Thiol/disulphide homeostasis and ischemia modified albumin levels in autoimmune gastritis and their relations with gastric emptying

Abstract

Background/Aims: Autoimmune gastritis is an autoimmune and inflammatory disorder. The aim of the study is to examine dynamic thiol/disulfide homeostasis, ischemia modified albumin levels and to analyze the association between thiol/disulfide homeostasis and gastric emptying time in autoimmune gastritis.

Materials and Methods: Thiol/disulphide homeostasis tests and ischemia modified albumin levels were determined in 50 autoimmune gastritis patients and 53 healthy subjects. Patients with delayed and normal gastric emptying were compared by means of thiol/disulphide homeostasis tests.

Results: It has been seen that native thiol (µmol/L), total thiol (µmol/L) and native thiol/total thiol ratio (%) of the patients with autoimmune gastritis decreased compared to control group (177.7 ± 34.18 vs 245.25 ± 33.83, p=0.001 , 227.25 ± 36.78 vs 284.20 ± 27.19 , p = 0.03, and 8.84 ± 1.1 vs 7.74 ± 1.3%, p=0.001). In addition, native thiol (µmol/L), total thiol (µmol/L) and native thiol/total thiol ratio (%) were found to be lower in patients with delayed gastric emptying (198.65±24.27 vs 167.12±20.51, 241.81±27.14 vs 213.92±26.35, 8.34±1.29 vs 7.20±1.83, p=0.001). Disulphide level, disulphide/native thiol, disulphide/total thiol (p=0.001) ratios and ischemia modified albumin levels (ABSU, 0.71±0.08 vs 0.83±0.07) were found to be higher in autoimmune gastritis patients with delayed gastric emptying (p=0.001).

Conclusion: It has been noted that thiol/disulphide homeostasis in patients with autoimmune gastritis has changed in the way that ischemia modified albumin and disulphide increased whereas thiols decreased. Altered thiol/disulphide balance was
also observed in patients with delayed gastric emptying. These results have suggested that the oxidative process is involved in patients with autoimmune gastritis.

**Key words**: Autoimmune gastritis, thiol, disulphide, gastric emptying, oxidative stress

1. **Introduction**

Autoimmune gastritis (AIG) is an autoimmune disorder. It mainly consists of chronic infiltration of the corpus mucosa of the stomach. It is marked by reduction or absence of parietal cells and autoantibodies against H^+·K^+ ATPase [1]. Some studies conducted in literature have revealed that oxidant radicals increase secondary to inflammation in some autoimmune and autoinflammatory disorders [2-4]. Reactive oxygen species (ROS) can produce molecules leading to cellular damage. The increase in ROS may react with cellular macromolecules and causes lipid peroxidation and nucleic acid damages [5]. Reactive oxygen species induce oxidation of disulphide groups into amino acids containing sulphur. This process is one of the first marks of protein oxidation [6]. Thiols are able to react with free radicals in order to provide defense mechanism against tissue damage [7]. Oxygen molecules oxidize thiol groups of proteins and therefore, reversible conversion into disulfide bonds is seen [8]. In case of oxidative stress, thiols form some products due to oxidative stress [9]. These disulfide bonds may be converted into thiol groups once again. A distortion in this homeostasis system may cause different disorders due to the antioxidant protection characteristic of thiol groups [10,11]. Ischemia modified albumin (IMA) is produced as a result of oxidative stress which could be used as an oxidative stress marker [12]. Ischemia modified albumin levels increase in conditions such as tissue damage caused by free radicals [13]. The hypothesis of the study is abnormal TDH and alteration of IMA level may have a place in the pathogenesis of this disorder. Direct measurement of thiol-disulfide levels with a new and automated method is already available [14]. It has been reported that there is a
significant relationship between autonomic dysfunction and elevated oxidative stress in
diseases such as hypertension and in patients with diabetic peripheric neuropathy [15-
17]. Moreover, it has been found that there a change in autonomic nerve function of
some of the AIG patients. This has revealed a close association between altered
autonomic nervous system function and delay in gastric emptying (GE) [18]. Therefore,
the aim of our research was to examine dynamic TDH and IMA levels in AIG and
specify possible factors associated with this oxidation. Moreover, the association
between TDH and GE time in AIG being one of the cause of delayed GE was addressed
[19].

2.Materials and Methods

2.1. Patients

The study is a prospective single center research including 50 AIG patients and 53
healthy individuals. The diagnostic criteria for AIG are: the presence of anti-parietal
cell antibodies, elevated blood gastrin levels and the presence of histology suggesting
chronic AIG which includes intestinal metaplasia or pseudopyloric metaplasia or
atrophy of the gastric fundus or body [1]. The subjects with concomitant disorders that
may influence TDH were not included in the study [2,20-23]. The patients having
atherosclerotic disorders, diabetes mellitus, kidney disorders, thyroid and liver diseases,
malignancy, and rheumatic disorders , systemic or other dermatologic diseases, acute or
chronic pancreatitis, psychiatric disorders and patients using antioxidant and anti-
lipid agents, any known autoimmune disorders and tobacco and alcohol users were
excluded from the study. The control group was selected from subjects who admitted
for screening and check-up purposes. Thiol/disulfide homeostasis parameters and IMA
levels were compared between patients and control group, patients with delayed and
normal GE. The relationships between serum gastrin and chromogranin A levels and TDH and IMA levels were analyzed.

2.2. Thiol/Disulfide Homeostasis and Ischemia Modified Albumin

Blood samples were drawn in the fasting state from patients and healthy subjects for the measurement of biochemical parameters and for TDH tests. The blood samples were centrifuged for 10 minutes at 1500 rpm, and serum was separated. Serum samples were stored at a temperature of −80°C. Thiol / disulfide homeostasis tests were carried out as developed by Erel et al. [14]. Briefly, disulfide concentrations were computed as the half of the difference between levels of the total thiol and native thiol. Then, disulfide / total thiol percent ratio, disulfide / native thiol percent ratio, and native thiol/total thiol percent ratio were computed [7]. Ischemia modified albumin was determined using a colorimetric cobalt-albumin binding assay as previously described [24].

2.3. Gastric Emptying Study

Gastric emptying time was performed using a 2-hour scintigraphic method [19]. In brief, subjects consumed an isotope-labeled (55 MBq Tc - 99 m macroaggregated albumin) scrambled egg, white meal of 300kcal. A GE half-time (GET ½) of longer than 110 minutes was accepted as delayed GE [25]. Thiol / disulfide homeostasis and IMA levels were compared between patients with AIG and control group. The factors that might affect these parameters were determined. Patients were further stratified into two groups: patients with normal GE and delayed GE. Then these two groups were taken under an analysis in order to see whether an abnormality in TDH had any effect on gastric emptying time. The study was approved by the local ethical committee of the related institution and informed consent was obtained from all subjects before conducting the study. Some of the data included in this research were used in some studies previously [18,19].
2.4. Statistics

Statistical analysis was performed by using SPSS 16.0 (SPSS, Chicago, IL, USA) for Windows. Results were expressed as percentage of the patients or mean ± SD where appropriate. The Shapiro-Wilk test was used to test the normality of the data, and according to the results, parametric or non-parametric tests were selected. Analysis were performed using paired Student’s t test, Mann-Whitney U-test, Pearson’s and Spearman’s correlation tests where appropriate. A p-value of < 0.05 was considered as significant. The standard deviation was found to be 0.3 and 0.4 for 53 patients and 50 healthy subjects, respectively with a type I error of α=0.05 and β=0.20. The power of this study was calculated as 86% (Power Analysis Statistical System 11.0, NCSS Statistical Software, Kaysville, UT, USA).

3. Results

Totally 50 patients (29 women, mean age 61.3 ± 8.17 years) with AIG and 53 healthy subjects (31 women, 59.5 ± 6.18 years, p = 0.443) were included in the study. It was found that the native thiol (μmol/L), total thiol (μmol/L) and native thiol / total thiol ratio (%) of the patients with AIG decreased compared to the control group (177.7 ± 34.18 vs 245.25 ± 33.83, p=0.001, 227.25 ± 36.78 vs 284.20 ± 27.19, p = 0.03, and 8.84 ± 1.1 vs 7.74 ± 1.3%, p = 0.001, respectively, Figure 1). Disulphide, disulphide / native thiol, disulphide / total thiol ratios and IMA of the patients with AIG were found to be higher compared to the control group (Table 1). Of the 50 patients with AIG, 26 (52%) patients showed delayed GE and 24 patients showed normal GE (GET ½: 152.61 ± 26.8 vs 90.5 ± 6.61 mins, p < 0.001). The native thiol (μmol / L), total thiol (μmol / L) and native thiol / total thiol ratio (%) were found to be lower in AIG patients with delayed GE than in patients with normal GE (p = 0.001) (Table 2). The disulphide level, the disulphide / native thiol, disulphide / total thiol (p = 0.001) ratios and IMA
level were found to be higher in AIG patients with delayed GE compared to the patients with normal GE (p = 0.001). The correlation analysis between TDH tests and other parameters within the AIG patients were shown in detail in Table 3 - 4. A positive correlation between disulphide, disulphide / native thiol, disulphide / total thiol and serum gastrin and chromogranin A levels was found. However, correlation analysis revealed a negative correlation between native thiol, total thiol, native thiol / total thiol, IMA and serum gastrin and chromogranin A levels. While there was a positive correlation between GE and disulphide level, we found negative correlations between GE and native and total thiol levels (Figure 2).

4. Discussion

It has been revealed that native thiol, total thiol and native thiol / total thiol ratio of AIG patients significantly decreased compared to the control group and disulphide, disulphide / native thiol, disulphide / total thiol ratios and IMA of AIG patients were found to be significantly higher compared to the control group. The TDH protects human body from oxidative stress and this balance plays a pivotal role in the detoxification and antioxidant protection. Provided that disulphide formation increases, functional and structural alterations are seen in most of the systems. This condition has an adverse impact on protection against oxidative stress [26,27]. Although the investigation of this subject is a matter of debate, various studies have been conducted using this method including inflammatory bowel diseases [26,27] diabetes mellitus [28], cardiovascular diseases [29], and a close relation with oxidative stress has been found. Ates et al showed that altered TDH in subclinical hypothyroidism and thyroid autoantibodies were positively correlated with thiol oxidation [2]. Although the literature data regarding the role of oxidative stress in the development of AIG is not sufficient, it is known that there is a greater increase in oxidant radicals than in
antioxidant molecules leading to oxidative stress in autoimmune thyroid diseases which is an organ specific autoimmune disorder similar to AIG [30-32]. Excessive production of reactive oxygen species and deranged redox state are accepted as one of the pathogenic mechanisms underlying systemic autoimmune response. The increase in the level of reactive oxygen species may cause oxidative alteration of lipids, proteins and carbohydrates. This oxidative alteration of proteins leads pathogenic antibodies in autoimmune diseases [33]. Baser et al examined oxidative status of autoimmune thyroiditis patients by means of total antioxidant status, total oxidant status and IMA. It has been found that oxidants increased while antioxidants decreased in patients with euthyroid autoimmune thyroiditis. They concluded that increased oxidative stress may play a role in autoimmune thyroid disorders [32]. Kaplan et al studied TDH in 73 patients with gluten sensitive enteropathy being a chronic and autoimmune disease by the same method [34]. They found an altered TDH in patients with gluten sensitive enteropathy compared to healthy subjects and concluded that this alteration was associated with autoimmunity and inflammation. Kalkan et al showed that native thiol and total thiol levels significantly higher in patients with lichen planus which describes as an autoimmune inflammatory disease of the mucocutaneous tissue [20]. Koseoglu et al showed that disulfide/total thiol percent ratios and disulfide/native thiol percent ratios were significantly higher in patients with acute pancreatitis, whereas the total and native thiol levels and native thiol/total thiol percent ratio were significantly lower. These changes indicate that the thiol/disulfide redox balance shifted to the disulfide bond side in acute pancreatitis [23].

Patients with AIG exhibited altered autonomic function indicating an important association between autonomic dysfunction and delayed [18]. This result and existence of a positive association between elevated oxidative stress and autonomic dysfunction
have led us to examine the relationship between GE and TDH [35,36]. The native thiol, total thiol and native thiol / total thiol ratio were found to be lower in AIG patients with delayed GE than in patients with normal GE. The disulphide level, the disulphide / native thiol, and disulphide / total thiol ratios were found to be higher in AIG patients with delayed GE than in patients with normal GE. In this regard, altered TDH may cause delayed GE due to autonomic nerve dysfunction. We have also examined IMA levels of patients and of control group. IMA is a modified form of albumin and may be used as an indicator of oxidative stress [37]. IMA is produced as a consequence of changes in albumin’s capacity in order to bind heavy metals. It is widely used to evaluate myocardial ischemia. However, the increase in IMA levels is also observed in disorders such as obesity, type 2 diabetes mellitus, hypercholesterolaemia, psoriasis and familial Mediterranean fever which are associated with oxidative stress [38-40]. As a conclusion, it has been suggested that IMA may have a role as an oxidative stress marker. In our study, higher levels of IMA were found in OIG patients than healthy controls. Kucuk et al studied IMA levels in FMF patients having an autoinflammatory disease and found that IMA levels were higher in the familial Mediterranean fever group than in healthy controls [41]. Furthermore, Capkin et al observed that IMA levels were higher in Behçet’s disease, which is an inflammatory disease similar to familial Mediterranean fever, than in control group [42]. Moreover, it was found in our study that IMA levels were higher in patients with delayed GE than in patients with normal GE. It was observed that there was a significant inverse association between IMA and serum gastrin and chromogranin A levels. It has been reported that essential oils and their secondary metabolites are related as potent antioxidants and free radical scavengers in chronic inflammation [43]. Furthermore, some products such as synthetic trans-Δ9-tetrahydrocannabinol dissolving in sesame oil have proven to
possess a potential antioxidative effect in inflammatory arthritis. Therefore, AIG patients with altered TDH homeostasis may benefit from agents having antioxidative properties [44].

As a conclusion, our study has revealed that TDH was altered and IMA levels increased in patients with AIG compared to healthy controls. Furthermore, the dynamic TDH shifted through disulphide form in AIG patients with delayed GE than in patients with normal GE. Altered TDH observed in these patients may shed light on the pathophysiology of this disorder and could suggest therapeutic options such antioxidant agents in the management of AIG.

References


Table 1: Thiol/sulphide hemostasis parameters in patients with autoimmune gastritis and control group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=53)</th>
<th>AIG (n=50)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native thiol (μmol/L) (mean±SD)</td>
<td>245.25±33.83</td>
<td>177.7±34.18</td>
<td>0.001</td>
</tr>
<tr>
<td>Total thiol (μmol/L) (mean±SD)</td>
<td>284.20±27.19</td>
<td>227.25±36.78</td>
<td>0.003</td>
</tr>
<tr>
<td>Disulfide (μmol/L) (mean±SD)</td>
<td>25.37±2.27</td>
<td>32.45±4.43</td>
<td>0.002</td>
</tr>
<tr>
<td>Disulfide/native thiol (%)</td>
<td>10.31±2.7</td>
<td>16.37±1.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Disulfide/total thiol (%)</td>
<td>8.06±2.3</td>
<td>12.8±2.07</td>
<td>0.002</td>
</tr>
<tr>
<td>Native thiol/total thiol (%)</td>
<td>8.84±1.1</td>
<td>7.74±1.3</td>
<td>0.001</td>
</tr>
<tr>
<td>IMA (ABSU)</td>
<td>0.61±0.07</td>
<td>0.77±0.05</td>
<td>0.001</td>
</tr>
</tbody>
</table>

(AIG: autoimmune gastritis, ABSU: absorbance unit)
Table 2: Thiol/sulphide hemostasis parameters among patients with delayed gastric emptying and normal gastric emptying

<table>
<thead>
<tr>
<th>Variables</th>
<th>AIG (GET ½&lt;110) (n=24)</th>
<th>AIG (GET ½≥110) (n=26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native thiol (µmol/L) (mean±SD)</td>
<td>198.65±24.27</td>
<td>167.12±20.51</td>
<td>0.001</td>
</tr>
<tr>
<td>Total thiol (µmol/L) (mean±SD)</td>
<td>241.81±27.14</td>
<td>213.92±26.35</td>
<td>0.001</td>
</tr>
<tr>
<td>Disulfide (µmol/L) (mean±SD)</td>
<td>30.49±4.42</td>
<td>34.41±3.79</td>
<td>0.001</td>
</tr>
<tr>
<td>Disulfide/native thiol (%)</td>
<td>13.28±2.36</td>
<td>20.29±1.94</td>
<td>0.001</td>
</tr>
<tr>
<td>Disulfide/total thiol (%)</td>
<td>9.73±2.28</td>
<td>16.13±1.66</td>
<td>0.001</td>
</tr>
<tr>
<td>Native thiol/total thiol (%)</td>
<td>8.34±1.29</td>
<td>7.20±1.83</td>
<td>0.001</td>
</tr>
<tr>
<td>IMA (ABSU)</td>
<td>0.71±0.08</td>
<td>0.83±0.07</td>
<td>0.001</td>
</tr>
</tbody>
</table>

(AIG: Autoimmune gastritis, GET ½: Gastric emptying half time, ABSU: Absorbance unit)

Table 3: The correlation analysis of thiol/disulfide homeostasis parameters and other risk factors in the autoimmune gastritis patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Native thiol</th>
<th>Total thiol</th>
<th>Disulfide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Gastrin (ng/L)</td>
<td>-0.757</td>
<td>0.001</td>
<td>-0.612</td>
</tr>
<tr>
<td>Chromogranin A (µg/L)</td>
<td>-0.644</td>
<td>0.007</td>
<td>-0.628</td>
</tr>
</tbody>
</table>
Table 4: The correlation analysis of thiol/disulfide homeostasis parameters and other risk factors in the autoimmune gastritis patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Disulfide / Native thiol</th>
<th>Disulfide / Total thiol</th>
<th>Native thiol / Total thiol</th>
<th>IMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Gastrin (ng/L)</td>
<td>0.681</td>
<td>0.005</td>
<td>0.745</td>
<td>0.001</td>
</tr>
<tr>
<td>Chromogranin A (μg/L)</td>
<td>0.764</td>
<td>0.002</td>
<td>0.866</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Figure 1: These plots show mean values of serum native thiol (μmol/L) and total thiol (μmol/L) in patients with AIG and control group. Differences of serum native thiol (p=0.001), and total thiol (p=0.03) levels between AIG and control groups were statistically significant. The native thiol and total thiol levels of the patients with AIG decreased compared to control group (AIG: autoimmune gastritis).
Figure 2: Correlations between GE and disulphide level and native thiol and total thiol levels. While there was a positive correlation between GE and disulphide level, negative correlations were found between GE and native and total thiol levels (GE: gastric emptying).