Selenium Supplementation Reduced Oxidative DNA Damage in Adnexectomized BRCA1 Mutations Carriers

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Abstract

Some experimental evidence suggests that *BRCA1* plays a role in repair of oxidative DNA damage. Selenium has anticancer properties that are linked with protection against oxidative stress. To assess whether supplementation of *BRCA1* mutation carriers with selenium have a beneficial effect concerning oxidative stress/DNA damage in the present double-blinded placebo control study, we determined 8-oxodG level in cellular DNA and urinary excretion of 8-oxodG and 8-oxoGua in the mutation carriers. We found that 8-oxodG level in leukocytes DNA is significantly higher in BRCA1 mutation carriers. In the distinct subpopulation of *BRCA1* mutation carriers without symptoms of cancer who underwent adnexectomy and were supplemented with selenium, the level of 8-oxodG in DNA decreased significantly in comparison with the subgroup without supplementation. Simultaneously in the same group, an increase of urinary 8-oxoGua, the product of base excision repair (hOGG1 glycosylase), was observed. Therefore, it is likely that the selenium supplementation of the patients is responsible for the increase of BER enzymes activities, which in turn may result in reduction of oxidative DNA damage. Importantly, in a double-blinded placebo control prospective study, it was shown that in the same patient groups, reduction in cancer incidents was observed. Altogether, these results suggest that *BRCA1* deficiency contributes to 8-oxodG accumulation in cellular DNA, which in turn may be a factor responsible for cancer development in women with mutations, and that the risk to developed breast cancer in *BRCA1* mutation carriers may be reduced in selenium-supplemented patients who underwent adnexectomy. (Cancer Epidemiol Biomarkers Prev 2009;18(11):2923–8)

Introduction

Breast cancer is one of the leading causes of death among women, and carriers of the *BRCA1* gene mutations face a life-time risk of developing breast and ovarian cancers (1). Therefore, it is of great importance to find agent(s) that can inhibit carcinogenesis in the carriers without symptoms of the disease or slow down the progression of the disease in cancer patients.

It is widely accepted that the proteins encoded by *BRCA1* gene participate in the monitoring and repair of DNA damage (2). Moreover, some experimental evidence suggests that BRCA1 plays a role in repair of oxidative DNA damage (3).

It is clear that oxidative DNA damage may be responsible for mutation, and elevated levels of oxidative DNA lesions have been noted in many tumors, strongly implica-ting such damage in the etiology of cancer [for review, see Cooke et al. (4)]. Lesions such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) are established biomarkers of oxidative stress/DNA damage, and linked with their potential mutagenicity in mammalian cells, this has led to their proposed potential as intermediate markers of cancer [for a review see Cooke et al. (4)].

Selenium has several anticancer properties that are linked with protection against oxidative stress. Namely, selenium is required to maintain the activity of some antioxidant enzymes and was found to scavenge free radicals. Several epidemiologic studies support the hypothesis that enhanced selenium status reduces the risk of cancer [review in L. Letavayova (5)]. Moreover, it was shown that selenium increases DNA repair capacity in human cells damaged by hydrogen peroxide and UV light (6).

When investigating the antioxidative effect of certain agents, it is important to apply an appropriate biomarker of oxidative stress. The most popular way of exploring oxidative stress includes measures of oxidative DNA damage, which can be assessed by determination of 8-oxodG level in cellular DNA. In addition, the whole-body burden of oxidative stress may be assessed by the determination of urinary excretion of oxidatively modified bases/nucleosides.

Therefore, to assess whether supplementation of *BRCA1* mutations carriers with selenium have beneficial effect concerning oxidative stress/DNA damage in the present double-blinded placebo control study, we determined 8-oxodG level in cellular DNA and urinary

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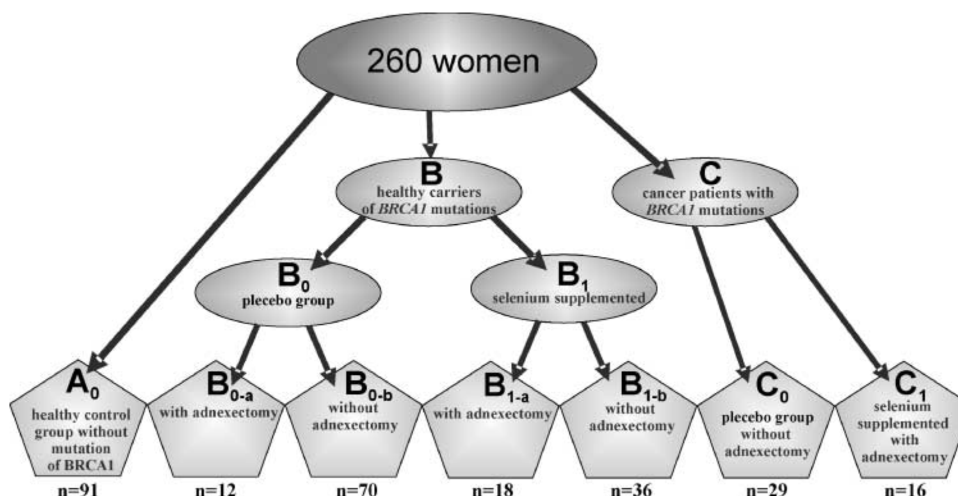

Figure 1. Study design (for a detailed description see also *Study Subjects* section of Materials and Methods).

excretion of 8-oxodG and 8-oxo-7,8-dihydroguanine (8-oxoGua). Because antioxidant vitamins and uric acid are the most effective free radical scavenger, in our study, the level of these compounds was analyzed in patients' blood.

Materials and Methods

Study Subjects. Female study subjects were recruited from among the attendees of a single familial cancer clinic of the Hereditary Cancer Center of the Pomeranian Academy of Medicine in Szczecin, Poland. Women were referred to this clinic because of a family history of breast or ovarian cancer (B and C groups). Control subjects (A-group) were recruited from among the family members of the carriers without symptoms of the disease, who had been determined not to carry the deleterious mutation. Every woman who participated in this study had previously been offered and had consented to genetic testing.

The study comprised 281 women divided into eight groups (see Fig. 1):

A_0 - the control group of healthy subjects without mutation of BRCA1 (n = 91)
B_0 - the placebo group; carriers of *BRCA1* mutations without symptoms of the disease (n = 82) who subsequently were divided in two subgroups:
  $B_{0\text{-}a}$ - with adnexectomy (n = 12)
  $B_{0\text{-}b}$ - without adnexectomy (n = 70)
B_1 - selenium-supplemented carriers of *BRCA1* mutations without symptoms of the disease (n = 54) who subsequently were divided in two subgroups:
  $B_{1\text{-}a}$ - with adnexectomy (n = 18)
  $B_{1\text{-}b}$ - without adnexectomy (n = 36)
C_0 - the placebo group of breast and ovarian cancer patients (n = 38) subsequently divided in two subgroups:
  $C_{0\text{-}a}$ - with adnexectomy (n = 9)
  $C_{0\text{-}b}$ - without adnexectomy (n = 29)
C_1 - selenium-supplemented group of breast and ovarian cancer patients (n = 16)

Three mutations were identified in the *BRCA1* mutation carriers (groups B and C); 5382insC (exon 20, codon 1756), C61G (exon 5, codon 61), and 4153delA (exon 11, codon 1345). The distribution of these mutations in each group (B and C) were 54%, 30%, and 12%, respectively. The details of each mutation can be found in Gorski B et al. (7)

The groups were chosen in such a way that the following criteria were matched: eating habits, age, body weight, and smoking status.

The study was approved by the medical ethics committee of the Pomeranian Academy of Medicine in Szczecin, Poland and all the patients gave informed consent.

Selenium Supplementation. To examine possible protective effect of selenium, a randomized, double blind, placebo-control clinical study of selenium supplementation was conducted. An oral selenium solution was in the form of sodium selenite (Na_2SeO_3 in 30%ethanol solution, from ADAMED). All subjects were randomized into either the sodium selenite–supplemented groups (300 μg of selenium per day) or the placebo groups.

The selenium concentration was determined in plasma of 181 cases, using graphite furnace atomic absorption spectrometry (8). The method was validated using reference material (lyophilized human reference serum samples of Seronorm from Nycomed Pharma AS) and through participation in interlaboratory comparison trials (9).

Urine Analysis. Spontaneously voided urine samples were collected. Standard, sterile, plastic cups were used for urine collection. Urine samples were frozen at −85°C and stored for no more than 1 month. The precise description of the method that was used for quantitative assessment was given previously (10). The levels of creatinine has been determined colorimetrically by the Jaffe reaction.

Isolation of Leukocytes from Venous Blood, DNA Isolation, and 8-oxodG Determination in DNA Isolates. Venous blood samples from the patients were collected in heparinized Vacuette tubes and centrifuged for 10 min, at 1,800 *g*, at 4°C to obtain plasma. The heparin-plasma samples were stored at −85°C for a maximum period of 3 mo. The blood with cells was carefully applied on top of Histopaque 1119 solution (Sigma), and leukocytes were isolated by centrifugation according to the procedure described by the manufacturer.

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Quantification of 8-oxodG in DNA isolates was described previously (10). Since it was recently shown that the extent of artifactual formation of 8-oxodGua is inversely dependent on the amount of extracted DNA, we used only those samples for which the quantity of DNA extracted was in the range 100 to 300 μg.

Determination of Plasma Vitamins A, E, C, and Uric Acid Concentration by High Performance Liquid Chromatography. Quantification of vitamin E (α-tocopherol), vitamin A (retinol), vitamin C (ascorbic acid), and uric acid by high performance liquid chromatography technique was described previously (10).

Statistical Analysis. For the statistical analysis, the STATISTICA (version 6.0) computer software (StatSoft, Inc.) was used. All results are expressed as median with interquartile range (IQR). For normal distribution, variables were analyzed by the Kolmogorov-Smirnov test with Lillefor's correction. For variables with nonparametric distribution, Mann-Whitney *U* test was carried out; for variables with normal distribution, Student's *t* test. Statistical significance was considered at $P < 0.05$.

Results

The median values of selenium in plasma were significantly higher in all the supplemented groups (group $B_{1\text{-a}}$, $B_{1\text{-b}}$, and C_1) in comparison with nonsupplemented counterparts (group $B_{0\text{-a}}$, $B_{0\text{-b}}$, and C_0). The respective values were 83.97, 71.97, and 93.32 μg/L versus 52.53, 63.02, and 53.64 μg/L (Fig. 2).

The levels of 8-oxodG in DNA isolated from leukocytes of *BRCA1* mutation carriers ($B_{0\text{-b}}$ group) and cancer patients (C_0 group) as well as in the supplemented carriers without adnexectomy (group $B_{1\text{-b}}$) were significantly higher than in DNA isolated from the control group (4.89, 6.35, and 5.34 versus 4.13 per 10^6 dG, respectively; Fig. 3.). The levels were reduced in both the supplemented groups with adnexectomy (groups $B_{1\text{-a}}$ and C_1) with respective values of 4.67 and 4.78 per 10^6 dG. However, only the decrease in group $B_{1\text{-a}}$ was statistically significant (Fig. 3).

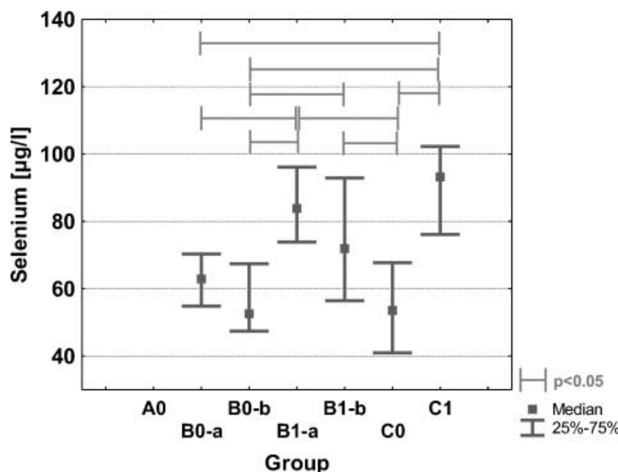

Figure 2. Median values of selenium in leukocytes DNA in different groups. Center square indicates the medians of the samples. The length of each bar (IQR) represents the range within which the central 50% of the values fell, with the edges placed at the first and third quartiles. For a detailed description of the groups, see *Study Subjects* section of Materials and Methods.

The median of 8-oxodG in urine samples of cancer patients (C_0) reached the value of 1.74 nmol/mmol of creatinine (Fig. 4A). This level was significantly higher than in the urine of the healthy carriers group (B_0) where the median reached the value of 1.25 nmol/mmol of creatinine ($P = 0.002$). In the control group, the median level was 1.44 nmol/mmol of creatinine. The level of 8-oxodG in urine in nonsupplemented, healthy carriers (B_0) was significantly reduced in comparison with the groups A_0 and $B_{1\text{-a}}$. No statistically significant changes were found among the other groups (Fig. 4A).

The concentration of the modified base in urine samples was similar in all groups of subjects with the exception of the adnexectomized, supplemented patients (group $B_{1\text{-a}}$) where the level was significantly higher in comparison with nonsupplemented carriers (group B_0): the respective values were 7.75 and 5.77 nmol/mmol of creatinine (Fig. 4B).

The endogenous concentrations of retinol were significantly reduced ($P < 0.005$) in the plasma of the group of healthy carriers (B_0) and the group of supplemented carriers without adnexectomy ($B_{1\text{-b}}$) when compared with the control group (A). The median values were 2.1 and 2.08 μmol/L versus 2.41 μmol/L, respectively. The levels were significantly higher in all the supplemented groups (groups $B_{1\text{-a}}$ and C_1) in comparison with nonsupplemented counterparts (groups B_0 and C_0.). The median values were 2.44 and 2.82 μmol/L versus 2.1 and 2.14 μmol/L, respectively. Moreover, in the groups of supplemented, adnexectomized patients ($B_{1\text{-a}}$ i C_1), the levels of retinol were significantly higher in comparison with the counterpart group ($B_{1\text{-b}}$) without adnexectomy. The respective values were 2.44, 2.82, and 2.14 μmol/L (Fig. 5A).

No statistically significant differences in the levels of plasma α-tocopherol and uric acid were observed. Ascorbic acid concentrations were similar in all the groups with the exception of the group of supplemented carriers without adnexectomy ($B_{1\text{-b}}$), where the level (38.1 μmol/L) was significantly reduced in comparison with the control group (52.9 μmol/L; Fig. 5C).

There were no differences in patients with breast and ovarian cancer concerning the analyzed parameters, i.e., antioxidants and those reflecting oxidatively damaged DNA (data not shown). No significant differences between adnexectomized ($B_{0\text{-a}}$) and nonadnexectomized ($B_{0\text{-b}}$) subgroups within nonsupplemented, healthy carriers (B_0), concerning the analyzed parameters were observed. Likewise, no significant differences between adnexectomized ($C_{0\text{-a}}$) and nonadnexectomized ($C_{0\text{-b}}$) subgroups within nonsupplemented, cancer patients (C_0), concerning the analyzed parameters were observed (data not shown).

No significant differences in urinary creatinine concentrations among the study groups were found.

Discussion

In our previous work, we found that 8-oxodG level in leukocytes DNA is significantly higher in *BRCA1* mutation carrier groups without symptoms of the disease and cancer patients in comparison with the control group, which comprised close relatives of the carriers (11).

It has long been known that the anticancer properties of selenium may involve inhibition of oxidative stress

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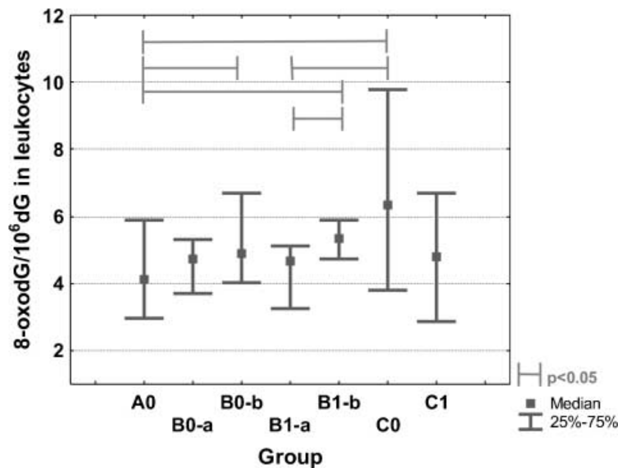

Figure 3. Median values of 8-oxodG in leukocytes DNA in different groups. Center square indicates the medians of the samples. The length of each bar (IQR) represents the range within which the central 50% of the values fell, with the edges placed at the first and third quartiles. For a detailed description of the groups see *Study Subjects* section of Materials and Methods.

(5, 12). Therefore, investigating the chemopreventing effect of this nutritional supplement in high-risk individuals and cancer patients by means of biomarkers that would signal changes in oxidative stress/oxidative DNA damage may be an important approach to understanding the mechanisms of selenium action and cancer prevention. The supplementation of the cancer patients resulted in ~26% reduction of background level of 8-oxodG in cellular DNA (however, because of the small size of the groups, this reduction was not significant) and a substantial increase in retinol concentration in the blood, in comparison with nonsupplemented cancer patients. It is noteworthy that the great majority of these patients (12 of 16) underwent adnexectomy.

Similarly, in the distinct subpopulation of 18 *BRCA1* mutation carriers without symptoms of cancer who underwent adnexectomy and were supplemented with selenium, the level of 8-oxodG in DNA decreased significantly in comparison with the subgroup without supplementation (Fig. 3). However, no reduction of the background level was observed in the subgroups of nonsupplemented carriers and cancer patients with adnexectomy nor in nonadnexectomized supplemented carriers in comparison with the appropriate counterpart group (Fig. 3). Furthermore, the levels of retinol increased substantially only in the supplemented individuals with adnexectomy. Of all antioxidants determined in this study, vitamin A level was the only one that responded to the supplementation and the level is inversely related to 8-oxodG background level (compare Fig. 3. with Fig. 5). Interestingly, our recently published study revealed that vitamin A has the strongest effect of all antioxidant components on oxidative DNA damage biomarkers (13). Similarly, Collins et al. (14) reported a significant negative correlation between basal concentration of total serum carotenoids and oxidative DNA damage measured as endonuclease III sensitive sites, in human lymphocytes. They did not find a similar association with concentrations of vitamin C. It is possible that vitamin A is simply a particularly good indicator of antioxidant status/redox tone of cell.

Altogether, these results suggest that in selenium-supplemented patients, oxidative stress/DNA damage may be substantially reduced in individuals with adnexectomy. Therefore, the obvious question is why selenium supplementation reduces oxidative DNA damage in *BRCA1* mutations carriers with adnexectomy. Adnexectomy is linked with estrogen deficiency. Strong biochemical evidence suggests that estrogen metabolites play some role in breast cancer initiation and progression and that estrogens metabolism includes an oxidative stress–mediated pathway (15, 16). Moreover, metabolic redox cycling between 4-hydroxyestradiol and its quinine was involved in the generation of oxidatively modified DNA bases (16, 17). Therefore, adnexectomy may be involved in the reduction of oxidative stress/oxidative DNA damage.

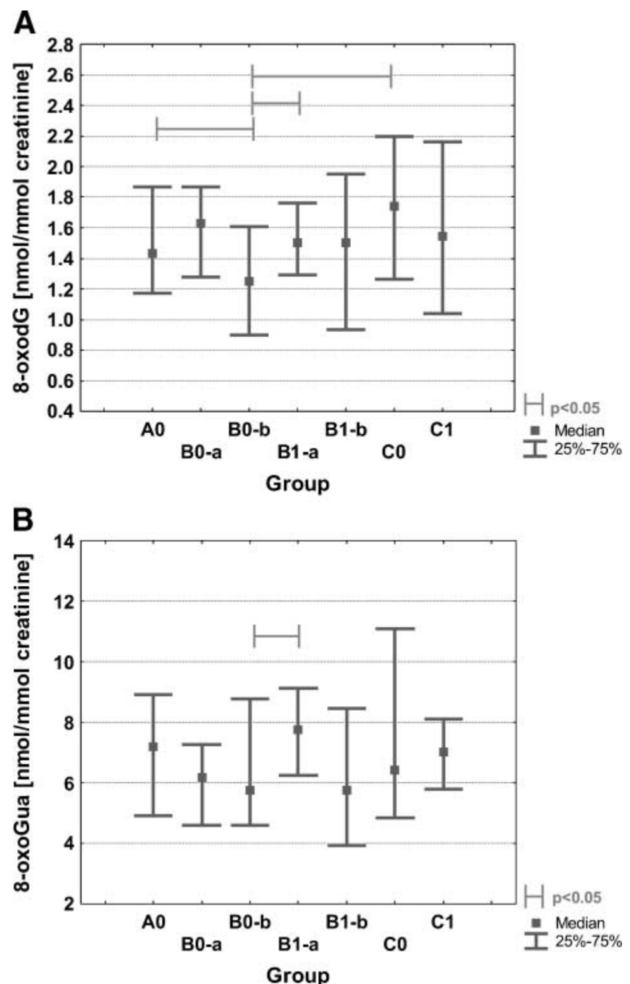

Figure 4. Median values of urinary 8-oxodG and 8-oxoGua in different groups. **A,** levels of urinary 8-oxodG; **B,** levels of urinary 8-oxoGua. Center square indicates the medians of the samples. The length of each bar (IQR) represents the range within which the central 50% of the values fell, with the edges placed at the first and third quartiles. For a detailed description of the groups see *Study Subjects* section of Materials and Methods.

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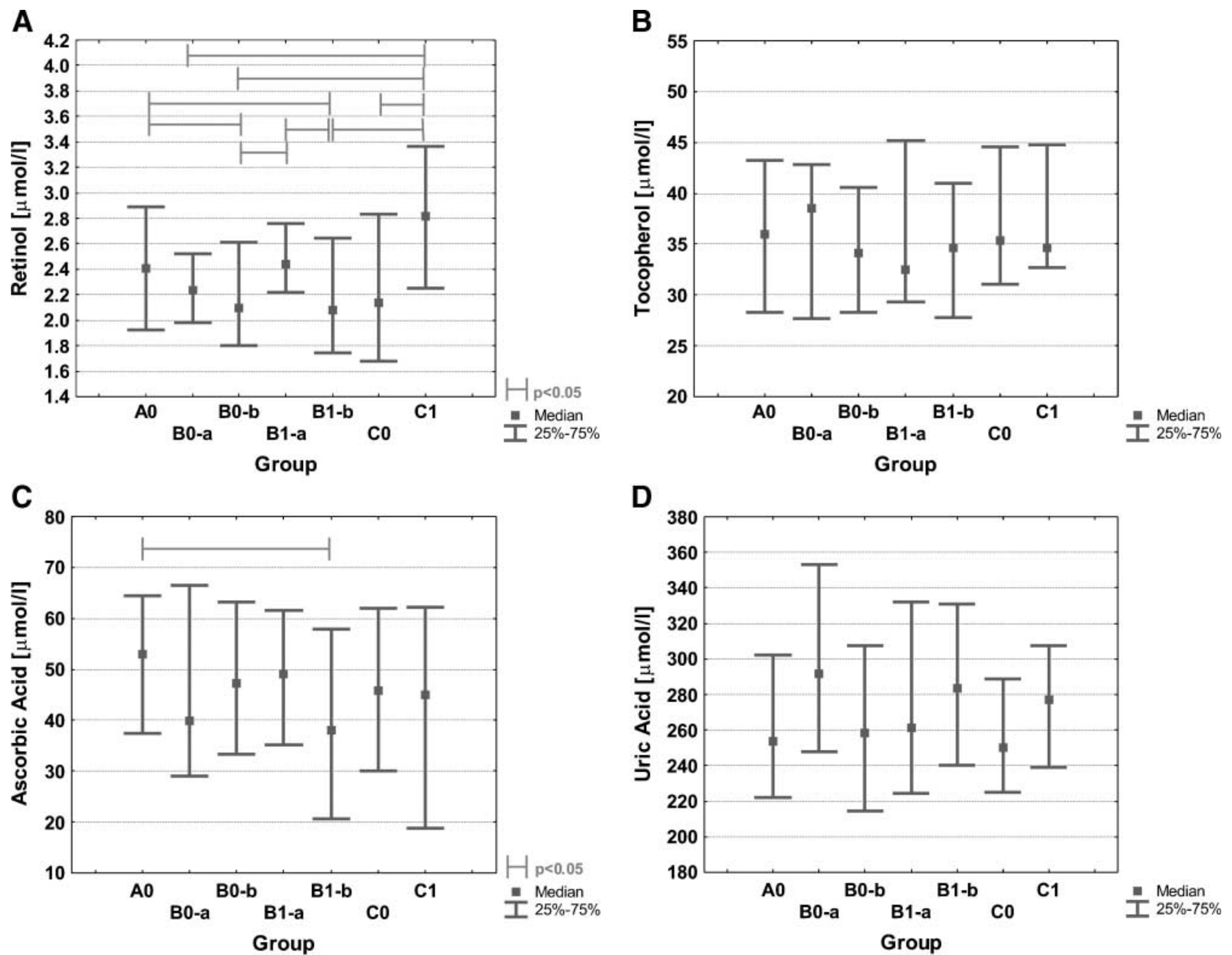

Figure 5. Median values of antioxidants in plasma in different groups. **A,** levels of retinol; **B,** levels of tocopherol; **C,** levels of ascorbic acid; **D,** levels of uric acid. Center square indicates the medians of the samples. The length of each bar (IQR) represents the range within which the central 50% of the values fell, with the edges placed at the first and third quartiles. For a detailed description of the groups see *Study Subjects* section of Materials and Methods.

Supplementation with selenium was shown to protect against several cancers, particularly prostate cancer and this property may be linked with oxidatived stress reduction (5, 12). Furthermore, it has been shown that selenium supplementation, which was provided in a way similar to this study, resulted in a significant reduction in the formation of chromosome breaks in *BRCA1* mutations carriers (18). This beneficial cancer chemopreventic effect of the supplementation is probably not directly linked with increased activity of selenium-dependent antioxidant enzymes, since numerous animal studies showed no change in the specific activities of the aforementioned enzymes with selenium supplementation dose comprising nutritional to supranutritional levels (12). Rather, the anticancer/antioxidative properties of selenium supplementation may be related to the alteration of cellular redox homeostasis in cells (12).

Since neither selenium itself nor adnexectomy without the supplementation are efficient in reduction of oxidative DNA damage (compare counterparts within B_0 and B_1 groups in Fig. 3), our results suggest that in the case of *BRCA1* mutation carriers, only the additive action of both adnexectomy and the supplementation may result in the modulation of redox tone toward its antioxidant/anticancer properties.

In this context, it is noteworthy that the excision activity of OGG1, the main enzyme responsible for 8-oxodG removal from DNA, is sensitive to alteration in the cellular redox equilibrium (19). Furthermore, OGG1 activity depends among other things on APE1/Ref-1. The aforementioned protein was shown to stimulate 8-oxoGua excision by OGG1, increasing enzyme turnover on damaged DNA (20). Importantly, APE1/Ref-1 apart from DNA repair participates in redox signaling. Moreover, selenium may operate through a redox cascade that includes Ape/Ref1 (for review, see ref. 21).

A number of literature reports and our data indicate that the base excision repair, namely hOGG1 glycosylase, which removes 8-oxoGua from cellular DNA, is responsible for its presence in urine (for a discussion, see ref. 22). It is possible

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that the observed increase of urinary 8-oxoGua in the supplemented adnexectomized carriers (Fig. 4) and simultaneous reduction of the background level to the control value (see also above) may reflect an increase in the activity of hOGG1 (restoration to the value characteristic for the controls). Therefore, it is likely that the selenium supplementation of the carriers is responsible for the increase of BER enzymes activities, which in turn may result in reduction of oxidative DNA damage. Supporting this notion are the results of a work that showed that selenium increases DNA repair capacity in human cells damaged with Reactive Oxygen Species-generating factors: hydrogen peroxide and UV light (6).

Collectively, the results of this and our recent work (11) show that *BRCA1* mutations carriers have elevated levels of promutagenic 8-oxodG in cellular DNA. This increase may be normalized with selenium supplementation in patients after adnexectomy. Importantly, in a double-blinded placebo control prospective study, it has been shown that in the same patients group, reduction in cancer incidents was observed.[5]

Altogether, these results suggest that *BRCA1* deficiency contributes to 8-oxodG elevation in cellular DNA, which in turn may be a factor responsible for cancer development in women with mutations, and that the oxidative DNA damage and the risk to developed breast cancer in *BRCA1* mutation carriers may be reduced in selenium-supplemented patients who underwent adnexectomy.

[5] J. Lubinski, personal communication.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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