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Angiogenic factors are associated with multiple sclerosis

Sajad Karampoor [a], Hamid Zahednasab [b], Sreeram Ramagopalan [c], Masoud Mehrpour [d], Hossein Keyvani [a,*]

[a] Department of Virology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[b] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[c] Evidera, London, United Kingdom
[d] Department of Neurology, Firoozgar Hospital, Firoozgar Clinical Research Development Center (FCRDC), Iran University of Medical Sciences (IUMS), Tehran, Iran

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ABSTRACT

A growing body of evidence suggests that angiogenesis plays a crucial role in the pathogenesis of multiple sclerosis (MS). Animal models of MS show a significant improvement when the process of angiogenesis is halted. In this study, we measured the serum levels of vascular–endothelial growth factor (VEGF), soluble Endoglin (sEng), angiopoietin 1 (Ang-1), angiopoietin 2 (Ang-2), and uric acid (UA) as well as serum anti-Epstein–Barr virus (EBV) EBNA-1 IgG in 50 MS patients (including 20 newly diagnosed and 30 patients taking IFN-beta for >6 months) and 40 healthy individuals. Enzyme-linked immunosorbent assays (ELISA) were used apart from UA where the uricase quantitative enzymatic assay was used. A significant increase of VEGF, Ang-1, Ang-2, and sEng in serum samples of MS patients with respect to healthy subjects was observed. VEGF was higher in newly diagnosed MS patients in comparison to patients taking interferon-beta and was associated with EDSS. The serum levels of UA were statistically lower in MS patients as compared to the healthy group. Higher levels of anti-EBV antibody titers were seen in MS patients than controls and anti-EBV titers correlated with angiogenic factors. It seems that in summary, angiogenesis may play an important role in MS and infection with EBV might be correlated with this phenomenon.

1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) in which the immune system is thought to attack myelinated axons in the CNS, demolishing myelin and axons that leads to varying degrees of disability (Goldenberg, 2012). MS mainly affects young adults with age range of 20–40 years of age (Trapp and Nave, 2008). The disease has a heterogeneous presentation including sensory disturbance, blurry vision, imbalance, fatigue, motor impairment, and cognitive deficits (Dendrou et al., 2015). Currently, there is no cure for MS. Treatment typically focuses on expediting recovery from relapses, decelerating the progression of the disease and managing symptoms. Interferon-beta (IFN-β) is one of the most common disease modifying therapies (DMTs) for MS (Group, I.M.S. S, 1993) displaying inhibitory effects on proliferation of leukocytes, antigen presentation, and modulation of cytokine production in favor of the anti-inflammatory phenotype (Paty et al., 1993).

Although the precise etiology of MS remains enigmatic, it has been postulated that interplay between genetic and environmental factors are involved in the development of this devastating disease (Ramagopalan et al., 2010). Numerous studies indicate that both human leukocyte antigen (HLA) (Hillert, 1994) and non-HLA (Bahreini et al., 2010) genes contribute to the development of MS in individuals bearing some alleles or single nucleotide polymorphisms (SNPs). Among environmental factors, Epstein–Barr virus (EBV) is a leading candidate. It has been shown that nearly all MS patients (>99%) are sero-positive for EBV with respect to age and sex matched controls (94%), implying that MS is very rare in adults who have not been infected with EBV (Pakpoor et al., 2012). In line with this, individuals with high titers of anti-EBV antibodies are more prone to develop MS in comparison to those having low titers of antibodies (Pakpoor et al., 2013).

Accumulative evidence suggests that angiogenesis plays a crucial role in the pathogenesis of MS disease (Hamid and Mirshafiey, 2016). Angiogenesis is primarily defined by the formation of new blood vessels from pre-existing ones such as capillaries and post-capillary venules (Otrock et al., 2007). Angiogenesis is a fine-tuned process being under delicate regulation between pro-angiogenic and anti-angiogenic factors. Any condition that tilts the balance in favor of pro or anti angiogenic agents can cause a disturbance in physiological condition leading to illness. It has been shown that there is a strong cross-talk between inflammation and angiogenesis implying that inflammatory reactions rely on angiogenesis (Jackson et al., 1997). A growing body of evidence reveals that angiogenesis plays a role in MS pathology, not only being present at the edge of acute MS lesions but also in the area surrounding the plaque, and it is often associated with areas of inflammation (Seabrook et al., 2010). It

* Corresponding author.
E-mail address: keyvanlab@yahoo.com (H. Keyvani).

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has been demonstrated that blood vessels within lesions comprise reactive endothelial cells representing an abnormal morphology, consistent with angiogenesis (Proescholdt et al., 2002). It has also been shown that some pro-angiogenic factors are elevated in post-mortem brain and in the animal model of EAE including vascular–endothelial growth factor (VEGF) (Proescholdt et al., 2002), angiopoietin 2 (Ang-2) (MacMillan et al., 2012), and soluble endoglin (sCD105, sEng) (Thone et al., 2016).

Apart from the association between angiogenesis and inflammation, some other factors have been investigated to stimulate angiogenesis. Epstein–Barr nuclear antigen 1 (EBNA-1) is capable of modulating the AP-1 transcription factor pathway and enhancing angiogenesis in nasopharyngeal carcinoma cells (NCC) (O'Neil et al., 2008a). Studies show increased anti-EBNA-1 IgG titers in serum of patients with MS in comparison to healthy individuals (Lunemann et al., 2008; Mameli et al., 2013). EBV is also able to promote angiogenesis by inducing the secretion of pro-angiogenic cytokines, such as the VEGF (Wakisaka et al., 2004).

On the other hand, some molecules possess anti-angiogenic effect which in turn can modulate the inhibitory cascades occurred in inflammation. IFN-β is one of those molecules that has been shown to halt angiogenesis through inducing genes involved in anti-angiogenesis (Taylor et al., 2008). Uric acid (UA) is decreased in the serum of patients affected by MS (Ashtari et al., 2013) and is able to dampen angiogenesis by diminishing VEGF. Based on these statements, we hypothesized that (i) pro-angiogenic factors maybe higher in the serum of MS patients (treatment naïve and IFN-beta-treated patients) than healthy individuals; (ii) EBV titers may be associated with high pro-angiogenic factors in the serum of MS patients; (iii) higher levels of pro-angiogenic factors might be correlated with the disability of patients afflicted with MS. In this study, we measured four factors attributed to pro-angiogenesis including angiopoietin 1 (Ang-1), Ang-2, sEng, and VEGF in the serum of patients with MS along with the titers and concentrations of anti-EBV antibodies and UA, respectively.

2. Materials and methods

2.1. Patient and sample collection

A case–control study was designed to understand the serum factors associated with angiogenesis in both newly diagnosed MS patients and MS patients who were on beta-interferon therapy for at least 6 months. In order to obtain legal and ethical permission for gathering the samples, informed consent was taken from all individuals who participated in this study. Additionally, this project was approved by the ethics committee of Iran University of Medical Sciences (ECIUMS; ethical code# 94-04-30-25961). A total number of 90 individuals who visited the department of Neurology, Firouzgar Hospital, Iran University of Medical Sciences (IUMS) were recruited in this research and categorized into three groups as follows: the first group comprised 20 individuals who were definitely diagnosed for the first time (new cases) as having active relapsing remitting MS (RRMS) and therefore had no experience of receiving immunosuppressive/immunomodulator treatment or/and vitamin D supplements; the second group consisted of 30 patients who had a diagnosis of active RRMS at least 1 year prior and were being treated with interferon-beta; the third group were healthy individuals. Individuals afflicted with any types of cancer, recent infection and/or cardiovascular disease were excluded from the study. The samples obtained from RRMS patients taking IFN-beta while they were at the remission stage of the disease and they were all Interferon-beta responders. The serum specimens of newly diagnosed MS patients were obtained when they were at relapse phase of the disease.

The diagnosis of MS was carried out by an expert and trained neurologist based on the revised McDonald's criteria (Polman et al., 2011). After complete neurological examinations, demographic data as well as the EDSS of the patients were obtained. All blood samples (5 ml from each patient) were collected between 2015 and 2016. Immediately after sample collection, the serum was separated by centrifugation and stored at −70C° until use.

2.2. ELISA for angiogenic factors

Serum levels of Ang-1, Ang-2, VEGF, and sEng were measured using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions (Abcam, Cambridge, MA, USA). All samples were examined in duplicate and the mean values of individual sera were applied for the statistical analysis. The intra-assay and inter-assay coefficients of variation (CV) were <12.0%, for all four measured factors.

2.3. Uric acid concentrations

The concentrations of UA were determined using the uricase quantitative enzymatic assay (Biolabo SA, Maizy, France). Measurements were carried out in accordance to the manufacturer's instructions. Specimens were tested in a Hitachi 7150 auto-analyzer (Hitachi, Tokyo, Japan).

2.4. Serology of Epstein–Barr virus

The serum titers of anti-EBNA-1 IgG were measured following the manufacturer's instructions (Abcam, Cambridge, MA, USA). Samples with values greater than the upper limit of detection were diluted to extend the range of detection. Based on the manufacturer's guidelines, the test results were interpreted as positive if >11 U/ml, equivocal if 9–11, and negative if <9 U/ml.

2.5. Statistical analysis

The Kruskal–Wallis test was used for the global hypothesis followed by Dunn–Bonferroni correction for the multiple comparisons. Fisher's exact test was used to compare the sex ratio. Correlations were assessed with Pearson's method. Linear regression was used to assess relationships between variables after adjusting for confounding factors.

3. Results

3.1. Demographic and clinical features

As shown in Table 1, all individuals including newly diagnosed MS patients, IFN-beta-treated MS patients (RRMS at remission phase), and healthy subjects were similar in terms of sex and age as there were no

Table 1
Demographic and clinical characteristics of cases and healthy individuals.

	Healthy individuals ($n = 40$)	Chronic MS patients ($n = 30$)	Newly diagnosed MS patients ($n = 20$)	Statistical test (P value)
Gender (female/male ratio)	31/9	24/6	15/5	Fisher's exact test[a] ($P = 0.07$)
Age (mean ± SD) (years)	30.7 ± 8.5	33.13 ± 9.2	31.27 ± 7.14	One-way ANOVA ($P = 0.52$)
Duration of disease (mean ± SD) (years)	–	2.5 ± 1.35	–	–
EDSS (mean ± SD)	–	1.65 ± 0.52	1.57 ± 0.49	Student t-test ($P = 0.47$)

[a] The P values shown have been calculated between controls and chronic MS patients.