

Angiogenic activity of diabetes type 2 patients sera – dependence on sex

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Abstract

Diabetes mellitus type 2 (DM2) is characterized by recurrent or persistent hyperglycemia and some alterations of angiogenesis, such as macro and microangiopathy (nephropathy, retinopathy). In our previous study performed with healthy people we have found negative correlation in case of men, between their age and serum in vivo activity, and negative correlation between their age and serum VEGF concentration. In case of women, no such relations were observed.

The aim of the present study was to evaluate total in vivo angiogenic activity as well as a concentration of VEGF in sera collected from 40 healthy and 64 DM2 people of various age (40-90 years old), and to establish whether some correlation exists between studied parameters and persons sex.

We have found for women higher DM2 sera angiogenic activity than the activity of sera collected from DM2 men and healthy women. Similarly, VEGF content was the highest in the sera collected from DM2 women.

Key words: diabetes type 2, sex, sera, angiogenesis, VEGF.

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Introduction

In physiological conditions, angiogenesis occurs primarily in embryo development, during wound healing and in response to ovulation. Pathological angiogenesis, or the abnormal rapid proliferation of blood vessels, is implicated in many diseases, including cancer, psoriasis, age-related macular degeneration and diabetes [1-3].

Diabetes mellitus type 2 (DM2) is due to insulin resistance or reduced insulin sensitivity, combined with relatively reduced insulin secretion which in some cases becomes absolute. In 2000, according to the World Health Organization, at least 171 million people worldwide suffered from diabetes (2.8% of the population). Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will almost double [4]. There are many complications of diabetes including macro and microangiopathy (nephropathy and

retinopathy). The main complications of retinopathy is arising poor-quality new blood vessels in the retina as well as macular edema (swelling of the macula), which can lead to severe vision loss or blindness. Retinopathy is the second microvascular complication of diabetes, leading to blindness in the United States among 20-64 aged people [5].

Human serum contains various factors able to induce or to suppress formation of new blood vessels. Among these angiomodulatory factors, VEGF (vascular endothelial growth factor) is the main proangiogenic one. Cells in the retina activated by a lack of oxygen (typical diabetic symptom in the retina) release this pro-angiogenic molecule, that attracts inflammatory and endothelial cells and promotes their proliferation. These changes lead to proliferative retinopathy and retinal detachment [6].

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Aim

The aim of the present study was to evaluate total in vivo angiogenic activity as well as concentration of VEGF in sera collected from 40 healthy and 64 DM2 people of various age (40-90 years old), and to establish whether some relation exists between studied parameters and persons' sex.

Material and Methods

Patients

The study was performed on sera collected from healthy people (20 men and 20 women) and from DM2 people (34 men and 30 women), 40-90 years old, without proliferative retinopathy. The mean duration of diabetes mellitus was 12 ± 2.4 years, HbA1c was $8.65 \pm 0.4\%$ (normal range $< 6\%$). Patients and control sera after separation were aliquoted and stored at -78°C until further examination.

Serum-induced cutaneous angiogenesis assay

Cutaneous angiogenesis assay (SIA) was performed according to Sidky and Auerbach method [7] with own modifications [8, 9]. Studies have been performed in 2-month old, female inbred Balb/c mice. Mice have been of local laboratory breed, weighing ca 20 g each. The sera of healthy subjects and DM2 patients were injected intradermally (0.05 ml per one injection, 3-6 injections per mouse) into regionally shaved, anaesthetized with chloral hydrate (POCH, Poland) groups of 3 or more mice. In order to facilitate the localization of injection sites later on, all injected samples were coloured with 0.1% of trypan blue. After 72 hours mice were killed with lethal dose of Morbital (Biowet, Poland). All newly formed blood vessels were identified and counted in dissection microscope in 1/3 central area of microscopic field, at $6 \times$ magnification. Identification was based on the

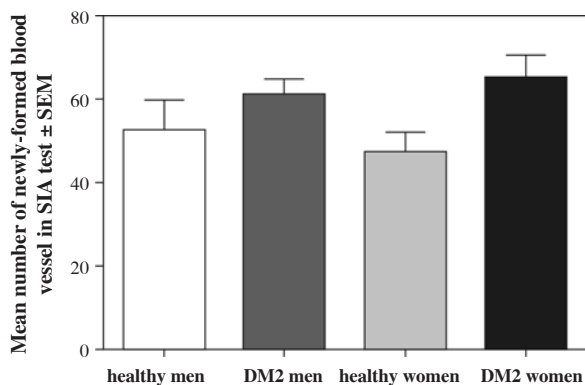


Fig. 1. Angiogenic activity of sera collected from healthy people and DM2 patients aged 40- 90 years. Total number of sera: 104.

fact that newly-formed blood vessels differ from background vasculature by their small size, tortuosity and divarications. Mean number of newly-formed blood vessels was calculated from a dozen or so separate readings and designated as “angiogenic activity” of tested sample.

Informed consent for blood drawing was obtained from each subject.

Experiments were approved by the Local Ethics Committee.

Measurement of VEGF concentration

Cytokine level was determined in examined sera using sandwich ELISA kits (R&D Systems, USA) for human VEGF, according to the producer instructions. Optical density was measured at 450 nm using spectrophotometric reader ELx 800 (Biotek Instruments, Inc., USA). VEGF concentration was expressed as pg/ml.

Statistical analysis

The results of experiments were verified statistically by unpaired T test(angiogenic activity) and Mann-Whitney test (VEGF concentration), using GraphPadPrism software package.

Results

The results of angiogenic activity of sera collected from healthy people (men and women) and DM2 patients (men and women) aged 40-90 are shown on Figure 1. DM2 women sera presented higher angiogenic activity than sera of healthy women ($p < 0.05$), Table 1. There are no statistically significant differences between angiogenic activity of sera from DM2 men and healthy men group (Table 1). No difference between DM2 men and DM2 women group was observed.

The results of VEGF content in sera from healthy people (men and women) and DM2 patients (men and women) are presented on Figure 2. DM2 women group sera

Table 1. Statistical analysis of sera angiogenic activity

	Healthy women vs. DM2 women	Healthy men vs. DM2 men
Unpaired t test		
p value	0.0109	0.3008
p value summary	*	ns
Are means signif. different? ($p < 0.05$)	yes	no
One- or two-tailed p value?	two-tailed	two-tailed

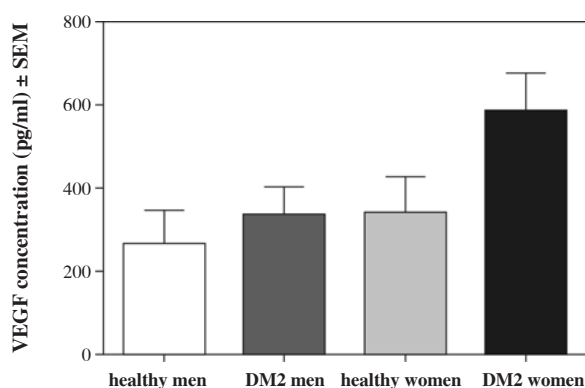


Fig. 2. VEGF content of sera collected from healthy people and DM2 patients aged 40-90 years. Total number of sera: 78.

had higher concentration of VEGF than other groups ($p < 0.02$), Table 2.

Discussion

In this paper we demonstrate for the first time sex-dependence of overall angiogenic activity of serum collected from diabetic (type 2) patients. Sera from diabetic women presented significantly higher angiogenic activity and significantly higher VEGF concentration than sera from corresponding healthy people. In the physiological conditions, angiogenesis is a dynamic process depending on slight games between pro and anti-angiogenic factors. According to Malamitsi et al. [10, 11], in the physiological conditions pro-angiogenic cytokines: bFGF – basic fibroblast growth factor and VEGF are elevated during human growth and development only.

These authors have not observed differences in sera level of bFGF between men and women. However, they showed higher level of VEGF in females than in the males sera. Another group of researchers [12] have found that serum levels of some angiomodulatory mediators (angiogenin, bFGF and leptin) depend on age and physical activity – the important observation in the clinical research on the context of sera samples standardization.

High blood glucose concentration induces hypoxia in retinal tissues, leading to the production of VEGF. Hyperglycaemia also increases inflammatory cytokines concentration in blood. The balance between VEGF and angiogenic inhibitors determines the proliferation of the new vessels in diabetic retinopathy [5].

VEGF was one the first identified pro – angiogenic growth factor, playing a key role in the development of diabetic complications, such as DME – diabetic macular edema and NV – neovascularisation of retina [13].

Table 2. Statistical analysis of sera VEGF content

	Healthy women vs. DM2 women	Healthy men vs. DM2 men
Mann Whitney test		
p value	0.0227	0.2738
Exact or approximate p value?	Gaussian approximation	Gaussian approximation
P value summary	*	ns
Are medians signif. different? ($p < 0.05$)	yes	no
One- or two-tailed p value?	two-tailed	two-tailed
Sum of ranks	245,575	180,561
Mann-Whitney <i>U</i>	109,0	114,0

Decreased levels of angiogenic inhibitors in the eye have been observed in diabetic patients. This conditions shift the balance between angiogenic factors and angiogenic inhibitors and lead to development of DME and retinal NV.

In our previous study we observed higher angiogenic activity of DM2 patients sera in comparison to healthy controls [14, 15]. We also observed in the healthy men negative correlation between the age and serum *in vivo* angiogenic activity and negative correlation between the age and serum VEGF concentration. In case of women, no such relation were observed [16].

Thus, it was very interesting to find answer to the arising question: Are there any differences in angiogenesis between female and male in pathological conditions such as diabetes? On the basis of the results obtained in this study we have found, that our examined DM2 female group presented higher sera angiogenic activity than control group. No differences between DM2 men and DM2 women group were observed. Accordingly, DM2 women group sera presented higher concentration of VEGF than other groups.

There is a group of papers showing that male and female hormonal status has a great influence on pro or antiangiogenic potential.

Some authors showed that the understanding of the basis of gender differences in vascular function is of a critical importance in order to establish gender targeted interventions in cardiovascular medicine [17]. Endothelial dysfunction may be manifested through an elevation of vascular tone and may be an initiating factor of hypertension and atherosclerosis. Jones et al. [18] reported influence of testosterone on regulation of vascular tone and vasodilatation. Other paper [19] described deleterious influence of endogenous and exogenous testosterone on mesenchymal stem cell (BMSCs) VEGF production. On the contrary, endogenous estrogen may enhance (BMSCs) VEGF production.

Our data obtained in this study agree with above mentioned papers. In general, females show more “pro-angiogenic” profile than males. It is probably connected with hormonal status and, as the last findings suggest, “angiogenic profile” may depend partially on psychosomatic status. Swedish group [20] investigated early plasma markers of prolonged stress (burn-out) in women. They found elevated levels of monocyte chemotactic protein (MCP-1), EGF and VEGF.

Final conclusion

Pathomechanism of diabetic retinopathy is multi-factorial and not homogeneous. It is confirmed by other authors' observations and our present study. This has a significant importance in therapy, which shall be adjusted to the particular patient's needs, depending on the age, sex and general health conditions.

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