

Original Research

Concentration analysis of hypoxia-inducible factor-1 α and vascular endothelial growth factor in patients with aortic aneurysm at different stages and its clinical significance

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Abstract: This study aims to investigate serum concentration of hypoxia inducible factor -1 α (HIF-1 α) and vascular endothelial growth factor (VEGF) in patients with aneurysms at different stages, and to determine critical role in early diagnosis and evaluation of severity. A cohort of 102 cases of patients diagnosed as aneurysms during May 2012 to April 2015 in our hospital were retrospectively analyzed. Patients were divided into early stage group (n=32), mid-term group (n=34), and late stage group (n=36) according to severity of aneurysms. The measurement of concentration of HIF-1 α and VEGF was detected by Enzyme-linked immunosorbent assays (ELISA). Serum level of HIF-1 α and VEGF was compared among different groups. The results showed that serum concentrations of HIF-1 α (t=25.53, $P<0.05$) and VEGF (t=12.10, $P<0.05$) in early stage group were significantly higher than those in normal group. Serum concentrations of HIF-1 α (t=25.63, $P<0.05$) and VEGF (t=9.71, $P<0.05$) in the mid-term group were significantly higher than those in the early stage group. Serum concentrations of HIF-1 α (t=14.61, $P<0.05$) and VEGF (t=21.42, $P<0.05$) in the late stage group were significantly higher than those in the mid-term group. In conclusion, serum HIF-1 α and VEGF has clinical value of early diagnosis and assessment of disease severity.

Key words: Aneurysm, hypoxia-inducible factor-1 α , endothelial growth factor, diagnosis.

Introduction

Aneurysm is associated with significant morbidity and mortality, mainly occurs in the trunk limb arteries, aortic and carotid artery and other parts caused by arterial wall disease or injury (1). Currently, early diagnosis and evaluation of the severity for patients with cerebral aneurysms remains a major challenge (2). Therefore, strategies for rapid and non-invasive early diagnosis of aneurysms were considered to be inevitable and necessary. HIF-1 α and VEGF have been reported to be related to the development and progression of aneurysm. However, little has been reported for HIF-1 α and VEGF as predictive biomarkers for early diagnosis and assessment of disease severity. In this study, we retrospectively analyzed 102 cases of patients with aneurysms at different stages during May 2012 to April 2015 in our hospital. The serum level of HIF-1 α and VEGF was compared among different staging groups.

Materials and Methods

Patients

In this study, a total of 102 cases of patients diagnosed with aneurysms during May 2012 to April 2015 in our hospital were enrolled. Additionally, forty-five healthy adults, who has not been diagnosed as aneurysms or other cardiovascular diseases, were accepted as normal control group. Patients were divided into early stage group (n = 32), mid-term group (n = 34), and late stage group (n = 36) according to the severity of the aneurysms and the following criteria. The measurement of aneurysm width or aneurysm neck-to-dome length was defined as the aneurysm size. Aneurysm size was divided into ≥ 7 mm (defined as late stage group), < 7 mm (defined as mid-term stage group) and ≤ 3 mm (defined as early stage group). The aneurysm width

was measured on a 0.1 mm scale. All of the individuals (both normal individuals and patients) included in this study were from Chinese population. The clinical features are shown in Table 1. This study was approved by the ethical committee of Shaoxing Hospital of Zhejiang University and all the participants were informed about study design. The present study was also approved by the ethics committee of Shaoxing Hospital of Zhejiang University, Shaoxing, China.

Inclusion criteria

Patients met the following inclusion criteria were included in this study: ① diagnosed of aneurysm; ② mainly were aortic aneurysm; ③ no other cardiovascular disease occur; ④ with age range of 40-60 years old; ④ no immunological diseases, digestive tract diseases and associated diseases that can make serum HIF-1 α and VEGF change, whereas pregnant or lactating patients were excluded from this study.

ELISA assay

Fasting venous blood samples were collected from individual patients, venous blood samples were subjected to centrifugation at 2500 revolutions per minute for 10 minutes. Supernatant was transferred into eppendorf tubes after centrifugation and stored at -80°C deep freeze until the measurement. Enzyme-linked immuno-

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sorbent assays (ELISA) were applied for concentration detections of HIF-1 α and VEGF. The concentrations of plasma HIF-1 α was determined by HIF-1 α SimpleStep ELISA™ kit (ab171577, Abcam), and the concentrations of plasma VEGF level was detected by Human VEGF ELISA kit (ab100663, Abcam).

Statistical analysis

Data are expressed as the mean \pm standard deviation or the median and range if applicable. The difference between groups was analyzed by Student's *t*-test, χ^2 -test, using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered as statistically significant.

Results

General clinical characteristics

The mean age, ratio of body trunk aneurysm and carotid artery aneurysm of patients was similar among three groups, indicating that the three groups were comparable (Table 1).

HIF-1 α and VEGF levels in patients group were higher compared to normal group

In order to observe the changes of biomarkers in the patients, the HIF-1 α and VEGF levels were examined. The results indicated that the serum concentrations of HIF-1 α and VEGF in the patients group (including early stage group, mid-term group and late stage group) were significantly higher compared to the normal group (Table 2, $P < 0.05$).

Significantly increased serum levels of HIF-1 α and VEGF in different stage of aneurysms patients

In order to observe the relationship between the different levels of HIF-1 α and VEGF and the severity of aneurysms, the HIF-1 α and VEGF were examined in different aneurysms stage. The results indicated that the serum concentrations of HIF-1 α ($t = 25.63$, $P < 0.05$) and VEGF ($t = 9.71$, $P < 0.05$) in the mid-term group were significantly higher than those in the early stage group. Serum concentrations of HIF-1 α ($t = 14.61$, $P < 0.05$) and VEGF ($t = 21.42$, $P < 0.05$) in the late stage group were significantly higher than those in the mid-term group

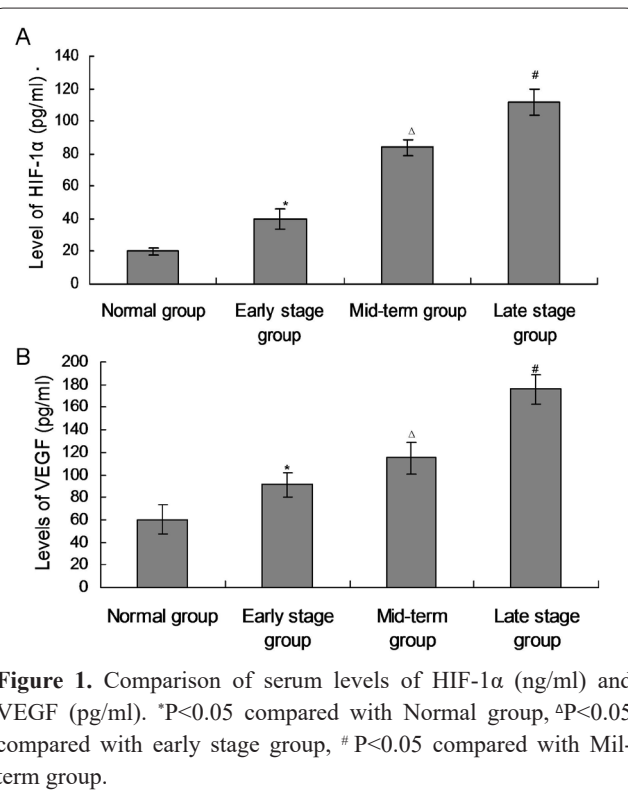


Figure 1. Comparison of serum levels of HIF-1 α (ng/ml) and VEGF (pg/ml). * $P < 0.05$ compared with Normal group, $\Delta P < 0.05$ compared with early stage group, # $P < 0.05$ compared with Mid-term group.

(Table 2, Figure 1).

Discussion

This study retrospectively analysed 102 cases of patients diagnosed aneurysm during May 2012 to April 2015 in our hospital. Blood samples were collected and serum was isolated. Serum levels of HIF-1 α and VEGF in each individual was detected by ELISA and compared among the early stage group, the mid-term group and late stage group. Serum concentrations of HIF-1 α ($t = 25.53$, $P < 0.05$) and VEGF ($t = 12.10$, $P < 0.05$) in the early stage group were significantly higher than those in the normal group. Serum concentrations of HIF-1 α ($t = 25.63$, $P < 0.05$) and VEGF ($t = 9.71$, $P < 0.05$) in the mid-term group were significantly higher than those in the early stage group. Serum concentrations of HIF-1 α ($t = 14.61$, $P < 0.05$) and VEGF ($t = 21.42$, $P < 0.05$) in the late stage group were significantly higher than those in

Table 1. General characteristics of patients.

Characteristics	Early stage group (n= 32)	Mid-term group (n=34)	Late stage group (n=36)	Control group (n=45)	χ^2/t	<i>P</i>
Male/Female	15/17	16/18	18/18	21/24	0.007	0.221
Mean Age	52.5 \pm 10.2	52.5 \pm 10.2	52.5 \pm 10.2	52.4 \pm 9.4	23.043	0.215
Limb trunk aneurysm	1	2	2	-	0.015	0.532
Aortic aneurysm	32	34	36	-	0.132	0.472
Carotid aneurysm	1	2	1	-	0.023	0.184

Table 2. Serum concentrations of HIF-1 α and VEGF in different groups.

	Normal group	Early stage group	Mid-term group	Late stage group
			group	group
HIF-1 α (pg/ml)	19.22 \pm 3.27	39.11 \pm 7.48*	83.36 \pm 6.93 Δ	113.72 \pm 10.23#
VEGF (pg/ml)	59.45 \pm 11.85	89.34 \pm 9.77*	115.47 \pm 12.04 Δ	175.83 \pm 11.51#

* $P < 0.05$ compared with Normal group, $\Delta P < 0.05$ compared with Early stage group, # $P < 0.05$ compared with Mid-term group.

the mid-term group. Data from this study suggested that HIF-1 α and VEGF concentrations in early stage patients with aneurysms were significantly higher than those in normal control subjects. Moreover, serum HIF-1 α and VEGF concentrations gradually increased along with the progression of aneurysms, indicating that HIF-1 α and VEGF concentration detection could have clinical value of early diagnosis and assessment of aneurysms severity.

HIF-1 α is a subunit heterodimer of 120 kDa, which is regulated by hypoxia signal to exert its biological functions through DNA binding with helix-loop-helix structure. HIF-1 α (a key subunit of HIF-1), in conjunction with β subunit, participates in various biological functions, including erythropoiesis, angiogenesis, energy metabolism of nucleosides, amino acids and sugars, cell survival, apoptosis and drug resistance etc., in order to maintain homeostasis to adapt to hypoxia. Meanwhile, HIF-1 gene and its downstream expression genes play an important role in physiological hypoxia, such as stem cells microenvironment, placental development, tissue during embryonic development cell differentiation, as well as in a variety of pathological conditions such as tumor cell proliferation and metastasis. The relationship between HIF-1 expression and tumor progression has become research hotspot in recent years. Previous studies have already indicated that HIF-1 α was correlated to the formation and progression of non-small cell lung cancer. Zhao et al. (3) identified that HIF-1 α expression was significantly higher in lung cancer tissues than those in para-carcinoma tissues and normal tissues, indicating HIF-1 α was associated the occurrence and development of non-small cell carcinoma. Previous studies also showed that HIF-1 α was associated with breast cancer, stomach cancer, liver cancer and so on. Fang et al. (4) showed that in breast cancer MCF-7 cells, HIF-1 α expression was significantly increased, which promoted the proliferation of MCF-7 cells. Zhang et al. (5) found by immunohistochemical method that expression of HIF-1 α protein in gastric cancerous tissue was increased, which promoted gastric carcinogenesis through upregulation of VEGF. Osman et al. (6) compared the expressions of HIF-1 α in liver carcinoma tissues, carcinoma margin tissue, and non-carcinomatous tissues and found that HIF-1 α expression was significantly increased in cancer tissue, indicating that the HIF-1 α was correlated with the development of liver cancer. However, little has been reported in the relationship of HIF-1 α and development and progression of aneurysm. This paper revealed the correlation of HIF-1 α and aneurysm, and found that, serum HIF-1 α in the early stage of aneurysm patients was significantly higher than that of normal controls, indicating that the concentration of serum HIF-1 α can be used as a biomarker for arterial aneurysm early diagnosis. Additionally, with the progression of the disease, a significant increase in serum HIF-1 α concentrations was found, indicating that HIF-1 α concentration can be used to assess the severity of the aneurysm.

VEGF participates in angiogenesis by binding to VEGF receptor to participate in the formation of new blood vessel. Tumorigenesis also depends on new blood vessels to provide oxygen and nutrients. Many studies have reported VEGF was involved in cancer and disease progression. Zhao et al. (7) found that in patients with

myocardial infarction, serum VEGF was significantly increased, and VEGF signaling pathways were associated with cardiovascular disease. Mylona et al. (8) found that, VEGF-C and VEGF-D may play a role in promoting cervical carcinogenesis progression, the combined detection of the expressions of VEGF-C and VEGF-D in biopsies may effectively predict lymph node metastasis of cervical carcinoma. Xie et al. (9) by interfering the expression of VEGF to inhibit the proliferation of human breast cancer cells. However, this article revealed for the first time that VEGF was correlated to the development and progression of aneurysms. Additionally, serum VEGF was significantly higher in the early stage of aneurysms patients than normal controls, indicating that serum VEGF concentration detector may be useful for arterial aneurysm early diagnosis. In addition, with a significant increase in the concentration of serum VEGF development of the disease, indicating that VEGF concentration can be used as a sensitive detector to assess the severity of the aneurysm.

Although this article revealed HIF-1 α and VEGF may have clinical value in early diagnosis and severity assessment, further studies were needed for elucidating the signaling pathway of serum HIF-1 α and VEGF underlying the pathogenesis of aneurysms.

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