by xylene, neutral balsam mounting, observation and capturing images under a microscope (Olympus, Tokyo, Japan). We continuously observed 3 sections, counted 4 non-overlap horizons randomly <400-fold enlarged horizon, and tested the ratio of the number of cells with positive response, which was stained pale brown, in each horizon. The averages were determined by using the Image-Pro Plus v6.0 image analysis system.

Western blot analysis involved extraction of general protein by conventional protein extraction kit (CWbio Co., Ltd., Beijing, China), BCA protein quantitation kit (Beijing ZS-Bio Co., Ltd.) testing, polyacrylamide gel electrophoresis (separation gel, stacking gel buffer, Beijing ZS-Bio Co., Ltd.), transmembrane (PVDF film; Millipore Corp., Billerica, MA, USA), closure, antibody incubation (first antibody is rabbit anti-rat PrP 1:2,000; FOXO 1:2,000; p-PI3K 1:1,000; p-Akt 1:1,000; GAPDH 1:500; second antibody is HRP-marked goat anti-rabbit, 1:500), developing and fixing, scanning and analyzing band density by using gel image analysis software, expressing relative content of target protein by using the ratio of target band and relevant GAPDH band signal intensity. The test was repeated 3 times and the average was calculated.

Statistical analysis. SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA) was utilized to analyze the data. Mean \pm standard deviation was used to represent the quantitative data and comparisons among various groups were analyzed by one-way

Table I. Comparison of the level of IGF-1 and A β .

Group	IGF-1, ng/ml	A β , pg/ml
A	160.3 \pm 42.1	268.7 \pm 78.5
B	113.4 \pm 34.6	184.7 \pm 62.3
C	94.7 \pm 25.8	105.6 \pm 41.4
D	82.6 \pm 20.3	112.5 \pm 50.6
F-value	10.325	22.624
P-value	<0.001	<0.001

Group A, senior patients with diabetes and dementia; group B, senior patients with dementia but no diabetes; group C, senior patients with diabetes but no dementia; group D, healthy seniors. IGF-1, insulin-like growth factor-1; A β , β -amyloid peptide.

ANOVA, while the qualitative data were expressed by the number of cases, and inter-group comparison was tested by χ^2 . $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of the level of serum IGF-1 and A β . The levels of IGF-1 and A β in group A were significantly higher than that in group B, which was significantly higher than that in groups C and D ($P < 0.05$). The differences between groups C and D were not statistically significant ($P > 0.05$; Table I).

Comparison of tau protein phosphorylation level. The tau protein positive rate of group A was $75.6 \pm 20.3\%$, which was significantly higher than that of group B, $46.9 \pm 15.6\%$, which was significantly higher than that of groups C and D, 12.3 ± 5.5 and $10.8 \pm 4.7\%$, respectively. The results were statistically significant ($F = 15.634$, $P < 0.001$). The difference between groups C and D were not statistically significant ($P > 0.05$; Fig. 1).

Comparison of the level of PrP, FOX protein, p-PI3K and p-Akt. The levels of PrP, FOX protein, p-PI3K and p-Akt of group A were significantly higher that of group B, which were significantly higher than that of groups C and D, and the results were statistically significant ($P < 0.05$). The difference between groups C and D were not statistically significant ($P > 0.05$; Table II and Fig. 2).

Discussion

AD and Creutzfeldt-Jakob disease (CJD) are neurodegenerative diseases that are caused by conformational change which result from the abnormal protein folding. In these diseases, normal and solvable protein transfers to unsolvable protein aggregation. In AD, this results in A β protein deposition and in CJD, this results in proteinase K resistant prion protein (PrP^{Sc}) deposition (6). It has been found that PrPs play an important role in the process where A β triggers AD (7). PrP, a highly conserved protein in animals, is highly expressed in

Table II. Comparison of the level of PrP, FOX protein, p-PI3K and p-Akt.

Group	PrP	FOXO	p-PI3K	p-Akt
A	0.63±0.07	0.74±0.08	0.82±0.06	0.76±0.05
B	0.44±0.05	0.48±0.06	0.53±0.07	0.50±0.06
C	0.18±0.03	0.20±0.04	0.21±0.05	0.19±0.04
D	0.15±0.03	0.17±0.03	0.16±0.04	0.15±0.03
F-value	9.637	8.457	13.265	11.527
P-value	<0.001	<0.001	<0.001	<0.001

PrP, prion protein; FOX, forkhead box.

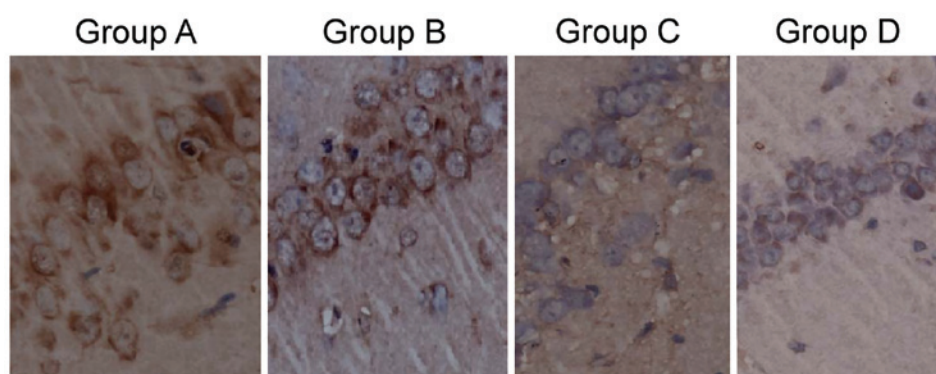


Figure 1. Immunohistochemical staining of tau protein (magnification, x400).

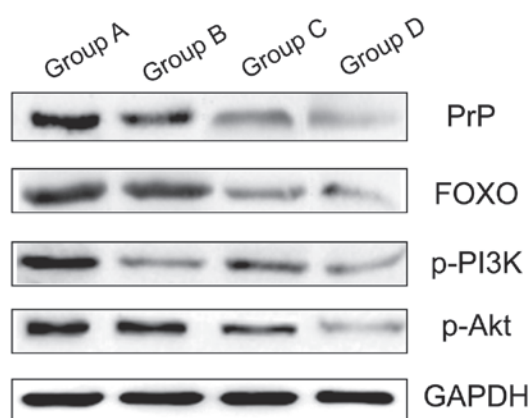


Figure 2. Western blot analysis. PrP, prion protein; FOX, forkhead box.

the nervous system. In normal conditions, PrP is glycoprotein coded by the housekeeping gene and located on the surface of cell membranes. The change in natural conformation featuring rich α -spiral to the wrong conformation featuring rich β -folding (that is PrP^C transferring to PrP^{Sc}) will lead to lethal neurodegenerative diseases such as CJD in mammals. The incidence of AD is accompanied by abnormal protein folding, conformational change, the formation of protein aggregation, and neuronal cell degeneration. Protein aggregation plays an important role in the death of cells (8). Currently, there are two opinions on the impact of PrP in the incidence

of AD: One is that the PrP, as the membrane-anchored protein, functions as signal molecular receptor and triggers a pathway in the cell leading to toxicity by combining with A β oligomer outside the cell (9). The second opinion is that the PrP can impact A β formation, and is neuro-protective (10). In fact, the two functions can coexist and function in the body due to the PrP structural features and multi-functions resulted from the complexity of glycosylation.

IGF-1, made up of 70 amino acids and also known as sulphited factor and growth regulator C is a member of the insulin family. It has the function of stimulating the production, differentiation, migration, survival and metabolism. In recent years, IGF-1 has been found closely related to the central nervous system, especially to AD (11). IGF-1 functions through the IGF-1R, which is prevalent in the body. IGF-1R belongs to receptor tyrosine kinase family and is phosphorylated by IGF-1, which can activate insulin receptor substrate serial and other signal transduction pathways, including mitotic active protein kinase pathway, and PI3K/PKB pathway (12). IGF-1 plays a significant role in A β metabolism and tau protein phosphorylation. Adlerz *et al* (13) found that by stimulating α -secretase, IGF-1 decreased the formation of A β . Zhang *et al* (14) utilized PC12 cells and found that by stimulating α -secretase, IGF-1 decreased the formation of A β and that IGF-1 treatment can significantly decrease β -secretase (BACE-1) mRNA and protein levels and further decrease the formation of A β by using quantitative polymerase chain reaction (PCR) and western blot method for analysis of the results. This process was related to PI3K/Akt and MAPK/ERK1/2 signal pathways

and the disorders of this signal system are involved in the formation of AD pathology. Wang *et al* (15) conducted IGF-1 intervention by using A β 25-35 protein to induce injured PC12 cells to establish a cell model for over-phosphorylated tau proteins and found that IGF-1 can repress apoptosis of A β 25-35-induced PC12 cells and tau protein phosphorylation, the action mechanism of which is activated through the signal transduction pathway.

FoxO family, a transcription factor, is a critical factor in INS/IGF-1 signal pathway. The upstream is adjusted by inter-connected pathway of PI3K-PKB and the downstream adjusted target genes are mostly related to cell cycle, apoptosis, aging and metabolism (16).

FoxO phosphorylation/dephosphorylation status is closely related to the transcription adjustment function. FoxO transcription activeness is subjected to adjustment of the complex signal pathway. There are mainly two types of FoxO transcription activeness adjusted by phosphorylation: one is PI3 relying on pathway for phosphorylation adjustment and the other is non-PI3 relying on pathway for phosphorylation adjustment. PI3K/Akt/FoxO is a verified signal pathway (17). Insulin, IGF-1 and other growth factors combine with tyrosine kinase receptor to activate PI3K. Then Akt, including protein kinase of Akt family and relevant serum and glucocorticoid, induces the activation of SGK. RT-PCR and northern blot display that FoxO transcription factor is highly expressed in tissues and organs of adults, including in the heart and brain (18). In recent years, the study on FoxO action mechanism has gradually transferred from tumor cells to neuronal cells and it was found that when PI3K/Akt is activated, it can phosphorylate and deacetylate under the control of Sirt1, which enhanced the survival and production of neuronal cells (19). When the PI3K/Akt pathway is repressed, it can dephosphorylate and acetylate under the control of P300, inducing neuronal cell apoptosis (20). Dick and Bading (21) reported that the neuronal cells in CA1 area of hippocampus in particular is extremely prone to damage, which is related to the highly expressed FoxO transcription factors in CA1 area (21).

It is concluded from the present study that the level of IGF-1 and A β , tau protein positive rate, and the level of PrP, FOXO protein, p-PI3K and p-Akt of group A is significantly higher than that of group B, which is higher than that of groups C and D, and the results are statistically significant. The difference between groups C and D is not statistically significant. In summary, IGF-1 is highly expressed in senior patients with diabetes and dementia and it can adjust the expression of PrP and FOXO through p-PI3K/Akt pathway and further impact the formation of A β and tau protein, leading to dementia.

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