Oxidative Stress in Children with Cutaneous Mastocytosis

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Background: Thiols and ischemia-modified albumin (IMA) are novel biomarkers for the evaluation of the oxidative stress (OS) that has been associated with the pathogenesis of various neoplastic and inflammatory diseases. The aim of this study was to find out the dynamic thiol–disulfide homeostasis and IMA levels in patients with cutaneous mastocytosis (CM) to demonstrate the presence of the OS.

Methods: A total of 36 patients with CM and 27 age- and sex-matched healthy participants were included in this study. Serum thiols and IMA levels were measured and compared between the 2 groups.

Results: Levels of native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol, and IMA were similar in both patients with CM and the control group. No significant difference was found with regard to mediator-releasing symptoms, clinical types, and remission status between the OS markers (thiol/disulfide variables and IMA levels) of the patients ($P > 0.05$).

Conclusions: To our knowledge, this study is the first study in the literature that investigated thiol–disulfide homeostasis and IMA levels in pediatric patients with CM. Our study indicated that OS plays no role in the course of the pediatric CM.

Keywords: cutaneous mastocytosis, disulfide, ischemia-modified albumin, thiols, oxidative stress, reactive oxygen species

Introduction

MASTOCYTOSIS IS A HETEROGENEOUS disease defined by clonal expansion and focal accumulation of tissue mast cells in different organs and systems. The classification of mastocytosis is basically divided into 2 groups: cutaneous form and systemic variants.¹ Cutaneous form is typically and most commonly observed in children in clinical practice and is highly heterogenous accordingly to the age of the disease onset and type of skin findings.² Broesby-Olsen et al. reported that “small monomorphic skin lesions; disease onset after 3 years of age; detection the *KIT* D816V mutation; high mast cell burden; and systemic disease” are the risk factors for the persistence of the cutaneous form.³

In both cutaneous and systemic manifestations, mastocytosis presents itself in the same way: activation and degranulation of mast cells. The severity of the symptoms is correlated with the high burden of the mast cells. The mediator-induced symptoms occur in both local and systemic tissue throughout blood circulation.⁴ The proinflammatory mediators released from mast cells cause inflammation. On the other hand, immunomodulatory effects of mast cells are demonstrated recently. The regulation of the mast cell differential response and the balance of immune regulation is still occult.⁵

Oxidative stress (OS) is a cell physiology emerging as a consequence of increased reactive oxygen species (ROS) due to oxidant–antioxidant imbalance. An increased level of ROS damages lipids, proteins, and DNA in the cells and causes cellular injury. Moreover, ROS is related to the immune system, signal transduction, and normal functions of organisms. There have been various complex antioxidant systems defined to work against ROS and reduce their damage.⁶ Thiols are important antioxidants that control ROS level. Thiols within human cells are mainly formed by albumin thiols, protein thiols, and low-molecular-weight thiols such as glutathione, which provides a regulatory mechanism for the ROS-related process. Thiols can undergo an oxidation reaction via oxidants and form disulfide bonds; on the contrary, disulfide bounds can be reduced to thiol groups, which is called dynamic thiol–disulfide homeostasis. This homeostasis is involved in the pathogenesis of many disorders such as neoplastic diseases, degenerative diseases, and inflammatory diseases. During OS, disulfide levels tend to increase whereas native thiol and total thiol levels decrease.⁷

One of the other commonly used OS markers is ischemia-modified albumin (IMA), which reflects hypo-oxygenation and increased hydroxyl free radicals. In the presence of hydroxyl free radicals, albumin structure is altered and albumin

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is unable to buffer the toxic molecules, which is called IMA.^{8,9} IMA is an early marker proven by US FDA to rule out acute coronary syndrome in low-risk patients in cardiology.¹⁰ There are reports that have shown the rise of IMA as a marker of OS in patients with obesity, gestational diabetes mellitus, preeclampsia, and surgery with general anesthesia.^{11,12} OS has been accompanied with these biomarkers in various diseases but in the literature there is only 1 study searching for the relation in mastocytosis and the report suggested that mastocytosis is associated with OS.¹³

The aim of this study was to evaluate the dynamic thiol-disulfide homeostasis and IMA concentration in children with cutaneous mastocytosis (CM) for the first time by using a novel method to demonstrate the OS.

Materials and Methods

Patients

Thirty-six pediatric patients with CM aged between 0 and 18 years were recruited in the study. Patients who fulfilled the World Health Organization (WHO) diagnostic criteria for CM with the proven histopathologic examination of skin lesions were included in the study. None of the patients had clinical findings pointing to extracutaneous disease. None of the patients underwent bone marrow biopsy. The WHO classified mastocytosis into 2 groups: CM, in which no systemic involvement is found with limited involvement in the skin, and systemic mastocytosis.¹⁴ A generally accepted approach is to divide CM into 3 subgroups: maculopapular cutaneous mastocytosis (MPCM), diffuse cutaneous mastocytosis (DCM), and mastocytoma of the skin (cutaneous mastocytoma)¹⁵ (Fig. 1). Twenty-seven of the total 36 patients (75%) were diagnosed with MPCM (96% of them

were polymorphic), and 9 patients (25%) were diagnosed with cutaneous mastocytoma. There were no cases of either DCM or systemic mastocytosis. There were no familial cases. There were 14 patients (39%) experiencing repeated episodes of the symptoms due to mast cell mediator release. Blood sampling was performed at least 4 weeks after the mast cell mediator releasing episode. The control group of the study was obtained from the healthy individuals who had been admitted to the pediatric clinic.

The study protocol was approved by Keçiören Hospital Research Ethics Comity, and written consent was obtained from the parents or guardians for this study.

Plasma samples and determination of thiols and IMA

Venous blood samples withdrawn from the participants were collected in tubes containing EDTA. To separate the plasma samples, centrifugation was performed at 1,500g for 10 min. The samples were stored at -80°C in Eppendorf tubes until assay. Thiol/disulfide homeostasis tests were analyzed by a novel automated colorimetric method developed by Erel and Neselioglu.⁷ Cobas c501 chemical analyzer (Roche Diagnostics, Mannheim, Germany) was used for the measurements.

Disulfide bonds in the sample were reduced to functional thiol groups by sodium borohydride. Formaldehyde was used to avoid extra reduction of 5,5-dithiobis-2-nitrobenzoic acid. The native thiol content of the sample was measured by using modified Ellman reagent. Native and total thiols, disulfide amounts were analyzed and the ratios (disulfide/total thiol percent ratios, disulfide/native thiol percent ratios, and native thiol/total thiol percent ratios) were calculated by multiplying with 100. Results were shown as μM .

FIG. 1. Maculopapular cutaneous mastocytosis (A–C), Darier's sign (D), cutaneous mastocytoma (E).

Albumin cobalt binding test was utilized for the evaluation of IMA. At first, plasma serum and 0.1% cobalt chloride were mixed and then dithiothreitol and 0.9% sodium chloride solution was added, respectively. The absorbance of samples was measured at 470 nm by using a spectrophotometer. The results were presented as absorbance units.

Statistical analysis

Analyses of the study were administered by using Statistical Package for Social Sciences 15 software. Categorical results were presented as frequency and percentage. Student *t*-tests (for parametric variables) and Mann–Whitney *U* tests (for nonparametric variables) were used to show the differences between the groups. For categorical variables, the Pearson Chi-square test was used for evaluation. A value of $P < 0.05$ was considered statistically significant. Correlations between the variables were assessed by using a Spearman's correlation analysis.

Results

The study population consisted of 36 patients and 27 healthy control subjects. The mean age of the patients was 58 ± 42 months, and the mean age of the control group was 71.5 ± 48.3 months. There were 22 males (61.1%) in the patients group and 17 (63%) in the control group. No significant differences were found in the demographic features between the patients and the control group ($P > 0.05$). All the patients were followed up for at least 6 months and a maximum of 122 months.

Demographic, clinical, and laboratory data of the patients are summarized in Table 1. Complete blood count, liver function tests, and lactate dehydrogenase levels were checked annually and results were normal in all patients. Tryptase levels were also checked annually unless there was an abnormal finding pointing to systemic disease during the follow-up of the patients. Levels of native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol, and IMA were similar in both patients with CM and the control group ($P > 0.05$). Levels of OS markers (thiol/disulfide variables and IMA levels) were similar in

both patients with CM and the control group when analyzed according to gender (male/female) separately ($P > 0.05$). The thiol/disulfide homeostasis variables of the participants are shown in Table 2.

No correlation was found between age, gender, tryptase level, vitamin D (total 25[OH]D), and OS parameters (thiol/disulfide variables and IMA levels) of the patients. No significant difference was found with regard to mediator-releasing symptoms, clinical types, and remission status between the OS markers (thiol/disulfide variables and IMA levels) of the patients ($P > 0.05$). Based on passive smoking, the OS parameters (thiol/disulfide variables and IMA levels) did not differ significantly among patients with or without secondhand smoke exposure.

Discussion

To the best of our knowledge, this is the first study that investigated thiol–disulfide homeostasis and IMA as a novel marker for the relationship between OS and CM in children. Our findings demonstrated that thiol–disulfide homeostasis or IMA levels do not change in children with CM when compared with healthy subjects.

Mastocytosis is a chronic disease with a broad clinical spectrum. The pathogenesis of both systemic and cutaneous forms is associated with excessive mast cell accumulation in one or more tissues and episodic mast cell-mediator release. With a favorable clinical course in children, CM has been considered a benign condition. In children, KIT mutations existing with a variable rate have been detected in skin mast cells and these mutations are capable of the persistence of the disease into adulthood. However, the molecular pathogenesis of mastocytosis is incompletely understood.¹⁶ Inflammation is considered one of the factors that might play a role in mastocytosis and/or contribute to clinical manifestations of the disease.¹³

OS parameters are a reliable sign of inflammation. Previous studies revealed a relationship between several systemic diseases and impaired thiol balance.^{17–19} There has been only 1 study found in the literature that researched the relationship between the OS and mastocytosis. Gangemi¹³ et al. evaluated advanced oxidation protein products

TABLE 1. CLINICAL AND LABORATORY CHARACTERISTICS OF PATIENTS WITH CUTANEOUS MASTOCYTOSIS

	Patients
Total number	36
Sex (female)	22 (61.1%)
Age, month	58 ± 42^a
Classification of the patients	
MPCM	27 (75%)
Cutaneous mastocytoma	9 (25%)
Presence of mediator-releasing symptoms	14 (38.9%)
Exposure to SHS	10 (27.8%)
Patients in remission	22 (61.1%)
Tryptase (ng/mL)	4.05 [3.2–5.4] ^b
Vitamin D (total 25[OH]D) (ng/mL)	21.3 ± 8.56^a

^aMean $\pm$ SD.

^bMedian [interquartile range].

MPCM, maculopapular cutaneous mastocytosis; SHS, secondhand smoke.

TABLE 2. THIOL–DISULFIDE HOMEOSTASIS PARAMETERS AND ISCHEMIA-MODIFIED ALBUMIN LEVELS IN PATIENTS WITH CUTANEOUS MASTOCYTOSIS AND CONTROL GROUP

	Patients (n = 36) (mean $\pm$ SD)	Controls (n = 27) (mean $\pm$ SD)	P value
Native thiol, $\mu\text{mol/L}$	393.97 ± 45.28	374.20 ± 86.47	0.290
Total thiol, $\mu\text{mol/L}$	439.44 ± 59.82	425.87 ± 91.33	0.480
Disulfide, $\mu\text{mol/L}$	23.06 ± 4.41	22.85 ± 4.98	0.857
Disulfide/native thiol, %	5.89 ± 0.98	6.13 ± 1.14	0.374
Disulfide/total thiol, %	5.25 ± 0.78	5.44 ± 0.92	0.388
Native thiol/total thiol, %	89.48 ± 1.56	89.11 ± 1.84	0.389
IMA	0.65 ± 0.21	0.74 ± 0.23	0.150

IMA, ischemia-modified albumin.

(AOPPs) and advanced glycation end products (AGEs) of OS in patients with mastocytosis. They indicated that mastocytosis is associated with a state of increased OS. In the study, they found higher levels of AOPPs in patients than the healthy control group, but surprisingly patients with recurrent mediator-related symptoms had lower levels of AOPPs and AGEs as compared with patients without symptoms. There are significant differences between our study group and those of Gangemi¹³ et al. All of our patients were pediatric, whereas only 29.4% of their patients were pediatric. Thirty-two percent of the patients were diagnosed with the cutaneous form; however, our entire study group consisted of the cutaneous form. It is clear that in the systemic form mast cell load and proinflammatory mediators released would be much higher. The method used to measure oxidative status was different from our study. No single parameter has yet been recommended as a gold standard for defining OS in clinical samples to establish the relationship between OS and disease status.²⁰ Therefore, methodology and OS measurement superiority could not be determined. Moreover, many variables can affect oxidative parameters. ROS are not only produced endogenously as a natural by-product of the normal cellular metabolism of oxygen but also can be stimulated exogenously with drugs, UV light, ionizing radiation, lifestyle, nutrition style, stress, heavy metals, and active smoking.²⁰ Since the variables that affect OS parameters are expected to vary greatly in pediatric and adult patients, we suggest that evaluating both groups together may give different and contradictory results. In addition, OS management and the metabolic process in children are not expected to be the same as those of adults. We think that the difference between the results of 2 studies may have been due to the reasons mentioned earlier.

In a report searching the effect of passive smoking on oxidant–antioxidant status in preschool children, passive smoking was found to be a potent oxidant.²¹ In our patients, no significant association was found between OS parameters and passive smoking.

CM, by nature, is self-limited and tends to disappear during adolescence.² The frequency of episodic symptoms and the severity of the disease gradually decrease in the majority of patients. Many children do not have clinical symptoms despite persistent skin lesions over time. In our study, serum samples were obtained from the patients in the stable period and if there were signs of active mediator release, a period of at least 1 month was intervened. Inflammation is expected in the presence of mast cell mediator-releasing episodes, but no chronic OS has been demonstrated in our patients. In our study, the fact that OS parameters were not different from the healthy group supports the benign course of the disease in children.

The most significant limitation of our study was the low number of participants. We believe that further studies with a greater number of patients are needed to reveal the relationship between CM and OS in children.

Conclusion

In conclusion, this is the first study demonstrating the thiol–disulfide homeostasis and IMA in children with CM. These OS markers cannot provide valuable information to differentiate CM in children.

Author Disclosure Statement

No competing financial interests exist.

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