

Evaluation of chronic inflammation in the aetiology of venous insufficiency by investigating cytomegalovirus DNA

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Abstract

Introduction: Lower extremity venous insufficiency is a significant health problem with economic and sociological consequences, lowering the quality of life, and sometimes leading to serious complications. The aim of this study is to evaluate the cytomegalovirus (CMV) effect on chronic inflammation in the aetiology of chronic venous insufficiency.

Material and methods: Between November 2017 and August 2018, 468 patients who underwent radio-frequency ablation therapy and phlebectomy were included in the study. PCR analyses for CMV DNA were performed on the venous tissue samples. Patients with post-thrombotic syndrome were excluded from the study. After ethical approval, the relationship between the presence of CMV DNA, gender, body mass index, and bilaterality of chronic venous insufficiency were investigated.

Results: When the relationship between CMV DNA and gender or body mass index was examined, a significant relationship was not detected. But when the patients with bilateral chronic venous insufficiency and patients with unilateral chronic venous insufficiency were compared regarding CMV DNA positivity, the patients with bilateral chronic venous insufficiency had significantly higher CMV DNA positivity ($p = 0.002$). Also, the incidence of venous ulcers in the CMV DNA exposed group was significantly higher.

Conclusions: In the literature there are many studies showing that CMV triggers atherosclerosis, but there is no study in which CMV directly produces chronic venous insufficiency. The high rates of positivity suggest that CMV, which is the basis of chronic inflammation, may be a significant factor in the aetiology of chronic venous insufficiency.

Key words: cytomegalovirus, venous insufficiency, chronic inflammation.

Table I. Characteristics of the patients

Parameter	N
Sex:	
Male	195
Female	273
BMI [kg/m ²]:	
< 25	270
> 25	198
Bilateral CVI	288
History of venous ulcer	49

BMI – body mass index, CVI – chronic venous insufficiency.

Introduction

Lower extremity venous insufficiency is a common and important health problem in the community, which effects quality of life and causes serious complications. The prevalence of varicose veins and chronic venous insufficiency has been reported between 30% and 40% in many studies [1, 2]. Venous insufficiency causes different findings ranging from telangiectatic veins that cause cosmetic discontent to painful varicose veins and even skin ulcers due to severe venous insufficiency [3].

The main mechanism of chronic venous insufficiency (CVI) is increased venous pressure due to valve insufficiency or venous obstruction. Superficial venous insufficiency is most commonly seen in the vena saphena magna (VSM) (60%) and less frequently in the vena saphena parva (VSP), perforating veins, and gonadal and pelvic veins, respectively. The main aetiological factors for venous insufficiency and varicose veins are genetic predisposition, age, gender, obesity, pregnancy, intraabdominal malignancies, thrombophlebitis, chronic inflammation, previous history of leg injury, and long standing time [4, 5].

Developments in the last 10 years have revolutionised the diagnosis and treatment of venous insufficiency. Thermal ablation methods such as endovenous laser (EVL) and radio-frequency (RF) have been developed and replaced surgical treatments all over the world. Thermal ablation with RF is the most commonly used treatment modality [6].

Cytomegalovirus (CMV) is a known cause of disease in the foetus, allograft recipients, and HIV-positive patients. It has been considered as a pathogen for intensive care units, the elderly, and the general population. The virus is able to account for varying clinical presentations. Cytomegalovirus may present anywhere in the human body, but only produces disease if the viral load reaches high levels. This is normally prevented by immune response, so the infection usually remains asymptomatic and causes disease especially in

immunosuppressed hosts [7]. Primary and latent CMV infections induce systemic inflammatory response [8].

In this study, we aimed to show the effect of CMV on chronic inflammation in the aetiological basis of chronic venous insufficiency, which is an important clinical condition with economic and socioeconomic consequences, and which may significantly affect the quality of life.

Material and methods

Between November 2017 and August 2018, 468 patients who underwent radio-frequency ablation therapy and phlebectomy were included in the study. Patients were diagnosed with unilateral and bilateral lower extremity venous insufficiency. The patients who had post-thrombotic syndrome were excluded from the study. Among the 468 patients included in the study, 195 were male and 273 were female. The median age was 47.2 ±13.1 (18–65) years. A total of 198 patients had body mass index greater than 25 kg/m², 288 had bilateral CVI, and 49 had a history of venous ulcer (Table I). The patients were not classified according to diabetes mellitus or hypertension, but none of them had diabetic neuropathy.

Varicose vein treatment preference in patients who underwent bilateral surgery was evaluated by a combination of current risk factors (age, gender, obesity, pregnancy) with the exception of additional immunosuppressive conditions (malignancy, chronic autoimmune disease, AIDS). Patients underwent radio-frequency ablation therapy and classic phlebectomy technique. Venous tissue samples were taken for CMV DNA examination. The relationship between the presence of CMV DNA and demographic data, the development of venous ulcer, and bilaterality of CVI were investigated.

CMV DNA analysis

The samples taken from patients after classical phlebectomy were subjected to physical fragmentation. After the fragmentation, 20-mg samples were placed in 2-ml sterile Eppendorf tubes, and 40 µl of tissue lysis solution (Qiagen, cat no. 939011) and 40 µl of proteinase K (Qiagen, cat no. 19133) were added and incubated at 56°C overnight. Then, 600 µl of AVE solution was added to the Eppendorf tubes and passed to the CMV DNA extraction phase. Extraction was performed on a QIASymphony SP/AS platform using a DSP Virus/Pathogen midi kit (Qiagen, cat no. 937055). PCR was performed on Rotorgene Q using an Artus CMV QS-RGQ kit (24) (cat no. 4503363). During evaluation, CMV DNA existence in the clinical samples was observed at the green channel and

the existence of any inhibition was observed in the yellow channel on the Rotorgene Q device.

Statistical analysis

All analyses were performed with the Statistical Product and Service Solutions (SPSS) software package (version 21.0, SPSS-IBM, Armonk, NY, USA) at the 95% confidence level and $p < 0.05$ significance level. Quantitative variables were reported as the mean and standard deviation (SD); qualitative variables were described as numbers and percentages. Quantitative variables were analysed by Friedman analysis for dependent groups. Subgroup analysis was performed by Wilcoxon analysis. The independent groups were compared with χ^2 analysis.

Results

A total of 468 patients participated in the study; 195 patients were male and 273 were female. The median age was 47.2 ± 13.1 (18–65) years. 288 patients had bilateral CVI, and 49 had a history of venous ulcer (Table I). When the relationship between CMV DNA and gender or body mass index was examined, a significant relationship was not detected.

While 10 of 409 patients had CMV DNA positivity in the non-venous ulcer group, 48 of 49 patients had CMV DNA positivity in patients with venous ulcer (Table II). The incidence of venous ulcer in the CMV DNA exposed group was significantly high (RR = 339.31, 95% CI: 47.4–2411.5, $p < 0.05$). Forty of 221 patients with unilateral CVI had CMV DNA positivity, and 104 of 247 patients with bilateral CVI had CMV DNA positivity. When the patients with bilateral chronic venous insufficiency and patients with unilateral chronic venous insufficiency were compared, the incidence of bilateral CVI was 1.636 times more than the unilateral CVI for the CMV DNA-positive group (95% CI: 1.396–1.918, $p = 0.002$) (Table II).

Discussion

Chronic venous insufficiency is an important clinical condition with epidemiological and socioeconomic consequences that can affect the quality of life. High prevalence, diagnosis, and treatment costs, causing loss of work and lowering the patients' quality of life, makes CVI a significant health problem. Although there are no definitive data about the incidence of varicose veins, it has been found that approximately 50% of people over 40 years old had varicose veins or telangiectasias in America and Europe. Similar studies revealed varicose vein incidence of 15–20% in adults, 0.5% of whom had deep vein insufficiency accompanied by venous ulcers [9].

Table II. Effect of cytomegalovirus (CMV) according to disease type and venous ulcer

Parameter	CMV DNA	
	Negative	Positive
Effected side:		
Unilateral	221	40
Bilateral	247	104
Venous ulcer:		
Negative	419	10
Positive	49	48

Venous diseases of lower extremities are mostly caused by VSM and VSP [3]. Age, gender, genetic predisposition, obesity, pregnancy, thrombophlebitis, and long standing time are known risk factors for CVI. While the most common clinical findings of CVI are telangiectasis and reticular or varicose veins, common symptoms are pain, cramping, itching, and complications related to venous ulcers [10]. Nowadays, there are an increasing number of studies in the literature, suggesting that infectious agents such as *C. pneumonia*, *H. pylori*, and some *Herpesvirus* subtypes may trigger the vascular inflammatory response like atherosclerosis and lead to venous insufficiency [11–14].

Venous ulcer is a common vascular pathology affecting 1% of the population, and its prevalence increases with age [15, 16]. Superficial, perforating, or deep vein insufficiency are risk factors for venous ulceration. When venous insufficiency increases, the risk of venous ulceration also increases. Chronic skin changes, oedema, and local trauma can be considered as risk factors for developing an ulcer. Although it is not clear that they act as primary causes, various neuropathies, autoimmune diseases, infection, chronic oedema, and obesity may predispose to venous ulceration [17]. The patient's age, body mass index, wound area, ulcer chronicity, impairment of calf muscle pump, deep venous reflux, and history of deep venous thrombosis (DVT) are also associated with delayed ulcer healing [18]. In some studies, venous ulcer prevalence is higher in women, which is thought to be due to chronic venous disease being more prevalent among women. However, there are contradictory studies that show small differences between genders [17]. In many studies, body mass index is the most important factor for recurrence of ulcers [18].

The CMV infections induce the pathogenesis of vascular damage and vascular stenosis through various mechanisms such as: cellular dysfunction or death, injury of the vessel wall, and increased procoagulant activity [8]. The CMV is also thought to be associated with thrombosis; the most accepted theory about the role of CMV in thrombosis pathophysiology is related to anti-phospholipid

antibodies. Moreover, in thrombosis patients, CMV mononucleosis, CMV colitis, retinitis, pneumonitis, encephalitis, and Guillain-Barré syndrome are the most prevalent CMV diseases [19]. The CMV retinitis occurs predominantly in human immunodeficiency virus (HIV)-infected patients [20]. Primary and latent CMV infections also induce systemic inflammatory response [8].

Cytomegalovirus has become the subject of an increasing number of studies associated with chronic inflammation, and it is an important human pathogen that causes disease especially in immune-suppressed hosts and remains in the host after primary infection, persistently. Although studies suggest that the vascular wall may be a latent infection area for CMV and reactivation of the virus in this area may be the mechanism of the development of vascular pathology, it has not been proven that this virus causes the vascular pathology as a primary or leading to the lesion on the vascular wall and causes pathology [10–12].

According to our study, there was no significant relationship between CMV DNA and gender or body mass index. Although in our study we showed an increased rate of CMV DNA positivity in patients with venous ulcer and bilateral venous insufficiency, we still could not detect whether CMV triggers the venous insufficiency by chronic inflammation. Also we did not classify patients according to diabetes mellitus and hypertension. These can be considered as limitations of our study.

In conclusion, in the literature, there are many studies showing that CMV triggers atherosclerosis, but there are no studies ascertaining whether CMV directly produces CVI by chronic inflammation. Our study suggests that CMV DNA positivity was significantly higher in patients with bilateral chronic venous insufficiency, and the incidence of venous ulcer in the CMV DNA-exposed group was significantly higher. The high rates of positivity suggests that CMV, which is the basis of chronic inflammation, may be significant in the aetiology of chronic venous insufficiency.

Conflict of interest

The authors declare no conflict of interest.

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