Implication of oxidative stress and its correlation with activity of matrix metalloproteinases in patients with Takayasu's arteritis disease

Nitin Mahajan [a], Veena Dhawan [a,*], Sonal Malik [a], Sanjay Jain [b]

[a] Department of Experimental Medicine & Biotechnology, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh-160012, India
[b] Department of Internal Medicine, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh-160012, India

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The aetiopathogenesis of Takayasu's arteritis (TA) disease remains enigmatic and various mechanisms such as post-infective, autoimmune, ethnic susceptibility and a genetic predisposition have been postulated [1]. The diagnosis of this disease is difficult, because only non-specific symptoms may be present during the early phase of TA, symptoms of arterial stenosis and occlusion are late phase manifestations and the current diagnostic criteria focus mainly on these manifestations [2]. The disease tends to progress despite treatment with glucocorticoid/immunosuppressive agents and selection of an appropriate therapeutic strategy is based on monitoring the severity of the disease [3,4].

Oxidative stress is a cardinal feature of the inflammatory process and has been generally considered to be uniformly deleterious in vascular abnormalities. An obvious mechanism of oxidative stress (e.g. in the form of reactive oxygen species (ROS) and reactive nitrosative species (RNS)) is through oxidative damage to cellular proteins, membranes and DNA [5,6]. Another mechanism is the potential of ROS and RNS to influence extracellular matrix remodeling through activation of matrix metalloproteinases (MMPs) [5,6]. 8-isoprostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) has been shown to be a sensitive and specific biomarker of the oxidative stress *in vivo* and is a member of isoprostane family which have been shown to exert potent biological actions [5,7].

Though, deeply explored in other diseases, the degree of oxidative stress and its relation to Takayasu's arteritis is unclear. Also, how oxidative stress and MMP activity are linked together in the aetiopathogenesis of TA disease is not known. The aim of the present study is to investigate whether oxidative stress indices i.e. (8-iso-$PGF_{2\alpha}$ and NO_2^-) and MMP activity could correlate with the disease activity.

40 patients with angiographically proven Takayasu's arteritis (Group I) and 40 normal healthy controls (Group II) were enrolled in the present study as described earlier [8]. The study has been approved by The Institutional Ethics Committee.

Plasma 8-iso-$PGF_{2\alpha}$ was determined in all the study subjects using commercially available immunoassay kit (Assay Designs, USA). Plasma nitrite (NO_2^-), as a stable end product of NO production and DNA damage was determined as described previously [9]. Gelatin zymography was done to determine the serum activity of MMP-2 and MMP-9 [10]. Statistical analysis was performed using SPSS 10.0.

The baseline characteristics of the subjects are shown in Table 1. We observed no significant difference between the two study groups as far as lipid and lipoprotein profile, blood urea, serum creatinine and uric acid levels are concerned ($p>0.05$; data not shown). Levels of 8-iso-$PGF_{2\alpha}$ and NO_2^- were found to be significantly higher in patients with TA as compared to the normal healthy subjects ($p<0.01$). Further, we also observed higher levels of 8-iso-$PGF_{2\alpha}$ and NO_2^- in TA patients with active disease as compared to those patients who were in remission ($p<0.01$) (Table 1).

Zymography pattern revealed two bands in the range of 85–92 kDa (MMP-9) and 65–72 kDa (MMP-2). We observed remarkably broad and intense clear zones of gelatinolytic activity in lanes with serum of TA subjects, as compared to the control subjects where the bands were less intense. Further, the samples from normal healthy controls and subjects of TA who were in remission showed small clear zones of comparable intensities and were not significantly different from each other (Fig. 1A) (Table 1).

Agarose gel electrophoresis patterns of DNA samples from TA patients showed a significant tailing pattern indicating augmented DNA damage as compared to clear and intact bands observed in control subjects. However, a more intense tailing pattern was observed in subjects with active disease vs those in remission (Fig. 1B).

Both MMP-2 and MMP-9 gelatinolytic activities were found to be positively correlated with plasma 8-iso-$PGF_{2\alpha}$ in subjects with TA (MMP-9, $r=0.81$, $p<0.01$; MMP-2, $r=0.60$, $p<0.01$). The observed positive correlation was also seen in TA subjects with active disease (MMP-9, $r=0.73$, $p<0.01$; MMP-2, $r=0.36$, $p<0.01$). However, a positive correlation of plasma nitrite levels was observed with MMP-9 activity ($r=0.35$; $p<0.05$) only, but not with MMP-2 activity in the case of TA patients.

Binary logistic regression analysis revealed that 8-iso-$PGF_{2\alpha}$ has 85% and 90% sensitivity and specificity respectively. MMP-2 activity showed 82.5% sensitivity and 100% specificity as compared to the 85% sensitivity and 92.5% specificity observed for the MMP-9 activity. Further, systolic blood pressure (SBP), 8-iso-$PGF_{2\alpha}$ and MMP-2 activity cumulatively showed 90% sensitivity and 95% specificity in predicting the disease status.

Takayasu's arteritis (TA) is a progressive inflammatory and occlusive disease of the unknown origin and mainly affects the aorta and its branches, as well as the pulmonary and coronary arteries [2]. However, the current focus of the research is on newer developments which have taken place with regard to etiology, diagnosis of disease activity and management aspects.

Augmented levels of 8-iso$PGF_{2\alpha}$ in TA patients reflects enhanced lipid peroxidation which may possibly be due to an acute inflammatory response and were able to discriminate TA subjects with active disease from those who were in remission phase. Oxidative injury caused by lipid oxidation has been considered a major factor in the development of temporal arteritis and ROS have an additional role as cell signaling mediators influencing biological process [11].

* Corresponding author. Department of Experimental Medicine & Biotechnology, Research Block 'B', Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh-160012, India. Tel.: +91 172 2747585x5235; fax: +91 172 2744401.
E-mail address: veenad2001@yahoo.com (V. Dhawan).

Table 1
Baseline characteristics of the study subjects.

Investigations	Controls ($n=40$)	TA ($n=40$)	TA	
			Active disease ($n=32$)	In remission ($n=8$)
Age range (years)	15–49	15–53	17–50	15–53
Sex ratio (M: F)	10:30	8:32	6:26	2:6
Blood pressure				
Systolic (mm Hg)	117 ± 6	134* ± 19	135 ± 19	128 ± 16
Diastolic (mm Hg)	76 ± 6	84* ± 14	85 ± 14	80 ± 14
BMI (kg/m^2)	21.5 ± 3.1	20.7 ± 3.6	20.46 ± 3.0	21.4 ± 4.1
Smokers	5[a]	5[a]	5[a]	1[a]
Alcohol use	5[a]	6[a]	5[a]	1[a]
8-iso $PGF_{2\alpha}$ (ng/ml)	79.4 ± 29.1	238.9 ± 125.8[b, **]	272.2 ± 117.3	105.9 ± 43.5[c, **]
Nitrite (NO_2^-) (μM)	3.3 ± 1.7	14.9 ± 15.6[b, **]	17.9 ± 16.05	3.1 ± 2.5[c, **]
MMP-9 Activity (AU)	19.5 ± 5.1	61.42 ± 28.1[b, **]	70.8 ± 22.8	24.1 ± 10.0[c, **]
MMP-2 activity (AU)	5.5 ± 1.8	25.4 ± 13.2[b, **]	30.2 ± 9.9	6.4 ± 3.1[c, **]

n = number of patients; results are expressed as mean ± S.D. AU = arbitrary units.
[a] Occasional.
[b] Takayasu's arteritis (TA) vs healthy controls (HC).
[c] TA subjects studied during active phase (TAA) vs and TA subjects studied during remission phase (TAR).
** $p<0.01$.
* $p<0.05$.

We also observed significantly augmented nitrite levels in TA patients which were clearly able to distinguish patients with active TA disease from those who were in remission. Previously, Parildar et al. had also, reported enhanced levels of nitrite production in TA [12]. Cross talk between ROS and RNS regulated pathways may occur at both the chemical levels and via their coordinate effects on their common cellular targets [6]. Oxidative stress and inflammation are inextricably linked; one begets the other to form a closed circuit. Oxidative stress induces heat shock proteins (HSP), which in turn stimulate production of proinflammatory cytokines and expression of adhesion molecules [13,14]. Though, we have not determined HSP in the present study, in our previous study we have reported enhanced levels of hRANTES and MCP-1 in subjects with TA, which strongly suggests existence of an inflammatory state in these subjects [8,9].

High gelatinolytic activity of both MMP-2 and MMP-9 in TA patients also showed a positive correlation with both 8-isoPGF$_{2\alpha}$ and nitrite levels and strengthens the relationship of 8-isoPGF$_{2\alpha}$ and MMP activity with the disease status. Rajgopalan et al. [15] reported ROS dependent increased activities of MMP-2 and MMP-9 in smooth muscle cells *in vitro*. There have been few *in vivo* and many *in vitro* studies which demonstrate a relationship between the generation of free radicals (ROS and RNS) and MMP induction [16,17].

In our previous study in essential hypertensives, we demonstrated that increased ROS and lipid peroxidation leads to a considerable oxidative damage to DNA [8]. Also, of significance is the fact that in another study (unpublished data), we observed a positive correlation of 8-OH-dG (a biomarker of oxidative DNA damage) with the extent of DNA damage in hypertensives. A similar pattern of DNA damage was also observed in TA subjects with active disease vs those who were in remission, thereby confirming that the patients were in a state of heightened oxidative stress.

Although, exact source of oxidative stress in TA is not known, there is sufficient evidence in literature pointing to clustering of sources of oxidative stress and their involvement with the pathophysiology of TA [18,19]. Ours is the first study to report increased milieu of oxidative stress prevailing in TA patients. Though, the commonest mode of presentation of TA was hypertension, our data indicates that the observed increase in 8-isoPGF$_{2\alpha}$, nitrite and MMPs' activity could be attributed to the heightened inflammatory and oxidative stress milieu in these patients. Also, it may not be a consequence of elevated blood pressure, as there were no significant differences in blood pressure recordings between subjects of TA, either in active or in remission phase. Also, we did not observe any significant correlation of blood pressure and age with either 8-isoPGF$_{2\alpha}$ or activity of MMPs. Our results clearly demonstrate that these molecules were able to discriminate the patients on the basis of severity of the disease.

However, some limitations of the study should also be considered. Firstly, we haven't taken follow-up samples of these subjects; therefore, we are not able to comment on the effects of drugs on these variables. Secondly, the subjects were only from the north Indian population, thus caution should be exercised for extrapolating the data to other ethnic groups.

In conclusion, our data provides strong evidence in support of the notion that oxidative stress and MMPs might contribute to the pathogenesis of TA. Further, oxidative stress seems to be a sequel of an already existing inflammatory condition prevailing in TA subjects which could further perpetuate inflammation.

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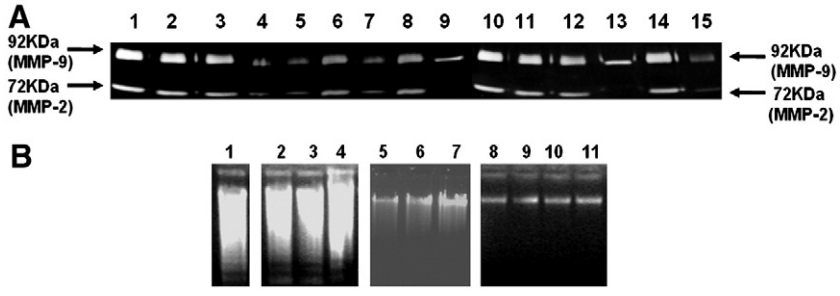

Fig. 1. A. Gelatin zymography of study subjects showing activity of gelatinase A (MMP-2) and B (MMP-9). Lanes 1–3, 10–12, 14: TA patients with active disease; Lanes 4–6: TA patients in remission; Lanes 7–8, 15: normal healthy controls; Lanes 9 and 13: standard MMP-9. B. Agarose gel electrophoresis revealing the DNA pattern of subjects with Takayasu's arteritis and their controls. Lane 1: positive control of damaged DNA; Lanes 2–4: TA patients with active disease; Lanes 5–7: TA patients in remission; Lanes 8–10: normal healthy controls; Lane 11: positive control for intact DNA.

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Inflammatory characteristics of premature coronary artery disease☆

R. Puranik [a,b,d,*], O.J. Fox [a], D.S. Sullivan [b], J. Duflou [a,c], S. Bao [a,b]

[a] Discipline of Pathology, School of Medical Science, University of Sydney, Australia
[b] Department of Medicine, University of Sydney, Australia
[c] Department of Forensic Medicine, Central Sydney Laboratory Service, Australia
[d] Department of Cardiology, Royal Prince Alfred Hospital, Australia

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Premature coronary artery disease (CAD) presents an important opportunity to understand mechanisms of accelerated atherosclerosis. We have previously reported that CAD is one of the most common cardiac causes of sudden natural death in young Australians aged 5–35 years of age [1]. The vast majority of these deaths occurred between the ages of 30 and 35 years. Further, although manifesting a severe coronary plaque phenotype, in the main this disease is clinically silent.

We report our initial experiments examining the patterns of traditional and novel inflammatory markers in men with premature coronary heart disease (<45 years). These studies present a "proof of concept" that age-related factors are an important aspect in the pathophysiology of this often fatal condition.

From 2001–2005, we collected coronary plaque samples from consecutive cases of CAD ($n=23$) reported to the Department of Forensic Medicine, Central Sydney Laboratory Service which led to unexpected death in men aged <45 years. All autopsies were performed within 48 h of collection. This facility serves a population of over 2.5 million people in the eastern part of Sydney, Australia. The population is demographically stable and representative of urban Australia.

Based on our previous studies [1], subjects were divided according to age < and >35 years, in order to ascertain if there were age-related inflammatory characteristics unique to this cohort. Immunohistopathologic characterization of coronary plaque (>50% stenosis) was performed. The presence of CD3+ T cells, macrophages, smooth muscle cells, FOXP3+ T cells, myeloperoxidase, ApoA-I and MMP-2 were determined by quantitative immunohistochemistry [2]. One-way ANOVA tests were used to determine significant differences ($p<0.05$).

Subjects we studied were 34.5 ± 5.9 years (range 25–45 years) with BMI of 27.7 ± 5.3 kg/m^2. There was no significant difference in BMI between groups. 13% of decedents were known to have a family history of vascular disease and 52% presented with symptoms. In decedents <35 years, there were lower numbers of CD3+ T cells ($p=0.03$) (Fig. 1B) and higher macrophage content ($p=0.01$) (Fig. 1C) in culprit coronary plaque when compared to decedents >35 years. There was no difference in the smooth muscle content in culprit plaque ($p=0.80$) (Fig. 1D). In CAD decedents <35 years, culprit plaque contained higher numbers of FOXP3+ T cells when compared to older decedents (>35 years) ($p=0.03$) (Fig. 2A). There was no significant difference in culprit plaque content of myeloperoxidase ($p=0.49$) (Fig. 2B), apoA-I ($p=0.63$) (Fig. 2C) and MMP-2 ($p=0.60$) (Fig. 2D) when younger decedents were compared with older decedents.

We have previously reported that CAD accounts for a large proportion of sudden deaths in young Australians <35 years [1]. The

☆ In memory of Dr Bruce Wilson, Consultant Cardiologist at Royal Prince Alfred Hospital.

* Corresponidng author. Department of Cardiology, Royal Prince Alfred Hospital, Missenden Rd, Camperdown, 2050, NSW, Australia. Tel.: +61 2 95156111; fax: +61 2 9550 6262.
E-mail address: rpuranik@mail.usyd.edu.au (R. Puranik).