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SERUM LEVEL OF VEGF-D IN PATIENTS WITH PRIMARY LYMPHEDEMA

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ABSTRACT

Recent studies have indicated that vascular endothelial growth factor-D (VEGF-D) stimulates lymphangiogenesis in humans. Furthermore, mutations of vascular endothelial growth factor receptor 3 (VEGFR-3) have been observed in families with hereditary lymphedema. The lack of stimulation of lymphangiogenesis could lead to production of even more VEGF-D to obtain stimulation of lymphangiogenesis resulting in a high serum level of VEGF-D.

The aim of the present study was to compare the serum level of VEGF-D in patients with primary lymphedema with healthy controls.

In a prospective study, the serum level of VEGF-D was determined by a solid phase ELISA in patients with primary lymphedema and compared with healthy controls.

In the group of patients with primary lymphedema the serum level of VEGF-D was significantly higher compared with controls (p=0.0047).

The increased levels of VEGF-D observed in the present study suggest that primary lymphedema may be based on defective stimulation of VEGFR-3.

Lymphatic vessels play the central role in maintaining the fluid balance of the interstitial tissues. The development of primary lymphedema is based on a congenital deformity of the lymphatic system that causes a decreased transport capacity of lymphatic vessels. Therefore, protein-rich fluid collects in the tissues causing chronic swelling (1).

Recent studies have focused on the molecular mechanism regulating the lymphatic vessels (2-4). The proteins that control lymphangiogenesis in humans are vascular endothelial growth factor-C (VEGF-C) and vascular endothelial growth factor-D (VEGF-D) (5-8). VEGF-C and VEGF-D are members of the VEGF- family. They control lymphangiogenesis in humans (6,7) by activating the vascular endothelial growth factor receptor 3 (VEGFR-3) (2,8-11), which in adults is mainly restricted to lymphatic endothelium (4,7). Both factors are the only known ligands for VEGFR-3 (6,12). When VEGFR-3 is stimulated, new lymph vessel formation occurs by sprouting from preexisting lymphatic capillaries (2). Additionally, it could be demonstrated that the VEGFR-3 stimulation protects the lymphatic endothelial cells from apoptosis and induces their growth and migration (3).

Recent clinical studies have shown mutations of VEGFR-3 in some families with hereditary lymphedema (13-16).

Thus, the question arises if there is a different serum level of VEGF-D in patients with primary lymphedema compared to those without.

Therefore, the aim of the present study was to compare the serum level of VEGF-D

in patients with primary lymphedema with healthy controls.

MATERIAL AND METHODS

Study Design

The study was conducted prospectively comparing the serum level of VEGF-D in patients with primary lymphedema and those without. Inclusion criteria for patients into the study was the presence of unilateral or bilateral primary lymphedema of the leg. Patients with secondary lymphedema and pregnant patients were excluded. Written informed consent was obtained from all included patients. The study was performed at the Department of Dermatology, Wilhelminenspital, Vienna Austria.

Patient Sample

Over a 6-month period (September 2002 to February 2003), 30 consecutive patients with lymphedema of the leg were referred to our department. The diagnosis of lymphedema was determined by clinical examination (characteristic clinical swelling of the leg, positive Stemmer sign) as well as by quantitative isotopic lymphoscintigraphy (17). In 12 of 30 patients, the lymphedema of the leg was based on secondary damage to the lymphatic vessels e.g., infection, radiation, malignancy or when lymph nodes were surgically removed. These patients were excluded.

A total of 18 patients with primary lymphedema of the leg (4 men and 14 women) with a mean age of 53.7 years (range: 20-79 years) were finally enrolled into the study. The clinical stage of primary lymphedema was moderately severe to severe grade 2 lymphedema in 13 patients and grade 3 lymphedema (elephantiasis) in 5 patients according to the classification of the International Society of Lymphology (18). In all included patients, family history, presence of unilateral or bilateral lymphedema, as well as the results of the quantitative isotope lymphography were evaluated.

Eighteen healthy patients matched for sex and age were used as controls.

The serum level of VEGF-D was evaluated in all patients. Blood from the cubital vein was taken in the morning to avoid a possible day dependent pattern of the serum level. The research investigators were unaware of any clinical information.

Evaluation of the Serum Level of VEGF-D

In all patients the serum level of VEGF-D was determined by the Quantikine VEGF-D Immunoassay (R&D Systems) according to the manufacturer's instructions. The assay employs the quantitative sandwich enzyme immunoassay technique. Standards and samples were pipetted in wells coated with a murine monoclonal antibody against VEGF-D. Any VEGF-D present in the probe was bound by the immobilized antibody. After washing away any unbound substances, an enzyme linked monoclonal antibody specific for VEGF-D was added to the wells. Following a second wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of VEGF-D within the probe. The intensity of the color was measured representing the amount of VEGF-D within the sample.

Statistical Analysis

Frequencies and percent are reported for categorical variables. Continuous variables are expressed as mean ± standard error, median, minimum and maximum and were compared between groups applying the two-sample t-test. The assumed significance level was 5%. Serum levels of VEGF-D are visualized with box plots.

For statistical analyses, SAS® Software, Version 8.2 (SAS Institute Inc., Cary NC) was used.

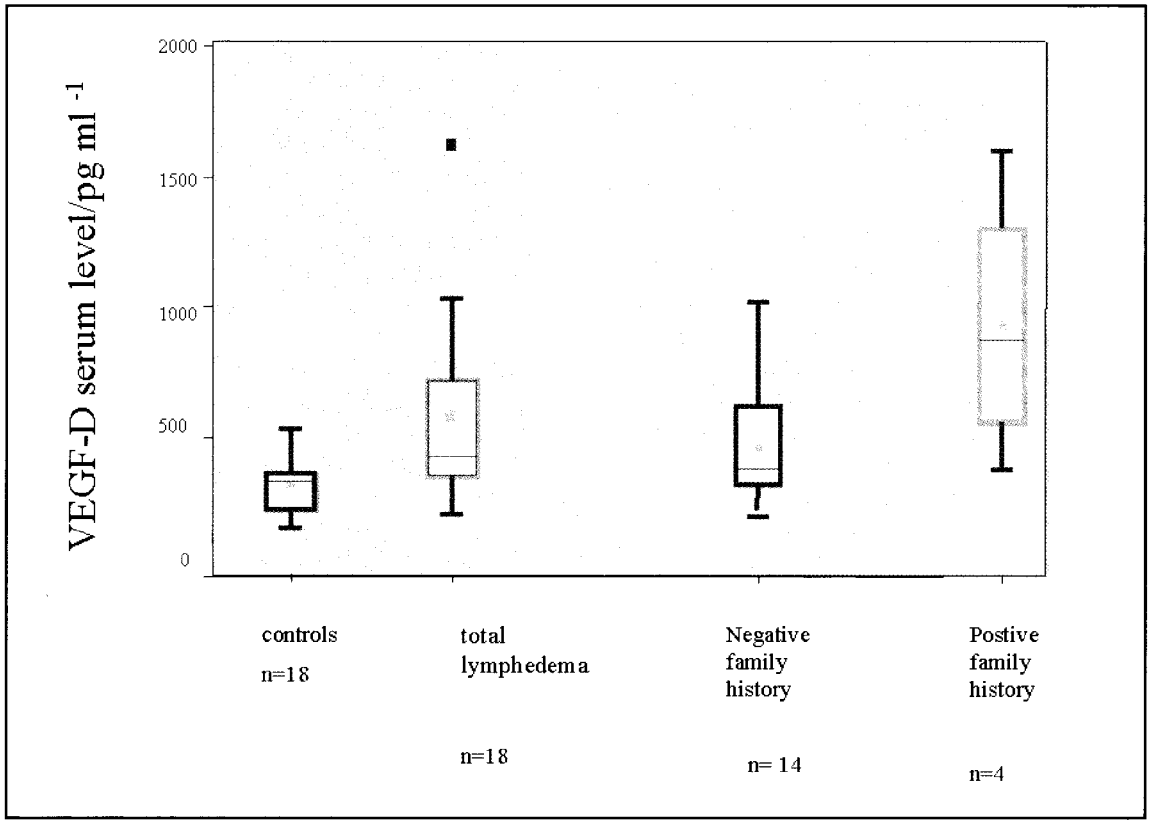

Fig. 1. Serum level of VEGF-D in patients with primary lymphedema and controls (dots represent mean values, faint lines in box represent median values, top and bottom of box plot represent the third quartile and the first quartile, the upper bar is largest data value that is less than the upper cut-off, and lower bar is the smallest data value that is greater than the cut-off). The black square represents an outlier.

RESULTS

Patient Characteristics

A total of 18 consecutive patients with primary lymphedema and 18 controls matched for sex and age were enrolled in the study. The mean age of the study population was 53.7 ± 15.2 years (range 20 to 79 years). A total of 8 men and 24 women were evaluated.

In the group of patients with primary lymphedema (n = 18), 11 patients (61%) suffered from unilateral lymphedema and 7 patients (39%) from bilateral lymphedema. Of the 18 patients, 4 patients (22 %) had a positive family history of primary lymphedema.

In the group of controls (n=18), none of the patients had a positive family history of primary lymphedema.

Serum Level of VEGF-D

In all included patients—18 patients with primary lymphedema and 18 controls— the serum level of VEGF-D was evaluated. In patients with primary lymphedema, the mean serum level of VEGF-D was 576.5 pg ml^{-1} while in the control group the mean serum level was 314.2 pg ml^{-1}. The serum level of VEGF-D was significantly higher in patients with primary lymphedema compared with controls (p=0.0047) *(Fig. 1)*. Furthermore, in the group of patients with primary

lymphedema, those patients with a positive family history of lymphedema had a significantly higher serum level of VEGF-D (942.9 pg ml^{-1}) compared to those without a positive family history (471.9 pg ml^{-1}) (p= 0.01) *(Fig. 1)*.

DISCUSSION

Primary lymphedema is a developmental disorder of the lymphatic vessels resulting in chronic swelling due to a decreased lymphatic drainage (1).

Vascular endothelial growth factor-D (VEGF-D) and vascular endothelial growth factor-C (VEGF-C) have been recently found to play an important role in lymphangiogenesis (5-8). They have a mitogenic effect for lymphatic endothelial cells by activating its receptor VEGFR-3 (2,9). When VEGFR-3 is stimulated, the growth and migration of lymphatic endothelial cells have been observed (3). In contrast, blocking VEGFR-3 signaling pathway by use of soluble VEGFR-3 results in a lymphedema-like phenotype in transgenic mice (19). Furthermore, the importance of VEGFR-3 for the development of the lymphatic vessels has been shown recently, where human hereditary lymphedema (14-16) was found to be linked to the VEGFR-3 locus on chromosome 5q. Furthermore, other missense mutations have been described interfering with the VEGFR-3 tyrosine kinase signaling pathway in lymphedema patients (13,20).

These observations lead us to the hypothesis that a lack of stimulation of VEGFR-3 results in an attempt to produce even more VEGF-D to maintain stimulation of lymphangiogenesis, leading to a high serum level of VEGF-D. The present study was designed to investigate this issue by comparing the serum level of VEGF-D in patients with primary lymphedema and healthy controls.

We demonstrated that the serum levels of VEGF-D in patients with primary lymphedema were significantly higher compared with healthy controls. Additionally regarding patients with primary lymphedema, those with a positive family history were found to have a significantly higher serum level of VEGF-D compared to those with a negative family history.

Although the results of the present study do not permit a definitive conclusion, they suggest that in these patients with primary lymphedema, increased levels of VEGF-D are due to defective stimulation of VEGFR-3.

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