Serum concentrations of metalloproteinase 2, metalloproteinase 9 and granzyme B in contact eczema patients

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Postep Derm Alergol 2013; XXX, 2: 73–76
DOI: 10.5114/pdia.2013.34154

Abstract

Introduction: Contact eczema is a common skin condition with complex etiology, variable clinical presentation and lengthy therapy duration. The mechanism of contact eczema is complex, since it is affected by multiple inflammatory mediators.

Aim: To assess concentrations of metalloproteinase 2 (MMP-2), metalloproteinase 9 (MMP-9) and granzyme B (GzmB) in patients with contact eczema.

Material and methods: Seventy patients with contact eczema and 30 healthy persons as controls were included in the study. In all subjects, MMP-2, MMP-9 and GzmB were determined using ELISA immunoassay. In study group patients, concentrations were assayed in periods of disease exacerbation and remission. Obtained results were analyzed statistically.

Results: Mean MMP-2 and GzmB concentrations were found to be significantly higher in the study group than in the control group. Mean MMP-2, MMP-9 and GzmB levels were also statistically significantly higher during skin lesion relapse compared to contact eczema remission periods.

Conclusions: The presented paper demonstrates that MMP-2, MMP-9 and GzmB are good markers of contact eczema exacerbations.

Key words: contact eczema, metalloproteinase, granzyme.

Introduction

The term "contact eczema" groups diseases of varied etiology that are similar in clinical presentation and course. In contact eczema, the pathogenic factor provokes the disease by direct skin contact. Factors causing contact eczema include, among others, irritating factors and allergens. The contact eczema group includes allergic contact dermatitis (ACD), irritant contact dermatitis (ICD), protein eczema and contact urticaria.

Irritant contact dermatitis is a local inflammatory skin reaction of non-immune mechanism that involves release of cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), which induce adhesion molecule expression on keratinocytes and vascular endothelium. Allergic contact dermatitis pathomechanism is based on Gell-Coombs' type IV immune reaction.

Skin symptoms and inflammatory lesions in type IV hypersensitivity reaction result from action of both Th and Tc cells. Tc lymphocytes, as part of the cytotoxic action, release, among others, granzymes, which induce apoptosis in the caspase-dependent and independent pathway. Granzymes are a family of serine proteases. They are indispensable in cytolytic granule-dependent cytotoxic reaction. They can directly induce target cell apoptosis through their proteolytic properties. Penetration of granzymes into the target cell is possible through canals created by perforin, endocytosis, and crossing the cell membrane due to

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Received: 20.03.2012, **accepted:** 28.02.2013.

presence of mannose-6-phosphate receptor in the membrane [1-4].

Some pathologic skin processes may arise from compromise of synthesis-degradation balance of extracellular matrix, caused by, among others, changes in metalloproteinase genes expression or individual matrix metalloproteinase (MMPs) activity [5, 6]. The MMPs are a family of metal-dependent endopeptidases, which break down extracellular matrix. Their activity is related to presence of active zinc (Zn^{2+}) and calcium (Ca^{2+}) ions [7, 8]. Inflammatory infiltration cells (monocytes, T lymphocytes, leucocytes) are the main source of MMPs. Known MMPs vary in specificity of substrates which they digest. The MMP-2 and MMP-9 are gelatinases, which break down type IV, V, VII, IX collagen, fibronectin, and elastin [9-12].

Aim

The aim of the paper was to assess serum concentrations of MMP-2, MMP-9 and GzmB in patients with contact eczema. Differences in assessed parameters in periods of disease exacerbation and remission were studied.

Material and methods

One hundred patients participated in the study. The study group included 70 contact eczema patients: 41 females and 29 males, aged 18-70 years (mean age: 40.3 years), who had no history of chronic and severe systemic diseases and therapies, which would influence the study results. The control group was composed of 30 healthy people: 25 females and 5 males, aged 20-60 years (mean age: 35.6 years).

To assess serum concentrations of MMP-2, MMP-9 and GzmB, venous blood was drawn twice from 50 patients out of a 70-patient study group (20 did not consent to repeated blood draw) during contact eczema exacerbation and remission and once from control group subjects (30 patients).

With use of Vacutainer closed system, 5 ml of venous blood was drawn to a tube containing clotting activator. The labeled tube was left for a few minutes in room temperature to form a clot. Then, tubes were centrifuged at 3000 rpm for 10 min. Obtained serum was frozen at a temperature below –20°C and stored in these conditions for further examinations. On the day of concentration assessment, tubes with serum were thawed gradually in room temperature.

Serum concentrations of MMP-2, MMP-9 and GzmB were determined with ELISA kits manufactured by R&D Systems, Inc., USA and eBioscience, USA. Assays were performed in accordance with manufacturer's guidelines. Reference ranges provided by the manufacturer were: 161-301 ng/ml, 2.0-139.4 ng/ml, and 0.8-24.1 pg/ml for MMP-2, MMP-9, and GzmB, respectively.

Statistical analysis

To verify the hypotheses put forward, parametric and nonparametric significance tests (*t*-Student test, modified Cochran-Cox test, Shapiro-Wilk test, F Snedecor test) were performed. A significance level of $p = 0.05$ was assumed as reliable for verifying the proposed hypotheses. A difference was assumed statistically significant at $p < 0.05$.

Table 1. Comparison of MMP-2 concentrations

Variables		Patients	
		Contact eczema patients	Control group
MMP-2	*n*	70	30
	Min	123.6	126.5
	Max	498.5	262.5
	Median	196.3	171.9
	Mean	219.6	171.9
	SD	74.2	27.8
Shapiro-Wilk test	*W*	–	0.940
	W_{kr}	–	0.927
	Normality	–	Yes
F Snedecor test ($F_{kr} = 1.74$)	*F*	7.13	
	p	< 0.0001	
Cochran-Cox test ($C_{kr} = 2.01$)	*C*	4.67	
	p	< 0.0001	

n – number of respondents, SD – standard deviation, W, F, C – value W_{kr}, F_{kr}, C_{kr} – critical value, p – significance level

Results

Mean MMP-2 concentrations were found to be significantly higher in the study group than in the control group (Table 1). Mean MMP-2 levels were also statistically significantly higher during skin lesion relapse compared to contact eczema remission periods (Table 2).

There were no statistically significant differences between mean concentrations of MMP-9 in the study and control groups (Table 3). Mean MMP-9 levels were statistically significantly higher during skin lesion relapse compared to contact eczema remission periods (Table 2).

Mean concentrations of GzmB were significantly higher in the contact eczema group than in the control group (Table 4). Mean GzmB concentrations were statistically significantly higher during skin lesion relapse compared to contact eczema remission periods (Table 2).

Discussion

Contact eczema is a chronic, recurring condition, and is often difficult to diagnose and treat. The mechanism of

Table 2. Mean differences of MMP-2, MMP-9 and GzmB concentrations during contact eczema exacerbations and remissions

		Gzm B (B-A)*	MMP-2 (B-A)	MMP-9 (B-A)
Mean changes of measured parameters	*n*	50	50	50
	Mean	–14.0	–29.4	–14.1
	SD	26.7	44.8	37.7
***t*-Student test (T_{kr} = 2.01)**	*T*	3.67	4.59	2.62
	p	0.0006	< 0.0001	0.011

**B – second serum draw (remission), A – first serum draw (exacerbation), T_{kr} – critical value, T – value*

Table 3. Comparison of MMP-9 concentrations

Variables		Patients	
		Contact eczema patients	Control group
MMP-9	*n*	70	30
	Min	56.2	82.0
	Max	312.5	176.9
	Median	136.2	144.6
	Mean	142.72	142.70
	SD	45.8	24.5
Shapiro-Wilk test	*W*	–	0.928
	W_{kr}	–	0.927
	Normality	–	Yes
F Snedecor test (F_{kr} = 1.74)	*F*	3.49	
	p	0.0002	
Cochran-Cox test (C_{kr} = 2.02)	*C*	0.003	
	p	0.998 (NS)	

NS – not statistically significant

Table 4. Comparison of GzmB concentrations

Variables		Patients	
		Contact eczema patients	Control group
GzmB	*n*	70	30
	Min	10.8	0.8
	Max	670.1	48.2
	Median	38.8	19.9
	Mean	72.8	22.1
	SD	98.7	13.4
Shapiro-Wilk test	*W*	–	0.955
	W_{kr}	–	0.927
	Normality	–	Yes
F Snedecor test (F_{kr} = 1.74)	*F*	54.2	
	p	< 0.0001	
Cochran-Cox test (C_{kr} = 2.00)	*C*	4.21	
	p	< 0.0001	

contact eczema is complex. The mechanism of clinical symptoms is different depending on allergic or non-allergic character of eczema.

Numerous studies revealed the role of selected MMPs in pathogenesis of several skin diseases, such as herpetiform dermatitis [9], atopic dermatitis [13] and systemic scleroderma [14]. The role of MMPs in contact eczema is poorly understood, and results of studies published to date in the field are scarce. The presented paper demonstrates that mean concentrations of both MMP-2 and MMP-9 are higher during recurrences than during remissions of contact eczema, what may stand for the role in disease pathomechanism. Khorramizadeh *et al.* [15] revealed a MMP-2 activity increase in skin biopsy specimens from ACD patients. Gianelli *et al*. [16] demonstrated an increased expression of MMP-2 and MMP-9 genes in ACD patients, and MMP-9 gene expression was higher than that of MMP-2 gene.

Studies on effects of GzmB on inflammatory processes in skin have been very innovative and very recent. Other authors reported participation of GzmB in pathogenesis of various dermatoses, including lupus erythromatosus, drug-induced toxic epidermolysis and Stevens-Johnson syndrome, and in lichen sclerosus et atrophicus [17, 18]. The presented paper reveals that mean levels of GzmB in serum from patients with contact eczema are higher than in control patients and that concentration of this protein increases as the disease relapses. Ku *et al.* [19] demonstrated usefulness of GzmB as an allergic reaction marker. They observed an increase in serum GzmB levels in mice with allergic hypersensitivity after prior allergen exposure. In a study by Senoh *et al.* [20], the authors demonstrat-

ed presence of GzmB in cellular infiltration in patients with erythema multiforme caused by occupational exposure to DBNPA (2,2-dibromo-3-nitrilopropionamide). Increased synthesis of GzmB and perforin was found in patients with psoriasis and atopic eczema [21].

Conclusions

The paper is one of few reports on concentration of the assayed markers in periods of exacerbation and remission of contact eczema. Significant differences of MMP-2, MMP-9 and GzmB in periods of exacerbation and remission of skin lesions were found. The obtained results may advocate developing of assessed marker inhibitors for therapy. Further studies in the field are needed.

Acknowledgments

The study funded by the "Scholarship for doctoral students – Integrated Development Regional Operational Programme".

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