Correlation of Sympathovagal Imbalance with Disease Activity and Inflammatory Markers in South Indian Patients with Rheumatoid Arthritis

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ABSTRACT
Background and Aim: Rheumatoid arthritis (RA) patients have higher risk of cardiovascular disease and mortality and that is not linked to traditional cardiovascular risk factors. Altered sympathovagal balance is associated with cardiovascular disease. In RA sympathovagal balance and inflammatory markers were reported to be increased, but there is inadequacy of data on its assessment and association with disease activity. This study was conducted to find the correlation of sympathovagal balance with disease activity and inflammatory markers in South Indian patients with RA. **Methods:** RA patients (diagnosis of RA made as per 2010 ACR/EULAR classification criteria) were enrolled from Department of Clinical Immunology, JIPMER. Participants were sub grouped as low disease activity (LDA), moderate disease activity (MDA) and severe disease activity (HDA) using disease activity score 28 with ESR. Frequency and time domain parameters of heart rate variability and inflammatory markers like Interleukin-1α (IL-1α), Interleukin-6 (IL-6), tumor necrosis factor-α (TNFα) and cortisol were measured for all the participants. **Results:** A total of 143 RA patients (23 in LDA group, 69 in MDA group and 51 in HDA group) were assessed during the study. LF-HF ratio (ratio of low frequency power to high frequency power) a marker of sympathovagal balance showed significant increase in MDA and HDA group compared to LDA group. LF-HF ratio correlated positively with DAS28 ($r=0.473$, $p<0.001$), cortisol ($r=0.363$, $p<0.001$), IL -1α ($r=0.379$, $p<0.05$). **Conclusion:** Sympathovagal imbalance was significantly increased in the MDA and HDA group compared to LDA group and positively correlated with disease activity, IL-1α and cortisol in the patients of RA. Assessment of sympathovagal balance may help to find the autonomic dysfunction and cardiovascular risk in RA.
Key words: Autonomic dysfunction, Cardiovascular risk, HRV, Inflammatory markers, Rheumatoid arthritis, Sympathovagal balance.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune inflammatory rheumatic disease. It involves the joints symmetrically and causes inflammation mediated damage. The disease also affects various organ systems causing cardiovascular, pulmonary, skin and ocular involvement.[1,2] Accumulating evidence suggests that chronic inflammatory nature of RA leads to the development of cardiovascular complications.[3,4] RA patients have higher risk of developing myocardial infarction, heart failure and cerebrovascular disease than the general population.[5-7] Traditional cardiovascular risk (CV) factors were not associated with higher cardiovascular complications in RA.[8,9]

Heart rate variability (HRV) is the variation of the time intervals between adjacent heartbeats. Assessment of HRV is a noninvasive method to measure various reflex autonomic changes that occur in the cardiac autonomic system.[10] Assessment of sympathetic and parasympathetic activity of autonomic nervous system has been accepted as a good predictor of cardiovascular risk. Important component of frequency domain analysis of HRV is LF-HF ratio (ratio of low frequency power which indicates sympathetic activity to high frequency power which indicates parasympathetic activity), a marker of sympathovagal balance.[11,12] Sympathovagal balance is the balance of autonomic functions of an individual at any time. Nerve supply from sympathetic and parasympathetic system regulate important functions of the body like circulatory, immune, excretory, reproductive, respiratory systems and metabolism.[13] Limited number of studies in RA have reported that high activity of sympathetic component leads to ventricular tachyarrhythmia and sudden death and reduced HRV in patients with heart disease is linked to elevated risk of CV incidents.[14,15]

Increased levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNFα) and acute-phase reactants

causes endothelial activation which alter the stability of atheromatous plaques and lead to higher CV risk in RA patients.[16] Cytokines like interleukin-1(IL-1), IL-6 and TNF-α released from inflamed joints increase sympathetic activation and affect myocardium.[17] Cytokines also affect the potassium and calcium channels causing extended action potential.[18-20] Studies are limited in RA assessing the relationship between disease activity, HRV and inflammatory markers.[21] Therefore, this study was planned to find the correlation of sympathovagal balance with disease activity and inflammatory markers in South Indian patients with newly diagnosed RA.

MATERIALS AND METHODS

This was a cross-sectional study conducted at the Department of Physiology in collaboration with Department of Clinical Immunology JIPMER. It was duly approved by the institute ethics committee (Reg. no. JIP/IEC/2015/14/554) and carried out between July 2015 to June 2018.

Participants

The diagnosis of RA was made according to the 2010 ACR / EULAR rheumatoid arthritis classification criteria.[22] RA patients attending the outpatient clinic of the Department of Clinical Immunology, JIPMER were screened for this study. All participants were explained about the study in their native language (Tamil). They were explained the study procedure, likely benefits and harms and those willing to participate were enrolled after obtaining written informed consent. The patients were classified as having low, moderate and high disease activity as per disease activity score 28 criteria. The study participants included both genders, between 30 to 60 years of age, who were on a stable dose of disease modifying antirheumatic drugs (single or combination therapy) in the last 3 months were included in the study.[23] RA patients with history of comorbidities like diabetes mellitus, uncontrolled hypertension, any other neuromuscular, auto immune disorder, on yoga therapy or any other bio-feedback techniques and history of alcoholism or drug abuse were excluded.

After the recruitment and collection of baseline information related to disease activity, damage etc. Disease activity of all the participants was calculated with the help of disease activity score 28 using ESR (DAS28ESR) by an independent treating physician not a part of the study team. Cardiovascular parameters were evaluated in the autonomic research laboratory of the Department of Physiology.

Sub Grouping of Participants

Using Disease Activity Score in 28 Joints (DAS28ESR), we had divided the participants into the following three groups:

Low disease activity (LDA) group: DAS28 >2.6 to ≤ 3.2

Moderate disease activity (MDA) group: DAS28 >3.2 to ≤ 5.1

High disease activity (HDA) group: DAS28 >5.1

Assessment of Anthropometric Parameters and Blood Pressure

All the participants were informed to report to autonomic function testing (AFT) laboratory maintained at controlled temperature (22–25°C) in the Department of Physiology JIPMER between 9.00 to 11 am, two hours after light breakfast. Participants were explained the procedure and precautions to be taken for autonomic function testing during the previous visit. Complete autonomic function test battery was conducted as per European task force guidelines.

Height was measured on a wall mounted stadiometer to the nearest millimetre and weight on a digital weight balanced to the nearest 0.1 kg. BMI was calculated using the formula weight in kilograms divided by

square of height in meters. Omron MX3 automated BP monitor (Omron healthcare co, Kyoto, Japan) was used to measure blood pressure after 10 min of rest in supine position.

Assessment of Heart Rate Variability

The analogue lead-II electrocardiogram (ECG) signals from physiograph was acquired by BIOPAC MP 100 data acquisition system (BIOPAC Inc, USA) and data was screened for the presence of ectopic defects, only normal beats were used for analysis. HRV was analyzed using Kubios HRV software (version 3.0, Bio-signal analysis group, University of Kuopio, Finland). In frequency domain analysis, the power was calculated for very low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0.04-0.15 Hz) and high frequency bands (HF, 0.15-0.40 Hz). Further, total power (TP), low frequency normalized unit (LFnu), high frequency normalized unit (HFnu) and LF-HF ratio (ratio of low frequency power to high frequency power) were recorded. Time domain indices of mean RR (mean-RR interval), RMSSD (square root of the mean squared differences of successive normal to normal intervals), SDNN (standard deviation of normal to normal interval), NN50 (number of interval differences of successive normal to normal intervals greater than 50 milliseconds) and pNN50 (proportion derived by dividing NN50 by the total number of NN intervals) were recorded, NN-intervals being the intervals between normal R-peaks in ECG.[24]

Assessment of Blood inflammatory markers and Cortisol Levels

Five milliliter of peripheral venous blood was obtained between 7.30 am and 8.30 am for the assessment of inflammatory markers and cortisol by enzyme-linked immunosorbent assay (ELISA) method. Serum was separated by centrifugation after blood sample were allowed to clot for thirty minutes. Serum samples were stored at −80°C until use. The serum IL-1 alpha, IL-6 and TNF alpha levels were measured by ELISA kits obtained from Korain Biotech Co Ltd, Shanghai, China. Serum Cortisol levels were measured by the ELISA kits obtained from Calbiotech, Inc (California, USA).

Statistical Analysis of Data

Using the Kolmogorov-Smirnov test all the data were checked for normality, data found to be non-normally distributed has been expressed as median (interquartile range) and normally distributed as median and standard deviation. Comparison of all the parameters in relation to different disease activity groups has been carried out by Kruskal–Wallis test or ANOVA based on the normality. Partial correlation analysis was used to identify the association between parameters after controlling for the age and disease duration. All statistical analysis was performed at a significance level of 5 percent and the $p<0.05$ was considered as significant. The statistical analysis was performed using SPSS version 19 (SPSS, Chicago, IL, USA).

RESULTS

A total of 143 patients with RA completed the study. There were 23 participants in LDA group, 69 participants in MDA and 51 in the HDA group (Figure 1). There was no significant difference in age, BMI, Heart rate, systolic and diastolic blood pressure between the three disease activity groups. However, the disease duration was significantly higher in the HDA compared to LDA group (Table 1).

HRV parameters are described in Table 2. LF-HF ratio showed significant increase in MDA group ($p<0.01$) and HDA group ($p<0.001$) compared to LDA group (Figure 2). When MDA and HDA groups were compared, it was significantly increased in HDA group as compared to MDA group ($p<0.01$). Total power was significantly reduced in MDA ($p<0.05$)

Table 1: Comparison of age, BMI, blood pressure parameters between LDA, MDA and HDA groups.

Parameters	LDA (*n*=23)	MDA (*n*=69)	HDA (*n*=51)
Age (years)	43.7 ±8.09	41.65 ± 8.44	41.94 ± 8.44
Gender (Male, Female)	Male (*n*=2) Female (*n*=21)	Male (*n*=6) Female (*n*=63)	Male (*n*=47) Female (*n*=4)
Disease duration (months)	12.47 ± 5.4	16.18 ± 6.92	17.17 ± 6.74*
BMI (kg/m²)	24.09 ±4.62	24.39 ± 3.92	24.56 ± 3.99
HR (per min)	82.7 ± 9.2	85.43 ± 8.24	85 ±9
SBP (mmHg)	116.6 ± 8.65	116.94 ± 9.62	117.24 ± 9.7
DBP (mmHg)	73.96 ± 6.44	75.42 ±7.49	74.6 ± 12.2

Values are expressed as mean ± standard deviation. BMI: Body mass index; HR: Heart rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; LDA: Low disease activity; MDA: Moderate disease activity; HDA: High disease activity. * Mark indicate comparison with LDA group, *$p<0.05$. $P<0.05$ is considered to be statistically significant.

Table 2: Comparison of frequency domain indices and time domain indices of HRV between LDA, MDA and HDA groups.

Parameters	LDA (*n*=23)	MDA (*n*=69)	HDA (*n*=51)
TP (ms²)	447 (562)	417 (367) *	339.3 (507) *
LFnu	56.5 (15.7)	64.1 (13.3) **	69.9 (12.2) ****#
HFnu	43.4 (16)	35.8 (13.1) **	30 (12.4) ****#
LF- HF ratio	1.3 (0.76)	1.79 (1.09) **	2.37 (1.33) ****#
Mean RR (ms)	780 (181)	713.4 (144)	693.2 (140)
RMSSD (ms)	25.6 (31)	15.7 (12.1) *	14.8 (17.1) **
SDNN (ms)	24.5 (22.2)	18.8 (11.2)	17 (17.2) *
NN50	13 (14)	8 (11.5) **	3 (10.6) **
pNN50 (%)	2.9 (8.4)	2 (3.7)	0.4 (3.9)

Values are expressed as median (interquartile range). TP: Total power; LFnu: Normalized low frequency component; HFnu: Normalized high frequency component; LF-HF ratio: Ratio of the low frequency component to the high frequency component of HRV; Mean RR: Mean-RR interval; RMSSD: Square root of the mean of the sum of the squares of differences between adjacent NN intervals; SDNN: Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording; NN50: Number of interval differences of successive NN intervals greater than 50 ms; pNN50: Proportion derived by dividing NN50 by the total number of NN interval; LDA: Low disease activity; MDA: Moderate disease activity; HDA: High disease activity. * Mark indicate comparison with LDA group and the # mark indicate comparison with MDA group. *$p<0.05$; **$p<0.01$; ***$p<0.001$; #$p<0.05$; ##$p<0.01$; ###$p<0.001$. $P<0.05$ is considered to be statistically significant

Table 3: Comparison of disease activity, inflammatory markers and cortisol parameters between LDA, MDA and HDA groups.

Parameters	LDA (*n*=23)	MDA (*n*=69)	HDA (*n*=51)
DAS28	2.87 (0.32)	4.72 (0.54) ***	5.39 (0.48) ***###
Interleukin 1α (pg/ml)	6.71 (10.8)	8.09 (13.2)	12.5 (19.8) ***#
Interleukin 6(ng/ml)	93.21 (53.8)	95.6 (52.1)	119.4 (63.5) *#
TNF-α (pg/ml)	78.6 (85)	86.5 (79.1)	97.9 (113)
Cortisol (ng/mL)	84.79 (70.2)	121.1 (70.8) *	144.2 (78.9) **

Values are expressed as Median (Interquartile range). DAS 28: Disease activity score 28; TNF-α: Tumor necrosis factor-α. * Mark indicate comparison with LDA group and the # mark indicate comparison with MDA group. *$p<0.05$; **$p<0.01$; ***$p<0.001$; #$p<0.05$; ##$p<0.01$; ###$p<0.001$. $P<0.05$ is considered to be statistically significant.

Table 4: Correlations of sympathovagal balance with disease activity and inflammatory markers among rheumatoid arthritis patients.

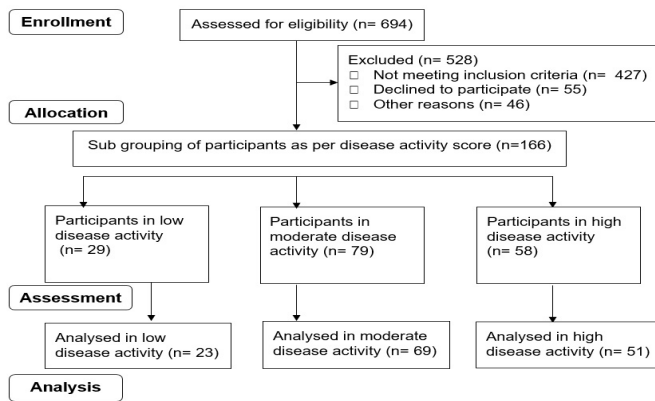| Parameters	LF-HF ratio	
	r	p
Disease duration	0.131	0.12
Disease activity score 28	0.473	0.000
Interleukin - 1α (pg/ml)	0.379	0.01
Interleukin - 6 (ng/L)	0.092	0.272
tumor necrosis factor-α (pg/ml)	0.199	0.17
Cortisol (ng/ml)	0.363	0.000

LF-HF ratio: Ratio of the low frequency power to the high frequency power. $P<0.05$ is considered to be statistically significant.

Correlation of sympathovagal balance with disease activity and inflammatory markers is shown in Table 4 and Figure 3. Correlation analysis of LF-HF ratio with DAS28 (r=0.473, p<0.001), Cortisol (r=0.363, p<0.001), IL -1α (r=0.379, p<0.05) showed significant positive association. RMSSD showed weak negative correlation with disease activity score 28 (r = -0.217, p= 0.009) and cortisol (r = -0.172, p= 0.04). SDNN also has weak negative correlation with disease activity score 28 (r = -0.205, p= 0.04) and TNF-α (r = -0.183, p= 0.04).
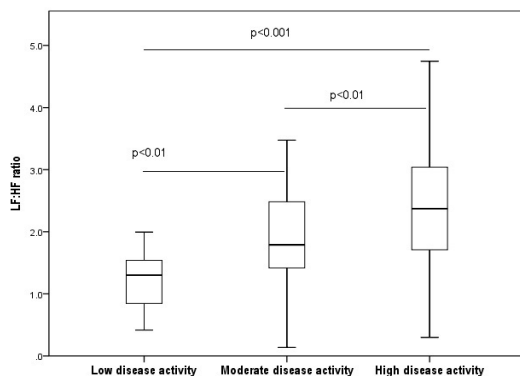
DISCUSSION

This study tried to correlate sympathovagal balance with disease activity and inflammatory markers in South Indian patients with newly diagnosed RA.

LF-HF ratio, represents the sympathovagal balance and LFnu, predominately expresses the sympathetic activity, showed significant increase in MDA and HDA group compared to LDA group. Parasympathetic activity represented by HFnu decreased in MDA and HDA groups. Increased LF-HF ratio, LFnu and reduced HFnu indicates that sympathetic activity was increased and parasympathetic activity was decreased in MDA and HDA patients. Evrengul *et al.* reported sympathovagal imbalance in terms of increased LF-HF ratio, LFnu and reduced HFnu in RA patients compared to healthy controls.[14] Previous study by Yadav *et al.* found that there was no difference in LF-HF ratio but decreased total power of HRV which represents overall autonomic variability in RA compared to healthy controls.[25]

and HDA group ($p<0.05$) compared to LDA group, but the difference between MDA and HDA groups was not significant. LFnu was increased in HDA group compared to LDA group (p<0.001). HFnu was decreased significantly in MDA ($p<0.01$) and HDA group ($p<0.001$) compared to LDA group. Time domain parameters of RMSSD, SDNN and NN50 were reduced in HDA group in comparison with LDA group.

Comparison of inflammatory markers between the three disease activity groups is shown in Table 3. Levels of IL-1α showed significantly higher levels in HDA compared to LDA ($p<0.001$). Similarly, IL-6 was increased in HDA compared to LDA ($p<0.05$) and MDA ($p<0.05$). Serum cortisol levels were significantly higher in the MDA ($p<0.05$) and HDA group ($p<0.01$).
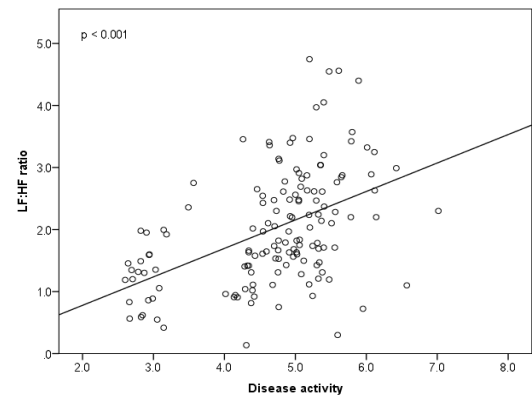
Figure 1: Study flow diagram showing the participants flow from screening to analysis.

Figure 2: Box plots of LF-HF ratio for LDA, MDA and HDA groups. Box plots of LF-HF ratio (ratio of low frequency power to high frequency power) for LDA, MDA; and HDA groups; LDA: Low disease activity; MDA: Moderate disease activity; HDA: High disease activity. The boxes show the data between the 25th and 75 Percentile; the middle line represents the median. P<0.05 is considered to be statistically significant.

Figure 3: The correlation between LF-HF ratio and disease activity controlling age and disease duration is shown by positive relationship (r=0.456, *p*<0.001) in the patients with rheumatoid arthritis. LF-HF ratio: Ratio of low frequency power to high frequency power. *P*<0.05 is considered to be statistically significant.

RMSSD and SDNN were decreased in patients with MDA and HDA compared to LDA. Since RMSSD indicates parasympathetic activity and SDNN indicates vagal modulation, results from our study show that the patients with moderate and high medium and high disease activity have reduced parasympathetic activity. A study by Anichkov *et al.* found that RMSSD and SDNN were lower in RA patients compared to normal controls, suggesting decreased vagal modulation.[26] Jahan *et al.* also reported reduced RMSSD, SDNN and pNN50% values in RA patients suggesting autonomic dysfunction.[27]

Correlation analysis of LF-HF ratio which represents sympathovagal balance showed moderate positive association with disease activity after controlling for age and disease duration. Therefore, higher the sympathovagal imbalance, higher the disease activity. Particularly, the causality of such an interaction could not be determined by this study and whether a gradual rise in disease activity could result in increased sympathovagal imbalance or excessive sympathovagal imbalance could cause or promote disease activity in RA, remains to be explained. Anichkov *et al.* reported that disease activity showed negative association with SDNN which represents vagal activity and positive association with disease duration.[26] In our study, there was no correlation between sympathovagal balance and disease duration. Disease duration of 15 months in this study indicates that autonomic dysfunction occurs earlier in rheumatoid arthritis than thought.

Sympathovagal imbalance is the condition where over activity or under activity of sympathetic or parasympathetic system leads to imbalance of autonomic functions. Commonly, in sympathovagal imbalance, the sympathetic component is more active than the parasympathetic component of ANS.[28,29] Maule *et al.* demonstrated autoantibodies against sympathetic and parasympathetic nerves in RA subjects compared to normal subjects, which was associated with sympathovagal imbalance.[30] Increased sympathovagal imbalance leads to alteration in circulatory and immune functions.[13] In the present study, weak correlation was found between sympathovagal imbalance and IL-1α and cortisol levels. Previous studies by Czura *et al.* reported that autonomic nervous system modifies inflammatory marker production via inflammatory reflex.[31] Increased level of sympathovagal imbalance and inflammatory markers may be responsible for the increased cardiovascular risk in RA patients, as altered HRV with increased sympathetic activity and reduced parasympathetic activity are associated with increased cardiovascular risk.[14,15]

Several confounding factors can affect HRV, including age, diabetes, hypertension, cardiovascular disease, renal failure, obstructive sleep apnoea, tobacco, caffeine and drugs.[32-34] Therefore, in our study we excluded patients with history of diabetes, hypertension, alcoholism, drug abuse and any condition or drug likely to affect test results. Correlation analysis was done after controlling for age and disease duration. However, some conditions like prediabetes and subclinical cardiovascular disease may affect HRV and single or combination therapy of disease modifying antirheumatic drugs affecting the test results cannot be ruled out.

Strength of this study is a relatively large sample size, inclusion of RA patients with early disease (within three years of disease onset) and inclusion of inflammatory markers. The disease activity correlated with inflammatory markers and HRV. Limitation of this study is that we could not include normal healthy controls, RA patients in remission and those with cardiovascular disease. Autonomic biomarkers like epinephrine, nor-epinephrine and neuropeptide Y also could not be assessed due to financial constraints. Future longitudinal studies with controls, adequate number of patients in different disease activity groups (including those in remission) and RA patients with cardiovascular disease may help to confirm the role of HRV as a predictor of cardiovascular risk, morbidity and mortality in RA.

CONCLUSION

Sympathovagal imbalance was significantly increased in the MDA and HDA group compared to LDA group and it was positively correlated with disease activity, IL-1α and cortisol in the patients of RA. Assessment of sympathovagal balance may help to find the autonomic dysfunction and cardiovascular risk in newly diagnosed patients with RA.

ACKNOWLEDGMENT

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

RA: Rheumatoid Arthritis; **LDA:** Low Disease Activity; **MDA:** Moderate Disease Activity; **HAD:** High Disease Activity; **IL-1α:** Interleukin-1α; **IL-6:** Interleukin-6; **TNFα:** Tumor Necrosis Factor-α; **HRV:** Heart Rate Variability; **AFT:** Autonomic Function Testing; **ECG:** Electrocardiogram; **VLF:** Very Low Frequency; **LF:** Low Frequency; **HF:** High Frequency; **RMSSD:** Square root of the mean squared differences of successive normal to normal intervals; **SDNN:** Standard deviation of normal to normal interval; **NN50:** Number of interval differences of successive normal to normal intervals greater than 50 milliseconds; **pNN50:** Proportion derived by dividing NN50 by the total number of NN intervals; **ELISA:** Enzyme-linked immunosorbent assay.

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