

## Glutathione concentration, glutathione peroxidase and glutathione reductase activity in elderly patients with type II diabetes compared to hypertensives

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**Rybka Joanna, Kupczyk Daria, Kędziora-Kornatowska Kornelia, Kędziora Józef**

Katedra i Zakład Biochemii, Uniwersytet Mikołaja Kopernika w Toruniu, Collegium

Medicum im. L. Rydygiera w Bydgoszczy, tel: (48) 52 585 3755

e-mail: joanna.rybka1@wp.pl

### Summary

*Age-related oxidative stress is generated by a combination of increased production of free radicals, decreased antioxidants levels, diminished activity of antioxidant enzymes and impaired repair of oxidative damages. Oxidative stress is associated with many diseases commonly present in elderly such as hypertension and diabetes. In our study we have observed significantly ( $p < 0,01$ ) increased level of reduced glutathione in treated hypertensive compared to treated diabetic patients ( $3,1 \pm 0,29$  mmol/L and  $2,72 \pm 0,4$  mmol/L, respectively) and significantly ( $p < 0,01$ ) increased activity of glutathione reductase ( $83,43 \pm 15,25$  U/g Hb and  $65,74 \pm 14,27$  U/g Hb, respectively).*

### 1. Introduction

The oxidative hypothesis of senescence has been introduced in 1956 by Harman and has become one of the most prolific theories of aging since its origin [1,2]. This age-related oxidative stress is generated by a combination of increased production of free radicals, decreased antioxidants levels, diminished activity of antioxidant enzymes and impaired repair of oxidative damages. There are many physiological processes in the human body which generate reactive oxygen species (ROS) which due to their unstability and high reactivity are harmful for the cells. To protect themselves from ROS, cells are equipped with antioxidant enzymes i.e. superoxide dismutase (SOD) catalyzes dismutation of  $O_2^{\cdot-}$ , catalase (CAT) scavenges  $H_2O_2$ , glutathione peroxidase (GPx) converts  $H_2O_2$  to water and neutralizes lipid peroxy radicals. Glutathione serves as a major thiol-disulfide redox buffer of the cell and its reduced form pool is maintained by

glutathione reductase (GR). When oxidants are produced in excess and overwhelm the cellular antioxidant capacity the state of oxidative stress occurs. This can result in molecular and cellular tissue damage as well as in severe metabolic malfunctions caused by oxygen radical-mediated toxicity [3]. It has been established that  $O_2^{\cdot-}$ ,  $H_2O_2$  and ONOO $^-$  which is formed in radical-radical coupling reactions, play an important role in the development of hypertension due to their effect on vascular tone [4] and the major source of vascular ROS production are NADP(H) oxidases, [5]. Furthermore decreased endogenous antioxidant status has been observed in essential hypertension [6]. Likewise, imbalance between oxidants and antioxidants has been reported in diabetes mellitus [7] and reactive oxygen metabolites increase the autooxidation of glucose and glycosylated proteins as well as activate polyol pathways [8]. Altogether previous results of oxidative stress parameters and antioxidant enzymes activity are divergent and so far we are not able to state explicitly how they change in particular health condition, therefore further studies are required. The aim of this study was to compare concentration of reduced glutathione and activity of glutathione reductase and glutathione peroxidase in diabetes mellitus type II and hypertensive elderly patients.

## 2. Materials and methods

30 elderly patients age above 60 were recruited to the Department of Gerontology and Clinic of Geriatrics, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Poland. There were 12 and 18 patients with type II diabetes and essential hypertension, respectively. Exclusion criteria for both experimental groups were cardiovascular diseases, cancer, dementia, Alzheimer disease, antioxidants supplementation, smoking and alcohol abuse. We distinguished HT group for hypertensive patients currently treated for and DM with no hypertension neither presently nor in medical record. Hypertensive as well as diabetic patients were treated for their condition. Groups were age and sex matched.

The study was approved by the Nicolaus Copernicus University in Toruń Human Ethics Committee. Written informed consent was obtained before inclusion in the study.

Venous EDTA anti-coagulated blood samples were taken from the cubital vein. GSH concentration was determined in whole blood (method described by Beutler, 1971), GPx1 and GR activities were determined in erythrocytes from hemolysed blood samples according to Paglia & Valentine, 1967; Flohe & Gunzler methods, respectively. We performed statistical analyses with Statistica version 9 software for the comparisons between measured parameters in experimental groups. P-values of less than 0.05 were considered to indicate statistical significance in differences between means of measured parameters.

## 3. Results

The antioxidant barrier defined as antioxidant enzymes activity and concentration of antioxidants in treated elderly patients with diabetes mellitus type two has been demonstrated to function unlike in hypertensive patients. First, in

order to analyse whether oxidative stress parameters varied between groups we have measured GSH concentration. The mean GSH concentration (mmol/L) was significantly lower in DM group compared to HT group ( $p < 0,01$ ) with values of  $2,7 \pm 0,38$  mmol/L and  $3,1 \pm 0,29$  mmol/L, respectively (Fig.1). Lower concentration of GSH in DM group when compared to HT group, was accompanied by decreased activity of GR (Fig. 2). Mean enzyme activity was significantly lower in diabetics ( $p < 0,01$ ) than in hypertensives. GR exhibited activities of  $63,8 \pm 15,15$  U/g Hb and  $83,4 \pm 15,25$  U/g Hb in DM and HT groups, respectively. We have also measured GPx activity, however no significant differences between analysed groups were discovered in this case. Activity of GPx1 was as high as  $12,76 \pm 2,65$  U/g Hb and  $13,16 \pm 1,95$  U/g Hb in patients treated for diabetes and for hypertension, respectively which again follows the pattern of decreased antioxidants in diabetes mellitus type II patients in comparison to hypertensive patients in our study.

Altogether this results suggest that diabetes mellitus type II and essential hypertension which both has been proven to be associated with oxidative stress may differ regarding to mechanisms implicating oxidative stress in their pathogenesis as well as response to medical treatment.

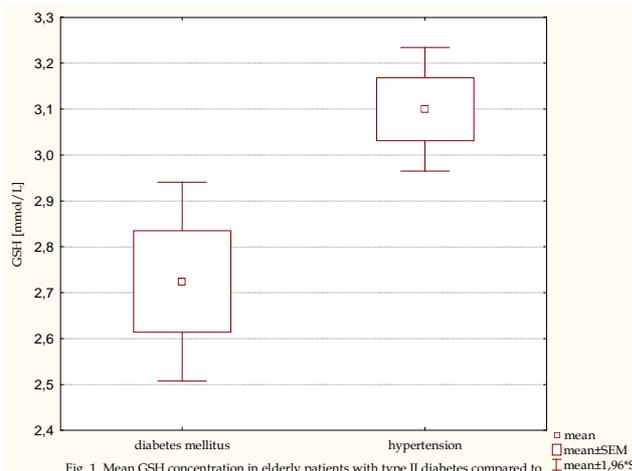


Fig. 1. Mean GSH concentration in elderly patients with type II diabetes compared to elderly patients with hypertension

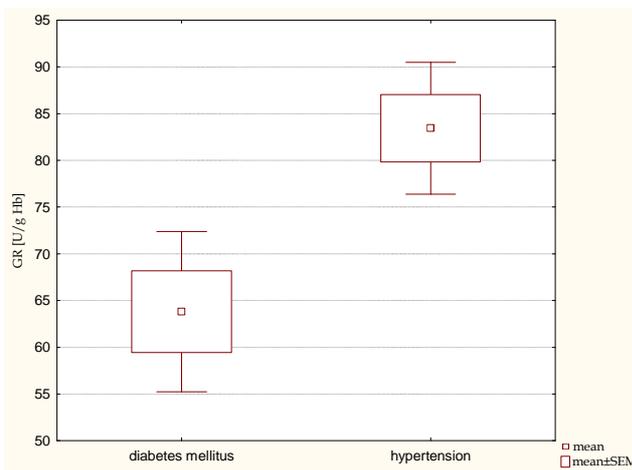


Fig. 2. Mean GR activity in elderly patients with type II diabetes compared to elderly patients with hypertension

from our groups with healthy controls at the same age. However we have done similar study before and interestingly when parallelly analyzed the same parameters in controls we observed that patients with treated hypertension had significantly higher GSH concentration than subjects from two other groups which have had similar level of GSH. The explanation of this phenomenon may be that antioxidant thiols concentration increase due to higher concentration of ROS which means that hypertensives are more vulnerable to oxidative stress. Glutathione deficiency contributes to oxidative stress, which plays important role in aging and the pathogenesis of many diseases such as diabetes and cardiovascular diseases [11, 12]. On the other hand higher level of reduced glutathione can be considered as better protection against oxidative stress since protective role of glutathione against oxidative stress among other important functions has been recognized [13].

In order to better understand the mechanisms of GSH increase in hypertension the values of other parameters of oxidative stress and antioxidant defense need to be taken into account as well as the fact that important confounder in our study was the medication. Antihypertensive drugs are indeed proven to have antioxidant properties [14, 15, 16, 17, 18]. Since hypertensive patients received different antihypertensive drugs with various combinations in this group we could look for the complete status of antihypertensive drugs effect over oxidative stress alone.

The relation between oxidative stress and blood pressure is explained through inactivation of the vasodilator nitric oxide (NO) by ROS and formation of peroxynitrite  $\text{ONOO}^-$  [15]. GSH plays important role in maintenance of NO pool. GSH reacts with peroxynitrite to produce S-nitrosoglutathione (GSNO) which extends the half-life of NO and in consequence contributes in relaxation of vascular tissue

#### 4. Discussion

Results from this research suggest significantly decreased level of glutathione (GSH) in treated diabetes type II elderly subjects when compared with these treated for hypertension. Majority of studies reveal decrease of GSH in pathological conditions analysed [9,10] but we can not conclude it from the data presented herein still we have not compared the result of patients

[20]. GSH enhances decomposition of nitrosothiols and release of NO [21]. Moreover nitrosothiols have the ability to inhibit NADPH oxidase and therefore have beneficial effect on vascular tension. Other important role of GSH is control of PDGF-mediated ROS production by NADH/NADPH oxidase which is considered to be the main source of vascular ROS [5, 15, 22].

Well-established role of GSH is reduction of hydrogen peroxide in the presence of GPx. Reduced form of glutathione is restored by glutathione reductase (GR) [13]. In our study GPx1 activity has not differed significantly while comparing two examined groups. Results of other studies concerning GPx activity in HT subjects are contradictory showing both increased and decreased activity of the enzyme [23]. The same conclusion can be made regarding review of diabetes mellitus studies [24, 25]. Although GPx1 remained unchanged, GR activity increased significantly in HT group. This observation has been interrelated with GSH concentration analyses. We assumed that elevated level of glutathione is the result of increased activity of GR. However, this parameters are not significantly correlated in our study.

Protective role of GPx1 in coping with oxidative injury and cell death mediated by reactive oxygen species in vivo has been supported by substantial body of evidence. Among other important functions in coping with oxidative stress GPx possesses the ability to decompose GSNO and is also responsible for lipid hydroperoxides reduction, thereby preventing inactivation of NO [26].

Nevertheless the ability to potentiate reactive nitrogen species stress has been also reported. GPx seems to play contradictory roles in coping with ROS vs. RNS [27].

Altogether our results suggest that the mechanisms which link oxidative stress with various pathological conditions may differ and may be associated with changes

of different parameters depending of the pathogenesis of the condition and furthermore the action of pharmaceutical agents.

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