Oxidative stress in common variable immunodeficiency

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Abstract
Common variable immunodeficiency (CVID) is a heterogenous group of immunologic disorders of unknown etiology. Alterations of the normal cellular balance due to an increase in reactive oxygen species and/or decrease in antioxidant defense may lead to increased oxidative stress. We aimed to evaluate the levels of oxidative stress biomarkers in patients with CVID who had different presentations. We investigated the serum catalase (CAT), erythrocyte superoxide dismutase (SOD), erythrocyte reduced glutathione as antioxidants and serum malondialdehyde levels as lipid peroxidation marker in patients with CVID in Uludag University Hospital Department of Pediatric Allergy and Immunology’s outpatient clinics. In the analysis, there were 21 patients and 27 matched healthy controls. The median levels of CAT in patients with CVID was significantly lower than in healthy controls (p = 0.04). Among the patients with CVID, 19% had autoimmune disease, one had Sjögren’s syndrome, one had autoimmune alopecia, one had juvenile rheumatoid arthritis, and one had chronic inflammatory demyelinating polyneuropathy. Patients with autoimmune complications had significantly lower CAT levels compared to the ones without autoimmune diseases (p = 0.03). The patients without non-infectious complications (NICs) had lower SOD levels than the patients with NICs (p = 0.05). The analysis of oxidative stress markers in the patients with CVID suggested a series of abnormalities in the anti-oxidant system. The clinical syndrome associations may be a useful tool for future studies to set prediction markers for the prognosis of patients with CVID.

Keywords
anti-oxidant system, common variable immunodeficiency, oxidative stress

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Introduction
Several pathological conditions such as cancer, atherosclerosis, diabetes, arthritis, neurodegenerative disorders, and pulmonary, renal, and hepatic diseases have been defined in association with oxidative stress. Satisfying evidence for the association of oxidative/nitrosative stress with acute and chronic diseases is based on validated biomarkers of oxidative damage.1,2 If the inflammatory response is not properly controlled, this becomes a chronic challenge for the organism and may be detected with oxidant and antioxidant markers.3 Many researchers tended to observe the inflammatory response with oxidative stress markers in different samples. For instance, patients with systemic lupus erythematosus had significantly higher serum levels of protein-associated nitrotyrosine associated with the disease score, while in patients with rheumatoid arthritis, serum/synovial nitrotyrosine concentrations became lower after...
6 months of anti-TNF treatment, which was associated with a change in the rheumatoid arthritis disease activity score.3,4

Common variable immunodeficiency (CVID) is a heterogeneous group of immunologic disorders of unknown etiology, which is characterized by impaired antibody response. The clinical spectrum of the disease mainly consists of two phenotypes: the first group with predominantly recurrent infections (26%) and the second group with additional autoimmune/inflammatory manifestations. Non-infectious complications (NICs) may be present upon presentation or may appear afterward, and they include progressive lung disease (30.3%), autoimmune disease (AID) (33.2%), gastrointestinal inflammatory disease (17.3%), granulomatous disease (9.3%), liver disease (12.7%), lymphoid hyperplasia (20.9%), and the development of cancer, especially lymphoma (6.7%).5,6

With regard to diverse presentations, until now, it has been difficult to identify the underlying pathophysiological mechanisms. Although a few early papers on this topic have been published, knowledge of the role of oxygen metabolism in the pathophysiology and clinical manifestations of CVID, as well as multiple complications is still limited. We sought to evaluate levels of oxidative stress biomarkers associated with different CVID presentations.

Methods

Ethical statement

The trial was approved by the local ethical committee of Uludag University (Approval number: 2009-9/44). At the time of enrollment of the patients and the healthy controls in the study, written informed consent was taken from the participants over 18 years of age, and the legally authorized representatives of the participants younger than and equal to 18 years of age.

Patient selection

This prospective study was conducted in Uludag University Pediatric Allergy and Immunology Department, Bursa, Turkey, between June and December 2010. A formal sample size calculation for this preliminary study was not conducted. Twenty-one patients with the diagnosis of CVID who were under regular outpatient follow-up, were involved in the study. Twenty-seven age- and gender-matched healthy controls were individuals who had admitted to our hospital for well-care visits. The patients with CVID were diagnosed according to the following criteria7: at least one of the clinical presentations of CVID (i.e., increased susceptibility to infection, autoimmune manifestations, granulomatous disease, unexplained polyclonal lymphoproliferation, a family member with antibody deficiency) and

(a) patients aged 4 years and older;
(b) serum IgG levels <4.5 g/l for adults or the 2.5th percentile for age, usually with levels of serum IgA below the lower limit of normal for age or serum IgM below the lower limit of normal for age;
(c) a significant lack of antibody responses to protein antigens following immunization or exposure antigens in at least two assays; and
(d) exclusion of all other known causes of immunoglobulin production failure, as defined by the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee.8

All the patients were under intravenous immunoglobulin (IVIG) replacement therapy at three-week intervals. None of the patients had any type of infection in the previous 3 weeks and at the time of sample collection. The patients were receiving neither corticosteroids nor other immunosuppressive medications. All of the patients had basal IgG levels of 400 mg/dl and higher in the last 3 months of follow-up. All the patients underwent detailed history taking and physical examination at the time of sample collection. Demographic and clinical data were extracted from the electronic medical records of the hospital.

Study protocol

The medical records of the patients were also analyzed for the presence of any type of NIC. Non-infectious complications were defined as manifestations such as malignancy, allergic reactions, inflammatory reactions, and autoimmune manifestations. A diagnosis of malignancy, lymphoma, and granulomatous disease was verified by the pathology reports. The determination of chronic lung disease was based on radiology and pathology results. Pulmonary infiltrates, nodules, fibrocystic parenchymal changes, granulomatous
or lymphocytic infiltrates, bronchiectasis, or a combination of these findings were evaluated as structural lung disease. Clinical history, stool and blood studies, and endoscopy with mucosal biopsy were required for the diagnosis of gastrointestinal disease. Liver function tests, imaging techniques, and specific polymerase chain reaction tests were used for the diagnosis of liver disease.

Patients included in the study were grouped according to the clinical findings (Figure 1). Patients with NIC (Group 1) were further divided into subgroups as patients with AID (Group 1a) and patients with NICs other than AID (Group 1b). Patients who did not have any non-infectious complications were designated as Group 2.

**Laboratory analysis**

To measure serum catalase (CAT) level, erythrocyte superoxide dismutase (SOD) levels, serum malondialdehyde (MDA) levels, and erythrocyte reduced glutathione (GSH) levels, samples were collected just before an IVIG treatment session. Serum samples were stored at −80°C until testing.

**Analysis of CAT.** Serum CAT levels were studied spectrophotometrically by utilizing ammonium molybdate to obtain a yellow stable complex with H₂O₂. The CAT activity was determined at 405 nm on a spectrophotometer and evaluated as mmol H₂O₂ reduced per minute. The results were expressed as kU/l.

**Analysis of SOD.** Erythrocyte SOD measurement was performed by enzyme-linked immune-sorbent assay (Cayman Chemical Company, Catalogue no. 706002, Ann Arbor, MI, USA). Erythrocyte superoxide activity was expressed as U/ml.

**Analysis of MDA.** For MDA, the high-pressure liquid chromatography method, which is based on lipid peroxidation of the thiobarbituric acid and the pink-colored complex production of the last product (MDA) in the acidic medium, was used. The data were expressed as nmol/ml.

**Analysis of erythrocyte reduced GSH levels.** Reduced GSH levels were determined using the methods proposed by Beutler et al. and were expressed as mg/dl.
Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (Windows, Version 22.0. Armonk, NY: IBM Corp.). Descriptive analyzes were performed including medians, minimum, and maximum values, interquartile ranges (IQR), standard deviations for continuous variables and frequency distributions for categorical variables. Chi-Square or Fisher exact tests were used to compare the categorical variables and the Mann-Whitney U test to compare the continuous variables ($p \leq 0.05$).

Results

There were 21 patients and 27 matched healthy controls included in the analysis. Among the patients with CVID and the controls, 11 (52.3%) and 12 (44.4%), respectively were males ($p > 0.05$). All the patients and controls were from the same ethnic group—the Turkish population. The median ages of the patients with CVID was 20 years (min–max: 6–44 years), while the median age of healthy controls was 21 years (min–max: 16–44 years) ($p > 0.05$). The immunoglobulin levels and percentages of lymphocyte subsets of CVID patients at the time of initial diagnosis before the start of IVIG prophylaxis.

Table 1. Immunoglobulin levels and percentages of lymphocyte subsets of CVID patients at the time of initial diagnosis before the start of IVIG prophylaxis.

| Gender $^b$ (Males) | N ($^a$) | Age $^a$ (years, min–max) | At the time of CVID diagnosis |
|---------------------|---------|---------------------------|------------------------------|
|                     | Overall CVID patients | 21 | 20 (6–44) | 11 (52) |
| Group 1a | 4 (19) | 10.5 (7–16) | 3 (75) |
| Group 1b | 9 (42) | 30 (16–43) | 5 (55.5) |
| Overall CVID patients | IgG [mg/dl]$^c$ (IQR) | 264 (165–379) | 16.1 (10.8–36.5) | 14.4 (8.8–16.6) |
| Group 1a | 305 (164–491) | 18.3 (11.9–35.9) | 15.7 (7.8–60.8) |
| Group 1b | 249 (153–356) | 14.3 (9.9–17.7) | 13.4 (6.3–17) |
| Overall CVID patients | IgM [mg/dl]$^c$ (IQR) | 14.4 (10.8–36.5) | 14.4 (8.8–16.6) | 14.4 (8.8–16.6) |
| Group 1a | 15.7 (7.8–60.8) | 15.7 (7.8–60.8) | 15.7 (7.8–60.8) |
| Group 1b | 13.4 (6.3–17) | 13.4 (6.3–17) | 13.4 (6.3–17) |
| Overall CVID patients | IgA [mg/dl]$^c$ (IQR) | 35.2 (26.3–42.7) | 32.6 (22.9–42.1) | 72.9 (66.1–79.8) |
| Group 1a | 30.5 (20.7–36.3) | 30.5 (20.7–36.3) | 30.5 (20.7–36.3) |
| Group 1b | 39.1 (21.5–63) | 39.1 (21.5–63) | 39.1 (21.5–63) |
| Overall CVID patients | CD4 [%]$^c$ (IQR) | 35.2 (26.3–42.7) | 32.6 (22.9–42.1) | 72.9 (66.1–79.8) |
| Group 1a | 30.5 (20.7–36.3) | 30.5 (20.7–36.3) | 30.5 (20.7–36.3) |
| Group 1b | 39.1 (21.5–63) | 39.1 (21.5–63) | 39.1 (21.5–63) |
| Overall CVID patients | CD8 [%]$^c$ (IQR) | 32.6 (22.9–42.1) | 32.6 (22.9–42.1) | 72.9 (66.1–79.8) |
| Group 1a | 30.5 (20.7–36.3) | 30.5 (20.7–36.3) | 30.5 (20.7–36.3) |
| Group 1b | 39.1 (21.5–63) | 39.1 (21.5–63) | 39.1 (21.5–63) |
| Overall CVID patients | CD3 [%]$^c$ (IQR) | 72.9 (66.1–79.8) | 72.9 (66.1–79.8) | 72.9 (66.1–79.8) |
| Group 1a | 66.7 (59.8–73.3) | 66.7 (59.8–73.3) | 66.7 (59.8–73.3) |
| Group 1b | 10.9 (6–17.3) | 10.9 (6–17.3) | 10.9 (6–17.3) |
| Overall CVID patients | CD19 [%]$^c$ (IQR) | 12 (7.2–18.9) | 12 (7.2–18.9) | 12 (7.2–18.9) |
| Group 1a | 22.4 (19–28) | 22.4 (19–28) | 22.4 (19–28) |
| Group 1b | 10.9 (6–17.3) | 10.9 (6–17.3) | 10.9 (6–17.3) |

IQR: interquartile range; CVID: common variable immunodeficiency; AID: autoimmune disease; NICs: non-infectious complications; Group 1a: patients with AID; Group 1b: patients with NICs other than AID.

$^a$Median values.

$^b$Percentages

Table 2. Comparison of CVID patients and healthy controls regarding serum catalase, serum malondialdehyde, erythrocyte superoxide dismutase, and reduced glutathione levels.

| Serum CAT levels (kU/l)$^g$ | 34.78 (18.84–60.86) |
|-----------------------------|-----------------------|
| Erythrocyte SOD (U/ml)$^g$ | 215.78 (190.81–235.67) |
| Serum MDA (nmol/ml)$^g$ | 1.11 (0.75–1.41) |
| Reduced GSH (mg/dl)$^g$ | 85.99 (76.55–102.33) |

CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde; GSH: glutathione.

$^g$Median values (IQR).
sub-analysis of parameters for healthy controls according to gender depicted no statistical difference ($p = 0.39, p = 0.3, p = 0.68, p = 0.23$, respectively).

Figure 1 shows the distribution of subgroups of patients with CVID. Thirteen patients, who had a type of NIC (Group 1), were further analyzed according to presence of AID. Among the patients with AID (Group 1a; $n = 4, 19\%$), one had Sjögren’s syndrome, one had autoimmune alopecia, one had juvenile rheumatoid arthritis, while one had chronic inflammatory demyelinating polyneuropathy. In Group 1b ($n = 9, 42\%$) with complications other than AID, three patients had splenomegaly, two patients had cytopenia associated with hypersplenism, one had granulomatous lung disease, one had splenic granuloma, while four had mediastinal lymphadenopathy without any infectious cause. A comparison of the patients with CVID based on presence of the complications is shown in Table 3.

Patients without a NIC (Group 2; $n = 8$) had erythrocyte SOD levels lower than the patients with NICs ($p = 0.05$). Patients with an autoimmune complication had significantly lower serum CAT levels compared to the ones without AID ($p = 0.03$).

**Table 3.** Comparison of CVID patients according to complications.

|                      | CVID patients ($n = 21$) | p     | CVID patients ($n = 21$) | p     |
|----------------------|--------------------------|-------|--------------------------|-------|
|                      | Group 1 $n = 13$         |       | Group 1a $n = 4$         |       |
| Serum CAT level (kU/l)* | 49.51 (12.08–69.56)     | 0.86  | 18.36 (16.9–27.05)       | 0.03  |
| Erythrocyte SOD level (U/ml)* | 224.08 (195.8–514.3)   | 0.05  | 207.2 (114.2–218.3)     | 0.36  |
| Serum MDA level (nmol/ml)* | 1.12 (0.69–2.14)       | 0.86  | 1.29 (0.8–1.95)         | 0.36  |
| Reduced GSH level (mg/dl)* | 85.36 (67.1–131.5)    | 0.80  | 96.23 (74.4–105.2)      | 0.41  |

|                      | Group 2 $n = 8$         |       | Group 1b $n = 2$         |       |
|                      | 34.3 (16.9–84.05)       |       | 18.36 (12.08–84.0)       |       |
|                      | 198.6 (114.1–258.6)     |       | 218.2 (139.4–514.3)     |       |
|                      | 1.11 (0.66–1.95)        |       | 1.1 (0.66–2.14)         |       |
|                      | 87.1 (65.74–129.9)      |       | 84.74 (65.74–131.5)     |       |

Group 1: patients with NIC; Group 1a: patients with AID; Group 1b: patients with NICs other than AID; Group 2: patients without NICs; NIC: non-infectious complication; AID: autoimmune disease; CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde; GSH: glutathione.

*Median values (min–max).

Discussion

Many defense systems involve nonenzymatic molecules such as glutathione, and vitamins as well as enzymatic scavengers of reactive oxygen species (ROS) such as SOD, CAT, and glutathione peroxidase being the best-known defense systems. Endogenous SOD and CAT are crucial enzymes to keep ROS in balance. In the present study, to unveil the disturbances of oxidative stress in CVID, which is a specific group of primary immunodeficiency with accompanying chronic complications, we studied the biomarkers of oxidative stress. Overall, we found that serum CAT levels were low in patients with CVID, which is suggestive of an altered antioxidant defense system in response to increased ROS. Past studies investigated ROS generation in CVID. Aukrust et al. found that monocytes from patients with CVID had significantly enhanced ROS generation both unstimulated and stimulated. In a later study, Aukrust et al. further demonstrated the increased levels of plasma reduced homocysteine levels and no corresponding rise in plasma levels of total homocysteine in patients with CVID, which might be related to a derangement in oxidative system response. In addition to disturbed homocysteine metabolism, they found significantly elevated levels of MDA in plasma as well. Consistent with this finding, though not statistically significant, in the present study, MDA levels were relatively higher in the CVID group compared to the healthy controls.

Very early reports have demonstrated that redox modulation has a regulatory role in the adaptive immune system and harmonization of T cell activation and proliferation. Dendritic cell and T cell interaction stimulate cystine consumption, so that an extracellular redox potential appropriate for T cell proliferation is produced by cysteine accumulation in the extracellular space. A more reducing extracellular redox potential is reflected in the increased T cell surface thiol status. Before, enhanced oxidative stress in association with persistent immune activation had been linked for patients with CVID and depicted that occurrence of splenomegaly was significantly correlated with the enhanced ROS generation. The findings of chronic activation in CVID have been noted on T cells. Perreau et al. showed the chronic activation on invariable natural killer T cells and Barbosa et al. displayed the activation of monocytes and elevated sCD14 levels in the blood of CVID...
patients as the serum evidence of chronic activation. In a report by Litzman et al.\textsuperscript{22} as an indication of chronic granulocytic activation, neutrophil elastase and myeloperoxidase plasma levels were significantly high in patients with CVID with exacerbations by IVIG administrations. Although CVID is predominantly an antibody production deficiency, it constitutes frequent autoimmune, inflammatory, and immunoproliferative presentations. These diverse spectra of presentations may advocate chronic immune activation. The association of lymphoproliferative phenotype with the imbalance of TH17 and Tregs\textsuperscript{23}; the association of autoimmunity with defects in regulatory T cells, and switched memory-B cells;\textsuperscript{24} uncontrolled T cell polarizations and altered cytokine production in patients with CVID with a high prevalence of bacterial infections\textsuperscript{25} can be considered as some evidence of immune dysregulation in patients with CVID. In the present study, as a sign of immune dysregulation, patients with an AID had significantly lower serum CAT levels. These findings might be associated with the disturbed anti-oxidant defense system due to the complications in CVID. Especially supporting our findings on CAT activity, there are reports showing the crucial role of these enzyme systems in immunity. Catalase transduction of human CD4\textsuperscript{+} T cells had increased intracellular expression and activity of catalase, which led CD4\textsuperscript{+} T cells to become less sensitive to H\textsubscript{2}O\textsubscript{2}-induced loss-of-function.\textsuperscript{26} Catalase was defined as a critical factor for maintaining activated T cells at high density in the expansion of T cells.\textsuperscript{27} In clinical studies, lupus erythematosus patients exhibited significantly lower levels of SOD and CAT in neutrophils/lymphocytes compared to healthy individuals,\textsuperscript{28} and decreased CAT activity has been reported in Crohn’s disease patients.\textsuperscript{29} These results show that catalase is an important modulator of T-cell-mediated immunity. For diseases with underlying chronic immune activation such as CVID and autoimmune diseases, further studies on treatments targeting anti-oxidant systems like catalase seem good candidates as future projections. Moreover, in the present study, the patients without any NIC displayed lower levels of erythrocyte SOD, which might be a sign of lower oxidative stress status compared to patients with NIC. Although not statistically, serum CAT levels of the patients without NIC were lower, as well. A more precise evaluation would be possible with the increment of number of patients in the following studies.

Studies recommended that other immunodeficiencies such as ataxia telangiectasia, Bloom Syndrome, and Nijmegen Breakage Syndrome multifaceted phenotypes were intricately linked to high levels of ROS and mitochondrial dysfunction with a continual activation of cellular stress responses,\textsuperscript{30,31} and impairment of anti-oxidant defenses.\textsuperscript{31,32} Whenever an evidence of oxidative damage in any disease has been found, the major focus of the investigation would be mitochondrial dysfunction, because mitochondria have a crucial role in oxygen metabolism and eventually are the major source of ROS formation.\textsuperscript{33,34} SOD and CAT changed quantitatively in various tissues and cells of individuals with mitochondrial diseases. Imbalance of ROS production induces mitochondrial permeability transition and damages the mitochondrial membrane potential, which triggers apoptosis and necrosis.\textsuperscript{35} Mitochondrial disorders, for which further studies are still required, might be a key point for evaluation of the source of oxidative stress in patients with CVID, with the present findings in erythrocyte SOD and CAT levels in serum.

Our study has several limitations. Firstly, findings from this study should be interpreted cautiously, since the study took place in an uncontrolled clinical setting using an observational design. Secondly, the number of patients was low, and all patients were from a single center. In the study, patients with CVID have a wide range of age groups, and they had different durations since the diagnosis and start of IVIG replacement therapy. Analysis of B cell subtypes at the time of initial diagnosis, which might define the distribution of immunologic characteristics of the patients better, were not performed due to lack of infrastructure. In addition, the oxidative stress markers were not evaluated before and after the complications. As a future projection, this type of evaluation may be valuable to foresee the complications of CVID. The long-term effect of IVIG therapy on the oxidative stress responses in CVID is not clear yet, which limited the interpretation of the cause-and-effect relation in the present study. Since CVID has diverse types of presentations including autoimmunity and malignancy, different cellular stress pathways such as endoplasmic reticulum stress responses, and cellular markers of oxidative responses may be investigated in the future.
Conclusion

Although a preliminary data, in the present study, the analysis of a markers of oxidative stress such as serum CAT and erythrocyte SOD levels in patients with CVID with and without complications suggested some abnormalities in responses to ROS. The present data with further clinical syndrome associations may be a beneficial tool for future studies, in order to set prediction markers for different clinical presentations of CVID.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval for this study was obtained from local ethical committee of Uludag University (Approval number: 2009-9/44)

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Informed consent

At the time of enrollment of the patients and the healthy controls in the study, written informed consent was taken from the participants over 18 years of age, and the legally authorized representatives of the participants younger than and equal to 18 years of age.

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