

# Correlation between insulin-degrading enzyme versus total tau and selected cytokines in patients with Alzheimer's disease compared to non-demented controls

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## Abstract

**OBJECTIVE:** It has been increasingly recognized that the pathological progress of Alzheimer's disease (AD) is connected to metabolic function and inflammation. Insulin-degrading enzyme (IDE) is essential for glucose metabolism and the degradation of amyloid- $\beta$ . We aimed to explore the associations between IDE, total tau, and cytokines levels in plasma from subjects with AD and non-demented controls.

**METHODS AND MATERIAL:** Plasma samples (18 patients diagnosed with AD and 6 non-demented controls) from the Netherlands Brain Bank were used to analyze IDE levels and total tau with an enzyme-linked immunosorbent assay. Cytokines were analyzed with Luminex custom plex assays for interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor-alpha (TNF- $\alpha$ ). Results were analyzed using the Mann-Whitney U and Spearman's rank correlation tests.

**RESULTS:** Total tau in plasma was significantly increased in AD subjects compared to non-demented control subjects ( $p = 0.044$ ). Total tau was positively correlated with IDE levels in plasma in all subjects ( $r = 0.494$ ,  $p = 0.017$ ). Significant correlations could be demonstrated between plasma levels of IDE and IL-6 ( $r = 0.546$ ,  $p = 0.019$ ), IL-8 ( $r = 0.664$ ,  $p = 0.003$ ), IL-10 ( $r = 0.833$ ,  $p < 0.001$ ), and TNF- $\alpha$  ( $r = 0.633$ ,  $p = 0.005$ ) in subjects with AD, but not in non-demented controls.

**CONCLUSION:** Results from this study suggest that plasma IDE levels may be associated with inflammation and neurodegeneration and could potentially be a target for future diagnostic and treatment strategies.

**Abbreviations:**

A $\beta$	- Amyloid beta
AD	- Alzheimer's disease
CSF	- Cerebrospinal fluid
ELISA	- Enzyme-linked immunosorbent assay
GM-CSF	- Granulocyte-macrophage colony-stimulating factor
GSK-3 $\beta$	- Glycogen synthase kinase 3-beta
IDE	- Insulin-degrading enzyme
IFN- $\gamma$	- Interferon-gamma
IL	- Interleukin
NBB	- the Netherlands Brain Bank
NINCDS-ADRD	- National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association
PET	- Positron emission tomography
T2DM	- Type 2 diabetes mellitus
TNF- $\alpha$	- Tumor necrosis factor-alpha

**INTRODUCTION**

Alzheimer's disease (AD) is the most prevalent neurocognitive disorder in the Western world and is becoming increasingly common worldwide. The disease is progressive and causes distress for both patients and their families. AD is also challenging for societies and care systems (World Health Organization [WHO], 2022).

The pathological process of AD may start as early as 15-20 years before clinical symptoms appear (Sperling *et al.* 2011). Therefore, identifying relevant risk factors and initiating early diagnostic markers has been emphasized (Kivipelto *et al.* 2018). An early diagnosis of AD could substantially benefit patients in prevention and disease-modifying treatment (WHO, 2022). AD is characterized by the accumulation of amyloid-beta (A $\beta$ ) in amyloid plaques and tau aggregation in neurofibrillary tangles in the brain that will impair neuronal function. These accumulations are accompanied by activated microglia, neuroinflammation, and increased oxidative stress, all contributing to cognitive decline (Selkoe & Hardy, 2016).

In later years, the interest in metabolic dysfunction as a part of the pathogenesis of AD has proliferated (de la Monte, 2014). Insulin signaling is essential for cognitive functions, and it has been recognized that AD and type 2 diabetes mellitus (T2DM) share pathophysiological features (Gasecka *et al.* 2020). The common denominator of these diseases has been suggested to be insulin resistance (Gonçalves *et al.* 2019). In addition, A $\beta$  and insulin are metabolized by the same enzyme, insulin-degrading enzyme (IDE). IDE is a zinc-metalloprotease essential for insulin metabolism and maintaining glucose homeostasis. Low peripheral levels of IDE have been demonstrated to promote glucose intolerance and hyperinsulinemia (Farris *et al.* 2003). IDE has a cell protective role by preventing amyloidogenic peptides from misfolding and clogging into plaques (Sharma *et al.* 2015; Sousa *et al.* 2021). This has led to the hypothesis that high insulin levels,

such as in the presence of insulin resistance, result in IDE favoring insulin degradation and are insufficient to degrade the A $\beta$  load. The excess A $\beta$  could then accumulate and form plaques (Kurochkin *et al.* 2018).

Insulin resistance is closely connected to inflammation, and pro-inflammatory cytokines, such as interleukin (IL)-6, IL-8, and tumor necrosis factor-alpha (TNF- $\alpha$ ), have been suggested to increase in the presence of insulin resistance, further aggravating the condition (Vinuesa *et al.* 2021). Earlier studies found that peripheral IL-6 and TNF- $\alpha$  were higher in patients with AD than those without cognitive impairment. At the same time, anti-inflammatory cytokines, such as IL-10, remained unchanged (Swardfager *et al.* 2010). In addition, both insulin resistance (Hobday & Parmar, 2021) and IL-8 (Vaz *et al.* 2020) have been suggested to affect glycogen synthase kinase 3-beta (GSK-3 $\beta$ ) in the insulin cascade, contributing to increased tau hyperphosphorylation. Hence, metabolic dysfunction creates a vicious circle that increases protein aggregation and inflammation (Kurochkin *et al.* 2018).

Over the years, the interest in blood-based markers in diagnosing AD has increased. Measuring AD-specific biomarkers in the cerebrospinal fluid (CSF) and with positron emission tomography (PET) is possible, but these methods are expensive and invasive for patients. The ability to analyze biomarkers in plasma could increase accessibility for patients while reducing costs for society (Zetterberg & Burnham, 2019). IDE is expressed in all tissues and can be found in extracellular fluids such as CSF, wound fluid, and blood (Tang, 2016). Compared to healthy controls, elevated levels have been detected in the blood of patients with metabolic syndrome (Sofer *et al.* 2021) and T2DM (Kullenberg *et al.* 2022). Metabolic syndrome and T2DM are both conditions characterized by insulin resistance. However, low levels of IDE have been reported in post-mortem brain tissue from patients diagnosed with AD (Miners *et al.* 2009), making it possible that IDE could be a predictive marker for the risk of AD.

Nevertheless, knowledge about the relationship between IDE, neurodegenerative markers, and cytokines in blood from patients with AD is limited. Therefore, this study aimed to explore the relationship between IDE levels, total tau, and inflammatory cytokines in plasma from subjects with AD and non-demented controls.

**MATERIALS AND METHODS**

Post-mortem plasma samples from 18 AD patients and 6 non-demented individuals were obtained from The Netherlands Brain Bank, Netherlands Institute for Neuroscience, Amsterdam (open access: [www.brainbank.nl](http://www.brainbank.nl)). All Material has been collected from donors for or from whom written informed consent for a brain autopsy, and the use of the material and clinical

**Tab. 1.** Description of the study sample. Differences between groups were analyzed with Chi2-test or Mann-Whitney U-test. A *p*-value > 0.05 was considered statistically significant.

	Subjects with Alzheimer's disease n = 18	Non-demented controls n = 6	<i>p</i> -value
Female sex, n (%)	11 (61.1)	3 (50.0)	0.633
Age at death years, m (IQR)	85.0 (82-88)	83.5 (77-92)	0.770
Postmortem delay hours, m (IQR)	5.7 (5.2-7.1)	5.0 (4.5-6.5)	0.343
Braak, n (%)			
0-3	-	6 (100)	
4-5	14 (77.8)	-	
6	4 (22.2)	-	< 0.001
IDE ng/ml, m (IQR)	83.5 (25.8-148.3)	30.1 (23.3-42.0)	0.280
t-Tau pg/ml, m (IQR)	958.9 (461.6-1142.0)	317.4 (65.4-781.1)	0.044
IL-6 pg/ml, m (IQR)	640.5 (51.8-2792.1)	307.3 (63.8-413.4)	0.454
IL-8 pg/ml, m (IQR)	136.3 (49.9-379.4)	80.7 (56.9-89.8)	0.537
IL-10 pg/ml, m (IQR)	24.0 (6.7-69.9)	27.8 (9.2-46.4)	0.923
TNF-α pg/ml, m (IQR)	31.0 (22.0-43.9)	28.0 (12.6-32.4)	0.310

Abbreviations: insulin-degrading enzyme (IDE), interleukin (IL), interquartile range (IQR), median (m), number (n), total tau (t-Tau), tumor necrosis factor-alpha (TNF-α)

information for research purposes had been obtained by the NBB.

The clinical diagnosis of AD patients was performed according to the NINCDS-ADRDA criteria (G. McKhann *et al.* 1984; G. M. McKhann *et al.* 2011). The severity of the neurocognitive disorder was estimated according to the Global Deterioration Scale (Reisberg *et al.* 1982). The control subjects had no known history or symptoms of neurological or psychiatric disorders. The NBB is known to be a high-quality brain bank with low post-mortem intervals. Still, the number of available plasma samples was limited.

The medical ethics review committee of the University Medical Center (Amsterdam, the Netherlands) has approved the tissue collection procedures. All samples were anonymous to the researchers in this study. An advisory opinion was requested from the Ethical Review Board in Sweden, and they found no obstacles with the analyses conducted (Dnr. 2023-02405-01).

Concentrations of specific proteins in blood plasma were determined by enzyme-linked immunosorbent assay (ELISA) or multiplex immunoassay. Commercially available kits were used. All samples were diluted, and reagents and buffers were prepared following the manufacturer's instructions. An automated HydroWash® wash station from Tecan was used in the required wash steps. We performed statistical analyses for biomarkers where at least 90% of included samples exceeded the detection limit in the laboratory analyses. This means that all analyzed biomarkers were not presented in the results. Standard curves for the assays were fitted by using logistic 5PL regression.

Plasma IDE levels were measured using a human IDE ELISA assay (Human Insulin-degrading enzyme ELISA Kit, AH Diagnostics, Solna, Stockholm) with a detection limit of 0.037 ng/ml. In these analyses, all samples were measured above the detection limit.

Total tau was measured with a total tau ELISA assay (Total tau, Abcam, Oxford, United Kingdom) with a 16 pg/ml detection limit. All samples were measured above the detection limit.

To measure cytokines, the Bio-Plex Pro Human Cytokines 8-Plex assay (Bio-Rad Laboratories, #M50000007A) was used, including granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ), IL-2, IL-4, IL-6, IL-8, IL-10, and TNF-α. Only IL-6, IL-8, IL-10, and TNF-α met the 90% detection criterion.

### Statistical analysis

Due to the small sample size and non-normal distribution, continuous variables were presented as median and interquartile range. Differences between groups were analyzed using the Mann-Whitney U test and correlations using Spearman's rank correlation test.

A *p*-value < 0.05 was considered statistically significant, and all *p*-values were two-tailed. All analyses were conducted in IBM SPSS version 27, and figures were produced in GraphPad Prism version 8.4.3.

## RESULTS

### Demographic data

There was no significant difference in age at death, post-mortem time, or distribution of gender between

**Tab. 2.** Correlation between insulin-degrading enzyme and other biomarkers in all subjects. Analyzed with Spearman's rank correlation.

	<b>r</b>	<b>p-value</b>
t-Tau	0.494	0.017
IL-6	0.585	0.003
IL-8	0.745	< 0.001
IL-10	0.757	< 0.001
TNF- $\alpha$	0.536	0.007

Abbreviations: Alzheimer's disease (AD), interleukin (IL), total tau (t-Tau), tumor necrosis factor-alpha (TNF- $\alpha$ )

the AD patients and non-demented controls (Table 1). The NBB defined all samples as AD patients and non-demented controls, and this classification was adopted in the data analyses in this study. All AD patients in the present study were defined as Braak stage 4-6 (Table 1). The Braak stage is a gradation of the severity of the disease based on neurofibrillary changes (Braak & Braak, 1991).

#### IDE and total tau in plasma measured by ELISA

ELISA determined IDE and total tau levels in plasma samples from AD patients and non-demented control subjects (Table 1). For plasma levels of IDE, we could not demonstrate a statistically significant difference between the groups (Table 1). However, plasma levels of total tau were significantly increased in the AD patients compared to the non-demented control subjects ( $p = 0.044$ , Table 1).

#### IL-6, IL-8, IL-10, and TNF- $\alpha$ levels in plasma measured by multiplex assay

There were no statistically significant differences between subjects with AD and non-demented controls regarding plasma levels of IL-6, IL-8, IL-10, or TNF- $\alpha$  (Table 1).

#### Correlation between IDE and total tau and cytokines

We separately examined the correlations between IDE, cytokines, and total tau plasma levels in all subjects, AD subjects, and non-demented controls. Plasma levels of IDE were significantly correlated with plasma levels of IL-6, IL-8, IL-10, and TNF- $\alpha$  for the total samples ( $r = 0.494-0.757$ ,  $p = 0.017- < 0.001$ ) (Table 2) and for subjects with AD ( $r = 0.546-0.833$ ,  $p = 0.019- < 0.001$ ) but not for non-demented controls (Figure 1). In addition, we could demonstrate a statistically significant correlation between plasma levels of IDE and total tau when analyzing all subjects (Table 2). However, this correlation did not withstand when analyzing subjects with AD ( $r = 0.135$ ,  $p = 0.377$ ) and non-demented controls ( $r = 0.771$ ,  $p = 0.072$ ) separately.

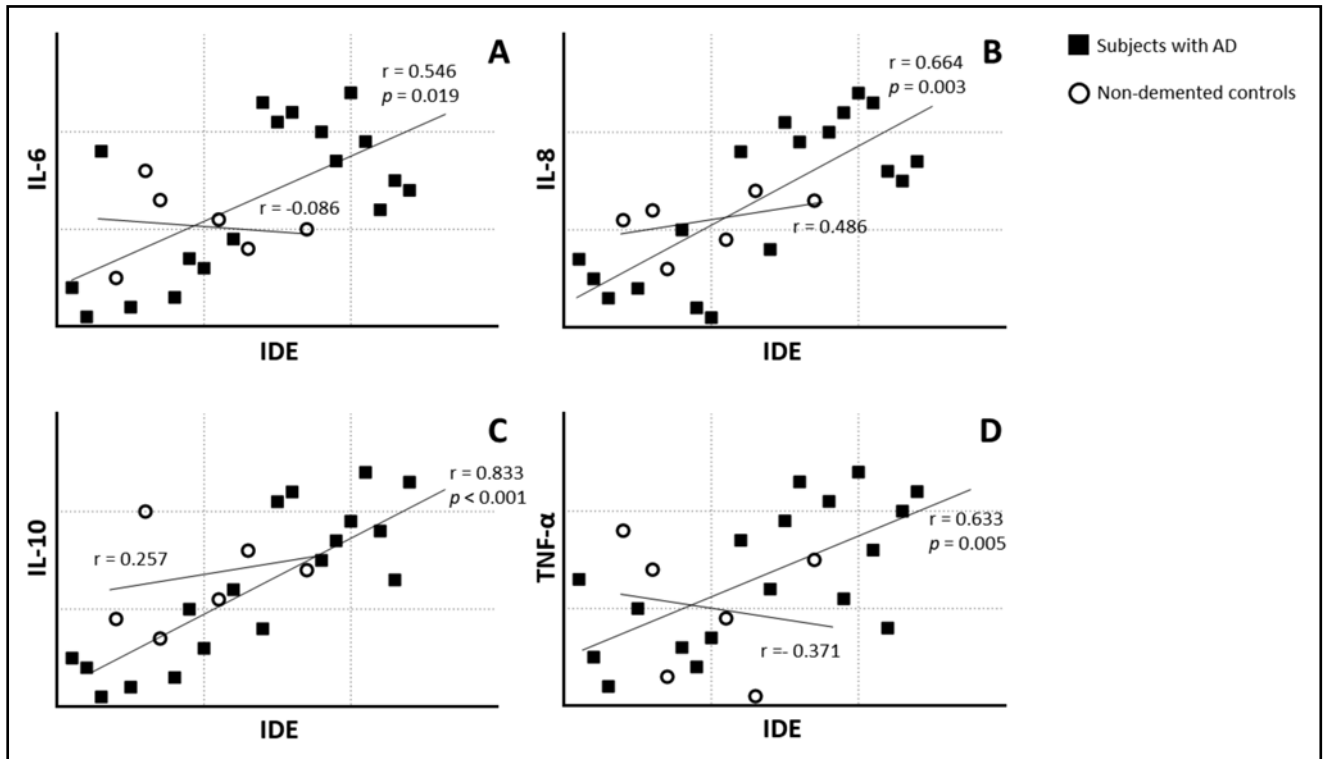
## DISCUSSION

In this study, we could demonstrate correlations between plasma levels of IDE and IL-6, IL-8, IL-10, and TNF- $\alpha$  in patients diagnosed with AD but not in non-demented controls. In addition, total tau was positively correlated with IDE levels in plasma when we analyzed the total sample but not in AD or non-demented controls separately.

To our knowledge, no earlier studies have demonstrated a correlation between levels of IDE and total tau in human plasma. IDE has been indicated as a marker of abnormal insulin metabolism (Sofer *et al.* 2021). Insulin resistance is closely associated with neurodegeneration, as high levels of insulin have been suggested to compete with the degradation of A $\beta$  (de la Monte, 2014), but also through over-activation of GSK-3 $\beta$  leading to increased hyperphosphorylation of tau (Hobday & Parmar, 2021). Overactivation in GSK-3 $\beta$  has been demonstrated in patients with T2DM and patients with AD (Khezri *et al.* 2022). In addition, total tau in CSF has been demonstrated to correlate with insulin resistance and cognitive function, even if the association was thought to be mediated by a specific form of A $\beta$  (A $\beta$ 42) (Woodfield *et al.* 2022). A correlation between total tau and IDE, as in this study, could indicate a correlation between insulin resistance and neurodegeneration.

Insulin resistance is strongly connected to inflammation in the periphery and the brain (Folch *et al.* 2019). Inflammatory responses are essential to protect, defend, and heal tissues (Kempuraj *et al.* 2016). Still, chronic peripheral low-grade inflammation can impair blood-brain-barrier function and allow immune and inflammatory cells to pass into the brain tissue, activating microglia and increasing neuroinflammation (Calsolaro & Edison, 2016). Neuroinflammation is thought to be a crucial part of the onset of AD and is mediated by cytokines such as IL-6, IL-8, and TNF- $\alpha$  (Kempuraj *et al.* 2016). In this study, we could not demonstrate statistically significant differences in cytokine levels between patients with or without AD. Nonetheless, we could demonstrate correlations between all measured cytokines and IDE in subjects with AD.

Insulin resistance has been demonstrated to increase IL-6 and TNF- $\alpha$  (Kandimalla *et al.* 2017). Also, increased levels of IL-10 have been linked to obesity and decreased sensitivity to insulin (Acosta *et al.* 2019). The most investigated marker is IL-6, and earlier studies have found correlations between plasma levels of IL-6 and IDE in humans (Kurauti *et al.* 2017) and mice (Li *et al.* 2022). IL-6 is essential in metabolic functions, especially glucose homeostasis. IL-6 stimulates insulin secretion and enhances insulin clearance, most likely by inducing the expression and activity of IDE (Kurauti *et al.* 2017). IL-6 has also been demonstrated to correlate with memory impairment (Lyra *et al.* 2021)



**Fig. 1.** Correlations between plasma levels of IDE and cytokines analyzed with Spearman's rank correlation test. In subjects with AD ( $n=18$ ), there were significant correlations between plasma levels of IDE and IL-6 ( $r = 0.546$ ,  $p = 0.019$ ) (A), IL-8 ( $r = 0.664$ ,  $p = 0.003$ ) (B), IL-10 ( $r = 0.833$ ,  $p < 0.001$ ) (C), and TNF- $\alpha$  ( $r = 0.633$ ,  $p = 0.005$ ) (D). No correlations were found in non-demented controls ( $n=6$ ). Abbreviations: Alzheimer's disease (AD), insulin-degrading enzyme (IDE), interleukin (IL), tumor necrosis factor-alpha (TNF- $\alpha$ ).

and A $\beta$  deposition in the brains of patients with AD (Oberlin *et al.* 2021). Consequently, IL-6 is connected to both T2DM and AD. The correlation between IL-6 and IDE has suggested that IDE could be a potential target for treating diseases related to hyperinsulinemia and inflammation (Kurauti *et al.* 2017). If IDE is also correlated with other pro-inflammatory cytokines, as in this study, it could further increase the potential for IDE as a treatment target.

However, clinical trials with IDE have yet to yield substantial results. This could be due to the versatile nature of the enzyme (Kurochkin *et al.* 2018). IDE inhibition increases insulin signaling and induces impaired glucose tolerance and hyperinsulinemia. At the same time, upregulation of IDE decreases A $\beta$  accumulation and ameliorates rodent cognitive functions but aggravates insulin resistance (Pivovarova *et al.* 2016). In addition, levels of IDE are most likely to be influenced by factors such as age and drug treatment (Kurochkin *et al.* 2018), and there is a lack of evidence on how levels of IDE in brain tissue, CSF, and blood are related. Hence, it has been suggested that it would perhaps be a better choice to target other markers that would indirectly affect IDE (Kurauti *et al.* 2017), and such other markers could be cytokines.

Nevertheless, many studies regarding inflammation and neurodegeneration have been performed in rodents. These experimental models are necessary for

basic research, but one limitation is that the animal models do not mimic the extensive neuroinflammation seen in neurodegeneration in human diseases (Kempuraj *et al.* 2016). In this study, we could only demonstrate an association between IDE and cytokines in plasma from subjects with AD. This could be due to the small sample size, but it could indicate that such association may only be present in the context of AD and not in non-demented individuals. This highlights the importance of considering the specific context in which a relationship is observed and suggests that further research is needed to understand the underlying mechanisms of the relationship between IDE, inflammation, and neurodegeneration in AD in humans.

#### Strength and limitations

This study has some limitations that must be considered when interpreting the results. As we used post-mortem samples, we depended upon available samples, limiting the number of study samples. The relatively small sample size may not be representative or provide enough statistical power to detect differences between our groups. Also, the restricted availability of plasma samples made it impossible to stratify patients with or without T2DM. Therefore, this study did not have information on the diabetic status of included subjects, which should be considered a limitation.

On the other hand, utilizing post-mortem samples confirmed that we had a general homogeneity in the sample collection and with certainty samples from subjects diagnosed with AD and subjects with no neurocognitive disorder. This can be considered a strength, as it is difficult to differentiate different neurocognitive disorders clinically. It has also been suggested that contradictory results of earlier studies could be due to misdiagnosis (Brunnström & Englund, 2009).

Furthermore, the fact that the samples are collected post-mortem can affect both the analytical results and the transfer of the results to living patients. For example, we chose not to analyze metabolic markers, such as blood glucose or insulin levels, as these markers can be challenging to interpret in plasma samples taken after death (Palmieri, 2015). Also, the cause of death can affect levels of, for example, cytokines (Mimasaka, 2002). In this study, we did not have information on the cause of death and, therefore, could not consider this.

## CONCLUSION

Both insulin resistance and inflammation in the periphery have been connected to an increased risk of developing AD. These conditions may intensify ongoing neurodegeneration, as they are linked to the accumulation of A $\beta$  and the hyperphosphorylation of tau. Results from this study suggest that plasma IDE levels may be associated with inflammation and neurodegeneration and could potentially be a target for future diagnostic and treatment strategies.

## CONFLICT OF INTERESTS

The authors have no conflict of interest to report.

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