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Research Brief

The study of novel inflammatory markers in takayasu arteritis and its correlation with disease activity

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ABSTRACT

Objectives: Currently, erythrocyte sedimentation rate (ESR) and highly sensitive serum C-reactive protein (hsCRP) levels are used to monitor disease activity and guide therapy in Takayasu Arteritis (TA). However, non-specificity of these markers suggests the need for novel biomarkers. In this pilot study, we explore the role of novel biomarkers for evaluating disease activity in TA.

Methods: A total of 40 patients with TA were divided into active and stable disease groups. Disease activity was assessed according to the National Institutes of Health criteria proposed by Kerr et al. Routine blood investigations were obtained and serum tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-18, ESR, hsCRP levels and NLR (neutrophil to lymphocyte ratio) were assayed at baseline and after 6 months.

Results: Among the 40 patients enrolled, 18 were classified as active while 22 were stable at baseline and with a similar pattern at 6 months. Along with ESR and hsCRP, IL-6 and IL-18 levels were significantly higher in the active disease group than in the stable disease group ($p < 0.005$). The levels of other novel biomarkers (IL-1, TNF-α) and NLR were not significantly higher in active disease group.

Conclusion: Serum IL-6 and IL-18 levels correlates well with disease activity in TA which suggests their important role in disease pathogenesis and may be helpful in guiding and monitoring therapy in active TA patients.

1. Introduction

Takayasu arteritis is a chronic inflammatory arteritis involving mainly the aorta and its major branches, including the coronary, carotid, pulmonary and renal arteries.[1,2] Inflammation of these arteries results in segmental stenosis, occlusion, dilatation and/or aneurysm. It has progressive course with relapses and remissions. The activity of disease is currently assessed by ESR and hsCRP.[3] The disease progresses despite normal ESR, suggesting that ESR may not be a reliable indicator of activity,[4] hence there remains an unmet need for an appropriate biomarker to assess disease activity.

Several novel biomarkers such as IL-6, IL-8, IL-18 and TNF-α have been suggested as candidate biomarkers.[5–7] However, there is paucity of data on the association of these biomarkers to disease activity in TA. In present study, we examined the levels of novel inflammatory biomarkers (IL-1, IL-6, IL-18, TNF-α) and neutrophil lymphocyte ratio (NLR) in TA patients and their correlation to disease activity along with conventional biomarkers (ESR and hsCRP).

1.1. Material and methods

This was a prospective observational study in which 40 TA patients during January 2018 to July 2019 were enrolled. All the patients underwent thorough clinical examination, blood investigations, MR (magnetic resonance)/CT (computed tomography)/conventional angiography at baseline and six months post procedure to assess restenosis/new lesions. The study population was divided into two groups: one with active disease and another

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one with inactive disease. Disease activity was assessed according to the National Institutes of Health criteria for disease activity.[8] All patients who had active disease were given standard treatment as per the guidelines. Commercial enzyme linked immunosorbent assay (ELISA) kits were utilized for the measurement of serum TNF-α, IL-1, IL-6, IL-18 (Diaclone kit). Patients who were pregnant, hemodynamically unstable and had systemic inflammatory disorders and connective tissue disease were excluded from the study.

1.2. Statistical analysis

Statistical analysis was done with SPSS 26.0 software. Depending upon the type of data distribution, the data was depicted as mean ± standard deviation or median (Inter quartile range). Mann Whitney *U* test was used for comparing levels of inflammatory biomarkers between two groups. Statistical significance was set at a probability level <0.05.

2. Results

Among 40 patients of diagnosed TA (old as well as new) enrolled, 18 patients had active disease while 22 had stable disease at baseline. These patients were followed up for 6 months and again 18 patients were found to be active and 22 had stable disease. Table 1 summarizes the baseline characteristics of the study subjects. At baseline, the active disease group had higher levels of acute phase reactants (ESR and hs-CRP) compared to stable disease group ($p < 0.005$) (Tables 2 and 3).

IL-6 and IL-18 values were significantly elevated in active disease group compared to stable disease group at baseline ($p = 0.001$). Levels of TNF-α, IL-1 and NLR were higher in patients with active disease as compared to stable TA patients but did not attain statistically significance with $p = 0.8$, $p = 0.42$ and $p = 0.06$ respectively. At six months of follow-up, ESR, hsCRP, IL-6 and IL-18 levels were significantly elevated in active group compared to stable group with $p < 0.05$ for all the variables.

At baseline, six patients of TA out of 18 in active disease group had normal ESR and hsCRP levels but IL-6 and IL-18 were raised in four out of these six patients. Similarly, at six months follow up, six patients in active disease group had normal conventional biomarkers but IL-6 and IL-18 were raised in two out of these six patients. It signifies that IL-6 and IL-18 levels were more sensitive than ESR and hsCRP in detecting the disease activity. As compared to the NIH criteria for disease activity, the sensitivity of IL-6 was 92.4 % with a specificity of 77.3 %, positive predictive value of. 77.3 % and negative predictive value of 94.4 %. Similarly, for IL-18, a sensitivity of 93.4 %, a specificity of 90.9 %, positive predictive value of 89.5 % and negative predictive value of 95.2 % was obtained. Both ESR and hsCRP had a lower sensitivity and specificity in assessment of disease activity (ESR: sensitivity - 88.9 %; specificity - 86.4 %, hs CRP: sensitivity - 77.8 %; specificity - 82.9 %). Seven patients in our study had restenosis in the initial visit with IL-6 elevated in six patients (85 %) and six had stenosis at six months, with IL-6 elevated in five patients (83 %). This suggests that patients with higher levels of IL-6, IL-18 were more likely to develop restenosis.

3. Discussion

Once diagnosis of TA is made, determining the degree of disease activity is mandatory before the decision is made to start treatment with immunosuppressive medications. Assessing disease activity in patients with TA is often challenging. The NIH criteria for active disease[8] have been conventionally accepted as reliable measures of disease activity. The NIH criteria proposed by Kerr et al comprises of clinical symptoms such as fever, musculoskeletal, raised ESR, vascular signs such as claudication, diminished/absent pulse or bruit, asymmetric BP in either upper or lower limbs and typical angiographic features.[8] Due to the incorporation of imaging findings and acute phase reactants, the NIH criteria often seems to be robust. However, several studies have recognized that patients thought to be in remission at the time of surgery can have evidence of acute and/or chronic inflammation at histopathological examination.[8–10] Sequential angiographic evaluation performed regardless of disease activity found new lesions in 61 % of patients who experienced prolonged remission by clinical criteria.[8] Angiography is still considered gold standard in delineating vascular lesions in TA patients however, its invasiveness and cumulative radiation toxicity limit its use in monitoring disease progression.

In our study, mean ESR and hsCRP levels in patients with active disease were significantly higher than those with stable disease ($p < 0.05$ for both). It was also seen that mean IL-6 and IL-18 levels of patients with active TA were significantly higher than those of

Table 1
Baseline characteristics of the study population ($n = 40$).

Age (years)	28.98 ± 5.51
Females (%)	39 (98.0 %)
Signs and symptoms	
Claudication	27 (72.5 %)
Peripheral pulse	
i) Absent upper limb pulses (%)	26 (65 %)
ii) Absent lower limb pulses (%)	1 (2.5 %)
iii) Absent carotid pulse (%)	22 (55 %)
Carotidynia (%)	23 (58 %)
Syncope (%)	13 (33 %)
Chest pain (%)	4 (10 %)
Dyspnoea (%)	20 (50 %)
Mean SBP/DBP (mm Hg)	148.9 ± 22/88 ± 8.35
Laboratory parameters	
Hemoglobin (g/dl)	11.65 ± 2.15
Total leucocyte count (cells/mm$^{3)}$ (median IQR)	8900 (7600–9860)
Serum Creatinine (mg/dl)	1.0 ± 0.28
Mean EF (echocardiography)	52 ± 8%
Inflammatory markers	
ESR	34 ± 17.9
hsCRP (mg/ml)	2.29 ± 1.3
IL-6 (pg/ml)	7.48 ± 5
IL-18 (pg/ml)	210 ± 123
TNF-α (pg/ml)	7.64 ± 3.4
IL−1 (pg/ml)	4.89 ± 1.47
NLR	2.82 ± 0.51
Vascular involvement in angiography	
1. Ascending Aorta	4 (10 %)
2. Arch of Aorta	30 (75 %)
3. Descending thoracic Aorta	29 (72.5 %)
4. Abdominal Aorta	28 (70 %)
5. Brachiocephalic Artery	13 (32.5 %)
6. Right Common Carotid Artery	15 (37.5 %)
7. Left Common Carotid Artery	22 (55 %)
8. Right Subclavian Artery	19 (47.5 %)
9. Left Subclavian Artery	30 (75 %)
10. Right Vertebral Artery	18 (45 %)
11. Left Vertebral Artery	17 (42.5 %)
12. Right Internal Carotid Artery	14 (35 %)
13. Left Internal Carotid Artery	17 (42.5 %)
14. Right Renal Artery	11 (27.5 %)
15. Left Renal Artery	14 (35 %)
16. Coeliac Artery	15 (37.5 %)
17. Superior Mesenteric Artery	9 (22.5 %)
18. Pulmonary Artery	4 (10 %)
Pattern of vascular involvement	
Long stenotic	37 (92.5 %)
Post-stenotic dilatation	15 (37.5 %)
Aneurysm	9 (22.5 %)

Abbreviations: DBP- diastolic blood pressure; EF- ejection fraction; ESR-erythrocyte sedimentation rate; hsCRP- highly sensitive C reactive protein; IL-interleukin; IQR-interquartile range; NLR-neutrophil to lymphocyte ratio; SBP- systolic blood pressure; TNF- tumor necrosis factor.

Table 2
Distribution of inflammatory markers in Takayasu arteritis at baseline and after six months.

Variables	Activity at baseline			Activity at six months		
	Present ($n = 18$)	Absent ($n = 22$)		Present ($n = 18$)	Absent ($n = 22$)	
	Mean ± SD	Mean ± SD	p-value	Mean ± SD	Mean ± SD	p-value
ESR (mm/hr)	46.17 ± 18.7	20.28 ± 10.2	**0.001**	55.61 ± 23.40	18 ± 7.95	**0.001**
hsCRP (mg/l)	3.40 ± 1.13	1.07 ± 0.16	**0.003**	3.05 ± 0.88	1.48 ± 0.84	**0.002**
NLR	2.86 ± 0.61	2.74 ± 0.39	**0.38**	3.31 ± 0.63	3.21 ± 0.63	**0.25**
IL-6 (pg/ml)	12.11 ± 4.10	3.01 ± 0.9	**<0.001**	8.0 ± 2.02	3.5 ± 1.20	**0.01**
IL-18 (pg/ml)	298 ± 90	119.17 ± 23.7	**0.002**	354 ± 92	101 ± 10.28	**0.02**
TNF-α (pg/ml)	8.33 ± 3.8	6.61 ± 3.25	**0.15**	9.17 ± 3.16	8.11 ± 2.76	**0.23**
IL-1 (pg/ml)	5.10 ± 1.50	4.31 ± 0.29	**0.07**	3.31 ± 0.63	3.21 ± 0.63	**0.25**

Abbreviations: ESR-erythrocyte sedimentation rate; hsCRP- highly sensitive C reactive protein; IL-interleukin; NLR-neutrophil to lymphocyte ratio; TNF- tumor necrosis factor.

Table 3
Comparison of biomarkers at baseline and after six months in active and inactive disease group.

		Activity at baseline		Activity at six months	
		Present ($n = 18$)	Absent ($n = 22$)	Present ($n = 18$)	Absent ($n = 22$)
ESR (mm/hr)	>30	12 (66.7 %)	5 (22.7 %)	14 (77.8 %)	3 (13.6 %)
	≤30	6 (33.3 %)	17 (77.3 %)	4 (22.2 %)	19 (86.4 %)
hsCRP (mg/l)	>3	12 (66.7 %)	3 (13.6 %)	13 (72.2 %)	5 (22.7 %)
	≤3	6 (23.4 %)	19 (86.4 %)	5 (27.8 %)	17 (77.3 %)
NLR	>3.5	3 (16.7)	2 (9 %)	6 (33.3 %)	12 (54.5 %)
	≤3.5	15 (83.3 %)	20 (91 %)	12 (66.7 %)	10 (45.5 %)
IL-1 (pg/ml)	>6.25	5 (27.8 %)	6 (27.3 %)	3 (16.7 %)	2 (9 %)
	≤6.25	13 (72.2 %)	16 (72.7 %)	15 (83.3 %)	20 (91 %)
IL-6 (pg/ml)	>5	16 (88.8 %)	4 (18.2 %)	15 (83.3 %)	5 (22.7 %)
	≤5	2 (11.1 %)	18 (81.8 %)	3 (16.6 %)	17 (77.3 %)
TNF-α (pg/ml)	>8	4 (22.2 %)	10 (45.5 %)	8 (44.4 %)	12 (54.5 %)
	≤8	14 (77.8 %)	12 (54.5 %)	10 (55.6 %)	10 (45.5 %)
IL-18 (pg/ml)	>215	16 (88.8 %)	2 (9.1 %)	16 (88.8 %)	3 (13.6 %)
	≤215	2 (11.1 %)	20 (90.9 %)	2 (11.2 %)	19 (86.4 %)

Abbreviations: ESR-erythrocyte sedimentation rate; hsCRP- highly sensitive C reactive protein; IL-interleukin; NLR-neutrophil to lymphocyte ratio; TNF- tumor necrosis factor.

stable disease ($p < 0.05$), but mean TNF-α, IL-1, NLR were not elevated to a significant level, suggesting the possible role of IL-6, IL-18 in pathogenesis of TA.

IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response and hematopoiesis. The finding of raised IL-6 levels in TA patients can be taken as indirect evidence of peripheral mononuclear cell activation whose cytokine product contributes to the inflammatory process cascade.[11,12] Evidence suggests that IL-6 is abundantly secreted not only from immune cells but also from the cells of aortic tissue, such as vascular smooth muscle cells and endothelium in TA patient and Tocilizumab, an IL-6 receptor blocker has been shown to control inflammation in this disease.[13]

Serum IL-6 levels have been shown to be elevated in TA patient with active disease in a previous study by Salvarani et al.[4] A recent Japanese study also observed raised IL-6 and TNF-α levels in TA patients during active phase.[14] However, in another study from Turkey, IL-18 but not IL-6 was found to be elevated in active disease.[8] Noris et al[15] and Saadoun et al[16] reported that the alteration of the serum IL-6 level might be correlated with TA disease activity.

Restenosis rate in previously revascularized patients of TA about 15–30 % in stable patients and 50 % in active patients.[17] In the present study, there was a subgroup where patients with active disease had high level of IL-6, IL-18 with a normal ESR and CRP. They had higher rate of restenosis, suggesting that these novel biomarkers can possibly be used in previously intervened patients for predicting the restenosis and early initiation of tocilizumab for its treatment.[16]

Our data indicates that IL-6 and IL-18 might be involved in the pathogenesis of TA and that measuring their levels by using a combination ELISA kit (IL-6 + IL-18) would be helpful in following disease activity and monitoring the therapeutic response in TA. This has previously been shown in one of the studies.[6]

3.1. Study limitation

It is a single centre study with a small sample size and shorter duration of follow-up which limited our ability to detect a response to treatment effects. This calls for larger multicentric studies to assess the utility of novel markers in determining the disease activity. In addition, newer modalities of imaging for activity like FDG PET-CT and MRI were not done.

4. Conclusion

The present pilot study revealed that both serum IL-6 and IL-18 levels have higher sensitivity and specificity than traditional biomarkers in assessing disease activity in patients of TA. Patients with higher level of these markers were more likely to develop restenosis after intervention.

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Conflict of interest

All the authors state that they have no conflict interest to declare with respect to the present article titled “Novel Inflammatory Markers in Takayasu Arteritis and its correlation with Disease Activity”.

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