Vascular endothelial growth factor and hypertrophic osteoarthropathy

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Abstract

Objective

Hypertrophic osteoarthropathy (HOA) is characterized by the coexistence of digital clubbing and periosteal proliferation of the tubular bones. Localized vascular proliferation associated with platelet/endothelial cell activation are recognized features of this syndrome. Current knowledge suggests that HOA develops from the presence in the systemic circulation of one or more growth factors that are normally inactivated in the lungs. The nature of these purported growth factors has not yet been identified. Vascular endothelial growth factor (VEGF) has several features that may fit in with the pathogenesis of HOA. The objective of our study was to measure serum and plasma levels of VEGF in different groups of patients with HOA.

Methods

We studied 24 patients with HOA; of these, in 12 the HOA was secondary to cyanotic congenital heart disease and in 7 to lung cancer, while 5 represented primary cases. As controls we studied 28 individuals without HOA; of these, 12 were apparently healthy individuals, 7 had cyanosis secondary to chronic obstructive pulmonary disease, and 9 had lung cancer. ELISA was used to measure serum and plasma levels of VEGF.

Results

Plasma levels of VEGF were significantly higher in the patients with primary HOA (median 46.2; range 19.4 - 398.8 pg/ml) and in those with lung cancer-HOA (median 75.5; range 24.6 - 166.7), compared to healthy controls (median 7.4; range: 0 - 26.1), $p < 0.05$.

Serum VEGF levels were higher in patients with lung cancer and HOA (median 411.4; range 164.2 - 959.5 pg/ml) compared with lung cancer patients without HOA (median 74.5; range 13.2 - 205.4), $p < 0.001$.

Conclusions

Patients with primary HOA and those with HOA and lung cancer have increased circulating levels of VEGF. This cytokine may play a role in the pathogenesis of HOA.

Key words

Hypertrophic osteoarthropathy, VEGF, lung cancer, cyanotic heart disease.

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Introduction

Hypertrophic osteoarthropathy (HOA) is a syndrome characterized by digital clubbing and periosteal proliferation of the tubular bones. This unique syndrome is frequently associated with a variety of internal illnesses, mostly hypoxic or neoplastic conditions. Nevertheless, in some instances this acropachy may present itself without any underlying illness (primary HOA) (1).

HOA is characterized by localised vascular hyperplasia associated with platelet/endothelial cell activation as demonstrated by histologic studies of clubbing showing vascular proliferation (2,3) with ultrastructural signs of endothelial cell activation (4). Edema and excessive connective tissue deposition accompany these alterations. There is also an increased number of arteriovenous anastomosis (2, 3). Vascular hyperplasia is also evident in the periosteoum of the tubular bones (3). The vascular proliferation of HOA has been confirmed by arteriographic and gammagraphic studies (5).

Patients with HOA associated with cyanotic heart disease have an abnormal platelet population characterized by larger than normal thrombocytes with aberrant volume distribution curves. These platelet abnormalities suggest that in cases of right-to-left shunts of blood, large platelets that under normal circumstances are fragmented in the lung microvasculature gain access to the systemic circulation, reach its most distal sites in axial streams, and there activate endothelial cell releasing growth factors, thus inducing acropachy (6). Further supporting the notion of an enhanced platelet/endothelial cell activation in HOA is the fact that patients with cardiogenic HOA, as well as patients with primary HOA, have raised circulating levels of von Willebrand factor antigen (7). For cases of HOA associated with lung cancer, it has been proposed that a tumor-derived growth factor that gains direct access to the systemic circulation is responsible for the development of HOA (8).

Nevertheless, the cytokine(s) responsible for the connective tissue abnormalities of HOA has not been identified. In the present study we focused our attention on vascular endothelial growth factor (VEGF), since this cytokine has several peculiarities that could fit in with the pathogenesis of HOA. For example, this growth factor is an angiogenic agent with specific action for endothelial cells. It is typically produced by neoplastic tumors as a mechanism for mass growth (9). Hypoxic induction of VEGF is a well-known fact. This appears to be a general response since many types of cultured cells have been observed to increase VEGF mRNA levels by approximately 10- to 50-fold as a consequence of a lowering of the oxygenation of the media. Similar induction of VEGF at reduced pO_2 levels has been demonstrated *in vivo* (9). Furthermore, it has been recently shown that VEGF is constitutively produced by megakaryocytes and platelets and that it is released upon the activation of these blood elements (10). Thus, the objective of our study was to measure serum and plasma levels of VEGF in patients with HOA of diverse etiologies.

Patients and methods

Patients

We studied 24 patients with HOA; of these 12 had HOA secondary to cyanotic congenital heart disease and 7 secondary to lung cancer, while 5 were primary cases. In all instances the diagnosis of HOA was based on the presence of digital clubbing on inspection, confirmed by a digital index greater than 10 (1), in addition to periosteal proliferation of the tubular bones as demonstrated by X-ray examination. The primary HOA cases had been under observation for a mean period of 7 years. None of the patients displayed any internal illness and none were cyanotic.

As controls we included 28 individuals without digital clubbing. Of these, 12 were apparently healthy individuals, 7 had cyanosis secondary to chronic obstructive pulmonary disease (COPD), and 9 had lung cancer.

Subjects with primary HOA were taking no medications. Six of the 12 patients with cyanotic heart disease were receiving drugs that included digoxin, diuretics, and allopurinol. Individuals with lung cancer were studied before being subjected to chemotherapy or radiotherapy. Patients with chronic obstruc-

tive pulmonary disease were in the stable phase of their illness. Their medication consisted of inhaled bronchodilators (salbutamol).

All patients and controls gave their informed consent before being enrolled in the study. The Ethics and Human Research Committee of our institution approved the study protocol.

Serum and plasma collection

We used the Schiebout-Clark method (11) in order to avoid artificial platelet activation during plasma separation. Venipuncture was performed without a tourniquet, 10 ml of blood were drawn and discarded to clear the system of activating factors, and then with a vacutainer system blood was collected into Thrombotect tubes. These tubes contained EDTA as anticoagulant and 2-chloroadenosine and procaine-HCL as platelet inhibitors. The tubes were gently inverted and placed in melting ice baths for 30 minutes. Then the samples were centrifuged at 2,500 x g for 20 minutes at 4°C. Two ml were removed from the center of the plasma and were stored at -70°C until they were used for analysis. Serum was separated by the conventional method.

For this study paired serum/plasma samples were available for 41 of the 52 individuals tested. In 4 instances our bank ran out of plasma samples. Serum samples were not available in 7 of the 52 cases (Fig. 1).

A commercial ELISA kit (R&D Systems, Minneapolis, MN) was used to measure the levels of VEGF. The GraphPad Prism program version 2.0 (GraphPad Software Inc, San Diego, CA) was used for the statistical analysis. The non-parametric Kruskal-Wallis test was utilized to compare VEGF levels among the different groups and Dunn's multiple comparison test to compare differences in the medians. The non-paired Student's t-test was used to compare serum levels with plasma levels. P values < 0.05 were considered significant. Pearson's correlation coefficient was used to correlate platelet count with VEGF levels.

Results

The demographic features of the patients and controls are shown in Table I. As expected, the group of patients with cancer and the group with COPD were older than the remaining groups. Subjects with primary HOA and cyanotic heart defects had their disease since birth.

Serum and plasma VEGF levels are shown in Figure 1. Patients with primary HOA and those with lung cancer-HOA had significantly higher plasma levels of VEGF when compared with healthy controls (Fig. 1a).

In healthy controls, serum VEGF levels were significantly higher (mean ± SD: 124.1 ± 83.2 pg/ml) than those detected in the plasma (9.8 ± 10.7) ($p < 0.0005$). Similar serum/plasma differences were found in the other groups, except for the group with primary HOA. In this group, although the serum levels were higher (209.1 ± 112.7 pg/ml), they were not significantly different from the plasma levels (128.9 ± 158.4). (Note that the values in Table I are different because they are expressed as the median/range).

There were no inter-group differences in the serum levels of VEGF, except when lung cancer patients without HOA (median 74.5; range 13.2 - 205.4 pg/ml) were compared with lung cancer patients with HOA (median 411.4; range 164.2 - 959.5) ($p < 0.001$) (Fig. 1b).

When the two groups of cyanotic patients (those with CCHD and those with COPD) were combined, plasma VEGF levels were significantly higher than in healthy controls (median 45.1, range 0 - 398.1 pg/ml vs median 7.4, range 0 - 26.1), $p < 0.05$.

There was no correlation between the platelet count and serum or plasma VEGF levels.

Discussion

Our studies found two variables associated with raised plasma levels of VEGF: the presence of HOA and the presence of cyanosis. Patients with the primary form of the syndrome and patients with HOA secondary to lung cancer had significantly higher VEGF plasma levels compared to healthy controls. VEGF levels in the subgroup of patients with CCHD-HOA, although higher, did not reach such significance.

As expected from previous basic research, cyanosis was also a variable associated with high VEGF plasma levels (9). One possible explanation for the divergent presence of HOA in the two cyanotic groups with similar VEGF plasma levels is that for the development of HOA, in addition to cyanosis, an abnormal platelet population (as reported in

Table I. Outstanding features of patients with hypertrophic osteoarthropathy (HOA) and controls.

Group	#	Sex (M/F)	Mean age (± SD)	Disease dur. (months)	Platelet count (x1000)	Plasma VEGF levels (pg/ml) (median/range)	Serum VEGF levels (pg/ml) (median/range)
HOA							
Primary	5	5/0	24.4 ± 8	292.8 ± 96.6	254 ± 38	46.2 / 19.4 - 398.8*	151.2 / 98.3 - 335.3
CCHD	12	4/8	33 ± 14.4	396 ± 172.2	59 ± 93	25.4/0-398.1	210.1/17.9-1138
Lung cancer	7	6/1	62.4 ± 17.1	5.4 ± 3.3	280 ± 81	75.5/24.6-166.7*	411.4/164.2-959.5^{+}
Controls							
Healthy	12	7/5	26.3 ± 6.2	—	—	7.4 / 0 - 26.1	102.2 / 8.9 - 250.2
COPD	7	4/3	69.4 ± 8.9	55.5 ± 48.3	171 ± 50	50.8 / 0 - 87.9	186.6 / 130.2 - 797
Lung cancer	9	7/2	57.6 ± 12.5	5.4 ± 3.3	290 ± 67	19.4 / 6.2 - 130.9	74.5 / 13.2 - 205.4^{+}

= number of patients; M = male; F = female; SD = standard deviation; CCHD = cyanotic congenital heart disease; COPD = chronic obstructive pulmonary disease. *$p < 0.05$ vs. healthy controls; $^{+}p < 0.001$

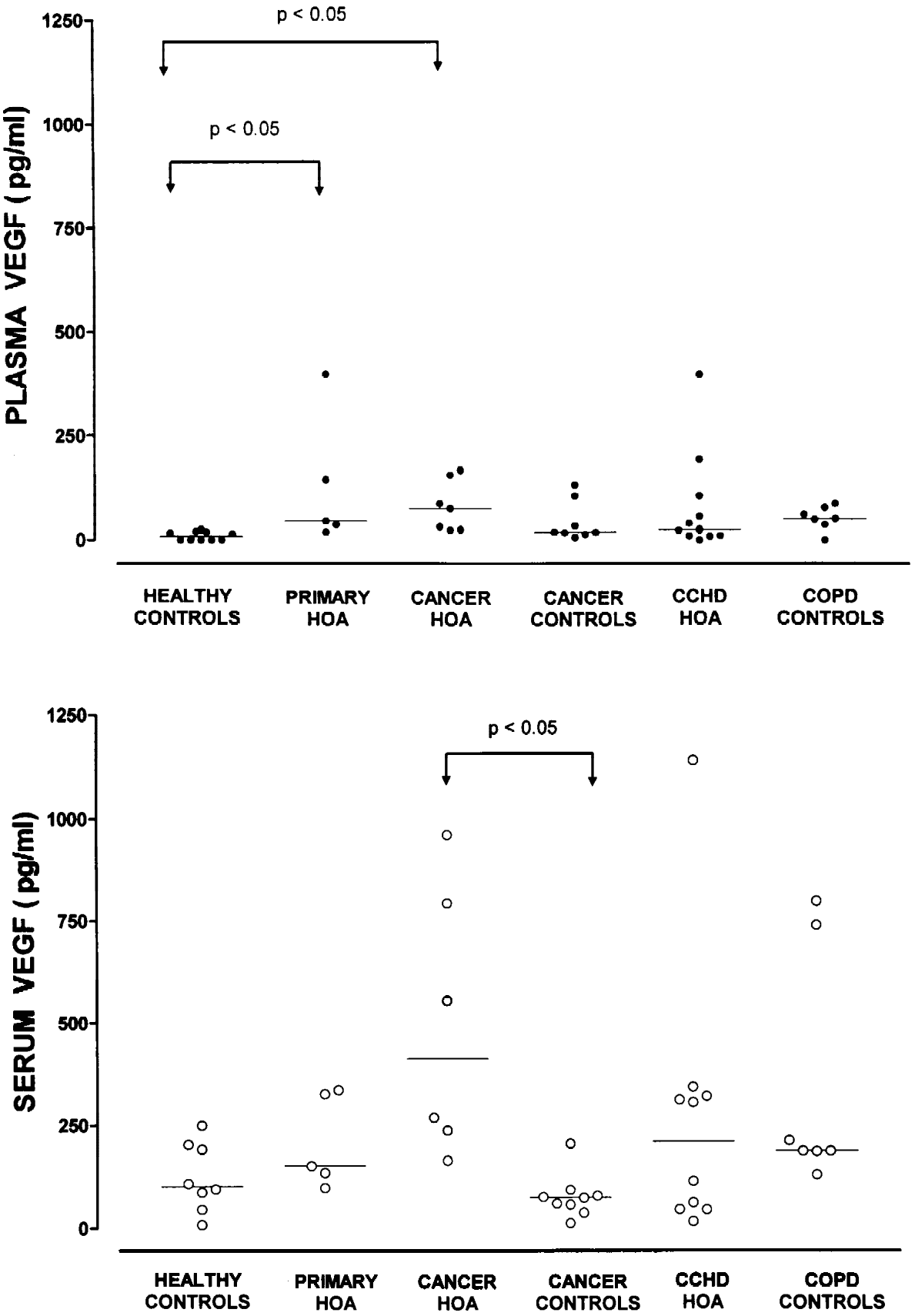

Fig. 1. Plasma and serum levels of vascular endothelial growth factor (VEGF) in patients with different types of hypertrophic osteoarthropathy (HOA) as compared with controls.

cases with CCHD) is needed for the unfolding of HOA. This abnormal platelet population is consequence of the right-to-left shunting of blood and could theoretically induce abnormal shear rates with endothelial cells, thus liberating VEGF.

Serum levels of VEGF were higher than plasma levels in practically all the groups under study. This agrees with the notion that VEGF is platelet-derived (10) and is thus liberated during *in vitro* clot formation. Therefore, measuring serum levels of a platelet-derived cytokine yields artificially higher values. Our interpretation of the finding that cancer patients with HOA have higher serum levels of VEGF than cancer patients who do not, is that in these neoplasic states VEGF is not only derived from platelets but is also generated by the lung tumor.

Previous studies have reported the finding of different circulating growth factors in HOA. Researchers from our institution found normal plasma levels of platelet derived growth factor (PDGF) (12). In contrast, Silveri *et al.* reported high PDGF levels in the serum of such patients (13), but they did not discuss the likely artifactual role of platelet degranulation during *in vitro* serum separation. There are reports of elevated serum levels of transforming growth factor beta 1 (another platelet-derived factor) (14) and hepatocyte growth factor (15) in patients with clubbing associated with different lung diseases.

A hyperproduction of VEGF, as found in our study, could explain the peculiar clinical features of HOA. It is a known fact that osteogenesis is strongly dependent on angiogenesis (16). In tissue cultures VEGF is a potent promoter of osteoblast differentiation (17). Thus VEGF could theoretically induce the periosteal proliferation of HOA. Clubbing could also be explained on the basis of VEGF-induced vascular neogenesis with the subsequent deposit of extracellular matrix and edema.

Besides the experimental evidence here presented, there are also clinical associations that may suggest a link between VEGF and HOA. Two independent groups have recently reported that patients with POEMS syndrome have greatly raised circulating levels of VEGF (18, 19). On the other hand, a metaanalysis has shown that the majority of the described cases of POEMS syndrome had the most conspicuous features of HOA namely, digital clubbing and pachydermia (20). POEMS syndrome most closely resembles the primary form of HOA, since this subset of HOA displays a more generalized skin hypertrophy as well as hyperhydrosis. In primary HOA there is no underlying illness, so we propose the possibility that in these cases a genetically determined overproduction of a growth factor (purportedly VEGF) could be the cause of the acropachy.

Interestingly, VEGF has been implicated in the pathogenesis of several other diseases associated with HOA such as pleural mesothelioma (21) and Graves' disease (22). These two entities are characterized by prominent neovascularization. VEGF also appears to be involved in the development of inflammatory bowel disease (23), another entity associated with HOA.

We recognize several limitations in our study. Ideally, the different groups of patients with HOA should have been matched with patients having the same underlying illness but without HOA. This was possible only in the subgroup of patients with lung cancer. The peculiar characteristics of the other HOA subgroups precluded such a comparison, since all the patients with cyanotic heart disease had at least digital clubbing and such matching would not have been possible in patients with primary HOA. In addition, larger numbers of patients should be studied, particularly with regard to the subgroup of primary HOA, of which we included only 5 cases.

HOA is a syndrome localized to the distal parts of the extremities, so that venous blood levels may not accurately reflect what is happening at the most distal sites of the extremities. The role of platelets in the pathogenesis of HOA should be better characterized. Finally, one important subgroup of patients, those with CCHD, did not reach significantly higher VEGF plasma levels.

Therefore, there is no doubt that much more research is needed in this area. Nevertheless, our exploratory study suggests the possibility that VEGF may play a role in the pathogenesis of HOA.

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