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Breast cancer - the role of free radicals and antioxidants in pathogenesis of benign dysplasia and breast carcinoma

Rola wolnych rodników i antyoksydantów
w patogenezie łagodnej dysplazji oraz raka sutka

Breast cancer is still the leading cause of the cancer mortality in women. Among the different agents which are discussed presently to play the role in the etiology and pathogenesis of the cancer disease, the role of reactive oxygen species and particularly the alteration of the prooxidation-antioxidation status appears to be very important. On the other hand early diagnosis of the disease, the distinction of cancer to non-cancer tumors and also the biochemical signs of healing are the serious clinical problem, so the new diagnostic markers are still being developed. Therefore the aim of our study was to investigate the intensity of the lipid peroxidation process in plasma and the antioxidant potential in patients with breast cancer, because we hope that the examined variables can become useful diagnostic markers.

MATERIAL AND METHODS

The study included two groups of women. The first (group I) contained 15 women with a diagnosis of benign dysplasia. All patients from group I were treated by

tumorectomy. The second group (group II) contained 48 women with breast cancer which were treated by modified radical mastectomy consisting in removal of only the breast and the axillary lymph nodes. The patients did not have other complaints. Before the surgical treatment the following examinations were performed in the two groups of women: ultrasonography, mammography, stereotactic fine-needle biopsy of the breast tissue and histopathological study.

We collected blood samples from the women, immediately after surgical treatment and 8 weeks after operation. After the treatment group I and group II signified I T and II T groups in our investigations.

The following variables were examined in the above mentioned groups: the level of malonyldialdehyde (MDA); activities of glutathione peroxidase (GPx) in whole blood; the level of total antioxidant status (TAS) in plasma. Plasma for MDA and TAS study was obtained by centrifuging blood for 10 min at 2000 rpm in the room temperature.

Concentration of malondialdehyde (MDA) was measured in plasma according to the Ledwożyw method (8) as follows: 0.5 ml of plasma was mixed with 2.5 ml of 1.22 mol/l trichloroacetic acid in 0.6 mol/l HCl and allowed to stand for 15 minutes. Then 1.5 ml of thiobarbituric acid (TBA) solution was added (TBA solution was obtained by dissolving 500mg of TBA in 6 ml of 1 mol/l NaOH and then adding 69 ml H_2O), and thereafter heating for 30 min in a boiling water bath. Then the mixture was cooled to the room temperature and 4 ml of n-butanol was added to it, and the mixture was virgously shaken for 3 minutes and centrifuged for 10 min at 1500 g. After that the organic layer was removed and its absorbance was measured at 532 nm against n-butanol. The concentration of MDA in the samples was determined from the standard curve plotted by using malondialdehyde bis-methyloacethal. Finally, the concentration of MDA in plasma was described in nM/ mg of protein.

The activity of glutathione peroxidase (GPx) in whole blood was determined by Ransel kits (RS 505, Randox Lab. Ltd., Ardmore Diamond Road, Crumlin, Co. Antrim UK). In this assay GPx catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide (ROOH) and oxidized glutathione (GSSG) is immediately reduced in the presence of glutathione reductase (GR) with an associated oxidation of NADPH to NADP+. The absorbance of the sample at 340nm decreases according to the conversion of NADPH to NADP+. The concentration of GPx was described in Units/g of Haemoglobin.

The level of total antioxidant status (TAS) in plasma was determined with TAS kits (Nx 2332, Randox Lab. Ltd., Ardmore Diamond Road, Crumlin, Co. Antrim UK). In this assay 2,2'-Azino-di-[3-ethylbenzthiazoline sulphate] (ABTS) is incubated with a peroxidase (metmioglobin) and hydrogen superoxide to produce the blue-green colour radical cation-ABTS*+. Antioxidants in the test plasma sample cause suppression of this colour production to the proportional degree to their concentration and the change of absorbance is measured at 600 nm. TAS was described in mmol/l of plasma (Trolox Units). Trolox is a water-soluble vitamin E analogue.

The absorbance of samples was measured using the Hitachi spectrophotometer. The results were expressed as arithmetical means ± standard deviation. Student-t test for unpaired data was employed for the statistical analysis and $p < 0.05$ was considered statistically significant.

RESULTS

In our investigations we noticed that before the surgical treatment the level of malonyldialdehyde (MDA) in plasma (Fig. 1) from the women with benign dysplasia was lower than in plasma from breast cancer subjects ($p<0.05$). After the operation we did not notice any significant differences between two groups.

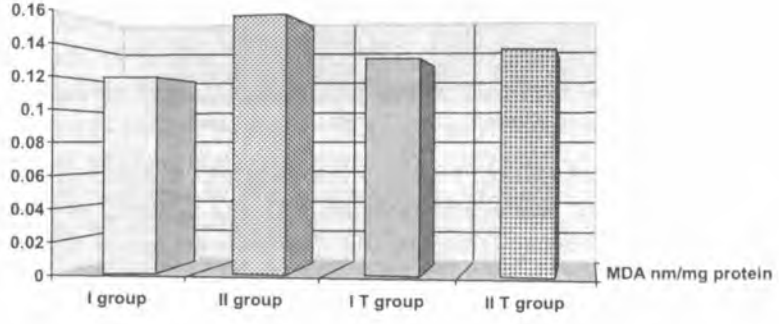

Fig. 1. The plasma level of MDA (nmol/mg of protein) in plasma of women with benign dysplasia before treatment (group I) and after operation (group I T), and in plasma of women with breast cancer before (group II) and after operation (group II T)

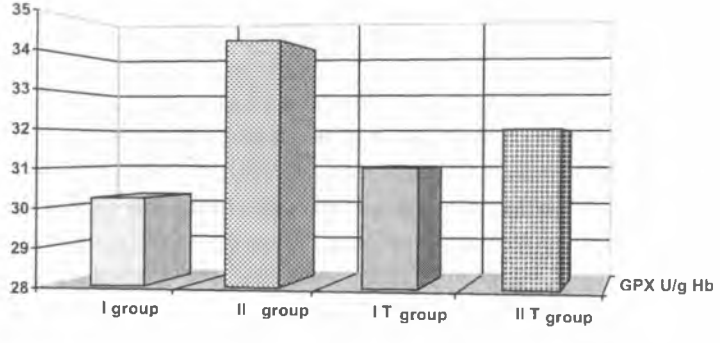

Fig. 2. The activity of GPx (U/gHb) in whole blood of women with benign dysplasia before treatment (group I) and after operation (group I T), and in plasma of women with breast cancer before (group II) and after operation (group IIT)

The activity of glutathione peroxidase (GPx) in whole blood (Fig. 2) was higher in the women with breast cancer than in those with benign dysplasia ($p<0.05$) before the treatment. After operations, GPx activity was still higher in the women with breast cancer.

The levels of total antioxidant status (TAS) (Fig. 3) in plasma only before surgical treatment was significantly lower in the breast cancer subjects in comparison to the women with benign dysplasia ($p<0.05$).

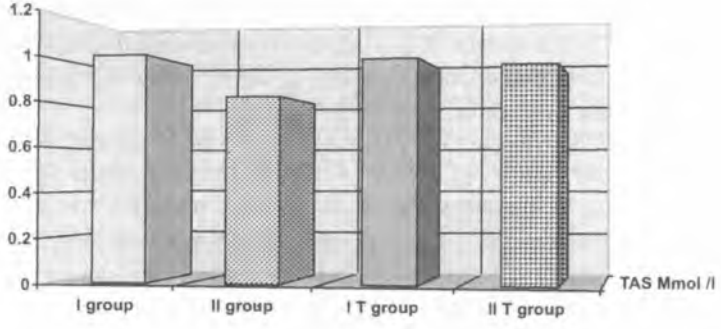

Fig. 3. The level of TAS (mmol/l in plasma of women with benign dysplasia before treatment (group I) and after operation (group I T), and in plasma of women with breast cancer before (group II) and after operation (group II T)

DISCUSSION

In our investigations we compared women with breast cancer with not healthy patients but with women with benign dysplasia because we looked for a possibly useful marker differing cancer from benign dysplasia before the surgical treatment. We noticed that imbalance between prooxidant and antioxidant status can play the role in the pathology of breast cancer. The intensity of lipid peroxidation process in women with breast cancer assessed by the level of malonyldialdehyde in plasma, was elevated in comparison to the women with dysplasia. Simultaneously the total antioxidant capacity containing mainly nonenzymatic free radical scavengers, has decreased. Contrary to this the activity of selenium dependent glutathione peroxidase in whole blood was higher in the women with breast cancer before and also after surgical treatment.

Many authors in their investigations comparing the oxidant status of healthy women and of breast cancer patients showed that the process of lipid peroxidation and the alteration of antioxidant mechanisms can play the role in the process of carcinogenesis. Huang et al. (6) demonstrated that the level of MDA in serum was significantly higher in women with cancer in comparison to the healthy control group. Also Look

and Mush (11) showed that the concentration of thiobarbituric-acid-reactive substances (TBARS) in plasma was generally significantly higher in patients with breast tumor than in healthy subjects and after polychemiotherapy TBARS level still increased. Kumar et al. (7) showed a significant increase in circulation peroxides in breast cancer women. They also observed the increase in erythrocyte membrane lipid peroxidation and the decrease in antioxidant vitamin levels in plasma.

Very interesting in our opinion are the results showed by Wang et al. (13). They noticed that normal breast tissue from women with breast cancer exhibited significantly higher levels of the putative MDA adducts than tissues from noncancer women, which received reduction mammoplasty. Simultaneously tumor tissue displayed lower levels of the putative MDA adducts than their corresponding normal adjacent tissue. Also Punnomen (11) showed that the content of TBARS was lower in the breast cancerous tissue than in the corresponding reference tissue, but serum TBARS levels were elevated in patients with breast cancer in comparison to the healthy subjects. In their observations the peroxyl-radical-trapping capacity did not differ in cancer and healthy women.

Contrary to the results described above, Gerber et al. (2,3,4) showed that the concentration of MDA is lower and vitamin E level is higher in patients with breast cancer in comparison to healthy women, and MDA content in plasma decreased proportionally with the severity of pathology and tumor size.

In our investigations we showed the high levels of GPx activities in whole blood obtained from the women with breast cancer. Doroshow (1) in his experiment noticed the overexpression of cDNA for cytoplasmatic peroxidase isoenzyme in human MCF breast cancer cells and this fact caused the significant increase in the tolerance of these cells to oxidative stress. Doroshow concludes that glutathione peroxidase activity can play a critical role in resistance of tumor cells against oxidative stress. Also Seven et al. (12) showed that the activity of GPx and also the concentration of vitamin C, Zn and Cu significantly increased, but MDA level decreased in the plasma of breast cancer patients in comparison to the women with benign dysplasia. Contrary, Pawlowicz et al. (10) showed that red cell and plasma GPx activities were lower in patients with cancer than in healthy women, but Hardel et al. (5) suggested that GPx activity cannot be a marker for the risk of breast cancer.

Different results described above can result from the fact that carcinogenesis is a multi-step process and many mechanisms may be involved in each step. Oxidative stress damages DNA, proteins and cell membranes and results in mutagenesis, loss of cells functions and finally in the development of cancer. On the other hand tumoricidal activity of free oxygen radicals is very useful during the treatment and the high activity of enzymes-free radical scavengers causes the resistance of tumor cell during the chemotherapy or radiotheraphy treatment. In the accessible bibliography we did not find many investigations comparing the oxidant status of

the women with benign dysplasia and breast cancer but our results show that high MDA and low TAS level in plasma, and high GPx activity in whole blood can be helpful in the diagnosis of breast cancer.

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STRESZCZENIE

Celem pracy była ocena zaawansowania procesu peroksydacji lipidów oraz wartości potencjału antyoksydacyjnego ustroju u kobiet z chorobami sutka. Pierwszą badaną grupę (15 kobiet) stanowiły pacjentki z łagodną dysplazją sutka, drugą grupę (48 kobiet) pacjentki z rakiem sutka. Pacjentki obu grup leczone były chirurgicznie: grupa I poprzez tumorektomię, grupa II metodą zmodyfikowanej radykalnej mastektomii, polegającą na usunięciu sutka oraz pachowych węzłów chłonnych. W obu grupach przed leczeniem chirurgicznym wykonywano rutynowe badania diagnostyczne: ultrasonografię, mammografię oraz stereoskopową biopsję cienkoigłową i badanie histopatologiczne. Badania doświadczalne obejmowały ocenę wartości całkowitego potencjału antyoksydacyjnego osocza (TAS), aktywności peroksydazy glutationu we krwi (GPx) oraz zawartości aldehydu malonowego w osoczu bezpośrednio przed zabiegiem chirurgicznym oraz po ośmiu tygodniach od zabiegu.

Przeprowadzone badania wykazały, iż całkowity potencjał antyoksydacyjny był niższy u kobiet z rakiem sutka w porównaniu z grupą z dysplazją, zaś po leczeniu nie stwierdzono istotnych różnic między obiema grupami. Aktywność peroksydazy glutationu we krwi była wyższa u pacjentek z rakiem sutka zarówno przed leczeniem chirurgicznym, jak i w osiem tygodni po leczeniu. Przed leczeniem zawartość aldehydu malonowego (MDA) w osoczu kobiet z rakiem przewyższała istotnie wartości uzyskane w grupie kobiet z łagodną dysplazją, po operacji zaś nie odnotowano różnic pomiędzy grupami.

Uzyskane przez nas wyniki wskazują na to, iż markery peroksydacji lipidów oraz wydolności antyoksydacyjnej ustroju mogą być przydatnymi pomocniczymi wskaźnikami w diagnozowaniu raka sutka.