Redox Biology 4 (2015) 34–39

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Urinary markers of nucleic acid oxidation and cancer in type 2 diabetes

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ARTICLE INFO

Article history:
Received 2 November 2014
Accepted 4 November 2014
Available online 2 December 2014

Keywords:
Type 2 diabetes
Cancer
RNA oxidation
DNA oxidation
8-oxo-7,8-dihydro-2′-deoxyguanosine
8-oxo-7,8-dihydroguanosine

ABSTRACT

Aims/hypothesis: We investigated whether urinary markers of nucleic acid oxidation are associated with an increased risk of cancer in type 2 diabetes patients.
Methods: Urine samples from 1381 newly diagnosed diabetes patients were assayed for the oxidatively modified guanine nucleosides 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo). Cox proportional hazards regression was used to examine the relationship between the urinary markers and cancer incidence.
Results: The crude analyses showed an association between overall cancer and urinary excretion of the RNA oxidation marker 8-oxoGuo (unadjusted hazard ratio for cancer per natural log increase in 8-oxoGuo 1.35 [95% CI, 1.01–1.81]), however, in the adjusted analyses, no significant associations between 8-oxodG or 8-oxoGuo and overall cancer were found. For site-specific cancers 8-oxodG was associated with breast cancer in the crude analyses (unadjusted hazard ratio for breast cancer per natural log increase in 8-oxodG was 2.37 [95% CI, 1.07–5.26]), although the association was attenuated in the adjusted analyses (sex- and age-adjusted hazard ratio 2.15 [95% CI, 0.92–5.02] and multivariate adjusted hazard ratio1.98 [95% CI, 0.95–4.10]).
Conclusions: Urinary excretion of the nucleic acid oxidation markers 8-oxodG and 8-oxoGuo at the time of diagnosis was not associated with cancer overall in type 2 diabetes patients. For site-specific cancers, risk elevations were seen for breast cancer (8-oxodG). These findings should be examined in future and larger studies.

Introduction

Epidemiological studies suggest that type 2 diabetes is associated with increased risks of several cancer types, including cancers of the liver, pancreas, breast, endometrium, kidney, bladder, and colorectum [1–4]. Although diabetes may influence carcinogenesis by several mechanisms, e.g., hyperinsulinaemia, hyperglycaemia, or chronic inflammation, any exact mechanisms underlying the association between type 2 diabetes and cancer remain to be established. The mutagenic properties of oxidatively damaged DNA and the fact that diabetes is associated with increased urinary excretion of the DNA oxidation marker 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) [5] suggest that DNA oxidation could be one possible biological link between diabetes and cancer risk, and that urinary 8-oxodG may predict development of cancer in diabetes patients.

No previous studies have explored the relationship between 8-oxodG excretion and cancer incidence in type 2 diabetes patients. In general, the evidence on a potential association between urinary 8-oxodG excretion and cancer is limited, although some studies have demonstrated elevated levels of urinary 8-oxodG in patients with various malignancies [6–13]. We recently showed

Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; DCGP, Diabetes Care in General Practice; UPLC, Ultra-performance liquid chromatography

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http://dx.doi.org/10.1016/j.redox.2014.11.010

that urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo), the ribonucleoside counterpart to 8-oxodG, is an independent predictor of mortality in type 2 diabetes patients [14,15]. Whether 8-oxoGuo is also associated with cancer incidence is unknown.

In this study, we investigated whether urinary markers of oxidative stress 8-oxodG and 8-oxoGuo are associated with an increased risk of cancer in type 2 diabetes patients.

Methods

Study population

In the Diabetes Care in General Practice (DCGP) study [16], 474 general practitioners agreed to include all subjects on their practice list who fulfilled the following criteria: newly diagnosed diabetes based on hyperglycaemic symptoms and/or raised blood glucose values, diagnosed between 1 March 1989 and 28 February 1992, and aged 40 years or over. The diabetes diagnosis was subsequently confirmed with a single fasting whole blood/plasma glucose value of $\geq$ 7.0/8.0 mmol/l, measured in a major laboratory. The protocol-based exclusion criteria were life threatening somatic disease, severe mental illness, or unwillingness to participate. After exclusion of 162 patients, the study population consisted of 1381 newly diagnosed diabetes patients. Based on the onset of insulin treatment within 180 days of diagnosis, approximately 97.5% were considered to have type 2 diabetes [16]. Freshly voided morning urine samples were collected from all patients at the time of diagnosis.

The protocol was approved by the ethics committee of Copenhagen and Frederiksberg and informed consent was obtained from all patients.

Assessment of urinary markers and covariates

The urine samples were assayed between 2009 and 2010 for the oxidatively modified guanine nucleosides 8-oxodG and 8-oxoGuo using a validated method of ultraperformance liquid chromatography (UPLC) and tandem mass spectrometry [17]. 8-oxodG and 8-oxoGuo were normalized against urinary creatinine concentration. The assessment of the remaining patient characteristics at baseline has been described elsewhere [16].

Ascertainment of cancer

Information on cancer incidence was obtained from the Danish Cancer Registry [18], which contains accurate and virtually complete records of cancer cases in Denmark. The patients were followed for cancer occurrence from date of diabetes diagnosis until 1 January 2009.

Statistical analysis

The patients were grouped according to the quartiles of their urinary 8-oxodG and 8-oxoGuo levels in order to examine the associations between patient characteristics at diagnosis and the corresponding levels of oxidative stress. The medians and interquartile ranges (for continuous characteristics) or percentages (for categorical characteristics) were reported for each quartile, and associations were assessed by Kruskal–Wallis or chi-square tests, respectively.

Associations between oxidative stress and cancer risk were estimated by Cox proportional hazards regression models based on time from diagnosis to cancer event or censoring. Oxidative stress was represented by the natural logarithm of 8-oxodG and 8-oxoGuo, and by a four-class ordinal variable corresponding to the quartiles of the distribution. Three models were estimated for each of the oxidative stress variables and each outcome: an unadjusted model, a model adjusted for age and sex, and a third model adjusting for sex, age, smoking status, physical activity, education, BMI, alcohol consumption and cohabitation status.

We restricted cancer site-specific analyses to the most common cancer types in the cohort (more than 20 events). In these analyses, the above-mentioned models were used, and oxidative stress was represented by the natural logarithm of 8-oxodG and 8-oxoGuo.

Cancer incidence was plotted against follow-up time using the Kaplan–Meier method.

Reported p values were two-sided and $p < 0.05$ was considered to be significant. Analyses were performed with SAS version 9.2.

Results

Patient characteristics

The median age at diagnosis of diabetes was 65.4 years (interquartile range, 55.7–73.6 years), with a slight male preponderance (53%). Tables 1 and 2 show the baseline characteristics according to quartiles of 8-oxodG and 8-oxoGuo, respectively. For both 8-oxodG and 8-oxoGuo, patients in the highest quartiles were older, more often women, more often living alone, and had higher levels of glycated haemoglobin. In addition, patients in the highest quartiles of 8-oxodG had lower BMI, total cholesterol and serum creatinine, and patients in the highest quartiles of 8-oxoGuo were more often highly educated, less often smokers, and less physically active.

Nucleic acid oxidation and overall cancer risk

We identified a total of 264 incident cancers during follow-up. Kaplan–Meier estimates of cancer incidence for all subjects according to quartiles of urinary 8-oxodG and 8-oxoGuo are shown in Fig. 1.

In the unadjusted Cox regression analyses, log8-oxoGuo was significantly associated with cancer (Table 3). The unadjusted hazard ratio for cancer per natural log increase in 8-oxoGuo was 1.35 (95% confidence interval [CI], 1.01–1.81; $p=0.04$). In the adjusted analyses, no significant associations between 8-oxodG or 8-oxoGuo and overall cancer were found. Both analyses using the quartiles of distribution and log8-oxodG and log8-oxoGuo as continuous covariates showed no association between urinary excretion of the two markers and risk of cancer.

Risk of site-specific cancers

For both 8-oxodG and 8-oxoGuo, the site-specific hazard ratios varied in direction and magnitude (Table 4), probably due to the small numbers of cancer cases. Elevated risk estimates were observed for breast cancer. The unadjusted hazard ratio for breast cancer per natural log increase in 8-oxodG was 2.37 (95% CI, 1.07–5.26; $p=0.03$). When adjusting for covariates in models 2 and 3, the corresponding hazard ratios were 2.15 (95% CI, 0.92–5.02; $p=0.08$) and 1.98 (95% CI, 0.95–4.10; $p=0.07$), respectively.

Discussion

This cohort study that included 1381 newly diagnosed type 2 diabetes patients observed for up to 20 years is the first prospective study to explore the association between urinary excretion of markers of oxidative stress and cancer in diabetes patients.