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Evaluation of dynamic serum thiol/disulfide homeostasis in locally advanced and metastatic gastric cancer

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ABSTRACT

Background: Gastric cancer is one the most diagnosed cancer and the third leading cause of death from cancer worldwide. As an indicator of antioxidant capacity thiol/disulfide homeostasis regulates detoxification, cell signal mechanisms, apoptosis, transcription and antioxidant defense mechanisms. Disregulation of thiol/disulfide homeostasis identified in other cancer types by recent data. In this study, we aimed to evaluate the thiol/disulfide homeostasis in advanced gastric cancer patients.
Methods: The patients who diagnosed with gastric cancer and healthy control subjects were included to study. Serum samples for the thiol-disulphide test were obtained at the time of diagnosis. Thiol-disulphide homeostasis tests were measured by the automated spectrophotometric method. Thiol-disulphide homeostasis was also measured according to clinical and laboratory features.
Results: Thirty newly diagnosed advanced gastric adenocarcinoma patients and 28 healthy controls were enrolled in the study. The native thiol (NT) and total thiol (TT) levels of patients' group were significantly lower compared with controls ($p = 0.001$ and $p < 0.001$). In the CEA high ($\geq$5.4 ng/ml) group, DS/NT ratio were higher compared with CEA low (<5.4 ng/ml) group ($p = 0.024$). In CA.19-9 high ($\geq$28.3 kU/L) group, both DS and DS/NT ratio were significantly higher compared with a CA19-9 low(<28.3 kU/L) group ($p < 0.05$ both). The correlation between CEA and DS levels was also significant ($p = 0.02$). There was also a positive correlation between CEA levels and DS/NT ratio ($p = 0.01$).
Conclusion: Derangements of thiol/disulfide homeostasis may have a role in gastric cancer pathogenesis and the higher level of oxidative stress may relate to extensive and aggressiveness of the advanced disease. The diagnostic and prognostic values of thiol/disulfide products need to identify with further studies.

1. Introduction

Gastric cancer is one the most diagnosed cancer and the third leading cause of death from cancer worldwide.[1] In 2017, an estimated 28,000 people will be diagnosed as gastric cancer and over 17,000 patients will die because of disease in the United States of America.[2] Some risk factors includes Helicobacter Pylori infection, smoking, high salt intake and some dietary factors as well as genetic mutations.[3] Despite the encouraging studies defining new mechanisms, the complexity of disease pathogenesis still needs to clarify.[4]

As a healthy physiological process, there is a balance between oxygen free radicals and antioxidant defence systems and the loss of this balance causes oxidative stress. It is well known that low amounts of reactive oxygen species (ROS) may have beneficial effects on important metabolic pathways whereas the supra-physiological levels of ROS can because oxidative tissue damage and dysregulate metabolic reactions.[5]

Loss of the physiological balance of redox signaling which is vital for regulating cell renewal, proliferation and differentiation contribute to the pathogenesis of gastric disorders. Derangements between oxidant and antioxidant status identified in chronic gastritis, intestinal metaplasia, peptic ulcer as well as gastric cancer with so many different pathologic pathways and end-products in a broad spectrum by recent data.[6]

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Thiol groups (mercaptans), are potent anti-oxidant molecules and including hydrogen and sulfhydryl groups which plays an essential role to neutralise ROS by either enzymatic or non-enzymatic way. Thiols can undergo oxidation reaction via oxidants and form disulphide bonds. The formed disulphide bonds can again be reduced to thiol groups under normal circumstances and dynamic thiol–disulphide homeostasis is continued to maintain.[7,8]

As an indicator of antioxidant capacity thiol/disulfide homeostasis regulates detoxification, cell signal mechanisms, apoptosis, transcription and antioxidant defense mechanisms.[9] Furthermore, dysregulation of thiol/disulfide homeostasis identified in prostate cancer, lung cancer and anthracycline-associated cardiotoxicity.[10–12] In this study, we aimed to evaluate the thiol/disulfide homeostasis in gastric cancer patients.

2. Materials and methods

Treatment naïve, newly diagnosed patients with advanced gastric adenocancer in Ankara Yildirim Beyazit University Medical Oncology Department, between 2015 and 2017 were prospectively analyzed. Patients were over 18 years old and the diagnosis of gastric cancer made pathologically with endoscopic biopsy, tru-cut needle biopsy from metastatic lesions or surgically resected specimens were enrolled in our study. American Joint Committee on Cancer (AJCC), TNM classification for carcinoma of the stomach 7th edition (2010) was used for staging. Patients with renal or liver disease, diabetes and active inflammatory or infectious disease were excluded from the study. Healthy subjects were also enrolled in our study as a control cohort.

Blood samples for thiol-disulfide homeostasis analyses were collected at the time of diagnosis for the patients with gastric cancer. Blood samples were collected from the control and patient groups in the morning and centrifuged at 1500*g* for 10 min. Serum samples were separated and stored at −80 °C until being used for the analysis. Thiol/disulfide homeostasis tests were measured using a novel automatic and spectrophotometric method developed by Erel and Neselioglu.[8] In this method, dynamic and reducible disulfide bonds in the samples were reduced to free functional thiol groups by using sodium borohydride. In order to prevent the reduction of unused reduced sodium borohydride to dithionite-2 nitrobenzoic (DTNB), NaBH4 was removed with formaldehyde. Native thiol (NT) and total thiol (TT) levels were determined after reaction with DTNB and their levels were measured ultimately. Half of the difference of the result obtained by the subtraction of native thiol amount from total thiol content indicated the disulfide (DS) level. Disulphide/Native thiol (DS/NT) ratio which is the best marker for reflection of thiol-disulfide homeostasis was also calculated.

University Clinical Research Ethics Committee's approval was obtained (No: 26379996/136).

The parameters were investigated using visual (histograms, probability plots and analytic methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. Differences in categorical factors were determined with Fisher's exact test. Differences in continuous values between two groups were assessed with Student's *t*-test for normally distributed variables and non-parametric Mann-Whitney *U* tests for non-normally distributed variables as appropriate and also multiple sets of comparisons performed using ANOVA. In normally distributed parameters, the correlation coefficients and their significance were calculated using the Pearson test and in non-normally or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test.

All statistical procedures were performed with SPSS 17.0 (SPSS Inc, Chicago, Illinois). A P value < 0.05 was considered to statistically significant. A 5% type-1 error level was used to infer statistical significant.

3. Results

Thirty newly diagnosed advanced gastric adenocarcinoma patients and 28 healthy controls were enrolled in the study. The median age of the patients was 63 (min-max: 27–90) and 62.5 (27–90) in the control group. There were 20 males (66.7%), 10 females (33.3%) in the patient' and 17 males (60.7%), 11 females (39.3%) in the control group. There was no significant difference between arms in age and sex status ($p = 0{,}98$ and 0,63 respectively).

In the patient's group, 26 (86.7%) participants were stage IV disease and 18 (60%) participants had visceral metastasis. The clinical and laboratory features of patients and controls summarised at Table 1.

The NT and TT levels of patient group were statistically significantly lower compared with controls. Likewise, the DS levels were also lower in the patient group but this difference had failed to reach statistical significance. There was no significant DS/NT ratio difference between groups. Detailed statistical data was shown in Table 2.

When we stratified the patient group with their clinical features according to primary tumor localization or visceral/non-visceral metastasis; any of the parameters (NT, TT, DS, DS/NT) have statistically significant difference between subgroups. There were also no significant relationships between thiol or disulfide levels with hemoglobin, white blood cells, platelets or lactate dehydrogenase levels.

When we divided the patients to albumin low and albumin high groups according to median, albumin high group (median TT: 454 and NT: 413.5) had significantly high levels of thiol groups compare with albumin low group (median TT: 380 and NT: 337.5; $p < 0.001$

Table 1
(A) Age and sex status of patients and controls groups, (B) Patient characteristics.

(A) Age and sex status of patients and controls groups			
Parameters	Patients	Controls	p Value
Age (median, min-max)	63 (27–93)	62.5 (27–93)	0.98
Sex (m/f, %)	20/10 (66.7%/33.3%)	17/11 (60.7%/39.3%)	0.63
(B) Patient Characteristics			
Primary Localization			
Cardia-Fundus (n, %)	8 (26.7%)		
Corpus (n, %)	15 (50%)		
Antrum-Pylorus (n, %)	7 (23.3%)		
Disease Stage			
Stage III (n, %)	4 (13.3%)		
Stage IV (n, %)	26 (86.7%)		
Metastasis			
Visceral (n, %)	18 (60%)		
Non-Visceral (n, %)	12 (40%)		
H. Pylori			
Yes (n, %)	4 (13.3%)		
No (n, %)	3 (10%)		
Unknown (n, %)	23 (76.7%)		
Laboratory Parameters	**Median, (min-max)**		
CEA	5.4 ng/ml (0.5–7585)		
CA 19-9	28.3kU/L (0.6–2099)		
Albumin	3.85 g/dL (2–4.89)		
Hb	11.15 g/dL (7.5–13.8)		
WBC	7650/mm^3 (3500–21000)		
Platelet	264 × 10^3/mm^3 (99–528)		
LDH	238 U/L (166–2561)		

Abbreviations: m: Male, f: Female, CEA: Carcinoembryonic Antigen, CA 19–9: Cancer Antigen 19–9, Hb: Haemoglobin, LDH: Lactate Dehydrogenase,WBC: White Blood Cells.

Table 2
Thiol and disulfide levels in patients and control groups.

Parameters	Patients, Median (min-max)	Controls, Median (min-max)	p Value
Native Thiol (μmol/L)	374.5 (209–473)	435.6 (364.7–493.6)	**0.001**
Total Thiol (μmol/L)	409 (246–507)	469.2 (403.6–532.5)	**<0.001**
Disulfide (μmol/L)	15.5 (3–30)	19.3 (5.8–33)	0.09
Disulfide/Native Thiol	0.044 (0.01–0.12)	0.045 (0.01–0.08)	0.9

$p < 0.05$ is statistically significant.

both of them respectively).

When we stratified 28 patients to two groups according to their median levels of tumor markers, there were no statistically significant NT or TT levels between groups. However, in the CEA high (≥5.4 ng/ml) group, DS levels (med: 18.9) and DS/NT ratio (med: 0.05) were higher compared with CEA low (<5.4 ng/ml) group (med. DS: 13.0 and med. DS/NT: 0.039). The DS difference has failed to reach statistical significance ($p = 0.060$) but DS/NT difference was statistically significant ($p = 0.024$). In CA19-9 high (≥28.3 kU/L) group, both DS (med: 19.9) and DS/NT ratio (med: 0.049) were significantly higher compared with a CA19-9 low (<28.3 kU/L) group (median DS: 13.7 and median DS/NT: 0.039; $p < 0.05$ both).

The correlation between CEA and DS levels was also significant ($p = 0.02$). There was also positive correlation between CEA levels and DS/NT ratio ($p = 0.01$).

4. Discussion

In this study, we showed the significantly lower levels of thiol groups in advanced gastric adenocancer patients compared with healthy controls. Also, we found significant higher levels of thiol groups in patients whose albumin levels were higher. Moreover, our study demonstrated significant changes of DS/NT ratio with higher levels of tumor markers CEA and CA19-9, as well as positive correlation with CEA levels.

As the products of cellular metabolism and environmental factors ROS, have potential to damage DNA and other vital structures of the cell and alter the functions of proteins and critical signal mechanisms. The rising of the amounts of ROS above physiological levels that overburden antioxidant defense results as oxidative stres.[13] The contribution of this process to the cancer pathogenesis defined by recent data.[14] A growing body of evidence demonstrated different pathogenetic pathways related to dysregulation of oxidant-antioxidant balance also studied in gastric cancer pathogenesis.[6]

One study found the higher levels of important antioxidant enzymes which includes glutathione reductase (GR), glutathione peroxidase (GPX), superoxide dismutase (SOD), malondialdehyde (MDA) and glucose 6 phosphate dehydrogenase (G6PD) in gastric cancer tissue compared with normal tissue as a reaction to increased oxidative stress.[15] Monari M. et al. demonstrated the higher activity of manganese superoxide dismutase (MnSOD) which is a mitochondrial form of SOD and catalase (CAT) antioxidant enzymes in gastric cancer patients.[16] Literature has multiple other studies evaluated the antioxidants in gastric cancer as well as NADPH oxidase, selenium-dependent GPX.[17–21] On the other hand, thiol groups have sulfhydryl (-SH) components and one of the most important members of antioxidant defense systems and they easily interact with different oxidative substances in a broad spectrum.[22] Similarly, in our study TT and NT levels were significantly lower in locally advanced and metastatic gastric cancer patients compared with controls. Our results about thiol parameters also compatible with two other studies' results in prostate and non-small cell lung cancer patients.[10,11] This result may reflect increased consumption of thiol groups as a consequence of increased oxidative stress in cancer patients.

We also found TT and NT levels in albumin level high patients were higher than albumin level low patients. Cysteine, glutathione, homocysteine and γ-glutamylcysteine constitute the low molecular weight thiol groups but the most important amounts of plasma thiol reserve provided by albumin thiols.[23] With this point of view, our results was similar with literature as well as other solid tumors. This result may consider with low albumin levels may reflect malnutrition and level of inflammation that can classify as bad prognostic factors in gastric cancer patients.

Under the effects of oxidants, thiol groups turn to disulfide with covalent bonds. This is not an irreversible reaction, and the formed disulfide bonds reduced to thiol groups back under convenient circumstances.[7,24] As a reflection of thiol/disulfide imbalance, the shift from thiol groups to disulfide defined well in diabetes mellitus, pneumonia or obesity. In contrast, disulfide levels tend to lower in proliferative diseases like multiple myeloma, bladder cancer, colon cancer and renal cancer compared with normal population. Furthermore, disulfide levels seem closely related to the proliferation rate of cancer type and aggressively growing tumors showed the lowest disulfide levels.[8] Similarly, in our study, gastric cancer patients had lower disulfide levels than healthy controls.

In contrast; in the patient's group, DS levels and DS/NT ratio levels associated with tumor markers. In CEA high-level patients DS/NT ratio was significantly higher compared with CEA low-level patients and also DS levels strongly tended to high even lack of significance. In CA 19-9 high groups both DS and DS/NT levels significantly higher than CA 19-9 low patients. Similarly to our study, the relationships between antioxidant capacity, oxidative stress and tumor markers in chronic pancreatitis, lung adenocarcinoma, prostate tumors as well as metastatic gastric cancer demonstrated by recent data.[25–28] CEA is strongly connected with stage disease and with liver or peritoneal involvement in metastatic disease setting. Likewise, CA 19–9 attributed to disease stage, tumor depth, nodal involvement and peritoneal metastases.[29] In a manner, both of this tumor markers seem related to extensity and aggressiveness of the gastric cancer.And our result may consolidate the contribution of oxidative stress to disease pathogenesis via this significant higher DS/NT ratios.

Moreover, we found a positive correlation between CEA levels with both DS and DS/NT levels. As a strong indicator of tumor burden in advanced disease, high CEA levels also related to tumor size, serosal involvement, lymphovascular invasion, lymph node involvement and hepatic metastases.[29,30] But the causality between oxidative stress and tumor markers still needs to clarify with new studies. The probable prognostic value of these oxidative stress markers in gastric cancer would identify in follow-up periods with survival parameters and also needs further studies.

In conclusion, derangements of thiol/disulfide homeostasis may have a role in gastric cancer pathogenesis and the higher level of oxidative stress may relate to extensive and aggressiveness of the advanced disease. The diagnostic and prognostic values of thiol/disulfide products need to identify with further studies.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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