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Peripheral blood heat shock protein 27 correlates with information processing speed and executive function, potentially serving as a marker for mild cognitive impairment in patients with type 2 diabetes mellitus

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Abstract

Background and aims Previous study found that interleukin 1β (IL-1β) is associated with diabetic cognitive dysfunction. Heat shock protein 27 (HSP27) is one of the factors related to IL-1β associated inflammation. Here, we aim to investigate the role of HSP27 in mild cognitive impairment (MCI) in patients with type 2 diabetes mellitus (T2DM).

Materials and methods In this study, individuals with T2DM with and without MCI were recruited and categorized into Control and MCI groups. Plasma HSP27 levels were assessed and compared between the Control and MCI groups. Furthermore, the relationship between HSP27 and diabetic dysfunction was elucidated through association and regression analyses. Finally, diagnostic values were determined using ROC curves.

Results In humans, individuals with T2DM and MCI exhibit decreased levels of HSP27 compared to those without MCI. Notably, the levels of HSP27 are associated with neuropsychological test scores that reflect cognitive preferences. Additionally, decreased HSP27 levels serve as one of the risk factors for MCI in T2DM patients (OR = 0.355, $P = 0.002$). Moreover, there is a defined cut-off point for HSP27 in diagnosing MCI, set at 3.51 pg/ml, with a sensitivity of 47.2%, a specificity of 94.4%, and an area under the curve (AUC) of 0.695.

Conclusions Generally speaking, HSP27 is linked to cognitive decline in individuals with T2DM. Decreased levels of HSP27 in plasma are identified as both a risk factor for MCI and a potential diagnostic biomarker for MCI in patients with T2DM. The diagnostic value of HSP27 in MCI is primarily reflected in its demonstrated true negative rate.

Keywords Type 2 diabetes mellitus, Heat shock protein 27, Mild cognitive impairment

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Introduction

Mild cognitive impairment (MCI) is one of the complications of diabetes mellitus [1]. It serves as a transitional phase between normal cognitive function and dementia, with the potential to progress into dementia if effective interventions are not employed. The scarcity of well-defined mechanisms hinders the development of intervention strategies, while the inconspicuous nature of MCI symptoms presents challenges for timely diagnosis [2]. Consequently, it becomes imperative to explore the underlying mechanisms and ascertain early biomarkers for MCI in order to inform the design of intervention strategies specific to patients diagnosed with type 2 diabetes mellitus (T2DM).

Neuroinflammation represents a significant feature of diabetic cognitive impairment [3]. Our prior investigation demonstrated that elevated interleukin-1β (IL-1β) levels in the plasma are linked to memory impairment in patients with T2DM [4]. In animal models, diabetic mice induced by streptozotocin exhibited impaired cognitive function accompanied by increased IL-1β levels in the hippocampus. Notably, the cognitive improvements observed following treatment were accompanied by a reduction in IL-1β levels [5]. Heat shock proteins (HSPs), a family of stress proteins with diverse roles in inflammation [6] have been implicated in the modulation of spatial memory associated with neuroinflammation in sleep-deprived rats [7]. Specifically, hippocampal tissue from rats with impaired spatial learning and memory capacity demonstrated heightened levels of HSPs [8]. Moreover, HSPs were found to exacerbate tau pathogenesis in aged wild-type mice with cognitive impairment [9].

Apart from IL-1β-associated neuroinflammation, HSPs are also implicated in various processes such as hypoxia, ischemia, cellular energy depletion, cellular damage resulting from calcium overload, and oxidative stress [10]. Additionally, HSPs also play roles in apoptosis [11] observed in aging-related diseases. Notably, diabetic cognitive impairment represents an aging-associated condition characterized by neuronal apoptosis [12–14]. The expression of HSP27, derived from the hspb1 gene, is associated with age-related changes [15] and leads to a reduction in reactive oxygen species (ROS) levels [16]. Thus, it has been demonstrated that HSP27 may have a significant role in oxidative stress, which is closely linked to diabetic cognitive impairment [17, 18]. In fact, an animal experiment has indicated that oxidative stress and Ferroptosis are associated with cognitive dysfunction in db/db mice [19]. Interestingly, HSP27 regulates iron homeostasis, thereby preventing ferroptosis caused by the excessive accumulation of intracellular iron [20]. Additionally, studies on the correlation between heat shock protein 27 and changes in the hippocampus and cortex of diabetic rats associated with cognitive function [21]. Moreover, there exists a strong association between HSP27 and specific changes observed in Alzheimer's disease, particularly with regards to the presence of tangles in the hippocampus [22].

Generally, it is postulated that HSP27, which is derived from the expression of the hspb1 gene, may have implications in the development of MCI and could potentially serve as a biomarker for MCI in individuals with T2DM. In this present work, we analyzed the correlation between the levels of HSP27 and cognitive preferences in patients with T2DM. Finally, the diagnostic values of HSP27 for MCI in patients with T2DM were calculated.

Materials and methods

Clinical study design and clinical ethical approval

A total of 108 patients were recruited from Department of Endocrinology at Taizhou People's Hospital, all of whom met the diagnostic criteria for T2DM. Out of these patients, 36 were identified as having MCI and were subsequently allocated to the MCI group. The remaining 72 patients who exhibited normal cognitive function were allocated to the control group. Prior to participation in the study, all participants were provided with information regarding the purpose and procedures of the investigation, and subsequently provided informed consent by signing their name. This study was ethically approved by the Ethics Committee for Medical Research at Taizhou People's Hospital (Approval No.: 2022-046-01) on April 28, 2022.

Inclusion and exclusion criteria

All study participants met the 1999 Criteria for diabetes as defined by the World Health Organization [23] and had a diabetes duration exceeding three years. Among the recruited individuals, 36 fulfilled the criteria for MCI according to the standards established by the MCI Working Group of the European Consortium on Alzheimer's disease [24]. The remaining 72 participants did not meet the MCI criteria but were included in the control group as they met the standards for T2DM. Exclusion criteria were consistent with those utilized in a previous study [25] and are specifically delineated below: (a) Recently diagnosed acute complications of diabetes; (b) Severe hypoglycemia; (c) Acute vascular diseases of the heart and brain; (d) Alcohol or drugs abuse; (e) Diagnosed thyroid diseases (with thyroid dysfunction or abnormal autoimmune antibodies); (f) Severe infections, major surgeries; (g) Visual or hearing impairments preventing completion of neuropsychological tests; (h) Depression and dementia (severe cognitive decline beyond the scope of MCI); (i) Other diseases (cancer, and autoimmune diseases, disease with inflammation, etc.) or drugs (glucocorticoid drugs, drugs with neurological damage, etc.) potentially affecting cognition.

Clinical data collection

The study gathered clinical information from patients, encompassing age, gender, duration of education, and diabetes status. Anthropometric assessments, such as weight and height, were conducted upon admission, and body mass index (BMI) was computed using the formula weight (kg)/height $(m)^2$. Blood samples were drawn on the second day of hospitalization to assess fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) at the Center Laboratory of Taizhou People's Hospital, for clinical purposes. All data were extracted from the patients' medical records. The procedure for measuring HSP27 levels is elaborated below.

Neuropsychological tests

The evaluation of global cognitive abilities in this study utilized MoCA scores. In instances where the education duration was under 12 years, an additional point was added to the MoCA scores. The calculation of MoCA scores adhered to a previously documented method [26]. Information processing speed was assessed through TMTA, following protocols outlined in prior research [27]. Executive function was appraised via the Digit Span Test (DST) [28], Verbal Fluency Test (VFT) [29] and Trail Making Test-B (TMTB) [27], based on previous studies. Scene memory function was assessed using the Logical Memory Test (LMT) [30].

Enzyme-Linked Immunosorbent Assay (ELISA)

In addition to the measurements mentioned above, we also obtained and processed blood samples simultaneously to extract plasma. To do so, the collected blood samples were centrifuged at 4 °C for 30 min with a relative centrifugal force of 1000 g. Following this, the resulting plasma samples were quantified for HSP27 levels using an ELISA kit following the manufacturer's protocol (Cloud-Clone Corp., Wuhan, China, Catalogue No.: SEA693Hu), which has a detection range from 0.78 ng/ml to 50 ng/ml.

Sample size calculation

The minimum sample size was determined using PASS V11.0.7 (NCSS, USA). Following the recruitment of participants, the minimum sample size was calculated considering the ratio of patients in the MCI group to those in the control group, along with the mean and standard deviation of HSP27 levels. With a 1:1 ratio between the MCI and control groups, the minimum sample size for each group is 22 participants.

Statistical methods

SPSS 22.0 (IBM, USA) was utilized for data analysis. Levels of LDL-C were normally distributed variables, thus their disparities in the two groups were depicted using mean and standard deviation and compared using Student's t-tests. Age, education levels, BMI, HbA1c, FPG, TG, TC, HDL-C, HSP 27, MoCA, DST, VFT, TMTA, TMTB, and LMT were asymmetrically distributed variables. Therefore, the disparity between them in the two groups was described using median and interquartile range and compared using nonparametric Mann–Whitney U tests. Gender was represented as a binary variable, with frequency and percentage used for description. The disparity in gender between the two groups was compared using a chi-squared test. Pearson and partial correlation analyses were performed with or without adjusting for factors (age and gender), respectively. Additionally, binary logistic analysis was employed to identify the risk factors for MCI. Furthermore, multiple linear regression was conducted to investigate the factors influencing information processing speed function and executive function. Finally, the diagnosis cut-off point and diagnostic values were evaluated using ROC curves.

Results

Comparison of clinical characteristics, HSP27 levels and cognitive preference in T2DM patients with and without MCI

In this present study, an initial examination was conducted on the clinical data of subjects diagnosed with T2DM. Employing a cross-sectional study design, notable distinctions in age and gender were observed between patients in the MCI group and the control group. Our findings revealed a significant disparity in age, with patients exhibiting MCI being notably older than those with normal cognitive function ($P = 0.020$). Additionally, a significant divergence in gender distribution was observed, with a higher percentage of females in the control group compared to the MCI group ($P = 0.017$). Notwithstanding, various pertinent factors, encompassing educational attainment, BMI, and levels of HbA1c, FPG, TG, TC, HDL-C, and LDL-C, demonstrated satisfactory homogeneity across both groups (all $P > 0.05$). This clinical segment of the study sought to elucidate the potential role of HSP 27 in individuals diagnosed with T2DM experiencing MCI. To fulfill this aim, a comparative analysis was undertaken concerning plasma HSP 27 levels and cognitive performance scores derived from various assessments, namely the MoCA, DST, VFT, TMTA, TMTB, and LMT, between T2DM patients with and without MCI. Significantly decreased levels of HSP27 were identified in patients exhibiting impaired cognition compared to those with normal cognitive function ($P = 0.025$). Furthermore, patients with MCI displayed

Table 1 Comparation of clinical parameters, cognitive function preference and HSP27 levels between control and MCI group in patients with T2DM

	Control ($n=72$)	MCI ($n=36$)	*P*
Age (year)	55.50 (49.00, 58.00)	58.50 (53.00, 64.75)	0.020[b*]
DM duration (year)	10.00 (5.00, 13.00)	9.00 (5.00, 13.75)	0.878[b]
Female (n, %)	21, 29.17	19, 52.78	0.017[c*]
Education	12.00 (9.00, 15.00)	12.00 (6.00, 12.00)	0.201[b]
BMI (Kg/m^2)	25.01 (22.39, 27.16)	23.98 (22.53, 25.95)	0.450[b]
HbA1c (%)	8.50 (7.03, 10.00)	8.90 (7.88, 9.60)	0.643[a]
FPG (mmol/l)	7.67 (6.02, 10.11)	7.39 (5.87, 8.99)	0.302[b]
TG (mmol/l)	1.43 (1.00, 2.45)	1.49 (0.79, 2.35)	0.912[b]
TC (mmol/l)	3.98 (3.54, 4.95)	4.34 (3.35, 5.36)	0.646[b]
HDL-C (mmol/l)	0.98 (0.85, 1.16)	1.01 (0.87, 1.20)	0.655[b]
LDL-C (mmol/l)	2.73 ± 0.81	2.71 ± 0.74	0.889[a]
HSP27 (pg/ml)	3.36 (2.86, 4.19)	2.97 (2.54, 3.29)	0.025[b*].
MoCA	28.00 (27.00, 28.00)	23.00 (21.25, 25.00)	< 0.001[b*]
DST	11.67 ± 1.85	10.72 ± 2.25	0.022[b*]
VFT	18.00 (16.00, 21.00)	14.00 (12.25, 17.00)	< 0.001[b*]
TMTA	53.00 (41.50, 69.75)	75.00 (50.75, 100.00)	0.001[b*]
TMTB	135.50 (108.50, 174.75)	176.50 (135.75, 240.00)	< 0.001[b*]
LMT	9.00 (6.00, 13.00)	7.50 (4.25, 12.00)	0.126[b]

Notes:

a Student's t test was employed for normally distributed variables

b The Mann-Whitney U test was employed for asymmetrically distributed variables

c The Chi-square test was employed for categorical variables

*$P < 0.05$

Abbreviations: HSP27, Heat shock protein 27; MCI, Mild cognitive impairment; BMI, Body mass index; HbA1c, Glycosylated hemoglobin; FPG, Fasting plasma glucose; TG, Triglycerides; TC, Total cholesterol; LDL-C, Low density lipoprotein cholesterol; HDL-C, High density lipoprotein cholesterol; MoCA, Montreal Cognitive Assessment; DST, Digit Span Test; VFT, Verbal Fluency Test; TMTA, Trail Making Test-A; TMTB, Trail Making Test-B; LMT, Logical memory test

diminished scores on MoCA, DST, and VFT, coupled with elevated scores on TMTA and TMTB, in comparison to their counterparts without MCI (all $P < 0.05$). While a decline in LMT scores was also noted in diabetic patients with cognitive impairment relative to those with T2DM and normal cognitive function, statistical significance was not attained ($P = 0.126$) (refer to Table 1).

Association between HSP 27 levels and cognitive preference in patients with T2DM

To scrutinize the potential correlation between HSP27 and cognitive function among individuals diagnosed with T2DM, Pearson association analyses were executed. The findings unveiled noteworthy associations between HSP 27 levels and MoCA scores ($R = 0.276$, $P = 0.004$), as well as TMTA and TMTB scores (R = -0.300, $P = 0.002$; R = -0.318, $P < 0.001$) in T2DM patients. Given the inherent disparities in age and gender between T2DM patients with and without MCI, coupled with the observed link between cognitive performance and the duration of DM, partial association analyses were additionally performed. These analyses controlled for age, gender, and DM duration. Intriguingly, the results demonstrated a positive correlation between HSP27 levels and MoCA scores ($R = 0.255$, $P = 0.008$), along with a negative association with TMTA and TMTB scores (R = -0.249, $P = 0.010$; R = -0.258, $P = 0.007$) in T2DM patients, adjusting for age, gender, and DM duration (see Table 2).

Table 2 Association between HSP27 and cognitive function in patients with T2DM

	Model 1		Model 2	
	R	*P*	R	*P*
MoCA	0.276	0.004*	0.255	0.008*
DST	-0.003	0.979	-0.049	0.621
VFT	0.148	0.127	0.104	0.289
TMTA	-0.300	0.002*	-0.249	0.010*
TMTB	-0.318	< 0.001*	-0.258	0.007*
LMT	0.088	0.365	0.088	0.368

Notes:

Model 1 showed the Pearson association between HSP27 and cognitive preference test scores in all patients and patients with MCI; Model 2 showed the partial association between HSP27 and cognitive preference test scores in all patients and patients with MCI adjusted for age and gender

*$P < 0.05$

Abbreviations: HSP27, Heat shock protein 27; MCI, Mild cognitive impairment; MoCA, Montreal Cognitive Assessment; DST, Digit Span Test; VFT, Verbal Fluency Test; TMTA, Trail Making Test-A; TMTB, Trail Making Test-B; LMT, Logical memory test

Analysis for the risk factor for MCI in patients with T2DM

As HSP27 levels are associated with MoCA scores, which is related to the global cognitive function, we guess that HSP27 may serve as one of the risk factors of MCI in patients with T2DM. In order to determine whether decreased levels of HSP27 serve as a risk factor for MCI in patients diagnosed with T2DM, binary logistic regression analysis was employed. The results indicate that decreased plasma concentrations of HSP27 are indeed a risk factor for MCI in T2DM patients (OR = 0.353, $P = 0.001$). Additionally, after adjusting for age and gender, decreased levels of HSP27 remained a risk factor for MCI in T2DM patients (OR = 0.355, $P = 0.002$) (Table 3).

Decreased HSP27 levels May influence TMTA and TMTB scores in patients with T2DM

To further explore the impact of HSP27 on cognitive function, a detailed investigation was undertaken employing multiple linear regression analyses. The objective was to elucidate the effect of HSP27 on TMTA and TMTB scores in patients with T2DM. Intriguingly, the analysis revealed that HSP27 not only exerted a statistically significant influence on TMTA scores ($P = 0.002$) but

Table 3 HSP27 is an independent risk factor for MCI in patients with T2DM

	β	OR	95% CI for OR		*P*
			Lower	Upper	
Model 1	-1.043	0.353	0.188	0.659	0.00[1*]
Model 2	-1.080	0.355	0.182	0.693	0.002*

Notes:

Model 1 showed that HSP27 is a risk factor for MCI without the adjusting for age and gender; Model 2 showed that HSP27 is a risk factor for MCI adjusting for age and gender

*$P < 0.05$

Abbreviations: HSP27, Heat shock protein 27; MCI, Mild cognitive impairment

Table 4 Analysis for factors influence the TMTA and TMTB scores of T2DM patients

	Model 1		Model 2	
	β	*P*	β	*P*
TMTA	-9.788	0.002*	-6.911	0.010*
TMTB	-19.485	< 0.001*	-13.632	0.007*

Notes:

Model 1 indicated that HSP27 was a significant factor that influenced both TMTA score and TMTB score when age and gender were not adjusted for; Model 2 indicated that HSP27 was a significant factor that influenced both TMTA score and TMTB score after adjusting for age and gender

*$P < 0.05$

Abbreviations: TMTA, Trail Making Test-A; TMTB, Trail Making Test-B; T2DM, Type 2 diabetes mellitus; Heat shock protein 27

also on TMTB scores ($P < 0.001$). Importantly, this effect was observed to be independent of other potentially confounding factors such as age and gender in patients with T2DM ($P = 0.010$ and 0.007, respectively), as presented in Table 4.

Evaluation of the diagnostic value of HSP27 for MCI in patients with T2DM

As a decreased level of HSP27 has been identified as a potential risk factor for MCI in patients with T2DM, we conducted a detailed investigation to ascertain the diagnostic utility of HSP27. Using ROC curve analysis, we determined the diagnostic cut-off value for HSP27 to be 3.51 pg/ml. Furthermore, our analysis revealed a sensitivity of 47.2%, a specificity of 94.4%, and an area under the curve (AUC) of 0.695 for the established cut-off value (Fig. 1).

Discussion

With the increasing prevalence of diabetes [31], diabetes-related cognitive impairment has emerged as one of the pressing issues requiring attention [32]. MCI represents an intermediary state between normal cognition and dementia, and at this stage, it is potentially amenable to intervention [33]. As it progresses over time to the dementia stage, cognitive impairment enters an irreversible phase [34]. However, due to the unclear pathogenic mechanisms and the lack of effective biomarkers, there is currently no fully effective means of intervention and early identification. Numerous studies suggest that various factors, including glucose metabolism [35], fatty acid metabolism [36], cholesterol metabolism [37], and even the occurrence of non-alcoholic fatty liver disease [38], are closely associated with diabetes-related cognitive impairment. Therefore, identifying biomarkers for patients at high risk of cognitive impairment is crucial. On the other hand, given the high prevalence and serious consequences of cognitive decline among individuals with diabetes, some patients experience significant psychological stress due to the fear of developing cognitive dysfunction. As a result, timely identification of both low-risk and high-risk individuals is equally important.

Neuroinflammation stands out as one of the extensively investigated mechanisms in the pathogenesis of cognitive dysfunction associated with diabetes. Our preliminary research [4, 39], along with existing studies [40–42], has elucidated the involvement of neuroinflammation in diabetes-related cognitive impairment. HSP27, particularly in non-type 2 diabetes populations, exhibits a close association with cognitive dysfunction. Notably, its relevance extends beyond oxidative stress [43, 44] and ferroptosis [45, 46], encompassing a significant connection with inflammatory responses. The explicit involvement of oxidative stress, ferroptosis, and neuroinflammation in the occurrence of cognitive dysfunction is well-established. Consequently, we hypothesize a close correlation between HSP27 and cognitive dysfunction in diabetic patients. Indeed, our study reveals that, compared to T2DM patients without MCI, those with MCI exhibit significantly decreased levels of HSP27 in peripheral plasma. Moreover, these levels correlate with the global cognitive function assessed by the MoCA score, and this correlation persists even after adjusting for age and gender. This suggests that decreased HSP27 levels may be linked to the occurrence of MCI in patients with T2DM, possibly representing a risk factor for MCI. Subsequently, our research employs binary logistic regression analysis to further investigate the risk factor for MCI in diabetic patients. The results consistently demonstrate that decreased peripheral blood level of HSP27 is a risk factor for MCI in patients with T2DM, irrespective of age and gender adjustments.

To further elucidate the relationship between peripheral blood HSP27 levels and cognitive dysfunction, we

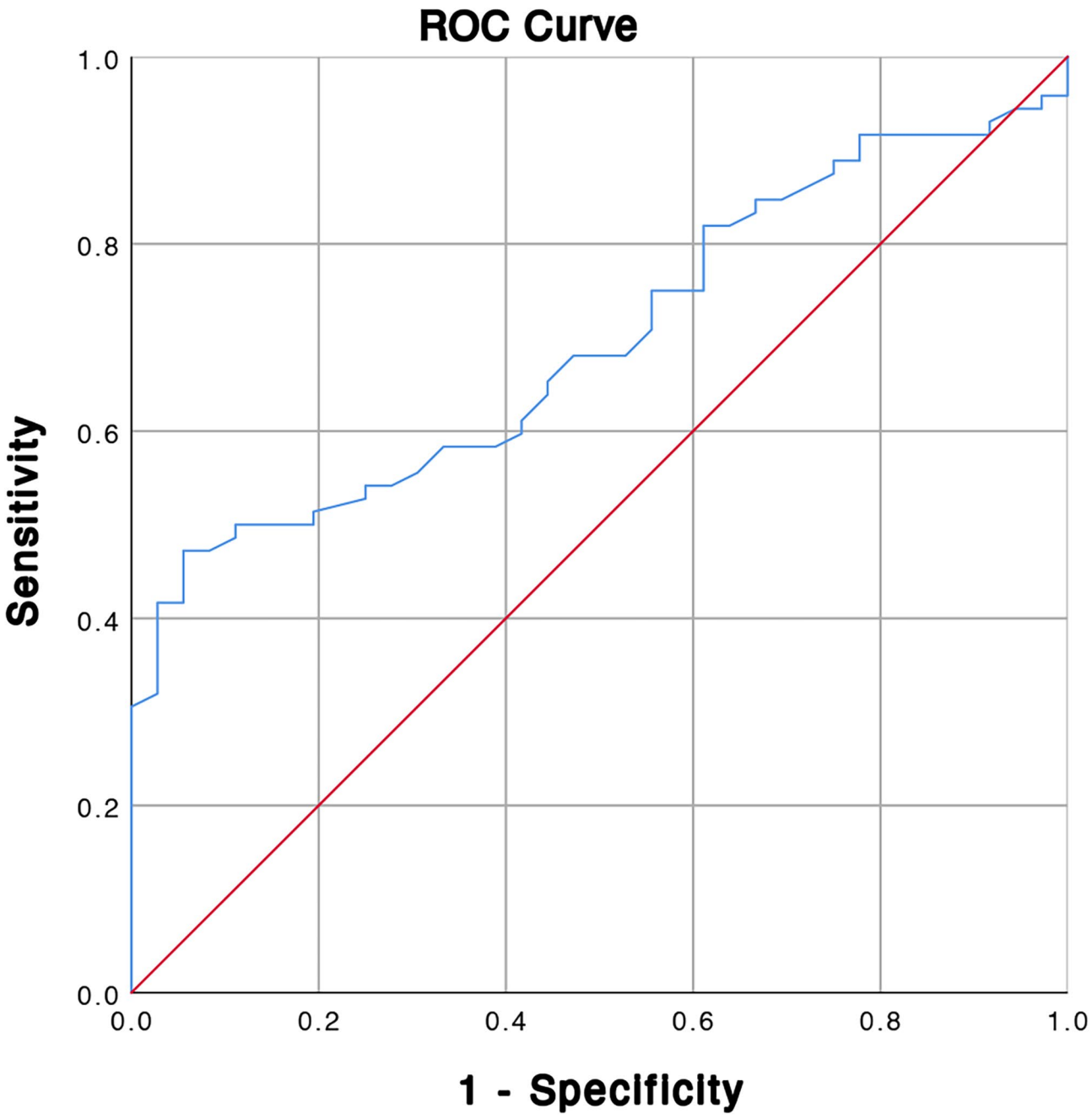

Fig. 1 ROC curve of HSP27 for the sensitivity and specificity of MCI
Notes for Fig. 1: It is determined that the diagnostic cut-off value for HSP27 to be 3.51 pg/ml, revealed a sensitivity of 47.2% and specificity of 94.4%. The area under the curve is 0.695
Abbreviations: ROC, Receiver operating characteristic; Heat shock protein 27; MCI, Mild cognitive impairment

conducted additional correlational analyses. The results revealed that, in patients with T2DM, irrespective of age and gender corrections, peripheral levels of HSP27 were not only associated with TMTA scores reflecting information processing speed function but also correlated with TMTB scores reflecting executive function. Further regression analysis indicated that decreased peripheral HSP27 is a contributing factor to the impairment of information processing speed and executive function in individuals with T2DM.

Currently, the diagnosis of MCI requires a series of complex cognitive function assessments, which are time-consuming and inherently subjective [47]. While neuroimaging studies may identify certain changes associated with cognition, their diagnostic criteria are challenging to quantify and involve substantial costs [48,

49]. Although some research has explored the relationship between imaging changes and cognitive impairment, there remains much work to be done in achieving a definitive diagnosis of cognitive dysfunction. Certain biomarkers, such as those found in cerebrospinal fluid, may be relevant to cognition, but their invasive nature poses significant risks to patients, limiting their widespread applicability [50, 51]. In contrast, peripheral blood biomarkers present a promising avenue of exploration. Peripheral blood biomarkers have the advantage of being obtained through routine clinical procedures with minimal trauma to patients, making them more widely accepted. Earlier, we mentioned that decreased level of HSP27 in peripheral blood are correlated with global cognitive function in patients and is a risk factor for MCI in T2DM patients. Therefore, we hypothesize that decreased level of HSP27 in peripheral blood may serve as a biomarker for early identification of cognitive impairment in patients with T2DM. To assess the diagnostic value of peripheral blood HSP27 levels as a biomarker for cognitive impairment in T2DM patients, we employed ROC curve analysis. The results revealed an area under the ROC curve of 0.695. Using a cutoff value of 3.51 pg/ml in T2DM patients, the sensitivity for diagnosing MCI based on peripheral blood HSP27 levels was 47.2%, with a specificity of 94.4%. Despite the ROC curve's area under the curve being 0.695, the sensitivity (47.2%) at this cutoff suggests limited value for HSP27 in early identification of MCI. However, this does not diminish the significance of the study's findings. Considering the heavy psychological burden borne by patients fearing cognitive impairment and subsequent dementia, early exclusion of the risk of cognitive impairment is also crucial. Informing individuals that fall into the low-risk category can alleviate their concerns, allowing them to focus more on managing diabetes and its associated complications. Our results indicate that when peripheral blood HSP27 levels are over 3.51 pg/ml in T2DM patients, the probability of developing MCI is extremely low. This implies that such patients can avoid excessive worry about cognitive impairment and redirect their energy towards diabetes treatment and management of its complications.

Although our research has, for the first time, revealed an association between peripheral blood HSP27 levels and cognitive function in patients with T2DM, with potential implications for information processing speed and executive function, as well as being an independent risk factor for MCI in diabetic patients. Furthermore, we have elaborated on its diagnostic value as a biomarker for MCI. However, Certainly, this research has some limitations that we must acknowledge. As a cross-sectional study, we did not strictly match participants by age and sex. Although we identified differences in the age between T2DM patients with and without MCI, there were no significant differences in gender. In our further correlation analyses, we not only adjusted for the age but also for gender to mitigate the limitations inherent in our cross-sectional study design. In addition, we did not include a control group without diabetes or a control group with MCI but without diabetes. Being a cross-sectional analysis, we cannot definitively establish causation between Hsp90α levels and cognitive dysfunction. Further cohort studies are necessary. We consider this a limitation of our research. Medication use, including diabetes treatments, may also impact cognitive function. Anti-diabetic therapy could influence cognitive function, mainly due to diabetes duration and severity, frequency of hyperglycemia, and specific drug effects (both positive and negative). Moreover, insulin therapy might indirectly be associated with worse cognitive outcomes because it usually reflects more severe diabetes and carries higher hypoglycemia risks [52–56]. it is essential to consider the patients' medication use. However, for the patients enrolled in our study, we only collected information on the medications they were taking at the time of enrollment, without detailed records of dosage, formulation, and duration of use. Due to the limited sample size, some medications were used by only a few patients. Consequently, a detailed analysis of medication use is not feasible in our current study, which should be acknowledged as a limitation. Nonetheless, in a separate study conducted by our former colleagues, the potential impact of various medications on cognitive function was investigated using network meta-analysis [57]. As a clinical research, while we speculate that the relationship between HSP27 and cognitive dysfunction may be linked to oxidative stress, ferroptosis, and neuroinflammation, the specific research mechanisms remain to be further confirmed through additional basic research in laboratory. Due to the presence of the blood-brain barrier, peripheral blood biomarkers inherently face disadvantages compared to specimens from the central nervous system. Nevertheless, in terms of accessibility, peripheral blood biomarkers have significant advantages. From the perspective of the results of this study, the value of HSP27 in blood as one of the markers for the occurrence of cognitive dysfunction is evident. This may be attributed to three reasons. First, peripheral blood and the central nervous system share similar environments, suggesting a potential correlation between peripheral and central factors levels. Second, in diabetic patients, the blood-brain barrier is compromised, allowing factors produced by the central nervous system to be detected in peripheral blood, and simultaneously, factors produced peripherally may influence the central nervous system through the compromised blood-brain barrier, contributing to the occurrence of cognitive dysfunction [58]. Third, certain structures, such as exosomes [59], not

only carry specific factors from the periphery into the central nervous system, affecting cognitive function, but also, during cognitive dysfunction, factors produced by the central nervous system can be transported by these structures through the blood-brain barrier into peripheral blood, where they can be detected.

Conclusion

In summary, we posit that elevated peripheral plasma HSP27 levels are associated with cognitive dysfunction in patients with T2DM and serve as an independent risk factor for the occurrence of MCI in these patients. Furthermore, in individuals with T2DM, HSP27 may function as a biomarker for diagnosing MCI in patients with T2DM. Its utility in early identification of MCI may be limited. However, when HSP27 levels are high, its value in excluding the presence of MCI in T2DM patients may be substantial. Clinically, for individuals overly concerned about the potential presence of cognitive dysfunction, it may be applied as a tool to rule out MCI in patients.

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None.

Author contributions
All authors made significant contributions to the work reported. Shaohua Wang contributed to the idea. Shufang Yang, Haoqiang Zhang, and Wenwen Zhu wrote the manuscript draft and revised the manuscript. Tong Niu, Huzaifa Fareeduddin Mohammed Farooqui, Jue Wang, Mingyue Yang, and Enlin Liu collected the data and performed the tests and (or) statistical analysis. All authors gave final approval of the version to be published, agreed on the journal to which the article has been submitted, and agreed to be accountable for all aspects of the work. Shufang Yang, Haoqiang Zhang, and Wenwen Zhu contributed equally to this work.

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Data availability
All data are available on reasonable request from corresponding author.

Declarations

Ethical approval
This study was ethically approved by the Ethics Committee for Medical Research at Taizhou People's Hospital (Approval No.: 2022-046-01).

Competing interests
The authors declare no competing interests.

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