Newly-Diagnosed Disturbed Glucose Metabolism is Associated with Atherosclerosis in Patients with Transient Ischemic Attack or Ischemic Stroke

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Abstract

Objective: Newly-diagnosed disturbed glucose metabolism is highly prevalent in non-diabetic patients with transient ischemic attack (TIA) or ischemic stroke, and increases the risk of recurrent stroke. Diabetes mellitus is associated with atherosclerosis. We aimed to assess whether newly-diagnosed disturbed glucose metabolism is associated with atherosclerosis as well.

Research design and methods: Patients with a recent TIA or ischemic stroke were classified in three groups based on glucose levels and use of antidiabetic drugs. Pre-existent diabetes mellitus was defined as the use of antidiabetic drugs prior to the event. Newly-diagnosed disturbed glucose metabolism was defined as two or more disturbed glucose tests: fasting plasma glucose level ≥ 5.6 mmol/L, 2-hour post-load glucose level ≥ 7.8 mmol/L, and/or glycosylated hemoglobin level ≥ 39 mmol/mol.

We used CT-angiography to assess stenosis in the carotid artery bifurcations and calcification volume in the aortic arch and carotid bifurcations and intracranial carotid arteries. The relation between glucose groups and measures of atherosclerosis was expressed as odds ratios and beta coefficients with corresponding 95% CI, adjusted for potential confounders.

Results: Of the 1217 patients, 384 (32%) had newly-diagnosed disturbed glucose metabolism, and 210 (17%) had pre-existent diabetes mellitus. Newly-diagnosed disturbed glucose metabolism was independently associated with stenosis ≥ 50% (aOR (95%CI) 1.15 (1.04-2.20)). Pre-existent diabetes mellitus was associated with stenosis ≥ 50% and with calcification volume in all regions, especially in patients without the use of cholesterol-lowering drugs prior to the event.

Conclusions: Our study shows that newly-diagnosed disturbed glucose metabolism in patients with a recent TIA or ischemic stroke is associated with more severe extra- and intracranial atherosclerosis, similar to pre-existent diabetes mellitus.

Keywords: Stroke, atherosclerosis, glucose, pre-diabetes

Introduction

Diabetes mellitus is a well-known risk factor for first stroke and stroke recurrence [1]. Pre-diabetes is a metabolic state with a high risk of developing diabetes mellitus in the future [2,3] and comprises impaired fasting glucose and/or impaired glucose tolerance and/or disturbed glycosylated hemoglobin levels [3,4]. Pre-diabetes and newly-diagnosed diabetes mellitus are highly prevalent (up to 79%) in patients with an ischemic stroke or transient ischemic attack (TIA) without known diabetes mellitus prior to the event [5-9] and is also associated with an increased risk of cardiovascular disease and recurrent stroke [10-12].

Patients with diabetes mellitus are highly susceptible for atherosclerosis, which is reflected in increased carotid intima media thickness (CIMT) [13-15] and severe calcification in the aortic arch and carotid arteries [16,17]. However, it is still unsure whether the association between diabetes mellitus and calcification volume is similar between men and women [16-20]. Diabetes mellitus is characterized by a clustering of vascular risk factors, including dyslipidemia, hypertension, obesity, and insulin resistance. Among these factors, dyslipidemia may play an important role in the development of atherosclerosis [21,22]. More severe atherosclerosis is presumed to be the main underlying mechanism of the increased risk of recurrent stroke in patients with diabetes mellitus. However, there is no consensus whether pre-diabetes is associated with atherosclerosis as well [13-15,23,24].

To the best of our knowledge, there are no studies on the association between newly-diagnosed disturbed glucose metabolism (pre-diabetes or newly-diagnosed diabetes mellitus) and atherosclerosis in patients with TIA or ischemic stroke. We therefore aimed to assess whether...
newly-diagnosed disturbed glucose metabolism, determined with fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels is associated with craniocevical atherosclerosis in patients with TIA or ischemic stroke compared with patients with pre-existent diabetes mellitus or normal glucose metabolism.

Research Design and Methods

Study population

Patients were derived from the Erasmus Stroke Study, a registry of patients with cerebrovascular diseases treated at the Erasmus Medical Center Rotterdam. All consecutive patients with a clinical diagnosis of acute ischemic stroke or TIA between December 2005 and October 2010 were included. Patients without a CT-angiography and/or missing glucose assessment were excluded. Our study was approved by the Institutional Ethics Committee and written informed consent was obtained from all participants.

Demographic data, vascular history (previous TIA or ischemic stroke and previous ischemic heart disease among others) and risk factors (current smoking and BMI among others), laboratory assessments, and data on event characteristics were collected. Hypertension was defined as the use of antihypertensive drugs prior to the event.

Glucose assessment

Pre-existent diabetes mellitus was defined as the use of oral and/or parenteral antidiabetic drugs prior to the TIA or ischemic stroke. In all patients, fasting plasma glucose and glycosylated hemoglobin levels were assessed on the 2nd or 3rd day of admission or when the patient visited the outpatient clinic in case of TIA patients who were not hospitalized. Subsequently, an oral glucose tolerance test (OGTT) was performed according to the World Health Organization protocol in all patients without pre-existing diabetes mellitus [25]. After overnight fasting, patients drank a solution of 75 grams glucose in 150 mL water, and 2-hour post-load glucose levels were assessed. Newly-diagnosed disturbed glucose metabolism was defined as 2 or more disturbed glucose tests according to the American Diabetes Association: fasting plasma glucose level ≥ 7.8 mmol/L, and/or 2-hour post-load glucose level ≥ 7.8 mmol/L, and/or glycosylated hemoglobin level ≥ 39 mmol/mol [3].

CT acquisition and analysis

CT Angiography (CTA) of the carotid artery was performed with a 16 slice, 64 slice or 128 slice multi-detector CT system (Sensation 16, Definition, Definition AS+ or Definition Flash, Siemens) a 16 slice, 64 slice or 128 slice multi-detector CT system (Sensation 16, Definition, Definition AS+ or Definition Flash, Siemens) a 16 slice, 64 slice or 128 slice multi-detector CT system (Sensation 16, Definition, Definition AS+ or Definition Flash, Siemens) a 16 slice, 64 slice or 128 slice multi-detector CT system (Sensation 16, Definition, Definition AS+ or Definition Flash, Siemens) a 16 slice, 64 slice or 128 slice multi-detector CT system (Sensation 16, Definition, Definition AS+ or Definition Flash, Siemens). All patients received 80 ml of contrast agent (320 mg/mL iodixanol, Visipaque, Amersham Health, Little Chalfont, above the sella turcica). The scan range was assessed on the 2nd or 3rd day of admission or when the patient visited the Erasmus Stroke Study, a registry of patients with cerebrovascular diseases treated at the Erasmus Medical Center Rotterdam. All consecutive patients with a clinical diagnosis of acute ischemic stroke or TIA between December 2005 and October 2010 were included. Patients without a CT-angiography and/or missing glucose assessment were excluded. Our study was approved by the Institutional Ethics Committee and written informed consent was obtained from all participants.

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CT Angiography (CTA) of the carotid artery was performed with a 16 slice, 64 slice or 128 slice multi-detector CT system (Sensation 16, Sensation 64, Definition, Definition AS+ or Definition Flash, Siemens Medical Solutions, Erlangen, Germany) using a standardized optimized contrast-enhanced CTA protocol (120 kVp, 180-200 mAs, collimation 16×0.75 mm; 32×2×0.6 mm; 64×2×0.6 mm, pitch<1). The scan range extended from the ascending aorta to the intracranial circulation (3 cm above the sella turcica). All patients received 80 ml of contrast agent (320 mg/mL iodixanol, Visipaque, Amersham Health, Little Chalfont, UK), followed by 45 ml saline bolus chaser, both at an injection rate of 4 or 5 ml/s. Image reconstructions were made with field of view of 120 mm, matrix size 512x512, slice thickness 0.75 or 1.0 mm, increment 0.4 – 1.0 mm and with an intermediate reconstruction algorithm.

Stenosis: The severity of stenosis in carotid bifurcations (within 3 cm proximal and distal of the bifurcation) was assessed with the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria [26]. A stenosis of 50% or more was considered significant. An intraclass correlation coefficient (ICC) for the degree of stenosis was based on the ratings of 3 independent observers (S.F., A.v.D. & E.v.d.H.) on 50 CT examinations: 0.96-0.99.

Volume of calcifications: Dedicated commercially available software (Syngo CalciumScoring, Siemens) was used to semi-automatically quantify calcifications in the aortic arch and both extracranial carotid arteries, expressed as calcification volume in mm³. The aortic arch was defined as the origin of the aortic arch to the first 1 cm of the common carotid arteries, the vertebral arteries and the subclavian arteries beyond the origin of the vertebral arteries. The carotid arteries were scored within 3 cm proximal and distal of the bifurcation. A threshold of 600 Hounsfield units (HU) was used to differentiate calcifications from contrast material in the lumen. A detailed description of the measurement is provided elsewhere [16].

A custom-made plug-in for the freely available software ImageJ (Rasband, National Institute of Mental Health, Bethesda, MD, available at http://rsb.info.nih.gov/ij) was used for quantification of intracranial internal carotid artery calcifications. The intracranial internal carotid artery comprised the horizontal segment of the petrous internal carotid artery to the top of the internal carotid artery. Due to the close relationship of calcium in the arterial wall to the skull base, the previous mentioned semi-automatic tool could not be used. With the plug-in, it is possible to draw regions of interest in axial multidetector (MD) CTA images and to calculate automatically the total number of pixels above a predefined threshold. The volume of the intracranial calcifications (in mm³) was calculated as the product of the number of pixels above the threshold (600 HU), the pixel size, and the increment [27]. ICC for extracranial and intracranial calcifications was assessed, based on the ratings of respectively 3 independent observers (S.F., A.v.D. & T.Z.) on 35 CT examinations (ICC 1.00) and the ratings of 2 independent observers (S.F. & T.Z.) on 40 CT examinations (ICC 0.99).

Statistical analysis

The analysis was carried out with STATA 12.1 statistical package (Statacorp, College Station, Texas). Missing variables were imputed with single imputation using relevant baseline patient characteristics and the outcome variable. In the analysis with severity of stenosis, we used the highest degree of stenosis in both carotid artery bifurcations. In the analysis with calcification as continuous measure, we used the natural log-transformed values to deal with participants with a calcium score of zero. Calcification volumes of the left and right side were summed for the extra- and intracranial carotid arteries separately.

We performed logistic and linear analyses to study the relation between glucose metabolism on the one hand and atherosclerosis on the other. Adjustments were made with a multivariable logistic and linear regression model that included age, sex, hypertension, dyslipidemia, previous TIA or ischemic stroke, previous ischemic heart disease, current smoking, and BMI.

Based on the previous conflicting results between men and women, we repeated these analyses in men and women separately. Furthermore, we tested the difference in the effect of glucose on atherosclerosis between men and women and between patients with and without cholesterol-lowering drugs use prior to the event with a test for interaction.

Results

Between December 2005 and October 2010, 1611 consecutive patients with a TIA or ischemic stroke were included in the Erasmus...
Table 1: Patient characteristics per glucose metabolism group (* defined as the use of antihypertensive drugs prior to the ischemic stroke/TIA, ‡ in patients with ischemic stroke only).

| Characteristic                  | Normal glucose metabolism N=623 | Newly-diagnosed disturbed glucose metabolism N=384 | Pre-existent diabetes mellitus N=210 | p-value |
|--------------------------------|---------------------------------|--------------------------------------------------|-------------------------------------|---------|
| **Demographic data**           |                                 |                                                  |                                     |         |
| Age, mean (SD) in years        | 59 (14)                         | 88 (13)                                          | 65 (12)                             | <0.001  |
| Men, n (%)                     | 316 (51)                        | 202 (53)                                         | 124 (59)                            | 0.112   |
| Caucasian, n (%)               | 492 (82)                        | 296 (62)                                         | 123 (63)                            | <0.001  |
| **Risk factors**               |                                 |                                                  |                                     |         |
| Hypertension*, n (%)           | 263 (42)                        | 233 (61)                                         | 153 (73)                            | <0.001  |
| Use of cholesterol-lowering drugs prior to the event, n (%) | 153 (25)                        | 131 (34)                                         | 126 (60)                            | <0.001  |
| Atrial fibrillation, n (%)     | 29 (5)                          | 30 (8)                                           | 17 (8)                              | 0.063   |
| Current smoking, n (%)         | 228 (37)                        | 99 (26)                                          | 48 (23)                             | <0.001  |
| BMI, mean (SD) in kg/m²†       | 26 (3)                          | 27 (4)                                           | 28 (4)                              | <0.001  |
| **Medical history**            |                                 |                                                  |                                     |         |
| TIA/Ischemic stroke, n (%)     | 139 (22)                        | 102 (27)                                         | 77 (37)                             | <0.001  |
| Ischemic heart disease, n (%)  | 82 (13)                         | 69 (18)                                          | 65 (31)                             | <0.001  |
| **Event characteristics**      |                                 |                                                  |                                     |         |
| TIA, n (%)                     | 287 (45)                        | 133 (35)                                         | 69 (33)                             | <0.001  |
| NIHSS, median (IQR)†           | 2 (1-5)                         | 3 (1-6)                                          | 3 (2-5)                             | 0.006   |
| TOAST classification           |                                 |                                                  |                                     | <0.001  |
| Large artery disease, n (%)    | 82 (13)                         | 94 (25)                                          | 39 (19)                             |         |
| Cardio-embolism, n (%)         | 68 (11)                         | 51 (13)                                          | 30 (14)                             |         |
| Small vessel disease, n (%)    | 131 (21)                        | 62 (16)                                          | 50 (24)                             |         |
| Other determined disease, n (%)| 47 (8)                          | 12 (3)                                           | 7 (3)                               |         |
| Undetermined disease, n (%)    | 295 (47)                        | 165 (43)                                         | 84 (40)                             |         |
| **Glucose assessment**         |                                 |                                                  |                                     |         |
| Fasting plasma glucose, mean (SD) in mmol/L | 4.9 (0.6)                        | 6.2 (1.4)                                         | 7.9 (3.0)                           | <0.001  |
| 2-hour post-load glucose, mean (SD) in mmol/L | 7.5 (2.4)                        | 11.2 (3.6)                                        | NA                                  | <0.001  |
| Glycosylated hemoglobin, mean (SD) in mmol/mol | 36 (4)                         | 43 (8)                                           | 58 (17)                             | <0.001  |

Table 1: Patient characteristics per glucose metabolism group (* defined as the use of antihypertensive drugs prior to the ischemic stroke/TIA, ‡ in patients with ischemic stroke only).
in population-based studies and measured CIMT with duplex ultrasound instead of stenosis degree and calcification volume with CTA. We used degree of stenosis and calcification volumes assessed with CTA as a proxy for atherosclerosis. Carotid artery stenosis is a well-known and important risk factor for ischemic stroke and a stenosis of 50% or more is therefore a good marker for atherosclerosis [30]. Furthermore, in large population-based studies calcification volume in aortic arch and extracranial carotid arteries was not only associated with vascular risk factors, but also with the presence of (silent) cerebral infarcts on MRI and prevalent stroke [16,27,31]. Also, diabetes mellitus and glucose levels are significantly associated with calcification growth in patients with a TIA or ischemic stroke [32].

In contrast with previous studies [16-20], we found no significant interaction between glucose and sex. We therefore conclude that there is no consensus on the role of sex in the association between disturbed glucose metabolism and atherosclerosis. It is known that women with diabetes mellitus have a three- to seven-fold increased risk for coronary heart disease, compared to a two- to three-fold increased risk in men with diabetes mellitus [33]. Therefore, it would be interesting to investigate the possible different effects of glucose metabolism on atherosclerosis in men and women.

Atherosclerosis is an important vascular complication in patients with diabetes mellitus. The pathogenesis of atherosclerosis in diabetes mellitus is multifactorial. Not only the co-existence of other
cardiovascular risk factors in patients with diabetes mellitus like hypertension and dyslipidemia, but also insulin resistance, endothelial dysfunction, dyslipidemia, chronic inflammation, procoagulability, and impaired fibrinolysis promote atherosclerosis [21,22]. In patients with pre-diabetes, these processes may already play a role. This is also reflected in the Oxford Plaque Study, in which histological features of symptomatic atherosclerotic carotid plaques were assessed in patients with impaired glucose tolerance and diabetes mellitus. Not only in patients with diabetes mellitus, but also in patients with impaired glucose tolerance, surface thrombus and plaque macrophages seemed to persist longer in plaques, compared to plaques from those with normal glucose tolerance [34]. A recently developed mouse model could be used to investigate the causal role of impaired glucose tolerance in the pathogenesis of atherosclerosis [35]. However, whether pre-diabetes is associated with atherosclerosis remains controversial [13-15,23,24,36-38]. Two recent meta-analyses found an association between impaired glucose tolerance and a small increase in CIMT [24,39].

Interestingly, we found that the association between pre-existing diabetes and calcification volume was true for patients without cholesterol-lowering drugs use prior to the event. This underlines the importance of cholesterol on the atherogenesis in these patients [40]. In patients with newly-diagnosed disturbed glucose metabolism this association was not found, which might indicate that cholesterol starts to play a role in patients with more overt diabetes. However, we should take into account that we did not use a pre-specified test for interaction, so our results should be interpreted as hypothesis-generating.

To our knowledge, this is the first study that assessed the association between newly-diagnosed disturbed glucose metabolism and atherosclerosis in patients with ischemic stroke or TIA. Strengths of this study are the large sample size, detailed clinical information, the use of three different glucose tests and two different methods to assess atherosclerosis.

Our study has also some limitations. The glucose tests were not repeated to rule out laboratory error and the acute phase effect. Nonetheless, according to the American Diabetes Association two different simultaneously disturbed glucose tests are sufficient to diagnose pre-diabetes and newly-diagnosed diabetes mellitus and repeating the tests is therefore not obligatory [3]. Due to the small sample size patients with newly-diagnosed diabetes and pre-diabetes were combined into 1 group, although it would be interesting to establish the difference in the severity of atherosclerosis between these groups. Third, we excluded patients with missing glucose assessment and missing CTA, which might compromise the generalizability of our findings. Reasons for missing glucose assessments were mainly the poor medical condition of the patient, and these patients might therefore benefit less of secondary stroke prevention.

In conclusion, newly-diagnosed disturbed glucose metabolism, just like pre-existent diabetes mellitus, is independently associated with atherosclerosis in patients after ischemic stroke or TIA. This association may partly explain the increased risk for recurrent stroke in these patients. This study therefore might give a rationale to treat patients with newly-diagnosed disturbed glucose metabolism after ischemic stroke or TIA more aggressively to lower glucose levels and with cholesterol-lowering drugs. Future studies may include the effects of treatment on the progression of atherosclerosis in these patients.

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S.F. researched data and wrote the manuscript. A.v.d.L. researched data. T.Z. researched data. E.v.d.H. researched data. H.L. reviewed the manuscript and contributed to the discussion. P.K. reviewed the manuscript and contributed to the discussion. A.v.d.L. reviewed the manuscript and contributed to the discussion. H.D.H. wrote the manuscript.

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