

Research Article

Oxidatively Damaged DNA/Oxidative Stress in Children with Celiac Disease

Anna Szaflarska-Popławska¹, Agnieszka Siomek², Mieczysława Czerwionka-Szaflarska¹, Daniel Gackowski², Rafał Różalski², Jolanta Guz², Anna Szpila², Ewelina Zarakowska², and Ryszard Oliński²

Abstract

Background: Because patients with celiac disease face increased risk of cancer and there is considerable circumstantial evidence that oxidatively damaged DNA may be used as a marker predictive of cancer development, we decided, for the first time, to characterize oxidative stress/oxidative DNA damage in celiac disease patients.

Methods: Two kinds of oxidatively damaged DNA biomarkers, namely, urinary excretion of 8-oxodG and 8-oxoGua, and the level of oxidatively damaged DNA in the leukocytes, as well as the level of antioxidant vitamins were analyzed using high-performance liquid chromatography (HPLC) and HPLC/gas chromatography with isotope dilution mass detection methods. These parameters were determined in three groups: (a) children with untreated celiac disease, (b) patients with celiac disease on a strict gluten-free diet, and (c) healthy children.

Results: The mean level of 8-oxodG in DNA isolated from the leukocytes and in the urine samples of the two groups of celiacs was significantly higher than in controls, irrespective of diet. There was no statistically significant difference in these parameters between treated and untreated celiacs. The mean plasma retinol and α -tocopherol concentration in the samples of untreated celiacs was significantly lower than in treated celiacs.

Conclusion: Our results suggest that although diet can be partially responsible for oxidative stress/oxidatively damaged DNA in celiac patients, there is a factor independent of diet.

Impact: It is possible that celiac disease patients may be helped by dietary supplementation rich in vitamin A (and E) to minimize the risk of cancer development. *Cancer Epidemiol Biomarkers Prev*; 19(8); 1960–5. ©2010 AACR.

Introduction

Celiac disease is a common disorder with a prevalence of 1:100 to 1:200 in European and American populations (1). It is assumed that celiac disease is a gluten-sensitive disorder characterized by toxic injury of gliadin, a component of gluten (or by similar molecules). Ingestion of these molecules in susceptible individuals gives rise to inflammatory lesions in the small intestine (2). The inflammatory condition may be directly linked to increased production of proinflammatory cytokines such as IFN- γ and tumor necrosis factor (TNF)- α in patients with active celiac disease (3). Interestingly, some of the cytokines can produce large amounts of reactive oxygen species (4, 5). It has been shown that an elevated plasma level of TNF- α is responsible for increased oxidative stress/oxidatively

damaged DNA of CD34⁺ cells (6), and some experimental evidence indicates that oxidative stress is one of the major mechanisms that may be involved in celiac disease pathogenesis (7).

The association between inflammation and oxidative stress is well documented (8, 9), with a number of studies on inflammatory conditions reporting elevated levels of 8-oxodG, an oxidative stress biomarker. The inflammatory response can lead to the recruitment of activated leukocytes, which may, in turn, give rise to a “respiratory burst,” which is an increased oxygen uptake that causes the release of high quantities of reactive oxygen species, such as superoxide and hydrogen peroxide, with possible subsequent DNA damage production (10). Proof of this proposal comes from the report of Dizdaroglu et al. (11), who showed that exposure to activated leukocytes caused DNA base modifications in human cells typical of those induced by hydroxyl radical attack. Mechanistically, chronic inflammation can be closely linked to carcinogenesis (12). Moreover, it has been estimated that chronic inflammation may be involved in the development of about one third of all cancer cases worldwide (13, 14).

Although celiac disease is triggered by a dietary gluten, it is clearly a genetic disorder and therefore persists throughout an individual's life span and thus can be

Authors' Affiliations: ¹Chair and Department of Pediatrics, Allergology and Gastroenterology, and ²Department of Clinical Biochemistry, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland

Corresponding Author: Ryszard Oliński, Department of Clinical Biochemistry, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, ul. Karłowicza 24, 85-092 Bydgoszcz, Poland. Phone: 48-52-5853770; Fax: 48-52-5853771. E-mail: ryszardo@cm.umk.pl

doi: 10.1158/1055-9965.EPI-10-0295

©2010 American Association for Cancer Research.

responsible for chronic oxidative stress and inflammation that in turn may be associated with increased risk of malignant neoplasms. There is considerable circumstantial evidence that oxidative DNA damage may be used as a marker predictive of cancer development later on (15).

To assess whether celiac disease may impose oxidatively damaged DNA, we decided, for the first time, to analyze two kinds of oxidatively damaged DNA biomarkers, namely, urinary excretion of 8-oxodG and 8-oxoGua, as well as the level of oxidatively damaged DNA in the leukocytes, which are often used as surrogate cells that are supposed to inform about oxidative stress, measured as a certain level of 8-oxodG, in other tissues (16). Because antioxidant vitamins and uric acid are the most effective free radical scavengers, and can decrease oxidative stress, in our study the level of these compounds was analyzed in patients' blood. The above described parameters were analyzed in three groups of children: (a) children with untreated histologically confirmed celiac disease; (b) patients with histologically confirmed celiac disease, recognized in early childhood, who are on a strict gluten-free diet; and (c) healthy children (control group).

Materials and Methods

Patients

The study was conducted in three groups of patients. Group 1 consisted of 45 patients, including 28 girls and 17 boys with untreated celiac disease. Mean age in this group was 14.1 years (range, 2-27 years).

In 23 children celiac disease was newly diagnosed on the basis of modified ESPGHAN (European Society of Paediatric Gastroenterology, Hepatology and Nutrition) criteria (17). All patients underwent esophagogastroduodenoscopy to provide at least three biopsy samples taken from the second part of the duodenum. Villous atrophy was assessed according to Oberhuber modification of Marsh classification (18). All patients had histopathologically documented villous atrophy: grade 3a ($n = 1$), grade 3b ($n = 10$), and grade 3c ($n = 12$). All children were serum antiendomysial antibody positive.

In 22 patients celiac disease was diagnosed in childhood on the basis of "old" (ref. 19; 8 patients) or modified (ref. 17; 14 patients) ESPGHAN criteria. All patients reported more than two to three dietary transgressions per week or following an unrestricted gluten-containing diet for at least last two years. By the time of sampling all patients were serum antiendomysial antibody positive.

Group 2 consisted of 78 patients, including 49 females and 29 males with histologically confirmed celiac disease diagnosed in childhood on the basis of old (19) or modified (17) ESPGHAN criteria, who were following a strict gluten-free diet. Mean age in this group was 15.4 years (range, 4-29 years). Based on the patients' medical history, all patients were fully compliant with the gluten-free diet and by the time of sampling were serum antiendomysial antibody negative. The mean time under strict gluten-free diet was 10.1 years (range, 1-25 years).

None of the patients in groups 1 and 2 had a positive history of cancer or chronic inflammatory disease other than celiac disease or actual acute infection.

Twenty-four healthy subjects without any known disease matching the patient groups according to age, sex, and eating habits served as a control group (group 3). All children were nonsmokers.

The study was approved by the medical Ethics Committee of Ludwik Rydygier Medical University Bydgoszcz, Poland, No KB/439/2006. All patients' parents and patients older than 16 years expressed informed consent.

Methods

Leukocyte isolation from venous blood. Venous blood samples from the patients were collected in heparinized Vacuette tubes. The blood was carefully applied on the top of Histopaque 1119 solution (Sigma-Aldrich) and the leukocytes were isolated by centrifugation according to the procedure laid down by the manufacturer.

DNA isolation and 8-oxodG determination in DNA isolates. DNA from the leukocytes was isolated using the method as described earlier. Determination of 8-oxodG by means of high-performance liquid chromatography (HPLC)/with electrochemical detection (EC) technique was described previously (20).

Our team has participated in the European Standards Committee on Oxidative DNA Damage. In several interlaboratory trials determining the levels of 8-oxoG in HeLa cell DNA, isolated from untreated cells or from cells treated with light in the presence of a photosensitizer to induce different amounts of 8-oxoG in DNA, our laboratory showed a low background level of 8-oxoG in DNA from untreated cells and an ability to detect a dose-response between the concentration of photosensitizer and the level of 8-oxoG in the DNA of treated cells (21). In the last trial of the European Standards Committee on Oxidative DNA Damage it was found that the mean value of 8-oxodG in lymphocytes DNA (4,24 8-oxodG per 10^6 unmodified dG) was very close to the level reported in this study (22). To the best of our knowledge no substantial differences between schoolchildren and adults concerning 8-oxodG level have been reported in the literature (23, 24). However, some studies have reported much lower levels of 8-oxodG than those detected in our work; for example, Buthumrung et al. (25) reported $0.8/10^6$ dG levels of 8-OH-dG in the leukocytes of controls. It is possible that these inconsistencies may be a result of preparing a calibration curve. Accurate calibration is vital, and the preparation of standard curves, based on the molar absorption (instead of preparation "by weight") of the target compound, is recommended, where possible (26). We realize, however, that we are not in a position to disqualify the other results.

Urine analysis. Spot urine samples were collected. Added to the 2 mL of human urine were 0.2 nmol of $\{^{15}\text{N}_3, ^{13}\text{C}\}$ 8-oxoGua, 0.05 nmol of $\{^{15}\text{N}_5\}$ 8-oxodG, and 10 μL of acetic acid (Sigma, HPLC grade). After centrifugation ($2,000 \times g$, 10 minutes), the supernatant was

filtered through a Millipore GV13 0.22- μ m syringe filter and 500 μ L of this solution were injected into the HPLC system. The urine HPLC purification of 8-oxoGua and 8-oxodG was done according to the method described previously (20).

Gas chromatography with isotope dilution mass detection analysis was done according to the method described by Dizdaroglu (27), adapted for additional $\{^{15}\text{N}_5\}$ 8-oxo-Gua analyses (m/z 445 and 460 ions were monitored).

Determination of the plasma α -tocopherol (vitamin E), retinol (vitamin A), ascorbic acid (vitamin C), and uric acid concentration. Quantification of α -tocopherol (vitamin E), retinol (vitamin A), ascorbic acid (vitamin C), and uric acid by HPLC technique was described previously (20).

Statistical analysis. For the statistical analysis the STATISTICA (version 8.0) computer software (StatSoft) was used. All results are expressed as means \pm SD for variables with normal distribution, and as median with interquartile range for variables with nonparametric distribution.

For normal distribution, variables were analyzed by the Kolmogorov-Smirnov test with Lillefor's correction. For variables with nonparametric distribution Mann-Whitney's U test was carried out; Student's t test was used for variables with normal distribution. Statistical significance was considered at $P < 0.05$.

Results

The mean level of 8-oxodG in DNA isolated from the leukocytes and in the urine samples of children with celiac disease was significantly higher than that in controls, irrespective of diet. There was no statistically significant difference in these parameters between treated and untreated celiac patients. The mean level of 8-oxoGua in the urine samples of untreated children with celiac disease was 14.38 ± 9.29 nmol/mmol of creatinine and was significantly higher than that in the urine of patients keeping a strict gluten-free diet (11.66 ± 9.37 nmol/mmol of creatinine) and of controls (7.78 ± 3.46 nmol/mmol of creatinine; Table 1).

The mean plasma retinol and α -tocopherol concentration in the samples of children with untreated celiac disease was significantly lower than that in treated celiacs ($P = 0.006$ and 0.002 , respectively). There was no difference in the mean plasma retinol and α -tocopherol concentration between treated celiac disease patients and controls. The mean plasma ascorbic acid concentration was very similar in untreated and treated celiac disease patients (59.66 ± 23.86 and 63.24 ± 27.38 μ mol/L, respectively) and was significantly lower than in controls (74.34 ± 17.55 μ mol/L). There was no statistically significant difference in the plasma uric acid concentration among the three studied groups of patients (Table 2).

Table 1. Parameters of oxidative DNA damage in the study groups

Group of patients/parameter	8-oxodG/ 10^6 dG level in the leukocytes' DNA	8-oxoGua excretion (nmol/mmol of creatinine)	8-oxodG excretion (nmol/mmol of creatinine)
Group 1	$n = 42$	$n = 40$	$n = 39$
Untreated patients with celiac disease	Mean \pm SD 6.80 ± 4.54 Me (Q1, Q3) $5.33 (4.14, 7.64)^*$	Mean \pm SD 14.38 ± 9.29 Me (Q1, Q3) $12.68 (7.48, 17.57)^{\dagger, \ddagger}$	Mean \pm SD 2.48 ± 1.57 Me (Q1, Q3) $2.14 (1.44, 2.95)^{\S}$
Group 2	$n = 75$	$n = 75$	$n = 74$
Treated patients with celiac disease	Mean \pm SD 6.16 ± 2.90 Me (Q1, Q3) $5.45 (3.97, 8.03)^{\parallel}$	Mean \pm SD 11.66 ± 9.37 Me (Q1, Q3) $8.68 (5.32, 13.81)$	Mean \pm SD 2.16 ± 1.28 Me (Q1, Q3) $1.81 (1.24, 2.74)^{\nabla}$
Group 3	$n = 24$	$n = 23$	$n = 23$
Control group	Mean \pm SD 4.39 ± 1.03 Me (Q1, Q3) $4.20 (3.78, 4.84)$	Mean \pm SD 7.78 ± 3.46 Me (Q1, Q3) $7.29 (4.67, 10.72)$	Mean \pm SD 1.59 ± 0.52 Me (Q1, Q3) $1.29 (1.08, 1.95)$

Abbreviation: Me, median value; Q1, the first quartile; Q3, the third quartile.

*Statistically significant difference versus control group ($P = 0.003$).

\dagger Statistically significant difference versus control group ($P = 0.0006$).

\ddagger Statistically significant difference versus group 2 ($P = 0.02$).

\S Statistically significant difference versus control group ($P = 0.005$).

\parallel Statistically significant difference versus control group ($P = 0.006$).

∇ Statistically significant difference versus control group ($P = 0.039$).

Table 2. Plasma antioxidant parameters in the study groups

Group of patients/ parameter	Plasma retinol concentration ($\mu\text{mol/L}$)	Plasma α -tocopherol concentration ($\mu\text{mol/L}$)	Plasma ascorbic acid concentration ($\mu\text{mol/L}$)	Plasma uric acid concentration ($\mu\text{mol/L}$)
Group 1	<i>n</i> = 43	<i>n</i> = 43	<i>n</i> = 40	<i>n</i> = 42
Untreated patients with celiac disease	Mean \pm SD 1.37 \pm 0.38 Me (Q1, Q3) 1.39 (1.03, 1.56)*	Mean \pm SD 23.00 \pm 6.63 Me (Q1, Q3) 22.22 (18.14, 28.04) [†]	Mean \pm SD 59.66 \pm 23.86 Me (Q1, Q3) 61.45 (43.75, 75.55) [‡]	Mean \pm SD 240.07 \pm 69.16 Me (Q1, Q3) 227.35 (191.66, 289.76)
Group 2	<i>n</i> = 78	<i>n</i> = 78	<i>n</i> = 74	<i>n</i> = 75
Treated patients with celiac disease	Mean \pm SD 1.68 \pm 0.59 Me (Q1, Q3) 1.58 (1.20, 2.10)	Mean \pm SD 26.76 \pm 5.34 Me (Q1, Q3) 27.25 (22.93, 29.77)	Mean \pm SD 63.24 \pm 27.38 Me (Q1, Q3) 61.49 (43.95, 84.14) [§]	Mean \pm SD 254.95 \pm 95.97 Me (Q1, Q3) 229.55 (192.35, 284.64)
Group 3	<i>n</i> = 20	<i>n</i> = 20	<i>n</i> = 17	<i>n</i> = 20
Control group	Mean \pm SD 1.53 \pm 0.49 Me (Q1, Q3) 1.50 (1.08, 1.79)	Mean \pm SD 24.55 \pm 4.93 Me (Q1, Q3) 24.76 (22.34, 27.09)	Mean \pm SD 74.34 \pm 17.55 Me (Q1, Q3) 74.75 (59.79, 83.95)	Mean \pm SD 271.46 \pm 66.19 Me (Q1, Q3) 271.46 (221.59, 322.78)

*Statistically significant difference versus group 2 ($P = 0.006$).

[†]Statistically significant difference versus group 2 ($P = 0.002$).

[‡]Statistically significant difference versus control group ($P = 0.01$).

[§]Statistically significant difference versus control group ($P = 0.04$).

There were no differences between the subgroups of patients classified to the group 1 concerning analyzed parameters, i.e., antioxidants and those reflecting oxidatively damaged DNA (data not shown).

Discussion

It has been assumed that oxidative stress is one of mechanisms that can play a role in celiac disease development (7). In agreement with this assumption, it has been reported that gliadin can alter an oxidative balance in colonic cell culture (CaCo-2) triggering oxidative stress, described by an increase of 4-hydroxy-nonenal (4-HNE) and a reduction of glutathione and protein sulphhydryl content (28). Lipid peroxidation products, such as 4-HNE, can form other than 8-oxodG DNA modifications (e.g., etheno adducts). However, the most widely studied and the most representative marker of oxidative stress/oxidative DNA damage is 8-oxodG (10). For this reason we decided to analyze 8-oxodG in our study. Moreover, studies with celiac disease patients have shown that antioxidant capacity (including antioxidant vitamins) in this group was severely reduced when compared with healthy controls (7, 29).

When investigating the involvement of oxidative stress in disease development the important question is, what are the most appropriate biomarkers of oxidative stress and what is the best way to measure them? Commonly used biomarkers of oxidative stress include measures of oxidative damage to DNA. Oxidatively damaged DNA

can be assessed by determination of 8-oxodG level in cellular DNA; this parameter is the most studied and is widely acknowledged as a robust biomarker of oxidative stress. An alternative approach to assess oxidative stress/DNA damage on the level of the whole organism is determination of the urinary excretion of oxidatively modified bases/nucleosides (30).

To the best of our knowledge, there have been no studies concerning oxidatively damaged DNA in celiac disease. It is an important issue because celiac disease patients face an increased risk of cancer (31) and there is considerable circumstantial evidence that oxidatively damaged DNA may be used as a marker predictive of cancer development later on (15, 32, 33).

In our study all the parameters that represent oxidative damage to DNA, i.e., the urinary excretion of modified base and nucleosides and the level of 8-oxodG in the leukocytes, were determined. If the gluten itself or gluten-derived peptides were solely responsible for the proinflammatory condition and oxidative stress/oxidatively damaged DNA, as suggested (see above), one could expect a significant difference in the markers that described damage between the group with treated celiac disease on a strict gluten-free diet, in which the gluten-derived peptides were not detected (group 2), and the group of patients with untreated celiac disease (group 1).

Indeed, analysis of other parameters (e.g., myeloperoxidase activity) in untreated celiacs and celiacs on the strict gluten-free diet could have also been useful as an indicator of inflammation. However, such analyses demand a

large amount of biological material (blood). In our approach we used noninvasive sampling of biological material (urine) and small amounts of surrogate tissue (leukocytes). This is especially important for pediatric patients who demand special care. However, as mentioned in Materials and Methods, children with reported chronic inflammatory disease other than celiac disease or actual acute infection were excluded from the investigation.

Surprisingly all of the markers of oxidative DNA damage were higher in celiac disease patients in comparison with matched controls, irrespective of the diet (although all the parameters that describe the damage were slightly higher in the untreated versus the treated group). Only urinary 8-oxoGua level was significantly lower in patients on a strict gluten-free diet than in untreated celiac patients.

Of all the parameters that describe oxidative damage to DNA the most important one is the background level of 8-oxodG in DNA because it can inform about a load of potentially mutagenic DNA modification. Because the background level of 8-oxodG in cellular DNA represents a delicate balance between formation and removal of a damage, the observed increase of the level in all groups of patients may be a result of repair deficiency of oxidative damage to DNA in celiac disease patients. Interestingly it has been shown that celiac disease patients who developed adenocarcinoma had defective mismatch repair, and this DNA repair mechanism may play a role in the prevention of mutagenic effect of 8-oxodG (34).

Still another reason for the observed oxidative damage to DNA may be the oxidative stress induced by a deficiency in antioxidant levels, which in turn may be a result of malabsorption of antioxidant vitamins that may persist even in periods of remission. To explore this possibility, we checked the levels of antioxidant vitamins (A, C, and E) and uric acid in the study groups. We found statistically significant differences in the levels of vitamins A and E between the groups of untreated and treated celiac patients and for vitamin C between control group and celiac disease group, irrespective of diet. Although uric acid is an effective free radical scavenger, no significant differences among the study groups in the level of this compound were found. Therefore, our results suggest that although diet can be partially responsible for oxidative stress/oxidatively damaged DNA in celiac patients, it is a factor independent of diet, which may likely be associated with genetic make-up, possibly linked with DNA repair.

Many studies show increased cancer risk and mortality, especially for B- and T-cell lymphoma in celiac

disease (35, 36). In this case, however, the mechanism(s) involved in cancer development are not fully recognized.

Although we do not have enough data to draw a definitive conclusion, we can hypothesize that long-lived B and T lymphocytes (or/and hematopoietic progenitor cells) may serve as target cells for developing malignancies like lymphoma in some patients with celiac disease. Some base modification with mutagenic properties that escape repair in lymphocytes DNA could lead to mutagenesis in critical genes and ultimately to malignancies. This notion stems from the promutagenic properties of 8-oxoguanine and some other oxidative base modifications.

One subject may be 10 to 200 times more sensitive to carcinogenic factors than another and may therefore develop cancer, whereas others at the same level of exposure will not (37). In this context it is worth mentioning that we observed substantial variations in the levels of potentially mutagenic 8-oxodG in cellular DNA, as well as vitamin levels in celiac disease patients. Individuals also vary in their disease severity and response to gluten-free diet (38). This variable response is a major problem in the sensitivity or resistance of individuals and these factors cannot be reliably predicted prior to therapy.

We recently showed that oxidatively damaged DNA is inversely correlated with the endogenous level of antioxidant vitamins and that vitamin A has the strongest effect of all antioxidant components on the damage (39). It is possible that other food components that can accompany vitamin A, such as polyphenols and phytochemicals, may be partially responsible for observed effects. As mentioned above, vitamin A level was significantly decreased in the group of untreated celiac patients in comparison with the treated one (Table 2). Therefore, it is possible that celiac disease patients may be helped by dietary supplementation rich in vitamin A (and E) to minimize the risk of cancer development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

R. Oliński acknowledges financial support from ECNIS (European Cancer Risk, Nutrition and Individual Susceptibility). Ministry of Science and Higher Education; Grant Number: N407025 32/0949.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 03/21/2010; revised 05/28/2010; accepted 06/07/2010; published online 08/09/2010.

References

- Book L, Zane JJ, Neuhausen SL. Prevalence of celiac disease among relatives of sib pairs with celiac disease in U.S. families. *Am J Gastroenterol* 2003;98:377-81.
- van Heel DA, West J. Recent advances in coeliac disease. *Gut* 2006; 36:864-74.
- Nilsen EM, Lundin KE, Krajci P, Scott P, Sollid LM, Brandtzaeg P. Gluten specific, HLA DQ restricted T cells from coeliac mucosa produce cytokines with Th1 or Th0 profile dominated by interferon γ . *Gut* 1995;37:766-76.
- Kayanoki Y, Fuji J, Suzuki K, Kawata S, Matsuzawa Y, Taniguchi N.

- Suppression of antioxidative enzyme expression by transforming growth factor- β 1 in rat hepatocytes. *J Biol Chem* 1994;269:15488–92.
5. Ohba M, Shibamura M, Kuroki T, Nose K. Production of hydrogen peroxide by transforming growth factor- β 1 and its involvement in induction of egr-1 in mouse osteoblastic cells. *J Cell Biol* 1994; 126:1079–88.
 6. Peddie CM, Wolf CR, McLellan LI, Collins AR, Bowen DT. Oxidative DNA damage in CD34+ myelodysplastic cells is associated with intracellular redox changes and elevated plasma tumour necrosis factor- α concentration. *Br J Haematol* 1997;99:625–31.
 7. Diosdado B, van Oort E, Wijmenga C. "Coelionomics": towards understanding the molecular pathology of coeliac disease. *Clin Chem Lab Med* 2005;43:685–95.
 8. Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. Oxford, UK: Clarendon Press; 1996.
 9. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J* 1996;313:17–29.
 10. Cooke MS, Olinski R, Evans MD. Does measurement of oxidative damage to DNA have clinical significance? *Clin Chim Acta* 2006; 365:30–49.
 11. Dizdaroglu M, Olinski R, Doroshow JH, Akman SA. Modification of DNA bases in chromatin of intact target human cells by activated human polymorphonuclear leukocytes. *Cancer Res* 1993;53:1269–72.
 12. Weitzman SA, Gordon LI. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. *Blood* 1990;76:655–63.
 13. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A* 1993;90:7915–22.
 14. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420: 860–67.
 15. Olinski R, Gackowski D, Rozalski R, Foksinski M, Bialkowski K. Oxidative DNA damage in a cancer patients: a cause or a consequence of the disease development? *Mutat Res* 2003;531:177–90.
 16. Collins AR, Gedik CM, Olmedilla B, Southon S, Bellizzi M. Oxidative DNA damage measured in human leukocytes: large differences between sexes and between countries, and correlations with heart disease mortality rates. *FASEB J* 1998;12:1397–400.
 17. Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Pediatric Gastroenterology and Nutrition. *Arch Dis Child* 1990;65:909–11.
 18. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999;11:1185–94.
 19. McNeish AS, Harms HK, Rey I, Shmerling DH, Visakorpi JK, Walker-Smith JA. The diagnosis of coeliac disease. *Arch Dis Child* 1979;54:738–46.
 20. Siomek A, Gackowski D, Rozalski R, et al. Higher leukocyte 8-oxo-7,8-dihydro-2'-deoxyguanosine and lower plasma ascorbate in aging humans? *Antioxid Redox Signal* 2007;9:143–50.
 21. European Standards Committee on Oxidative DNA Damage (ESCODD). Measurement of DNA oxidation in human cells by chromatographic and enzymic methods. *Free Radic Biol Med* 2003;34: 1089–99.
 22. Gedik CM, Collins A. European Standards Committee on Oxidative DNA Damage (ESCODD). Establishing the background level of base oxidation in human lymphocyte DNA: results of an interlaboratory validation study. *FASEB J* 2005;19:82–4.
 23. Pagano G, Degan P, d'Ischia M, et al. Gender- and age-related distinctions for the *in vivo* prooxidant state in Fanconi anaemia patients. *Carcinogenesis* 2004;25:1899–909.
 24. Izzotti A, De Flora S, Cartiglia C, et al. Interplay between *Helicobacter pylori* and host gene polymorphisms in inducing oxidative DNA damage in gastric mucosa. *Carcinogenesis* 2007;28:892–8.
 25. Buthumrung N, Mahidol C, Navasumrit P, et al. Oxidative DNA damage and influence of genetic polymorphisms among urban and rural schoolchildren exposed to benzene. *Chem Biol Interact* 2008; 172:185–94.
 26. ESCODD. Inter-laboratory validation of procedures for measuring 8-oxo-7,8-dihydroguanine/8-oxo-7,8-dihydro-2'-deoxyguanosine in DNA. *Free Radic Res* 2002;36:239–45.
 27. Dizdaroglu M. Chemical determination of oxidative DNA damage by gas chromatography-mass spectrometry. *Methods Enzymol* 1994; 234:3–16.
 28. Rivabene R, Mancini E, De Vincenzi M. *In vitro* cytotoxic effect of wheat gliadin-derived peptides on the Caco-2 intestinal cell line is associated with intracellular oxidative imbalance: implications for coeliac disease. *Biochim Biophys Acta* 1999;1453:152–60.
 29. Stojiljkovic V, Todorovic A, Pejic S, et al. Antioxidant status and lipid peroxidation in small intestinal mucosa in children with coeliac disease. *Clin Biochem* 2009;42:1430–37.
 30. Olinski R, Rozalski R, Gackowski D, Foksinski M, Siomek A, Cooke MS. Urinary measurement of 8-OxodG, 8-OxoGua, and 5HMUra: a noninvasive assessment of oxidative damage to DNA. *Antioxid Redox Signal* 2006;8:1011–19.
 31. Logan RFA. Malignancy in unrecognized coeliac disease: a nail in the coffin for mass screening? *Gut* 2009;58:618–19.
 32. Olinski R, Gackowski D, Foksinski M, Rozalski R, Roszkowski K, Jaruga P. Oxidative DNA damage: assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. *Free Radic Biol Med* 2002;33:192–200.
 33. Djuric Z, Heilbrun NK, Lababidi S, Berzinkas E, Simon MS, Kosir MA. Levels of 5-hydroxymethyl-2'-deoxyuridine in DNA from blood of women scheduled for breast biopsy. *Cancer Epidemiol Biomarkers Prev* 2001;10:147–9.
 34. Boiteux S, Gellon L, Guibourt N. Repair of 8-oxoguanine in *Saccharomyces cerevisiae*: interplay of DNA repair and replication mechanisms. *Free Radic Biol Med* 2002;32:1244–53.
 35. Freeman HJ. Malignancy in adult coeliac disease. *World J Gastroenterol* 2009;15:1581–83.
 36. Gao Y, Kristinsson SY, Goldin LR, Björkholm M, Caporaso NE, Landgren O. Increased risk for non-Hodgkin lymphoma in individuals with coeliac disease and a potential familial associations. *Gastroenterology* 2009;136:91–8.
 37. De Jong MM, Nolte IM, Te Meerman GJ, et al. Genes other than BRCA1 and BRCA2 involved in breast cancer susceptibility. *J Med Genet* 2002;39:225–42.
 38. Di Sabatino A, Corazza GR. Coeliac disease. *Lancet* 2009;373: 1480–93.
 39. Foksinski M, Gackowski D, Rozalski R, et al. Effects of basal level of antioxidants on oxidative DNA damage in humans. *Eur J Nutr* 2007; 46:174–80.