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ORIGINAL ARTICLE

Role of Oxidative Stress Associated Molecular Diagnostic Signatures in the Personal Identification of Rheumatoid Arthritis Patients

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ABSTRACT

CONTEXT: Early interpretation of toxic effect of free radicals leading to cardiovascular diseases (CVDs) in autoimmune disorders is a quench thirst of therapeutic intervention and personal identification. **AIM:** The study aims to evaluate the expression of Nrf2, NQO-1 and LpPLA2 genes along with marker of endothelial dysfunction and oxi-inflammatory stress in active RA patients and to enlighten the assessment of study group markers in personal identification of RA patients for their early therapeutic intervention and prevention of vascular complications.

MATERIALS & METHOD: 64 active RA patients between 35-55 years and 64 healthy controls were recruited from North India region. Using specific primers, mRNA expression of Nrf2, NQO-1 & LpPLA2 genes were evaluated in blood by qPCR. 2 –ΔΔCT method was used to determine the fold change. Brachial artery flow mediated diameter (FMD), total antioxidant activity, malondialdehyde, TNF-α, IL-6 & hs-CRP levels were estimated by using standard methods followed by appropriate statistical analysis of data. **RESULTS:** Increased expression of NQO-1, Nrf2 and LpPLA2 was observed in RA patients along with marked altered levels ($p<0.05$; significant) of oxi-inflammatory markers which may be due to compensatory activation of antioxidant defense mechanism. Remarkably, FMD% was significantly low ($p<0.05$) and inversely associated with the expression of NQO-1, Nrf2 and LpPLA2, which highlighted the culprit effect of oxi-inflammatory stress in inducing altered vascular homeostasis. **CONCLUSIONS:** Thus, combinational analysis of molecular diagnostic signatures associated with toxic free radicals along with FMD measurement exhibits a great promise in personal identification of RA patients for early therapeutic intervention, mainly, by targeting the oxi-inflammatory stress mediated cytoprotective pathway, and thereby, reducing the burden of CVD morbidity and mortality in RA patients.

KEYWORDS | oxi-inflammatory stress, lipoprotein-associated phospholipase a2, keap1/nrf2 endothelial dysfunction, flow mediated diameter

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INTRODUCTION

RHEUMATOID ARTHRITIS (RA) is a multifaceted systemic inflammatory autoimmune disorder, driven by inexorable and stochastic accumulation of damage of biomolecules vital for proper synovial function. The incidence of RA increases during every decade of life and affects the quality and expectancy of human life in developed and developing countries as well.[1] Due to augmented toxic free

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radicals and inflammatory insult with aging process, the joints or bone become damaged causing the physical disability and restricted motion. In addition, in the wake of urbanization, altered metabolic profile, sedentary life habits, increased body weight, aging, smoking, positive rheumatoid factor, disease severity, formation of anti-cyclic citrullinated peptide (CCP) antibodies and genetic factors have a significant impact in establishing CVD risk in RA patients.[2,3] However, the exact clinico-molecular mechanistic pathway underlying this curse is still obscure.

Interestingly, oxi-inflammatory stress in combination with regulation of gene expression involved in cytoprotective pathway has been suggested as a common denominator and contributing factor in inducing endothelial dysfunction followed by atherosclerosis in rheumatological conditions.[4] Recently, a lot of interest has been generated to understand the role of Keap1/Nrf2/ARE pathway in elucidating the relationship between chronic oxi-inflammatory stress and altered vascular homeostasis in active RA patients. This pathway regulates cellular detoxification process and redox status.[5]

Previous studies have considered the role of Nrf2 as a multi-organ protector and its involvement in various diseases associated with oxi-inflammatory stress such diabetes, arthritis, obesity, hypertension and metabolic dysregulation.[6]

NADPH quinine oxidoredcutase (NQO-1) gene is an important downstream target of Nrf2 and involved in cellular antioxidant response by encoding NADPH oxidase, which acts as a potent antioxidant.[7] Thus, expression of NQO-1 may affect the production and removal of reactive oxygen and nitrogen species (RONS). Although, NQO-1 is shown to have an effect on RA pathology in rodents,[8] there is a scarcity of literature on human studies highlighting the role of Nrf2, NQO-1 and Total antioxidant activity (TAA), at a single platform, in maintaining the redox homeostasis.

Apart from Keap1/Nrf2/ARE pathway and TAA, lipoprotein-associated phosholipase A2 (LpPLA2) is a circulating inflammatory biomarker secreted by macrophages and monocytes, and exhibits proinflammatory and proatherogenic role. Independent studies on human and experimental animal models revealed the increased activity of LpPLA2 and expression, as well, in inflammatory conditions.[9] However, not enough literature regarding the expression studies of Keap1/Nrf2/ARE pathway along with NQO-1 and LpPLA2 in active RA is available.

Early pathophysiological alterations in vascular disease arise due to RONS mediated endothelial dysfunction.[10] Interestingly, a repertoire of rheumatic disease researches revealed the importance of non-invasive approach in the detection of vascular health.[11] Recently, the assessment of brachial artery flow mediated dilation (FMD) in RA patients is a subject of research interest for cardio-rheumatologists. Altered expression of genes of cytoprotective pathway, inflammation and endothelial dysfunction may be an interconnected link in accelerated vascular complications; however, the toxic effect of free radical in inducing endothelial dysregulation in rheumatic complications has not been fully elucidated. Therefore, the objectives of present study aimed to enlighten the plausible connecting link between expression of genes of cytoprotective pathway against toxic free radicals and inflammation along with marker of endothelial dysfunction in active RA patients; and to provide a great promise in personal identification of RA patients not only for early interpretation of CVD risk by assessing toxic free radicals mediated destruction but also pave the way of therapeutic intervention as well.

METHOD AND MATERIALS

Subjects

The present study protocol was approved by the research and ethics committee of School of Medical Sciences and Research, Sharda Hospital, India. A total 157 active RA patients

visited outpatient clinic of the hospital with joint complaint over a period of two years (October 2017 to September 2019). In the present case-control study, only 64 active RA patients of either sex belonging to age group 35-55 years were recruited from North India region. 68 patients did not meet the inclusion criteria and 25 patients refused to participate. 64 age-matched healthy individuals were recruited as controls from college staff and their family members, after taking their informed consent.

Inclusion Criteria: Criteria recommended by the American Rheumatism Association were used for the diagnosis of RA.[12] Written informed consent was obtained from all the subjects included in the study. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination including blood pressure measurement was completed from all the subjects The recruited patients had active RA, defined as the presence of at least three of the following criteria: six or more tender joints; three or more swollen joints; ≥30 minutes of morning stiffness and an erythrocyte sedimentation rate of ≥28 mm/h. The number of swollen and tender joints (28 joint count) and patient's assessment of pain on Visual Analog Score (VAS) were registered. Disease activity score-28 (DAS28) were calculated using erythrocyte sedimentation rate.[13] The level of RA disease activity was interpreted as low (DAS28 ≤3.2), moderate (3.2 <DAS28 ≤5.1) and severe disease activity (DAS28 >5.1).

Height was measured by using wall mounted scale where weight was measured with subjects standing barefoot and lightly dressed by using digital weighing machine. The Body Mass Index (BMI) was calculated as [BMI = Weight (kg) / Height (metre 2)]. Blood pressure was measured by mercury sphygmomanometer using auscultatory method. To diminish any confounders developed by other arthritic complications, patients with positive rheumatoid factor were recruited and their disease duration was recorded. However, RA patients with family history of arthritis and hypertension were not excluded. In addition, RA patients who had previously under any medical treatment including supplementation of antioxidants or non-steroidal anti–inflammatory drugs were not excluded from the study if the subject agreed that no supplements or analgesic drug would be taken in the seven days before entry into the study. However, there was no restriction or withdrawal on the conventional anti-rheumatoid drugs treatment.

Exclusion Criteria: None of the patients and control subjects had family history of concomitant diseases, such as diabetes mellitus, hepatitis, renal failure and neurological disorder. In addition, patients with established cardiovascular complications, pregnancy, lactation, obesity (BMI>25), Stage I and stage II hypertension (BP>129/89 mmHg), smoking habit, renal failure, liver disease, hypothyroidism or who did not follow study instructions were also excluded from the study.

Methods

Fasting venous blood sample was collected into EDTA (8ml) and plain (2ml) vials from the study group subjects after confirming their inclusion criteria. Whole blood (2ml) was used for gene expression analysis. For the estimation of study group parameters, plasma and serum were separated from rest of the collected blood sample by centrifugation at 1000g for 15 minutes at room temperature and stored at -80°C until use.

Gene Expression Analysis

Total RNA was isolated from whole blood using TRI-reagent BD from Sigma Chemicals, USA; as per manufacturers' instruction. The quality of RNA was checked by taking the optical density ratio at 260/280; a ratio of 1.8-2.0 was considered adequate. The reaction for cDNA synthesis was carried out using 200U reverse transcriptase (Revert Aid from Thermo Scientific, Inc., USA), 500ng of RNA, 40 U ribonuclease (RNase) inhibitor (Thermo Scientific Inc., USA), 10 mM dNTPs, 100pM

random hexamer and oligo dT in the ratio 1:1 (Sigma Aldrich, India), 4µl of 5 x reaction buffer and the final volume made to 20 µl with diethyl pyrocarbonate (DEPC) treated water. Incubation was carried out at 25ºC for 10 min, followed by 45ºC for 60 min. After that, reverse transcriptase was inactivated at 70ºC for 10 min in a thermocycler (Biorad CFX Connect), and resultant cDNA was stored at -20ºC and used as template sample for qPCR. In order to analyze the relative expression of the genes by real time polymerase chain reaction (RTPCR), specific primers are used.[14] The primer sequences of all the genes are as below:

1) Nrf2 (forward-5'ACACGGTCCACAGCTCATC-3' and Reverse-5'-TGTCAATCAAATCCATGTCCTG-3')
2) NQO-1 (forward-5'GGCAGAAGAGCACTGATCGTA-3'and Reverse-5'-TGATGGGATTGAAGTTCATGC -3')
3) LpPLA2 (forward-5'CCACCCAAATTGCATGTG-3' & Reverse-5'-GCCAGTCAAAAGGATAAACCACAG-3'
4) α-actin (forward 'TCATGAAGTGTGACGTTGACATCCGT-3' and Reverse-5'-CCTAGAAGCATTTGCGG TGCACGATG-3')

For real time PCR of Nrf2, NQO-1 and LpPLA2 along with internal reference gene or housekeeping gene (β-actin), 0.5µl of above mentioned forward and reverse primers, 10µl of Hot-Start PCR master mix (Thermo Scientific Inc., USA), 1µl of diluted cDNA, 1µ l of 1:100 diluted syto9 dye and final volume made upto 20µl with DEPC treated water were used. For all the genes, cycling conditions were the same but annealing temperature was different (hold 95ºC for 4mins, cycling for 35 cycles; 95ºC for 15 sec, annealing at 54ºC temperature for 30 sec and 72ºC for 30 sec). All the reactions were run in duplicates and fluorescence was obtained at 72ºC. ΔΔcT method was used to analyze the relative expression of the gene and 2-ΔΔcT method was used to calculate the fold change.

Biochemical Analysis

Plasma hs-CRP (Calbiotech, USA; sensitivity less than 0.005mg/ml), TNF-α (Diaclone, France; sensitivity less than 8pg/ml), IL-6 (R&D Systems, USA; sensitivity less than 0.7 pg/ml) levels were measured using commercially available ELISA kits, according to manufacturer's instructions. Routine biochemical parameters were assayed in automated analyzer using commercial kits. All these investigations were carried out once at the time of entry into the study. Plasma lipid profile contents (Total Cholesterol, Triglycerides and HDL-cholesterol) were analyzed enzymatically using kit obtained from (Randox Laboratories Limited, Crumlin, UK). LDL-cholesterol levels were calculated by Friedwald's formula.[15]

LDL-C = TC - [(TG/5)+HDL-C]

Serum MDA levels were estimated by thiobarbituric acid (TBA) reaction.[16] Serum lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid (pH 2-3) and boiled with thiobarbituric acid which reacts with Malondialdehye, forming a MDA-TBA to get pink color. The pink colored complex that occurred was refrigerated to room temperature and measured by using a spectrophotometer at 530 nm.

Plasma total antioxidant activity was estimated spectrophotometricaly by the method involving reaction of standardized solution of iron EDTA complex with hydrogen peroxide, i.e. Fenton type reaction, leading to the formation of hydroxyl radicals. This reactive oxygen species degrades benzoate, resulting in the release of Thiobarbituric acid reactive substances (TBARS). Antioxidants from the added plasma cause the suppression of produc*tion of TBARS. The reaction was measured spectrophotometrically at 532 nm.*[17]

Radiological vascular analysis:

To determine endothelial function, brachial artery flow mediated diameter percent (FMD%) was performed in a subject with overnight

fasting (of at least 10 hours) in the morning, in a quiet and dark room under controlled ambient temperature (20°C to 26°C). After 10 minutes of rest in a supine position, the right arm of the subject was comfortably immobilized in the extending position. Approximately 5-10 cm above the antecubital fossa, ultrasound scanning of brachial artery was performed. After inflation of a cuff to a suprasystolic pressure (40-50 mmHg above systolic pressure) for about 5 minutes, the vessel images were recorded. Post dilation of a cuff, the brachial artery diameter image was taken and recorded for 3 minutes. Brachial artery FMD% more than 10% is considered as normal response whereas FMD% values less than 10% is considered as endothelial dysfunction and subject is susceptible to develop future CVD complications.[2]

Statistical Analysis

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD. To compare the parametric data between two groups, paired Student's t test was performed whereas Whitney U test was performed for non-parametric data. The distribution of 't'-probability was calculated depending on 'n' and significance of test was obtained. Correlation studies were carried out to evaluate the relationship between different parameters. For parametric data, Pearson correlation coefficient was used whereas Spearman rho's correlation was used for non-parametric data. 2-ΔΔcT method was used to calculate the fold change in expression and ΔcT was taken as one of the variable to perform correlation studies by using Pearson correlation coefficient. P value <0.05 and <0.001 were considered as significant and highly significant respectively.

RESULTS

Demographic and biochemical profile of the patients:

The anthropometric, clinical and biochemical parameters are shown in Table 1. All the 64 active RA patients were aged 35-55 years and there was no significant difference in mean age, BMT and blood pressure between the patient and control group. Among recruited 64 active RA patients, 67% were females and 33% were males whereas in control group 64% were females and 36% were males. Out of 64 patients, 14 patients were overweight and 48 patients (75%) were pre-hypertensive as per JNC 7th guidelines. However, they were not taking any antihypertensive drug and were being managed by diet and exercise. The ESR level of RA patients was significantly high ($p<0.001$; 28% high) and disease duration was 28.7 ± 3.5 months. RA patient population had a moderate disease activity with a mean DAS28-ESR of 4.27 ± 0.26.

As compared to normal healthy controls, marked occurrence of atherogenic profile along with abnormalities in lipid profile contents were observed in active RA patients (Table 1.0). Plasma total cholesterol ($p < 0.05$), triglycerides ($p < 0.05$) and LDL cholesterol ($p < 0.001$) levels were found to be increased significantly in active RA patients as compared to that of healthy controls Moreover, statistically significant low HDL-cholesterol levels ($p < 0.05$) along with, high atherogenic index (TC/ HDL-C ratio was higher than five) in active RA patients revealed the increased risk of atherosclerotic complication.

Comparative analysis of the markers of oxi-inflammatory and endothelial dysfunction:

The results of present study revealed statistically significant changes in the marker of endothelial dysfunction and oxi-inflammatory stress in the study group patients. The changes in FMD% and plasma TAA along with serum MDA levels in active RA patients and control group were represented in Fig. 1. FMD% value (35.90% low; $p = 0.001$; Fig. 1) and plasma TAA level (31.62% low; $p = 0.004$; Table/Fig. 5) were significantly low whereas serum MDA level was found to be significantly high (29.96% high; $p = 0.002$) in RA patients as compared

to controls. Similarly, plasma hs-CRP (47.05% high; p = 0.000) and TNF-α (42.06% high; p = 0.002) and IL-6 (33.58% high; p = 0.001) levels were found to be increased significantly in RA subjects as compared to healthy controls (Fig 1) which reflect the etiopathological role of inflammation in active RA patients.

Gene Expression Analysis

We observed higher level of mRNA expression of Keap1/Nrf2/ARE pathway and LpPLA2 gene in active RA patients as compared to healthy controls. Taking α-actin as reference gene, it was observed that the fold change in mRNA expression of Nrf2 and NQO-1 in whole blood was 1.7 and 1.9 respectively. Moreover, mRNA expression of LpPLA2 was 3.8 times higher in active RA patients. Interestingly, previous studies revealed that Nrf2 has a role in the maintenance of vascular integrity; we therefore compared Nrf2 expression in active RA patients with normal blood pressure (BP >120/80 mmHg) and pre-hypertension (BP < 129/89 mmHg). Increased blood pressure was found to be associated with 4.5 times higher mRNA expression of Nrf2 in active RA patients.

Correlational analysis of the biochemical parameters

Correlation studies revealed that mRNA expression of Nrf2 was significantly correlated with expression of NQO-1 (r = 0.533, p = 0.05) and LpPLA2 (r = 0.658, p = 0.02), as shown in Figure 2. Remarkably, we observed a negative correlation between Nrf2 gene expression with FMD% and TAA, whereas marker of lipid peroxidation (MDA) and inflammation such as hs-CRP, TNF-α, IL-6 and ESR levels were positively correlated with Nrf2 (Figure 3, p<0.05) which indicates the association of Keap1/Nrf2/ARE pathway induction with oxi-inflammatory stress and altered vascular homeostasis in active RA patients. Similarly, mRNA expression of Nrf2 was positively correlated with VAS pain score, DAS-28 score and disease duration (Figure 3). These results clarify the role of Nrf2 gene expression in the pathophysiological manifestation of active RA most probably by its relation with pain sensation, clinical symptoms with severity of disease.

DISCUSSION

Over the past several decades, a myriad of studies on RA patients emphasized the need of effective diagnostic approach for early interpretation and therapeutic intervention to mitigate the risk of cardiovascular disease (CVD) in RA.[18] Unfortunately, despite massive efforts, scientists have failed to reveal the secrets of CVD complications in RA patients and CVD threat among rheumatic disease is still looming large. Recently, studies focused on regulatory modulation of the expression of genes involved in cyto-protection have received much attraction among cardio-rheumatologists. In this context, the cytoprotective role of Keap1/Nrf2/ARE pathway by means of activating the antioxidant signaling network against oxi-inflammatory stress mediated cytotoxicity has opened new avenues to enlighten the mechanistic pathway of rheumatic diseases & its related drug development. In the present study, we evaluated the expression of genes involved in cytoprotective pathway which include Nrf2 and its downstream target NQO-1. mRNA expression of Nrf2 and NQO-1 were higher in active RA patients. It could be explained on the basis of oxi-inflammatory stress mediated induction of Nrf2/ARE pathway as a result increased expression of Nrf2 gene takes place in combination with reduced ubiquitination and proteasomal degration of Nrf2 which inturn facilitates the enhanced expression of NQO-1 in RA patients.

NADPH quinine oxidoredcutase (NQO-1) is an important antioxidant enzyme which not only contributes significantly in providing protection against augmented oxidative stress but also assigned with multiple protective roles.[7] Higher expression of NQO-1 along with its positively correlation with Nrf2 expression authenticate the contention that activation of antioxidant defence mechanism takes place

in active RA patients. Moreover, DAS28, VAS and disease duration of RA were positively correlated with Nrf2 expression and appeared as independent predictors of cytoprotective pathway activation in RA patients (Figure 3). Similarly, Wruck et al documented the activation of Nrf2 gene in both the joints of antibody induced arthritic mice and RA patients in order to maintaining the cellular defense against oxidative stress.[19] According to Bozbus and Sendur, ozone therapy inhibits chronic inflammation in disorders such as RA. The effect could be attributed to the activation of antioxidant defense through the Nrf2 which is characterized by increased transcription of various antioxidant and phase II detoxification enzymes.[20]

Existence of oxi-iflammatory stress is a common thread which connects RA pathology and cardiovascular complications. Pro-inflammatory cytokines such as IL-6, TNF-α, produced by T-lymphocytes stimulate the release of tissue-destroying matrix metalloproteinases and pro-inflammatory enzymes which eventually lead to degeneration of cartilage extracellular matrix and thereby play a crucial role in RA pathology.[21,22] In addition, oxidative stress triggered by activated neutrophils during inflammatory reactions leads to lipid peroxidation of chondrocytes, mediates collagen degradation, promotes atherosclerotic plaque formation, prostacyclin synthesis, enhancement of cytosolic free calcium and peripheral vascular resistance.[23] Interestingly, alteration in total antioxidant activity and increased levels of lipid peroxides induces the loss of homeostasis in vascular system that eventually elaborates the degeneration of vascular endothelium followed by cardiac complications.[10]

In the present study, plasma TAA levels were decreased significantly whereas serum MDA levels along with markers of systemic inflammation (hs-CRP, IL-6 and TNF-α) were significantly high in RA subjects (Figure 1), which suggest the role of enhanced lipid peroxidation and systemic inflammation along with reduced antioxidant status in the progression and development of CVD complications in RA patients. Correlation studies also revealed that hs-CRP, ESR, IL-6 TNF-α and MDA were positively correlated with Nrf2 expression and TAA was inversely associated with Nrf2 which supports the substantial involvement of cytoprotective pathway activation due to oxi-inflammatory stress in active RA patients (Fig. 2 and 3). Similarly, Saxena et al. estimated the level of C-reactive protein, superoxide dismutase, catalase, glutathione peroxidase and ceruloplasmin in active RA patients and suggested that combined effect of inflammation and free radical generation is involved in the pathogenesis of active RA.[24] Consistent findings have been reported by Dudeja et al.[25] They evaluated the plasma total antioxidant activity along with the markers of systemic inflammation, oxidative stress and metabolic profile in RA patients. In addition to dyslipidemia, they observed a marked reduction in plasma TAA along with enhanced CRP, MDA and synovial IL-6 levels in RA patients. They also suggested that these inexorable alterations contribute significantly to the progression of vascular complications in rheumatic diseases and future drugs of RA could be developed to target the non-traditional CVD risk factors also.

Moreover, LpPLA2, a marker of vascular inflammation, has been found to be associated with cardiovascular complications. It circulates with low density lipoproteins LDL) and high density lipoproteins (HDL) and acts on the oxidized phospholipids, ensuing the production of lysophospholipids and oxidized fatty acids. It has a direct role in the causal pathway of plaque inflammation and implicated in inducing enhanced risk of atherosclerotic event.[9] Sodergren et al. in their cohort of early RA patients from Northern Sweden also observed that the increased concentration of LpPLA2 was associated with enhanced inflammation leading to both subclinical atherosclerosis and disease activity.[26] In the present study, mRNA expression of LpPLA2 was higher in active RA patients and was positively correlated with

PARAMETER	CONTROL GROUP (N=64)	PATIENT GROUP (N=64)	P-VALUE
Age (years)	43.5 ± 5.0	46.4 ± 4.8	0.158
M:F ratio	23/41	21/43	-
Height (meter)	1.58 ± 0.029	1.59 ± 0.030	0.201
Weight (Kg)	59.4 ± 1.6	62.5 ± 2.5	0.069
BMI (Kg/m2)	23.2 ± 1.4	27.6 ± 1.5	0.050
Systolic blood pressure (mm Hg)	109.5 ± 4.58	119.42 ± 5.42	0.042
Diastolic blood pressure (mm Hg)	75.8 ± 3.9	80.5 ± 4.85	0.030
VAS (mm)	0.0	37.08 ± 4.5	0.001
ESR (mm/h)	15.7 ± 2.30	34.4 ± 3.48	0.004
DAS28	0.0	4.27 ± 0.26	0.078
Total Cholesterol (mg/dl)	154.78 ± 7.64	195.36 ± 11.21	0.050
Triglycerides (mg/dl)	107.25 ± 7.9	132.5 ± 9.0	0.071
HDL cholesterol (mg/dl)	44.2 ± 3.15	33.54 ± 3.20	0.004
LDL cholesterol (mg/dl)	91.54 ± 7.67	138.50 ± 7.52	0.001
Atherogenic index	3.47 ± 0.73	5.94 ± 1.52	0.052

Table 1: Anthropometric, clinical and biochemical profile of Patient and Control groups (Mean ± SD)

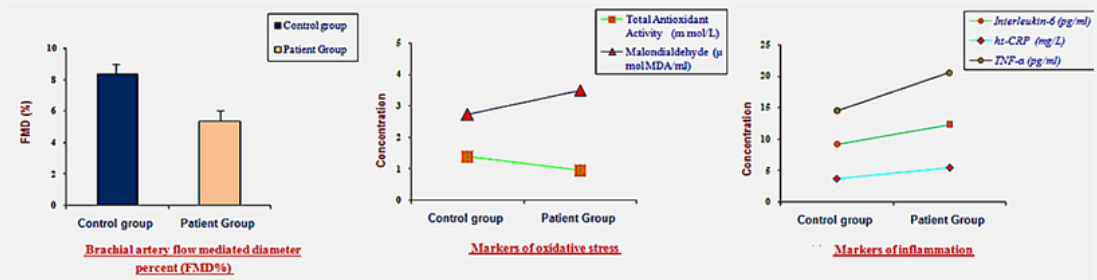

Figure 1: Endothelial dysfunction and oxi-inflammatory markers in study group subjects

where,
*p<0.1: Non-significant,
**p<0.05: Significant,
***p<0.001: Highly significant
BMI: Body mass index;
ESR: Erythrocyte sedimentation rate.
DAS: Disease activity score;
VAS: Visual analogue scale
HDL: High density lipoprotein;
LDL: Low density lipoprotein.

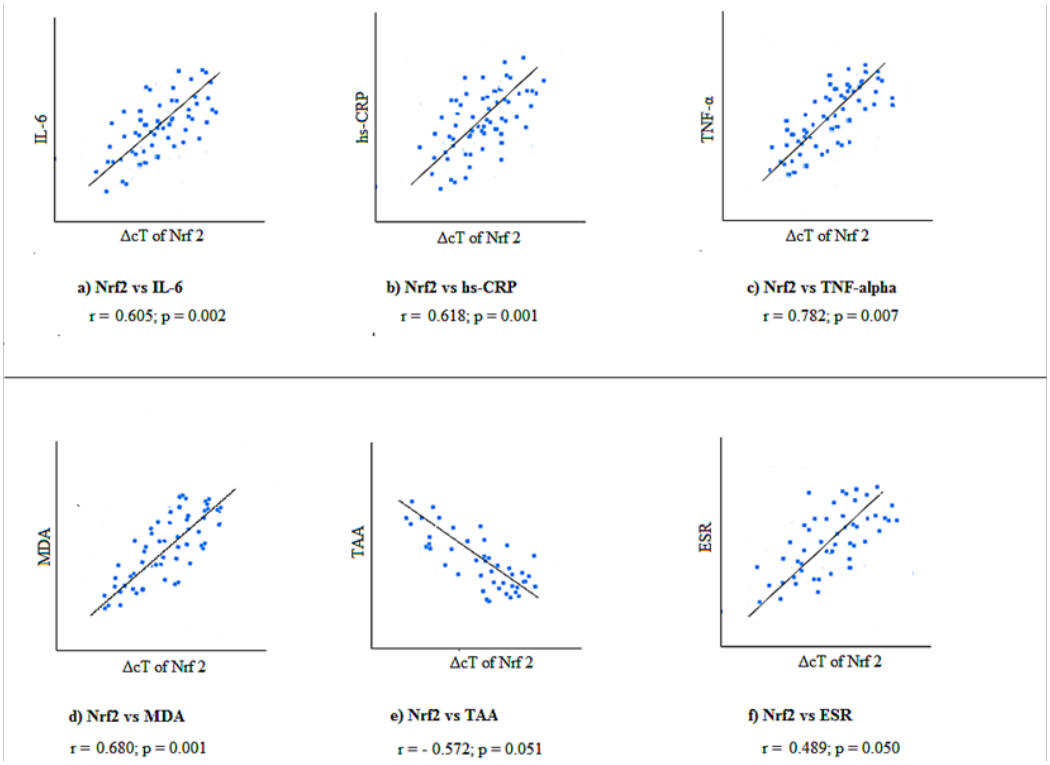

Figure 2: Correlation of Nrf2 expression with markers of Oxi-inflammatory stress in active Rheumatoid arthritis patients

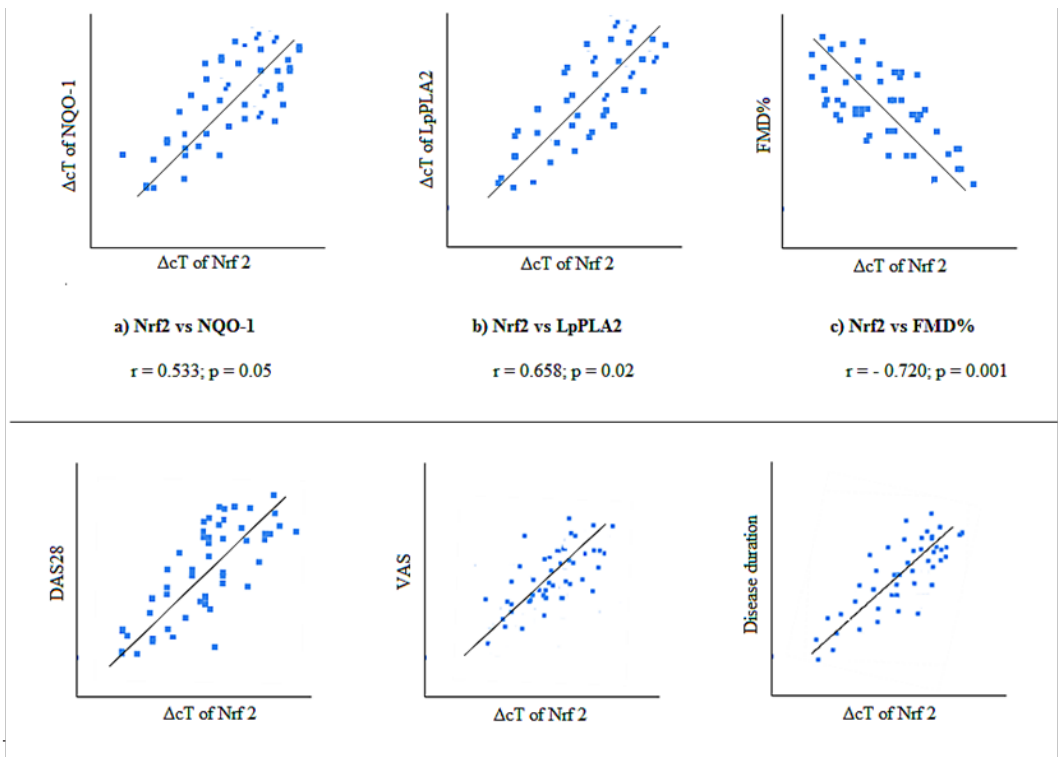

Figure 3: Corelation of Nrf2 expression with several variables in active RA patients.

Nrf2 expressions (Fig. 3) which reflect the role of enhanced inflammation in induction of cytoprotective pathway. Moreover, previous studies pertaining to association of expression of LpPLA2 with hypertension, hyperglycemia, insulin resistance, dyslipidaemia, and abdominal obesity have been documented.[14] These results suggest the regulation of LpPLA2 expression is an attractive therapeutic target candidate as its inhibition not only mitigate the production of pro-inflammatory factors but also its associated consequent sequels such as vascular complication and RA pathogenesis as well.

Our study also revealed that the mRNA expression of Nrf2 was 4.5 times higher in blood in pre-hypertensive RA patients as compare to normotensive RA patients indicating that Nrf2 have some role in endothelial vascular redox homeostasis. Moreover, altered endothelial redox homeostasis as observed in present study and characterized by low FMD% in active RA patients (Figure 1), and its inverse relation with Nrf2 expression (Figure 3) could be explained on the basis of the compensatory vasculo-protective effect of Nrf2 against augmented oxi-inflammatory stress. Similarly, Adawi et al. showed marked reduction in brachial artery flow mediated dilation in RA patients and emphasized the assessment of FMD% as an early predictor of atherosclerosis in RA patients.[2] Matinez-Hernandez et al. also analysed the association between metabolic syndrome (a constellation of cardiovascular risk factors) and genes involved in oxidative stress among Mexican Mestizos. They suggested that NQO-1 gene polymorphism is associated with a high risk of metabolic disorders, including high blood pressure, hypertriglyceridemia and low HDL-c levels.[27] Apart from this, Zakkar et al. reported that activation of Nrf2 reduces the endothelial cell from exhibiting a proinflammatory state at atherosusceptible sites via suppression of p38-VCAM-1 (adhesion molecule) signaling and may provide a therapeutic strategy to halt atherosclerotic complication.[28] Nevertheless, our study revealed the expression of cytoprotective and inflammatory genes along with oxi-inflammatory markers in whole blood in RA patents only. However, the cause and effect relationship between product of these genes and various other markers in synovium needs to be evaluated further to shed more light in personal identification of RA patients.

CONCLUSION

Thus, it can be inferred that higher expression of LpPLA2 and enhanced free radical mediated cellular toxicity leads to the compensatory induction of genes of cytoprotective pathway, i.e. Nrf2-ARE axis in active RA patients. Nevertheless, this study also reveals that induction of cytoprotective pathway has a role in alteration of vascular homeostasis and metabolic profile in RA patients. In toto, it is obvious that assessment of oxi-inflammatory stress markers produced due to toxic free radicals, is responsible for altered gene expression, vascular homeostasis and elevated atherogenic index and thus, used as a tool for personal identification of RA patients to identify the risk of CVD complications. Moreover, Keap1/Nrf2/ARE axis may be an effective "treat to target" approach from a lens of therapeutic intervention strategy in treating RA and its associated complications. Nevertheless, more investigation and continued international collaboration are required to adopt evidence based molecular diagnostic signatures for the personal identification of CVD risk in the patients of rheumatic diseases. IJFMP

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Conflict of Interest:

The authors declare that there is no commercial or financial links that could be construed as conflict of interests.

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