The influence of crenotherapy with SHS water on the concentration of glutathione in blood of people with rheumatoid arthritis

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Abstract: The tripeptide-glutathione (GSH, γ-L-glutamyl-L-cysteinyl-glycine) is one of the most important low molecular antioxidant in human body. Enhancing GSH and associated enzymes represents an aim in the search for cytoprotective strategies against cancer, neurologic degeneration, pulmonary and inflammatory conditions, as well as rheumatoid arthritis (RA). The objective of the study was to agree whether crenotherapy (drinking therapy) with sulfide/hydrogen sulfide (SHS) waters from “Zuzanna” spring located in the area of Busko-Zdrój in Poland leads to increasing of reduced glutathione (GSH) content in human blood. SHS water in distinct from mineral water is characterised by specific pharmacokinetic, invariable content and natural microbiological purity. SHS waters contain at least 1 g of total sulfur per kilogram of water and a treatment effect also depends on other bioelements. The method employing capillary electrophoresis with UV detector for the analysis of glutathione in human blood was developed. The group of 106 volunteers consisted of both women and men, in different age range. The therapy with SHS waters lasted 2 weeks. We recently demonstrated that the administration of hydrogen sulfide (H2S) in SHS waters increases GSH concentration in blood, and therefore crenotherapy could be used in therapeutics.

Keywords: crenotherapy, reduced glutathione, rheumatoid arthritis, hydrogen sulfide

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent inflammation of synovial joints with pain, often leading to joint destruction. The tissues of the musculoskeletal system are damaged, the collagen and hyaluronic acid are degraded, as well as the immunoglobulin amino acid composition, which is probably the cause of autoimmune reactions in RA. Free- radical reactions are an important factor in the pathogenesis of rheumatic diseases, including RA. The numerous products that appear to be generated by reactive oxygen species have been identified in clinical samples including peripheral blood and fluid from the joints in patients with RA. RA is associated with significant depletion in GSH levels compared with healthy subjects [1-3].

GSH protects organism against reactive forms of oxygen, exogenous and endogenous electrophilic substances and oxidized forms of other antioxidants [4]. It is also presented in specific selenoenzyme -glutathione peroxidase, protecting organism from hydrogen peroxide and lipid peroxides [5]. Moreover, GSH can be bound to the proteins, leading to the formation of glutathionylated proteins. This connection is able to stabilize some proteins and protect them from oxidative stress [6].
GSH also regulates cell proliferation, apoptosis, immune function, and fibrogenesis. This compound participates in cytokine production, humoral immune response and cell-mediated response by antigen presentation to T lymphocytes [7]. GSH also involved in course and modification of viral diseases such as: influenza [8], HIV [9], herpes simplex [10] and hepatitis C virus infection [11], both in vitro and in vivo by regulating viral replication and infectivity.

Dysregulation of GSH synthesis contributes to the pathogenesis of variety of apparently unrelated clinical conditions and diseases including; RA [12,13] muscular dystrophy, alcoholic liver damage disease, liver fibrosis, cholestatic liver injury, endotoxemia [14], neurodegenerative diseases (Alzheimer disease, Parkinson disease, multiple sclerosis), schizophrenia, autism [15], diabetes mellitus [16], asthma, chronic obstructive pulmonary [17] and drug-resistant tumor cells [18].

The relative proportion of glutathione GSH/GSSG, is calculable value that provides information about the current state of the redox environment and cellular health. High levels of GSH, total glutathione (GSH + 2 GSSG), and GSH/GSSG are associated with enhanced antioxidant activity [19].

Despite the fact, the impact of GSH in various physiological processes is well known, there are still no specific reports concerning the purposefulness and effectiveness of supplementation this substance. The most obvious strategy is to directly administer glutathione. This can be done orally, topically, intravenously, intranasally, or in nebulized form [20].

The most popular modulators of glutathione content are: glutathione esters [21], amino acids (cysteine, glutamine) [22,23], their analogues (S-adenosylmethionine, N-acetylcysteine [24]) and a lot of other substances such as: vitaminum C [25] and E [26], D3 [27], silymarin [28],α-lipoic acid [29], whey protein [30].

The objective of the study was to agree whether crenotherapy with SHS waters leads to increasing of GSH content in human blood. Crenotherapy is a branch of balneotherapy- the method generally applied to everything relating to spa treatment, including the drinking of waters and the use of hot baths and natural vapor baths, as well as of the various kinds of gases or peloids such as: mud, sand used for hot applications. It is defined as: drinking medicinal water for a specified period of time, dosed in terms of quantity, temperature, time in relation to consumed meals and in accordance with medical recommendations. Crenotherapy, a neologism created by Prof. Ladouzy, (from the Greek “Krenos”, which means fountain or source) meaning treatment using mineral water has a long history. It began with the Celts and continued with the Romans and Greeks, through the Dark and Middle Ages, Renaissance and nineteenth and twentieth centuries[31-33]. The most valuable are waters obtained directly from springs, but also stored in bottles [34].

In this study the SHS water from “Zuzanna” spring located in the area of Busko-Zdrój (Winiarski Forest) was used. The Busko-Zdrój health resort is situated in central Poland and its SHS waters were already discovered in the Middle Ages. The physicochemical and microbiological analyses confirmed that it meets the requirements for medicinal water and the stability of its mineral composition (certificate number HU-1/WL/2010 issued by the National Institute of Public Health-National Institute of Hygiene in Poland).

SHS waters are used in “Busko-Zdrój” mostly for sulfur bathing but also in crenotherapy (gastrointestinal disorders, rheumatic diseases). In rheumatology they are used to treat: RA, psoriatic arthritis, ankylosing spondylitis, gouty arthritis and other disorders of muscles and bandapparat, in post-traumatic and post-operative disorders of locomotor [35,36].

SHS waters contain at least 1 g of total sulfur per kilogram of water. “Zuzanna” water contains both divalent sulfur compounds as well as sulfates (1617,2 mg/l). The other main components are sodium (3897 mg/l), magnesium (221,18 mg/l), calcium and bicarbonate. The treatment effect also depends on trace elements such as: bromine, iodine, fluorine, potassium, lithium, selenium, silicon and boron. The total amount of dissolved minerals is 12700-14100 mg/l. The water temperature is 13,5-13,8°C, and the pH is 7,0-7,3.

Despite of its major chemistry, therapeutic benefits of SHS water are related to the presence of reduced chemical species, such as HS- and H2S, the former being dominating in the pH. Its content in the water is about 42-54 mg / l. Such waters are regarded as unique in Europe.
H₂S is an endogenous gas with important physiologic functions in the brain, vasculature and other organs. It protects organism from oxidative stress by increasing the levels of GSH through enhancing the cystine transport and redistributing the localization of GSH to mitochondria. H₂S produced in mitochondria also may directly suppress oxidative stress [37]. Several authors have found that H₂S exhibited anti-inflammatory, anti-catabolic and/or anti-oxidant effects in rodent models of acute arthritis and in in vitro models using human synoviocytes and articular chondrocytes from RA tissues. The earliest studies used fast-dissolving salts, such as NaSH. Positive results have also been obtained when H₂S is administered as a sulfur water bath [38].

In vivo and in vitro studies suggest that H₂S takes part in reduction and reversing inflammation in RA. Exogenous administration of H₂S donor, significantly upregulates the gene expression of cystathionine-γ-lyase, L-cysteine (LC) transporter (Slc7a11/xCT), and the genes involved in GSH biosynthesis. Additionally, it reduces homocysteine, reactive oxygen species production, decreases formation of pro-inflammatory mediators in macrophages (PGE₂, NO•,IL-1b, IL-6), enhances cellular LC, H₂S, activity of NF-kb and glucose uptake and utilisation in myoblasts. Synovial fluid aspirates from RA patients contained up to fourfold-higher concentrations of H₂S in plasma samples. H₂S shrinks vessels localized in knee joints. This mechanism probably protects them against mediators such as: PGE₂, NO• and histamine. Controlling H₂S synthesis may represent a novel target for therapeutic intervention in human chronic inflammatory diseases [39,40-43].

2. Materials and Methods

Various methods could be used for glutathione determinations in physiological fluids, including high pressure liquid chromatography (HPLC) with different kind of detectors: electrochemical [44-46], UV [47-49], fluorometric [50,51], mass spectrometer [52,53], gas chromatography with mass spectrometry [54,55]. HPLC methods are the ones most frequently applied because of their specificity and sensitivity. However, HPLC methods are disadvantaged by the long time required to complete each assay, materials consumed and high costs of instrumentation. During the last decade increased use of capillary electrophoresis (CE) occurred while a lot of attempts have been focused on glutathione separations [56-59]. Main advantages of CE are low consumptions of buffers and solvents; requirement of a small sample volume and fast separation.

Glutathione concentration in human beings and animals has been analyzed mostly in plasma, whole blood and erythrocytes because GSH to GSSG ratio in those structures represents sensitive indicator of oxidative stress in whole organism. In this study GSH and GSSG ratio were analyzed in whole blood, using CE.

2.1. Chemicals

For determination of GSH and GSSG CExofix™ GSH KIT from Analis was used. CExofix™ contains: initiator (borax solution at pH 9, 2 ± 0, 2), accelerator (acid boric NaOH pH 8, 2±0, 2), conditioner solution (0,1 M NaOH) and sample diluents (naphthalene sulfonic acid). Glutathione (both GSH and GSSG) was purchased from Sigma Aldrich, metaphosphoric acid (MPA) was purchased from Fluka. All the solutions and samples were prepared in redistilled water daily.

2.2. Instrumentation

All CE experiments were performed on a Beckman P/ACE MDQ system (Beckman Coulter USA) equipped with a UV–Vis detector, an auto- sampler and a temperature controller. Instrument control and data analysis were carried out by Beckman P/ACE system soft- ware.

2.3. Preparation of blood samples and standard samples

Detailed method for preparing blood samples for the determination of GSH and GSSG by CE was developed on the basis of general literature information. One ml of venous blood was collected in tubes with EDTA. Samples were made homogenous by gentle up and down shaking. Blood was transferred to snap-cap conical-bottom centrifuge vial and added with 600 ml of 5% MPA.
immediately, to precipitate proteins. Fresh solution of acid was prepared every day. Next, samples were chilled in ice cold water and vortex-mixed for one minute. The precipitated proteins were removed by centrifugation (4000g ×15min) at 4°C. A 40-μL aliquot was diluted with 160 μL of deionized water before injection. Supernatant was collected to air tight closed vials and stored at -20°C before CE analysis which was run the same day.

Standard stock solutions of GSH and GSSG were prepared quantitatively with water and stored at 4°C until use. The working standard solutions were used after the dilution of stock solutions with MPA (Table 1). Standard solution or sample solution were made from sample diluent and treated sample or standard and mixed. The results are expressed in µmol/L according to the calibration curve and the dilution factor of 20.

Table 1. The quantities of reagents for the preparation of working solutions and final concentrations of GSH and GSSG.

<table>
<thead>
<tr>
<th>level</th>
<th>MPA</th>
<th>Stock</th>
<th>Stock solution</th>
<th>Water [μl]</th>
<th>GSH [μM/l]</th>
<th>GSSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7,5</td>
<td>1000</td>
<td>1000</td>
<td>500</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>7,5</td>
<td>750</td>
<td>1000</td>
<td>1250</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>7,5</td>
<td>500</td>
<td>1000</td>
<td>1900</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>7,5</td>
<td>250</td>
<td>1000</td>
<td>2000</td>
<td>10</td>
<td>2,5</td>
</tr>
<tr>
<td>5</td>
<td>7,5</td>
<td>125</td>
<td>1000</td>
<td>375</td>
<td>5</td>
<td>20</td>
</tr>
</tbody>
</table>

2.4. Electrophoresis procedure

All the separations were performed on a fused silica capillary of effective length 30 cm, ×75μm I.D. The samples were injected in pressure mode at inlet (0, 2 psi for 8s). The detection wavelength was set to 200 nm, voltage was +6, 5 kV, and the temperature was: 25 °C. The separation time was less than 9, 5 min. Before each run, the capillary was rinsed with water, initiator and accelerator at 20 psi for 1 min and 0, 5 psi for 4 min. to equilibrate the capillary inner wall. Typical electrophoregrams of a whole blood sample and a calibrator are shown in Fig. 1 and Fig. 2.

![Electrophoregram of selected working solution of GSH/GSSG.](image)
Fig. 2. Electrophoregram of selected blood sample.

2.5. Subjects

The study was approved by the Medical Ethics Committee of the Jan Kochanowski University in Kielce (26/2011, 18.03.2012). Written, informed consent was obtained from all volunteers. The subjects consisted of 106 volunteers (61 women and 45 men), in different age range (32-82 years). They had detailed medical questionnaire and consultation with a physician. Eligible candidates were those who were apparently healthy, without signs of serious illnesses, such as heart disease or cancer, or of diabetes and hepatitis. Volunteers were either non-responsive, intolerant, or had a contraindication to crenotherapy. They did not take any other substances that could modify GSH or GSSG concentration in organism and had commonly used diets. The study excluded people treated in Busko-Zdrój in the last 10 months, using drugs with a recognized antioxidative properties, and smokers.

The volunteers were divided into three groups. The first group consisted of people with classical RA, according to the criteria of the American College of Rheumatology, treated in a health resort in Busko-Zdrój. Patients were in Steinbrocker functional classes III to IV, in remission of inflammation and they did not use drug therapy. In the first group of patients was 31 women and 27 men. Age of women in this group ranged from 35 years to 82 years (mean 51.9 years) and male of 32 years to 70 years (mean 51.5 years).

The second group included healthy people, which were treated with SHS water as a group of sick people. This group consisted of 20 women aged between 29 years to 78 years (mean 50.9 years) and 10 males aged 23 years to 56 years (mean 38.6 years).

The third group consisted of healthy people, 10 women aged 21 years to 76 years (mean 48.5 years) and 8 men aged 27 years to 67 years (mean age 46.13 years) which do not was subjected to treatment by SHS water. The control group also was used to determining the physiological norms of glutathione in the blood. The control group consisted of persons clinically healthy, never previously using the spa treatment. The control group drank “Buskowianka-Zdrój” water (without H₂S) instead of “Zuzanna” SHS water (Table 2). The regimen was identical.

<table>
<thead>
<tr>
<th>Table 2. The ionic composition of the water “Buskowianka-Zdrój”</th>
<th>Concentration in milligrams</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
2.6. Statistical analysis

Statistical calculations were done using the Statistica Software Package, version 13.0 (StastSoft® Polska). The data are expressed as mean values. The normality of variables was tested by the Shapiro–Wilk W-test and Kolmogorov–Smirnov test. Differences between the groups were tested using Student’s t-test. In case of a lack of normal distribution and variance uniformity, the differences between groups were analyzed by means of a non-parametric Mann–Whitney U-test. When comparing more than two groups, the Kruskal–Wallis test was used. The significance coefficient value, p < 0.05, was considered statistically significant.

3. Results

The standard curve for GSH displayed remarkable linearity. The correlation coefficient was high ($R^2 = 0.999$), which confirms the method’s proper analytical performance. A statistically significant increase in GSH concentration was identified in group of patients with RA (Table 3). Before treatment, the average concentration of GSH was $942.25 \, \mu M / L$, and after treatment was $1041.11 \, \mu M / L$ which means a 10.49% increase, p <0.05. A statistically significant increase in GSH concentration was also observed separately in the groups of men (9.2 %) and women (12.05 %) with RA, p <0.05. A significant increase in R ratio in whole group of patients with RA was observed. R ratio before crenotherapy was 27.46 and after treatment 31.39, which means a 14.31% increase, p <0.05. A statistically significant increase in R ratio was also observed separately in the groups of men (9.2 %) and women (12.05%) with RA, p <0.05.

Table 3. Concentration of GSH and R ratio in the group of people with RA before and after crenotherapy.

<table>
<thead>
<tr>
<th></th>
<th>GSH concentration in $\mu M / L$ before crenotherapy</th>
<th>GSH concentration in $\mu M / L$ after crenotherapy</th>
<th>R ratio before crenotherapy</th>
<th>R ratio after crenotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=58 whole group of patients with RA</td>
<td>$942.25 \pm 215.84$ ($513.91 \div 1379.29$)</td>
<td>$1041.11 \pm 228.15$ ($579.83 \div 1484.18$)</td>
<td>$27.46 \pm 8.37$ ($16.26 \div 59.1$)</td>
<td>$31.39 \pm 10.17$ ($18.2 \div 61.59$)</td>
</tr>
<tr>
<td>n=31 women with RA</td>
<td>$796.56 \pm 146.63$ ($513.91 \div 1118.18$)</td>
<td>$892.57 \pm 164.37$ ($579.83 \div 1205.67$)</td>
<td>$26.13 \pm 6.69$ ($16.26 \div 42.95$)</td>
<td>$31.01 \pm 9.01$ ($19.66 \div 50.1$)</td>
</tr>
<tr>
<td>n=27 men with RA</td>
<td>$1109.52 \pm 151.53$ ($875.15 \div 1379.29$)</td>
<td>$1211.65 \pm 162.59$ ($924.54 \div 1484.18$)</td>
<td>$28.96 \pm 9.88$ ($17.77 \div 59.1$)</td>
<td>$31.82 \pm 11.52$ ($18.2 \div 61.59$)</td>
</tr>
</tbody>
</table>

A significant increase in GSH concentration was also identified in group of healthy volunteers which were treated with SHS water as a group of sick people (Table 4). Before treatment, the average...
concentration of GSH was 1094.04 μM/L, and after treatment was 1117.05 μM/L which means a 2.1%, increase, p < 0.05. A statistically significant increase in GSH concentration was also observed separately in the groups of men (1.52%) and women (3.45%) with RA, p < 0.05. A significant increase in R ratio in whole group of healthy patients was observed. R ratio before crenotherapy was 25.76 and after treatment 30.04, which means a 16.61% increase, p < 0.05. A statistically significant increase in R ratio was also observed separately in the groups of men (8.1%) and women (23.54%) with RA, p < 0.05.

Table 4. Concentration of GSH and R ratio in the group of healthy people before and after crenotherapy.

<table>
<thead>
<tr>
<th></th>
<th>GSH concentration in μM/L before crenotherapy</th>
<th>GSH concentration in μM/L after crenotherapy</th>
<th>R ratio before crenotherapy</th>
<th>R ratio after crenotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=29 whole group of healthy people</td>
<td>1094.04 ± 166.22 (711.5 ± 1410.1)</td>
<td>1117.05 ± 141.85 (779.94 ± 1426.75)</td>
<td>25.76 ± 10.74 (12.17 ± 57.37)</td>
<td>30.04 ± 11.98 (12.36 ± 63.05)</td>
</tr>
<tr>
<td>n=19 women with RA</td>
<td>1017.35 ± 133.11 (711.5 ± 1306.55)</td>
<td>1044.93 ± 141.85 (779.94 ± 1377.21)</td>
<td>21.71 ± 9.3 (12.17 ± 49.53)</td>
<td>26.82 ± 13.00 (12.36 ± 63.05)</td>
</tr>
<tr>
<td>n=10 men with RA</td>
<td>1239.76 ± 120.49 (1019.11 ± 1410.1)</td>
<td>1254.08 ± 116.58 (1028.37 ± 1476.8)</td>
<td>33.44 ± 9.25 (21.1 ± 57.37)</td>
<td>36.15 ± 6.69 (27.23 ± 51.3)</td>
</tr>
</tbody>
</table>

In the control group of women and men changes in GSH concentration and R ratio as a result of drinking “Buskowianka-Zdrój” were irrelevant and they were not related to drinking this water. In each of the three separate groups of volunteers, a higher concentration of GSH was observed in men in relation to women. In the group of patients with RA, the concentration of GSH before drinking SSH water was higher in men by 28.2% compared to women (p < 0.05), while after drinking “Zuzanna” water, the average GSH concentration was higher in men by 26.33% (p < 0.05).

In the group of healthy people drinking SSH water the concentration of GSH before crenotherapy was higher in men, on average, by 17.94%, (p < 0.05) in relation to women. After crenotherapy, GSH concentration was higher on average by 16.2% (p < 0.05). In the control group, the concentration of GSH before drinking was higher in men by 23.4% (p < 0.05) and after drinking water the mean concentrations of GSH was higher in men by 23.42% (p < 0.05).

In order to determine the relationship between GSH concentration and age, subjects were divided into four different age groups: 20-35, 36-50, 51-65, > 66 years (Table 5). The assessment of the influence of age on the content of GSH in the blood was based on the average concentration of GSH before drinking water. Both in the group of women and men, the highest concentration of GSH was observed in the group of the youngest (20-35 years) and the lowest in the group of the oldest (> 66 years). In the group of men a decreasing concentration of GSH was observed with age, however, in the group of women the concentration of GSH in the age range (51-65 years) was slightly higher than in the range (36-50 years).

In order to investigate the influence of the disease duration on the concentration of GSH, volunteers were divided into four subgroups, due to disease duration in years: <10, 11-20, 21-30, > 31 (Table 6). The concentration of GSH was measured before drinking SHS water. Both in the group of women and men, the average concentration of GSH was the highest in people with shorter disease duration. The average concentration of GSH decreased with the duration of the disease in the group of women while in the group of men a lower concentration of GSH was observed in the range of 11-20 years than 21-30 years. However, none of the differences showed statistical significance (p > 0.05).
Table 5. The influence of age on the concentration of GSH.

<table>
<thead>
<tr>
<th>Age range in years</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 20-35</td>
<td>n=7</td>
<td>n=9</td>
</tr>
<tr>
<td></td>
<td>GSH (1063.46±120.3)</td>
<td>GSH (1245.00±119.69)</td>
</tr>
<tr>
<td>2 36-50</td>
<td>n=25</td>
<td>n=22</td>
</tr>
<tr>
<td></td>
<td>GSH (827.17±161.38)</td>
<td>GSH (1162.71±153.06)</td>
</tr>
<tr>
<td>3 51-65</td>
<td>n=18</td>
<td>n=12</td>
</tr>
<tr>
<td></td>
<td>GSH (929.91±131.43)</td>
<td>GSH (1065.68±130.52)</td>
</tr>
<tr>
<td>4 &gt; 66</td>
<td>n=11</td>
<td>n=2</td>
</tr>
<tr>
<td></td>
<td>GSH (794.16±153.21)</td>
<td>GSH (888.64±17.97)</td>
</tr>
</tbody>
</table>

Table 6. The influence of disease duration on the concentration of GSH in patients with RA.

<table>
<thead>
<tr>
<th>Disease duration in years</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>n=6</td>
<td>n=4</td>
</tr>
<tr>
<td>GSH (832.66±209.7)</td>
<td>GSH (1212.14±160.27)</td>
<td></td>
</tr>
<tr>
<td>11-20</td>
<td>n=17</td>
<td>n=14</td>
</tr>
<tr>
<td>GSH (809.38±140.73)</td>
<td>GSH (1096.38±144.24)</td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>n=5</td>
<td>n=8</td>
</tr>
<tr>
<td>GSH (750.14±121)</td>
<td>GSH (1101.32±168)</td>
<td></td>
</tr>
<tr>
<td>&gt; 31</td>
<td>n=3</td>
<td>n=1</td>
</tr>
<tr>
<td>GSH (729.09±91.54)</td>
<td>GSH (988.67)</td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

The aim of the study was to evaluate the effects of SHS water in RA patients. Hydrotherapy is frequently indicated for the rehabilitation of patients with RA, nevertheless, there has been inadequate appraisal of its effectiveness [43]. There are very few published studies about the use of crenotherapy with SHS water in RA treatment. SHS water with a similar composition to water “Zuzanna” was applied in the form of crenotherapy in patients with obliterating atherosclerosis of lower limbs arteries and with hyperlipoproteinemia type IIa and IIb. Such a treatment resulted in statistically significant decrease of blood levels of cholesterol, triglycerides and LDL cholesterol. Moreover, therapy with sulphur water resulted in decreasing of platelet aggregability evoked by ADP and collagen, spontaneous platelet aggregability and increasing fibrinolytic activity of the blood (elongation of euglobulin clot lysis time) [60].

Most of the latest research concerns bathing. Karagülle et al. [61] described a 2-week spa therapy adjunct to usual pharmacological therapy provided beneficial clinical effects compared to usual pharmacological therapy alone, in RA patients treated with traditional disease-modifying antirheumatic drugs. These beneficial effects may last for 6 months. Sukenik wrote that the sulfur mineral water has special proprieties to rheumatologic diseases, including in the course of active inflammatory phases in RA balneotherapy could be used in acute phases of RA or active disease [62].
In our experiment statistically significant increase in GSH concentration and R ratio was identified in group of patients with RA and in the group of healthy patients. Increase of GSH concentration is a parameter of improvement of health in patients which take part in experiment and it has an influence on immune system and antioxidative activity. The subjective wellbeing of patients was also observed. A higher increase in GSH concentration occurring in the group of RA patients may be related to the initially reduced concentration of chemical compounds containing -SH groups in these individuals. The phenomenon of lowering the concentration of thiol compounds, including GSH is common in people suffering from diseases related to oxidative stress [63,64].

It is commonly known that a lot of diseases depend on gender and age. These study confirmed the significant influence of age on the concentration of GSH in human body. Ageing is the cumulative result of oxidative damage to the cells and tissues of the body: increased oxidation of proteins and fats, damage to DNA and limiting the functioning of mitochondria. The activity of enzymes: glutathione peroxidase and gamma-glutamyl-cysteine synthetase is limited with age. It was shown that every cell of an average 20-year-old is exposed to about 100,000 attacks of oxygen free radicals per day, their number at the age of 75 increases three-fold [65].

We showed that GSH levels in human blood decreased with age in both males and females. Similar results were found by Lang et al. [63] who described a significant increase in the proportion of elderly individuals with low blood GSH values compared with younger adults. Also plasma and human lymphocytes GSH levels were found to decrease with increasing age. We also observed the increase in GSH levels in men when compared to women, what was observed in other experiments [66–68].

5. Conclusions

Using CE method, an increase of GSH and R ratio was observed in the whole blood of patients with RA and healthy subjects drinking SHS water compared with the control group. This experiment confirms H,S influence on the concentration of the GSH in blood and indirectly in the entire body, as well as huge meaning of balneotherapy in the treatment of rheumatic diseases. Crenotherapy with SHS water could be the effective replenishment to persons suffering from RA, in cases, when the pharmacological treatment is contraindicated, or has strong side effects. The presented research confirmed significal influence of gender and age on GSH concentration and R ratio. The average concentration of GSH decreased with the duration of the disease but none of the differences showed statistical significance.

Funding: This research received no external funding.

Conflicts of Interest:

The authors declare no conflict of interest. Authors declare to have no financial or personal relationships with other people or organizations that could inappropriate influence (bias) the results presented in this manuscript.

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