DISSERTATION ON

AN ASSESSMENT OF HEART RATE VARIABILITY AND BIOMARKERS IN NEWLY DIAGNOSED RHEUMATOID ARTHRITIS

SUBMITTED TO

THE TAMIL NADU DR MGR MEDICAL UNIVERSITY

In partial fulfillment of the requirements

For the award of degree of

MD PHYSIOLOGY (BRANCH V)

STANLEY MEDICAL COLLEGE

THE TAMILNADU DR MGR MEDICAL UNIVERSITY

CHENNAI,TAMIL NADU

APRIL2016

CERTIFICATE

This is to certify that the dissertation **"AN ASSESSMENT OF HEART RATE VARIABILITY AND BIOMARKERS IN NEWLY DIAGNOSED RHEUMATOID ARTHRITIS"** presented here in by **DR R ABIRAMASUNDARI,** is an original work done in the Department of Physiology, Government Stanley medical college hospital,Chennai in partial fulfillment of regulations of the Tamilnadu DR MGR Medical University for the award of degree of MD (Physiology) Branch-V during the academic period 2013-2016.

Dr.Issac Christian Moses, M.D, FICP, FACP
DEAN
Government Stanley Medical College & Hospital,
Chennai - 600 001.

Dr. K. Balasubramanian M.D
The Professor and Head of Department,
Department of Physiology
Stanley Medical College
Chennai - 600 001.

DECLARATION

I**, Dr.ABIRAMASUNDARI. R.,**solemnly declare that this dissertation, titled **``AN ASSESSMENT OF HEART RATE VARIABILITY AND BIOMARKERS IN NEWLY DIAGNOSED RHEUMATOID ARTHRITIS"** is a bonafied record of work done by me in the Department of Physiology, Government Stanley medical college hospital,Chennai, under the guidance of **Dr.K.Balasubramanian M.D**., Head of Department of Physiology, Stanley Medical College & Hospital, Chennai-600001.

This dissertation is submitted to the TamilnaduDr.M.G.R. Medical University,Chennai in partial fulfillment of the University regulations for the degree of M.D. (Physiology), Branch V, examination to be held in April 2016.

Place: Chennai

Date: **Dr.ABIRAMASUNDARI.R**

ACKNOWLEDGEMENT

I express my profound gratitude to **Prof.Dr.Isaac Christian Moses,M.D, FICP,FACP**, The Dean of Govt. Stanley Medical College and Hospital, Chennai-600001 for permitting me to use all the needed resources for this dissertation work.

Isincerely express my grateful thanks to my **Prof. Dr. K. Balasubramanian,M.D.,**Head of Department of Physiology, Stanley Medical College who has been a great pioneer and without whom it would have been totally impossible to work on this subject. I thank him for being a constant source of encouragement, inspiration, not only in this study but in all my professional endeavors.

It is with deep sense of gratitude; I acknowledge my profound indebtedness to **Prof.Dr.R.Jayanthi, M.D.,** Head of department of Medicine, **Dr. M.Hema M.D.,D.M**. (Rheumatology) Assistant prof. in Medicine for providing cases for my study.

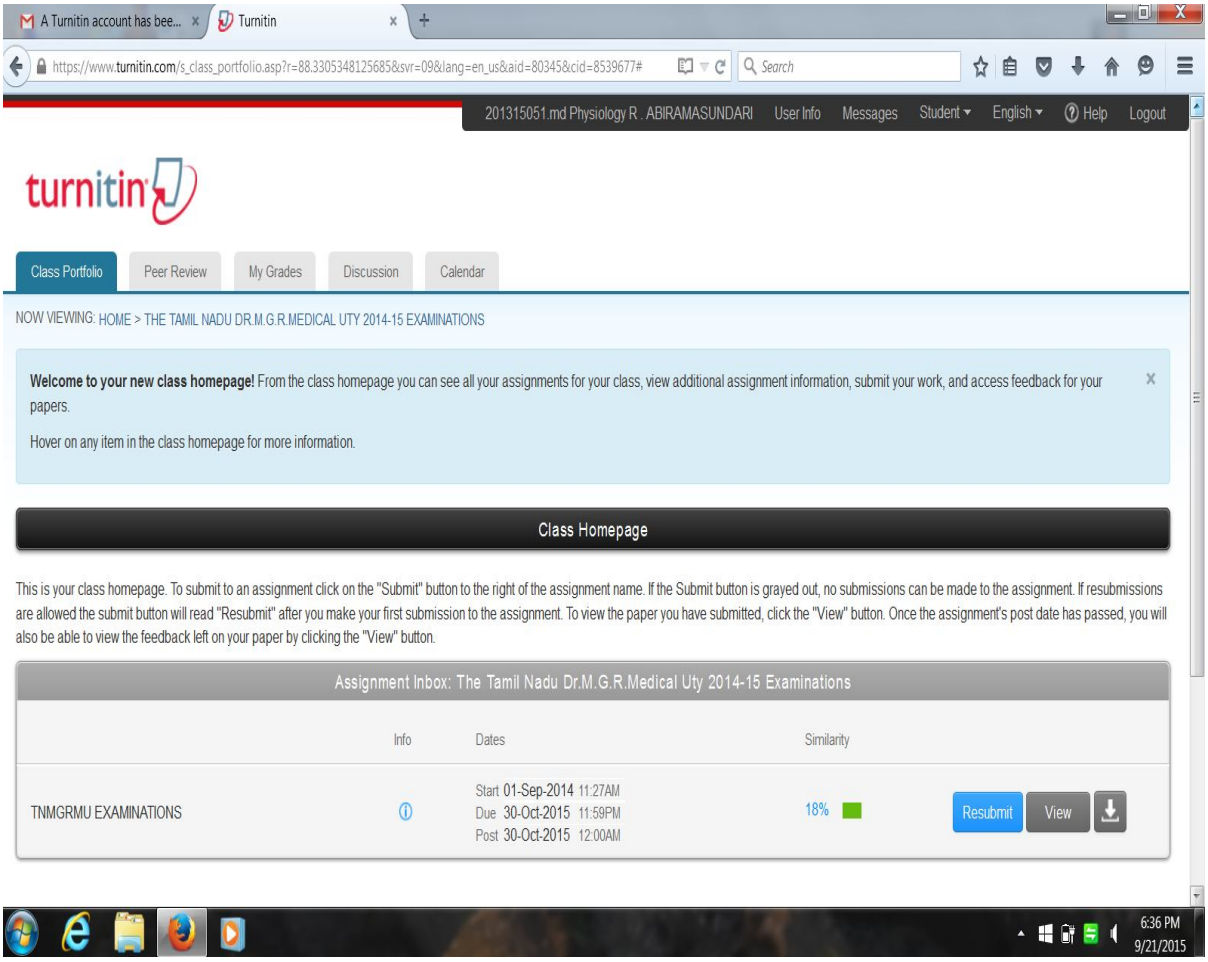
I sincerely thank **Dr.S. Meenakshi M.D.** Prof. of Immunology, **Dr.Vasumathy M.D.** assistant professor of Immunology and **Dr. S. Mary Lilly M.D.**(pathology) Prof. of pathology,for guiding and permitting me to carry out the biomarkers tests.

I express my profound thanks to **Dr.VijiDevanand M.D.,Dr. D. Celine M.D., Dch., Dr.C.C.Umayal M.D.**for their valuable guidance and moral support.

I express my sincere thanks to all the Asst.Professors, and Staff members, Dept.of Physiology,SMC,Chennai-1.

I whole heartedly thank my subjects without whom this study would have been impossible.

I extend my sincere thanks to my Post Graduate colleagues for their valuable support, encouragement and help during this study.


A Turnitin account has bee...
Turnitin
https://www.turnitin.com/s_class_portfolio.asp?r=88.3305348125685&svr=09&lang=en_us&aid=80345&cid=8539677#
Search
201315051.md Physiology R . ABIRAMASUNDARI
User Info
Messages
Student
English
Help
Logout
turnitin
Class Portfolio
Peer Review
My Grades
Discussion
Calendar
NOW VIEWING: HOME > THE TAMIL NADU DR.M.G.R.MEDICAL UTY 2014-15 EXAMINATIONS
Welcome to your new class homepage! From the class homepage you can see all your assignments for your class, view additional assignment information, submit your work, and access feedback for your papers.
Hover on any item in the class homepage for more information.
Class Homepage
This is your class homepage. To submit to an assignment click on the "Submit" button to the right of the assignment name. If the Submit button is grayed out, no submissions can be made to the assignment. If resubmissions are allowed the submit button will read "Resubmit" after you make your first submission to the assignment. To view the paper you have submitted, click the "View" button. Once the assignment's post date has passed, you will also be able to view the feedback left on your paper by clicking the "View" button.
Assignment Inbox: The Tamil Nadu Dr.M.G.R.Medical Uty 2014-15 Examinations
Info
Dates
Similarity
TNMGRMU EXAMINATIONS
Start 01-Sep-2014 11:27AM
Due 30-Oct-2015 11:59PM
Post 30-Oct-2015 12:00AM
18%
Resubmit
View
6:36 PM
9/21/2015

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANFT	Autonomic Function Test
ANS	Autonomic Nervous System
bpm	Beats Per Minute
BRS	Baroreflex Sensitivity
BMI	Body Mass Index
BP	Blood Pressure
CNS	Central Nervous System
CRP	C-Reactive Protein
CVLM	Caudal ventro lateral medulla
DBP	Distal Blood Pressure
DMV	Dorsal Motor Nucleus of Vagus
EC	Endothelial Cell
ECG	Electro Cardio Gram
FFT	Fast Fourier Transform
HF	High Frequency
HR	Heart Rate
HRV	Hear Rate Variability
LF	Low Frequency
NN50	Normal to Normal RR interval deviation more than 50 ms
NTS	Nucleus TractusSolitarius
n.u.	Normalised Units

PNS	Parasympathetic Nervous System
PSD	Power Spectral Density
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
RMSSD	Root Mean of the Sum of Squares of Difference between adjacent NN intervals
RVLM	RoastralVentro Lateral Medulla
SA	Sino Atrial node
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDNN	Standard deviation of average Normal to Normal RR intervals
SMC	Stanley Medical College
SNS	Sympathetic Nervous System
TP	Total Power
ULF	Ultra Low Frequency
VLF	Very Low Frequency
VLM	Ventrolateral Medulla

ABSTRACT

AN ASSESSMENT OF HEART RATE VARIABILITY AND BIOMARKERS IN NEWLY DIAGNOSED RHEUMATOID ARTHRITIS

INTRODUCTION: Rheumatoid arthritis (RA) is the most common chronic systemic inflammatory arthritis. In extra articular manifestations of RA cardiovascular system involvement is the most important complication which may lead to sudden cardiac death. The sudden cardiac death in RA is due to arrhythmia and myocardial infarction. The cardiovascular events are due to altered autonomic nervous system function. In RA the inflammatory mediators, endocrine abnormalities and autoantibodies lead to autonomic imbalance.**Aim & Objective** :To assess the cardiovascular autonomic functional status in the Newly diagnosed rheumatoid arthritis subjects by using Resting Heart Rate Variability analysis as a tool. To evaluate the individual contribution of inflammatory biomarkers such as Rheumatoid factor (RF), C-reactive protein (CRP) and platelet count in the pathogenesis of cardiovascular autonomic imbalance. **Materials & Methods**: The age and gender matched 40 study group diagnosed by using EULAR classification criteria 2010and 40 healthy controls were recruited from the Stanley Medical College, Hospital. The short term Heart rate variability analysis in supine position was taken in the neurophysiology laboratory, Department of physiology, SMC. RF, CRP, and platelet count were estimated. The overall autonomic tone, parasympathetic drive, sympathetic drive and sympatho-vagal ratio were quantified by using various parameters. It included

standard deviation of all R-R intervals (SDNN), root-mean square of successive differences (RMSSD), and number of R-R intervals differing by >50 ms from adjacent intervals (NN50) in the time domain analysis. In frequency domain analysis, low frequency (LF) and high frequency (HF), LF/HF and total power were assessed. Data was analyzed by using SPSS version 17. For statistical analysis Independent Student t test, Chi- Square test and Pearson's Coefficient were applied. **Result:**Heart rate and systolic BP were higher in patients with RA. SDNN, SDSD, RMSSD, NN50, LF and HF power in nu and total power (ms x ms) were significantly lower in patients with RA versus healthy controls ($P<0.001$).LF power in normalized unit was increased in the seropositive patients (70.43±15.26) than seronegative patients (52.09±26.5). LF/HF ratio increased in seropositive (2.81±2.4) when compared with seronegative patients (2.7±1.7). HF power in normalized unit was found decreased in the seropositive subjects (29.19±14.25) than seronegative subjects (46.08±24.6).Among the study group 62.3% showed CRP positive in their sera. None of the healthy controls showed positivity for both rheumatoid factor and C-reactive protein. In the frequency domain measures LF power in normalized unit was increased in the CRP positive patients(65.77±20.61) than CRP negative patients(63.20±21.8). HF power in normalized unit was found decreased in the CRP positive subjects (33.63±19.16) than CRP negative subjects (35.53±20.28). There was a statistically significant increase in platelet count in the study group (3.14±0.5) when compared with the controls (2.5±0.2). It was statistically significant ($P \leq 0.000^{**}$). There was a significant positive correlation found between platelet count and LF nu ($r =0.313$, $p< 0.01^{**}$) LF/HF ratio($r = 0.168, p <0.01^{**}$).Thus,We observed reduced HRV in RA

patients which denotes altered cardiovascular autonomic function. We also observed that RF, CRP and platelet count were increased in the study group and associated with increased sympathovagal imbalance. This signifies the importance of these factors in the pathogenesis of cardiovascular risk in RA patients**Conclusion**: The simple, noninvasive resting HRV analysis can be included in the routine basic investigation of RA. The periodic assessment of the biomarkers RF, CRP and platelet count may help in the early diagnosis of cardiovascular complication in RA patients.

Key words: cardiovascular status - rheumatoid arthritis - short term heart rate variability-RF-CRP-platelet count.

INTRODUCTION

Rheumatoid arthritis (RA) is the most common chronic systemic inflammatory arthritis. It is characterized by deforming symmetrical polyarthritis of varying extent and severity, associated with synovitis of joint and tendon sheaths, articular cartilage loss, erosion of juxta-articular bone. In extra articular manifestations of RA cardiovascular system involvement is the most important complication which may lead to sudden cardiac death[1]. Median life expectancy is shortened by an average of 7 years for men and 3 years for women compared to control population[2]

The sudden cardiac deathin RA is due to arrhythmia and myocardial infarction. The cardiovascular events are due to altered autonomic nervous system function. Autonomic dysfunction is because of the presence of auto antibodies against nerve growth factor, superior cervical ganglion and vagus nerve which leads to arrhythmia and myocardial infarction.The ischemia is due to inflammatory mediators and immune complex. In chronic arthritis such as RA, decreased responsiveness of hypothalamic-pituitary-adrenal axis causes inadequate production of cortisol in relation to inflammation that consequently leads to increased sympathetic activity, increased circulating cytokines, decreased local synovial sympathetic innervation, altered metabolism of estrogen in the synovium and high expression of estrogen receptors in synovial cells; all leading

to exacerbation of neuroendocrine abnormalities in RA. Thus in RA the inflammatory mediators, endocrine abnormalities and autoantibodies lead to autonomic imbalance.

Among the tests for cardiovascular autonomic function assessment, heart rate variability (HRV) is a simple, noninvasive study. It is easy to perform.It is a quantification measurement of sinus rhythm variability. It depends on the balance between sympathetic and parasympathetic activity of the heart. It detects the early impairment of the autonomic balance.[3] A high HRV indicates good adaptability of the heart. Reduced HRV denotes impaired autonomic function.

HRV may reflect changes in body to stress, while other physiological parametersare still in "normal" accepted ranges. Some HRV changes maybe a first sign ofdistress, reflecting energy more dependent sympathetic system involvement. Thedecrease in biological signals variability is a warning sign of a homeokinetic self-regulation loss.

Many studies are available in western countries relating to the cardiovascular happenings in RA. However autonomic imbalance evaluating studies in Indian population are scanty and the data available are differing.[4] Discrepancies might be related to the use of different criteria for the diagnosis of autonomic dysfunction. The range of abnormality could due to the inclusion of variousnumbers of tests.

Our study was planned to assess the autonomic function in patients with RA by using resting HRV analysis. To evaluate the association of biomarkers rheumatoid factor, C-reactive protein and platelet count with HRV indices.

REVIEW OF LITERATURE

Rheumatoid arthritis

In 1876 Sir Alfred Garrod an English physician introduced the term rheumatoid arthritis.0.5 -1% of adult population is affected by rheumatoid arthritis worldwide. In India the prevalence rate is 0.2-0.4% [5]. The incidence increases between 25 -55 years of age. After that the incidence plateaus until the age of 75,then it decreases.[2]

The female group affected more than the male with a ratio of 2-3:1.This preponderance in female is due to the hormone estrogen which stimulates Tumor Necrosis Factor α (TNFα) a major cytokine involved in the pathogenesis of rheumatoid arthritis.The combined factors of genetic, environmental and immunologic factors play an important role inthe etiology[6]

Genetic factor:

First degree relatives of the patients show 2-10 times risk of developing rheumatoid arthritis thanthe general population. The alleles are located in the Major Histocompatibility Complex class II. The allelic variation in the HLA-DRB1 gene which is associated with the etiology of rheumatoid arthritis encodes the MHC class II β chain.The shared epitopes (SE) present in HLA DR β chain increase the risk of developing the disease by increasing the production of anti CCP antibodies.

HLA DR β allele with high risk is * 0401 and associated with moderate risk are* 0101,* 1001 and* 0901. High prevalent areas show association with * 1042.

Genome wide association studies (GWAS) identified non MHC related genes that cause RA susceptibility. GWAS based on Single nucleotide polymorphisms(SNPs). There are 3 billion base pairs. The encoding protein tyrosine phosphatase non-receptor 22 (PTPN22)is an example of non MHC gene.

Environmental factors:

Cigarette smoking contributes risk for developing rheumatoid arthritis by 1.5 - 3.5. It is related to rheumatoid factor and anti CCP antibody positive disease. In 1931 relationship between rheumatoid arthritis and infectious diseases were identified. The Epstein Barr virus (EBV), parvo virus b- 19 and mycoplasma antibodies are present in serum synovial fluid of rheumatoid arthritis patients.

Immunological factors:

RA is an autoimmune disorder mediated by local immune complex formation and complement consumption. Self-reactive antibodies like rheumatoid factor and anti CCP antibodies present in sera of patients before the onset of clinical symptoms. Sero-positive patients have severe disease activity than sero-negative patients. Enhanced T-cell function has been associated with the spontaneous production of antibodies.

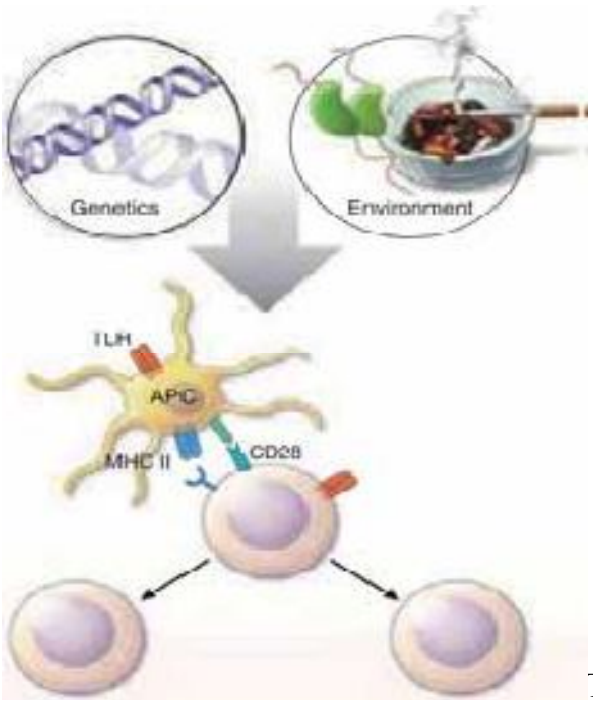
The pathogenesis involved in rheumatoid arthritis are synovial inflammation, proliferation and focal bone erosion. The T cell constitutes 30 – 50 % of inflammatory infiltrate .The cortical bone layer separate the bone marrow from the invading pannus is thin. So the inflammation from synovium penetrates the bone marrow easily.

Pathogenesis:

The etiological factors genetic, environmental and immunological factors produce dysregulation of the immune system. It was proved by the presence of RF and anti CCP antibodies in the sera of the patient inthe sub clinical condition.

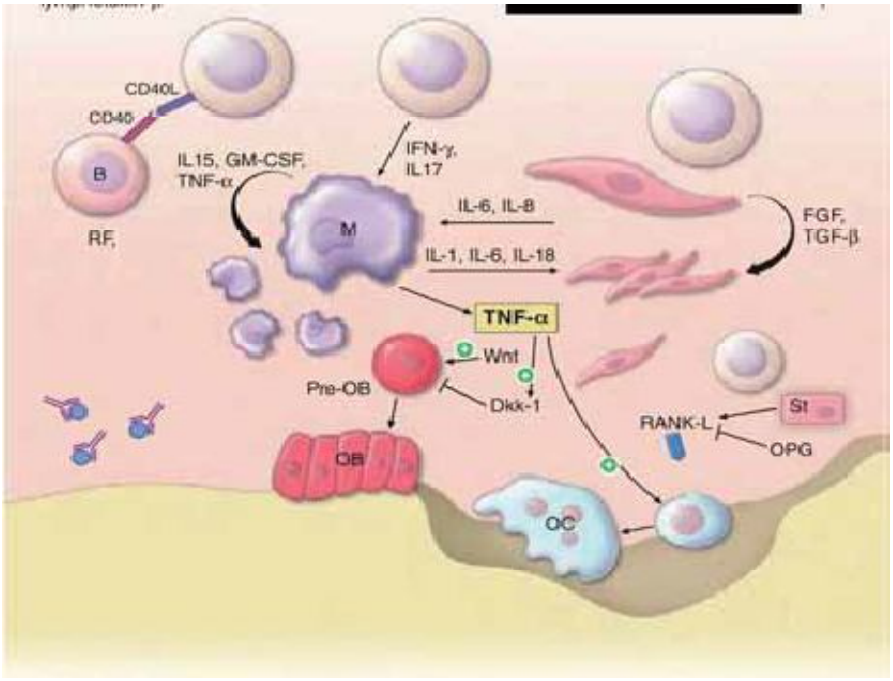
Tobacco smoking inducescitrullination of proteins in the lungs which induce self- reactivity.[7]The infections alter the immune system through Toll like receptors (TLRs). The activated T-cell stimulates the inflammatory mediators and destroys the cartilage and the bone. CD4+T-cells stimulate B cells which in turn produce antibodies.

Receptor activator of nuclear factor κ B ligand (RANKL) stimulates osteoclast and cause bone resorption. Increased expression of RANKL found in rheumatoid arthritis. The Wnt system is a family of glycoprotein which promotes cell growth.[8] The DKK-1 (dickkopf-1) inhibitor of Wnt system thus inhibits bone formation. The cytokine TNF α enhances the expression of DKK-1.



TH1 TH17 IL17 A

IFN γ, TNF αIL17F,TNFα,IL



Pathogenesis of rheumatoid arthritis

Diagnosis:

In the past 1987 ACR classification was used for diagnosis of RA. Now the criteria for rheumatoid arthritis was revised by ACR and European league against Rheumatism (EULAR) [9] 2010.This criteria gives a score of 0-10. The requirement for definite rheumatoid arthritis should fulfill the score of ≥ 6.

The radiographic findings inform the diagnosis in the later stage. The new classification criteria differ in several ways from the older criteria set. The new criteria include a positive test for serum anti-CCP antibodies (also termed ACPA, anti- citrullinated peptide antibodies) as an item, which carries greater specificity for the diagnosis of RA than a positive test for RF. The newer classification criteria also do not take into account whether the patient has rheumatoid nodules or radiographic joint damage because these findings occur rarely in early RA. It is important to emphasize that the new 2010 ACR-EULAR criteria are "classification criteria" as opposed to "diagnostic criteria" and serve to distinguish patients at the onset of disease who have a high likelihood of evolution to chronic disease with persistent synovitis and joint damage

TABLE 1

EULAR CLASSIFICATION CRITERIA 2010

Classification criteria for rheumatoid arthritis		
joint involvement	1 large joint (shoulder elbow,hip,knee,ankle)	**0**
	2-10 large joints	**1**
	1-3 small joints(MCP,PIP,ThumbIP,MTP, Wrist)	**2**
	4-10 small joints	**3**
	>10 joints (at least one small joint)	**5**
Serology	negative RF and negative ACPA	**0**
	low positive RF low positive anti-CCP antibodies(≤3times ULN)	**2**
	high positive RF high positive anti-CCP antibodies(>3times ULN)	**3**
Acute phase reactants	normal CRP and normal ESR	**0**
	abnormal CRP and abnormal ESR	**1**
Duration of symptoms	< 6 weeks	**0**
	> 6 weeks	**1**

Laboratory findings:

- IgM,IgG and IgA isotypes of RF may present in sera of RA.The IgMisotypeis the frequently measured RF.
- Anti CCP antibodies also present
- Slight leukocytosis with normal DC count
- Thrombocytosis
- Mild anemia (Hb 10 gm/dl)
- Elevated ESR and CRP
- Normal renal, hepatic and metabolic function
- Normal urine analysis
- Synovial fluid analysis shows WBC count 5000 to 50000.
- Antibodies may be found.

Radiography- Juxta- articular osteopenia is the early finding.

MRI–hasthe greatest sensitivity for detecting synovitis.

USG detects more bone erosion than radiography.

Self- reactiveIgM antibody presents in the sera of the patients subclinically. Sero-positive patient shows high disease activity than sero negative. Rheumatoid factor willfix and activate the classic pathway of complement system. Among the IgG, IgM andIgA, IgM antibody is the most common laboratory useful antibody. RF found in synovial fluid and sera of the patient.

Serum IgM RF has been found in 75-80% of patients with RA; therefore, a negative result does not exclude the presence of the disease. It is also found in other connective tissue diseases, such as primary sj□gren's syndrome, systemic lupus erythematous, and type II mixed essential cryoglobulinemia, as well as chronic infections such as subacute bacterial endocarditis and hepatitis B and C. Serum RF may also be detected in 1-5% of the healthy population.

C-reactive protein is an acute phase reactant. It is an inflammatory markerfor rapid diagnosis of RA. It is synthesized in liver in response to cytokines, particularly IL6. CRP has been suggested to stimulate the complement activation. It indicates the disease activity. The test is easy to perform. Low cost. CRP associates with therisk ofdeveloping myocardial infarction.

High platelet count indicates the increased activity of platelets which leads tothrombosis. The intensive stimulation of the bone marrow and increase platelet turn over may occur in response to an excess production of inflammatory cytokines .The platelets produce clot forming protein.[10]

Forerunners in the research field of autonomic function:

- 1898- Langley coined the term Autonomic nervous system
- 1920 –Herring explained Baroreceptor reflex
- 1936- Jordan explained the clinical findings in autonomic neuropathy

- 1948-Alquist divided the adrenergic receptors into alpha and beta receptors
- 1960-SharpeySchafer Taylor identified the symptom of autonomic neuropathy, the orthostatic hypotension. 1967 –Bannister observed the same finding.
- 1966- Albert BLevin described the heart rate changes occur during valsalva maneuver
- 1970-Ewing and Clark introduced noninvasive cardiovascular tests to study autonomic functions
- 1973-Timothy wheeler introduced the study for vagal function
- 1975-Lipski et al explained the role of NTS
- 1977-Page and Watkins described the orthostatic test
- 1991- Corellic demonstrated the spectral analysis of HRV in rats
- 1992- Ziegler explained spectral analysis of HRV in humans
- 1994- Boo tama et al described parasympathetic and sympathetic influence on the heart.

Autonomic nervous system:

The autonomic nervous system is otherwise called involuntary nervous system. It is a part of the peripheral nervous system. It controlsthe subconscious level functions like heart rate, digestion, salivation, respiratory rate, pupillary dilation, micturition and sexual function. The autonomic nervous system assists the body in maintaining a constantinternal environment (homeostasis). Whenever the internal stimuli signals aboutderangement of the body's internal environment, its autonomic nerves and thecentral nervous system (CNS) commands compensatory actions.

The term autonomic nervous system generally refers to the sympathetic andparasympathetic nervous systems, their preganglionic and postganglionicneurons and the central components which include the hypothalamus and higherlevels of the limbic system. The sympathetic and parasympathetic nerves have preganglionic cell bodies, which are located in brain stem and spinal cord.

Axons leave the central nervous system and synapse in specialized ganglia.

Second order neurons emerging from the ganglia directly innervate the smooth muscle and cardiac muscle.

The Sympathetic and parasympathetic nervous system are the two types of autonomic nervous system. The sympathetic gives Flight and Fight reactions and consists of thethoroco lumbar spinal nerves. The parasympathetic system controls slow active reactions and consists of 9, 10, and 11 cranial nerves and sacral spinal nerves.The sympathetic and parasympathetic nervous system organs work in acoordinated manner-sometimes acting reciprocally and sometimessynergistically-to regulate visceral functions.

Heart is innervated by both sympathetic and parasympathetic fibers. The parasympathetic functions in the heart are prominent. The preganglionic sympathetic fibers reach the cardiac ganglion. The post ganglionic fibers supply the cardiac tissues, muscles of atria and ventricle. The parasympathetic vagal nerves from the vasomotor center medulla supply the Sino- atrial node which contains the firing pace maker cells.[11]The blood vessels are supplied by sympathetic nerve fibers. The sympathetic tone controls the blood pressure by the action on arteries and vein.

The sympathetic fibers release neurotransmitter noradrenaline which binds with beta 1 receptor in the cardiac muscle fiber. After the binding of neurotransmitter,calcium is released and it causes depolarization and generates action potential.

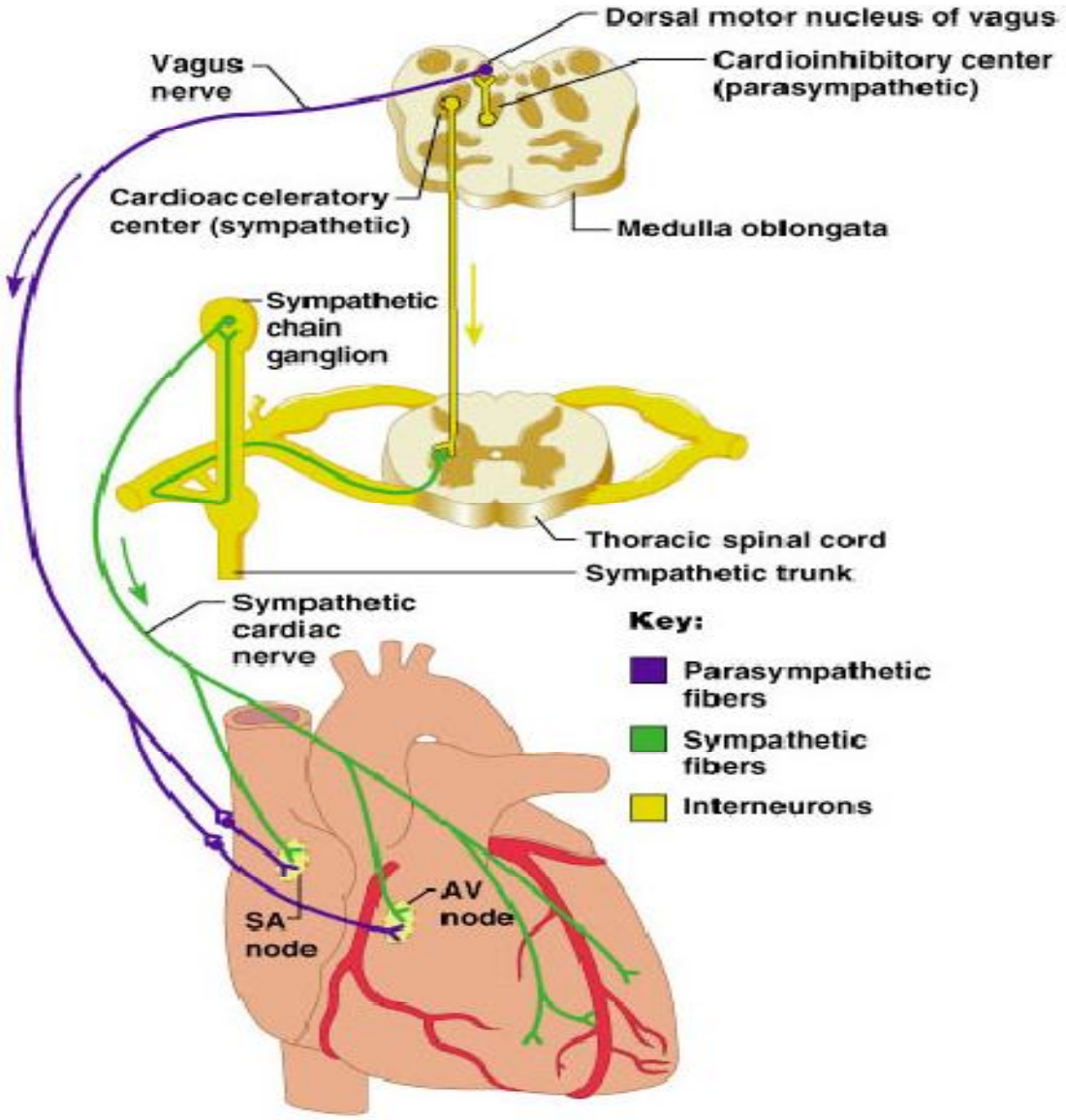
Ach neurotransmitter is released from the parasympathetic fibers. They combine with M2 muscarinic receptors and cause hyperpolarization by K channel opening.Noradrenaline acts on both alpha 1 and 2 receptors and produces biphasic response of vasoconstriction and vasodilatation.

The vasomotor center medulla has three myocardial centers which control cardiovascular functions. They are the Nucleustractus solitaries (NTS), the cardiovascular excitatory fibers and theinhibitory center.The major sensory nucleus of the autonomic nervous system is the nucleus tractus solitaries. It is a paired structure present in the lateral areas of medulla. It lies close to the paired dorsal motor vagal nucleus ambiguous. In this motor vagal nucleus ambiguous the primary sensory information from the baroreceptor integrated. The baroreceptors receive signals from the hypothalamus and the cortical areas.

The excitatory center is situated in the reticular formation of the rostral ventrolateral medulla. In this area most of the cells have pace maker properties. They provide a phasic excitatory drive to the sympathetic preganglionic neurons through reticular spinal pathways. The NTS and the hypothalamus control the rate of discharge of the excitatory cells.

The inhibitory cells are the preganglionic cells situated in the ventral part of the nucleus ambiguous and the dorsal motor nucleus of vagus.Both peripheral and central signals are connected by brainstem cardiovascular centers. They

formulate appropriate motor response and relay them to theheart motor neuronsof the ANS.[12]When both divisions of the ANS are blocked, the heart rate averages about 100beats/min which is called as intrinsic heart rate. However the average resting heart rate isabout 70 beats/min in normal adults. The Heart Rate estimated at any given timerepresents the balance of the parasympathetic (vagus) nerves, which slows HR,and the sympathetic nerves, which accelerate it. The heart response time tosympathetic stimulation is relatively slow. The heart response time toparasympathetic stimulation is almost instantaneous. Parasympathetic toneusually predominates in healthy, resting individuals. The heart rate isconventionally measured by noting the number of heart beats perminute.



Autonomic innervations of the heart

Baroreceptors:

Baroreceptors arethe stretch receptors monitor the arterial circulation. They are called as carotid sinus and aortic arch receptors.The afferent fibers pass through the glossopharyngeal and vagus nerves. They terminateat NTS and the neurotransmitter is glutamate which is excitatory.The glutamate stimulates GABA secreting neurons which inhibit the tonic discharge of vaso constrictor nerves. This causesvasodilation which leads to decreasein blood pressure.

The excitatory fibers from the NTS pass to the vagal motor neurons and cause bradycardia.[11]The baroreceptors sense the arterial pressure changes and pass it to the vasomotor and cardiac inhibitory areas thus it produce a reflex feedback mechanism to balance out BP and HR. Any drop in systemic arterial pressure decreases the baroreceptor inhibitory discharge and this in turn increases the blood pressure and vice versa. The baroreceptor system maintains the short term regulation of BP.

ASSESSMENT OF CARDIOVASCULAR AUTONOMIC SYSTEM

The test used to assess the cardiovascular autonomic system should have acceptable sensitivity, specificity and reproducible. Simple tests are available for evaluation of suspected autonomic dysfunction. They are blood pressure and heart rate response to standing, head –up to tilt, carotid sinus massage, sustained isometric handgrip,cold presser test,heart rate variation during deep breathing,valsalva maneuver and mental arithmetic. These testsexplain the baroreceptor reflexes. However these tests are not commonly performed outside of specialist units.

In view of Ewing et al[12] RR interval variations during deep breathing, valsalva maneuver and the blood pressure response to standing are the most widely used methods for the assessment of cardiovascular autonomic system. In many tests these measures have been combined with measurements of catechol amines and their metabolites and plasma renin activity to demonstrate the correlation between cardiovascular activity and sympathetic nervous system activity.

To interpret the significance of blood pressure and heart rate variation measurement of muscle sympathetic nerve activity has been used.The other pharmacological tests have been used to study the functioning of baroreceptors are blockade with atropine,beta blockers or provocations with norepinephrine[13]

So,this field of clinical assessment of cardiovascular autonomic function, in rheumatoid arthritis patients, has been of great interest in research people. To assess the sympathetic activity in skeletal muscle we can use microneurography. It allows a precise, reproducible and quantitative assessment of sympathetic activity.Pagane et al [14] in their study demonstrated that the sympathetic outflow to skeletal muscle will not reflect the sympathetic activity of other organs, which are also important for autonomic circulatory control. This is the limitation of the microneurographical method.

For the last two decades, other methods of studying and quantifying cardiovascular autonomic function have become popular. They are heart rate variability (HRV), baroreceptor sensitivity and blood pressure variability.

Heart rate variability:

HRV is a physiological phenomenon of variation in the time interval between heart beats. The oscillation in the interval between consecutive heart beats as well as the oscillation between consecutive instantaneous heart rate.(Task force 1996).

Usually, heart rate variability analysis attempts to assess cardiac autonomic regulation through quantificationof sinus rhythm variability. The main inputs tosinus nodeare from the sympathetic nervous system and the parasympathetic nervous system. Other factors that affect the inputs are the baroreceptor reflex,

thermo regulator, hormones, sleep-wake cycle, meals and stress.The sinus rhythm time series is derived from the R-R interval sequence of the electrocardiogram; by extracting only normal sinus to normal sinus(NN) inter beat intervals.

History:

- 18th century - Albrecht Von Haller noticed the regularity of heart beat
- 1965 - Hon and Lee noted the clinical relevance of HRV by appreciated thefetal distress which was preceded by changes in inter beat intervals before the appearance of changes in heart rate.
- 1971 - Sayers and others focused on rhythm imbedded in beat-to-beat heart rate
- 1977 - Wolf et al showed association of heart rate to sudden death in post MI
- Late 1980's -HRV wasconfirmed as a strong predictor of mortality after an acute MI
- 1981 - Axelrod introduced Power Spectral Analysis
- 1996 - Task Force published Standards of Measurement for HRV.

Measurement of HRV:

HRV analysis is done with the derived R-R intervals mainly by three methods.

- Time domain
- Frequency domain
- Non-linear methods

Time Domain Methods:

In this method, either the instantaneous heart rate or normal-to-normal (NN) intervals, the intervals between successive normal QRS complexes are determined.Time domain measures are the simplest to calculate and include the meannormal-to-normal (NN) intervals during the entire recording and statistical measures of the variance between NN intervals.

The majority of time domain metrics are statistical methods. It should be calculated over a specific and fixed period of time, or epoch, to carry any significance. The two epoch "windows" most often used are 24 hours (long term) and 5 minutes (short term). Significant results are achieved only by comparing SDNN valuesmay be unreliable if they are calculated over epochs that are too short.

SDNN-reflects the variability in the period of recordings. As SDNN gets Reduced, HRV gets reduced

SELECTED TIME DOMAIN MEASURES

VARIABLE	UNITS	DESCRIPTION
SDNN	ms	Standard deviation of all NN intervals
SDANN	ms	Standard deviation of averages of NN intervals in all 5 min segments
RMSSD	ms	The square root of the mean of the sum of the squares of differences between adjacent NN intervals.
SDNN index	ms	Mean of the standard deviation of all NN intervals for all 5 min segments
NN50 count	ms	No:of pairs of adjacent NN intervals differing by more than 50 ms
pNN50	%	NN50 count divided by the total number

SDNN - the standard deviation of NN intervals. Often it is calculated over a 24-hour period. It indicates the parasympathetic activity.

SDANN - the standard deviation of the average NN intervals calculated over short periods, usually 5 minutes.

RMSSD- the square root of the mean squared difference of successive NNs.

NN50 – the number of pairs of successive NNs that differ by more than 50ms.

pNN50 – the proportion of NN50 divided by total number of NNs.

Frequency Domain Methods:

Power spectral density (PSD) analysis provides the basic information of how power distributes as a function of frequency.For short term recording (about 5 minutes), frequency-domain methods are generally preferred. In humans the following frequency bands have been defined.

High Frequency band (HF) between 0.15 and 0.4 Hz.

HFis contributed mainly by **vagal activity** (parasympathetic nervous system) and itsoscillations depend primarily on respiration.

Low Frequency band (LF) between 0.04 and 0.15 Hz.

LFis driven by baroreflex mediated as well as centrally generated RR oscillations. It is considered as **Sympathetic marker** by some while others determine it to be a combination of sympathetic and parasympathetic activity.

Very Low Frequency band (VLF) band between 0.0033 and 0.04 Hz.

Though VLF is attributed to thermal regulation of the body's internal systems, the origin is not very clear.

Ultra LowFrequency (ULF) band between 0 and 0.0033 Hz.

This ULF is always expressed in 24 hour recordings. It depicts day night variations.

LF/HF ratio:

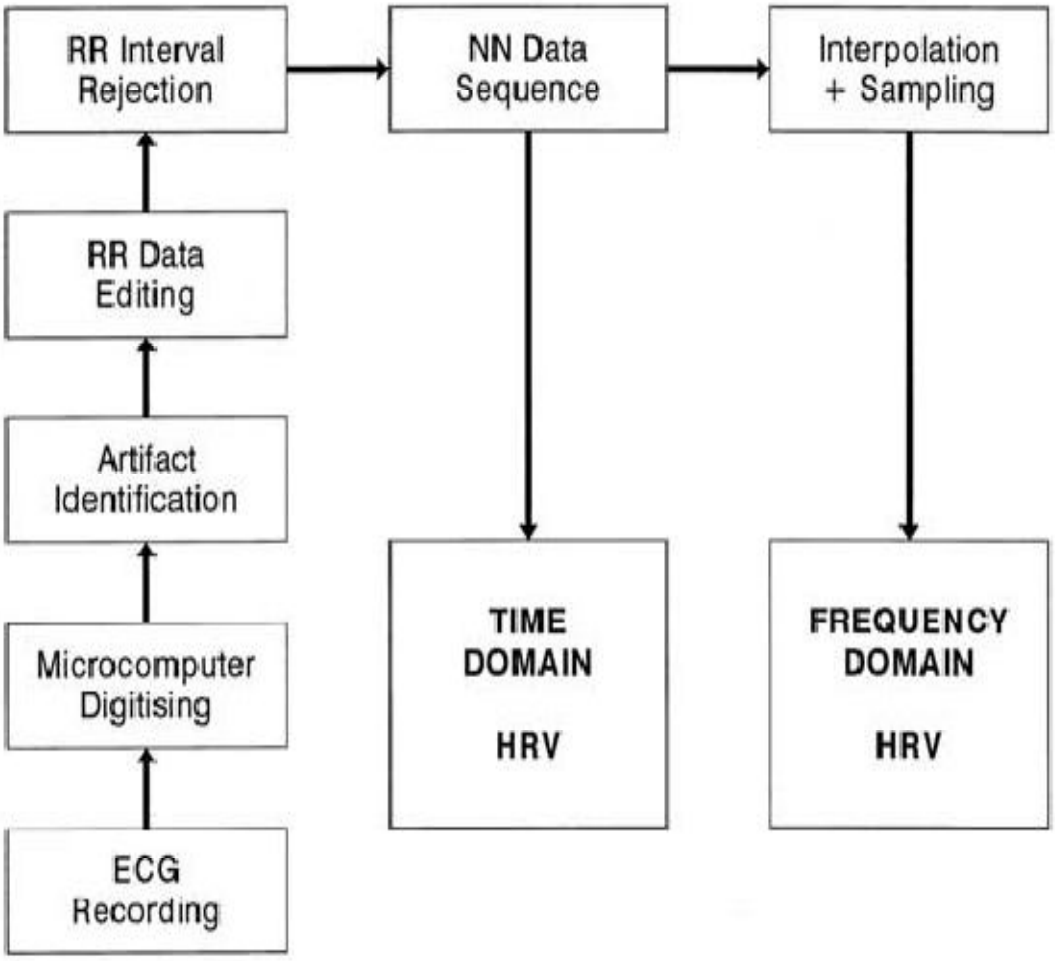
The ratio of low-to-high frequency spectral power (LF/HF) has been used as an index of sympathetic to parasympathetic balance of heart rate fluctuation. This is controversial because of the lack of understanding of the mechanisms for the LF component.

The measurement of VLF, LF and HF power components are measured in absolute values of power (milliseconds squared). LF and HF may also be measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the VLF component.Total power is the ultimate measure in PSD which is an indicator of autonomic modulation as a whole.

Non- linear methods:

1/f scaling of Fourier spectra, fractal scaling exponents in linear methods provides more powerful prognostic information than the traditional HR variability indexes.

Poincare plot where two successive R-R intervals are plotted as points. SD1 and SD2 represent dispersion of points along the line of identity and perpendicular to the line of identity respectively.



Conversion of ECG signal to HRV

Clinical Uses of HRV:

 HRV is the noninvasive simple test for measuring both cardiovascular andnon-cardiovascular autonomic function.

 To find out the early signs of development of pathological processes or the presence of a functional disorder

 To evaluate the treatment effectiveness and prognosis

 HRV is used for exercise training in sports physiology

 To confirm the effect of stress relaxation program (massage, exercise, meditation, light therapy and others)

 HRV analysis is a predictor of risk after MI, arrhythmias.

 HRV analysis is an early warning sign of diabetic autonomic neuropathy

HRV and Rheumatoid arthritis:

Fischer KM [15]in his hypothesis confirmed the etiologic factors smoking related alterations to the cytokine balance, stress to the immune system, and modifications of autoantibodies are strongly associated with development of rheumatoid arthritis. Peripheral neuropathy may be due to vasculitis in rheumatoid arthritis. The autonomic neuropathy may develop due to the same

pathophysiology and the presence of autoantibodies against nervous tissue or amyloidosis .This explanationwas given by Appenzeller 0 et al[16] in his study.

Mayumi Nagata –Sakurai, et al conducted a study in female subjects with stable disease activity of RA and found that the patients with RA have a higher rate of increase in thickening of the arterial wall. Inflammation and calcium mobilization are factors closely associated withthe accelerated arterial wall changes[17]

Geneon et al.[18] found there was a decreased autonomic nervous system function in patients of rheumatoid arthritis with duration of the illness less than 1 year. This decrease was associated with severity of pain and also related to the pathophysiology of RA.The auto antibodies against nerve growth factor, cervical ganglia and vagus nerve were present in rheumatoid arthritis patients who had cardiovascular ANS dysfunction[19]. Maule et al assessed the antibodies by using rabbit tissue as substrate. They performed four standard cardiovascular function tests. Confirmed the autonomic dysfunction in connective tissue disorders and suggested autoantibodies against ANS structures [20].

Stevens RJ et al.[21]studied the similarity of inflammatory processes occurring in RA and cardiovascular disease suggesting RA itself is the risk factor for CVD.Levy at al. Conducted a meta-analysis study to evaluate more accurately the incidence of cardiovascular events in RA. 17 publications and abstracts were

identified and 15 were selected for the study. He concluded RA patients had an excess risk of fatal myocardial infarction compared to general population. The prevention of cardiovascular complication should be taken into account by the rheumatologist.[22]

In the study done by Prior P et aldiscussed that the most common complication of RA was cardiovascular dysfunction and it leads to high mortality. In his study 489 patients with definite or classical RA was followed for a mean of 11.2 years. Cohort analysis showed a 3 fold increase in mortality. [23]

Turesson et al [24] suggested" Development of rheumatoid arthritis is thought to be an inflammatory process from early arthritis through rheumatoid arthritis and possibly to severe extra-articular rheumatoid arthritis" He observed autoantibodies associated with the development of extra articular manifestations.

In 1994 Huikuri HV et al [25]showed the relationship between Heart rate variability and progression of atherosclerosis. No correlation was found between HRV parameters and age factor of rheumatoid patients in the study done by Anichkov et al[26]

Lombardi F et al[27]in their study suggested reduced heart rate variability associated with increase in sympathetic and decreased vagal tone of sinus node. Laden et al.[28]found increased resting heart rate in patients with rheumatoid

arthritis. The increased resting heart rate in rheumatoid arthritis was confirmed by Pihaetal.[29] Theyfound there was no parasympathetic cardiovascular reflex test abnormalities.

The elevated heart rate in RA may be due to decreased parasympathetic tone. This decrease is due to increased central sympathetic activity which inhibit the parasympathetic vasomotor center was suggested by Loutherenoo W et al [30]

Saraswathi et al in their study confirmed the presence of parasympathetic nervous system dysfunction in rheumatoid arthritis patients. Also suggested seropositive or seronegative will not affect the severity of the disease[31]

Spectral analysis of heart rate variability (HRV) in RA patients have revealed a decrease in high frequency (HF) power which indicating vagal inhibition in addition to an increase in low frequency (LF) power indicating sympathetic activation. It was observed by Kamal [32] in his study.

Yadav and colleagues[33]have studied in detail about the HRV indices in RA patients and correlated with immunological and biochemical parameters. They observed a decrease in total power (TP) of HRV in RA patients of Indian population. This indicates poor cardiovascular health of these patients

From the studies carried out among western population, it has been observed that sympathetic nervous system activity is significantly elevated in RA patients.

Spectral analysis of heart rate variability (HRV) in RA patients have revealed a decrease in high frequency (HF) power representing vagal inhibition in addition to an increase in low frequency (LF) power indicating sympathetic activation. It was suggested that the increased incidence of sudden cardiac death in these patients could have been due to the decreased vagal drive to the heart. The magnitude of cardiovascular autonomic imbalance was linked to cardiovascular risks in patients suffering from RA. Reduction in HRV, prolongation in QTc interval and higher sympathetic and decreased vagal drive were proposed as significant risk predictors for onset of sudden cardiac death in RA. The cardiovascular autonomic dysfunction was suggested to stem from the underlyingproinflammatory cytokines in RA.

Del Rincon ID et al[34] in their study did not find any traditional risk factors associated with cardiovascular events in rheumatoid arthritis.Both seropositive and seronegative patients showed autonomic neuropathic changes in the study done by Edmonds et al [35].

80% of all patients with rheumatoid arthritis will be seropositive for rheumatoid factor. But only 40% are showed seropositive at clinical onset of the disease. This is the conclusion attained by Kuriyaet al[36]in their study.

In the study by Richardson C[37] depicted the importance of CRP as a serological marker for evaluating acute disease activity. Wolfe F in his study reported aboutthe association of CRP with factors such as age, smoking, coronary artery disease, increased cholesterol and glucose levels. He concluded positive correlation between them.[38]

Rantapaa-Dahlqvist S et al [39]supportedthe earlier studies finding that elevated levels ofrheumatoid factor can be present many years before the clinical manifestation of arthritis

S Van Doormen et al[40] depicted oxidative stress, hyperinsulinemia, oxidized low density lipoprotein (oxLDL), C-reactive protein (CRP), tumor necrosis factor alpha (TNFα), interleukins -1, -6, -18, RANK ligand, matrix metalloproteinase, and adipocytokines as platelet agonists. They all increased in RA patients.

Farr M et al in their study found out an increased number of platelets and platelet-derived proteins (growth factors) within the synovium and synovial fluid.[41] Andresen GK and his colleagues suggested high platelet counts in synovial fluid and rheumatoid factor (RF) associated with inflammatory arthritis, but not osteoarthritis [42]

Schmitt-Soddy M et al their study in the murine confirmed activated platelets, alone or together with other inflammatory cells and mediators play a significant role in thrombus formation, synovial microcirculation, and destruction of cartilage.[43]

Markers of inflammation such as CRP, ESR and WBC and platelet count have been significantly and positively correlated with cardiovascular disease. This result was obtained by Huang ZS et al in their study.[44]

AIM AND OBJECTIVE OF STUDY

- The aim of the study was
    1. To assess the cardiovascular autonomic nervous system function in the newly diagnosed rheumatoid arthritis subjects.
    2. To evaluate rheumatoid factor, C-reactive protein and platelet count in RA subjects.
- The objective of the study was
    1. To assess the cardiovascular autonomic system by using the resting heart rate variability analysis.
    2. To assess the individual contribution of inflammatory markers rheumatoid factor, C-reactive protein and platelet count to the genesis of sympathovagal imbalance.

MATERIALS AND METHODS

Study Design: Case control study

The study was conducted at Neurophysiology laboratory, Department of Physiology, Govt. Stanley Medical College, Chennai. The Institutional Ethical Committee approval was obtained.

Instrument:

The Heart Rate Variability recording and analysis was done by using RMS polyrite D 2.2 hardware which was connected with a window based PC. Using 2.5.2 software the instantaneous heart rate at RR intervals were continuously plotted. This software is provided with data base, filter settings and calculation stools. It stores records.

Selection of subjects:

Cases:

40 newly diagnosed rheumatoid arthritis patients from the Rheumatology outpatient department, Govt. Stanley Medical College, Hospital, and Chennai were recruited.

Controls:

40 apparently healthy, age and sex matched controls were selected from the hospital and college staffs and also healthy persons accompanying the patients.

Inclusion Criteria:

- Age group of 20 to 60 years including both gender.
- Diagnosed rheumatoid arthritis by using the EULAR classificationcriteria-2010.
- Patients who were newlydiagnosed and not yet started treatment

Exclusion Criteria:

- Smoker and alcoholic.
- Subjects with chronic diseases such as diabetes mellitus, renal failure and other diseases
- Subjects who are taking drugs which are known to affect the autonomic nervous system.
- Subjects with hypertension, Cardiovascular disorders, Endocrine disorders,
- Bronchial asthma and Neurological diseases
- Pregnant subjects.
- Subjects with hemoglobin value less than 10 gm. /dl.

Methodology:

- The study was done between 10 A.M. and 1 P.M in the neurophysiology laboratory.
- The lab was kept calm, the temperature was maintained between 25 to 28^0 C with minimal lighting.
- A 2hour fasting was ensured prior to recording including liquids.
- Subjects were asked to empty the bladder.
- A complete instruction about the study was given to the subject and their doubts cleared.
- After that the informed and written consent were obtained from the subjects. A brief preliminary general and clinical examination of the subjects was made.

The subjects were made to relax and comfortable. 2ml of blood for RF and CRP tests, 1.8ml of blood in EDTA coated test tube for platelet count were collected. Height in centimeters and weight in kilogram were taken.

In all subjects in supine position,the blood pressure, heart rate, respiratory rate were recorded after 10-15 minutes rest of the subjects.

The electrodes were fixed after cleaning with spirit in the following places-

ELECTRODE	**POSITION**
Exploring Electrode	Right forearm
Exploring Electrode	Left forearm
Reference Electrode	Right leg

The continuous lead II Electrocardiography was acquired for 10 minutes. During the recording the subjects were awake with eyes closed. The ECG findings with normal sinus rhythm of 5 minutes were taken for analysis. Careful analysis was done to exclude intervals between ectopic beats and artifacts.

The recording and analysis were done as described by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.

Heart rate variability was assessed by time domain and frequency domain analysis. The mean heart rate, standard deviation of all R-R intervals (SDNN), root-mean square of successive differences (RMSSD), and number of R-R intervals differing by >50 m sec from adjacent intervals (NN50) were measured in

the time domain analysis. Spectral measures were obtained by the fast-Fourier transform method. The power in the heart rate spectrum between 0.003 and 0.40 Hz was defined as total power (ms×ms), and was specified as low frequency [LF, (0.04-0.15 Hz), predominantly marker of sympathetic activity] and high frequency [HF, (0.16-0.4 Hz), marker of parasympathetic activity]. Also the ratio of low-to-high frequency power (LF/HF), reflecting the sympathovagal balance was measured, where a high value of this ratio indicated sympathetic dominance of cardiac autonomic drive.

Measurement of Biomarkers:

Evaluation of serum rheumatoid factor and C - reactive protein were done at immunology lab in Stanley Medical College. The estimation of platelet count was done at clinical pathology lab in Stanley Medical College.

Serum rheumatoid factor and C - reactive protein were estimated by Latex agglutination test. Platelet count estimation was done by using automated cell counter.

CRP usually appears in the sera of patients in the acute stages of a number of inflammatory conditions such as most bacterial and some viral infections; acute rheumatoid fever with or without carditis; rheumatoid arthritis and most other collagen diseases; and other conditions characterized by inflammation. CRP is considered to be a sensitive indicator of inflammation. Changes in the serum

level of CRP with time from the same patient can be used as an index of recovery. The use of the CRP test to measure the effectiveness of therapy is of great clinical significance.

Since the discovery that rabbits form precipitating antibodies against CRP, various immunoprecipitation techniques have been applied for its detection. The PATHOZYME CRP TEST is based on the latex-agglutination method. The principle of this test is based on the immunological reaction between CRP as an antigen and the corresponding antibody coated on the surface of biologically inert latex.

Procedure:

Contents of RF& CRP latex agglutination kit

CRP (or) Rheumatoid Factor latex 50 tests,

- Concentrated glycine buffer,
- Positive control,
- Negative control,
- 50 pipette stirrers and
- Agglutination slide.

By using Qualitative agglutination test method serum CRPand Rheumatoid Factor estimation were done. Serum from the fresh blood sample was

obtained by centrifugation. 50µl of sample was placed and one drop of positive and negative controlswas placed into separate circles on the slide test. The latex reagent was swirled gently before using and added one drop (50µl) to the samples to be added. Mixed the drops with a stirrer and spreaded them over the entire surface of the circle. Weused different stirrers for each sample. After that the slide was placed on a mechanical rotator at 80-100 rpm for 2 minutes and examined macroscopically forthe presence or absence of the agglutination immediately.

The presence of agglutination indicates a CRP concentration equal or greater than 6 mg/l, in case of Rheumatoid factor greater than 14 IU/L.

Alpha granules and dense bodies of platelets, activated by systemic rheumatoid Inflammation, may release their own inflammatory and immune mediators, facilitatinginitiation and propagation of synovitis. Inhibition of platelets with subsequent decrease ofplatelet-derived inflammatory markers may have beneficial effect on the course of arthritis.

The platelet count was obtained by automated cell counter by the principle of comparing the low angle and high angle light scatter created by each particle from the blood collected in the EDTA coated tube. This study did not involve administration of any drugs at any stage.

Statistical analysis:

The acquired data were analyzed by using SPSS version 17. Descriptive statistics mean, standard deviation were used to explain the characteristics of the data.

Student Independent t test:

As the cases and the controls were independent samples we applied independent student t test to find out the significant difference. Here $P<0.05$ taken as significant* and $P<0.01$ was taken as highly significant**

Pearson correlation:

For assessing correlation between two randomvariables Pearson correlation was applied. In our test to find out the linear relationship between biomarkers for rheumatoid arthritis and Heart rate variability parameters Pearson's correlation analysis was utilized

RESULTS

TABLE 2

Baseline characteristics of the studyand the control group

	Group	N	Mean	Std Deviation	Student independent t test
Age in years	Cases	40	41.57	7.82	t =0.298 p =0.767
	Control	40	41.25	7.88	
BMI	Cases	40	24.48	3.19	t =0.831 p = 0.934
	Control	40	24.43	2.79	

Observation

The age and BMI in the study and the control group do not show any statistically significant difference. Hence both the study and the control groups are comparable.

TABLE 3

Comparison of mean HR, SBP and DBP in the study and the control group

	Group	N	Mean	Std deviation	p value
Mean HR(bpm)	Cases	40	79.55	7.78	p =0.000**
	Control	40	70.83	7.52	
SBP (mmHg)	Cases	40	118.3	7.102	p =0.001**
	Control	40	108.3	10.104	
DBP (mmHg)	Cases	40	71.6	6.03	p =0.931
	Control	40	71.7	4.08	

** Highly significant

Observation:

The mean HR was significantly increased in the study group than control. The Systolic Blood Pressure was significantly increased in the study group than control.The Diastolic blood pressure did not show any statistically significant variation.

FIGURE 1

Comparison of heart rate between the study and the control group

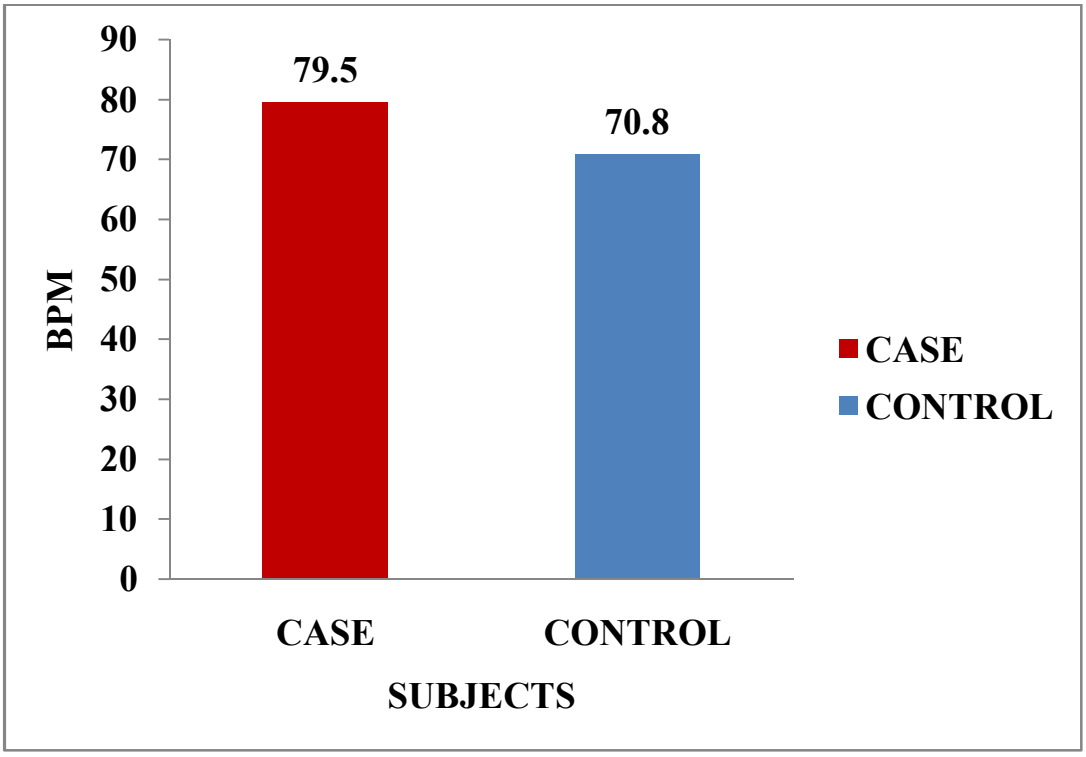


FIGURE 2

Comparison of SBP and DBP between the study and the control group

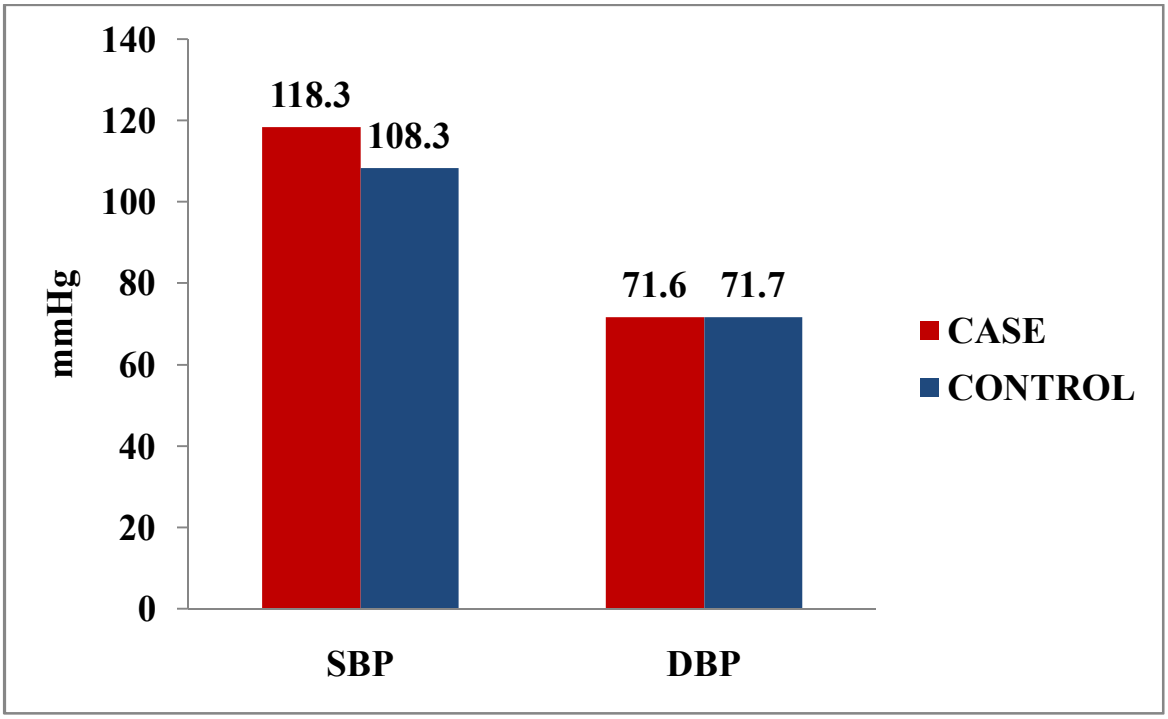


TABLE 4

Comparison oftime domain measures in supine positionbetween the study and the control group

Parameter	study		control		Independent t value	P value
	Mean	**SD**	**Mean**	**SD**		
Mean HR(bpm)	79.55	7.78	70.83	7.52	5.09	0.000**
Mean RR (ms)	731.08	167.8	837.96	89.35	3.55	0.001**
SDNN (ms)	24.97	8.9	51.64	25.35	6.27	0.000**
RMSSD (ms)	22.92	12.77	55.73	33.44	5.79	0.000**
NN 50	246.32	252.4	106.85	76.41	6.04	0.000**

** Highly significant

Observation:

There was a significant reduction of SDNN and RMSSD in the study group compared to controls. There was a significant increase in mean HR in the study group than control.

FIGURE 3

Comparison of time domain measures in supine position between the study and the control group

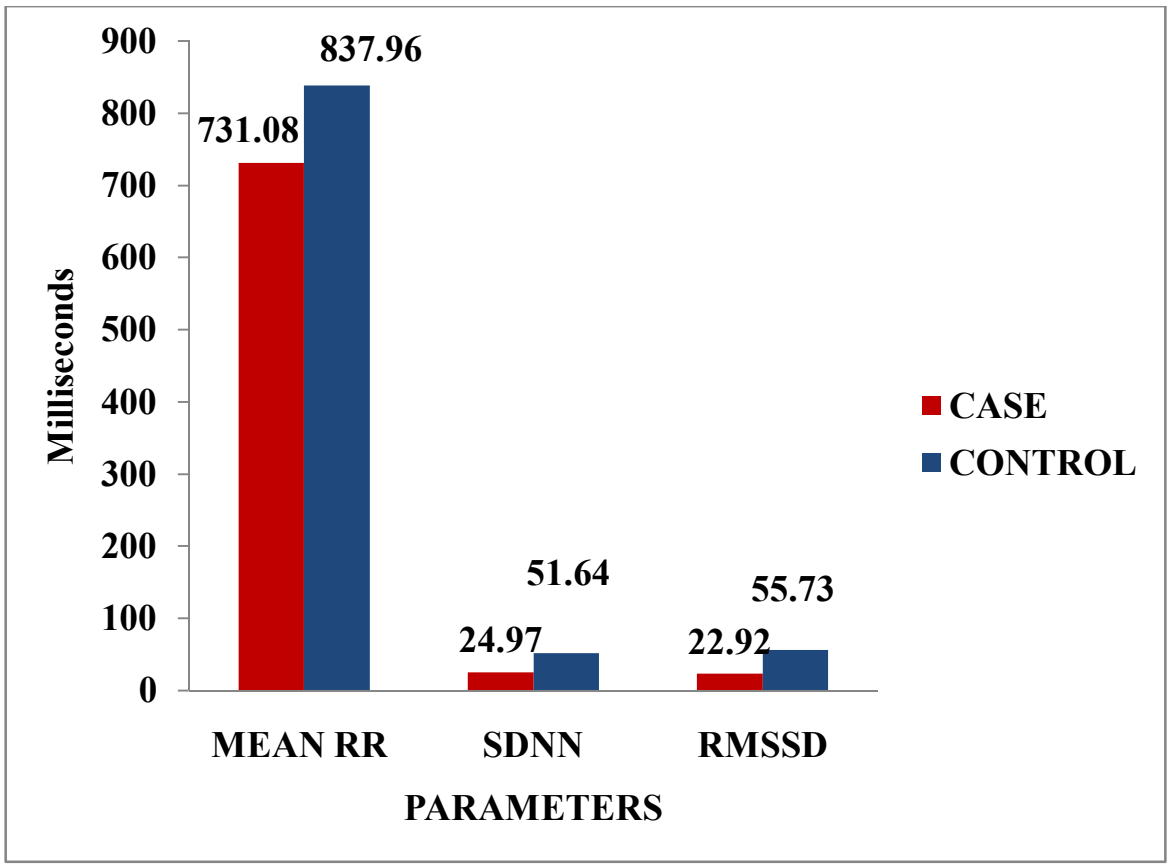


TABLE 5

Comparison of frequency domain measures between the study and the control group

Parameter	study		control		Independent t value	P value
	Mean	SD	Mean	SD		
LF n.u.	64.74	20.87	52.02	14.63	4.98	0.000**
HF n.u.	34.39	19.38	45.94	14.68	5.91	0.000**
LF/HF	2.79	2.33	1.04	0.66	5.83	0.000**

** Highly significant

Observation:

The LF power in n.u. and LF/HF ratio were increased in the study group than control. The HF power in n.u was decreased inthe study group than control.

FIGURE 4

Comparison of frequency domain measures betweenthe study and the control group

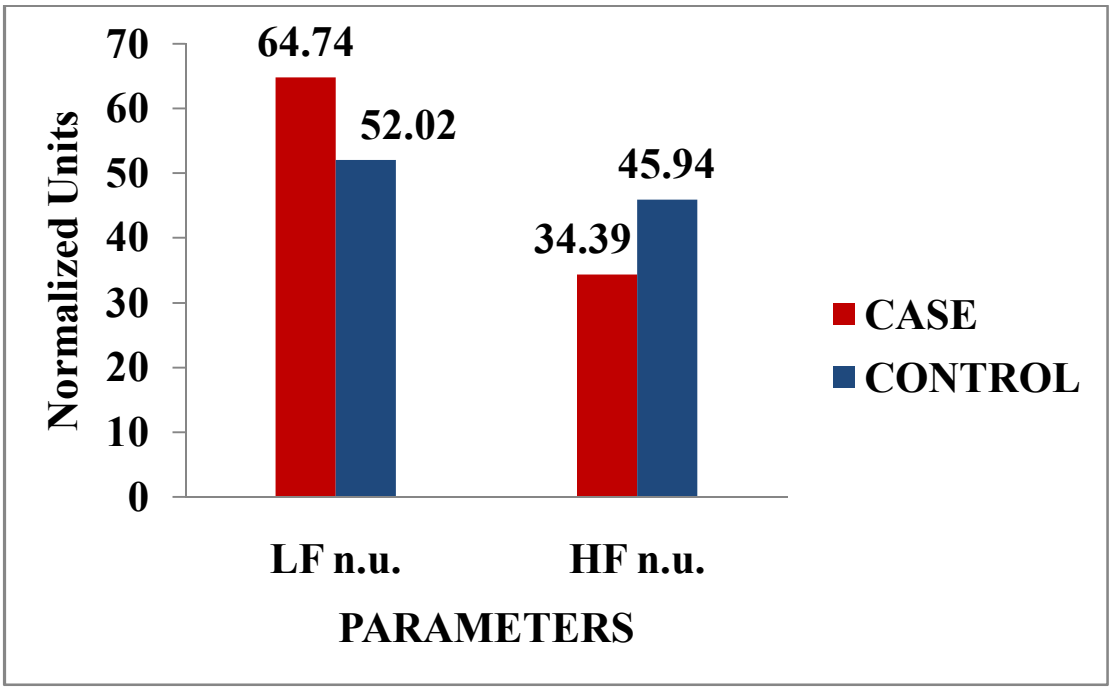


TABLE 6

Comparison of time domain measures in sero positive and sero negative subjects in study the group

	Sero-positive		Sero-negative		t test	P value
	Mean	**SD**	**Mean**	**SD**		
HR(bpm)	79.97	7.8	77.88	7.7	0.676	0.5
RR (ms)	755.34	83.16	634	335.8	1.88	0.06
SDNN (ms)	24.6	8.89	26.44	9.55	0.516	0.6
RMSSD (ms)	20.76	11.26	31.6	15.47	2.25	0.03*

* significant

Observation:

The RR interval, SDNN and RMSSD were decreased in the sero positive subjects than sero negative.

FIGURE 5

Comparison of heart rate in sero-positive and sero-negative subjects in the study group

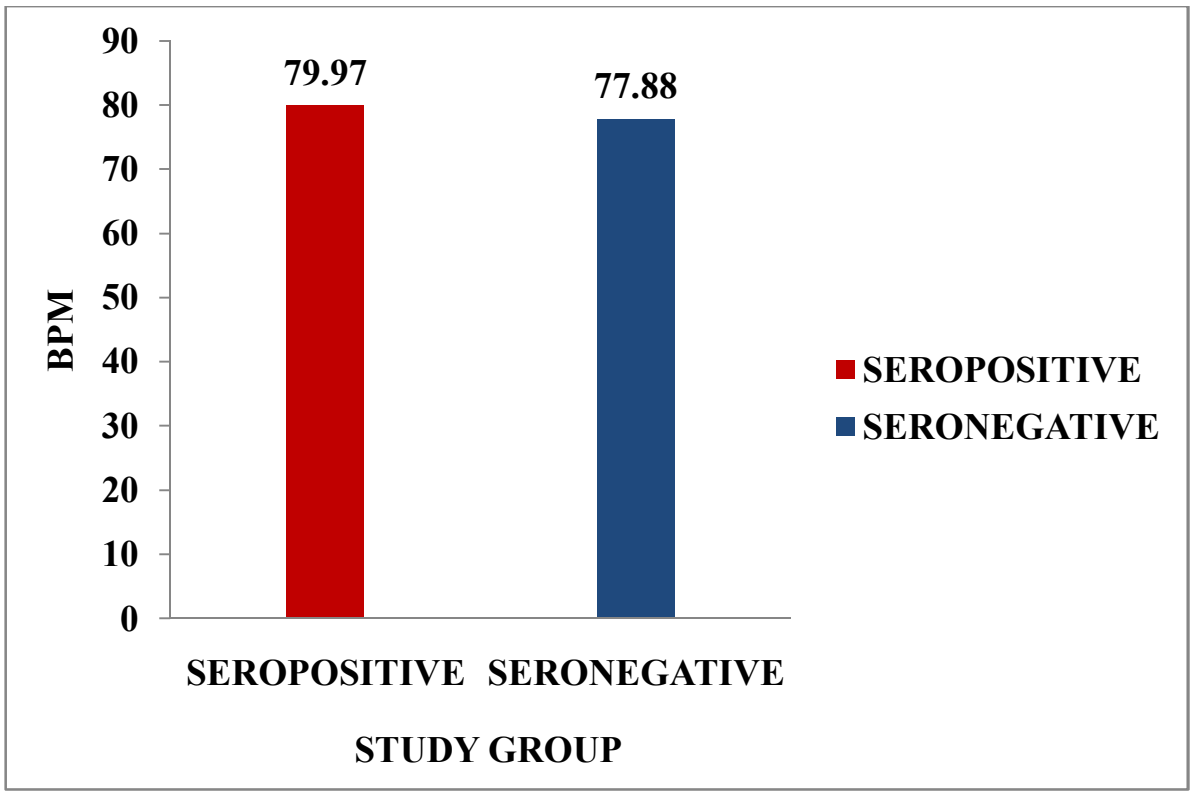


FIGURE 6

Comparison of SDNN AND RMSSD in sero positive and sero negative subjects in the study group

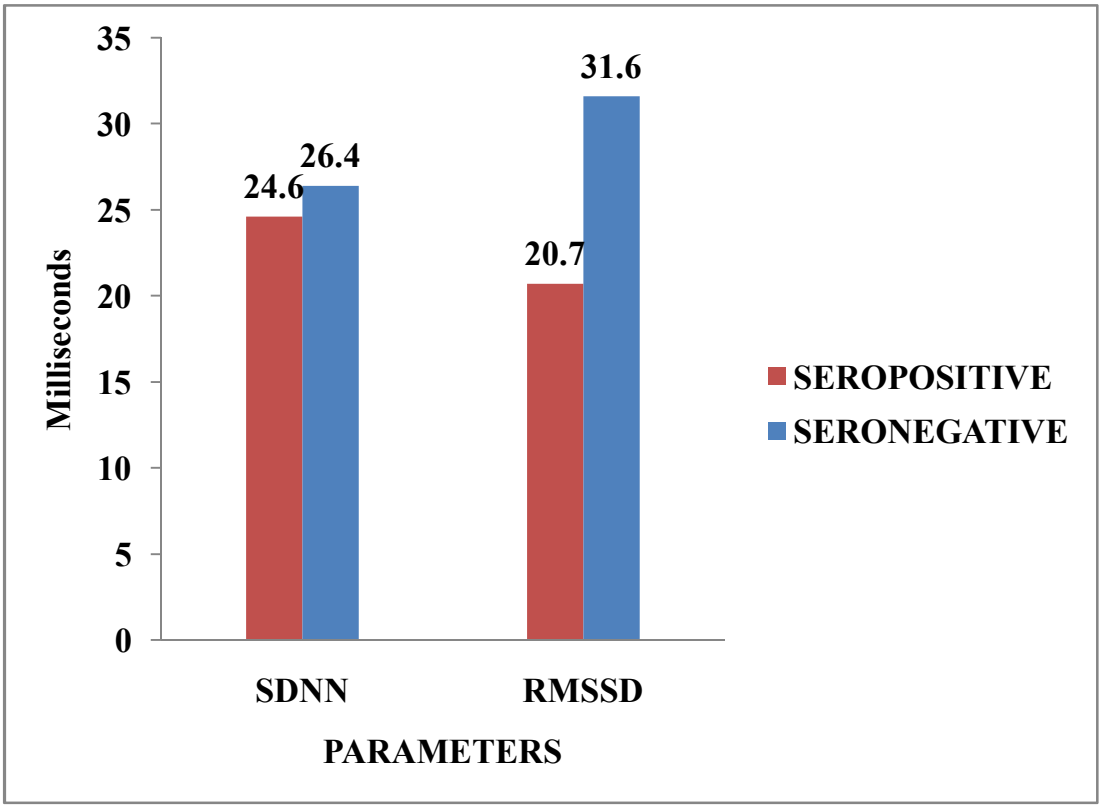


TABLE 7

Comparison of frequency domain measures in sero positive and sero negative subjects in the study group

	Sero-positive		Sero-negative		T test	P value
	Mean	**SD**	**Mean**	**SD**		
LFn.u.	70.43	15.26	52.09	26.5	1.59	0.111
HFn.u.	29.12	14.25	46.08	24.6	1.472	<0.01**
LF/HF	2.81	2.4	2.7	1.7	1.46	0.944

** Highly significant

Observation:

The LF power in n.u.and LF/HF were increased in sero positive when compared withsero negative subjects. The HF power in n.u. was decreased in the sero positive when compared with sero negative subjects.

FIGURE 7

Comparison of frequency domain measures in sero positive and sero negative subjects in the study group

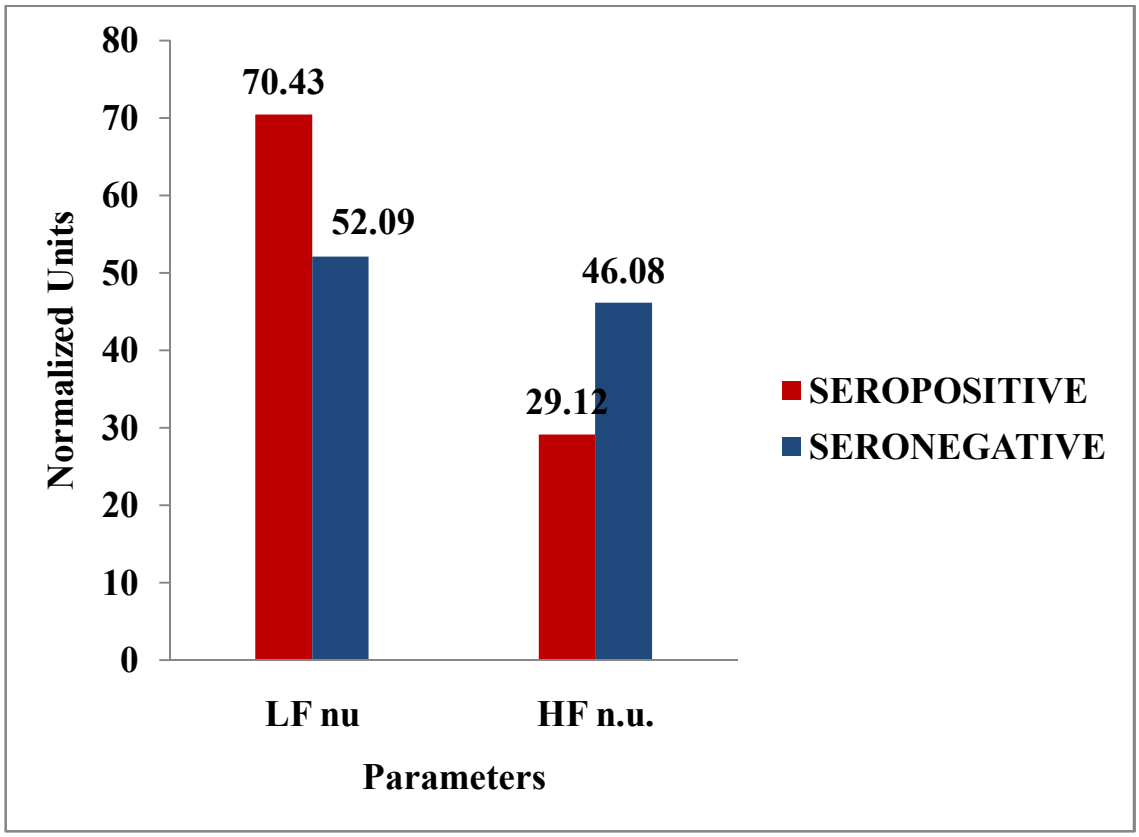


FIGURE 8

Comparison of LF/HF ratio in sero positive and sero negative subjects in the study group

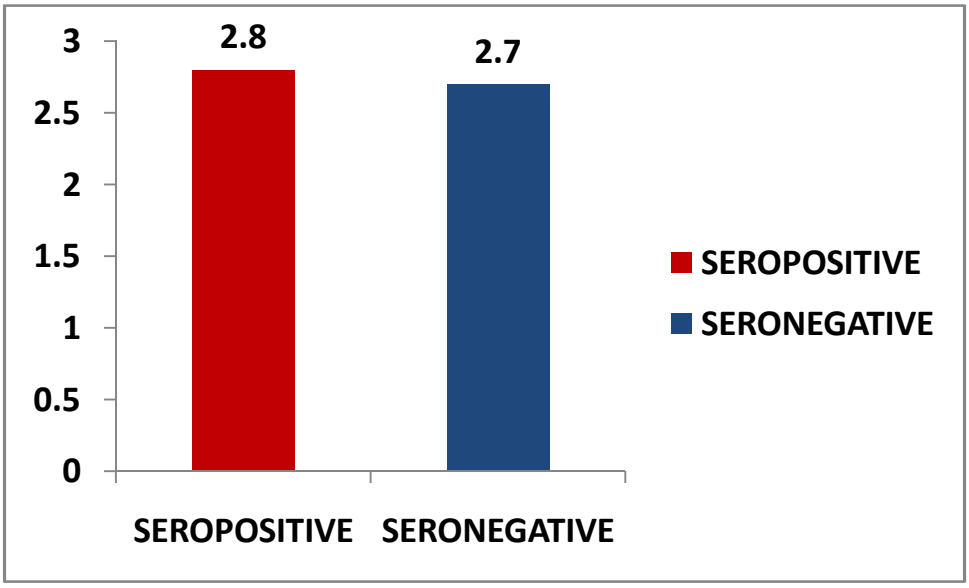


TABLE 8

Comparison of time domain measures in CRP positive and CRP negative subjects in the study group

	CRP-positive		CRP-negative		P value
	Mean	**SD**	**Mean**	**SD**	
HR(bpm)	79.36	6.78	79.86	9.47	0.857
RR (ms)	735.28	154.91	724.06	192.92	0.849
SDNN (ms)	24.62	8.82	25.56	9.39	0.754
RMSSD (ms)	22.28	13.05	24.01	12.68	0.68

Observation:

No significant difference was found in time domain measures between CRP positive and CRP negative subjects

FIGURE 9

Comparison of time domain measures in CRP positive and CRP negative subjects in the study group

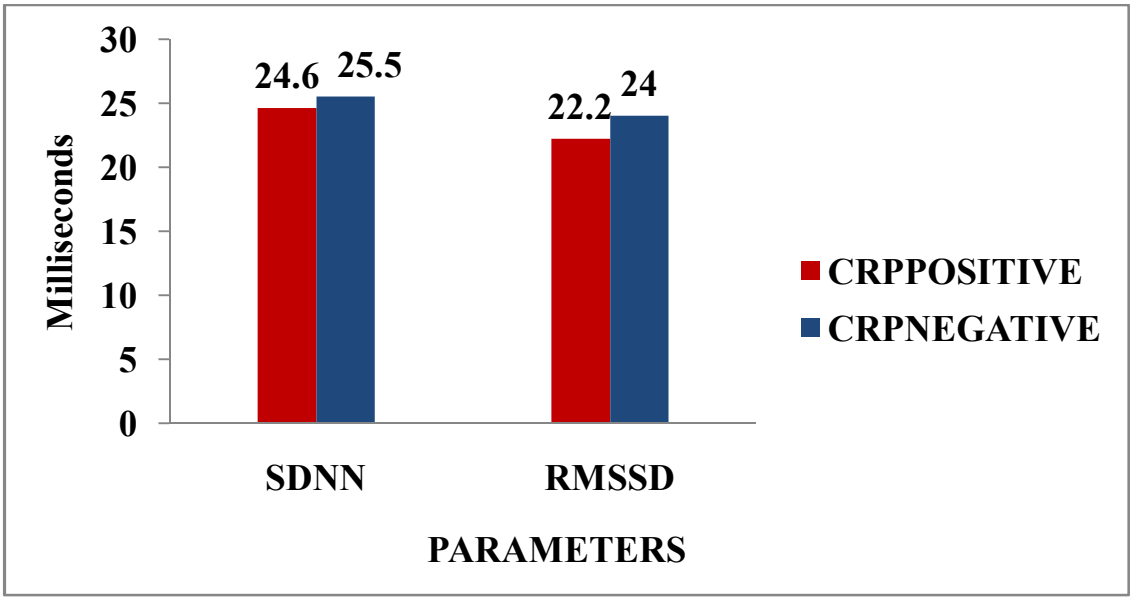


TABLE 9

Comparison of frequency domain measures in CRP positive and CRP negative subjects in the study group

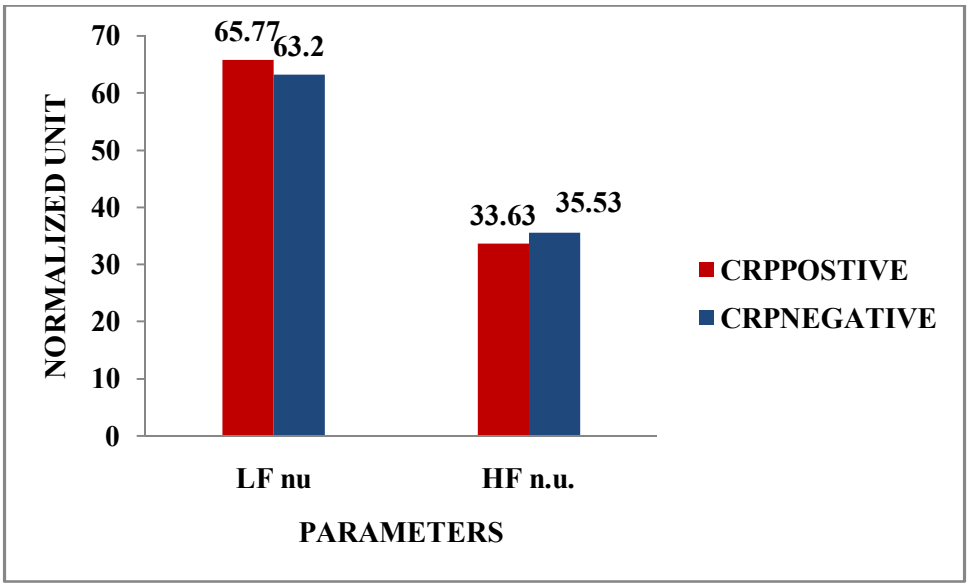
	CRP-positive		CRP-negative		P value
	Mean	**SD**	**Mean**	**SD**	
LF nu	65.77	20.61	63.20	21.8	0.628
HF nu	33.63	19.16	35.53	20.28	0.37
LF/HF	2.76	2.4	2.76	2.28	0.93

Observation

There was increased LF power in normalized units and LF/HF ratio in the CRP positive subjects. There was decreased HF power in normalized unit found inthe CRP positive subjects. However those were not statistically significant.

FIGURE 10

Comparison of frequency domain measures in CRP positive and CRP negative subjects in thestudy group



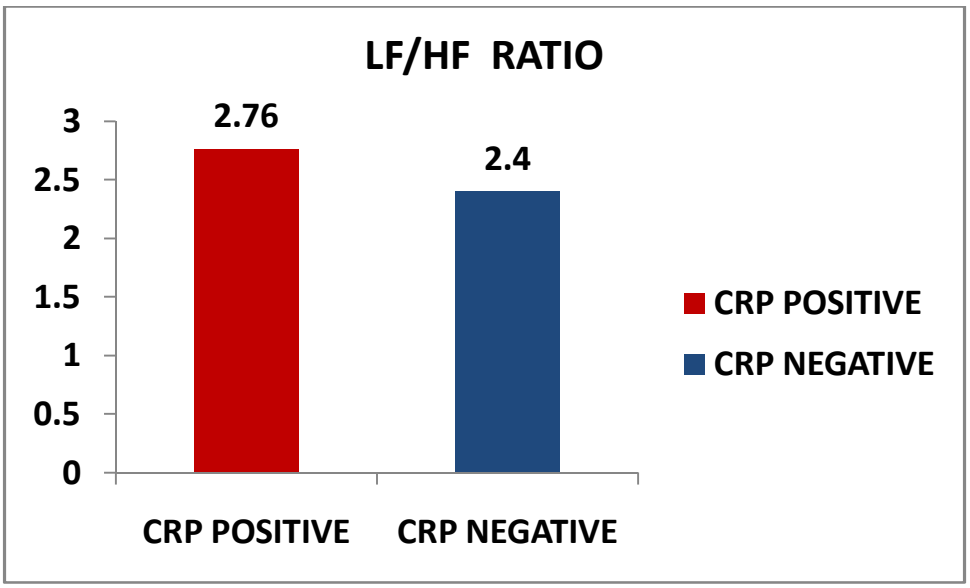


FIGURE 11

Comparison of platelet count between the study and the control group

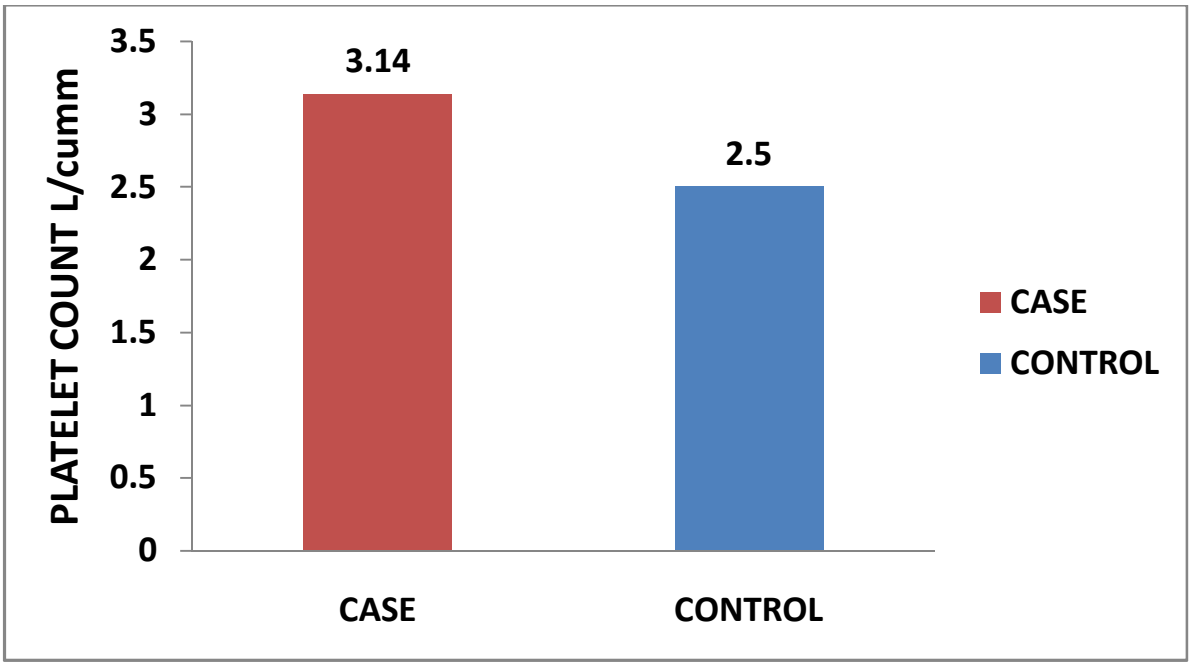


TABLE 10

Correlation between mean RR and platelet count in the study group

	Correlation coefficient and p value	**Interpretation**
Correlation between mean RR and platelet count	r = 0.093 P = 0.341	Positive correlation Not significant

TABLE 11

Correlation between SDNN and platelet count in the study group

	Correlation coefficient and p value	**Interpretation**
Correlation between SDNN and platelet count	r = -0.138 P = 0.000**	Negative correlation Highly significant

TABLE 12

Correlation between RMSSD and platelet count in the study group

	Correlation coefficient and p value	**Interpretation**
Correlation between RMSSD and platelet count	r = -0.218 P = 0.000**	Negative correlation Highly significant

TABLE 13

Correlation between LF nu and platelet count in the study group

	Correlation coefficient and p value	**Interpretation**
Correlation between LF nu and platelet count	r = 0.313 P = 0.000**	positive correlation Highly significant

TABLE 14

Correlation between HF nu and platelet count in the study group

	Correlation coefficient and p value	**Interpretation**
Correlation between HF nu and platelet count	r = - 0.194 P = 0.000**	Negative correlation Highly significant

TABLE 15

Correlation between LF/ HF Ratio and platelet count in the study group

	Correlation coefficient and p value	**Interpretation**
Correlation between LF/HF ratio and platelet count	r = 0.168 P = 0.000**	Positive correlation Highly significant

RESULTS

Statistical Package for Social Sciences (SPSS) 17 version was used for statistical analysis. An Independent Student t test, Chi- square test andPearson's coefficient were applied for analysis.

Table- 2 shows no significant difference in age and BMI between the study and the control group.

Table-3 shows significant increase in HR in the study group than the control. The Systolic Blood Pressure was significantly increased in the study group than the control.The Diastolic blood pressure did not show any statistically significant variation.

Table-4 There was a significant reduction of SDNN and RMSSD in the study group compared to controls. There was a significant increase in mean HR in the study group than control.

Table-5 shows the LF power in n.u. and LF/HF ratio were increased in the study group than the control. The HF power in n.u was decreased in the study group than the control.

Table-6 shows the R-R interval, SDNN and RMSSD were decreased in the sero positive subjects than thesero negative.

Table-7 showsthe LF power in n.u. and LF/HF were increased in sero positive when compared with sero negative subjects. The HF power in n.u. was decreased in the sero positive when compared with sero negative subjects.

Table-8 shows nosignificant difference in time domain measures between CRP positive and CRP negative subjects.

Table-9 shows increased LF power in normalized units and LF/HF ratio in the CRP positive subjects. There was decreased HF power in normalized unit found in the CRP positive subjects. However those were not statistically significant.

Table- 10 shows weekly positivecorrelation between mean RR and platelet count in the study group.

Table-11 shows significant negative correlation between SDNN and platelet count in the study group.

Table-12 showssignificant negative correlation between RMSSD and platelet count in the study group.

Table-13 shows significant positive correlation between LF nu and platelet count in the study group.

Table-14 shows significant negative correlation between HF nu and platelet count in the study group.

Table-15 shows significant positive correlation between LF/HF ratio and platelet count in the study group.

DISCUSSION

The most common extra articular complication of RA is cardiovascular involvement. The pathogenesis is mediated by the involvement of inflammatory mediators and auto antibodies. An early diagnosis and management of the cardiovascular complication in RA improves prognosis and the quality of life. HRV is a noninvasive technique to assess the autonomic nervous system function in both normal and diseased subjects [12]

The resting short term heart rate variability analysis in supine position showed the following results.

Baseline parameters

The mean age of the study group was 41.01±7.94. The mean age of control was 41.58±7.8. The mean BMI of study group was 24.48±3.19. The mean BMI of control was 24.43±2.79. Both parameters were statistically comparable. Thus the influence of age, BMI and gender on the cardiovascular autonomic system was alleviated by selecting age, BMI and gender matched subjects for the study.

The resting heart rate in the study group was 79.55±7.7. The control group was 70.83±7.5. The resting heart rate was found tobe significantly increased (p value <0.001**) in the study group than the control. The mean systolic blood pressure of study group was 118.3±7.06.The mean SBP of control group was

108.35±10.1. The SBP was significantly increased (p value 0.001**) in the study group than control. These findings indicate the presence of sympathetic over activity in the RA patients. These findings were in accordance with the findings of Laden et al[45]Piha et al[29] studies. The reason for increased heart rate can be due to the parasympathetic efferent vagal damage which was suggested by Ewing et al[12] in his study. HRV is a valuable, noninvasive tool to assess the autonomic neural function.

Any alteration in resting HRV is associated with increased risk of adverse cardiac events. Analysis of the resting heart rate variability of a 5 minutes recording done using the HRV analysis software version2.2 among both the study and the control groups showed the following results.

Frequency domain parameters

In HRV, the frequency domain parameter LF power in normalized unit was increased in the study group(64.74±20.87) than the control(52.02±14.64).This was statistically significant($p<0.01^{**}$) This indicates the sympathetic over activity in the RA patients. The HF power in normalized unit was significantly lower in the study group (34.39±19.38) than the healthy controls (45.94±14.68). This was statistically significant ($p<0.01^{**}$). This indicates the parasympathetic withdrawal in RA patients. The total power also reduced in the study group when compared to control but it was not statistically significant. This denotes the depressive state

of the cardiovascular autonomic nervous system in the RA patients. LF/HF ratio was increased in the study group (2.79±2.33) than the controls (1.04±0.66) and it was statistically significant ($p<0.01$**). This indicates the sympathovagal imbalance in the RA patients. The overall frequency domain parameters in our study indicate the state of depressed vagal tone on the heart and the altered sympathovagal balance in RA patients.

Our study findings were in accordance with the findings of Everngul et al [47] study. He observed a significant decrease in HF and increase in LF and LF/HF ratio, signifying predominance of the sympathetic activity of the heart. Conversely in the study done by Yadavet al.[33] in Indian population, he observed a decrease in both $LFms^2$ and HFms2 and concluded reduced HRV in RA patient.

Sandhuetal[46] has suggested that increased heart rate may be due to increased sympatheticactivity and decreased parasympathetic vagal tone. This decreased parasympathetic tone may lead to increased heart rate.

In our study 80 % of the study group was positive for RF and 20% were sero negative. This may be due to the selection of the study group from Rheumatology outpatient department. Kuriya B (36),Boultry N,[48] and Machold KP[49] concluded in their studies 80% of all patients with rheumatoid arthritis will

eventually be seropositive for rheumatoid factor. But only 40% are positive at clinical onset of the disease.

Sune F Nielsen et al did a prospective cohort study, for 20 years since 1981 to 2010 august.9712 individuals from the general population were included. In that study the authors came to a conclusion that individuals in the general population with elevated rheumatoid factor have up to 26-fold greater long term risk of rheumatoid arthritis.

In the frequency domain measures LF power in normalized unit was increased in the seropositive patients (70.43±15.26) than seronegative patients (52.09±26.5). LF/HF ratio increased in seropositive (2.81±2.4) when compared with seronegative patients (2.7±1.7). These indicate the sympathetic over activity in the seropositive patients. HF power in normalized unit was found decreased in the seropositive subjects (29.19±14.25) than seronegative subjects (46.08±24.6). It was statistically significant ($p<0.01^{**}$).It indicates the parasympathetic withdrawal status in the seropositive patients.

Among the study group 62.3% showed CRP positive in their sera. None of the healthy controls showed positivity for both rheumatoid factor and C-reactive protein. In the frequency domain measures LF power in normalized unit was increased in the CRP positive patients (65.77±20.61) than CRP negative patients (63.20±21.8). These indicate the sympathetic predominance in the CRP positive

patients. HF power in normalized unit was found decreased in the CRP positive subjects (33.63±19.16) than CRP negative subjects (35.53±20.28). It indicates the parasympathetic withdrawal status in the CRP positive patients. It was not statistically significant (p =0.765).This may be due to small sample size.

There was a statistically significant increase in platelet count in the study group (3.14±0.5) when compared with the controls (2.5±0.2). It was statistically significant (P $\leq$0.000**). A significant positive correlation was found between platelet count and LF nu (r =0.313, p< 0.01**) LF/HF ratio(r = 0.168,p <0.01**). These results indicate an association of increased platelet count with impaired sympathovagal balance.

Time domain parameters

In our study the resting heart rate was significantly increased in the study group (79.55±7.78) than control group (70.83±7.52).It was statistically significant (p<0.01**). It indicates the increased sympathetic activity in the RA patients. The SDNN in the study group (24.97±8.9) was found to be decreased than control (51.64±25.35). It was statistically significant (p<0.01**). The RMSSD was found reduced in the rheumatoid arthritis patients (22.92±12.77) than the healthy controls (55.73±33.44).It was statistically significant (p<0.01**). It indicates the parasympathetic withdrawal in RA patients. Similar findings were observed in Evrengal H et al[46], Maule S et al [20]Louthrenoo W[30] and Yadav[33] studies.

In case of RF the RR interval was reduced in the seropositive subjects (755.35±83.16)and seronegative subjects (634±335).The SDNN was reduced in the seropositive subjects (24.6±8.89) than theseronegative subjects (26.44±9.55). The RMSSD was decreased in the seropositive patients (20.76±11.26) when compared with sero negative (31.6±15.47). It was statistically significant ($p<0.03^{*}$). These results indicate the parasympathetic withdrawal status in the CRP positive patients. The findings were in accordance with the findings of previous studies done by Sandhu V et al[47], and Castro EM et al[50].

The SDNN was reduced in the CRP positive subjects (24.6±8.89) than CRP negative subjects (25.54±9.55). The RMSSD was decreased in the CRP positive patients (22.26±11.26) when compared with CRP negative (24.01±15.47). The RR interval was variable in the CRP positive subjects (735.35±83.16) when compared with the CRP negative subjects .

These results reveal that C-reactive protein is associated with cardiovascular autonomic imbalance in the RA patients. CRP is an important inflammatory marker in RA patients and might be involved in the pathogenesis of cardiovascular complication. Similar result was observed in the study of R.T.Keenanet al.[51]

A significant negative correlation was found between platelet count and SDNN ($r = -0.138$, $p < 0.01^{**}$) and RMSSD ($r = -0.21$, $p < 0.01^{**}$) of time domain measures. Platelets are involved in the inflammation, atherosclerosis and thrombosis in RA patients. Cardiovascular and RA-associated factors can increase circulating platelet count and alter the structure and function of platelets, by reactive megakaryocytopoiesis. Hyperactive platelets target synovial membranes with subsequent local rheumatoid inflammation. Hyperactive platelets interact with other cells, and target the vascular wall and lead to cardiovascular risk in RA subjects.

Schmitt-sody M[43] conducted his study in murine. In that study he concluded that P-selectin, an adhesion molecule produced by platelets and ECs, play a vital role for the interaction of platelets, leukocytes, and ECs in the inflamed joints.

The results of our study indicate the presence of biomarkers RF, CRP and increased platelet count had influence on a reduced HRV and sympathovagal imbalance in the form of parasympathetic withdrawal and sympathetic over activity in the rheumatoid arthritis patients.

We assessed the association between biochemical markers RF, C-reactive protein and platelet count with autonomic balance in cardiovascular system. We did not correlate the clinical parameters like DAS28 (Disease Activity Score 28), fatigue, joint involvement, joint tenderness with HRV parameters.

In future, increasing number the number of patient recruitment and continuation of the study may provide more insight into the significance of biochemical markers like RF, CRP & Platelet count etc.

CONCLUSION

The sympathetic activity was increased in the rheumatoid arthritis patients as measured by the increased resting heart rate and increased Systolic Blood Pressure. The resting HRV in RA subjects indicates that there is a definite sympathovagal imbalance in patients with RA in the form of sympathetic over-activity and parasympathetic withdrawal. The significant decrease in RR interval, SDNN and RMSSD parameters indicate parasympathetic withdrawal in the study group. Increased LF power and decreased HF power in study group indicate the increased sympathetic activity with parasympathetic withdrawal. Increased LF/HF ratio also denotes impaired sympathovagal balance with predominant sympathetic activity.

The serum RF and CRP positive RA patients had reduced HRV when compared with that of sero-negative RA patients. Elevated platelet count observed in RA patients, showed a positive correlation with autonomic imbalance.

These results showed that the positive RF, positive CRP and the increased platelet count may play a significant role in the pathogenesis of cardiovascular complication in RA patients.

Hence, periodical evaluation of these biomarkersand assessment of cardiovascular status by HRV analysis may helpin theearly diagnosis of cardiovascular complicationin RA patients.

SUMMARY

The aim of the study was to assess the cardiovascular autonomic functional status in the newly diagnosed rheumatoid arthritis (RA) individuals by using resting Heart Rate Variabiliy (HRV) analysis. Along with this we assessed the association of rheumatoid factor (RF), C - reactive protein (CRP) and platelet count with cardiovascular autonomic function.

The age and gender matched 40 study subjects and 40 healthy controls were recruited. The short term Heart Rate Variability in supine position was taken. Serum RF, CRP, and platelet count were evaluated.

We observed reduced HRV in RA patients, which denotes altered cardiovascular autonomic function. We also observed that the positiveRF, CRP andincreased platelet count were associated with sympathovagal imbalance. This signifies the association of these factors in the pathogenesis of cardiovascular risk in RA patients.

As the resting HRV analysis is a simple, noninvasive tool, it can be included in the routine basic investigation of RA subjects. The periodic assessment of RF, CRP and platelet count may help in the early diagnosis of cardiovascular complication in RA patients.

BIBLIOGRAPHY

1. **Mutru O, LaaksoM,IsomakiH,Koota K**. Ten year mortality and causes of death in patients with rheumatoid arthritis.Br Med J 1985;290:1797-9.

2. **Harrison2nd volume**, part 15 ,chaptor 321 page no 2738-52.

3. **Stein PK, Bosner MS, Kleiger RF, Conger BM**. Heart rate 10. variability: a measure of cardiac autonomic tone. *Am Heart J* 1994; *127* : 420-4.

4. **Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, 8. Pizzinelli P, *et al***. Power spectral analysis of heart rate and arterial pressure variability as a marker of sympato-vagal interaction in man and conscious dog. *Circ Res* 1986; *59* : 178-93.

5. **MYASOEDOVA E et al**: Epidemiology of rheumatoid arthritis; Rheumatoid arthritis and mortality. CorrRheumatol Rep2010; 12:379.

6. **MICHOUL L et al**: Associations between genetic factors, tabacco smoking and auto antibodies in familial and sporadic rheumatoid arthritis.Ann Rheum Dis 2008;67:466.

7. **HOLERS VM**:Antibodies to citrullinatedproteins:Pathogeneic and diagnostic significance. CurrRheumatol Rep2007; 9:396.

8. **GOLDRING SR,GOLDRING MB**: Eating bone or adding it: The Wnt pathway decides.

9. **NEOGI T et al**:2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology / European League Against Rheumatism Collaborative initiative. Arthritis Rheum2010; 62:2569.

10. **Lerkevang Grove E,Hvas AM, DalbyKristensen S**. Immature platelets in patients with acute coronary syndrome.ThrombHaemost 2009;101:151-6.

11. **Ganong WF**.Review of Medical Physiology.23rd edition. Graw Hill 2005.

12. **Ewing DJ, Martyn CN, CN, oung RJ and Clarke BF**. The value of cardiovascular autonomic function tests:10 years' experience in diabetes. Diabetes care. 1985;8:491-8.

13. **ES Prakash et al**.2006.Autonomic functions in borderline and established hypertension.

14. **MallianiA, Pagani M, Lombardi and Cerullis**.1991.Cardiovascular neural regulation explored in the frequency domain. Circulation 84,482-492.

15. **Fischer KM,Hypothesis**: tobacco use is a risk factor in rheumatoid arthritis. Med Hypothesis 1991;34:116-7.

16. **Appenzellaro,Arnason BG, Adams RD**. Experimental autonomic neuropathy: an immunological induced disorder of reflex vasomotor function. J NeuroNeurosurg psychiatry 1965;28:510-5.

17. **Mayumi Nagata –Sakurai, Masaaki Inaba, Hitoshi Goto, YasuroKumeda, Yutaka Furumitsu and YoshikiNishizawa et al**. Inflammation and Bone resorption as Independent Factors of Accelerated arterial wall thickening in patients with Rheumatoid arthritis. ARTHRITIS & RHEUMATISM. Vol.48,No 11,November 2003;pp3061-3067.

18. **Geenen R, Godart GLR, Jacobs JWG, Peters ML, Mijlsma JWJ**. Diminished autonomic nervous system responsiveness in Rheumatoid Arthritis of recent onset. *J Rheumatol*1996; 23: 258.

19. **Dicoue, HurezD,Nerriere V**.Natural autoantibodies against the nerve growth factor in acute immune dieases. Neuroimmunol 1993;47:159-68.

20. **Maule S, Quadri R, Mirante D, et al**. Autonomic nervous dysfunction in systemic lupus erythematosus (SLE) and Rheumatoid Arthritis possible pathogenic role of autoantibodies to autonomic nervous structures. *ClinExpImmunol*1997; 110: 423–427

21. **Stevens RJ ,Douglas KM, saratzis AN, Kitas GD**: Inflammation an atherosclerosis in rheumatoid arthritis .Expert Rev Mol Med 2005; 7:1-24.

22. **Levy L, Fautral B, BarnetcheT,Schaeverbeke T**:Incidence and risk of fatal myocardial infarction and stroke events in rheumatoid arthritis

patients. A systemic review of the literature. ClinExpRheumatol 2008; 26; 673-9.

23. **Prior P, Symmons DP, Scott DL, Brown R, Hawkins CF**. 4. Cause of death in rheumatoid arthritis. *Br J Rheumatol*1984; *23* : 92-9.

24. **Turesson C, Jacobsson LTH, Sturfelt G,MattesonEL, Mathsson L, Ronnelid**. Rheumatoid factor and antibodies to cyclic citrullinated peptides are associated with severe extra- articular menifestations in rheumatoid arthritis. Ann Rheum Dis 2007; 66:59-64.

25. **Huikuri HV, Jokinen V, Syvanne M, Nieminen MS, Airaksinen12. KE, Ikaheimo MJ, *et al***. Heart rate variability and progression of coronary atherosclerosis. *ArteriosclerThrombVascBiol*1999; *19* : 1979-85.

26. **Anichkov DA, Shostak NA, Ivanov DS**. Heart rate variability 27. is related to disease activity and smoking in rheumatoid arthritis patients. *Int J ClinPract*2007; *61* : 777-83.

27. **Lombardi F, Malliani A, Pagani M, Cerutti S**. Heart rate 19. variability and its sympatho-vagal modulation. *Cardiovasc Res* 1996; *32* : 208-16.

28. **Leden I, Erikson A, Lilja B, Sturfelt G, SundkvistG**. Autonomic Nervous function in Rheumatoid arthritis of varying severity. *Scand J Rhematol*1983; 12(2): 166–170

29. **Piha SJ, VoipioPulkki LM**. Elevated Resting heart rate in Rheumatoid Arthritis possible role of physical deconditioning. *Br J Rheumatol*1993; 32: 212–215.

30. **Loutherenoo W, Ruttanaumpawan P, Aramrattana A and Sukitawut W**. Cardiovascular autonomic nervous system dysfunction in patients with Rheumatoid Arthritis and systemic lupus erythematosus. *QJ Med* 1999; 92: 97–102.

31. **Saraswathi P.V.,Neelambigai N.,Arjun Mahesh and Govindarajan K**. Cardiovascular parasympathetic nervous system dysfunction in female Rheumatoid arthritis patients. India n J physiolpharmacol 2013; 57(1):23-30.

32. **Kamal A**. Assessment of autonomic function in patients with rheumatoid arthritis using spectral analysis and approximate entropy method. *Neurosciences (Riyadh)* 2007; *12* : 136-9.

33. **Yadav RK, Gupta R, Deepak KK**. A pilot study on short term 13. heart rate variability & its correlation with disease activity in Indian patients with rheumatoid arthritis. *Indian J Med Res* 2012; *136* : 593-8

34. **Del Rincon ID, Williams K, Stern MP, Freeman GL, Escalante17.**A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum* 2001; *44* : 2737-45.

35. **Edmonds ME, Jones TC, Saunders WA, Sturrock RD**. Autonomic neuropathy in Rheumatoid Arthritis. *BMJ* 1979; 2: 173–175

36. **Kuriy b,Cheng CK, Chen HM, Bykert VP**,Validation of a prediction rule for development of rheumatoid arthritis in patients with early undifferentiated arthritis. ANN rheum Dis 2009;68:1482.

37. **RICHARDSON C, EMERY**;Laboratory markers of diease activity. J Rheumatol 1996;23(suppl.44):23-30.

38. **WOLFE F**: The C-reactive protein but not erythrocyte sedimentation rate is associated with clinical severity in patients with osteoarthritis of the knee or hip. J Rheumatol1997 ;24:1486-8.

39. **Rantappa –Dalquist S,de Jong BAW, Berglin E, Hallmans G, Wallell G,Stenlund H, et al**. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48:2741-9.

40. **Van Doornum S, McColl G, Wicks IP**. Accelerated atherosclerosis: an extraarticularfeature of rheumatoid arthritis? Arthritis Rheum 2002;46:862-73.

41. **Farr M, Wainwright A, Salmon M, Hollywell CA, Bacon PA**. Platelets in the synovialfluid of patients with rheumatoid arthritis. RheumatolInt 1984;4:13-7.

42. **Endresen GK**. Investigation of blood platelets in synovial fluid from patients withrheumatoid arthritis. Scand J Rheumatol 1981;10:204-8.

43. **Schmitt-Sody M, Metz P, Gottschalk O, Birkenmaier C, Zysk S, Veihelmann A, JanssonV**. Platelet P-selectin is significantly involved in leukocyte-endothelial cell interaction inmurine antigen-induced arthritis. Platelets 2007;18:365-72.

44. **Huang ZS, JengjS, Wang CH,YipPK, WuTH, Lee TK**. Correlations between peripheral differential leukocyte counts and carotid atherosclerosis in non-smokers.Atherosclerosis 2001:1258:431-6.

45. **Leden I, Erikson A, Lilja B, Sturfelt G, Sundkvist G**. Autonomic Nervous function in Rheumatoid arthritis of varying severity. *Scand J Rhematol*1983; 12(2): 166–170

46. **Evrengul H, Dursunoglu D, Cobankara V, Polat B, Seleci13. D, Kabukcu S, *et al***. Heart rate variability in patients with rheumatoid arthritis. *RheumatolInt*2004; *24* : 198-202.

47. **Sandhu V, Allen SC**. The effects of age, seropositivity and disease duration on autonomic cardiovascular reflexes in patients with Rheumatoid Arthritis. *Int J ClinPract*2004; 58(8): 740–745

48. **Boultry N,do carmpoccm, Flipo RM, Cotton A**. Early rheumatoid arthritis and its differentiation from other joint abnormalities.Eur J Radial 2009; 71:217-24.

49. **MacholdKP,Stamm TA, Ebert GJM, Nell VKP, DunkyA,Uffmann M et al**.Very recent onset arthritis –clinical,laboratory and radiological findings during the first year of diease.JRheumatol 2002;29:2278-87.

50. **Castro EM, Marques AO, Llorach MB, et al**. Rheumatoid arthritis and Sjogren'ssyndromewith special reference to the duration of rheumatoid arthritis. *Med Clin (Barc)* 1990; 94: 655–659.

51. **R.T.Keenan,C.J. Swearingen, Y.Yazici**. Erythrocyte sedimentation rate and C-reactive protein levels are poorly correlated with clinical measures of disease activity in rheumatoid arthritids, systemic lupus erythematosis and osteoarthritis patients. Clinical and Experimental Rhematology.2008;26:814-819.

PROFORMA

1. S.no. : Date:

2. I.D :

3. Name : Address&contact No:

4. Age :

5. Sex :

6. Religion : Hindu / Muslim / Christian / Others (specify)

7. RA –OPD no. :

8.Occupation : None / Housewife / labour /Clinical /Professional /Others

9.Per Capita Income :
Number of family members :
Net family income :

10.Educational Qualification :No education /Class I-V/VI-XII/College/ Professional

11.Family history:

12.Duration of illness :

13.Social habits: H/o Alcohol/ Smoking/ Betal nut chewing/Others

14.Past history:

15.Treatment history: :

16.Associated comorbidities:

17.Clinical details:

Joint pain Duration

On Examination

Ht: Wt:

Built & Nourishment:

Respiratory rate: Pulse: BP:

Clinical Examination:

CVS:

RS:

CNS:

Investigations:

Blood:

Hb gm%

Sugar mg/dl

Platelet Count lakh/cu mm

Serology:

Rheumatoid factor

C-reactive protein

Others:

.

ஒப்புதல் படிவம்

திரு/திருமதி/செல்வன்/செல்வி/ _____

ஆகிய நான் டாக்டர்.xxxxxxxxxxx, பட்டமேற்படிப்பு மாணவி உடல் இயங்கியல் துறை, ஸ்டான்லி மருத்துவக்கல்லூரி, மருத்துவமனையில் நடத்தும் ஆராய்ச்சியில் யாருடைய வற்புறுத்தலும் இன்றி என்னுடைய முழுசம்மதத்துடன் பங்கேற்க சம்மதம் தெரிவிக்கிறேன். இந்த ஆராய்ச்சி என்னுடைய இருதயம் மற்றும் இரத்தக் குழாய்களின் செயல்பாட்டை அறிந்து கொள்ள உதவியாக இருக்கும் என்பதை நான் அறிந்து கொண்டேன். ஆராய்ச்சியின் செயல்பாடுகளை ஆராய்ச்சியாளர் மூலம் அறிந்துகொண்டேன்.இந்த ஆராய்ச்சியில் எந்தவித மருந்துகளோ ஊசிகளோ அளிக்கப்படமாட்டாது எனவும் நான் இந்த ஆராய்ச்சியில் இருந்து எந்தவித முன்னறிவிப்புமின்றி விலகிக்கொள்ள எனக்கு உரிமை உண்டு எனவும் இந்த ஆராய்ச்சியின் ஏடுகள் இரகசியமாக வைக்கப்படும் என்பதையும் நான் அறிவேன்.

கையெழுத்து

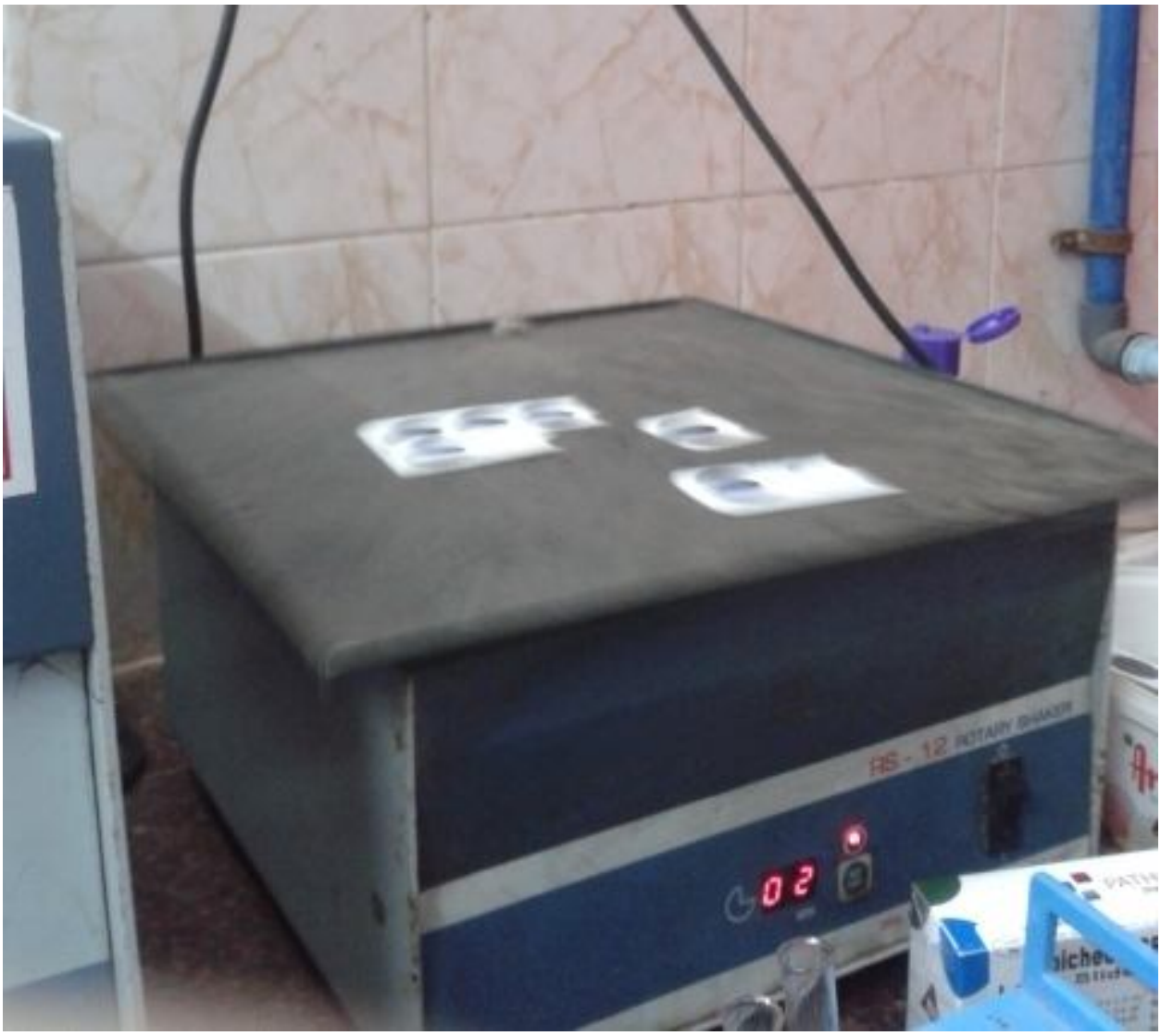
CONSENT FORM

IMr/Ms _____ understand that Dr.xxxxxxxxxxxx a postgraduate student in Stanley Medical College and Hospital, Chennai is doing the study on Rheumatoid arthritis subjects and control group. I have been made to understand that these tests will assess the functioning of my heart. These tests are simple, involve taking ECGs, and blood pressure. They donot involve injections or taking any medicines and are risk free.I have been familiarized with the testing procedures. I am participating in this study willingly. I have not been forced to do so.I have also been told clearly that I could withdraw from this study without any prejudice.

Date: Signature

HRV RECORDING IN SUPINE POSITION

MECHANICAL ROTATOR FOR LATEX AGGLUTINATION TEST



AUTOMATED CELL COUNTER

STUDY GROUP																				
S.NO.	AGE (yrs)	SEX	BMI	BLOOD SUGAR mg/dl	SBP mmHg	DBP mmHg	MEAN HR(bpm)	MEAN RR(ms)	SDNN (ms)	RMSSDN (ms)	NN 50	LF ms2	HF ms2	TOTAL POWER	LF nu	HF nu	LF / HF	RF	CRP	Platelet count (L/cumm)
P1	55	F	25.7	112	112	70	71	851	19.41	22.1	9	352	232	3075	78.3	23.2	1.5	neg	pos	2.8
P2	43	F	22.7	98	122	70	74	816	29.86	26.02	13	308	27.3	2822	72.2	27.8	11	pos	pos	3.2
P3	40	F	21.8	108	112	68	69	873	24.85	27.9	21	612	286	1242	69.4	28.6	2.1	neg	neg	3.2
P4	35	F	30.8	122	134	68	77	784	21.25	18.32	1	547	206	1945	79.4	20.6	2.6	pos	neg	3
P5	45	M	26.0	124	120	70	85	707	17.14	13.13	0	128	253	474	75.3	25.3	0.5	pos	pos	3.6
P6	53	F	26.0	118	116	72	96	626	9.67	5.9	0	15	112	27	88.8	11.2	0.1	pos	neg	3.7
P7	29	M	23.7	92	124	74	78	769	26.27	15.39	3	358	223	1889	78.5	22.3	1.6	pos	pos	3
P8	30	M	24.8	98	130	70	76	792	40.13	18.58	5	301	148	1768	85.2	14.8	2	pos	neg	3.1
P9	55	F	22.6	124	126	72	88	678	22.66	11.84	0	372	240	256	76.4	24	1.6	pos	pos	3.4
P10	53	M	22.0	122	140	72	82	732	18.39	9.8	0	267	195	764	80.5	19.5	1.4	pos	pos	3
P11	53	F	23.7	110	118	70	81	104	41.13	56.73	141	950	274	10765	72.6	27.4	3.5	neg	neg	2.6
P12	52	F	24.0	100	124	74	80	107	36.39	52.29	119	930	233	10532	75	23.3	4	neg	pos	3
P13	35	F	24.8	98	106	70	75	797	28.53	18.1	0	1269	220	2463	78	22	5.8	pos	pos	3.7
P14	35	F	28.4	114	116	70	73	825	26.4	17.8	2	401	214	3444	78.6	21.4	0.2	pos	pos	3
P15	50	F	24.3	126	118	76	69	867	28.99	29.1	37	477	256	5188	73.3	25.6	1.9	pos	pos	3.2
P16	45	M	22.9	108	100	80	78	898	33.14	34.09	55	229	39.6	4392	60.4	39.6	5.8	neg	pos	3.1
P17	45	F	20.0	96	112	74	72	829	14.85	17.65	2	350	98	3481	79	21	3.6	pos	pos	3.5
P18	45	F	27.1	98	110	72	90	666	15.34	5.03	0	286	192	71	80.4	19.2	1.5	pos	pos	3
P19	42	M	25.4	113	118	80	89	671	11.52	5.58	0	304	201	103	79.1	20.1	1.5	pos	pos	3.2
P20	32	F	27.1	93	100	70	93	647	24.92	27.47	32	213	67.7	155	32.3	67.7	0.3	neg	neg	3.5

STUDY GROUP																				
S.NO.	AGE (yrs)	SEX	BMI	BLOOD SUGAR mg/dl	SBP mmHg	DBP mmHg	MEAN HR(bpm)	MEAN RR(ms)	SDNN (ms)	RMSSDN (ms)	NN 50	LF ms2	HF ms2	TOTAL POWER	LF nu	HF nu	LF / HF	RF	CRP	Platelet count (L/cumm)
P21	42	F	23.8	102	108	80	93	643	27.32	24.6	24	189	67.5	144	51.1	40.8	2.8	pos	neg	3.8
P22	46	M	22.6	118	112	78	79	840	25.96	19.8	3	128	40.6	890	59.4	40.6	3.1	pos	neg	3.5
P23	44	F	22.8	125	114	82	81	805	25.81	17.3	3	598	301	928	69.9	30.1	2	pos	pos	2.8
P24	47	F	23.4	117	116	74	71	843	16.78	13.8	0	674	228	3796	78	22	3	neg	neg	2.5
P25	36	F	20.6	98	120	72	71	844	27.88	21.36	6	568	233	4327	76.7	23.3	2.4	pos	neg	3.5
P26	37	F	31.6	100	108	72	75	795	25.67	17.5	6	278	348	1654	76	24	0.8	pos	pos	3.6
P27	39	F	26.9	102	110	70	73	825	18.24	12.9	2	920	203	1669	79.5	20.5	4.5	pos	neg	3.8
P28	38	M	22.6	104	112	74	72	831	15.51	12.28	0	2165	217	3497	78.3	21.7	10	pos	neg	3.7
P29	30	M	23.2	96	122	70	71	847	41.8	41.27	30	1236	367	2475	66.7	36.7	3.4	pos	neg	3.6
P30	28	F	26.2	96	106	68	71	849	34.14	36.78	43	564	262	2321	73.8	26.2	2.2	pos	pos	3
P31	37	F	21.0	117	116	72	73	821	37.71	36.65	63	932	218	8563	79.5	21.8	4.3	pos	pos	2.6
P32	38	M	22.9	102	112	74	71	842	33.64	36.75	62	1130	275	2656	71.3	27.5	4.1	pos	pos	2.8
P33	35	F	23.6	98	120	70	89	674	24.26	21.14	12	645	662	1289	33.6	66.2	1	pos	pos	2.7
P34	42	M	18.1	106	112	70	85	710	31.98	31.82	48	534	515	435	48.5	51.5	1	pos	pos	1.8
P35	48	M	22.6	102	122	80	92	652	23.5	26.8	38	438	242	203	35	60.7	1.8	pos	neg	3.4
P36	29	F	24.7	90	112	72	80	749	14.97	18.42	1	990	721	1092	20.3	74.8	1.4	neg	pos	2.9
P37	35	M	23.3	117	120	70	84	710	24.6	32.4	46	960	740	832	20.5	74	1.3	pos	neg	3.3
P38	42	F	26.8	97	102	70	82	729	38.43	48.79	164	673	251	2067	68.4	25.2	2.7	pos	pos	2.5
P39	46	F	29.3	100	118	70	83	602	8.89	6.67	0	1246	221	3089	67.0	22.2	5.6	pos	pos	2.1
P40	52	F	27.3	96	120	74	90	593	11.17	7.34	1	2592	1176	2989	73	17.5	2.2	pos	pos	3.9

CONTROL GROUP																				
S.NO.	AGE (yrs)	SEX	BMI	BLOOD SUGAR mg/dl	SBP mmHg	DBP mmHg	MEAN HR(bpm)	MEAN RR(ms)	SDNN (ms)	RMSSDN (ms)	NN 50	LF ms2	HF ms2	TOTAL POWER	LF nu	HF nu	LF / HF	RF	CRP	Platelet count (L/cumm)
C1	35	F	26.7	100	120	70	64	931	65.48	73.29	341	262	313	760	47.5	52.7	0.83	neg	neg	2.6
C2	39	M	22.4	112	122	70	77	778	20.92	13.84	0	26	15	91	64.4	35.6	1.73	neg	neg	3.3
C3	26	F	23.2	108	110	68	68	777	45.14	49.26	140	456	260	6534	67.6	32.4	1.75	neg	neg	3
C4	33	F	32.4	98	100	60	67	885	51.06	56.6	158	668	380	5696	65	35	1.75	neg	neg	2.4
C5	38	M	26.6	100	120	72	81	741	20.89	19.2	4	103	56	831	67.4	32.6	1.83	neg	neg	2.3
C6	27	F	29.9	102	100	60	90	596	15.01	15.03	1	10	16	55	46.2	53.8	0.62	neg	neg	1.9
C7	49	F	24.0	97	110	68	73	819	36.63	38.06	72	326	169	2569	69.7	30.3	1.92	neg	neg	2.5
C8	42	F	27.4	1.4	110	70	70	715	35.62	19.12	69	316	172	2601	68.1	33.2	1.83	neg	neg	3.1
C9	28	M	21.5	100	100	80	68	885	51.06	56.65	158	317	261	2626	55.8	44.2	1.21	neg	neg	2.8
C10	47	F	22.3	96	10	80	64	937	67.17	75.72	170	251	206	1686	56.9	43.1	1.21	neg	neg	2.3
C11	36	F	27.6	98	110	68	68	885	51.06	56.06	158	668	380	5696	65	35	1.75	neg	neg	1.1
C12	44	F	22.6	98	128	68	71	842	63.02	56.38	135	934	415	6111	71.1	28.9	2.25	neg	neg	3.5
C13	52	M	26.4	89	128	80	69	870	77.93	55.13	105	985	508	8345	71.3	28.7	1.93	neg	neg	2.6
C14	53	F	24.8	93	124	84	90	631	19.76	10.63	0	30	13	87	30	13	2.3	neg	neg	3.6
C15	36	F	27.8	88	114	76	80	746	27.78	30.84	43	180	118	1298	63.6	37	1.52	neg	neg	1
C16	45	F	21.6	98	112	86	75	805	21.08	19.02	6	146	84	2490	67.8	30.5	1.73	neg	neg	2.3
C17	48	M	19.2	100	120	80	68	881	38.42	30.11	28	328	195	4680	73.2	26.8	1.68	neg	neg	4.5
C18	53	F	24.2	89	120	70	67	891	147.2	198	45	195	298	845	49.5	51.2	0.65	neg	neg	2.5
C19	52	F	28.1	96	120	70	63	927	46.92	48.4	86	272	341	784	40.1	58.2	0.79	neg	neg	4.3
C20	35	M	24.5	118	100	60	62	910	38.78	48.7	5	170	211	1389	50.8	49.2	0.8	neg	neg	2.2

CONTROL GROUP																				
S.NO.	AGE (yrs)	SEX	BMI	BLOOD SUGAR mg/dl	SBP mmHg	DBP mmHg	MEAN HR(bpm)	MEAN RR(ms)	SDNN (ms)	RMSSDN (ms)	NN 50	LF ms2	HF ms2	TOTAL POWER	LF nu	HF nu	LF / HF	RF	CRP	Platelet count (L/cumm)
C21	29	F	22.9	93	120	72	64	802	84.12	73.2	68	212	320	985	46.8	53.7	0.66	neg	neg	2.3
C22	39	F	19.7	118	110	70	65	896	24.76	22.7	17	98	212	1712	56	44	0.46	neg	neg	2.6
C23	37	M	24.3	102	118	74	80	754.3	47.3	53.8	63	548	1321	5234	35.2	67.5	0.41	neg	neg	2.8
C24	38	F	19.6	100	120	74	72	830	41.2	43.9	88	976	962	2018	52.4	50.1	1.01	neg	neg	1.9
C25	43	M	17.9	87	100	70	65	860	70.32	70.43	143	452	521	1097	45.3	44.7	0.83	neg	neg	3
C26	52	M	30.8	103	108	70	68	873	24.66	34.31	40	118	564	678	17.2	73.1	0.02	neg	neg	2.7
C27	29	F	24.2	96	104	64	74	797	49.88	64.36	205	843	1356	3245	37.6	62.2	0.62	neg	neg	2.3
C28	46	M	22.3	110	114	70	69	865	70.23	75.24	174	2088	1687	5342	62.3	35.2	1.23	neg	neg	1.9
C29	44	F	23.5	84	118	76	66	921	52.13	58.32	142	936	834	4237	59.8	40.1	1.12	neg	neg	2.5
C30	47	F	27.6	98	110	70	83	723	35.4	34.9	54	367	545	1954	44.4	55.7	0.67	neg	neg	2.9
C31	55	F	24.8	97	112	72	62	976	64.12	76.87	173	959	2035	3897	35.6	65.3	0.47	neg	neg	1.8
C32	37	F	22.3	100	108	70	65	943	80.23	100.3	202	1102	2564	5598	53.7	48.1	0.42	neg	neg	3
C33	44	F	25.5	96	108	70	69	861	33.24	36.73	67	357	586	1670	45.3	52.3	0.6	neg	neg	2.6
C34	45	F	20.2	102	110	68	86	696	47.3	57.52	123	513	1324	2023	27.4	72.3	0.38	neg	neg	3.2
C35	42	M	21.8	97	126	80	76	784	35.8	40.91	118	241	727	1038	27.2	71.9	0.33	neg	neg	2.4
C36	32	F	25.0	93	110	72	70	831	72.2	70.12	159	879	1842	3531	52.9	46.1	0.48	neg	neg	2.6
C37	43	F	26.5	102	130	80	63	945	84.15	105.3	192	608	2543	5758	52.5	41.7	0.23	neg	neg	2.3
C38	37	F	24.9	87	120	70	65	922	53.81	55.81	139	476	853	2217	58.6	41.0	0.55	neg	neg	1.4
C39	35	F	26.7	107	110	68	73	823	69.68	90.17	180	689	789	1578	45.7	56.3	0.87	neg	neg	3
C40	50	F	25.6	126	130	68	63	964	84.15	94.35	203	753	2364	5785	53.5	48.0	0.31	neg	neg	2.5