Alteration of T-cell subpopulations and lipid peroxidation in the blood of patients with vulvar non-neoplastic epithelial disorder

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Summary

Objective: To determine the relationship between vulvar non-neoplastic epithelial disorder and thymus-dependent lymphyocyte levels and lipid peroxidation. *Materials and Methods*: the authors measured the levels of CD3+, CD4+, CD8+, CD16+ T cell, and the concentration of superoxide dismutase (SOD) and malondialdehyde (MDA) in the blood of 62 patients with vulvar non-neoplastic epithelial disorder. A control group consisted of 30 normal women from the present hospitals. *Results:* The level of CD4+/CD8+ T-lymphocytes and SOD in the blood of the patients with vulvar non-neoplastic epithelial disorder was significantly lower than that in control subjects, but the level of MDA was higher as compared with normal women. *Conclusion:* There is increased immune activation and lipid peroxidation in patients with vulvar non-neoplastic epithelial disorder, which could contribute to destruction of vulvar tissue.

Key words: Vulvar non-neoplastic epithelial disorder; Thymus-dependent lymphyocyte; Lipid peroxidation; Superoxide dismutase.

Introduction

Non-neoplastic epithelial disorder of vulva, also known as vulvar white lesions, including squamous cell hyperplasia, lichen sclerosus (LS), lichen planus and lichen simplex chronicus, etc., is a form of dermatosis characterized by ivory-colored, severe pruritus and decreased tissue elasticity of the vulva and perianal skin. The cause remains uncertain, and the disorder is difficult to cure [1,2]. To investigate immune status and potential mechanisms of tissue injury in patients with vulvar non-neoplastic epithelial disorder, the authors determined levels of CD3+, CD4+, CD8+, and CD16+ T cell subsets, and assessed parameters relevant to peroxidation, including expression levels of superoxide dismutase (SOD) and malondialdehyde (MDA). The purpose of this study was to expand the authors' understanding of the potential role of the immune system and lipid peroxidation in patients with vulvar non-neoplastic epithelial disorder, to find the evidence of immune and lipid peroxidation-mediated impairment of cells, and to explore the pathogenic mechanism of vulvar non-neoplastic epithelial disorder.

Materials and Methods

The authors recruited 62 patients with vulvar non-neoplastic epithelial disorder who had been treated in the vulvar disease policlinic of the present hospitals from June 2001 to December 2002. Mean age of patients was 37.8 ± 9.2 years. All cases were identified histologically, and included 26 cases of squamous hyperplasia (SH), 23 cases of lichen sclerosus (LS), and 13 cases mixed histological type. In all cases, immune disease and acute and chronic diseases affecting patients' lipid peroxidation were excluded by clinical history and physical examination. Patients had no drug history affecting their immune status or lipid peroxidation condition. Mean age of the control group of 30 normal women was 39.2 ±10.6 years, which was not statistically different from patients ($p > 0.05$).

In all patients, six ml of venous blood was obtained by phlebotomy in the morning after an overnight fast. Of the six ml total, three ml was treated with an anticoagulant for detection of T-cell subsets (CD3+, CD4+, CD8+ and CD16+). The remaining three ml of blood was centrifuged, and serum was separated and stored in a refrigerator at 0-4^{0}C for measurement of SOD and MDA.

CD3+, CD4+, CD8+, and CD16+ T cells were examined with an immunofluorescence flow cytometer; using the fluorochrome propidium iodide and an RNA enzyme.

SOD activity was determined by the nitrite method. Nitrite unit per milliliter of serum acts as active unit of SOD. Reagent and colored immune board were provided by the Molecular Biology Center of China PLA Navy General Hospital.

MDA activity was examined using thiobarbituric acid (TBA) methods. The red product derived from compound of MDA and TBA has the highest absorption peak at 532 nm, the content (μmol/L) and can be measured with a 722 spectrometer.

Statistical analysis included Student's t-test, analysis of variance and linear correlation. A p value less than 0.05 was considered significant.

Results

There appeared to slightly lower levels of CD3+ and CD4+ T cells and slightly higher levels of CD8+ and CD16+ T cells in patients compared to controls, but these differences were not statistically significant. However, the CD4+/CD8+ ratio was significantly lower in patients compared to controls ($p < 0.05$, Table 1).

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Table 1. — *T lymphocyte subsets in patients with vulvar non-neoplastic epithelial disorder compared to control subjects.*

	CD3+	CD4+	CD8+	$CD4^+/CD8^+$	CD16
Control	67.63±9.65	37.01±7.69	25.35±6.75	1.55±0.58	16.53±7.64
Vulvar white lesions	63.58±10.49	34.5±6.58	28.57±9.37	1.23±0.51	18.24±6.7
p	>0.05	>0.05	>0.05	<0.01	>0.05

Serum levels of SOD in patients were significantly less than those of the control group, however serum MDA levels were higher in patients compared to controls ($p < 0.05$ for both comparisons, Table 2).

There were no significant differences detected among different types of vulvar non-neoplastic epithelial disorder in blood level of T cell subsets, serum SOD, or serum MDA ($p > 0.05$ for all comparisons).

Linear correlation analysis demonstrated a significant positive correlation between serum levels of SOD and CD4+/CD8+ ratio, CD3+ or CD4+, as well as MDA and CD8+ or CD16+ ($r = 0.66$, $p < 0.05$ for all comparisons).

Table 2. — *Serum levels of SOD and MDA in patients with vulvar non-neoplastic epithelial disorder compared to control subjects.*

	n	SOD	MDA
Control	30	23.81±4.53	3.25±0.58
Vulvar white lesions	62	18.24±3.36	4.01±0.64
p		<0.01	<0.01

Discussion

Vulvar non-neoplastic epithelial disorder is a degeneration and pigmental change caused by dystrophy of the skin and mucous membranes of the vulva. The exact cause is unclear, but is probably related to localized nerve and vascular dysfunction, stimulation of epidermal metabolites, and lack of estrogen, but the potential relationship with infection, immunity, and abnormal expression and/or function of SOD has not been well-studied and available data are conflicting [1-4].

Cluster of differentiation (CD) antigens are cell surface proteins or glycoproteins appearing or disappearing in lineage-specific leukocytes during specific stages of differentiation and activation. Besides serving to identify cell lineage and stage of development and activation, CDs extensively participate in development, maturation, differentiation, growth, migration and activation of cells, promote the interaction of immune molecules, and regulate cell-matrix adhesive interactions [5,6]. The principle function of CD3 in leukocytes is to stabilize the structure of TCRs and transmit signals activating T cells. When T cell receptors (TCRs) recognize and bind antigens, cells expressing CD3 participate in delivering signals to cytoplasm of T cells as the first signal inducing T cell activation. CD4 antigen is expressed on the cell surface of some T lymphocytes, thymus cells, B lymphocytes, B cells transformed by Epstein-Barr (EB) virus, monocyte / macrophages and brain cells, regulates adhesion and signal transduction, and participates in the pathogenesis of most autoimmune diseases. CD4 antigen mainly plays a role in the elimination of activated activated protein Cs (APCs) and T cells, thereby limiting the immune response. CD8+ cells can induce adhesion of cells and act as signal transduction molecules, but mainly recognize and kill tumor cells and virus-infected cells. With the development of modern immunology, much has been regarding about T cells surface markers and their biological function. Both CD4+ and CD8+ cells include subsets which can induce either up- or down-regulation. By interactions among various cell types (especially various T cells subsets), the organism can maintain normal immune status and induce an appropriate immune response to eliminate foreign pathogens, while not causing harm to the host organism. Usually, the total numbers of T cells and T cell subsets is relatively constant. If the total T cells or the ratio of CD4+/CD8+ changes, the function of immune regulation can be considered abnormal. CD16 antigen is a surface marker expressed by natural killer (NK) cells. NK cells are special lymphocytes, are different from T and B lymphocytes, and play an important role in killing target cells. The lethal effect on target cells of NK cells is rapid; NK cells can destroy target cells in less than four hours, and this process does not require advance sensitization. The target cells include tumor cells, viruses, bacterium-infected cells, and some normal cells [7].

Our study found a trend towards a decrease in levels of CD3+ and CD4+ T cells in patients with vulvar non-neoplastic epithelial disorder, though the difference compared to control subjects was not statistically significant. This may be related to an insufficiency in immune ability to remove activated APCs and T cells, leading to local pathological changes. The present results indicate there may be an excessive immune response that leads to pathologic damage and is reflected by increasing levels of CD8+ and CD16+ cells, and decreasing CD4+/CD8+ cell ratios in patients with vulvar non-neoplastic epithelial disorder.

In the course of normal metabolism, oxygen-free radicals are produced as the byproducts of enzymatic and non-enzymatic reactions, and can attack unsaturated fatty acids in cell membranes and cause lipid peroxidation. Lipid peroxides may interfere with normal cell metabolism and function. Antioxidants that protect against oxygen-free radical damage include SOD and various superoxidases. SOD has an extremely important effect on the balance of oxidation and reduction, and can rapidly dismute 2 superoxide anion radicals, thus reducing oxidant stress and minimizing the

potential for cellular damage. When local expression of SOD in skin tissues is decreased, free radicals can more readily form and accumulate. Increased local concentrations of free radicals will tend to damage cells and tissues of collagenous fibers, the reticular fibers, the elastic fibers, and a number of macromolecules within blood vessels and nerves including proteins, nucleic acids, and lipids, and this in turn could promote dystrophy of vulva by destroying the physiological structure, metabolism, and sources of nutrition for vulvar tissues [8-11]. In this study, levels of MDA were significantly increased and SOD was obviously decreased in patients with vulvar non-neoplastic epithelial disorder. These results suggest that lipid peroxidation was promoted, and antioxidation defenses diminished in patients with vulvar non-neoplastic epithelial disorder. The increase in oxygen-free radicals and their metabolites that would be expected to result from this imbalance would tend to damage the skin and mucus of the vulva of patients.

In conclusion, the results of the present study suggest that patients with vulvar non-neoplastic epithelial disorder simultaneously exhibit excessive immunoreaction and increased production of free radicals, raising the possibility that these two effects combine to importantly contribute to pathologic features of vulvar non-neoplastic epithelial disorder. The authors found no significant differences in these parameters between different tissue types of vulvar non-neoplastic epithelial disorder, suggesting that they may similar immune mechanisms contribute to different forms of vulvar non-neoplastic epithelial disorder. Differences in pathological behavior are likely to related to other factors in the progression of the disease.

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