Brief Report

DOI: 10.5582/bst.2018.01118

Serum cytokine profiles are altered in patients with progressive infantile hemangioma

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Summary **Infantile hemangioma sometimes grows rapidly to a significant size around the first 2 months of life, which can be problematic and even destroy normal tissue. However, it is very difficult to predict the tumor growth at the first visit and to decide necessity of treatment. Therefore the identification of the biomarkers that can indicate a tendency to grow is clinically very important. In the present study, we evaluated the possibility that serum cytokine levels are available as the marker of hemangioma growth. Progressive hemangioma was defined as a lesion showing increased tumor size and/or coloration two weeks before and after the serum sampling, and we used membrane array to compare the twenty cytokine profiles between the sera of 3 progressive hemangioma patients and sex-/age-matched non-progressive hemangioma patients. As a result, many of the 20 cytokines were detected in the patients' sera. When a 2-fold difference in the mean levels of each group was considered meaningful, 6 of the 20 cytokines (IGF-1, IL-6, IL-8, PIGF, RANTES, TGF-β1) were down-regulated in the progressive hemangioma group compared to the non- progressive hemangioma group, and there were statistically significant difference ($p < 0.05$): especially, IGF-1, IL-6, IL-8, PIGF, and TGF-β1 did not expressed in all 3 progressive hemangioma patients. Accordingly, complicated cytokine network by these multiple cytokines may control the pathogenesis, and these cytokine levels may become clinically useful tumor markers. Furthermore, immunotherapy against them will be novel therapeutic approach.**

Keywords: Cytokine, IGF-1, IL-8, Infantile hemangioma, RANTES

1. Introduction

Infantile hemangiomas ("strawberry marks") are a vascular tumor caused by endothelial cell proliferation, typically appear on head or face around the second week of life (*1*). The lesions grow till one year (proliferating phase), and slowly regress over several years (involuting phase) (*2*). Solitary and small lesions without cosmetic or functional problem can be managed according to 'wait and see policy' without treatment because of the spontaneous regression. On the other hand, a part of hemangiomas grows rapidly to a significant size, which can be problematic and even destroy normal tissue (*3*). Such lesions cause complication (*e.g.* skin ulcer, a visual impairment, airway obstruction, and heart failure), or threaten life. Furthermore, larger lesions may result in the persistent scar formation, telangiectasia, fibrofatty tissue, or skin slackening (*4*).

As one of the clinical issues of this skin tumor, the most proliferation occurs around the first two months of life, and the majority of hemangioma growth is completed by five months of age (*5*). However, because the first visit to the general dermatologist may be around one month after birth, and it is very difficult to predict the tumor growth at that time and decide necessity of treatments. We often experience that a small lesion at the first visit become large and problematic at the next visit. Therefore the identification of the biomarker that can indicate a tendency to grow

Released online in J-STAGE as advance publication August 26, 2018.

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Table1. Clinical features of infantile hemangioma cases in this study

cases	age	sex	site	type	feature
1	1 month	F	cheek	superficial	progressive
2	3 month	F	eyelid	mixed	progressive
3	2 month	F	forearm	superficial	progressive
4	1 month	F	cheek	superficial	non-progressive
5	3 month	F	eyelid	deep	non-progressive
6	3 month	F	eyelid	superficial	non-progressive

is clinically very important. In the present study, we evaluated the possibility that serum cytokine levels are available as the marker of hemangioma growth using sera of patients with progressive lesions.

2. Materials and Methods

2.1. *Clinical assessment and patient material*

Serum samples were obtained from 3 progressive infantile hemangioma patients. Control serum samples were obtained from 3 non-progressive infantile hemangioma patients (Table 1). Progressive hemangiomas are defined as lesions showing increased tumor size and/or coloration two weeks before and after the serum sampling. Institutional review board approval and written informed consent were obtained before patients were entered into this study according to the Declaration of Helsinki. The informed consent from the guardian of the children to use the photographs for publication was also obtained.

2.2. *Measurement of serum cytokine levels*

Serum levels of 20 cytokines (Angiogenin, EGF, ENA-78, bFGF, GRO, IFN-γ, IGF-1, IL-6, IL-8, LEPTIN, MCP-1, PDGF-BB, PIGF, RANTES, TGF-β1, TIMP-1, TIMP-2, Thrombopoietin, VEGF, VEGF-D) were measured with Human Angiogenesis Antibody Array-Membrane (Abcam, Cambridge, UK). Monoclonal antibody for each cytokine was precoated onto microtiter wells. Aliquots of serum were added to each well, and then incubated with cocktail of biotin-conjugated antibodies to each cytokine, after that incubated with labeled streptavidin.

2.3. *Statistical analysis*

Statistical analysis was carried out with Mann-Whitney's *U* test for the comparison of medians. *P* values less than 0.05 were considered significant.

3. Results and Discussion

In this study, three infants with progressive hemangioma and sex-/age-matched three non-progressive hemangioma were included: Progressive

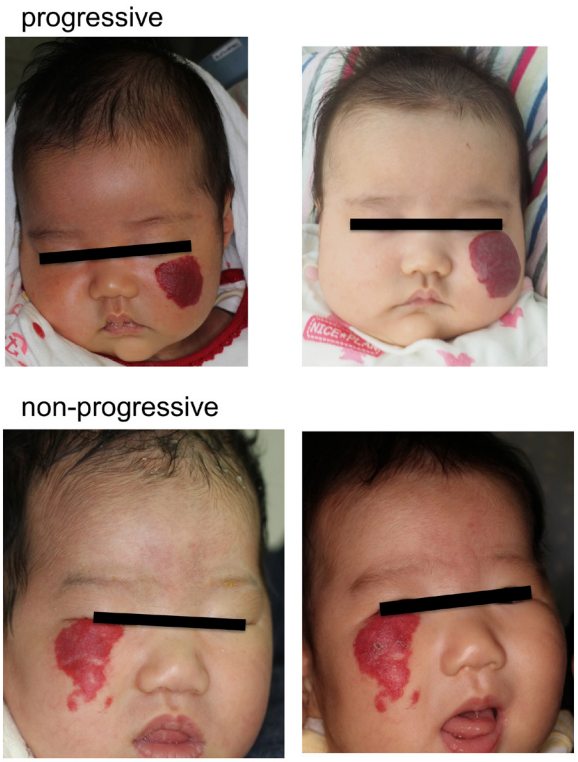

Figure 1. Clinical pictures of infantile hemangioma patients in this study. (upper left) case 1 with progressive hemangioma at 1 month after birth; **(upper right)** case 1 with progressive hemangioma at 2 month after birth; **(lower left)** case 4 with non-progressive hemangioma at 1 month after birth; **(lower right)** case 4 with non-progressive hemangioma at 2 month after birth.

hemangiomas showed increased tumor size and color during two weeks before and after the serum sampling (Figure 1). Progressive hemangiomas were found in one-month girl on her cheek (superficial type), three-months girl on her eyelid (mixed type), and two-months girl on her forearm (superficial type). On the other hand, non-progressive hemangiomas were in one-month girl on her cheek (superficial type), three-months girl on her eyelid (deep type), and three-months girl on her eyelid (superficial type). The clinical characteristics of all patients were summarized in Table 1.

Cytokine expression profiles in sera of infantile hemangioma infants were analyzed using commercially available membrane array kits (Figure 2). Many of the 20 cytokines were detected in the patients' sera. Signal densities for each antigen-specific antibody spot were

POS	POS	NEG	NEG	angiogenin	EGF	ENA-78	bFGF
POS	POS	NEG	NEG	angiogenin	EGF	ENA-78	bFGF
GRO	IFN-γ	IGF-1	IL-6	IL-8	LEPTIN	MCP-1	PDGF-BB
GRO	IFN-γ	IGF-1	IL-6	IL-8	LEPTIN	MCP-1	PDGF-BB
PIGF	RANTES	TGF-β1	TIMP-1	TIMP-2	TSP	VEGF	VEGF-D
PIGF	RANTES	TGF-β1	TIMP-1	TIMP-2	TSP	VEGF	VEGF-D
BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	NEG	POS
BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	NEG	POS

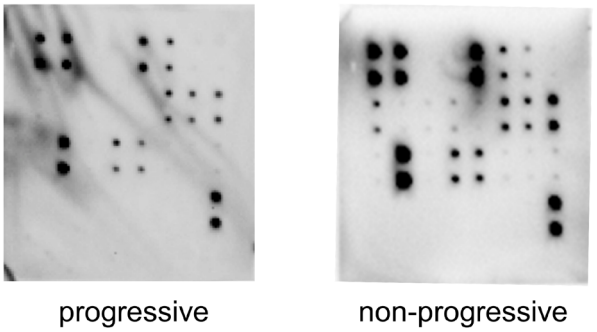

Figure 2. Results of human angiogenesis antibody array. (upper panel) array maps used in this study; **(lower panel)** representative membrane in each group.

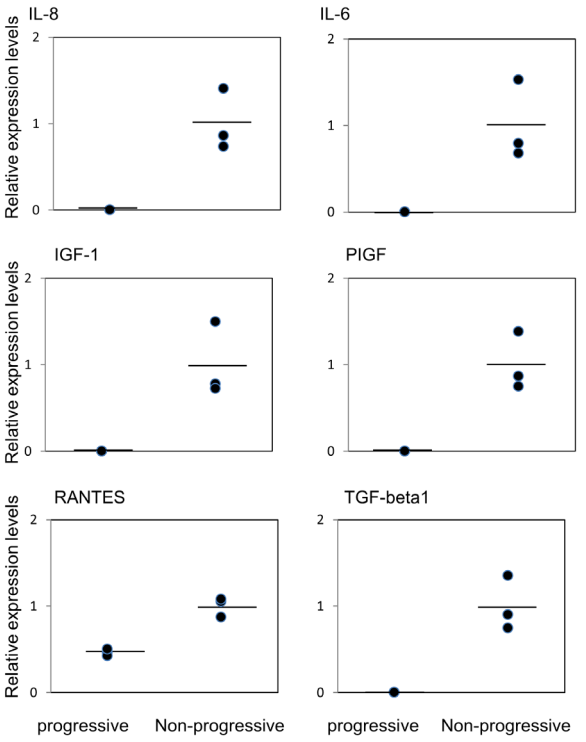

Figure 3. The cytokine expression levels measured by the array. The relative expression levels of six cytokines measured by the array using sera of progressive or non-progressive hemangioma patients are shown on the ordinate. Bars show means. *$p < 0.05$.

obtained using 2-D densitometry, and quantitated. When a 2-fold difference in the mean levels of each group was considered meaningful, 6 of the 20 cytokines (IGF-1, IL-6, IL-8, PIGF, RANTES, TGF-β1) were down-regulated in the progressive hemangioma group compared to the non- progressive hemangioma group, and there were statistically significant difference ($p < 0.05$, Figure 3): notably, levels of five cytokines except for RANTAS were not detected in all of progressive hemangioma group.

Various cytokine may participate in the pathogenesis of the infantile hemangioma. As an example, the involvement of VEGF, angiopoietin (*6*), TGF-β (*7*), TNF-α, and IL-1 (*8*) have already been reported. Furthermore, clinical significance of serum cytokine levels has also been evaluated in this disease. Serum levels of VEGF were elevated in infantile hemangioma at the proliferative or involuting phase. Serum MCP-1 and MIP-1β can be the marker of regression (*9*). bFGF levels were not available for the prediction of the clinical course (*10*). Furthermore, Jiang *et al.* reported that angiogenin levels are increased in the sera of proliferative hemangioma (*11*), and suggested as the biomarker. The authors defined proliferative hemangioma or involuting hemangioma as 1-6 month age or 13-36 month age after birth, respectively, and did not describe the tendency of tumor growth. However, the duration of proliferation phase varies by individual patient.

Accordingly, the serum levels of single cytokine may not be useful to predict tumor growth, but the profile of multiple cytokines can be more sensitive tumor marker. In the present study, we tried to determine multiple serum cytokines at the same time using array experiment. Furthermore, we defined progressive hemangioma as lesions showing increased tumor size and coloration two weeks before and after the serum sampling, and compared the cytokine profiles between progressive hemangioma and non-progressive hemangioma at the similar age to really identify clinically useful tumor marker.

As a result, we demonstrated that IGF-1, IL-6, IL-8, PIGF, RANTES, and TGF-β1 levels were significantly decreased in patient with progressive lesions: especially, IGF-1, IL-6, IL-8, PIGF, TGF-β1 did not expressed in all 3 progressive hemangioma patients. So far, there has been no report describing involvement of IGF-1, IL-8, or RANTES in the pathogenesis of hemangioma. However, IGF-1 is reported to promote migration and tube formation of endothelial cells (*12*). IL-8 directly enhanced endothelial cell survival, proliferation, and regulated angiogenesis (*13*). RANTES is thought to have pro-angiogenic effect (*14*). Thus, these cytokines may positively affect tumor growth. On the other hand, IL-6 suppresses endothelial proliferation (*15*), whereas the expression of IL-6 is increased in tissues of proliferative hemangioma in comparison with involuting hemangioma (*16*). In addition, the activation of VEGF receptor 1 by PIGF promotes signal transduction of VEGF receptor 2 by VEGF and cooperatively stimulates angiogenesis. The

mRNA levels of PlGF tended to be lower in infantile hemangioma when compared to other vascular lesions (*17*). TGF-β1 expresses in infantile parotid hemangioma (*7*), although the role are still unknown. The response of endothelial cells to TGF-β1 is dependent upon cell types or proliferative state (*18*). Accordingly, complicated cytokine network by these multiple cytokines may control the pathogenesis. Unexpectedly, cytokines that positively control endothelial proliferation (IGF-1, IL-8, RANTES) is decreased in serum of the hemangioma of the proliferative phase. Although its mechanism cannot be explained by our result, for example, we speculated that negative feedback mechanism against their increased expression in tumor tissue may cause the down-regulation of serum levels.

Because this is a pilot study using small number of patient samples, larger study with increased number of cases will be necessary in future. Furthermore, immunotherapy against these cytokines may become novel therapeutic approach.

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(Received May 27, 2018; Revised August 13, 2018; Accepted August 17, 2018)