Different Patterns of Cerebral Injury in Dementia With or Without Diabetes

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Background: Diabetes mellitus (DM) increases the risk of dementia in the elderly. However, its underlying mechanisms, its connection with Alzheimer disease and vascular cognitive impairment, and effects of therapy remain unclear.

Objective: To test the hypothesis that DM promotes specific neuropathologic processes that contribute to dementia and that these processes may be suppressed by antidiabetic therapy.

Design: A comprehensive neuropathologic assessment of all cases from a community-based study of incident dementia (Adult Changes in Thought Study) that underwent autopsies (n=259) and had information on DM status (n=196). Biochemical analysis was conducted on a subset of these cases with rapidly frozen brain tissue (n=57).

Participants: Autopsy cases were divided into 4 groups: no DM/no dementia (DM−/dementia−), DM/no dementia (DM+/dementia−), no DM/dementia (DM−/dementia+), and DM/dementia (DM+/dementia+). Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) diagnosis of dementia was assigned through a consensus of experts following biennial cognitive and physical evaluations. Diabetes was diagnosed based on information obtained from participants’ extensive medical records.

Results: In cases without dementia (n=125), neuropathologic and biochemical end points did not differ significantly by DM status. However, we observed 2 patterns of injury in patients with dementia (n=71) by their DM status. Individuals without DM but with dementia (DM−/dementia+) had a greater amyloid-β peptide load and increased levels of F2-isoprostanes in the cerebral cortex, while DM+/dementia+ patients had more microvascular infarcts and an increased cortical IL-6 (interleukin 6) concentration. The number of microvascular infarcts was greater in deep cerebral structures in patients with dementia whose diabetes was treated, whereas amyloid plaque load tended to be greater for untreated diabetic patients with dementia.

Conclusions: These novel characterizations of 2 different patterns of cerebral injury in patients with dementia depending on DM status may have etiologic and therapeutic implications.

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The association between diabetes mellitus (DM) and increased risk for dementia in the elderly is well documented. Multiple possible mechanisms for this association have been proposed, including direct effects of hyperglycemia, insulin resistance, and insulin-induced amyloid-β peptide (Aβ) amyloidosis in the brain as well as indirect ischemic effects of DM-promoted cerebrovascular disease. Numerous clinical and autopsy studies have attempted to clarify the mechanism(s) through which DM increases dementia risk. Indeed, DM increases risk for strokes and lacunar infarction; however, findings from prior studies have been inconsistent with respect to the association of DM with clinical dementia from either Alzheimer disease (AD) or vascular dementia. Autopsy studies have been partially illuminating. Heitner and Dickson failed to demonstrate an association between DM and the histopathologic features of AD—neuritic plaques and neurofibrillary tangles—in a study of 49 cases of diabetes and 52 age- and sex-matched nondiabetic control subjects. A recent, large autopsy series reported a negative association between the histopathologic features of AD and a diagnosis of DM. Other investigators re-
port no association, while others have reported an association of DM with microvascular infarcts (MVI), but not with macroscopic infarcts or the histopathologic features of AD. Microvascular infarcts are thought to mostly represent damage secondary to intrinsic microvascular disease, but they may also derive from emboli. Although relatively few in number, these studies support the view that the cerebral dysfunction associated with DM is at least partially mediated by cerebrovascular disease, especially small-vessel disease.

These discrepant results from clinicopathologic studies may be due to a lack of consideration of the potential influences of DM treatment. This may be important, as 2 population-based studies without autopsy end points have suggested that either treatment or the indications for treatment influence the strength of the association between DM and dementia. The Rotterdam Study reported an increased risk of clinical AD in patients who underwent oral antidiabetic therapy and a further increased risk of clinical AD in those taking insulin. In a population of Mexican Americans aged 60 years or older with diabetes, the Sacramento Area Latino Study on Aging reported that antidiabetic medication use decreased cognitive decline during a 2-year period and that this effect was more pronounced in those with diagnosed DM for longer than 5 years. Furthermore, combination therapy was associated with less decline in cognition than monotherapy.

Another possible contributor to the discrepancies in earlier clinicopathologic studies is that all of them relied exclusively on histologic end points to assess neurodegenerative changes in the brain. Although this approach is consistent with standard neuropathologic practice, it has limitations. Assessment of neurotoxic stress from Aβ accumulation by plaque density is likely an inaccurate reflection of this disease process, which can be quantified more robustly by measuring detergent-insoluble Aβ42. Additionally, our understanding of the pathogenesis of AD has expanded beyond evaluation of insoluble protein aggregates by light microscopy to include biochemical processes not represented by these histopathologic end points, such as activation of neuroinflammation and free radical–mediated injury. Furthermore, there are no widely accepted criteria for the neuropathologic assessment of vascular injury to the brain or vascular dementia; discrepancies may result from methodological differences in studies. Our study uses the number of microscopic infarcts as the metric of microvascular brain injury as previously described in our population and in the Honolulu-Asia Aging Study, as it is the single strongest correlate of vascular dementia and cognitive impairment in both populations.

We examined comprehensive histopathologic data from a community-based study of brain aging and incident dementia, and to our knowledge, it is the first investigation of human biochemical data to test the hypothesis that DM promotes specific processes that contribute to dementia in the elderly. We further examined whether or not these processes are similar for persons in whom DM was treated with medication compared with those not treated with medication.

METHODS

STUDY

The Adult Changes in Thought Study is an ongoing, prospective, longitudinal, population-based study of aging and cognitive decline that has been extensively described in our previous work. At entry, individuals were aged 65 years or older and received a cognitive assessment with the Cognitive Abilities Screening Instrument. Participants with scores of 85 or less received a protocol-based neuropsychological assessment and standardized clinical, laboratory, and neuroimaging examinations. Only those found to be free of dementia using Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) criteria were enrolled in the longitudinal cohort.

SUBJECT CLASSIFICATION

Individuals were divided into 4 groups: no DM/no dementia (DM−/dementia− [n=92]), DM/no dementia (DM+/dementia− [n=33]), no DM/dementia (DM−/dementia+ [n=45]), and DM/dementia (DM+/dementia+ [n=26]). Clinical diagnosis of dementia was assigned as previously described. Assignment of DM occurred if any of the following were present in the medical record: International Classification of Diseases, Ninth Revision (ICD-9) code indicating diabetes, any use of antidiabetic medication, a random glucose level higher than 200 mg/dL (to convert to millimoles per liter, multiply by 0.0555), or a mean hemoglobin A1c percentage greater than 6.5%. Individuals for whom none of these variables were reported in their medical records were eliminated from further analysis. To explore the effects of DM treatment, the DM+/dementia− and DM+/dementia+ groups were further divided into treated (TX+) any documented treatment with insulin and/or sulfonylureas and/or metformin) and untreated (TX−) groups.

AUTOPSIES

All autopsies were performed according to our previously described methods. In cases obtained within 8 hours of death, the brain was divided by a midsagittal section and half was fixed in formalin. The other half of the brain was immediately dissected and the following regions, among others, were flash frozen in liquid nitrogen and stored at −80°C: the middle frontal gyrus and superior and middle temporal gyri. The remaining tissue was placed in formalin. In cases obtained more than 8 hours after death, the whole brain was fixed. The same tissue blocks were obtained from all cases regardless of whether or not regions were dissected for freezing. Pathologic classifications followed established protocols.

TISSUE EXTRACTION

Tissue was extracted exactly as previously described. Full-thickness grey matter was dissected from underlying white matter and solubilized in buffer (10 mM Tris, 1 mM ethyleneglycol tetraacetic acid, 1 mM dithiothreitol, 10% sucrose; pH 7.5) plus Triton X-100, and sedimented; supernatant fluid was used to measure detergent-soluble proteins. The detergent-insoluble pellet was extracted with formic acid, 70%, dried by vacuum centrifugation, and solubilized by sonication in 20 volumes of 5-mol/L guanidine hydrochloric acid and 100-mmol/L Tris (pH 7.4). Interleukin 6 (IL-6) was measured in the detergent-soluble fraction using a kit from Invitrogen (Carlsbad, California.). Antibody capture assay for Aβ42 used a carboxy terminal-specific antibody (Signet Laboratories, Dedham, Massachusetts), secondary antibody/alkaline phosphatase conjugate from Am-
ersham (Piscataway, New Jersey), and tetramethylbenzidine as chromogen substrate with absorbance determined at 405 nm.

**F₂-ISOPROSTANES**

F₂-isoprostanes were quantified in modified Folch extracts of cerebral cortical grey matter using a stable isotope-dilution assay with gas chromatography/mass spectrometry and selective ion monitoring. We have recently reviewed this method in detail.²⁷

**STATISTICAL ANALYSIS**

Neuropathologic measures were divided into 4 categories: (1) standard indices of neuritic plaques and neurofibrillary tangles (Consortium to Establish a Registry of Alzheimer Disease [CERAD] and Braak scores rather than counts); (2) indices of vascular injury (atherosclerosis: mild, present at branch points in the cerebral arterial circle; moderate, present at additional points in the cerebral arterial circle and extending into interhemispheric fissures; and severe, involving vessels on the cerebral convexity), number of macroscopic infarcts (identified at the time of gross dissection), amyloid angiopathy index, number of cerebral MVIIs (infarcts identified by microscopy but not identified visually), and number of deep MVIIs; (3) soluble and formic acid–extracted insoluble Aβ in the frontal and temporal cortex; and (4) F₂-isoprostanes and IL-6 in the frontal cortex, which we associated with DM cerebrospinal fluid in previous work.²⁸

For each type of measure, multivariate analysis of covariance (MANCOVA) was carried out with group (control, DM−/dementia−, DM−/dementia+, and DM+/dementia+) as the independent factor and age at death as the covariate. Missing data were replaced with the mean value for the diagnostic group of the case with missing values. For Aβ assay results, a repeated-measures MANCOVA was conducted, with solubility (detergent-soluble and formic acid–soluble) and brain region (temporal and frontal) as repeated factors. Amyloid-β values were log-transformed to reduce variability. Following the ascertainment of a significant effect for each MANCOVA, individual measures were examined separately with analysis of covariance. If the analysis of covariance result was significant, post hoc t tests were conducted between groups. To explore the effects of DM treatment, MANCOVA was conducted for standard AD and vascular damage markers, with age as the covariate and group as the independent factor (control, DM+/dementia−/TX+, DM+/dementia−/TX+, DM−/dementia+, DM+/dementia+/TX+, and DM+/dementia+/TX−). Insufficient cell sizes prevented the analysis of treatment effects on biochemical measures. Statistical analyses were carried out using SAS, version 9.1 (SAS Institute Inc, Cary, North Carolina).

Histopathologic and molecular markers of AD, vascular disease, and vascular brain injury were stratified by DM and dementia. As of August 2007, 259 participants in the Adult Changes in Thought Study had died and undergone autopsy. Data on DM status were available in 196 of these individuals and their demographic characteristics are summarized in the Table. The median interval between last evaluation and death was 371 days for individuals without dementia and 366 days for patients with dementia. Individuals with DM data available did not differ significantly from other individuals with autopsies with respect to age, sex, Cognitive Abilities Screening Instrument score, or dementia status. Subjects with dementia were significantly older than those without; thus age was included as a covariate in all analyses.

Indices of AD are shown in Figure 1. The overall MANCOVA for the histopathologic features of AD demonstrated a significant effect for group (F₆,₇₇₈=8.23, P<.001). Neuritic plaque accumulation as indexed by CERAD score was significantly higher in the DM−/dementia+ group relative to both control and DM+/dementia− groups (Figure 1A). The DM+/dementia+ group had intermediate values that did not differ from any other group. Severity of cerebral amyloid angiopathy (CAA) as assessed by histopathologic grading showed a distribution similar to neuritic plaque score, with the DM+/dementia+ group having intermediate values that did not differ from any other group (Figure 1B). The DM−/dementia+ and DM+/dementia+ groups had similar levels of neurofibrillary tangle distribution as indexed by Braak stage; both groups had higher Braak stages than either group without dementia (Figure 1C).

Of the 196 cases listed in the Table, 82 underwent autopsy within 8 hours of death and had frozen tissue available from the superior and middle temporal gyri and the middle frontal gyrus for biochemical analyses. The overall repeated-measures MANCOVA for Aβ₄₂ that was insoluble in detergents but extracted into formic acid was

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**Table. Demographic and Clinical Characteristics of Individuals Undergoing Autopsy**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Without Dementia</th>
<th>With Dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without DM</td>
<td>With DM</td>
</tr>
<tr>
<td>No. of cases</td>
<td>92</td>
<td>33</td>
</tr>
<tr>
<td>Age at death, y</td>
<td>86 (7.1)</td>
<td>85 (6.5)</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>41</td>
<td>45</td>
</tr>
<tr>
<td>CASI score</td>
<td>90 (7.1)</td>
<td>90 (6.6)</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>105 (18)</td>
<td>145 (44)</td>
</tr>
<tr>
<td>Hb A₁c, %</td>
<td>5.9 (0.3)</td>
<td>7.4 (1.5)</td>
</tr>
</tbody>
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Abbreviations: CASI, Cognitive Abilities Screening Instrument; DM, diabetes mellitus; Hb, hemoglobin; TX, treatment for DM. SI conversion factor: To convert glucose to millimoles per liter, multiply by 0.0555.
significant \( (F_{12,110} = 2.69, P = .003) \). Levels of formic acid–extracted Aβ42, in the superior and middle temporal gyri were higher in DM−/dementia− cases compared with all other groups (Figure 1D). The DM+/dementia− cases also had higher formic acid–extracted Aβ42 levels in the middle frontal gyrus than the control or DM+/dementia− groups, but they did not differ from the DM+/dementia− group (Figure 1E). Levels of formic acid–extracted Aβ42 in the middle frontal gyrus were higher for the DM+/dementia− group than for the 2 groups without dementia, but this effect did not reach significance.

A different pattern was observed for histopathologic markers of vascular disease and vascular brain injury. The overall MANCOVA for these markers was also highly significant \( (F_{15,510} = 4.90, P < .001) \). As expected, both diabetic groups had more extensive atherosclerosis than the control or DM−/dementia− groups (Figure 2A). The DM+/dementia− group had the highest mean number of gross infarcts (Figure 2B), significantly higher than the DM+/dementia− group \( (P < .046) \), but this number did not differ significantly from either nondiabetic group. The DM−/dementia− and DM+/dementia− groups had similar numbers of cerebral MVIs compared with the groups without dementia (Figure 2C). In contrast, the DM+/dementia− group had significantly more deep MVIs than all other groups (Figure 2D), a finding consonant with previous work showing that the basal-penetrating vessels may be especially vulnerable to damage from DM.

In the 82 autopsies noted previously, we measured 2 molecular markers that are related to neurodegeneration from either AD or vascular brain injury and whose levels were elevated in a human model of insulin resistance: \( \text{F}_2 \)-isoprostanes, a quantitative in vivo marker of free radical–mediated injury, and IL-6, a marker of neuroinflammation. These 2 markers differed among groups, resulting in a significant MANCOVA result \( (F_{6,120} = 3.07, P = .01) \). Concentrations of \( \text{F}_2 \)-isoprostanes were greater in the DM−/dementia− group than in all other groups (Figure 3A). In contrast, IL-6 levels were increased in DM+/dementia− cases compared with all other groups (Figure 3B). These molecular data suggest that increased cerebral free radical–mediated damage is a feature of dementia without DM, whereas neuroinflammation is a feature of dementia with DM.

Treatment for DM was associated with a different pattern of neuritic plaque and deep MVI burden in dementia cases. Group differences were observed for CERAD and Braak scores, as reflected in an overall significant MANCOVA result \( (F_{10,374} = 3.07, P < .001) \). Subjects with dementia and untreated diabetes (DM+/dementia+)/TX−) had CERAD scores that were virtually identical to those in the DM+/dementia− group (Figure 1 and Figure 4A) and showed a trend toward increased neuritic plaque burden compared with the DM−/dementia− group \( (P = .07) \). In contrast, CERAD scores in treated diabetic cases with dementia (DM+/dementia+/TX+) were reduced relative to those in the DM−/dementia− group (Figure 4A). Both treated and untreated diabetic cases with dementia had higher Braak scores than controls (DM−/dementia−) (Figure 4B).

Results for vascular disease and MVIs revealed differences in the severity of pathology according to treat-
The DM/dementia/TX cases had more deep MVIs relative to all other groups (Figure 5A). Both treated and untreated diabetic cases with dementia had similar patterns of CAA severity and atherosclerosis (Figure 5B and C). No overall effect reached significance for cerebral MVIs or gross infarcts (data not shown).

**COMMENT**

Our goal was to investigate neuropathologic and neurochemical characteristics of DM and dementia in a series of autopsies from a community-based study of incident dementia. We observed 2 distinct patterns of cerebral damage associated with dementia in individuals with or without DM. In nondiabetic subjects, dementia was associated with a greater Aβ load and free radical damage, while...
in diabetic subjects, dementia was associated with more MVIs and activation of neuroinflammation. We observed a broadly consistent pattern for all indices of Aβ deposition: CERAD neuritic plaque score, CAA score, and concentration of detergent-insoluble Aβ42 in regions of the cerebral cortex. First, the groups of individuals without dementia, with and without DM, were very similar in both histopathologic scores and biochemical concentrations. Second, the DM−/dementia+ group had significantly higher histopathologic scores and biochemical concentrations than groups without dementia did. Third, the DM+/dementia+ group had scores and middle frontal gyrus formic acid–extracted Aβ42 concentrations that were intermediate between those of the groups without dementia and the DM−/dementia+ group; their superior and middle temporal gyrus formic acid–extracted Aβ42 concentrations were significantly lower than that in the DM−/dementia+ group and similar to the groups without dementia, a finding that parallels others’ observations. Indeed, this pattern of changes in Aβ deposition matches the changes in senile plaques observed in a recent case-control autopsy study. Together, these data support the conclusion that patients with DM and dementia die with a lower Aβ burden in brain parenchyma and cerebral blood vessels compared with their counterparts who die with dementia but do not have diabetes. We observed a different pattern for Braak staging of neurofibrillary tangles—the dementia groups scored significantly higher than the nondementia groups regardless of DM status, a finding similar to that in the Jewish Home and Hospital Study. The mean Braak stage in the subjects with dementia, who often have combined etiologies, was somewhat lower than that of cases selected for pure AD. Stratification of diabetic cases into treated and untreated groups did not reveal any significant differences in CAA score or Braak stage. The CERAD neuritic plaque scores trended toward lower values in the DM+/dementia+/TX+ group compared with the DM+/dementia+/TX− group, a result consistent with a recent report from a large autopsy series that showed that decreased senile plaque burden was associated with insulin therapy. In summary, individuals with dementia and DM had indices of Aβ deposition in brain parenchyma and cerebral blood vessels that were intermediate between patients with dementia and without DM and individuals in both nondementia groups.

Next, we examined indices of severity of atherosclerosis and vascular brain injury. As expected, individuals with DM had higher indices of cerebral atherosclerosis regardless of dementia status. We did not observe an association between the number of gross infarcts and DM status. In a smaller subset of the Adult Changes in Thought Study, we previously reported that in multivariate logistic regression the number of MVIs, but not the number of gross infarcts, is a significant independent risk for dementia. Similar observations have been made in the Honolulu-Asia Aging Study. Although an increased number of cerebral cortical MVIs was a feature of dementia in the present study, we observed that deep cerebral MVIs were strongly associated with dementia plus DM but not with dementia without DM. Indeed, others have hypothesized that DM preferentially increases the risk for infarction of subcortical structures perfused by small penetrating arteries that precipitously decrease in luminal diameter. The blood-flow dynamics of this transition may play an important role in mediating susceptibility to damage or dysfunction of these vascular territories in DM. Similar to our findings, a recent retrospective case-control study demonstrated increased MVIs in individuals with diabetes, but it did not relate this pattern to the presence of dementia. Our data suggest that MVIs are strongly associated with the presence of dementia in diabetic individuals and thus may play an important role in dementia pathogenesis for these people.

There were significantly more deep cerebral MVIs in the DM+/dementia+/TX+ group than in the DM+/dementia+/TX− group. If therapy was successful in suppressing the effects of DM on the microvasculature, then one would expect a decrease in MVI. Instead, we observed the opposite. Rather than showing that therapies have paradoxical effects on MVI, our findings may reflect the greater severity of DM in treated patients with
dementia compared with untreated patients with dementia, as indicated by higher random glucose levels and hemoglobin A1c values (Table). It is interesting to note, however, that none of the other histopathologic variables that were stratified by treatment status (CERAD neuritic plaque score, Braak stage, CAA score, extent of atherosclerosis, number of gross infarcts, and number of cerebral cortical MVIs) showed this likely confounded increase in treated patients. Perhaps these data again underscore the special vulnerability of cerebral basal- penetrating vessels to damage from DM.

We analyzed 2 molecular markers of pathogenic processes shared by AD and vascular brain injury: free radical-mediated injury and activation of neuroinflammation. The biochemical mechanisms through which aggregated Aβ42 or ischemia produce increased free radical damage to the brain as assessed by F2-isoprostanes or other measures have been well described. Interleukin 6 levels are known to be elevated in neuroinflammatory conditions, including AD and ischemia. Increased cerebrospinal IL-6 levels have been observed when hyperinsulinemia is induced to levels observed in adult-onset DM.28 Our results suggest that individuals with dementia in the absence of DM have higher levels of free radical damage, while individuals with dementia and DM have greater neuroinflammation. There are several processes that lead to increased free radical damage to the brain in AD, including Aβ accumulation.32 Our data suggest that these sources may differ in patients with dementia with and without DM and may be related to differences in the amount of Aβ accumulation. In contrast, inflammation is known to be a key modulator of vascular injury, and thus IL-6 elevations may be associated with the increased numbers of MVIs observed in diabetic cases with dementia.

Our investigation had several strengths. First, we were able to comprehensively evaluate key pathologic features associated with dementia in this group, including markers of AD and vascular brain injury. Second, ours is the first study to examine diabetes-related differences in biochemical markers of neurodegeneration, including detergent-insoluble Aβ42 accumulation, free radical-mediated damage assessed by cerebral cortical F2-isoprostanes, and activation of neuroinflammation assessed by cerebral cortical IL-6 concentration. Third, our pharmacy database allowed the most extensive analysis of diabetes treatment performed to date. Fourth, individuals were evaluated for dementia with a standardized protocol at regular intervals and had a median interval between last evaluation and death of just over a year. A weakness of our study was the limited numbers of DM+/dementia+ cases available for analysis of treatment effects, which made it impossible to analyze specific diabetes treatments. Despite the relatively small numbers, however, clear differences emerged between treated and untreated diabetic individuals with dementia, which suggests that treatment is an important factor to consider in neuropathologic investigations of diabetes. The complexity associated with treatment may play a role in contradictory results from studies in which the role of treatment cannot be evaluated.

In summary, we observed 2 patterns of cerebral damage associated with dementia in individuals with and with-


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