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PRELIMINARY STUDY OF ERYTHROCYTE GLUTATHIONE-S-TRANSFERASE ACTIVITY IN PATIENTS WITH SKIN MELANOMA

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ABSTRACT

Development and progression of skin melanoma is associated with impaired red/ox balance in tissues due to enhancement in melanin synthesis, tyrosinase upregulation and ROS production. Intracellular red/ox balance maintaining, xenobiotics and oxidants detoxification are due to glutathione (GSH) redox system which is essential for protecting the skin against photooxidative stress. In this respect we aimed to evaluate GST activity in erythrocyte lysates and total thiols in plasma from patients with skin malignant melanoma in comparison to healthy controls.

Our preliminary study involved 36 healthy individuals aged between 30 and 80 years and 43 patients with malignant melanoma (aged from 20 to 81 years). The patients are enrolled at the Oncology Canter of Stara Zagora, Bulgaria.

The concentrations of total thiols in plasma and the GST activity in erythrocyte lysates were measured spectrophotometrically in accordance to published in the literature methods modified by us.

Assessed concentration of total thiols (-SH) in plasma of the control group was significantly higher (0.447±0.094 mmol/l (GSH)) than that obtained in the group of patients with skin melanoma (0.321±0.123 mmol/l (GSH), $p<0.0001$), whereas the obtained glutathione-S-transferase activity of erythrocyte lysates from the control group (2.002±0.529 U/gHb) was slightly higher (1.815±0.883 U/gHb, $p=0.269$) than that assessed in the group of patients with skin melanoma. We did not observe a correlation between erythrocyte GST activity and plasma concentration of total thiols in the group of controls ($R=0.062$, $p= 0.772$) as well as in the group of patients, although there was a very weak tendency for enhancing of thiols when GST activity increases ($R=0.188$, $p=0.232$). It is interesting to note that a difference, even though not significant, of the GST activity was seen between genders. We found also a slight decrease of GST activity with age.

In conclusion, our preliminary results suggest that GST activity in erythrocytes is not severely influenced by the development and progression of skin malignant melanoma, as opposed to the concentration of total thiols in plasma.

In this respect, confirmation of these findings requires to perform a large prospective study.

Key words: melanoma, GST activity, total thiols, age, sex, glutathione

INTRODUCTION

During the past 20 years the number of new melanoma cases has doubled and it is supposed to be the most lethal cancer of skin [14, 15]. Enhancement in melanin levels, tyrosinase upregulation and ROS production is observed in human melanoma cells [29, 30].

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Intracellular red/ox balance maintaining, xenobiotics and oxidants detoxification are due to glutathione (GSH) redox system which is essential for protecting the skin against photooxidative stress [22, 26]. This is confirmed by the fact that human melanoma cells express high levels of both glutathione-S-transferases (GSTs) and multidrug resistance-associated proteins (MRPs) [24, 37] showing an inverse relationship between melanogenesis and GSH in melanoma cells [30, 38, 43], but the complicated correlation between

melanogenesis and cellular red/ox state is not well clarified, yet.

Glutathione-S-transferases (GSTs) are enzymes from the transferase family classified as EC 2.5.1.18 [9, 46]. They are ubiquitously distributed in microbes, animals and plants, as they are really important for maintaining the red/ox equilibrium in organisms. GSTs can be found extracelluraly in body fluids or in each cell where they are present in different subcellular compartments including cytosol, mitochondria, endoplasmic reticulum, nucleus and plasma membrane [32]. Each member of the family has multiple isoenzymes with overlapping substrate specificity grouped in several classes (alpha, kappa, mu, omega, pi, theta, delta, sigma, zeta), according to their distribution in cells and tissues, forming a multigene family [1, 9, 35, 40, 46].

GSTs catalyze conjugation of a variety of endogenic and exogenic electrophilic compounds with glutathione (GSH) which is critical for cellular functions, cell growth and cell death, regulating signal pathways mostly by maintaining redox status, ROS detoxification, reduced form of sulfhydryl groups of cellular proteins, and oxidative stress which on turn activates various signal transduction and transcriptional pathways [2, 7, 16, 19, 25]. GSH is a cellular antioxidant which contributes to the reduction of physiological hydroperoxides, including the products of lipid peroxidation, through glutathione peroxidase (GSHPX) and GSTs [11, 23].

The regulation and function of GSTs have implications in cell growth, oxidative stress as well as disease progression and prevention. GSTs are part of the II phase cellular detoxification, soluble or membrane-bound multifunctional enzymes catalyzing conjugation of GSH with various hydrophobic electrophiles, physiological metabolites and xenobiotics including drugs, carcinogens, herbicides, and insecticides – compounds which are causing oxidative stress and cell toxicity [5, 6, 13, 20, 21, 31, 39, 40, 41, 44]. GSTs have role in cellular protection of the erythrocytes against oxidative damage [28, 35]. GST isoenzymes have also been implicated in the progression of cancer [1, 9, 45, 46]. Some of GSTs play an important role as inhibitors of signals mediated by mitogen-activated protein (MAP) kinases that are involved in regulation of apoptosis and cell survival; other GSTs also have a role in glutathionylation of cellular proteins in cancer, which is considered as an important protective mechanism against oxidative stress [32, 40, 42, 45].

The aim of the current pilot study was to evaluate GST activity in erythrocyte lysates and total thiols in plasma from patients with skin malignant melanoma in comparison to healthy controls.

METHODS

Patients and controls

The current study was conducted in the Laboratory of Biochemistry of tumor growth in Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, Stara Zagora. Peripheral blood was collected then plasma was separated from blood cells and both fractions were kept till the time of assessment at -20^{o}C.

The control group consisted of 36 healthy individuals: 17 males with a mean age of 61.06 ±10.25 years, ranging between 44 and 80 years and median of 60 years, and 19 females with a mean age of 51.11 ±7.77 years, ranging between 30 and 62 years and median of 52 years.

Our preliminary study involved 43 patients with malignant melanoma: 19 males with mean age of 62.0 ±13.47 years, ranging between 39 and 78 years and median of 60 years, and 24 females with a mean age of 60.92 ±17.90 years, ranging between 20 and 81 years and median of 65.5 years. The patients are enrolled at the Oncology Center of Stara Zagora, Bulgaria.

Total thiols

Determination of total thiols in plasma was conducted according adapted by us for microanalysis *Ellman's* method. The thiol group of reduced thiols (i.e. glutathione) binds to *Ellman's* reagent (DTNB: [5,5'-dithiobis-(2-nitrobenzoic acid)]) by formation of 2-nitromercaptobenzoic acid anion which has an intensive yellow color and absorbs light at 412 nm. The mixture has to be incubated for 10' at 37^{o}C. Total thiols concentration is determined over a standard curve drawn by measuring aqueous solutions of glutathion with known concentration, in the range of 0.25 – 2 mM GSH (*SERVA Electrophoresis GmbH*, Germany) simultaneously prepared with each measurement. The product was extracted from

solution with methanol and measured spectrophotometrically. Results are represented as mmol/l (GSH).

GST activity

GST activity in erythrocyte (Er) lysates was assessed in accordance to Habig and Jakoby's method with modifications of Obrosova et al. (2000) [27]. Before measurement, hypotonic blood cell lysis on ice was performed and the lysates were diluted four times. The increase in absorbance of the conjugates between GSH and CDNB (1-chlor-2,4-dinitrobenzol) was measured spectrophotometricaly at 340 nm. The mixture has to be incubated for 12' at 37°C Results were calculated by using the absorption coefficient (9.6 $mM^{-1}.cm^{-1}$) of the conjugated product and represented as U/gHb. The hemoglobin concentration was determined by semimicro method with a commercial kit (Human).

Statistical methods

Statistical analyses were carried out using StatView v.4.53. for Windows (Abacus Concepts, Inc.). The ANOVA test was applied for comparing the continuous variables in independent groups. Regression analysis was used for evaluation of the correlation coefficient between the obtained GST activity and assessed total thiols concentration. The collected data were applied for drawing a regression plot.

RESULTS

Assessed concentration of total thiols (-SH) in plasma of the control group was 0.447 ± 0.094 mmol/l (GSH), ranging between 0.235 and 0.585 mmol/l (GSH) (median of 0.446 mmol/l (GSH)). These values were significantly higher ($p<0.0001$) than that obtained in the group of patients with skin melanoma where values are as follows: mean of 0.321 ± 0.123 mmol/l (GSH), ranging between 0.016 and 0.551 mmol/l (GSH) (median of 0.301 mmol/l (GSH)) **(Figure 1A)**.

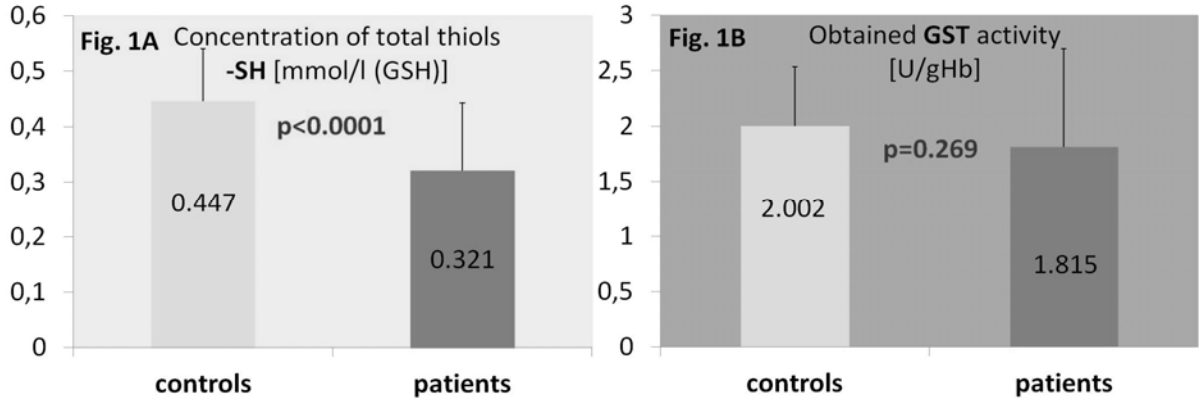

Figure 1. Erythrocyte activity of GST enzyme and plasma concentration of total thiols. Comparison of **GST** activity between controls and patients (**A**) and comparison of **total thiols** levels in plasma between controls and patients (**B**).

The obtained glutathione-S-transferase activity of erythrocyte lysates from the control group was 2.002 ± 0.529 U/gHb, ranging between 0.997 and 3.696 U/gHb (median of 2.019 U/gHb) which is slightly higher (p=0.269) than that assessed in the group of patients with skin melanoma (mean of 1.815 ± 0.883 U/gHb, ranging between 0.428 and 4.236 U/gHb and median of 1.478 U/gHb, p=0.269) **(Figure 1B)**.

We did not observe a correlation between erythrocyte GST activity and plasma concentration of total thiols in the group of controls (R=0.062, p= 0.772) **(Figure 2A)** as well as in the group of patients, although there was a very weak tendency for enhancing of thiols when GST activity increases (R=0.188, p=0.232) **(Figure 2B)**.

We did not observe also a correlation between GST activity and the age of the tested individual both in control group (R=0.047, p=0.786) **(Figure 3A)** and in the patients group (R=0.105, p=0.502) **(Figure 3B)**. Although that, it can be hypothesized that GST activity decreases with age.

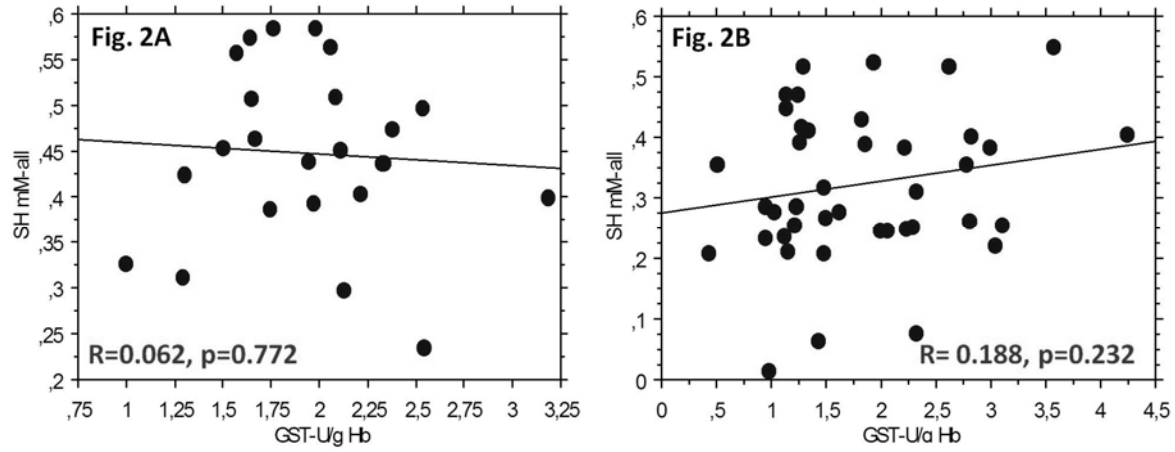

Figure 2. Regression plot of erythrocyte GST activity and plasma total thiols amount in the group of **controls** (**A**) and **patients** with skin malignant melanoma (**B**).

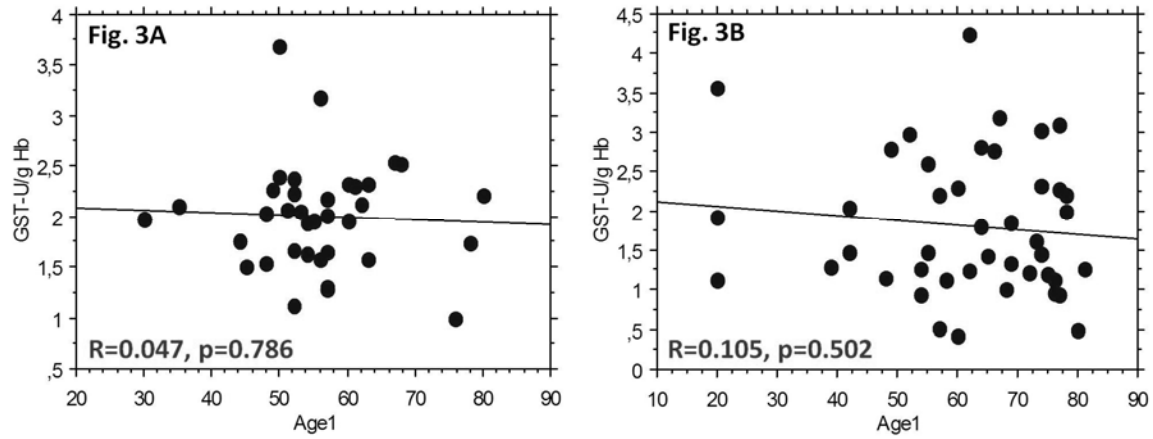

Figure 3. Regression plot of erythrocyte GST activity and the age of the individuals in the group of **controls** (**A**) and **patients** with skin malignant melanoma (**B**).

It is interesting to note that a difference, even though not significant, of the GST activity was seen between genders. Healthy male controls showed mean GST activity of 1.874 ± 0.460 U/gHb, ranging between 0.997 and 2.534 U/gHb (median of 1.759 U/gHb) whereas healthy female controls exhibited mean GST activity of 2.116 ± 0.571 U/gHb, ranging between 1.122 and 3.696 U/gHb (median of 2.054 U/gHb). In the patients group data display was as follows: for male patients mean GST activity was 1.572 ± 0.654 U/gHb, ranging between 0.506 and 3.097 U/gHb (median of 1.464 U/gHb), and for female patients mean GST activity was 2.008 ± 1.001 U/gHb, ranging between 0.428 and 4.236 U/gHb (median of 1.886 U/gHb). Considering this data it is seen that in both groups higher GST activity was obtained in female individuals (controls p=0.174 and patients p=0.109) **(Fig. 4)**.

DISCUSSION

The registered decrease of total thiols concentration in plasma of patients may contribute to confirm the presence of antioxidant system deterioration due to cancer development and therapy.

Our results suggest that GST activity in erythrocytes is not severely influenced by the development and progression of skin malignant melanoma. It was postulated that GST activity decreases with age, although in this preliminary study we did not find an inverse correlation between GST activity and the age, which was previously confirmed by van Lieshout and Peters (1998) [17]. Recently, it has been scientifically proved, that aging is associated with disturbances in glutathione metabolism [18, 34, 35]. Both decreased [8] and increased [10] as well as unaltered [12] levels of glutathione with age have been demonstrated in different studies.

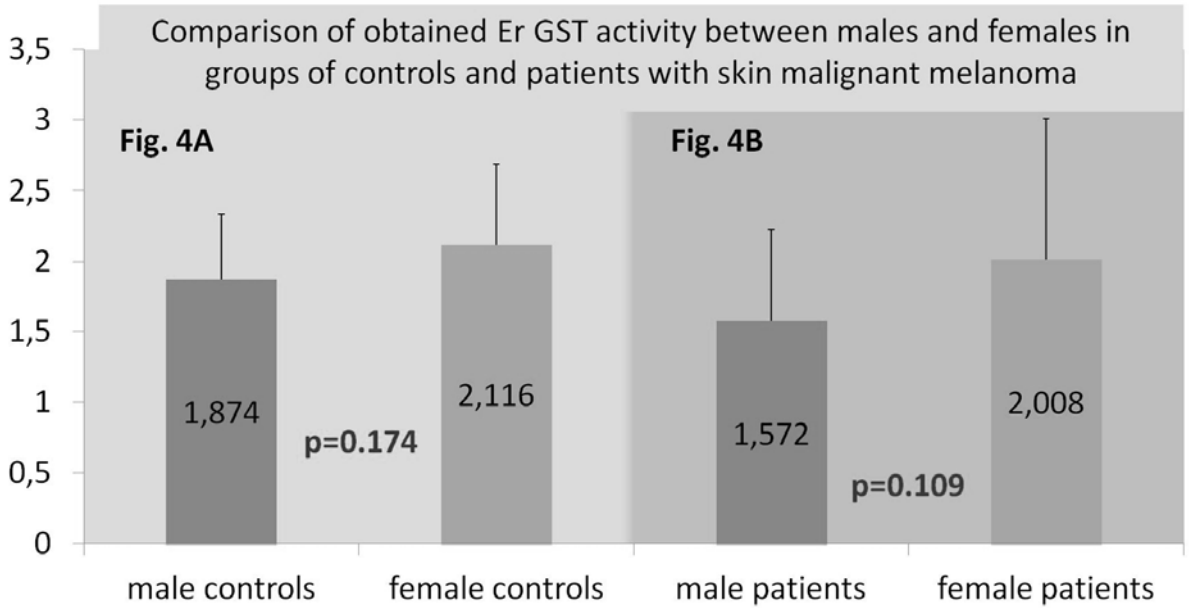

Figure 4. Comparison of GST activity between the genders in the group of **controls** (**A**) and **patients** with skin malignant melanoma (**B**).

The slightly higher GST activity in females may be explained by the different hormonal influence between sexes, as far as the metabolism of estrogens and androgens varies [4].

The melanogenesis and intercellular red/ox state are proved to be correlated and primarily maintained by thiol-based antioxidants, in particular GSH, GSH–γ-glutamate cysteine ligase (GSH-GCL) system and GSTs. Thus they play a crucial role both in regulation of pigmentation and in adaptation to melanogenesis-mediated oxidative stress [29]. In this respect, a large prospective study is necessary to be performed for confirmation of this notion.

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