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Oxidative damage DNA: 8-oxoGua and 8-oxodG as molecular markers of cancer

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Background:

The broad spectrum of oxidative damage DNA biomarkers: urinary excretion of 8-oxodG (8-oxo-7,8-dihydro-2'-deoxyguanosine), 8-oxoGua (8-oxo-7,8-dihydroguanine) as well as the level of oxidative damage DNA in leukocytes, was analyzed in cancer patients and healthy subjects.

Material/Methods:

222 cancer patients and 134 healthy volunteers were included in the analysis, using methodologies which involve HPLC (high-performance liquid chromatography) prepurification followed by gas chromatography with isotope dilution mass spectrometry detection and HPLC/EC.

Results:

For the whole patient population (n=222) the median values of 8-oxoGua and 8-oxodG in urine samples were 12.44 (interquartile range: 8.14–20.33) [nmol/24 hr] and 6.05 (3.12–15.38) [nmol/24 hr], respectively. The median values of 8-oxoGua and 8-oxodG in urine samples of the control group (n=85) were 7.7 (4.65–10.15) [nmol/24 hr] and 2.2 (1.7–2.8) [nmol/24 hr], respectively. The level of 8-oxodG in DNA isolated from leukocytes of the patient population (n=179) and of the control group (n=134) was 4.93 (3.46–9.27) per 10⁶ dG and 4.46 (3.82–5.31) per 10⁶ dG, respectively.

Conclusions:

The results suggest that oxidative stress in cancer patients, demonstrated by augmented amounts of these modifications in urine, could be typical not only for affected tissue but also for other tissues and even the whole organism. An assay that enables the determination of levels of basic markers of oxidative stress might be applied in clinical practice as an additional, helpful marker to diagnose cancer.

key words:

damage DNA • molecular markers • cancer patients • 8-oxoGua • 8-oxodG

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BACKGROUND

Oxidatively modified bases

Hydroxyl radical attack on DNA most frequently leads to base damages that result in generating a range of derivatives [1–3]. Aerobic organisms, within the course of evolution, developed a range of adaptive mechanisms inducing synthesis of anti-oxidative enzymes and/or enzymes repairing oxidative damages of DNA [5–7].

Oxidative damage to nucleic acid has been associated with a number of pathologies including cancer and neurodegenerative and cardiovascular diseases [8–10].

To date, more than 20 different types of oxidative modifications of bases have been identified [4].

Of all the modified bases, the processes which repair 8-oxoGua are perhaps best understood, and may be regarded as a template for the processes which repair other lesions. To combat the deleterious biological effect of the presence of 8-oxoGua, cells have developed specific mechanisms to remove this lesion from cellular DNA [11].

In mammalian cells, 3 enzymes form the equivalent of the bacterial “GO” system. The first level of this protection is human Mut T homologue (hMTH1) which hydrolyses 8-oxodGTP (a potential substrate for DNA polymerase), thereby eliminating it from the nucleotide pool. The second level of defence is specific glycosylases that initiate base excision repair (BER). Finally, human Mut Y homologue (hMYH) removes adenine that is mis-paired with 8-oxoGua. Most recently, we proposed that nucleotide excision repair (NER), which involves the removal of a lesion-containing oligonucleotide, may compliment the “GO” system [12,13], based upon evidence that oxidative DNA damage may be repaired by this route [14,15].

However, there is very little evidence that 8-oxodG is a direct product of DNA repair itself (ie, released as the deoxynucleoside, rather than the base, from DNA) [16].

It is generally accepted that products of cellular repair of oxidatively damaged DNA, such as modified bases and nucleosides: 8-oxo-7,8-dihydroguanine (8-oxoGua) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) are excreted in urine.

Diet as a potential source of 8-oxoGua and 8-oxodG in urine

An assay was performed to analyze levels of 8-oxoGua and 8-oxodG in urine samples with regard to diet. In case of the examined group, a conclusion was drawn that diet does not determine excretion of these biomolecules in urine [17]. In another study, different amounts (up to 25 mg) of ¹⁵N labeled oxidatively modified DNA were absorbed orally by volunteers. Throughout 2 weeks, blood and urine samples were collected.

No ¹⁵N 8-oxoGua or 8-oxodG were detected either in urine and or in DNA of monoclonal cells in venous blood obtained from the same subjects taking part in the study [18], demonstrating that diet has no influence on the level of these damages.

Cell death as a potential source of 8-oxoGua and 8-oxodG in urine

In both known works by Faure et al. [19] and Erhola et al. [20], no rise of levels in 8-oxodG excretion in urine was observed despite unequivocal evidence for mass reduction of treated tumors. In certain reports, a clear rise of 8-oxodG in urine excretion was observed after radio-chemotherapy or radiotherapy itself [21–23].

However, measuring excretion of repair product in urine exclusively may be misleading since it provides no information on the oxidative state of the organism (damage rate *vs.* repair rate) in cellular DNA and reports only the mean value of damage repair in the past.

Purpose

The aim of the work was to investigate whether the levels of markers of oxidative damages of DNA: excreted in urine 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanine (8-oxoGua) as well as the level of 8-oxodG in DNA of venous blood leukocytes differ in a population of healthy subjects when compared with cancer patients.

MATERIAL AND METHODS

Patients

Analysis of daily excretion of 8-oxoGua and 8-oxodG with urine was done in a study group consisting of 222 patients with malignant cancer (III and IV degree of clinical stage). Leukocytes from peripheral blood samples for analysis of 8-oxodG level were obtained from 179 patients from among the study group. Control peripheral blood samples for analysis of 8-oxodG in leukocytes were obtained from 134 healthy volunteers. From this group, 85 urine samples were taken for the measurement of daily excretion of 8-oxoGua and 8-oxodG in urine. The patients had various malignant tumors: , head and neck cancer (n=45), breast cancer (n=32), colon cancer (n=25), lung cancer (n=37), uterine cancer (n=15), ovarian cancer (n=39), testicular cancer (n=7), prostate cancer (n=11), gastrointestinal cancer (n=11).

Spot urine samples and blood were collected before the treatment. The patients were asked to abstain from vitamin supplementation for at least 1 month before the chemotherapy started and during the course of the treatment, and only these patients qualified. The control group was chosen in such a way that the following criteria matched the patient group: eating habits, age, body weight, sex, and smoking status.

The study was approved by the medical ethics committee of The Collegium Medicum Nicolaus Copernicus University Bydgoszcz, Poland, (in accordance with Good Clinical Practice, Warsaw 1998), and all the patients gave informed consent.

Isolation of leukocytes from venous blood

Venous blood samples (18 ml) from the patient and volunteer groups were collected. The blood was carefully applied on top of Histopaque 1119 solution (Sigma-Aldrich Inc.; St.

Table 1. The level of 8-oxogua, 8-oxodg in the urine, and 8-oxodg in the leukocytes' DNA.

	Median (interquartile range)	Mean (\pm SD)
	Healthy group (n=134)	Cancer group (n=222)
Urinary 8-oxoGua (nmol/24h)		
Median	7.7 (4.65–10.15)	12.44 (8.14–20.33)
Mean	7.8 (\pm 4.21)	15.25 (\pm 10.91)
Urinary 8-oxodG (nmol/24h)		
Median	2.2 (1.7–2.8)	6.05 (3.12–15.38)
Mean	2.3 (\pm 0.84)	13.05 (\pm 20.46)
Leukocytes' 8-oxodG/106dG		
Median	4.46 (3.82–5.31)	4.93 (3.46–9.27)
Mean	4.73 (\pm 1.11)	7.24 (\pm 5.59)

Louis, MO) and leukocytes were isolated by centrifugation according to the procedure specified by the manufacturer.

DNA isolation and 8-oxodG determination in DNA isolates

DNA from leukocytes was isolated using the method as described earlier [24]. Determination of 8-oxodG by the mean of HPLC/EC technique was as described previously [24,25].

Urine analysis

Urine sample preparation, HPLC purification and GC/MS analysis were conducted as described earlier [24].

Statistical analysis

All results are expressed as median (interquartile range). STATISTICA (data analysis software system), version 9.0. *www.statsoft.com*. (lic. no: JXVP002E256522ARE-E) was used for the statistical analysis. Mann-Whitney testing for independent groups with abnormal distribution was performed. For normal distribution, variables were analyzed by the Kolmogorov-Smirnov test with Lillefor's correction. Statistical significance was considered at $P < 0.05$.

RESULTS

Significantly elevated levels of 8-oxoGua and 8-oxodG excreted in urine daily as well as 8-oxodG in DNA of leukocytes in venous blood were observed in cancer patients as compared with healthy subjects, with considerable statistic significance (Table 1).

For the whole patient population (n=222), the median values of 8-oxoGua and 8-oxodG in urine samples were 12.44 (interquartile range: 8.14–20.33) [nmol/24 hr] and 6.05 (3.12–15.38) [nmol/24 hr], respectively.

The median values of 8-oxoGua and 8-oxodG in urine samples of the control group (n=85) were 7.7 (4.65–10.15) [nmol/24 hr] and 2.2 (1.7–2.8) [nmol/24 hr], respectively.

The level of 8-oxodG in DNA isolated from leukocytes in the patient group (n=179) and of the control group (n=134) was 4.93 (3.46–9.27) per 10^6 dG and 4.46 (3.82–5.31) per 10^6 dG, respectively.

DISCUSSION

Certain amounts of oxidatively modified bases are present in every cell, reflecting the balance between ROS attacking DNA in the course of many metabolic processes and damage repair of these molecules by specific enzymes repairing DNA. It is not known at present what is the endogenous level of these potentially mutagenic damages. According to the reports of authors applying different analytical techniques, the values range from 0.2 to several modifications/ 10^6 pairs of bases for healthy cells [26,27]. It seems, however, that the level differs considerably among subjects [28,29]. They are removed in the process of repair and excreted in urine in unchanged state. A balance between producing ROS which induce oxidative DNA damages and removing these damages was observed in a cell (background level) [30]. According to some authors, the levels of these modifications do not depend on the type of cancer and histopathologic diagnosis [23,31–33].

It is possible to estimate the extent of repair on the level of the whole organism while analyzing the amount of oxidative DNA damages in urine. High values of oxidative damages of DNA excreted in urine indicate an intensified level of oxidative stress, but they may also reflect high efficiency of repair systems of these damages (oxidative stress may be high, yet repair mechanisms remove its effects). However, combining the background level specific for every patient and analyzing 8-oxoGua and 8-oxodG in excreted urine may clearly reflect information about DNA repair mechanisms.

The results presented in the present study comparing amounts of 8-oxoGua and 8-oxodG excreted in urine daily as well as amounts of 8-oxodG in venous blood DNA leukocytes in patients with diagnosed cancer when compared with healthy subjects demonstrate higher levels of these

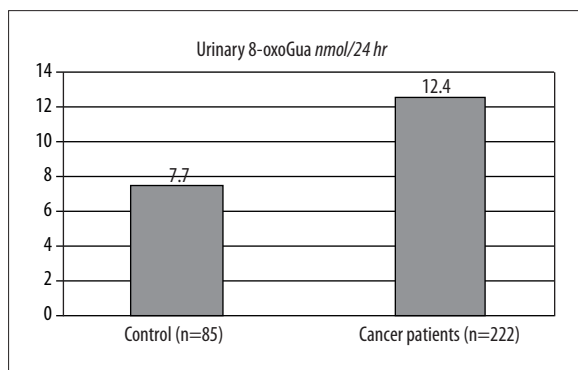


Figure 1. Daily urine excretion of 8-oxoGua in control group of healthy and all patients before treatment (median values). Mann-Whitney Test $p=0.004$.

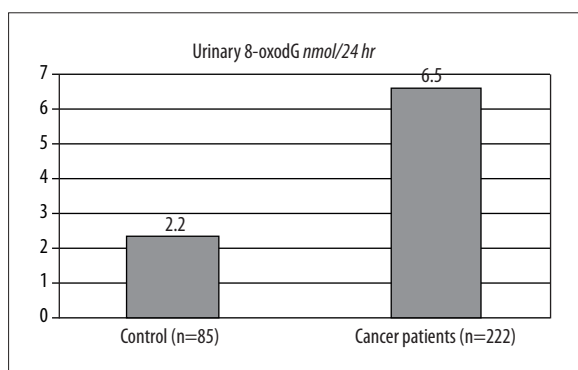


Figure 2. Daily urine excretion of 8-oxodG in control group of healthy and all patients before treatment (median values) Mann-Whitney Test $p=0.0001$.

modifications in cancer patients (Figures 1–3). Significantly elevated levels of analyzed markers of oxidative damage DNA may reflect the oxidative stress situation which accompanies cancer [34,35].

Several reasons account for this phenomenon. Several studies [19,20,23,31] have analyzed 8-oxoGua and 8-oxodG in urine in different types of cancer patients and control groups, reporting elevated level of 8-oxoGua in urine of cancer patients and in a control group of smoking subjects, and the level of 8-oxodG in DNA isolated from venous blood leukocytes was higher in the patient group.

These findings suggest that in cancer patients repair mechanisms of oxidative damage to DNA are less efficient (the concentration of modified nucleoside/base in urine reflects DNA damages of the whole organism, and the level of 8-oxodG in cellular DNA reflects balance between processes generating damages and the ability of their repair).

Rozalski et al. [34] compared a group of cancer patients and healthy volunteers with regard to the amount of 8-oxoGua and 8-oxodG excreted in urine, demonstrating elevated levels of oxidatively modified base in patients when compared with healthy subjects by 50%.

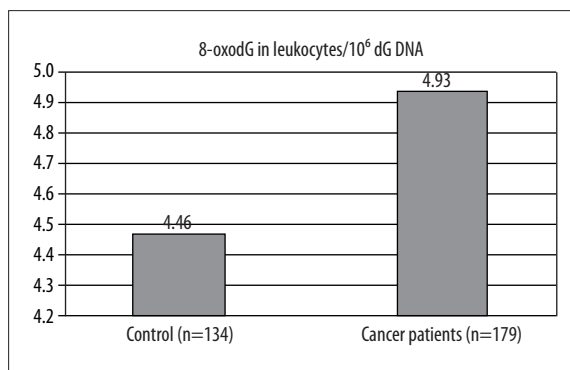


Figure 3. Levels of 8-oxodG in DNA isolated from venous blood leukocytes in control group of healthy subjects and cancer patients before treatment (median values). Mann-Whitney Test $p=0.0017$.

CONCLUSIONS

Our work indicates the levels of these derivatives elevated to such an extent cannot be related to its increase in cancer cells exclusively. The results suggest that oxidative stress in patients with cancer, demonstrated by elevated levels of these modifications in urine, may be typical not only of affected tissue but also of other tissues and even the whole organism.

From practical point of view, a test that would enable determination of background levels of basic markers of oxidative stress (8-oxoGua and 8-oxodG in urine and 8-oxodG in DNA in leukocytes), might be applied as an additional and helpful marker for early detection of the development of cancer.

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