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Association of VEGF and ANGPT1 Gene Expression with Microvessel Density of Synovial Tissues in Rheumatoid Arthritis

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ABSTRACT

Angiogenesis plays a crucial role in synovial cell proliferation and considered to be an essential process in chronic/destructive arthritis and in rheumatoid arthritis. VEGF (vascular endothelial growth factor) and ANGPT1 (Angiopoietin 1) plays an important role in blood vessel formation in synovial tissues results in persistence of inflammation. VEGF and ANGPT1 gene expressions were evaluated by quantitative Real Time PCR (qRT-PCR). Microvessel density (MVD) of synovial tissues were assessed by CD34 staining using immunohistochemistry. VEGF and ANGPT1 gene expression significantly associated with DAS28 score, bone erosion of RA patients. Up regulated gene expression was also correlated with elevated microvessel density of synovial tissues. Our results suggested that elevated VEGF and ANGPT1 gene expression might be involved in microvessel density and disease progression in RA patients.

INTRODUCTION

Rheumatoid arthritis (RA), a chronic inflammatory autoimmune disease affects synovial tissues of multiple joints. RA is characterized by infiltration of inflammatory cells which results in cartilage and bone destruction. Clinical manifestations of RA include joint swelling, stiffness, deformity and pain. The major sites of the tissue damage are joints with rheumatoid synovial microenvironment consisting intense immunological activity.

Angiogenesis is the formation of new blood vessels from pre-existing vasculature which plays an important role in the pathogenesis of several inflammatory autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, spondyloarthropathies, and atherosclerosis[1,2,3]. In RA, accumulation of circulating leukocytes at the inflamed joint requisites the formation of neovascularization to provide nutrients and oxygen to the hypertrophic joint[4,5.] The elevated pro-angiogenic factors act as an antagonist to the angiogenic inhibitors thereby support the excessive transendothelial leukocyte infiltration that promotes the synovial inflammation as well as the cartilage/bone destruction[4,5.] Previously, several studies were demonstrated that genetic abnormalities in angiogenic pathway genes significantly associated with disease development and progression in various diseases including cancers[6,7.]

Involvement of several endothelial growth factors have been reported in RA synovitis, among, VEGF (vascular endothelial growth factor) is the most endothelial cell growth factor which induces vascular permeability in synovial tissues. In RA synovium, Interleukin 1(IL-1) and Tumour Necrosis Factor (TNF) might be involved in up regulation of fibroblast VEGF expression which induces the neovascularisation results in leukocyte aggregation and synovial inflammation[8,9.] Another angiogenic growth factor, Angiopoietin (ANGPT-1) had been demonstrated to be involved in neovascularization in several pathological conditions. Gravallase EM et al.[10] reported that ANGPT-1 mRNA levels were upregulated in synovial tissues of RA patients and suggested ANGPT-1 might be an important angiogenesis regulator in inflammatory arthritis. These reports imply active involvement of VEGF and ANGPT1 in the angiogenesis of RA, however, their precise role in the development of synovial angiogenesis remains to be determined.

The aim of the present study is to evaluate the expression of VEGFA and ANGPT1 genes in synovial tissues of the RA patients and correlate the expression levels with microvessel density (MVD) of synovial tissues.

MATERIALS AND METHODS

Patients and study design

The study consisted of 50 Rheumatoid arthritis patients from Regional Orthopaedic and Arthritis Centre, Hyderabad, India. This study was approved by the Institutional Ethical Board Regional Orthopaedic and Arthritis Centre, Hyderabad. Before recruitment, an informed consent was taken from all patients. Demographical and Clinical characteristics of RA patients such as age, gender, age at onset, disease duration, DAS28, Rheumatoid factor, Erosive RA were collected. Synovial tissue specimens were obtained from RA patients during total knee joint arthroplasty. All participants fulfilled the diagnostic criteria of the American College of Rheumatology. For RNA isolation tissue samples were stored in RNAlater solution (Thermo Fisher Scientific, USA) and for immunohistochemistry, tissues were stored in 10% formalin then embedded in paraffin.

RNA isolation and gene expression analysis

Isolation of RNA was carried out by using TRIzol reagent (Thermo Fisher, USA). The quality and quantity of isolated RNA was assessed by Nano Drop spectrophotometer (Thermo Fisher Scientific, USA) followed by cDNA conversion using High capacity cDNA conversion kit (Thermo Fisher). The expression levels of VEGF and Ang-1genes assessed by Taqman gene expression assays (Thermo Fisher, USA). Assay ID are VEGFA Hs00900055_m1; ANGPT1 Hs00919202_m1. All experiments were carried out in triplicate using ABI PRISM 7500 (Applied Biosystems, USA) and GAPDH was used as an internal control to normalize the expression levels. The data for gene expressions were analysed with the $\Delta\Delta$Ct method.

Assessment of Microvessel Density (MVD) using immunohistochemistry

Development of neovascularization can be determined by assessing the microvessel density (MVD) using CD34 staining. Staining procedures and evaluation of MVD was carried out as previously described11.

RESULTS

The demographic and clinical characteristics of RA patients were summarized in table1. Sixty percentage of RA patients were above the age of 40 years at the time of diagnosis. The mean age of the RA patients was 57.18±14.15 whereas the mean of age at onset was 41.13±12.89. Seventy six percent of patients were diagnosed with erosive arthritis whereas 82 percent of patients were positive with rheumatoid factor.

Elevated VEGF and ANGPT1 gene expression was observed in patients with above 5 years disease duration compared to patients with below 5 years disease duration (p= 0.05). Similar trend was observed in patients with erosive arthritis (p= 0.03) and patients with high DAS28 score (p= 0.05). The association between gene expression and microvessel density (MVD) with respect to clinical parameters was illustrated in table2. Increased gene expression was significantly associated with elevated microvessel density (MVD) in patients with above 5 years disease duration (32.93±11.56; 43.13±18.58, p= 0.03), higher DAS score (25.11±12.05; 33.56±14.29, p= 0.05) and erosive arthritis (35.05±14.38; 27.46±11.83, p= 0.05) respectively.

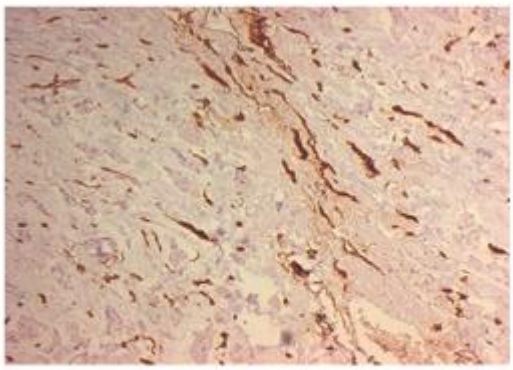
CD34 staining of Microvessel density in synovial tissues.

Table 1. Demographic data of RA patients

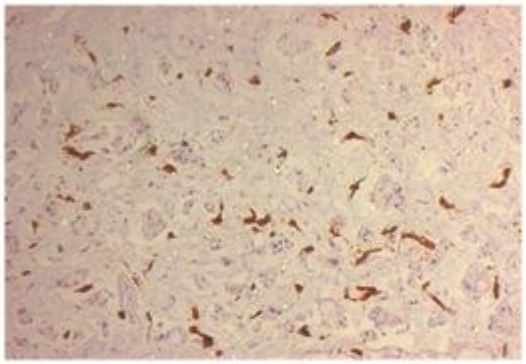
Parameter	Percentage Or Mean
Gender	
Male	29 (58%)
Female	21 (42%)
Age	
<40	20 (40%)
>40	30 (60%)
Age (mean)	57.18±14.15
Age at onset (mean)	41.13±12.89
Disease duration(mean)	11.21±6.89
Rheumatoid factor (Positive)	
Positive	41 (82.0)
Negative	9 (18.0)
DAS28	
<2.5	27 (54%)
>2.5	23 (46%)
Erosive RA	
Yes	38 (76.0)
No	12 (24.0)

Table 2. Association of Microvessel density (MVD) with demographic and clinical parameters of RA patients

Parameter	Mean±SD
Gender	
Male	31.81±17.55
Female	29.07±26.42
	P = 0.12
Age at onset	
<40	29.11±12.05
>40	33.56±14.29
	0.19
Disease duration	
<5 years	32.93±11.56
>5 years	43.13±18.58
	0.03
DAS28	
<2.5	25.11±12.05
>2.5	33.56±14.29
	0.05
Erosive RA	
Yes	35.05±14.38
No	27.46±11.83
	0.05



Synovial tissue with elevated VEGF Expression showing high MVD



Healthy control

DISCUSSION

Angiogenesis plays an important role in synovial cell proliferation considered to be an essential underlying process in chronic/destructive arthritis and in rheumatoid arthritis. Several soluble and cell- surface bound factors stimulate neovascularization. The perpetuation of neovascularization involves numerous soluble and cell surface-bound mediators had been associated with rheumatoid arthritis. Increased angiogenesis (high

microvessel density) have important clinical relevance for different rheumatic diseases including RA. Regulation of inflammatory neovascularization significantly attenuates synovitis in RA patients.

In our study, VEGF and ANGPT1 gene expression significantly associated with clinical characteristics of the RA patients. We observed increased VEGF and ANGPT1 gene expression in patients with above 5 years disease duration. Similar trend was also observed in high DAS28 score patients and erosive positive patients. Ozgonenel L et al.[12] reported that elevated VEGF associated with high DAS scores of the RA patients. Single nucleotide polymorphisms of VEGF gene found to be associated with increased serum VEGF levels and DAS score in RA patients[7]. It can be attributed that elevated VEGF and ANGPT1 levels might be promote the neovascularization that allows the leukocyte aggression in synovial tissues and ultimately results in persistence of the inflammation in joints of the RA patients. Further, increased VEGF and ANGPT1 expression correlated with elevated microvessel density of the synovial tissues of RA patients. Previously, several studies reported that dysregulated angiogenic growth factors associated with elevated microvessel density in several inflammatory diseases and cancers. Increase microvessel density was associated with VEGF levels in RA patients compared to osteoarthritis patients. In our study, elevated microvessel density significantly associated with disease duration, DAS score and bone erosion of RA patients suggesting that neovascularization might be perpetuated the leukocyte ingress into synovial tissue results persistence inflammatory conditions.

In conclusion, our results suggest that elevated VEGF and ANGPT1 gene expression might be contributed in elevated microvessel density of RA patients and might be involved in the persistence of inflammatory conditions.

CONFLICT OF INTEREST

None

REFERENCES

1. Szekanecz Z, Koch AE. Vascular involvement in rheumatic diseases: ‘vascular rheumatology’. Arthritis Res Ther. 2008;10:224.
2. Szekanecz Z, Koch AE. Mechanism of disease: angiogenesis in inflammatory diseases. Nat Clin Pract Rheumatol. 2007;3:635–643.
3. Lainer-Carr D, Brahn E. Angiogenesis inhibition as a therapeutic approach for inflammatory synovitis. Nat Clin Pract Rheumatol. 2007;3:434–442.

4. Agarwal SK, Brenner MB. Role of adhesion molecules in synovial inflammation. Curr Opin Rheumatol .2006;18:268–276.
5. Szekanecz Z, Koch AE. Macrophages and their products in rheumatoid arthritis. Curr Opin Rheumatol. 2007;19:289–295.
6. Meka PB, Cingeetham A et al. HIF-1α (1772C>T) polymorphism as marker for breast cancer development. Tumour Biol. 2015; 36(5):3215-20
7. Jin-Ping Yi, Yu-Zhang Wu et al. VEGF Gene Polymorphisms Affect Serum Protein Levels and Alter Disease Activity and Synovial Lesions in Rheumatoid Arthritis. Med Sci Monit. 2016; 22: 316–324.
8. Koch AE, Harlow LA, Haines GK, et al. Vascular endothelial growth factor. A cytokine modulating endothelial function in rheumatoid arthritis. J Immunol. 1994;152:4149–4156.
9. Kiselyov A, Balakin KV, Tkachenko SE. VEGF/VEGFR signaling as a target for inhibiting angiogenesis. Expert Opin Investig Drugs. 2007;16:83–107.
10. Gravallese EM, Pettit AR et al. Angiopoietin-1 is expressed in the synovium of patients with rheumatoid arthritis and is induced by tumour necrosis factor alpha. Ann Rheum Dis. 2003;62(2):100-7.
11. Meka Pb et al. LCN2 Promoter Methylation Status as Novel Predictive Marker for Microvessel Density and Aggressive Tumor Phenotype in Breast Cancer Patients. Asian Pac J Cancer Prev. 2015;16(12):4965-9.
12. Ozgonenel L[1], Cetin E, Tutun S. The relation of serum vascular endothelial growth factor level with disease duration and activity in patients with rheumatoid arthritis. Clin Rheumatol. 2010;29(5):473-7.
13. Giatromanolaki A[1], Sivridis E. The angiogenic pathway "vascular endothelial growth factor/flk-1(KDR)-receptor" in rheumatoid arthritis and osteoarthritis. J Pathol. 2001;194:101-108.