2014

Effect of Yoga on Endothelial Function, Vascular Compliance and Sympathetic Tone in Elderly Subjects with Increased Pulse Pressure: A Randomized Clinical Study

Thesis submitted to Faculty of Medicine

BLDE University, Bijapur, Karnataka, India

For the Award of the Degree of

Doctor of Philosophy (Medical)

Subject: Physiology

Satish G. Patil

Department of Physiology,
Shri B.M.Patil Medical College, Hospital & Research Centre,
Bijapur, Karnataka.
September 2014.

Effect of Yoga on Endothelial Function, Vascular Compliance and Sympathetic Tone in Elderly Subjects with Increased Pulse Pressure: A Randomized Clinical Study

Thesis submitted to Faculty of Medicine

BLDE University, Bijapur, Karnataka, India

For the Award of the Degree of

Doctor of Philosophy (Medical)

Subject: Physiology

Satish G. Patil

INSPIRE Research Fellow
Department of Science & Technology (DST)
Government of India

Department of Physiology
Shri B.M.Patil Medical College, Hospital & Research Centre
Bijapur, Karnataka

September 2014

BLDE UNIVERSITY

Bijapur, Karnataka, India

Certificate

This is to certify that this thesis entitled "*Effect of yoga on endothelial function, vascular compliance and sympathetic tone in elderly subjects with increased pulse pressure: A randomized clinical study*" is a bonafide work of Mr. Satish G. Patil and was carried out under our supervision and guidance in the Department of Physiology, Shri B.M.Patil Medical College, Hospital & Research Centre, Bijapur, Karnataka, India.

Prof. Kusal K. Das
Supervisor
Professor, Department of Physiology
Shri B.M.Patil Medical College, Hospital & Research Centre
Bijapur, Karnataka

Dr Manjunatha R. Aithala
Co-supervisor
Professor & Head
Department of Physiology
Shri B.M.Patil Medical College, Hospital
& Research Centre
Bijapur, Karnataka

Dr M. S. Biradar
Principal
Shri B.M.Patil Medical College, Hospital
& Research Centre
Bijapur, Karnataka

DECLARATION

I declare that the thesis entitled *"Effect of yoga on endothelial function, vascular compliance and sympathetic tone in elderly subjects with increased pulse pressure: A randomized clinical study"* has been prepared by me under the guidance of Professor Kusal K. Das and Dr Manjunatha R. Aithala, Department of Physiology, BLDE University's Shri B.M.Patil Medical College, Hospital and Research Centre, Bijapur, Karnataka, India. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

Satish G. Patil
INSPIRE Research Fellow (DST),
Department of Physiology,
Shri B.M.Patil Medical College, Hospital & Research Centre
BLDE University,
Bijapur, Karnataka
Date: 08.09.2014

ACKNOWLEDGEMENT

I sincerely take an opportunity to acknowledge gratitude to all the people including study participants without whom this thesis would not have been possible.

Firstly, I express my sincere gratitude to my supervisor Prof. Kusal K. Das, Professor of Physiology, BLDE University's Shri B.M.Patil Medical College, Hospital & Research Centre, Bijapur and Visiting Professor, School of Medicine, University of Leeds, UK for his constant support, guidance and blessings. His full faith and trust in me have contributed immensely in successfully completing this journey. He is easily approachable and venerable teacher with vast research experiences. The successful completion of this research work may be attributed to the critical evaluation of manuscripts and thesis by my supervisor and I really feel proud and blessed to be a student of Prof K.K. Das.

I am sincerely indebted to my co-supervisor Dr Manjunatha R. Aithala, Head of the Department of Physiology, BLDE University's Shri B.M.Patil Medical College, Hospital & Research Centre, Bijapur for his support and invaluable suggestions during the planning and development of this research work. He has provided all the necessary facilities in the lab and department required for my research works. He has also placed a full faith and undoubted trust in me which helped me a lot in carrying out my research work successfully. More than a co-supervisor- he was my guardian.

I am extremely thankful to Prof G.B.Dhanakshirur, Retired Professor of Physiology, BLDE University's Shri B.M.Patil Medical College, Hospital & Research Centre, Bijapur for guidance and constructive advices for initial planning and preparation of this thesis. He helped me in all possible ways to conduct the research work in a systematic manner.

I owe my most sincere gratitude to Dr M. S. Biradar, Principal, BLDE University's Shri B.M.Patil Medical College, Hospital & Research Centre for providing an

excellent research environment and facilities to work in the institute. He has provided academic and administrative help whenever I needed.

I would like to express my very great appreciation to the Vice-principal, Shri B.M.Patil Medical College, Hospital & Research Centre and Chairman of Doctoral committee, BLDE University Dr Tejashwini Vallabha, who was a constant source of inspiration during my entire thesis. She too rendered her support whenever I needed, whether in academic or in administration.

I would like express my sincere gratitude to Prof Basavaraj Devarnavadgi, Head of the Department of Biochemistry for providing the facilities to work in their laboratory. My special thanks to Mr Sanjay Walvekar, Dr Baswaraj Aski, Dr Nilima Dongre, Dr Indira H & Dr Anand Pyati for their valuable suggestions while working on biochemical investigations. It would be injustice if I don't express my gratitude to the great contribution and assistance of Mr Govindanagouda Naregal, Postgraduate of the Department of Biochemistry for all the biochemical investigations.

I would like to offer my special thanks to Dr M Rameshwarudu, Dean, SVS Medical College, Mahbubnagar, Andhra Pradesh and Dr Rameshwari Reddy, Professor & Head of the Department of Physiology, SVS Medical College, Mahbubnagar, Andhra Pradesh, my PG teachers, whose teachings made me sustainable in this competitive world. I am indebted to their care and moral support. Not only during my post-graduation but even during my entire thesis they were a constant source of inspiration.

I wish to acknowledge the help provided by my all colleagues of Department of Physiology. I extend my thanks to all the non-teaching staff of Department of Physiology specially Mr G.M. Mathpati and Mr Shivling G Biradar for assistance during my thesis.

Dr Subramanya, Assistant Director for ICMR Advanced Centre for Research in Yoga and Neurophysiology, Bangalore helped in preparation of Integrated Yoga

module for elderly with hypertension. I remain grateful to him for his very important support.

I also wish to thank Dr Shailaja Patil, Deputy Dean (R & D), BLDE University for her timely suggestions while preparing the thesis.

Advice given by Mrs Vijaya Sorganvi and Dr Shahnawaz, Statisticians of Department of Community Medicine were really a great help in data analysis and data interpretations.

Assistance provided by all faculty of Central Library was greatly appreciated.

I would like to thank the following organizations for their financial assistance: (1) I sincerely acknowledge Department of Science and Technology (DST), Government of India for the INSPIRE fellowship support provided for pursuing full-time PhD program. (2) My special thanks to BLDE University for providing financial assistance to this thesis (3) I express my gratitude to CSIR for providing foreign travel grant to present my research findings at 9th International congress of European Geriatric society held in Venice, Italy. (4) I would like to offer special thanks to Centre for International Cooperation in Science, Chennai for an award of Travel fellowship for presentation of research findings in Italy.

My special thanks are extended to the all the staff of BLDE University for providing academic facilities.

Last but not least, I would like to extend my warm thanks to my entire family, without their support and help; I could not dedicate to research and it would have been extremely difficult for me to accomplish this thesis. During this entire tenure of thesis, I hardly had given my time to them.

DEDICATION

I dedicate this thesis to

My Parents

Late Shri Gurunathrao Patil & Late Smt Paregbai Patil

&

My Teachers

"Longevity is a vascular question, which has been well expressed in the axiom that man is only as old as his arteries. To a majority of men death comes primarily or secondarily through this portal. The onset of what may be called physiological arteriosclerosis depends, in the first place, on the quality of arterial tissue which the individual has inherited, and secondarily on the amount of wear and tear to which he has subjected it"

_ Sir William Osler

ABSTRACT

Objectives: We aimed to determine the effect of yoga on vascular function in elderly with increased pulse pressure (PP) and to explore the yoga induced mechanism of control of blood pressure (BP) in elderly.

Methods: An open parallel arm randomized controlled study design was adopted. The participants were elderly subjects with PP>60mmHg (n=60). Subjects with Systolic BP > 159 mmHg and Diastolic BP > 99 mmHg was one of the major exclusion criteria. Yoga group (n=30) was assigned for yoga training and control group (n=30) for brisk-walk with stretching exercise for one hour in the morning for 6 days in a week for twelve weeks. The following parameters were tested before and after intervention: Arterial stiffness measures: Brachial-ankle pulse wave velocity (baPWV), Carotid-femoral pulse wave velocity (c-f PWV), augmentation index (AIx@75), arterial stiffness index at brachial (bASI) and tibial arteries (aASI); Endothelial function indices: Total serum nitric oxide concentration (NOx), augmentation index (AIx@75); Heart rate variability (HRV) measures: Low frequency (LF), high frequency (HF) and LF/HF ratio; Oxidative stress measure: serum malondialdehyde (MDA) concentration; and antioxidant capacity: serum superoxide dismutase (SOD) activity, erythrocyte reduced glutathione (GSH), serum ascorbic acid or vitamin C.

Results: We found a significant decrease in c-f PWV by 7.89% ($p<0.001$), baPWV by 7.74% ($p<0.001$), aASI@75 by 15.09% ($p<0.001$), LF by 3.07% ($p=0.012$), LF/HF ratio by 13.46% ($p<0.001$), SBP by 9% ($p<0.001$), PP by 16.71% ($p<0.001$) and MAP by 5.08% ($p<0.001$), and significant increase in HF by 12.65% ($p=0.008$) and NOx by 23.26% ($p=0.001$) in the Yoga group, whereas no significant difference was observed in control group. Yoga had also significantly reduced serum MDA level ($p<0.001$) and enhanced SOD activity ($p=0.007$), serum GSH ($p=0.002$) and vitamin C ($p=0.002$). While in control group, we observed a significant increase in serum MDA level ($p=0.04$) and reduction in serum vitamin C level ($p=0.015$) with no significant difference in the SOD activity and GSH level.

Conclusion: These findings suggest that yoga module tested in the present study is an effective physiological means to control hypertension along with arterial stiffness in elderly. Yoga also induces beneficial changes in endothelial function, cardiac autonomic nervous system, oxidative stress and antioxidant defense.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviations	
ACEIs	Angiotensin converting enzyme inhibitors
ADMA	Asymmetrical dimethylarginine
AGE	Advanced Glycation End product
ANCOVA	Analysis of Covariance
AHA	American Heart Association
AIx	Augmentation Index
AIx@75	Augmentation Index normalized for a heart rate of 75 beat per minute
ASI	Arterial Stiffness Index
aASI	Arterial Stiffness Index at tibial artery
baPWV	Brachial-Ankle pulse wave velocity
bpm	beat per minute
bASI	Arterial Stiffness Iindex at Brachial artery
BP	Blood pressure
cGMP	Cyclic guanosine monophosphate
CAD	Coronary artery disease
c-f PWV	carotid-femoral pulse wave velocity
CV	Cardiovascular
CV%	Co-efficient of variability
CHF	Congestive heart failure
CKD	Chronic kidney disease
CT	Computed Tomography
CONSORT	Consolidated Standards of Reporting Trials
CHOD-PAP	Cholesterol Oxidase-Peroxidase
DASH	Dietary approaches to stop hypertension
DBP	Diastolic blood pressure
DV	Dependent variable
ECG	Electrocardiogram
ECAM	Endothelial leucocyte adhesion molecule

ED	Endothelin
EDRF	Endothelium derived relaxing factor
eNOS	Endothelium Nitric Oxide Synthase
ESH	European Society of Hypertension
ESC	European Society of Cardiology
ESRD	End stage renal disease
FFT	Fast Furrier Transform
FMD	Flow mediated dilatation
GSH	Reduced glutathione
GPO-PAP	Glycerol Phosphatase-Oxidase
ICAM	Intercellular adhesion moledule
ICMR	Indian Council of Medical Research
IHD	Ischemic heart disease
ISH	Isolated systolic hypertension
H_2O_2	Hydrogen peroxide
HDL	High density lipoprotein
H0	Null hypothesis
H1	Alternate hypothesis
HRV	Heart rate variability
HF	High frequency of heart rate variability spectrum
JNC	Joint National Committee
LDL	Low density lipoprotein
LF	low frequency of heart rate variability spectrum
LF/HF	Ratio of Low frequency and high frequency of heart rate variability spectrum
MDA	Malondialdehyde
MMPs	Matrix metalloproteinases
MHC II	Major Histocompatability Complex II
NO	Nitric Oxide
NOx	Total Nitric Oxide Concentration
NCDs	Non-Communicable Diseases

NSAID	Non Steroid Anti-inflammatory Drug
nu	Normalized units
$O_2^{\cdot -}$	Superoxide radicals
OD	Optical density
PP	Pulse pressure
PGI_2	Prostacyclin or Prostaglandin I_2
PWV	Pulse wave velocity
PTT	Pulse transit time
QCA	Quantitative Coronary Angiography
ROS	Reactive oxygen species
RI	Reflection index
SBP	Systolic blood pressure
SHEP	Systolic Hypertension in Elderly Program
SOD	Superoxide dismutase
SV	Stroke volume
TONE	Trial of Nonpharmacologic Intervention in the Elderly
TXA_2	Thromboxane A_2
VCL_3	Vanadium chloride
VCAM	Vascular cell adhesion molecular
VEGF	Vascular endothelial growth factor
VLDL	Very low density lipoproteins
VOP	Venous Occlusion Plethysmography
VSMCs	Vascular smooth muscle cells
WHO	World health organization
WR	Working reagent

CHAPTER I

PURPOSE OF THE STUDY

1. INTRODUCTION

Aging is an established cardiovascular (CV) risk factor. Hypertension is becoming an important medical and public health problem all over the world and is found to be one of the common disorders of ageing (Fagard RH., 2002). According to World Health Organization (WHO), the most common cause of preventable death in developed countries is hypertension, which is significantly increasing in developing countries (Ezzati M et al., 2002). Hypertension along with aging is a major risk factor for cardiovascular (CV) morbidity and mortality (Supiano MA., 2009).

There are diverse mechanisms and age-related factors involved in the development of hypertension in older individuals. The major contributing factors and predominant mechanisms that develop hypertension in elderly are vascular stiffness and endothelial dysfunction. Two major age-related structural changes that take place in elastic arteries are stiffness and dilatation. These changes results in decline or failure in expansion of aorta in response to ventricular systole which leads to elevation in systolic blood pressure (SBP) (isolated systolic hypertension) and failure to recoil leads to decrease in diastolic blood pressure (DBP) thus causing widening of pulse pressure (PP). Hence, PP is a best tool for measuring vascular aging and a good marker for CV risk in elderly. Pulse pressure is an independent indicator of arterial stiffness. Another factor related to arterial stiffness that elevates SBP in elderly is early arrival of wave reflection during systole (Lim MA & Townsend RR., 2009; Laurent S & Boutouyrie P., 2007; Ghiadoni L et al., 2009). PP, a pulsatile component of blood pressure is more closely associated to CV events than SBP or DBP alone (Franklin SS et al., 2001). A meta-analysis of several studies with data of 8,000 elderly patients found that a 10mmHg increase in PP increased the risk of major CV complications and mortality by nearly 20% (Blacher J et al, 2000).

Arterial stiffness is an independent and strong predictor of CV morbidity and mortality in hypertensive without any overt CV disease (Blacher J et al., 1999; Laurent S et al., 2001) and also in well-functioning older adults (Sutton-Tyrrell K et al., 2005). Studies have shown a positive correlation between PP and arterial stiffness (Safar ME., 2000; Safar ME et al., 2003; Cecelja M et al 2009). Pulse wave velocity (PWV), augmentation index (AIx) and arterial stiffness index (ASI) are recommended measures of arterial stiffness

(Laurent S et al., 2006; Kaibe M et al., 2002). PWV is a measure of regional arterial stiffness. An increase in PWV indicates an increase in arterial stiffness or decrease in vascular compliance. AIx is a measure of wave reflection which elevates with an increase in arterial stiffness (Laurent S et al., 2006).

The age-related endothelial dysfunction associated with decreased bioavailability of nitric oxide (NO), a potent vasodilator, contributes to vascular stiffness and hypertension (Jin RC et al., 2010). Oxidative stress is also implicated in the development of hypertension. Increased vascular oxidative stress damage the endothelium causing reduction in NO production and its bioavailability which leads to impairment in endothelium-dependent vasodilation with resultant enhanced vascular tone and hypertension (Briones AM., et al 2010; Schultz E et al., 2011). Other age-related physiological changes that contribute to hypertension in elderly are increased sympathetic activity, decreased baroreceptor sensitivity, decreased alpha- and beta adrenergic receptor responsiveness and low plasma renin activity (Supiano MA., 2009).

As the elderly individuals suffering from isolated systolic hypertension are often resistant to pharmacological treatment, so any attempt to reduce the SBP aggressively lowers DBP (decreased with age) to such an extent to compromise coronary blood flow (Calhoun DA et al., 2008; Vongpatanasin W., 2014; Satoshkar RS et al., 2005). Moreover, it has also been reported that arterial stiffness increases at a faster rate even in treated hypertensives with well controlled blood pressure (BP) than in a normotensives (Benetos A et al., 2002). These findings necessitate an alternative approach that controls hypertension along with the progression of arterial stiffness with age in order to prevent the CV mortality and morbidity.

Among the life-style modalities, yoga has been known to have established health benefits. We have found a significant reduction in SBP and PP following yoga practice for 6 weeks in elderly subjects with Grade-I hypertension in a preliminary study (Patil SG et al., 2014). But, the exact underlying mechanism of benefit remains unknown. Therefore, we aimed to determine the effect of yoga on vascular function in elderly with increased pulse pressure and to explore the benefits of mechanism of yoga on hypertension.

2. OBJECTIVES OF THE STUDY

The objectives of the study in elderly individuals with increased pulse pressure are as follows:

i. To determine whether there is any significant effect of yoga on vascular compliance or arterial stiffness.
ii. To determine whether there is any significant effect of yoga on endothelial function.
iii. To determine whether yoga training can significantly modulate autonomic activity.
iv. To determine whether there is any significant effect of yoga on oxidative stress and antioxidant defense.
v. To explore the possible yoga induced mechanism of control of BP in elderly.

3. HYPOTHESIS

a. H0:Null Hypothesis

i. There will be no statistically significant difference in vascular compliance or arterial stiffness before and after yoga.
ii. There will be no statistically significant difference in endothelial function before and after yoga.
iii. There will be no significant beneficial alteration in the activity of cardiac autonomic nervous system following yoga practice.
iv. There will be no statistically significant difference in oxidative stress and antioxidant defense before and after yoga.
v. There will be no statistically significant difference between effects of yoga and walking on vascular stiffness, cardiac autonomic nervous system activity, oxidative stress and antioxidant capacity.

b. H1:Alternate hypothesis

i. Yoga training will lead to increase in vascular compliance or decrease in arterial stiffness,
ii. Yoga training will lead to improvement in endothelial function.
iii. Yoga practice will lead to decrease in sympathetic activity, increase in parasympathetic dominance and balance the sympathovagal balance.
iv. Yoga practice will lead to decrease in oxidative stress and increase in antioxidant defense.
v. Yoga intervention will be more effective than walking on vascular stiffness, endothelial function, cardiac autonomic nervous system activity, oxidative stress and antioxidant capacity.

4. REFERENCES

- Benetos A, Adamopoulos C, Burean JM, Temmar M, Labat C, Bean K et al. Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. *Circulation* 2002;105(10): 1202-7.
- Briones AM, Touyz RM. Oxidative stress and hypertension: current concepts. Curr *Hypertens Rep* 2010;12(2):135-42.
- Blacher J, Asmar R, Djane S. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1999; 33(5): 1111-7.
- Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, et al. American Heart Association Professional Education Committee. Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation* 2008; 24;117:e510-26.
- Cecelja M, Jiang B, McNeill K, Kato B, Ritter J, Spector T et al. Increased wave reflection rather than central arterial stiffness is the main determinant of raised pulse pressure in women and relates to mismatch in arterial dimensions: a twin study. *J Am Coll Cardiol* 2009;54(8): 695-703.
- Ezzati M, Lopez AD, Rodgers A, Van der Hoorn S, Murray CJ. Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. *Lancet* 2002; 360 (9343):1347-60.
- Fagard RH. Epidemiology of hypertension in the elderly. *Am J Geriatr Cardiol* 2002;11(1): 23-8.
- Faulkner, E.A. (1969): Introduction to the Theory of Linear Systems; Chapman & Hall; ISBN 0-412-09400-2 pp 89.
- Franklin SS, Larson MG, Khan SA, Wong ND, Leip EP, Kannel WB et al. Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. *Circulation* 2001;103(9): 1245-9.
- Jin RC, Loscalzo J. Vascular nitric oxide: formation and function. *J Blood Med* 2010;2010(1): 147-62.

- Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; 37(5): 1236-41.
- Laurent S, Cockroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D et al. on behalf of the European network for Non-Invasive Investigation of Large arteries. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006; 27(21): 2588-605.
- Patil SG, Dhanakshirur G, Aithala MR, Das KK. Comparison of the effects of yoga and lifestyle modification on grade-I hypertension in elderly males: A preliminary study. *Int J Clin Exp Physiol* 2014;1(1):68-72.
- Safar ME. Pulse pressure, arterial stiffness and cardiovascular risk. Curr Opin Cardiol. 2000;15: 258-63.
- Safar ME, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation* 2003;107(22): 2864-69.
- Schultz E, Gori T, Munzel T. Oxidative stress and endothelial dysfunction in hypertension *Hypertens Res* 2011;34(6):665-73.
- Satoshkar RS, Bhandarkar SD, Rege NN. Pharmacology and pharmacotherapeutics. 18th edn. Popular prakashan private limited, India. 2005:pp 421-22.
- Supiano MA. Hypertension. In: Halter JB, Ouslander JG, Tinetti ME, Studenski S, High KP, Asthana S, eds. Hazard's Geriatric medicine and Gerontology. 6th edn. McGraw Hill Medical publishers. 2009:pp. 975-82.
- Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM et al. Health ABC Study. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation* 2005;111(25): 3384-90.
- Vongpatanasin W. Resistant hypertension: a review of diagnosis and management. *JAMA* 2014; 4;311(21):2216-24.

CHAPTER II

REVIEW OF LITERATURE

1. ELDERLY AND AGING

Globally, 10 % (600 million) of the world's population is elderly and it is expected to increase to 21 % (1.97 billion) in 2051 (Department of economic and social affairs, New York, United Nations. World Population Ageing; 1950-2050). Demographically, Asia is the most important continent in the world, where the population is growing both larger and older. The population of elderly aged above 65 years in Asia is expected to increase by four fold to about 1 billion by 2050 (National Research Council., 2012). About 34% of the world's older population is present in India and China; the two most populous countries in the world. India is the second largest country in the world with about 76 million elderly persons above 60 years of age compared to China's 127 million (National Research Council., 2012; Census of India., 2001). India's older population is estimated to grow from close to 8% (76 million) to about 9% (113 million) in 2016, and almost 20% in 2050 (Kowal P et al., 2012).

There are medical conditions due to age-related physiological changes that occur exclusively among the elderly which affect the quality of life. The diseases associated with older age groups are often non-communicable diseases (NCDs) that include CV diseases (hypertension, heart attacks and stroke), cancers, chronic respiratory diseases (such as chronic obstructed pulmonary disease and asthma) and diabetes (Boutayeb A & Boutayeb S., 2005; Hunter DJ & Reddy KS., 2013). The NCDs were once more prevalent in industrialized countries, but is now greater in the low and middle-income countries than high income countries. The rapid economic growth accompanied by rapid urbanization with unhealthy diet and life-style may contribute to the increase of non-communicable diseases in rapidly developing countries like India and China (Kowal P et al., 2012). NCDs are the leading cause of death in older individuals. Among the NCDs, CV diseases account for the largest fraction of deaths followed by cancer, chronic respiratory diseases and diabetes (Hunter DJ & Reddy KS., 2013). Hypertension is one of the major risk factor and treatable cause for CV morbidity and mortality in older individuals (Fagard RH., 2002; National High Blood Pressure Education Program Working Group., 1994; Hypertension Study Group., 2001). Hypertension in most of the elderly individuals is accompanied by multiple comorbidities, which tremendously affect their management. The recent emphasis on studies pertaining to the elderly in the developing world is attributed to the increasing number of older individuals and

their associated deteriorating conditions. Hence, the scientific understanding to improve the quality of life in elderly is the need of the hour.

2. HYPERTENSION IN ELDERLY

2.1. Introduction

Increased age is an established CV risk factor. High blood pressure is the most common cause of CV morbidity and mortality. Aging and high BP leads to structural and functional changes in the heart and vascular system. Hence, aging along with hypertension is a major & strong risk factor for CV morbidity and mortality (Lewington S et al., 2002; Fagard RH., 2002). It has the greatest impact on globally attributable mortality of any other risk factor and accounts for the 3rd leading cause of global burden of disease (Supiano MA., 2009). A change in the patterns of hypertension with age has been observed. In elderly, SBP increases without much change in DBP, which is categorized as isolated systolic hypertension (ISH) leading to widening of PP. Systolic hypertension may lead to stroke, myocardial infarction, dementia, renal failure and death (Zeiman SJ et al., 2005). These clinical complications affect the quality and longevity of life in elderly. According to World Health Organization, the most common cause of preventable death in developed countries is hypertension, which is significantly increasing in developing countries (Ezzati M et al., 2002). Reduction of SBP by 10 mmHg and DBP by 5 mmHg at age 65 years is associated with a decrease in myocardial infarction by 25%, stroke by 40%, congestive heart failure (CHF) by 50%, and overall mortality by 10-20% (Law M et al., 2003; Supiano MA., 2009).

2.2. Epidemiology

The prevalence of hypertension in elderly ranges from 50% to 75% and it is estimated that two out of three individuals over 75 years of age suffer from hypertension (Supiano MA., 2009; Lloyd-Sherlock P et al., 2014). According to the Framingham Heart Study, about 60% of the population by age 60 develops hypertension. In the same study, it was also estimated that the prevalence of hypertension may increase to about 65 % in men and 75 % in women by age 70. Also, it has been observed that nearly 85% of individuals with normal BP upto the age of 55 were later developed hypertension over 20-25 years (their residual lifetime risk) of follow-up study (Levy D et al., 1996; Vokonas PS et al., 1988). According to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-7), two thirds of individuals after 65 years have hypertension

(Chobanian AV, JNC 7 ., 2003). From an Indian perspective, the prevalence of hypertension in elderly above 60 years was reported between 40% and 60% (Radhakrishnan S et al., 2013; Kalavathy MC et al., 2000; Chinnakali P et al., 2013). There was wide difference in the prevalence rates reported from various regions of India. The prevalence rates were shown higher in elderly women compared to men (Supiano MA., 2009; Chinnakali P et al., 2013).

2.3. Classification of hypertension

There are two classifications of hypertension proposed by two societies in their guidelines for management of hypertension: (1) European Society of Hypertension and European Society of Cardiology (ESH/ESC-2007 and 2009 update) and (2) Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure.

Table 1 Classification of blood pressure for adults according to ESH/ESC 2007 guidelines

Classification	SBP (mmHg)	DBP (mmHg)
Optimal	≤ 120	And ≤ 80
Normal	120-129	80-84
High normal	130-139	85-89
Hypertension		
1. Grade 1 (mild)	140-159	90-99
2. Grade 2 (moderate)	160-179	100-109
3. Grade 3 (severe)	≥ 180	≥ 100
Isolated systolic hypertension	≥ 140	≤ 90

SBP: Systolic blood pressure; DBP: Diastolic blood pressure

As per the guidelines of ESH and ESC (2007 and 2009 update), hypertension has been classified into Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe) and isolated systolic hypertension (Table 1). Isolated systolic hypertension should be graded (grades 1, 2 and 3) on the basis of SBP values in the ranges indicated in the Table 1.

Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure in its 7th report (JNC-7) defined criteria for normal BP and classified hypertension into prehypertension, Stage 1 hypertension and Stage 2 hypertension (Table 2). As JNC-7

omitted the isolated systolic hypertension and since isolated diastolic hypertension is so uncommon among older individuals, one may correctly classify an older patient's hypertension based entirely on the level of their SBP into: Stage 1 hypertension between 140 and 159 mmHg systolic and Stage 2 hypertension, ≥ 160 mmHg systolic.

Table 2 Classification of blood pressure for adults according to JNC7 guidelines

Classification	SBP (mmHg)	DBP (mmHg)
Normal	≤ 120	And ≤ 80
Prehypertension	120-139	Or 80-89
Stage 1 Hypertension	140-159	Or 90-99
Stage 2 Hypertension	≥ 160	Or ≥ 100

SBP: Systolic blood pressure; DBP: Diastolic blood pressure

2.4. Types and Definitions of hypertension

2.4.1. Essential hypertension

Essential, primary or idiopathic hypertension can be defined as a rise in BP of unknown cause that increases risk for cerebral, cardiac, and renal events (Messerli FH et al., 2007). It accounts for 95% of all cases of hypertension. Essential hypertension is a heterogeneous disorder, with different patients having different causal factors that lead to high BP (Carretero OA & Oparil S et al., 2000).

2.4.2. Secondary hypertension

Secondary hypertension is a type of hypertension with an underlying, potentially curable cause (Table 3). The prevalence of secondary hypertension varies by age group. The prevalence of secondary hypertension ranges between 5 & 10% (Chiong JR et al., 2008). The etiology for secondary hypertension also varies by age group. The most common secondary cause for hypertension in young adults (particularly women) is renal artery stenosis, in

middle-aged adults is aldosteronism and in older adults is atherosclerotic renal artery stenosis (Viera AJ et al., 2010).

Table 3 Causes of secondary hypertension

	Causes
1.	Coarctation of aorta
2.	Renal artery stenosis
3.	Thyroid disorders
4.	Aldosteronism
5.	Obstructive sleep apnea
6.	Pheochromocytoma
7.	Cushing syndrome
8.	Drugs (NSAID, alcohol, estrogen)

2.4.3. White-coat hypertension

It is defined as the presence of an elevated BP (≥140/90 mmHg) in an office/clinic setting or in medical environment, but with normal BP when measured at home or normal day time ambulatory BP (≤ 135 mmHg systolic & ≤ 85 mmHg diastolic). It is also called as 'isolated office or clinic hypertension'. It is more common in the elderly (Celis H & Fagard RH., 2004; Verdecchia P et al., 2002).

2.4.4. Isolated ambulatory or Masked hypertension

It is defined as the presence of a normal BP in an office/clinic setting or in medical environment, but with elevated BP when measured at home or day time ambulatory BP (≥135 mmHg systolic & ≥85 mmHg diastolic) (Pickering TG et al., 2007). It is associated with an increased risk of CV events. It is frequent in the elderly and is associated with a high vascular profile, so measurement of BP at home is suggested in this age segment (Caddiolati C et al., 2011).

2.4.5. Pseudohypertension

It is a condition in which indirect BP measured by the cuff method (Osler's Sign) overestimates the true intra-arterial BP (Kuwajima I et al., 1990). Systolic BP is falsely increased by atherosclerotic and other vascular changes associated with age (Foran TG et al., 2004). As, the measurement of BP depends on measuring on how much force it takes to compress an artery, so to compress the stiffened arteries the sphygmomanometer reading is falsely increased. Psudohypertension is suspected when we found very high BP without any signs of organ damage or other complications, or occurrence of features of hypotension (dizziness, confusion or decreased urine output) when treated with antihypertensive. It occurs frequently in the elderly irrespective of them being hypertensive. The Osler Manaeuver, a sphygmomanometric procedure can be performed if pseudohypertension is suspected in the elderly, but it has low sensitivity and specificity. If the radial artery pulse remains palpable even after inflating the cuff above systolic pressure indicates false hypertension (Wright JC & Looney SW et al., 1997). Psuedohypertension can be confirmed by direct intra-arterial measurement of BP (Spence JD., 1997; Foran TG et al., 2004).

2.4.6. Resistant hypertension

It is defined as BP that remains uncontrolled despite of the concurrent use of 3 optimally dosed antihypertensive agents of different classes (Vongpatanasin W., 2014). One of the three antihypertensive agents should be diuretic. It is prevalent among all ages, but is more prevalent in elderly hypertensive patients (Calhoun DA et al., American Heart Association Statement., 2008). Patients who are well controlled but require four or more medications were also considered as resistant hypertension as per American heart association (AHA) statement and JNC-7 guidelines. There are several factors and causes which contribute to resistant hypertension (Table 4).

2.4.7. Dipper or non-dipper patient

Normally, the BP falls at night compared to daytime. The individuals who fail to decrease their nocturnal BP by at least 10% relative to their daytime BP are referred to as non-dippers. They have been shown to have greater CV disease risk compared to those with the normal dipper pattern (decrease in blood pressure at night compared to daytime). This diurnal

variation correlates with variations in sympathetic nervous activity associated with other factors such as age, hypertensive status, quality of sleep, marital status and socioeconomic status (Holt-Lunstad J et al., 2009). The prevalence of non-dippers is higher in the elderly population (Aronow WS et al., 2011).

Table 4 Factors those contribute to resistant hypertension

1	Patient characteristics associated with resistant hypertension	Older age High baseline blood pressure Obesity Excessive dietary salt ingestion Chronic kidney disease Diabetes Left ventricular hypertrophy Black race Female sex
2	Factors contributing resistant hypertension	Poor patient adherence Physical inertia Lack of adherence to life-style modifications Inadequate doses Inappropriate combinations of antihypertensive drugs Excess alcohol intake
3	Secondary causes of resistant hypertension	**Common** Obstructive sleep apnea Renal parenchymal disease Primary aldosteronism Renal artery stenosis **Uncommon** Pheochromocytoma Cushing's disease Hyperparathyroidism Aortic coarctation Intracranial tumor

2.5. Pathophysiology of hypertension

Homeostatic regulation of BP within its normal range to ensure an adequate tissue blood flow requires co-ordination of several complex interacting physiological systems. Perturbation in this complex regulatory system results in change in the normal baseline of BP. There are diverse mechanisms (Figure 1) and age-associate physiological changes that likely contribute to the development of essential hypertension in elderly (Table 5 & Figure 2). Lifestyle factors such as high sodium containing diet, being sedentary and obesity also contributes to an elevation in BP in older individuals. The hallmark of hypertension in the elderly is increased vascular resistance.

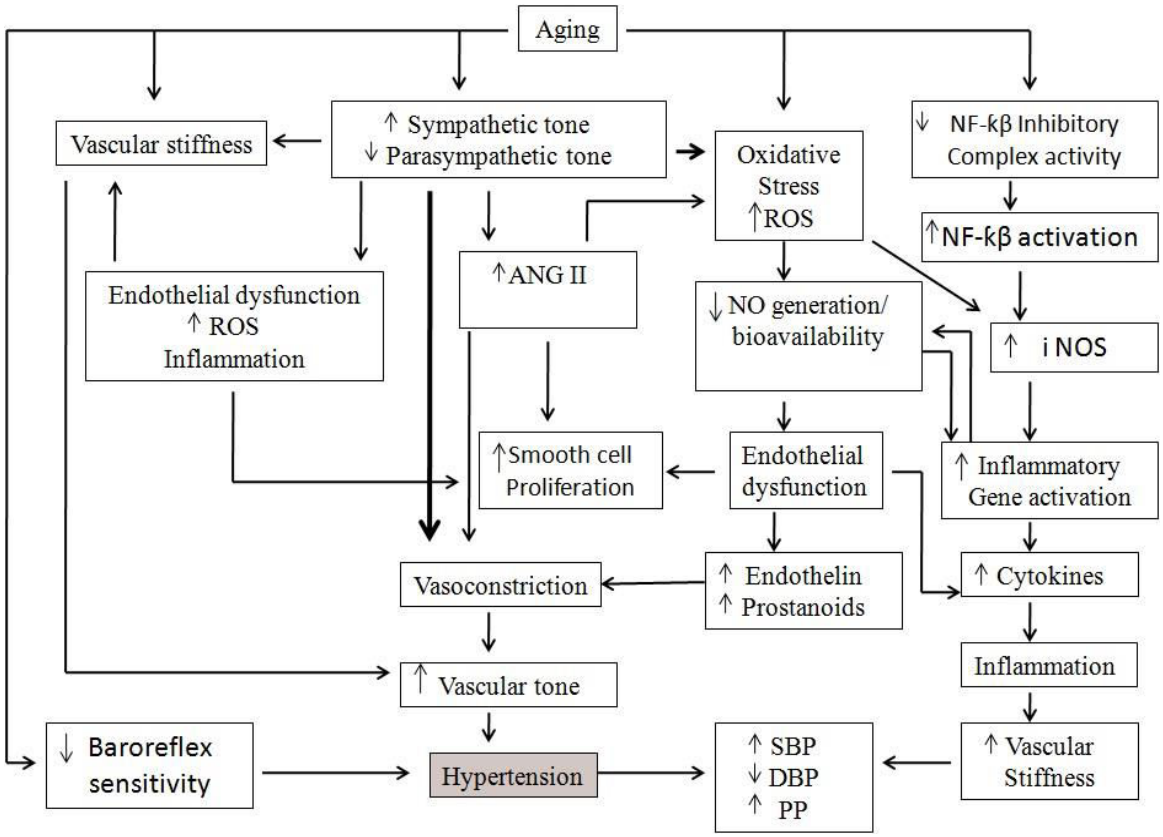


Figure 1 Pathophysiology of hypertension

Table 5 Age-associated factors contributing to hypertension

1	Arterial stiffness or decreased vascular compliance
2	Endothelial dysfunction
3	Increased sympathetic nervous system activity
4	Decreased baroreceptor sensitivity
5	Decreased alpha- and beta-adrenergic receptor responsiveness
6	Decreased ability to excrete sodium load (sodium sensitivity)
7	Low plasma renin activity
8	Resistance to insulin's effect on carbohydrate metabolism
9	Increase in aldosterone
10	Increase in oxidative stress
11	Central adiposity

(Reference: Supiano MA., 2009)

2.5.1. Age-associated structural change in arterial system: Arterial stiffness

The idea of Sir William Osler (1898) that has been stated 100 years before still holds true on association of vascular health and longevity. Sir William Osler states that "Longevity is a vascular question, which has been well expressed in the axiom that man is only as old as his arteries. To a majority of men death comes primarily or secondarily through this portal. The onset of what may be called physiological arteriosclerosis depends, in the first place, upon the quality of arterial tissue which the individual has inherited, and secondly upon the amount of wear and tear to which he has subjected it."

Blood vessel walls, especially large elastic arteries stiffen with age. In younger individuals, aorta and the proximal elastic arteries dilate by approximately 10% in response to each beat, while the muscular arteries dilate by only about 3% with each beat (O'Rourke MF & Hashimoto J., 2007). The heterogeneity in stiffness process with age between proximal and distal arteries can be explained on the basis of severity of fatigue exerted by the different degree of stretch (O'Rourke MF & Hashimoto J., 2007; Lionakis N et al., 2000).

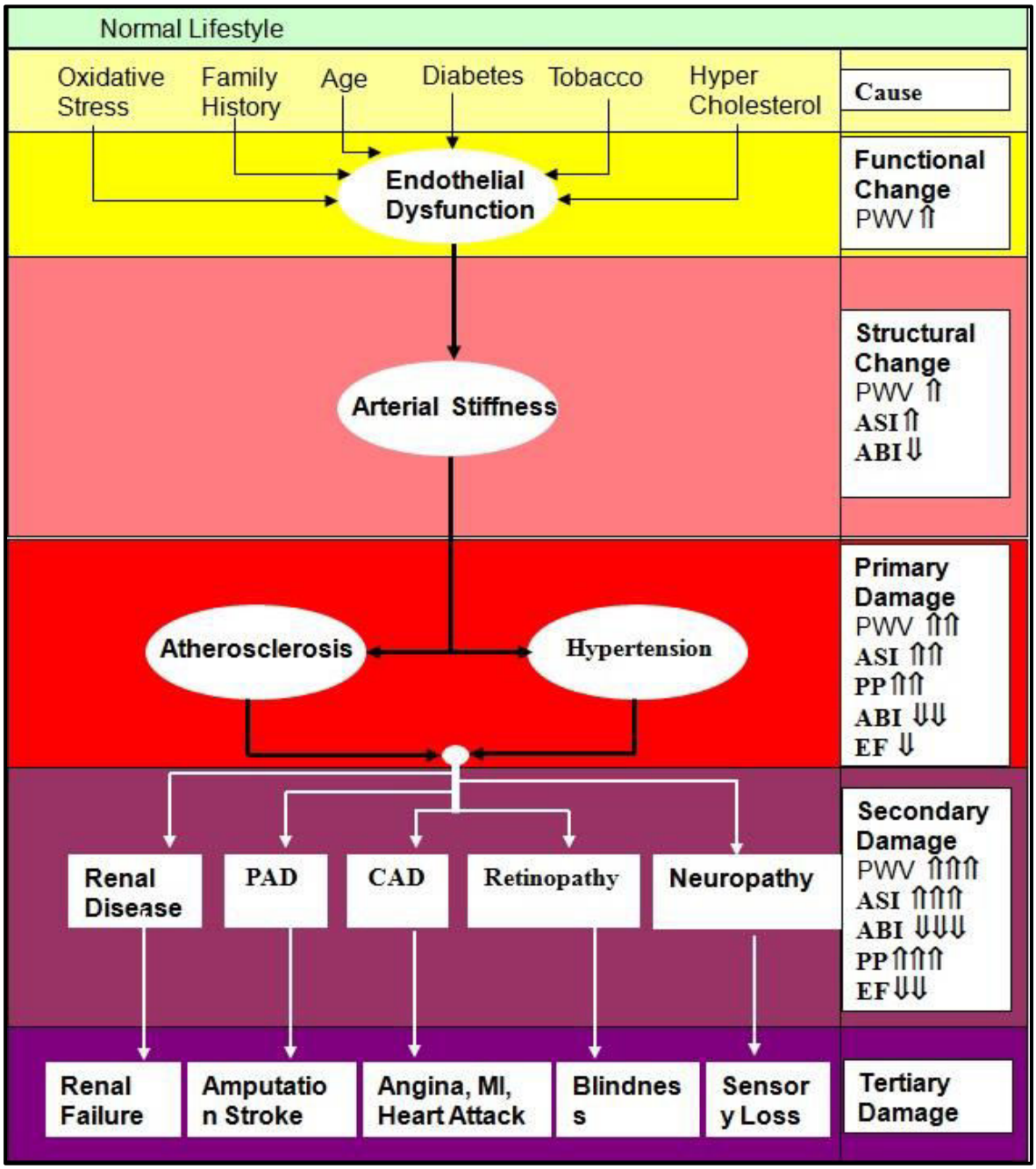


Figure 2 Causes and consequences of hypertension in elderly (PAD: Peripheral arterial disease, CAD: Coronary artery disease, PWV: Pulse wave velocity, ASI: Arterial stiffness index, ABI: Ankle brachial index, PP: Pulse pressure, EF: Ejection fraction)

The reduction of vascular compliance with age due to stiffening of arteries is the major contributor for elevation of BP, especially systolic pressure resulting in isolated systolic hypertension in elderly. Aging has been associated with both structural and

functional changes in the arterial system. Two major age-related structural changes that take place in elastic arteries are stiffness and dilatation. These changes results in decline or failure in expansion of aorta in response to ventricular systole which leads to elevation in systolic pressure and failure to recoil results in reduction in DBP thus causing widening of PP (Lee HY & Oh BH., 2010). Hence, an increase in PP, a pulsatile component creates a greater pulsatile stress on the arterial system even in the normotensive individuals (Millar JA et al., 2000).

The causes of arterial stiffness are summarized in the figure 3. The principal structural change with age occurs in the intima (hyperplasia) and the media (degeneration). The structural changes in the media of elastic arteries (medial degeneration) includes increase in collagen content and cross linking, increase in elastin fragmentation and decrease in elastin content (Lim MA & Townsend RR et al. 2009). The age-related structural changes in the elastin (thinning and fragmentation) and collagen are not seen in the muscular arteries. These changes in media are associated with increased expression of matrix metalloproteinases (MMPs). Matrix metalloproteinases regulate collagen and elastin molecules of the vessel wall. The factors those determine the stiffness of arteries and its ability to expand and recoil are structural proteins and pressure exerted by blood on their wall (Cecelja M &

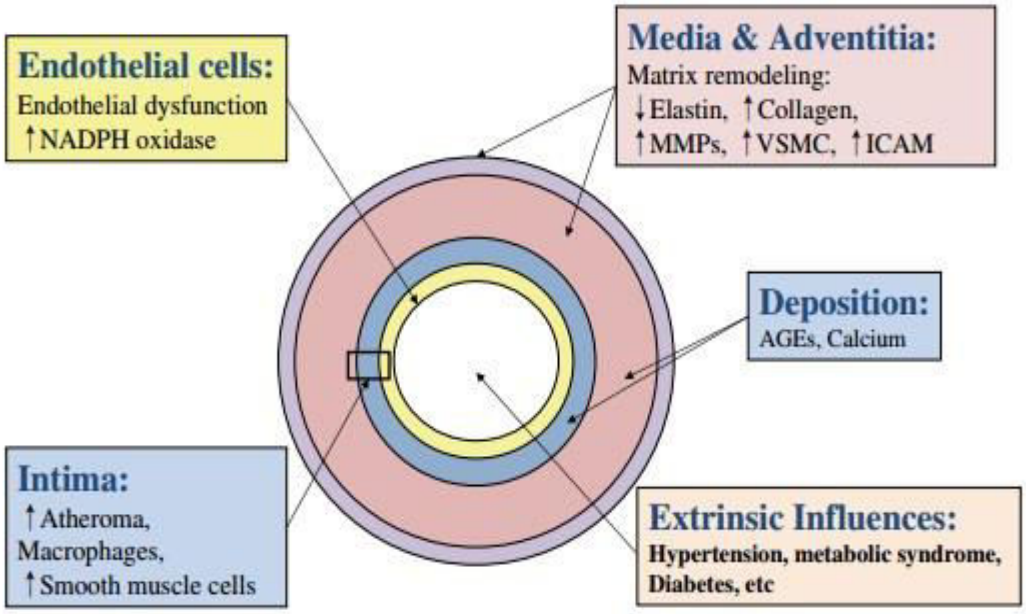


Figure 3 Causes of arterial stiffness (Reference: Lee HY & Oh BH., 2010)

Chowienczyk P., et al 2012). The direct effect of long standing pulsatile stress on the structural matrix proteins, collagen and elastin in the arterial wall results in disruption of muscular attachments and fracture of elastin fibers (Lee HY & Oh BH., 2010).

Arterial stiffness also occurs from deposition of advanced glycation end products (AGE) on the proteins leading to alteration in their physical properties. Calcium deposition in the arterial wall might also contribute to reduction in the vascular compliance with age, particularly after the 5^{th} decade (Atkinson J., 2008).

The functional change in the arterial system that contributes to stiffness is age-associated deterioration in endothelial function (Jin RC & Loscalzo J., 2010). Impaired vasomotor function associated with endothelial dysfunction leads to thickening of the intima-media layer, especially in the peripheral muscular arteries and can contribute to increase in peripheral vascular resistance, a pathognomonic characteristic of hypertension in the elderly population (Taddei S et al., 2001; Torregrossa AC et al., 2011). It has been reported that aside from extracellular matrix, increased vascular stiffness with aging is also attributable to intrinsic changes in vascular smooth muscle cells (VSMCs) by increasing the expression of adhesion molecule (Qiu H et al., 2010).

Arterial stiffness is an independent and strong predictor of CV morbidity and mortality in hypertensives without any overt CV disease (Blacher J et al., 1999; Laurent S et al., 2001) and also in well-functioning older adults (Sutton-Tyrrell K et al., 2005).

A number of genetic factors which influences arterial stiffness have also been identified. Polymorphic variation in the fibrillin-1 (Medley TL et al., 2002), angiotensin II type-1 receptor (Lajemi M et al., 2001) and endothelin receptor genes were found associated with vascular stiffness (Lajemi M et al., 2001).

2.5.2. Age-associated functional changes in arterial system: vascular endothelial dysfunction

Age also affects the regulation of vascular resistance by vascular endothelium. Vascular endothelium is a thin single layer of endothelial cells that lines the innermost surface of the entire vascular system i.e. all the blood vessels. In adults, approximately ten trillion (10^{13})

cells form an 'organ' with a large surface of approximately about 350m^2 area and about 110 g weight (Pries AR & Kuebler WM., 2006). Endothelial cell structure and functional integrity are important for various vital CV functions and integrity (Galley HF & Webster NR., 2004). The vasodilator function of endothelium was first demonstrated by Furchgott and Zawadki in 1980. They demonstrated that the removal of endothelial layer of isolated arteries prevents the in vitro dilator response to acetylcholine (Furchgott RF & Zawadzki JV., 1980). The key factor responsible for arterial relaxation was first discovered as endothelium derived relaxing factor (EDRF) and later identified it as NO (Vanhoutte PM et al., 2009). Nitric oxide, a key determinant of vascular homoeostasis, is a simple molecule that regulates vascular tone, vascular permeability and antithrombotic properties (Jin RC & Loscalzo J., 2010).

A. Functions of vascular endothelium

The endothelium is a highly dynamic cell layer that is involved in a multitude of physiological functions, including regulation of perfusion, fluid and solute exchange, haemostasis and coagulation, inflammatory responses, vasculogenesis and angiogenesis (Aird WC., 2004; Pries AR & Kuebler WM., 2006). Endothelium by secreting various mediators is involved in both synthetic and metabolic functions (Table 6).

1. **Vascular homoeostasis**: Vascular endothelium regulates several physiological properties of the blood vessel, including vasodilation, vascular permeability and antithrombotic properties. Nitric oxide is key determinant of vascular health (Jin RC & Loscalzo J., 2010).
2. **Haemostasis and coagulation**: Vascular endothelium is critical for protecting against vascular injury and maintaining blood fluidity. Normal endothelium produces a number of substances which regulate haemostasis and coagulation: (a) Prostacyclin and nitric oxide are vasodilators and potent inhibitors of platelet and monocyte activation. Normal endothelial surface inhibits platelet aggregation. (b) Thrombomodulin serves as a binding site for thrombin to activate protein C and heparin-like molecules serve as a cofactor for antithrombin III. (c) Tissue plasminogen activator activates the fibrinolysis system. (d) von Willebrand factor mediates platelet adhesion and shear-stress-induced

Table 6 Products of vascular endothelial cells

1.	**Vasodilator Factors**	Nitric oxide
		Prostacyclin (PGI_2)
		Endothelium derived hyperpolarization factor (EDHF)
2.	**Vasoconstricting factors**	Endothelin (ET)
		Thromboxane A_2 (TXA_2)
		Angiotensin converting enzyme
		Leukotrienes
		Free radicals or Reactive oxygen species (ROS)
3.	**Procoagulant factors**	Von Willebrand factor
		Thromboxane A2
		Thromboplastin
		Factor V
		Platelet activating factor
		Plasminogen activator inhibitor
4.	**Antithrombotic factors**	Prostacyclin
		Thrombomodulin
		Antithrombin
		Plasminogen activator
		Heparin
5.	**Growth factors**	Insulin like growth factor
		Transforming growth factor
		Colony stimulating factor
6.	**Lipid metabolism**	LDL-receptor
		Lipoprotein lipase
7.	**Matrix products**	Fibronectin
		Laminin
		Collagen
		Proteoglycans
		Proteases
8.	**Inflammatory mediators**	Interleukins 1,6,8
		Leukotrienes
		Major histocompatability complex class II (MHC II)

(Reference: Modified, Galley HF & Webster NR., 2004)

aggregation. Endothelial injury results in loss of protective substances and expression of adhesive molecules, procoagulant activities, and mitogenic factors leading to thrombosis formation and atherosclerosis (Wu KK & Thiagarajan P., 1996).

3. **Vascular tone & blood pressure**: Endothelial cells by secreting a number of vasodilators (NO, prostacyclin) and vasoconstrictors (endothelin, thromboxane A_2) regulates vascular tone and BP.
4. **Angiogenesis**: Angiogenesis refers to the growth of new blood vessels (or damaged blood vessels) from pre-existing endothelium. Vascular endothelium produces vascular endothelial growth factor (VEGF) which mediates angiogenesis.
5. **Barrier function**: Tight junction between endothelial cells acts as a 'gate' or semi-selective barrier between the blood and surrounding tissue, and controls the passage of substances, leucocytes, ions and water into and out of the blood stream. Increased vascular permeability leads to oedema.
6. **Anti-inflammation**: Endothelium produces various inflammatory mediators and prevents inflammation.

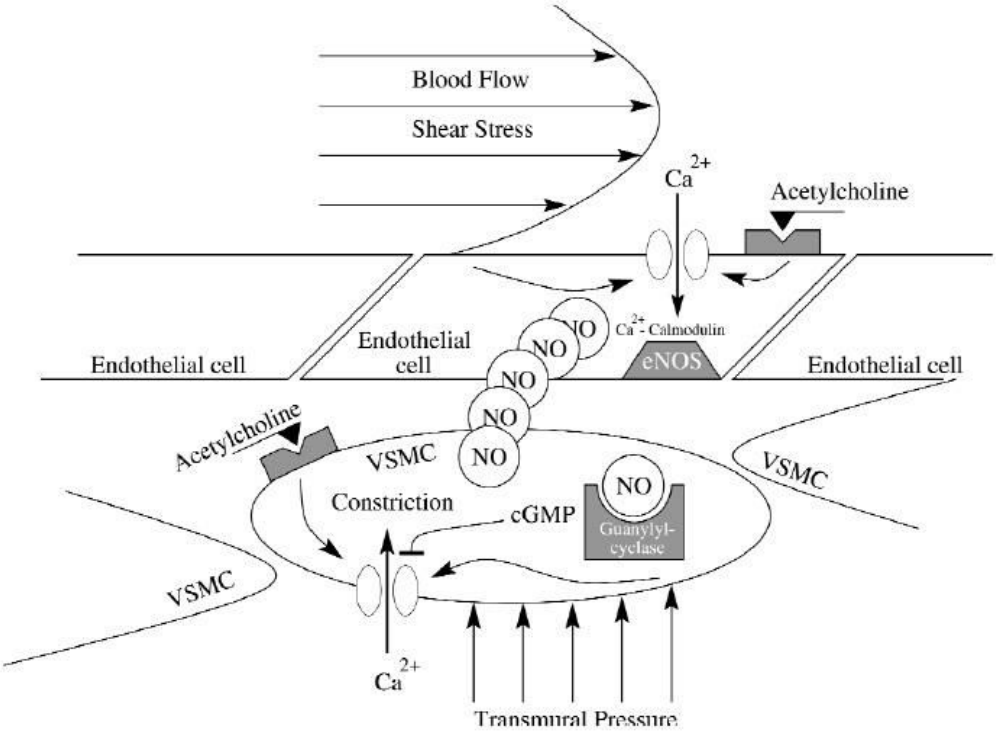
B. Regulation of blood pressure by vascular endothelial system

Endothelial system plays an important role in short-term regulation of BP like baroreceptor reflex (Stauss HM & Persson PB., 2000). Normal levels of NO produced by endothelial cells is critical for the maintenance of basal vascular tone and BP (Jin RC & Loscalzo J., 2010).

The mechanism of regulation of blood pressure by endothelial system is as follows (Figure 4)

i. Elevation in BP increases vascular shear stress.
ii. Vascular shear stress, a mechanical stimulus causes an increase in concentration of cytosolic Ca^{2+} in the endothelial cells.
iii. Ca^{2+} binds with calmodulin and forms a Ca^{2+}- calmodulin complex. This complex increases the activity of endothelial isoform of nitric oxide synthase (eNOS).
iv. Nitric oxide produced by eNOS diffuses into the adjacent VSMCs and activates an enzyme guanylyl cyclase (paracrine effect).

v. Activated guanylyl cyclase increases the synthesis of 3,5-cyclic guanosine monophosphate (cGMP).
vi. cGMP reduces the intracellular (VSMCs) Ca^{2+} concentration.
vii. Reduction in intracellular Ca^{2+} concentration results in inhibition of Ca^{2+}- calmodulin myosin light chain kinase complex formation in the VSMCs promoting relaxation.
viii. Relaxation of VSMC decreases the vascular resistance, tone and thus reduces BP.



(Reference: Stauss HM & Persson PB., 2000)

Figure 4 Mechanism of the vascular blood pressure control system

C. Ageing and endothelial dysfunction

Endothelial dysfunction is characterized by a shift of the normal endothelial function towards reduced vasodilation, a pro-inflammatory state and pro-thrombic properties (Endemann DH & Schiffrin EL., 2004). The age-related endothelial dysfunction associated with decreased bioavailability of NO contributes to increase in vascular tone, arterial

stiffness and hypertension (Matz RL et al., 2000; Torregrossa AC et al., 2011; Jin RC & Loscalzo J., 2010). A shift in endothelial function towards the vasoconstrictor dominance

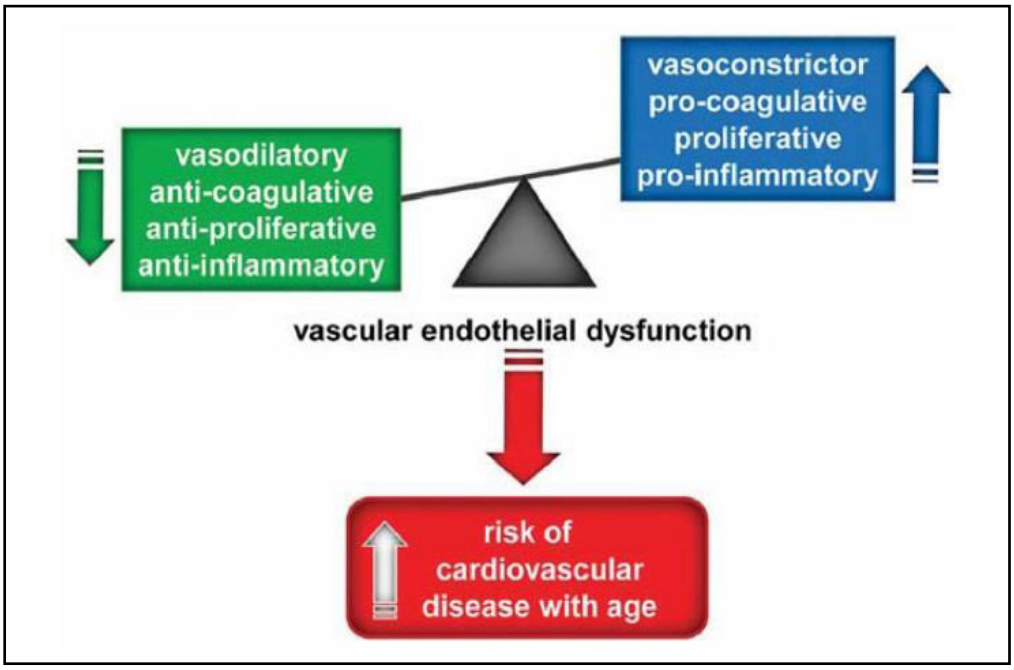


Figure 5 Endothelial dysfunction

increases the peripheral vascular resistance, a pathognomonic characteristic of hypertension in the elderly (Figure 5). The endothelial-dependent vasodilator function is reduced with aging and this impaired NO-mediated vasodilatation is a potential contributor to the age-related increase in arterial stiffness and peripheral vascular resistance (Wilkinson IB et al., 2002; Fitch RM et al., 2001). Coronary endothelial dysfunction is an independent predictor of all-cause and CV mortality (Schachinger V et al., 2000; Suwaidi JA et al., 2000). It has been demonstrated that local arterial stiffness increases by blocking NO synthesis (Wilkinson IB et al., 2002) and removal of vascular endothelium in animal models (Boutouyrie P et al., 1997) indicating that endothelium derived NO contributes to the regulation of large artery stiffness in vivo.

2.5.3. Age-related changes in autonomic nervous system

The autonomic nervous system maintains vascular homeostasis through pressure, volume and chemoreceptor signals. The three endogenous catecholamines which play important roles in cardiovascular regulation are nor-epinephrine, epinephrine and dopamine.

The regulation of vascular resistance is also affected by age-related changes in the autonomic nervous system. An age-related increase in sympathetic nervous system activity has been demonstrated by higher plasma nor-epinephrine levels (Seals DR, Esler MD., 2000) and muscle sympathetic nerve activity (Malpas SC., 2010; Supiano MA., 2009). This rise in plasma nor-epinephrine levels with age is thought to be a compensatory mechanism for age-related decrease in beta-adrenergic response (Seals DR, Esler MD., 2000). Arterial baroreceptor sensitivity declines with age. This age-related decline in baroreceptor sensitivity leads to relatively greater activation of sympathetic nervous system (compensatory mechanism) for a given level of BP (Supiano MA., 2009).

Sympathetic nervous system maintains vascular tone. Its overactivity increases vascular tone, vascular stiffness and thus hypertension. Age-related arterial stiffness was shown to be associated with increased sympathetic activity in hypertensive (Mancia G et al., 1999) and also in healthy subjects (Dinenno FA et al., 2000). Studies have also shown an association between increased sympathetic activity and endothelial dysfunction (Hijmering ML et al., 2002; Thijssen DHJ et al., 2006). Thijssen et al. demonstrated that sympathetic activation results in decrease in endothelial-dependent flow mediated dilatation (FMD) in superficial femoral artery in older persons and attenuation of this sympathetic activity restores the FMD (Thijssen DHJ et al., 2006).

2.5.4. Oxidative stress

Age-associated increase in oxidative stress has been implicated as one of the underlying causes of hypertension (Ceriello A., 2008; Mateos-Caceres PJ et al., 2012; Briones AM et al., 2010; Grossman E., 2008). An increase in production of ROS such as superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen causes oxidative stress. Although ROS are generated in multiple compartments and by multiple enzymes within the cell, but the majority of ROS are produced within the mitochondria during ATP production by oxidative phosphorylation contributing to aging and age-related disorders. If ROS are not removed or neutralized, it can target various cellular constituents like lipid membranes, proteins, DNA and RNA. Our body has evolved complex antioxidant defense mechanism to prevent the deleterious effects of ROS. An imbalance between ROS and antioxidants results in oxidative stress (Kohen R et al., 2002). Oxidative

stress contributes to inactivation of NO resulting in its reduction in bioavailability and endothelial dysfunction (Schulz E et al., 2011; Silva BR et al., 2012). Endothelial dysfunction associated with decreased NO production results in impaired vasodilation and increased BP.

Reactive oxygen species influences cardiovascular structure and function by modulating cell growth and inflammatory responses via reduction-oxidation-dependent signaling pathways. Increased vascular oxidative stress damage the endothelium, reduces nitric oxide production by inhibiting eNOS pathways and impairs endothelium-dependent vasodilation with resultant enhanced vascular tone and thus hypertension (Briones AM et al., 2010; Grossman E ., 2008). Further, oxidative stress causes thickening of the vascular media by promoting smooth muscle cell proliferation and hypertrophy with collagen deposition resulting in narrowing of vascular lumen (Grossman E ., 2008; Schulz E et al., 2011). These evidences suggest that oxidative stress may play an important role in the development of hypertension.

2.5.5. Neurohormonal changes

Aging also declines the neurohormonal mechanisms such as the renin-angiotensin-aldosterone system and contributes to elevation in BP. In general, the elderly population has low levels of plasma renin activity, i.e. about 40%-60% of the levels found in younger individuals (Epstein M., 1996). This decreased plasma renin activity has been attributed to the effect of age-related nephrosclerosis on the juxtaglomerular apparatus (Lionakis N et al., 2000). Plasma aldosterone levels also declines with age. Age-related changes in kidney function associated with decreased ability to excrete sodium load may also contribute to an elevation of BP in elderly.

2.5.6. New molecular mechanisms associated with hypertension in elderly

The new proposed mechanisms involved in the development of hypertension in elderly are as follows (Figure 6):

a. Telomere shortening: Studies have shown a strong association between shorter telomere length and hypertension. Telomeres are the ends of the chromosomes that protect the end

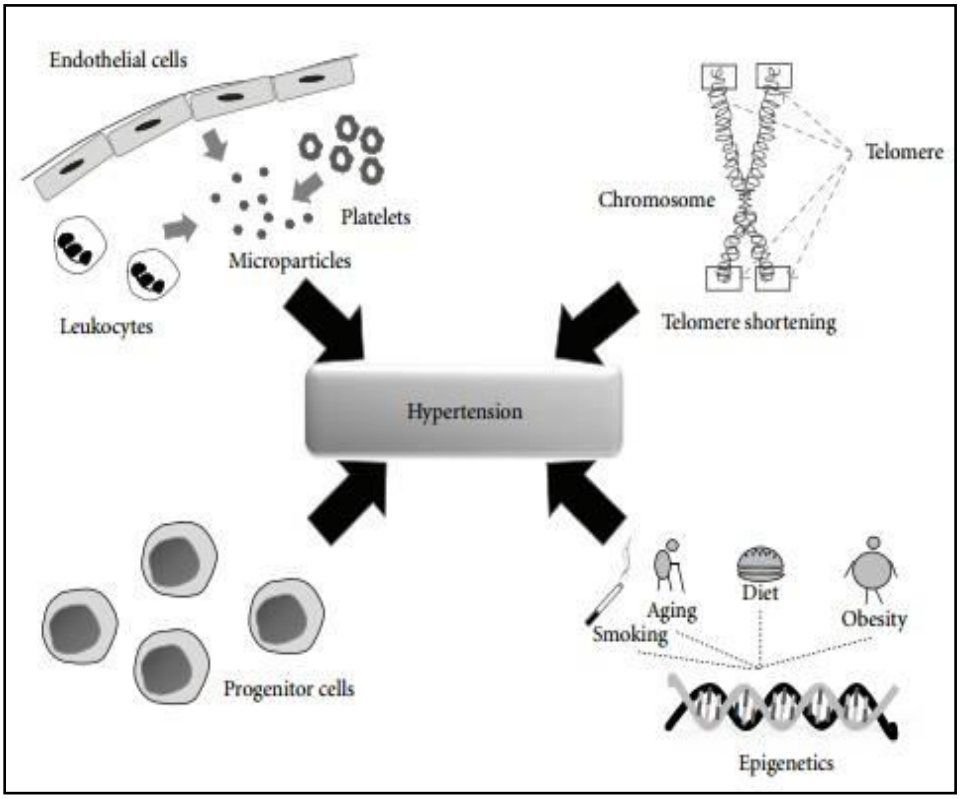


Figure 6 New proposed mechanisms involved in hypertension in the elderly population (Reference: Mateos-Caceres PJ et al., 2012)

of the chromosome from deterioration and preserve genomic integrity. The length of telomeres gets shortened progressively with replications. Growing evidence suggests that telomere shortening can be used as a marker of biological aging of the cardiovascular system and predictor for developing hypertension (Mateos-Caceres PJ et al., 2012).

b. Increase of deleterious micro particles: Small circulating procoagulant, prothrombotic and pro-inflammatory particles in plasma are called microparticles. These circulating microparticles are shed from the surface of different types of cells (platelet, leucocyte, erythrocyte and endothelial cells) in response to activation, injury and/or apoptosis. They are found associated with arterial thrombotic processes and increased in patients with hypertension. The deleterious effect of circulating microparticles on vascular function leads to endothelial dysfunction with impairment in NO production and release (Mateos-Caceres PJ et al., 2012).

c. Epigenetics and lifestyle: Epigenetics studies the interaction of DNA and its expression with the environment. Environmental factors such as diet, stress, obesity, smoking aging, and inactivity or sedentary lifestyle directly affect the incidence of hypertension (Mateos-Caceres PJ et al., 2012).

2.6. Pathologic consequences of hypertension in elderly

Heart: Cardiovascular disease is most common cause of death in hypertensive patients. Hypertension doubles the risk of coronary artery disease (CAD), ischemic heart disease (IHD), congestive heart failure (CHF) and peripheral arterial disease (PAD) (Figure 2). According to the American Heart Association Statistics Committee and Stroke Statistics Subcommittee, the prevalence of myocardial infarction was higher in elderly with hypertension than normal BP (Lloyd-Jones D et al., 2009). About 83% of deaths occurred due to CAD above 65 years of age (Franklin SS et al., 2001). Aortic stiffness is an independent predictor of CAD in patients with essential hypertension (Boutouyrie P et al., 2002). Active treatment leads to reduction in 25% of myocardial infarction and significant decrease in CHF (Supiano MA., 2009).

Chronic Kidney disease: Ageing is a risk factor for chronic kidney disease. Hypertension along with aging is a major risk for chronic kidney disease (CKD) (Figure 2). Systolic blood pressure is an independent predictor for CKD in elderly individuals with hypertension (Young AJH et al., 2002).

Cerebrovascular disease: In the elderly population, hypertension is a major risk factor for brain infarction and cerebral hemorrhage. The important component of BP related stroke risk is isolated systolic hypertension. Active treatment leads to reduction in 35% of stroke and significant decrease in dementia (Supiano MA., 2009). Systolic hypertension in elderly program (SHEP) has demonstrated that reduction of BP by active treatment resulted in reduction of incidence of both ischemic stroke by 37% and hemorrhagic stroke by 54% (Perry HM et al., 2000). Another study, The Systolic Hypertension in Europe Trial has also confirmed the stroke prevention by active treatment of ISH (PROGRESS Collaborative

Group., 2001). Age and hypertension are also an important risk factor for vascular dementia and Alzheimer's disease (Rosendorff C et al 2007).

2.7. Diagnostic evaluation

Hypertension should never be diagnosed on the basis of a single measurement of BP. In the elderly population, the BP is more variable, so single measurement of BP leads to misdiagnosis of hypertension. A strong association between arterial stiffness and auscultatory gap has been noticed, especially in the elderly. Systolic BP is falsely increased by atherosclerotic and other vascular changes associated with age (Foran TG et al., 2004). As the measurement of BP depends on measuring on how much force it takes to compress an artery, so to compress the stiffened arteries the sphygmomanometer reading is falsely increased leading to false measurement of BP and misdiagnosis of hypertension. Hence, it has been recommended that the diagnosis of hypertension should be based on the average of a minimum of nine BP readings that have been measured on three separate visits (Supiano MA., 2009). More than 90% of older individuals suffer from essential hypertension. A diagnostic evaluation for finding the secondary causes for hypertension should be done as per the standard guidelines (The task force for the management of arterial hypertension of the ESH & ESC., 2007).

2.8. Management of Hypertension in elderly

There are evidences from the studies that lowering of BP in elderly hypertensive patients reduces CV morbidity and mortality. According the 2007 ESH/ESC Guidelines, the initiation of antihypertensive treatment should be based on the level of BP and total CV risk. When to initiate an intervention for blood pressure (life-style modality or antihypertensive intervention) has been summarized in Figure 7 (The task force for the management of arterial hypertension of the ESH & ESC., 2007). In general, the recommended target of treatment is to reach goal of SBP below 140 mmHg and DBP below 90 mmHg in hypertensive patients.

Blood pressure (mmHg)					
Other risk factors OD or disease	Normal SBP 120–129 or DBP 80–84	High normal SBP 130–139 or DBP 85–89	Grade 1 HT SBP 140–159 or DBP 90–99	Grade 2 HT SBP 160–179 or DBP 100–109	Grade 3 HT SBP≥180 or DBP≥110
No other risk factors	No BP intervention	No BP intervention	Lifestyle changes for several months then drug treatment if BP uncontrolled	Lifestyle changes for several weeks then drug treatment if BP uncontrolled	Lifestyle changes + Immediate drug treatment
1–2 risk factors	Lifestyle changes	Lifestyle changes	Lifestyle changes for several weeks then drug treatment if BP uncontrolled	Lifestyle changes for several weeks then drug treatment if BP uncontrolled	Lifestyle changes + Immediate drug treatment
≥3 risk factors, MS or OD	Lifestyle changes	Lifestyle changes and consider drug treatment	Lifestyle changes + Drug treatment	Lifestyle changes + Drug treatment	Lifestyle changes + Immediate drug treatment
Diabetes	Lifestyle changes	Lifestyle changes + Drug treatment			
Established CV or renal disease	Lifestyle changes + Immediate drug treatment	Lifestyle changes + Immediate drug treatment	Lifestyle changes + Immediate drug treatment	Lifestyle changes + Immediate drug treatment	Lifestyle changes + Immediate drug treatment

Figure 7 When to initiate intervention for hypertension. (SBP: systolic blood pressure; DBP: diastolic blood pressure; CV: cardiovascular; HT: hypertension; OD: subclinical organ damage; MS: metabolic syndrome) (Reference: The task force for the management of arterial hypertension of the ESH & ESC., 2007)

2.8.1. Non-pharmacological approach

Lifestyle modifications are widely accepted as a very important aspect not only for prevention of hypertension and CV risk but also for management of hypertension. It is to often overlooked in the management of hypertension in elderly. According to ESC/ESH 2007 guidelines, an appropriate lifestyle measures should be instituted in all patients, even with high BP including those who require drug treatment. The purpose of lifestyle changes is to control BP and CV risk factors, and to reduce number of doses of antihypertensive drugs. The recommendations as illustrated in Figure 7 are to begin with appropriate lifestyle modifications for upto 6-month period for Grade-I hypertension and for several weeks for Grade-II hypertension if not associated with any CV risk factors. In cases of Grade-I and II

hypertension with 1 or 2 CV risk factors then the intervention can be tried with life-style modality for several weeks. However, if life-style modality fails to reduce the BP to the level of recommended target then drug (antihypertensive) treatment can be commenced. Lifestyle changes should be used as an adjunctive, even if the drug therapy is needed (The task force for the management of arterial hypertension of the ESH & ESC., 2007).

The lifestyle modalities that are known to reduce BP or CV risk factors are smoking cessation, moderation of alcohol consumption, weight reduction in the overweight, reduction of salt intake, decrease in saturated and total fat intake, exercise, yoga, meditation and acupuncture. The JNC 7 gives an estimate of SBP changes for various lifestyle interventions. SBP decreases approximately by 5-20 mmHg per 10 Kg loss of weight in overweight, 2-8 mmHg by dietary sodium restriction, 4-9 mmHg by physical activity, 2-4 mmHg by moderate alcohol consumption and 8-14 mmHg by Dietary approaches to stop hypertension (DASH) diet (Chobanian AV et al., 2003).

The Trial of Non-pharmacologic Intervention in the Elderly (TONE) studied the effect of dietary sodium restriction, weight loss or combination of both in obese and non-obese patients with hypertension (BP<145/85 mmHg) while taking one antihypertensive. The participants were weaned from their antihypertensive drug with a goal of discontinuing the drug altogether following 90 days intervention (first session). The primary end points were the finding of elevated BP after drug weaning or discontinuation, the need to reinstitute antihypertensive therapy, CV events and death. The intervention led to fairly modest declines in dietary sodium (average of 40mmol/day) and body weight (average 3.5 Kg). About 40% participants did not experience a rise in BP and there was no need to reinitiate antihypertensive therapy for about 30 months (Whelton PK et al., 1998).

A regular aerobic exercise for 30 min for 12 week program has lowered SBP by 8.5 mmHg, DBP by 5.1 mmHg and PP by 3.2 mmHg (Westhoff TH et al., 2007). Dietary modification is also an important lifestyle modality to lower BP in elderly. DASH diet with rich fruits, vegetables and low-fat dairy foods was shown as an effective and beneficial in Stage-1 Isolated systolic hypertension (Moore TJ et al 2001). In this study, Dash diet lowered SBP by 11.2 mmHg when compared to control group.

Yoga is emerging as an important lifestyle modality and physiological means for prevention and management of CV risk. Yoga is spiritually based, so elderly population may be more interested in practicing and following its lifestyle. It has many established health benefits. There are growing evidences that Yoga effectively controls hypertension and improves CV function (Refer section 5.3 for details).

2.8.2. Pharmacological approach

There are evidences from the studies that antihypertensive drug treatment in elderly patients benefitted in terms of reduced CV morbidity and mortality. A reevaluation of trials has found that no single trial has enrolled patients with Grade-1 hypertension. Results from meta-analysis of eight trials on elderly hypertensive patients has shown a reduction in total mortality by 13%, CV deaths by 18%, stroke by 30%, and coronary events by 23% following antihypertensive therapy (Staessen JA et al., 2000). The most common adverse effect caused by antihypertensive treatment is the development of postural hypotension. Therefore, it is recommended and important not to treat elevated BP too aggressively. The pharmacological drugs for hypertension in elderly with its main action and CV benefits are summarized in Table 7.

It has also been noticed that the elderly individuals suffering from isolated systolic hypertension are often resistant to pharmacological treatment, so any attempts to reduce the SBP aggressively lowers DBP (decreased with age) to such an extent to compromise coronary blood flow (Calhoun DA et al., 2008; Vongpatanasin W., 2014; Satoshkar RS et al., 2005). Aggressive approach to reduce the BP may also harm auto regulation of blood flow. Moreover, it has also been reported that arterial stiffness increases at a faster rate even in treated hypertensives with well controlled BP than in a normotensives (Benetos A et al., 2002). These reports indicate that an adequate approach that controls hypertension along with the progression of arterial stiffness with age is the need of the hour, in order to prevent the CV mortality and morbidity.

Table 7 Pharmacological agents for hypertension: Main action and cardiovascular benefits

	Agent	Main action	Cardiovascular Benefits
1.	Thiazide (diuretic)	Inhibit reabsorption of sodium (Na^+) and chloride (Cl^-) ions from the distal convulated tubules in the kidneys.	Reduces BP, Stroke & CV mortality.
2.	Angiotensin converting enzyme inhibitors (ACEIs)	Block the conversion of angiotensin I to angiotensin II.	Decreases systemic vascular resistance, BP, mortality in patients with MI and left ventricular dysfunction and progression of diabetic renal disease.
3.	Angiotensin receptor blockers	Direct blockage of angiotensin II receptors	Causes vasodilation and decreases systemic vascular resistance, decreases secretion of vasopressin and aldosterone, lowers BP and stroke.
4.	Calcium antagonists	Disrupts the movement of calcium through calcium channels in cardiac muscle and peripheral arteries.	Vasodilation and decrease in systemic vascular resistance, lowers BP, decreases CV complications in elderly patients with ISH.
5.	β blockers	Lowers heart rate, decreases cardiac contractility and cardiac output, inhibit renin release, increase nitric oxide production, and reduces vasomotor tone.	Lowers BP
6.	Other Agents: Renin inhibitors, Aldosterone receptor antagonists, Centrally acting agents, direct vasodilators	Lowers BP	

3. METHODS FOR ASSESSMENT OF ARTERIAL STIFFNESS

There are many invasive and non-invasive methods for the evaluation of arterial stiffness in the human beings.

3.1. Pulse pressure

Pulse pressure is a simple and best tool for measuring arterial stiffness and a good marker for CV risk in the elderly. Pulse pressure is an independent indicator of arterial stiffness. Studies have shown a positive correlation between PP and arterial stiffness (Safar ME., 2000; Safar ME et al., 2003; Cecelja M et al 2009). Systolic and diastolic pressure tends to increase with age upto 50-55 years. After 50-55 years, in most of the individuals the diastolic pressure falls and only systolic pressure rises with age thus causing widening of PP. Moreover, measurement of only PP is not adequate to assess arterial stiffness. Since age-related stiffness is greater in the aorta (elastic artery) than peripheral arteries, central aortic PP is a good marker than brachial PP to assess arterial stiffness.

Growing evidence suggests that PP is an important predictor of risk in elderly. Pulse pressure is more closely associated to CV events than SBP or DBP alone (Franklin SS et al., 2001). A meta-analysis of several studies with data of 8,000 elderly patients found that a 10mmHg increase in PP increased the risk of major CV complications and mortality by nearly 20% (Blacher J et al, 2000). Moreover, PP was an independent predictor of stroke and all-cause mortality in the SHEP study (Domanski MJ., 1999).

3.2. Pulse Wave velocity (PWV)

Pulse wave velocity measurement is the most simple, accurate and reproducible method for the assessment of regional arterial stiffness. It is the speed at which the forward pressure wave is transmitted from the aorta through the arterial tree (Mackenzie IS et al., 2002). It is widely used as an index of large artery elasticity and stiffness. PWV can be calculated in any segment of the circulation, provided the pulse waveform at two arterial sites is possible to record and time elapsed between the travels of waves and distance between them can be measured (Deloach SS & Townsend RR., 2008). There are various established methods for measuring PWV. The pulse waves can be recorded in different arteries using various sensors, transducers or probes, among which the most common are pressure sensitive transducers,

applanation tonometry (Nelson MR et al., 2002), Doppler ultrasound (Calabia J et al., 2011), piezoelectric transducers (Willum-Hansen T et al., 2006) and photoelectric transducers. The PWV of the given artery can be calculated by measurement of the transit time of the pulse waves (Δt) and the distance between two recording points (d) as follows:

$$PWV\ (cm/m) = \frac{\Delta t}{d}$$

The pulse transit time is the time taken by pulse wave to travel (between points) from peripheral wave to distal wave. The pulse transit time can be calculated by measuring time in seconds elapsed between the peak of the R-wave of ECG and the foot or onset of the pulse waves or between the foot of peripheral and distal wave (foot-foot method).

The PWV can be measured at different regions such as:

- Carotid- femoral PWV
- Carotid-radial PWV
- Femoral-tibial PWV
- Heart-brachial PWV
- Heart-ankle PWV
- Brachial-ankle PWV

Arterial stiffness increases with age due to decrease in elasticity and aorta is the major component of arterial elasticity. Hence, aortic PWV is the most preferred measure of arterial stiffness. The carotid-femoral PWV (c-f PWV) or aortic PWV is the gold standard method for assessment of aortic stiffness. Aortic PWV can also be measured non-invasively by using MRI. The accurate measurement of path length is an advantage of using MRI, however its usage is limited due to high cost and lack of availability (Mohiaddin RH et al., 1993). Brachial-ankle PWV (baPWV) was also shown as an independent predictor of carotid atherosclerosis in the elderly (Li JY & Zhao YS., 2010). The baPWV is strongly correlated with c-f PWV. Aortic stiffness is an independent predictor of all cause and CV mortality (Laurent S et al., 2001). It has been shown that, the aortic PWV is more predictive of CV mortality compared with PWV measured in the brachial or femoral circuits in end stage renal disease (ESRD) (Pannier B et al., 2005).

The determinants of the PWV are the elastic properties of the arterial wall, the geometry of the artery and the blood viscosity. Mathematically, Moens-Korteweg defined the PWV as follows (Bramwell JC, Hill AV., 1922):

$$\text{PWV} = \sqrt{Eh/2\rho r}$$

Where E is Young's elastic modulus in the circumferential direction, h is the wall thickness, r is the radius of the vessel and ρ is the blood density. Moens-Korteweg equation can be used to calculate the PWV.

3.3. Arterial distensibility and compliance

The change in diameter of an artery in relation to distending pressure provides a direct measure of arterial stiffness. Distensibility and compliance of a number of arteries such as carotid, brachial, radial and aorta can be assessed. To evaluate these parameters, the diameter of the artery and its pressure is required to be measured. Ultrasound is commonly used imaging technique to measure the arterial diameter. While evaluating the local arterial stiffness of carotid or aorta, brachial BP is most frequently used, assuming that the BP of the aorta and carotid arteries is similar to the brachial artery (Rhee MY et al., 2008). There are conflicts on whether local arterial stiffness reflects the stiffness of other arteries.

3.4. Stiffness index and Reflection index

Stiffness index and reflection index (RIx) reflects systemic arterial stiffness. They are usually measured from the digital volume pulse waveform recorded using Finger Photoplethysmograph (Mackenzie IS et al., 2002).

Reflection index reflects the peripheral vascular tone. A comparative study revealed that pulse wave velocity correlated more closely with the expected influences on vascular compliance (age and atherosclerosis) than photoplethysmography of the digital volume pulse.

3.5. Arterial stiffness index

Arterial stiffness index is estimated by quantifying the oscillometric envelopes derived from the oscillations in the respective artery (Naidu MUR et al., 2012).

3.6. Systemic arterial compliance

Arterial compliance is defined as the relationship between the change in volume and the change in the distending pressure. The simplest method to measure systemic arterial compliance is the ratio of the stroke volume (SV) to the pulse pressure (PP).

$$\text{Compliance} = \frac{\text{SV}}{\Delta \text{P}}$$

The stroke volume can be measured invasively or non-invasively (Rhee MY et al., 2008). Brachial BP is most frequently used, assuming that the Central PP is similar to the brachial artery. Most of the investigators assess the carotid and aorta BP with applanation tonometry using a transfer function. Other method is 'area method' for measuring systemic arterial compliance.

3.7. Augmentation index (AIx)

The arterial pulse wave is composed of a forward pressure wave that arises from the left ventricular output and a backward pressure wave (wave reflection) reflected from the point of impedance mismatch (arterioles). Though, there are many reflection points in the body at various distances from the heart, the reflected waves act like a single wave arising from one functional reflection point. The velocity of pressure wave along the arterial depends on the elasticity of the vessel wall. More the stiffness (less elasticity), higher is the velocity. Normally, the wave reflection arrives at the aortic root during diastole which augments the diastolic pressure and enhances the myocardial perfusion. In the stiffened arteries, the pressure wave travels at high speed along the arterial tree and reflected wave arrives earlier during systole, when the ventricle is still ejecting blood, adding the reflected wave to the forward wave resulting in augmentation of the central systolic pressure. Early arrival of reflected wave during systole leads to decrease in diastolic pressure causing reduction in myocardial perfusion. The rise in the systolic pressure is called an augmentation pressure. The aortic AIx is the ratio of augmentation pressure to the aortic PP and is expressed in percentage. So, the AIx is a simple method to measure the wave reflection, which reflects the arterial stiffness. More the stiffness, higher is the augmentation index (Rhee MY et al., 2008; Mackenzie IS et al., 2002; Laurent S., 2006).

4. METHODS FOR EVALUATING ENDOTHELIAL FUNCTION

There are many established invasive and non-invasive methods for the evaluation of endothelial function in the human beings.

4.1. Flow mediated dilatation

Conduit vessels respond to increase in blood flow (shear stress) by increasing vessel diameter. This phenomenon of vasodilation in response to alterations in blood flow is called as flow-mediated dilatation (FMD). Flow-mediated dilatation is endothelial dependent and is mainly mediated by endothelial-derived NO (Lekakis J et al 2011).

The FMD technique measures changes in conduit artery (mostly brachial artery) diameter by ultrasound in response to two stimuli: endothelial-dependent stimulus (shear stress) and endothelial-independent stimulus (Nitroglycerine). The vasodilation response to the shear stress reflect local bioactivity of endothelial-derived NO while to the nitroglycerine reflect vascular smooth muscle function (Corretti MC et al., 2002). Due to its non-invasive nature and reliability, it is widely used in the study of endothelial physiology. But, to obtain accurate and reproducible measurements, highly trained operators are most essential.

4.2. Coronary endothelial function

Coronary endothelial function can be assessed by both invasive and non-invasive techniques. Quantitative Coronary angiography (QCA) is an invasive technique that measures changes in the epicardial coronary arteries diameter in response to the pharmacological stimuli such as intracoronary infusion of endothelial agonists (acetylcholine, metacholine or papaverine) and vascular smooth muscle relaxants (nitroglycerine). Non-invasive methods have been developed to assess coronary endothelial function using computed tomography (CT) (Husmann L et al., 2008) imaging or magnetic resonance imaging (MRI) (Terashima M et al., 2008).

4.3. Venous occlusion Plethysmography

This is the oldest method (established more than 100 years ago) used to assess the blood flow in humans. Venous occlusion plethysmography (VOP) is an invasive technique to assess endothelial function. It is based on the measurement of tissue (usually muscular) blood flow by the assessment of the tissue volume change. Strain-guage technique is a highly reproducible and minimal invasive VOP method that is applied in the forearm to investigate *in vivo* endothelial function in the human microcirculation. This technique requires brachial artery cannulation for intra-arterial infusion of endothelial agonists and vascular smooth muscle factors in order to assess endothelial-dependent and independent vasodilation respectively (Lekakis J et al., 2011).

4.4. Pulse wave analysis

Endothelial function can be assessed by quantifying the changes in waveform (pressure waveform or digital volume pulse waveform) in response to the endothelial-dependent agonist (salbutamol) and vascular smooth muscle dilators. The arterial pulse wave or digital volume pulse is composed of a forward pressure wave that arises from the left ventricular output and a backward pressure wave (wave reflection) reflected from the point of impedance mismatch (mainly arterioles). This waveform contains important information about the arterial stiffness and endothelial function.

Wave reflection can be quantified by determining AIx or RIx, which represents the difference between the first and second systolic peaks (Chowienczyk PJ et al 1999). Impedance of the small arteries and arterioles depends to a large extent on smooth muscle tone which is mainly mediated by endothelium-derived NO. Thus, changes in small artery or arteriole tone affect wave reflection, so vasodilation reduces AIx or RIx while vasoconstriction increases them (Lekakis J et al., 2011).

4.5. Peripheral arterial tonometry

Recently, a simple, non-invasive technology based on measurement of peripheral vasodilator response at fingertip to reactive hyperaemia induced by temporary arterial

occlusion (digital reactive hyperaemia) known as peripheral arterial tonometry (EndoPAT) has been developed to assess peripheral vascular endothelial function (Kuvin JT et al., 2003).

4.6. Laser Doppler flowmetry

This technique is based on monitoring of skin microvascular blood flow with the assumption that the response noticed in the cutaneous circulation is a window towards the responses that should be observed in other vascular beds (Lekakis J et al., 2011). Laser Doppler flowmetry measures the changes in skin blood flow in response to the acetylcholine (endothelial agonist) delivered through iontophoresis or micro-dialysis, post-occlusive hyperaemia or local skin heating.

4.7. Biochemical markers

The biomarkers used to examine endothelial function are plasma asymmetrical dimethylarginine (ADMA) concentrations, oxidized low-density lipoprotein, vascular cell adhesion molecular (VCAM)-1, intercellular adhesion moledule (ICAM)-1, endothelial leucocyte adhesion molecule (ECAM)-1, total serum nitric oxide concentration and eNOS activity (Burger D & Touyz RM., 2012; Lekakis J et al., 2011).

5. YOGA

5.1. Introduction

Yoga is an ancient system having a psycho-somatic discipline, comprising physical and mental techniques that help to achieve a harmony between our mind and body. It is a tradition of lifestyle, health and spirituality. The term 'Yoga' is derived from Sanskrit word 'Yuj' which means joining. It is joining of individual self with universal self. Yoga is a conscious process of gaining mastery over the mind. It is a special skill to calm down the mind. According to Swami Vivekananda, yoga is a means of compressing one's evolution into a single life or a few months or even a few hours of one's bodily existence. Sri Aurobindo considers it as a means for self-perfection (Nagendra HR., 2004).

Yoga is originated in India and has a history of about 5000 years. Its roots are found in the Vedic period. After the period of Vedas, one of the great Seers, Maharishi Patanjali systematized yoga by compiling the essential features and principles of Yoga in the form of aphorisms (Sutras) about 5000 years ago. 'Yoga Sutra' was the text written by Maharishi Patanjali on classical yoga, the origin of which is estimated to date back to the period between 200 BC and 300 AD. After Maharishi Patanjali, many seers have contributed for the development of Yoga worldwide. Yoga includes diverse practices such as maintenance of posture (asanas), breathing practices (Pranayama), spiritual lectures, and meditation including prayer and devotional songs.

5.2. Streams of Yoga

There are four streams of yoga. They are as follows

i. Jnana Yoga

Jnana Yoga is the yoga of knowledge. Jnana is the Sanskrit word which means 'knowledge'. It is a means to inquire into its own nature. Yoga practitioner (Yogi) uses his mind to inquire into its own nature. It sharpens the mind and helps to discriminate between the real and the unreal, the permanent and the transitory.

ii. Bhakti Yoga

Bhakti Yoga is the science of emotion culture. It is the path of worship. This path of worship is a boon to gain control over emotional instabilities by properly harnessing the energy involved in it. This path overcomes our selfishness, hatred, greed, jealousy and raises us to the highest levels of universal brotherhood and oneness. In the path of workship or bhakti, we surrender in total ourselves physically, mentally and intellectually.

iii. Karma Yoga

Karma Yoga is the path of work or action. It involves doing action selflessly without thought of gain or reward. By detaching ourselves from the fruits of our actions and offering them up to God, we learn to sublimate the ego. Bhagavad Gita defines as"Karma Yoga is the selfless devotion of all inner as well as the outer activities as a sacrifice to the Lord of all works, offered to the eternal as master of all the soul's energies and austerities".

iv. Raja Yoga

Raja Yoga is the path of will power. It is the science of physical and mental control. Raja Yoga is a conscious process of gaining mastery over the mind. It is based on Astanga Yoga (referring to the eight limbs) described in Yoga Sutras by Maharishi Patanjali. One can reach to the higher states of consciousness through eight limbs of Astanga Yoga. They purify the body and mind. The eight limbs are broadly divided in two categories: Bahiranga Yoga (used for indirect control of mind) and Antaranga Yoga (mind is used directly for culturing itself). They are summarized in Table 8.

5.3. Yoga and cardiovascular health

Yoga has many established health benefits and is emerging as an important lifestyle modality for prevention and management of CV risk. Yoga has been shown to control hypertension and improves CV function in middle-aged subjects (Selvamurthy W et al 1998; Murugesan R et al., 2000; Anand MP., 1999; Bharshankar JR et al., 2003). A meta-analysis of 3168 participants showed evidence for clinically important effects of yoga

Table 8 Eight limbs of Astanga Yoga

No.	Limbs	Description
1.	Yama	Set of prohibitions or Don'ts to gain mastery over mind: • Ahimsa or non-voilence (absence of violence), • Satya or truth (not to speak untruth), • Asteya or non-stealing (not to steal), • Brahmacarya (control of all senses) • Aparigraha or non-possession.
2.	Niyama	A set of Do's: • Saucha or purity- this internal and external cleanliness. • Santosha or contentment • Tapas or austerity • Swadhyaya or study of the sacred texts • Ishwara Pranidhana which is constantly living with an awareness of the divine Presence (surrender to God's Will).
3.	Asana	Yoga postures
4.	Pranayama	Regulation or control of the breath.
5.	Pratyahara	Mastery through senses- withdrawal of the senses in order to still the mind
6.	Dharana	Concentration, focussing of mind or fixing the mind on an object. When dharana is achieved it leads to the next step.
7.	Dhyana	Meditation: is that state of pure thought and absorption in the object of meditation. There is still duality in Dhyana. When mastered Dhyana leads to the last step:
8.	Samadhi	The super conscious state. In Samadhi non-duality or oneness is experienced. This is the deepest and highest state of consciousness where body and mind have been transcended and the Yogi is one with the Self or God.

on CV risk factors and suggested that yoga can be considered as an ancillary intervention for patients with or without CV risk (Cramer H et al., 2014).

Recently, a systematic review (6 studies involving 386 patients) on effect of yoga on essential hypertension shown yoga as an effective modality for lowering BP. The studies included in this review were having a wide variation in the age of subjects from 20-75 years and total duration of intervention ranged from 6 to 12 weeks (Wang J et al., 2013). They reported that yoga significantly lowered SBP (-2 to 29.17) and DBP (-0.74 to -23.67) when compared to conventional treatment or no treatment.

The mechanism of yoga-induced BP reduction in young and middle-aged subjects may be attributed to its beneficial effects on the autonomic neurological function. Various studies have shown that yoga significantly modulates the autonomic nervous system activity. Yoga practice and meditation reduces sympathetic activity and shifts the autonomic balance towards the parasympathetic dominance (Patil SG et al., 2013; Pal GK et al., 2013). Yoga based meditation was also shown to reduce sympathetic activity and increase vagal tone (Pailoor S et al., 2009). Restoration of baroreflex sensitivity (decreased in in patients (middle-aged) with essential hypertension following yoga practice has been reported (Selvamurthy W et al 1998). Recently, we have reported a significant reduction in SBP, DBP and PP following yoga practice in elderly with grade-I hypertension (Patil SG et al., 2014). The mechanism of yoga-induced regulation of BP remains unknown and has to be determined.

There are conflicting results on yoga effects on vascular function. In a cross-sectional study, Duren CM et al. demonstrated a lowering effect of yoga on arterial stiffness in healthy subjects aged between 40 and 65 years (Duren CM et al., 2008). In this study, Yoga group subjects performed yoga at least 2 days a week in the previous year (n=8) and aerobic group subjects performed aerobic exercise for three or more days a week for at least 30 minutes a day for the last year (n=10). In contrast, Hunter SD et al. did not find any significant changes in the arterial stiffness in the yoga practitioners (Hunter SD et al., 2013). This study assessed arterial stiffness in healthy middle-aged and older subjects in two settings: a cross sectional (n=34) and interventional (n=13). They have given 12 weeks of Hatha yoga intervention for sedentary subjects.

However, we could not find any randomized controlled studies that assessed the effect of yoga on vascular stiffness, especially in elderly population with CV risk.

6. REFERENCES

- Aird WC. Endothelium as an organ system. *Crit Care Med* 2004;32(5):S271-9.
- Anand MP. Non-pharmacological management of essential hypertension. *J Indian Med Assoc* 1999;97(6): 220-25.
- Aronow WS, Fleg K, Pepine CJ, Artinian NT, Bakris G, Brown AS, et al. ACCF Task Force. ACCF/AHA 2011 expert consensus document on hypertension in the elderly: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. *Circulation* 2011;31;123(21):2434-506.
- Atkinson J. Age-related medial elastocalcinosis in arteries: Mechanisms, animal models and physiological consequences. *J Appl Physiol* 2008;105(5):1643-51.
- Benetos A, Adamopoulos C, Burean JM, Temmar M, Labat C, Bean K et al. Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. *Circulation* 2002;105(10): 1202-07.
- Bharshankar JR, Bharshankar RN, Deshpande VN, Kaore SB, Gosavi GB. Effect of yoga on cardiovascular system in subjects above 40 years. *Indian J Physiol Pharmacol* 2003;42(2):202-6.
- Blacher J, Asmar R, Djane S. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1999; 33: 1111-1117.
- Boutayeb A, Boutayeb S. The burden of non communicable diseases in developing countries. *Int J Equity Health* 2005;14;4(1):2.
- Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002;39(1):10-5.
- Boutouyrie P, Bezie Y, Lacolley P, Challande P, Chamiot-Clerc P, Benetos A et al. In vivo/in vitro comparison of rat abdominal aorta wall viscosity. Influence of endothelial function. Arterioscler Thromb Vasc Biol 1997;17(7):1346-55.
- Briones AM, Touyz RM. Oxidative stress and hypertension: current concepts. *Curr Hypertens Rep* 2010;12(2):135-42.
- Bramwell JC, Hill AV. The velocity of the pulse wave in man. *Proc Soc Lond (Biol)* 1922;93:194-99.

- Burger D, Touyz RM. Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells and circulating endothelial cells. *J Am Soc Hypertens* 2012;6(2):85-99.
- Calabia J, Torgnet P, Garcia I, Martin N, Guash B, Faur D, Valles M. Doppler ultrasound in the measurement of pulse wave velocity: agreement with the Complior method. *Cardiovascular Ultrasound* 2011; 9:13.
- Caddiolati C, Hanon O, Alperovitch A, Dufouil C, Tzourio C.Masked hypertension in the elderly: cross-sectional analysis of a population-based sample. *Am J Hypertens* 2011;24:674-80.
- Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, White A, Cushman WC, White W, Sica D, Ferdinand K, Giles TD, Falkner B, Carey RM; American Heart Association Professional Education Committee. Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation* 2008; 24;117(25):e510-26.
- Carretero OA, Oparil S. Essential hypertension. Part I: definition and etiology. *Circulation* 2000; 25;101(3):329-35.
- Ceriello A. Possible role of oxidative stress in the pathogenesis of hypertension. *Diabetes Care* 2008;2:S181-4.
- Cecelja M, Chowienczyk P. Role of arterial stiffness in cardiovascular disease. *J R Soc Med Cardiovasc Dis* 2012;1:11.
- Census of India 2001. Office of the Registrar General and Census Commissioner of India. Ministry of Home Affairs. Government of India. Available from: http:// www.censusindia.gov. (Accessed January 20, 2012).
- Celis H, Fagard RH. White-coat hypertension: a clinical review. *Eur J Intern Med* 2004;15(6):348-57.
- Cecelja M, Jiang B, McNeill K et al. Increased wave reflection rather than central arterial stiffness is the main determinant of raised pulse pressure in women and relates to mismatch in arterial dimensions: a twin study. J Am Coll Cardiol. 2009;54: 695-703.

- Chowienczyk PJ, Kelly RP, MacCallum H, Millasseau SC, Andersson TL, Gosling RG. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. *J Am Coll Cardiol* 1999;34(7):2007-14.
- Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr et al. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee. Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003;42(6):1206-52.
- Chinnakali P, Mohan B, Upadhyay RP, Singh AK, Srivastava R, Yadav K. Hypertension in the elderly: prevalence and health seeking behavior. *N Am J Med Sci* 2012;4(11):558-62.
- Chiong JR, Aronow WS, Khan IA, Nair CK, Vijayraghavan K, Dart RA, Behrenbeck TR, Geraci SA. Secondary hypertension: current diagnosis and treatment. *Int J Cardiol* 2008;20;124(1):6-21.
- Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. A report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;39(2):257-65.
- Cramer H, Lauche R, Haller H, Steckhan N, Michalsen A, Dobos G. Effects of yoga on cardiovascular disease risk factors: a systematic review and meta-analysis. *Int J Cardiol* 2014;173(2):170-83.
- Department of economic and social affairs, New York, United Nations. World Population Ageing 1950-2050. Available: http://www.un.org/esa/population/publications/worldageing19502050/pdf/prefaceweb.pdf
- Domanski MJ, Davis BR, Pfeffer MA, Kastantin M, Mitchell GF. Isolated systolic hypertension: prognostic information provided by pulse pressure. *Hypertension* 1999; 34(3):375–80.
- Duren CM, Cress ME, McCully KK.The influence of physical activity and yoga on central arterial stiffness. Dyn Med 2008;7:2.

- Decker WW, Godwin SA, Hess EP, Lenamond CC, Jagoda AS. Clinical policy: critical issues in the evaluation and management of adult patients with asymptomatic hypertension in the emergency department. *Ann Emerg Med* 2006;47:237-49.
- Dinenno FA, Jones PP, Seals DR, Tanaka H. Age-associated arterial wall thickening is related to elevations in sympathetic activity in healthy humans. *Am J Physiol Heart Circ Physiol* 2000;278: 1205-10.
- Deloach SS, Townsend RR. Vascular stiffness: Its measurement and significance for epidemiologic and outcome studies. *Clin J Am Soc Nephrol 2008;*3(1):184-92.
- Endemann DH, Schiffrin EL. Endothelial dysfunction. *J Am Soc Nephrol* 2004;15(8):1983-92.
- Epstein M. Aging and the kidney. *J Am Soc Nephrol* 1996;7(8):1106-22.
- Ezzati M, Lopez AD, Rodgers A, Van der Hoorn S, Murray CJ, Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. *Lancet* 2002; 360(9343):1347-60.
- Fagard RH. Epidemiology of hypertension in the elderly. *Am J Geriatr Cardiol* 2002;11(1): 23-8.
- Fitch RM, Vergona R, Sullivan ME, Wang YX. Nitric oxide synthase inhibition increases aortic stiffness measured by pulse wave velocity in rats. *Cardiovasc Res* 2001; 51: 351-8.
- Foran TG, Sheahan NF, Cunningham C, Feely J. Pseudo-hypertension and arterial stiffness: a review. *Physiol Meas* 2004;25(2):R21-33.
- Franklin SS, Larson MG, Khan SA, Wong ND, Leip Ep, Kannel WB et al. Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. *Circulation* 2001;103(9):1245-49.
- Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288(5789):373-6.
- Galley HF, Webster NR. Physiology of the endothelium. Br J Anaesth 2004;93:105-13.
- Grossman E. Does increased oxidative stress cause hypertension? *Diabetes Care* 2008;31:S185-9.

- Hijmering ML, Stroes ES, Olijhock J, Hutten BA, Blankestijn PJ. Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilation. *J Am Coll Cardiol* 2002;39(4): 683-88.
- Holt-lunstad J, Jones BQ, Birmingham W. The influence of close relationships on nocturnal blood pressure dipping. *Int j Psychophysiol* 2009;71:211-17.
- Hunter DJ, Reddy KS. Noncommunicable diseases. *N Engl J Med* 2013 3;369(14):1336-43.
- Hunter SD, Tarumi T, Dhindsa MS, Nualnim N, Tanaka H. Hatha yoga and vascular function: results from cross-sectional and interventional studies. J Bodyw Mov Ther 2013;17:322-27.
- Husmann L, Gaemperli O, Husmann L, Gaemperli O, Schepis T, Scheffel H, valenta I, Hoeffinghaus T. Accuracy of quantitative coronary angiography with computed tomography and its dependency on plaque composition: plaque composition and accuracy of cardiac CT. *Int J Cardiovac imaging* 2008 Dec;24(8):895-904.
- Hypertension Study Group. Prevalence, awareness, treatment and control of hypertension among the elderly in Bangladesh and India: A multicentre study. *Bull World Health Organ* 2001;79:490‑500.
- Jin RC, Loscalzo J. Vascular nitric oxide: formation and function. *J Blood Med* 2010;2010(1): 147-62.
- Kalavathy MC, Thankappan KR Sarma PS, Vasan RS. Prevalence, awareness, treatment and control of hypertension in an elderly community-based sample in Kerala, India. *Natl Med J India* 2000;13(1):9-15.
- Kowal P, Williams S, Jiang Y, Fan W, Arokiasamy P, Chatterji S. Aging, Health and Chronic conditions in China and India: Results from the multinational study on Global ageing and adult health (SAGE). In National Research Council. Aging in Asia: Findings from new and emerging data initiatives. Smith JP & Majmundar, eds. The national academic press, Washington DC. 2012; pp 415-37.
- Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* 2002;30(6):620-50.

- Kuwajima I, Hoh E, Suzuki Y, Matsushita S, Kuramoto K. Pseudohypertension in the elderly. *J Hypertens* 1990;8(5):429-32.
- Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Hear J* 2003;146(1):168-74.
- Lajemi M, Labat C, Gautier S, Lacolleya P, Safara M, Asmar R et al. Angiotensin II type 1 receptor^{153}A/G and ^{1166}A/C gene polymorphism and increase in aortic stiffness with age in hypertensive subjects. *J Hypertens* 2001;19:407-13.
- Lajemi M, Gautier S, Poirier S, Baquet JP, Mimran A, Gosse P et al. Endothelin gene variants and aortic and cardiac structure in never-treated hypertensives. *Am J Hypertens* 2001;14(8):755-60.
- Laurent S, Cockroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D et al. on behalf of the European network for Non-Invasive Investigation of Large arteries. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006; 27(21): 2588-605.
- Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; 37(5): 1236-41.
- Law M, Wald N, Morris J. Lowering blood pressure to prevent myocardial infarction and stroke: a new preventive strategy. *Health Technol Assess* 2003;7 (31): 1–94.
- Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. JAMA 1996;275(20):1557-1562.
- Lewington S, Clarke R, Qizilbash N, Peto R, Collins R; Prospective studies collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002;360(9349):1903-13.
- Lee HY, Oh BH. Aging and arterial stiffness. *Circ j* 2010;74(11):2257-62.
- Lekakis J, Abraham P, Balbarini A, Blann A, Boulanger CM, Cockcroft J. Methods for evaluating endothelial function: a position statement from the European Society of

Cardiology Working Group on Peripheral Circulation. *Eur J Cardiovasc Prev Rehabil* 2011;18(6):775-89.

- Lionakis N, Mendrinos D, Sanidas E, Favatas G, Georgopoulou M. Hypertension in the elderly. *World J Cardiol* 2012;4(5):135-47.
- Lim MA, Townsend RR. Arterial compliance in the elderly: its effect on blood pressure measurement and cardiovascular outcomes. *Clin Geriatr Med* 2009;25(2):191-205.
- Lloyd-Sherlock P, Beard J, Minicuci N, Ebrahim S, Chatterji S. Hypertension among older adults in low- and middle-income countries: prevalence, awareness and control. *Int J Epidemiol* 2014;43(1):116-28.
- Lloyd-Jones D, Adams R, Carnethon M, De Simone, Ferquson TB, Flegal K. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2009;119(3):e21-181.
- Li JY, Zhao YS. Brachial-ankle pulse wave velocity is an independent predictor of carotid artery atherosclerosis in the elderly. *J Geriatr Cardiol* 2010;7: 157-160.
- Malpas SC. Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. *Physiol Rev* 2010; 90(2):513–557.
- Mancia G, Grassi G, Giannattasio C, Seravalle G. Sympathetic activation in the pathogenesis of hypertension and progression of organ damage. *Hypertension* 1999;34(4 Pt 2): 724-728.
- Matz RL, Schott C, Stoclet JC, Andriantsitohaina R. Age-related endothelial dysfunction with respect to nitric oxide, endothelium-derived hyperpolarizing factor and cyclooxygenase products. *Physiol Res* 2000;49(1): 11-8.
- Mateos-Caceres PJ, Zamorano-Leon JJ, Rodriquez-Sierra P, Macaya C, Lopez-Farre AJ. New and old mechanism associated with hypertension in the elderly. *Int J Hypertens* 2012;2012:150107.
- Mackenzie IS, Wilkinson IB, Cockcroft JR. Assessment of arterial stiffness in clinical practice. QJM 2002;95(2):67-74.

- Messerli FH, Williams B, Ritz E. Essential hypertension. *Lancet* 2007 18;370(9587):591-603.
- Medley TL, Cole Tj, Gatzka CD, Wang WY, Dart AM, Kingwell BA. Fibrillin-1 genotype is associated with aortic stiffness and disease severity in patients with coronary artery disease. *Circulation* 2002;105(7):810-5.
- Millar JA, Lever AF. Implications of pulse pressure as a predictor of cardiac risk in patients with hypertension. *Hypertension* 2000;36(5):907-11.
- Moore TJ, Conlin PR, Ard J, Svetkey LP. DASH (Dietary Approaches to Stop Hypertension) Diet Is Effective Treatment for Stage 1 Isolated Systolic Hypertension. *Hypertension* 2001;38(2):155-8.
- Mohiaddin RH, Firmin DN, Longmore DB: Age-related changes of human aortic flow wave velocity measured noninvasively by magnetic resonance imaging. *J of applied physiology* 1993;74:492-97.
- Murugesan R, Govindarajalu N, Bera TK. Effect of selected yogic practices in the management of hypertension. *Indian J Physiol Pharmacol* 2000;44: 207-10.
- National Research Council. Aging in Asia: Findings from new and emerging data initiatives. Smith JP & Majmundar, eds. The national academic press, Washington DC. 2012; pp vii.
- National High Blood Pressure Education Program Working Group. National High Blood Pressure Education Program Working Group Report on Hypertension in the Elderly. *Hypertension* 1994;23:275‑85.
- Nagendra HR. Yoga its basis and applications. Swami Vivekananda Yoga Prakshana. 1st edition. 2004.
- Nelson MR, Stepanek J, Cavette M, Covalciuc M, Hurst T, Tajik AJ. Non Invasive measurement of Central Vascular Pressure with arterial tonometry: clinical revival of the Pulse pressure Waveform. *Mayo clin Proc* 2010;85(5):460-72.
- O'Rourke MF, Hashimoto J. Mechanical factors in arterial aging: a clinical perspective. *J Am Coll Cardiol* 2007;50:1-13.

- Patil SG, Mullur L, Khodnapur J, Dhanakshirur GB, Aithala MR. Effect of yoga on short-term heart rate variability measure as an index of stress in subjunior cyclists: A pilot study. *Indian J Physiol Pharmacol* 2013;57:81-6.
- Pal GK, Ganesh V, Karthik S, Nanda N, Pal P. The effects of short-term relaxation therapy on indices of heart rate variability and blood pressure in young adults. *Am J Health Promot* 2013 [Epub ahead of print].
- Pailoor S, Telles S. A review of the scientific studies on cyclic meditation. Int J Yoga. 2009;2: 46-8.
- Patil SG, Dhanakshirur G, Aithala MR, Das KK. Comparison of the effects of yoga and lifestyle modification on grade-I hypertension in elderly males: A preliminary study. *Int J Clin Exp Physiol* 2014;1:68-72.
- Perry HM, Davis BR, Price TR, Applegate WB, Yields WS, Gurlnik JM et al. Effect of treating isolated systolic hypertension on the risk of developing various types and subtypes of stroke: the Systolic Hypertension in the Elderly Program (SHEP). *JAMA* 2000;284:465-71.
- Pickering TG, Equchi K, Kario K. Masked hypertension: a review. *Hypertens Res* 2007;30(6):479-88.
- Pries AR, Kuebler WM. Normal endothelium. *Handb Exp Pharmacol* 2006;(176 Pt 1):1-40.
- PROGRESS Collaborative Group. Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6,105 individuals with previous stroke or transient ischaemic attack. *Lancet* 2001;358:1033-41.
- Pannier B, Guerin AP, Marchais SJ, Safar ME and London GM. Stiffness of capacitive and conduit arteries: Prognostic significance for end-stage renal diseases patients. *Hypertension* 2005;45:592-96.
- Qiu H, Zhu Y Sun Z, Trzeciakowski JP, Gansner M, Depre C, Resuello RR et al. Short communication: vascular smooth muscle cell stiffness as a mechanism for increased aortic stiffness with aging. *Circ Res* 2010;107(5):615-9.
- Radhakrishnan S, Balamurugan S. Prevalence of diabetes and hypertension among geriatric population in a rural community of Tamilnadu. Indian J Med Sci 2013;67:130-6.

- Rhee MY, Lee HY, Park JB. Measurements of arterial stiffness: Methodological aspects. *Korean Circ J* 2008;38:343-50.
- Rosendorff C, Beeri MS, Silverman JM. Cardiovascular risk factors for Alzheimer's disease. Am J Geriatr Cardiol 2007;16:143-9.
- Safar ME. Pulse pressure, arterial stiffness and cardiovascular risk. Curr Opin Cardiol. 2000;15: 258-63.
- Safar ME, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. Circulation 2003;107: 2864-9.
- Schachinger V, Britten MB, Zeither AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000;101:1899-906.
- Schulz E, Gori T, Munzel T. Oxidative stress and endothelial dysfunction in hypertension. *Hypertens Res* 2011;34:665-73.
- Seals DR, Esler MD. Human ageing and the sympathoadrenal system. *J Physiol* 2000;528:407-17.
- Silva BR, Pernomian L, Benchack LM. Contribution of oxidative stress to endothelial dysfunction in hypertension. *Front Physiol* 2012;3:441.
- Spence JD. Pseudo-hypertension in the elderly: still hazy, after all these years. *J Hum Hypertens* 1997;11:621-3.
- Supiano MA. Hypertension. In: Halter JB, Ouslander JG, Tinetti ME, Studenski S, High KP, Asthana S, eds. Hazard's Geriatric medicine and Gerontology. 6th edn. McGraw Hill Medical publishers. 2009:pp. 975-82.
- Sutton-Tyrrell K, Najjar SS, Boudreau RM et al. Health ABC Study. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation* 2005;111: 3384-90.
- Stauss HM, Persson PB. Role of Nitric Oxide in Buffering Short-Term Blood Pressure Fluctuations. *New Physiol Sci* 2000;15:229-33.
- Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 2000;101:948-54.

- Staessen JA, Gasowski J, Wang JG, Thijs L, Den Hond E, Boissel JP. Risk of untreated and treated isolated systolic hypertension in the elderly: meta-analysis of outcome trials. *Lancet* 2000;355(9207):865-72.
- Satoshkar RS, Bhandarkar SD, Rege NN. Pharmacology and pharmacotherapeutics. 18th edn. Popular prakashan private limited, India. 2005:pp 421-2.
- Selvamurthy W, Sridharan K, Ray US, Tiwary RS, Hegde KS, et al. A new physiological approach to control essential hypertension. *Indian J Physiol Pharmacol* 1998;42:205-13.
- Stanley S F. Arterial stiffness and hypertension: A two way street? *Hypertension* 2005;45:349-51.
- Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, Salvetti A. Age-related reduction of NO availability and oxidative stress in humans. *Hypertension* 2001;38:274-9.
- Terashima M, Nguyen PK, Rubin GD, Iribarren C, Courtney BK, Go AS. Impaired coronary vasodilation by magnetic resonance angiography is associated with advanced coronary artery calcification. *JACC Cardiovasc Imaging* 2008;1(2):167-73.
- The Task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) 2007 Guidelines for the management of arterial hypertension. *Eur Heart J* 2007;28: 1462-536.
- Thijssen DHJ, Groot PD, Kooijiman M, Smits P, Hopman MTE. Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. *Am J Physiol Heart Circ Physiol* 2006;291: H3122-9.
- Torregrossa AC, Aranke M, Bryan NS. Nitric oxide and geriatrics: Implications in diagnostics and treatment of the elderly. *J Geriatr Cardiol* 2011;8: 230-42.
- Vanhoutte PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. *Acta Physiol* 2009;196:193-222.
- Verdecchia P, Staessen JA, White WB, Imai Y, O'Brien ET. Properly defining white coat hypertension. *Eur Heart J* 2002;23(2):106-9.
- Viera AJ, Neutze DM. Diagnosis of secondary hypertension: an age-based approach. *Am Fam Physician* 2010;15;82(12):1471-8.
- Vokonas PS, Kannel WB, Cupples LA. Epidemiology and risk of hypertension in the elderly: The Framingham Study. *J Hypertens Suppl* 1988;6:S3‑9.

- Vongpatanasin W. Resistant hypertension: a review of diagnosis and management. *JAMA* 2014; 4;311(21):2216-24.
- Wang J, Xiong X, Liu W. Yoga for essential hypertension: A systematic Review. PLoS One 2013;8(10):e76357.
- Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolio AP, Cockroft JR. Nitric oxide regulates local arterial distensibility in vivo. *Circulation* 2002;105: 213-7.
- Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thij SL, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* 2006;113:664-70.
- Whelton PK, Appel LJ, Espeland MA, Applegate WB, Ettinger WH Jr, Kostis JB et al. Sodium reduction and weight loss in the treatment of hypertension in older persons: a randomized controlled trial of non-pharmacologic interventions in the elderly (TONE). *JAMA* 1998;18;279(11):839-46.
- Westhoff TH, Franke N, Schmidt S, Vallbracht-Israng K, Meissner R, Yildirim H. Too old to benefit from sports? The cardiovascular effects of exercise training in elderly subjects treated for isolated systolic hypertension. *Kidney Blood Press Res* 2007;30(4):240-7.
- Wright JC, Looney SW. Prevalence of positive Osler's maneuver in 3387 persons screened for the Systolic Hypertension in the Elderly Program (SHEP) *J Hum Hypertens* 1997;11:285-89.
- Wu KK, Thiagarajan P. Role of endothelium in thrombosis and hemostasis. *Annu Rev Med* 1996;47:315-31.
- Young AJH, Klag MJ, Muntner P, Whyte JL, Pahor M, Coresh J. Blood pressure and decline in kidney function: findings from the systolic hypertension in the elderly Program (SHEP). *J Am Soc Nephrol* 2002;13:2776-82.
- Zeiman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. Arterioscler Thromb Vasc Biol 2005;25:932-43.

CHAPTER III

STUDY PLAN & PROCEDURE

1. STUDY DESIGN

An open parallel-group randomized controlled study was conducted on elderly subjects between 60 and 75 years with increased PP (> 60mmHg) over a period of 3 months. Volunteers were screened at Visit 1-3. At visit 4, the baseline examination and recordings followed by randomization of selected subjects to either yoga group (Yoga intervention) or control group (walking intervention) was done. Post-intervention examination and recordings were made at Visit 5. No intervention was given on the day of investigation. Persons handling data analysis were kept blinded.

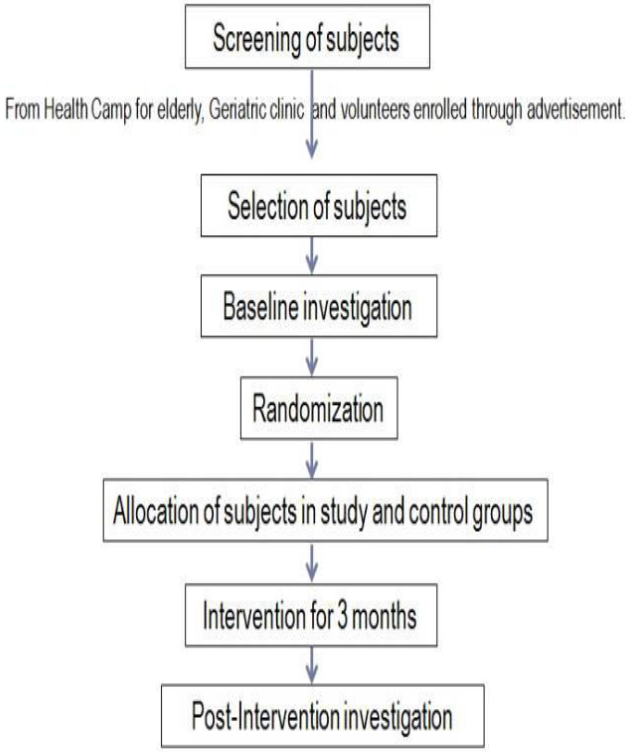


Figure 8: Study protocol

2. STUDY POPULATION

2.1. Participants

The study participants were elderly volunteers with increased PP, who were recruited from the health camp for elderly and Geriatric clinic of Shri B.M.Patil Medical College, and volunteers enrolled through advertisement.

2.2. Sample size

The sample size was calculated on the basis of the difference in means and standard deviation of PP obtained from the pilot study (Patil SG et al., 2014). A total of 60 elderly subjects included in the study. The probability was 80 % that study will detect a treatment difference at a two-sided 0.05 significant level, if the true difference between treatments was 4 units and standard deviation of the outcome variable was 5.11.

3. INCLUSION AND EXCLUSION CRITERIA

3.1. Inclusion criteria

Subjects who have fulfilled the following criteria were enrolled at Visit 3:

- Either sex between 60 and 75 years with pulse pressure > 60mmHg.
- The subject was expected to stay within driving distance of study for at least 5 months.
- No significant diseases or clinically significant abnormal laboratory values during screening.

3.2. Exclusion criteria

Any of the following was regarded as a criterion for exclusion from the study:

- Subjects with SBP > 159mmHg and DBP > 99mmHg.
- Subjects with secondary hypertension
- Subjects on any regular medical treatment.
- Subjects with CV risk factors such as diabetes mellitus, hypercholesterolemia and high triglyceride level.
- 12-lead ECG with any significant abnormalities
- Subjects with neuromuscular disorders.
- Subjects with joint pains.
- History of alcoholism (alcohol abuse) and cannot refrain from alcohol consumption during the study period.
- History of Smoking and cannot refrain from smoking during the study period.
- Subjects who do regularly yoga practice.

3.3. Justification for inclusion & exclusion criteria

The inclusion and exclusion criteria for selection of elderly subjects for life-style changes intervention for three months were as per the 2007 guidelines of the task force for the management of arterial hypertension of the European Society of hypertension and of the European Society of Cardiology (Task force for the management of arterial hypertension of the ESH and ESC., 2007).

4. CRITERIA FOR DISCONTINUATION

- Participant refusal
- Participant who could not sustain the intervention
- Participant who's SBP raised above 165 mmHg and DBP raised above 100mmHg
- Participant who develops any adverse affect

5. ETHICS

5.1. Informed consent

Informed written consent was obtained for participation in the study (Appendix I).

5.2. Institutional approval

The study was approved by the institutional ethical committee of Shri B.M.Patil Medical College, Hospital and Research Centre, BLDE University, India, as per the guidelines (2006) of Indian Council of Medical Research (ICMR ethical guidelines for biomedical research on human participants., 2006).

5.3. Declaration of Helsinki & ICMR guidelines

We followed the declaration of Helsinki during the entire study.

5.4. Study Registration

The study was registered retrospectively in the Clinical Trial Registry-India (CTRI/2011/10/002077).

5.5. CONSORT statement

The study was reported as per the recommendations of the CONSORT group (Schulz KF., 2010).

6. STUDY SUBJECTS SELECTION PROCEDURE

The subjects were screened (n=242) from the health camp for elderly, Geriatric clinic of Shri B.M.Patil Medical College and volunteers enrolled through advertisement. Screening for subjects was done from 15th October 2012 to 15th December 2012. Those subjects with PP > 60mmHg were selected for the study after thorough examination as per our inclusion and exclusion criteria.

7. RANDOMIZATION

Subjects who were selected from screening at Visit 1, 2 & 3 were allocated a subject number. The allocated volunteer randomization numbers were used to identify the volunteer during the entire study. They were then allocated for either yoga group (n=30) for yoga intervention or control group (n=30) for walking intervention using the random number table following baseline recordings.

8. INTERVENTION

8.1. Yoga Intervention

The participants allocated for yoga group were assigned for yoga practice for 6 days in a week for one hour daily in the morning from 06:00 hrs to 07:00 hrs for twelve weeks under the supervision of authorized yoga instructor. The yoga training included Loosening practices, Asanas (maintaining postures), Pranayama (breathing exercises) and Cyclic Meditation: yoga based guided relaxation technique (Pailoor S & Telles S et al., 2009). The integrated yoga module for elderly participants is shown in Table 9. Yoga intervention was given from 15th January to 17th April 2013 at Shri P.G.Halkatti Hall, BLDE University Campus.

8.2. Walking intervention

The protocol for the control group consisted of flexibility or loosening practices for 15-20 minutes followed by brisk-walk for 35-40 minutes and rest for 5 minutes for 6 days in a week for one hour daily in the morning from 06:00 hrs to 07:00 hrs for twelve weeks under the supervision of authorized instructor (Table 10). Walking intervention was given from 15th January to 17th April 2013 at BLDE University Campus.

Table 9 Integrated yoga module for elderly subjects with hypertension

Sl. No	Practice		Duration
1.	Opening Prayer		1 min
2.	Sukshma Vyayama (Loosening Practices)	Loosening of Fingers Loosening of Wrist Shoulder rotation Ankle stretch/rotation Drill walking	5 min
3.	Breathing Practices	Hands in and out breathing Ankle stretch breathing Straight leg raising breathing Lumbar stretch breathing	5 min
4.	Asana (Maintaining Postures)	Utkatasana Padhastasana Ardhachakrasana Shashankasana Ardha Ustrasana Bhujangasana Ardha Salabasana Trikonasana	15 min
5.	Pranayama	Anuloma Viloma Pranayama Brahmari Pranayama	5 min
6.	Cyclic Meditation [CM]		23 min
7.	Devotional Session – Chanting / Bhajans		5 min
8.	Closing prayer		1 min

Table 10 Practices for control group participants

Sl. No.	Practice		Duration
1.	Loosening practices	Neck flexion/extension stretch	15-20 min
		Neck lateral flexion stretch	
		Shoulder stretch	
		shoulder rotation	
		wrist stretch/rotation	
		Arm/trunk stretch	
		Hip stretch	
		Side bend	
		Forward bend	
		Lumbar extension stretch	
		Lumbar flexion stretch	
		Adductor stretch	
		Ankle rotations	
		Hamstring stretch	
		Calf stretch	
2	Brisk-Walk		35-40 min
3	Rest		05 min

9. MEASUREMENTS AT EACH VISIT

Visit 1: Screening-1st Day

- Medical history, including history of past use of medications, demographics (date of birth & sex) and personal history of alcohol & tobacco consumption.
- Measurement of brachial blood pressure (mmHg)

Visit 2: Screening-2nd Day

- Measurement of brachial blood pressure (mmHg).

Visit 3: Screening-3rd Day

- Measurement of brachial blood pressure (mmHg).
- General physical examination
- Estimation of blood glucose and lipid profile

Visit 4: Baseline investigation

- Anthropometric measurements such as height, weight & body mass index (BMI).
- Physiological parameters such as BP & heart rate.
- Measurement of arterial stiffness
    - ✓ Brachial-ankle pulse wave velocity (m/s)
    - ✓ Carotid-Femoral pulse wave velocity (m/s)
    - ✓ Aortic augmentation index
    - ✓ Arterial stiffness index at Brachial and Tibial arteries.
- Evaluation of cardiac autonomic activity
    - ✓ Heart rate variability analysis
- Estimation of serum nitric oxide concentration
- Estimation of serum malondialdehyde
- Estimation of reduced glutathione, serum vitamin C, serum super oxide dismutase.

Visit 5: Post-intervention investigation

- Anthropometric measurements such as height, weight & body mass index (BMI)
- Physiological parameters such as BP & heart rate.

- Measurement of arterial stiffness
  - ✓ Brachial-ankle pulse wave velocity (m/s)
  - ✓ Carotid-Femoral pulse wave velocity (m/s)
  - ✓ Aortic augmentation index
  - ✓ Arterial stiffness index at Brachial and Tibial arteries.
- Evaluation of autonomic activity
  - ✓ Heart rate variability analysis
- Estimation of serum nitric oxide concentration
- Estimation of serum malondialdehyde.
- Estimation of Erythrocyte reduced glutathione, serum vitamin C, serum super oxide dismutase

10. DETAILS OF MEASUREMENTS

All the recordings were made in the morning between 8.00hrs to 10.00hrs after supine rest for 10 minutes.

i. Anthropometric measurements

a. Height

Height was measured using a device (BIOCON^{TM}) mounted on the wall and is expressed in centimeters (cms).

b. Weight

Weight was measured using a weighing machine and is expressed in Kilograms (Kg).

c. Body Mass Index (BMI)

Body Mass Index was estimated from weight in Kilograms (Kg) divided by height in meters squared (m^2) and was expressed as Kg/m^2.

ii. Physiological parameters

a. Heart rate (bpm)

Heart rate was determined using a digital physiograph (Physiopac, Medicaid Systems Ltd, India). It was calculated by using R-R Interval of electrocardiogram (ECG) and was expressed as beat per minute (bpm). ECG was recorded for five minutes using limb leads.

b. Measurement of blood pressure

- Systolic & Diastolic blood pressure (mmHg): As BP is more variable in older people, so we have taken average of nine BP readings (Supiano MA., 2009). Brachial BP was measured thrice with an interval of one minute on every visit for three consecutive days in a sitting posture using mercury sphygmomanometer (Pickering TG et al., 2005).

- Pulse pressure (mmHg): It is the pulsatile component of the blood pressure. It was estimated as the difference between systolic and diastolic blood pressure and expressed in mmHg.

- Mean arterial pressure (mmHg): It is an average arterial pressure in an individual during single cardiac cycle. It was estimated by adding 1/3rd of PP (mmHg) to the DBP (mmHg).

iii. **Assessment of arterial stiffness:**

Vascular function was assessed by oscillometric method using a non-invasive automatic device (Periscope, Genesis Medical Systems, India). Periscope is a validated 8-channel real time PC-based simultaneous acquisition (200 samples per second) and analysis system (Naidu MU et al., 2005). According to Nyquist's criterion the minimum sampling rate should be twice the maximum input frequency which is sufficient to avoid aliasing and preserve all the input signal information (Faulkner EA et al., 1969). The significant frequency content of the pressure as well as ECG waveform was not more than 40 Hz; hence, a sampling rate of 200 samples per second was optimum. This device uses four BP cuffs and two-channel ECG leads to record arterial pressure waveforms and ECG (Lead I & II) simultaneously.

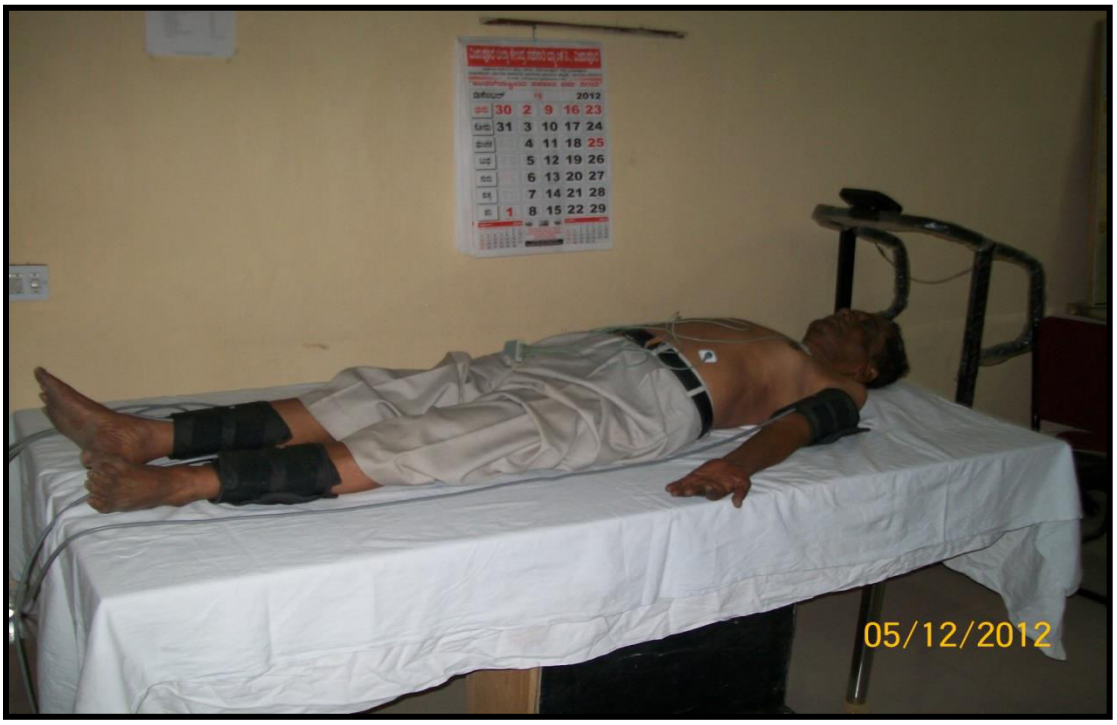


Figure 9a Blood pressure cuffs and ECG electrodes placement for vascular analysis using Periscope.

Figure 9b Vascular analysis using Periscope

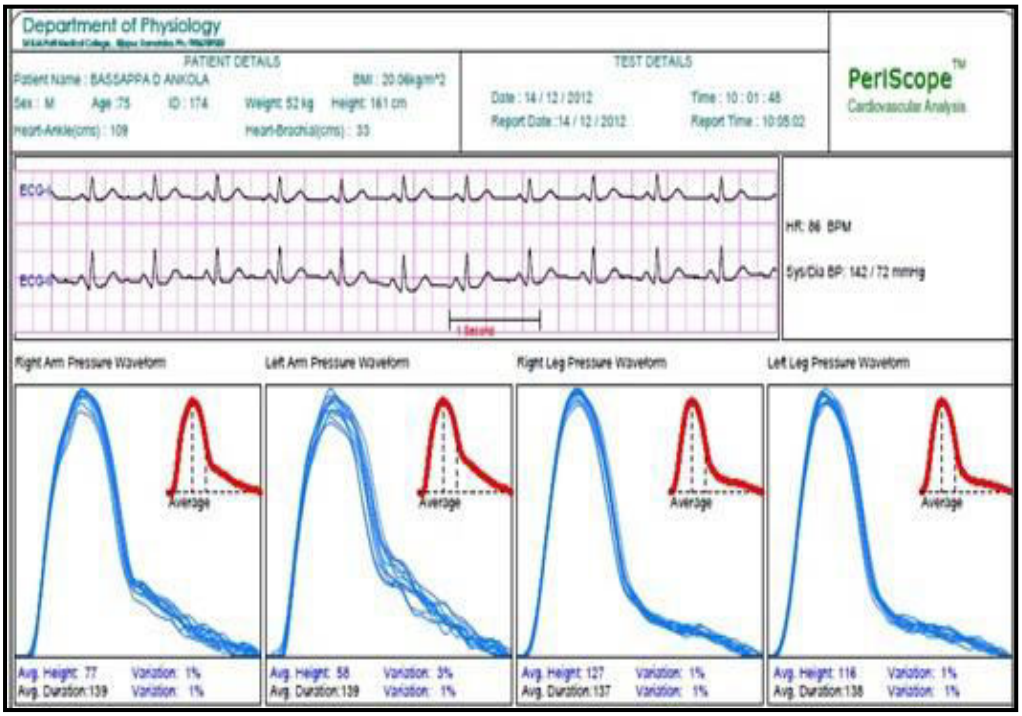


Figure 10 Sheet 1: Results of vascular analysis given by Periscope at a glance

The recordings were made in supine position. BP cuffs were wrapped on both upper arm brachial artery and tibial artery above ankles. ECG electrodes were placed on the ventral surface of both wrists and medial side of the ankles (Figure 9a & b). The BP cuffs were connected to oscillometric pressor sensor and plethysmographic sensor located on the hardware of the system (Periscope) to determine pressure waveforms and volume pulse waveform. The data obtained in 10 seconds was stored in the computer for further analysis and to detect various arterial stiffness parameters. Periscope supports a sophisticated digital-signal algorithm to calculate all the results. As the device is fully automated and does not require any operator for handling any probe to record the waveforms, so it is devoid of any operator bias. Periscope is fully automatic, so once the test is started, the recording completes itself by displaying the results directly (Figure 10a & b).

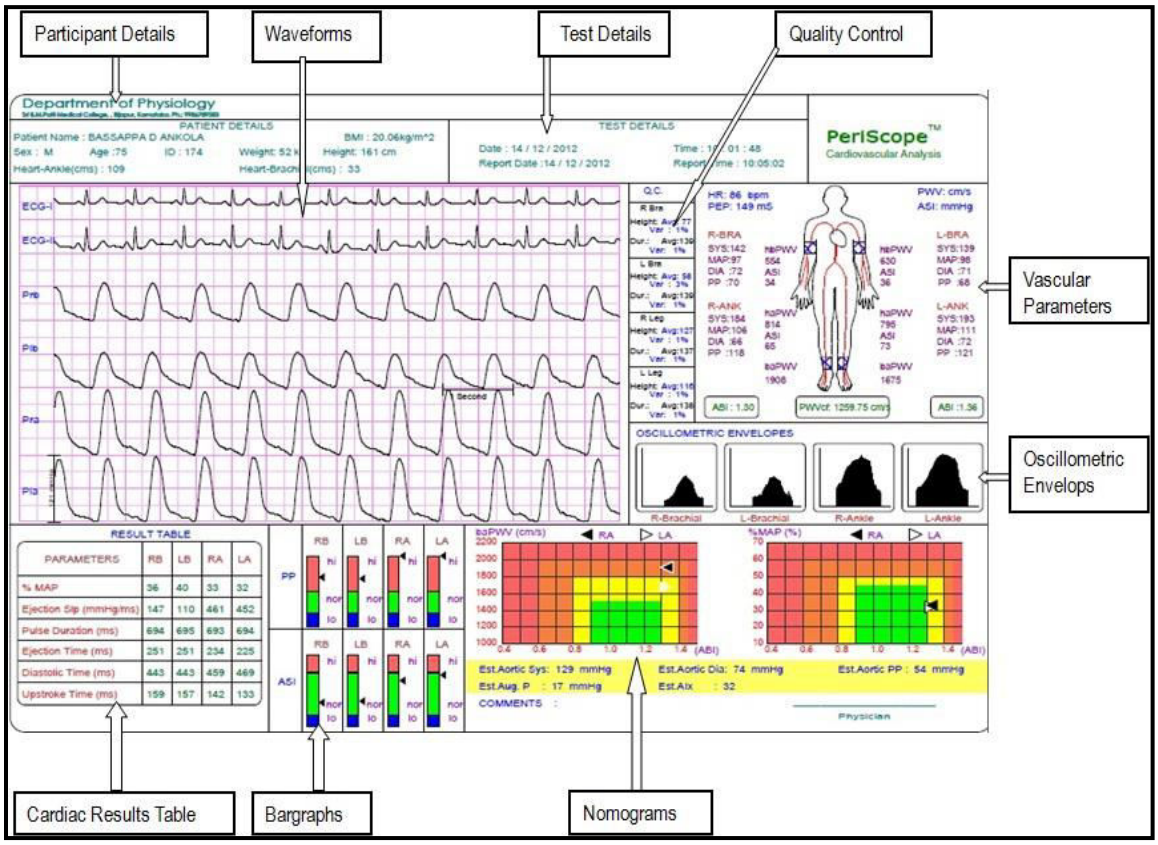


Figure 10b Sheet 2: Results of vascular analysis given by Periscope at a glance

a. **Estimation of pulse wave velocity**

- Brachial-ankle PWV (baPWV), a measure of arterial stiffness (central artery & peripheral semi-muscular arteries) was estimated using arterial pressure waveforms (Brachial and Tibial artery) and ECG recordings (Lead I & II). The pulse transit time (PTT) in seconds elapsed between brachium and respective ankle was calculated as the time difference between the R-wave of ECG and foot of respective pulse wave. The distance between the brachium and ankle was calculated automatically according to the height of the subject. The PWV was calculated by dividing the distance by PTT (Figure 11).

$$\boldsymbol{brachial - ankle\ PWV = \frac{Lba}{PTTba}}$$

Where Lba = Distance between respective brachium and ankle

PTTba = Pulse Transit Time (PTT) between brachium and respective ankle was calculated as the time difference between the feet of respective pulse wave originated from R-wave (QRS complex) of ECG.

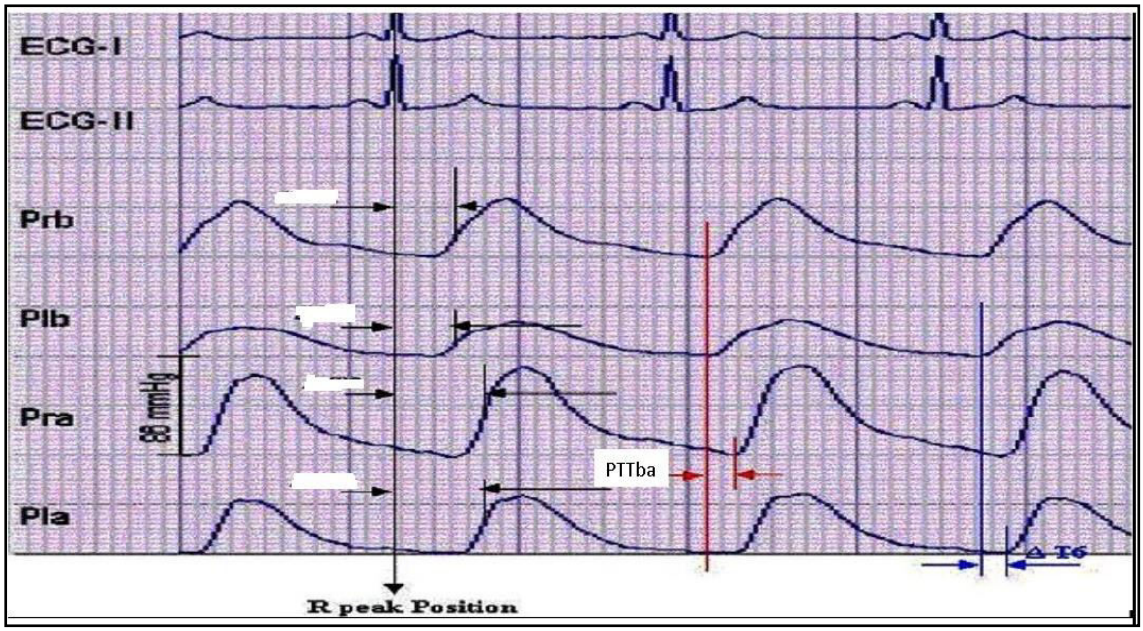


Figure 11 Pulse wave form and ECG and calculation of pulse transit time

- The carotid-femoral PWV (c-f PWV), a measure of central arterial (aortic) stiffness was calculated by the composite baPWV found out by averaging left and right baPWV. Periscope estimates the c-f PWV on the basis of equation (0.8333*Avg.baPWV-233.33) derived by regression analysis between baPWV and c-f PWV from the studies conducted elsewhere (Yamashina A et al., 2002).

$$\boldsymbol{Carotid - femoral\ PWV = 0.8333 * Avg.baPWV - 233.33}$$

b. Estimation of augmentation index

Periscope determines aortic pressure by Oscillometric PWV method. It estimates aortic pressure on the basis of regression equation derived by multivariate statistical analysis of invasive aortic pressure values (found by a fluid-filled catheter method) with respect to the brachial pressure and c-f PWV values obtained non-invasively by Periscope (Naidu MUR et al., 2012).

Measurement of aortic pressure by Oscillometric PWV method:

Aortic root pressure gradient is composed of two major components:

I. Systolic Pressure gradient – The rapid rise of pressure at the aortic root is contributed by the left ventricular pressure during systole. As soon as the left ventricle is emptied into the aorta, the aortic pressure falls rapidly. This gradient does not contribute to the aortic root pressure during diastole.

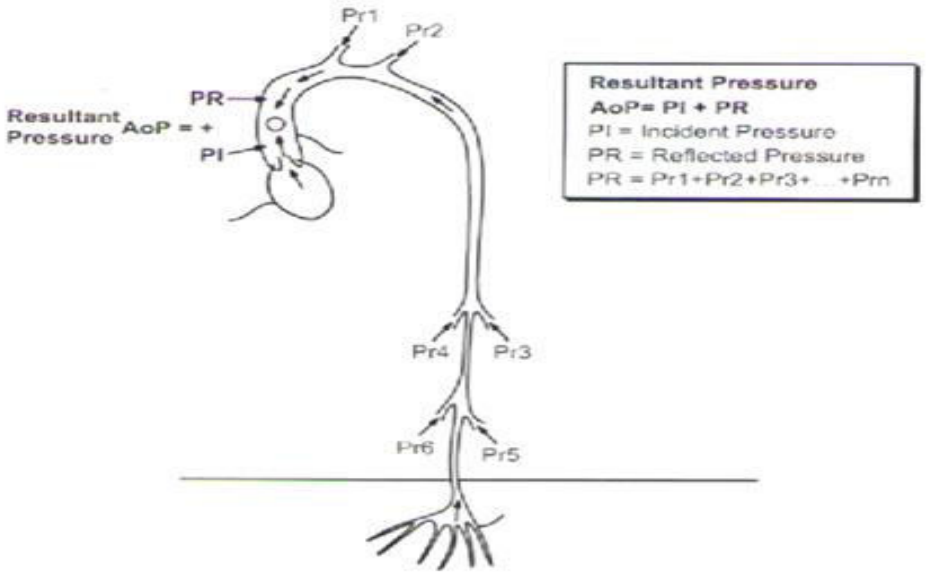


Figure 12 Wave reflection

II. Diastolic Pressure gradient – The pressure wave generated in the aorta during systole is propagated along the arterial tree which is resisted by the systemic vascular resistance from the branches at various points. From this various points of impedance mismatch at different arterial branches, the waves reflect back as a single wave (wave reflection) to the aorta during diastole and contribute for diastolic pressure gradient (Figure 12).

Thus, the aortic root pressure is mainly dependent on two components: Left ventricular systolic pressure and wave reflection pressure.

The timing of arrival of wave reflection at the aortic root is dependent on the arterial stiffness. The wave reflection arrives earlier during systole in the stiffened arteries due to increase in PWV and contributes to augmentation of aortic systolic pressure. Thus, the resultant aortic root pressure increases in proportion with the arterial stiffness.

Periscope uses brachial BP and c-f PWV to determine the aortic root pressure. It is based on the mathematical analysis of invasive aortic pressure values (Fluid-filled catheter method) with respect to the brachial BP and PWV found non-invasively. Aortic root pressure values are directly proportional to a combination of both the brachial pressure value and c-f PWV. A significant correlation was found in these parameter values when multivariate regression analysis was carried out. Equation relating aortic pressure value, brachial pressure and c-f PWV with respective coefficients was derived from this and added in the Periscope to determine equivalent aortic pressure.

The rise in the systolic pressure is called an augmentation pressure. The augmentation index (AIx) is the ratio of augmentation pressure to the aortic PP and is expressed in percentage. This oscillometric PWV method used for estimation of AIx by periscope has been validated (Naidu MUR et al., 2012). As it was reported that AIx is influenced by heart rate, an index normalized for a heart rate of 75 bpm (AIx@75) was used in this study (Wilkinson IB et al., 2000).

c. Calculation of arterial stiffness index

Arterial stiffness index (ASI), an another measure of local and peripheral arterial stiffness was estimated at brachial artery (bASI) and tibial artery (aASI) by quantifying the oscillometric envelopes derived from the oscillations in the respective artery (Naidu MUR et al., 2012).

ASI = (Systolic side Value of cuff pressure at 80% of maximal oscillation amplitude of cuff) - (Diastolic side Value of cuff pressure at 80% of maximal oscillation amplitude of cuff).

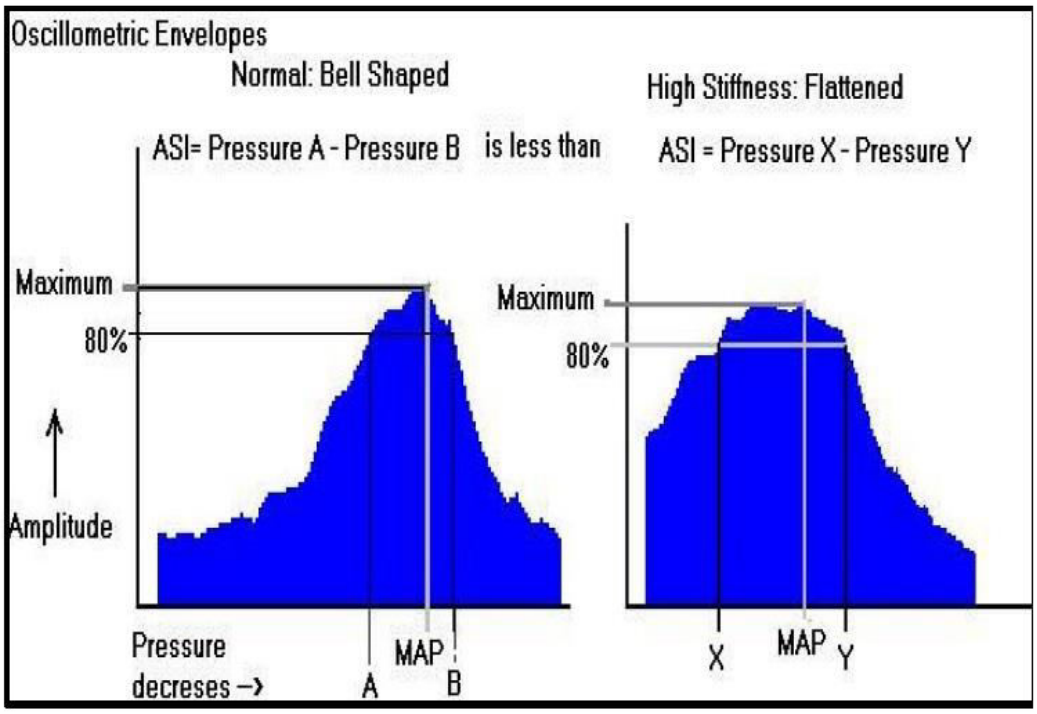


Figure 13 Oscillometric Envelope

Oscillometric envelope

An oscillometric envelope is a graph constructed by mapping the change in arterial pulse amplitude in response to changing cuff pressure (Acton A., 2013) (Figure 13). It is a graphical depiction of compressibility of the artery. It is derived from the oscillations in the artery during the deflation of BP cuffs while recording BP by

oscillometric method. The shape of the oscillometric envelope is bell-shaped in normal artery where as it is flattened in stiffened artery. It becomes harder to collapse the stiffened arteries by applying external pressure; hence the oscillometric envelope becomes flatter as the stiffness increases. The ASI value gives a clear indication of this flattening process (Figure 13). The ASI values increases with an increase in arterial stiffness.

iii. Assessment of heart rate variability

Heart rate variability (HRV) is an established tool for evaluation of autonomic activity (sympathetic and parasympathetic tone). HRV is the physiological variation in time interval between heart beats. It is measured by the variation in the beat-to-beat interval.

Procedure

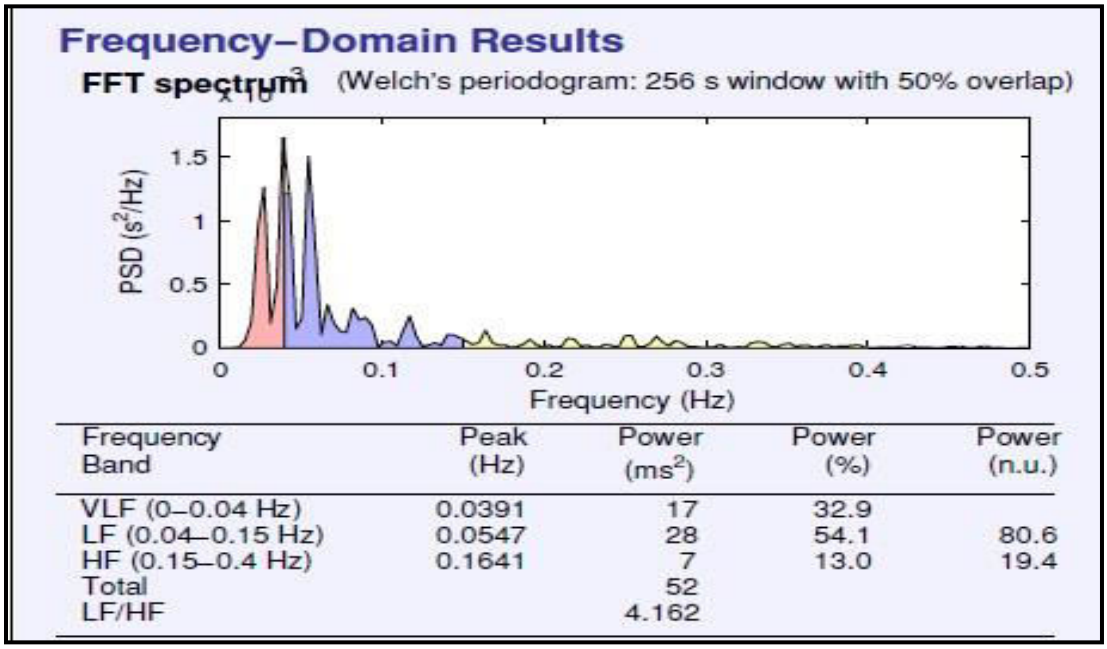
ECG was recorded in the standard limb lead II configuration for five minutes using a four channel digital polygraph (Medicaid systems Pvt Ltd, Chandigarh, India). The subjects were asked to breathe normally during the ECG recording. ECG recordings were exported from the digital polygraph for HRV analysis. The recorded data were visually inspected off-line and only noise free data were included for analysis. No ectopic beats were found on offline scrutiny. HRV analysis software version 2.0 developed by the Biomedical Signal Analysis group, University of Kuopio, Finland was used for HRV analysis (Tarvainen MP et al., 2014). HRV analysis was done by Frequency domain method. A non-parametric Fast Furrier Transform (FFT) technique was used to obtain the Power spectral density of the RR Series (Figure 14).

Total power in the frequency range (0-0.40Hz) was divided into very low frequency (VLF: 0.0-0.04), low frequency (LF: 0.04-0.15Hz) and high frequency (HF: 0.15-0.40Hz).

- LF is a measure of both sympathetic and parasympathetic activity, but mainly reflects sympathetic activity.

- HF measure reflects parasympathetic activity. The LF and HF components were expressed in normalized units (nu).
- LF/HF ratio was calculated to assess overall balance between the sympathetic and the parasympathetic systems (sympathovagal balance).

HRV analysis was done as per the guidelines of a Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Task force of the ESC and the NAS of Pacing and Electrophysiology., 1996).



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0391	17	32.9	
LF (0.04–0.15 Hz)	0.0547	28	54.1	80.6
HF (0.15–0.4 Hz)	0.1641	7	13.0	19.4
Total		52		
LF/HF		4.162		

Figure 14 Fast Furrier Transform (FFT) spectrum

iv. Evaluation of endothelial function

a. Estimation of serum nitric oxide concentration

Total serum nitric oxide concentration (NOx) was measured as an index of endothelial function. Serum NOx was estimated by improved Griess method using vanadium chloride as a reducing agent for reduction of nitrate to nitrite (QuantiChrom™ Nitric Oxide Assay Kit: D2NO-100, BioAssay Systems, USA).

The subjects were advised to abstain from foods such as cured meat, fish, cheese, herbal or black tea, beer, wine and malted beverages on the previous day to avoid dietary effect on NOx (Choi JW et al., 2001). To avoid change in the serum NO levels secondary to physical activity, subjects were given rest for at least 10 minutes before collection of blood sample.

Principle

Since NO is unstable and oxidized to nitrite and nitrate, it is common practice to quantitate total NO_2/NO_3 as a measure for NO level. Nitrate was reduced to nitrite by vanadium chloride (VCl_3) after deproteinization of serum sample by somogyi reagent (NaoH & $ZnSO_4$). The nitrite produced was determined by diazotization of sulfanilamide and coupling to napthylethyline diamine.

Reagents

1. $ZnSO_4$ Solution (75mMol/L)
2. NaOH solution (55mMol/L)
3. Vanadium chloride III
4. Griess reagent
    a. Sulfanilamide
    b. N-Naphthylethylene diamine
5. $NaNO_2$ standard (1.0 mM/L)

Procedure

1. Deproteination

   150 µl of sample was mixed with 8 µl $ZnSO_4$ in 1.5 ml eppendorf tube. 8 µl of NaOH was added following vortex for one minute. The mixture was vortexed again and centrifuged for 10 min at 14,000 rpm. Clear supernatant obtained was transferred to a clean tube.

2. Standards

   1.0 ml of working standard (100 µM/L) was prepared by mixing 0.1 mL of 1.0 mM/L $NaNO_2$ standard with 0.9 mL of distilled water.

Following calibrators were prepared from the working standard.

No.	Working standard	Distilled water	Nitrite (µmol/L)
1	500 µL	----	100
2	300 µL	200 µL	60
3	150 µL	350 µL	30
4	---	500 µL	0 (blank)

3. Reaction

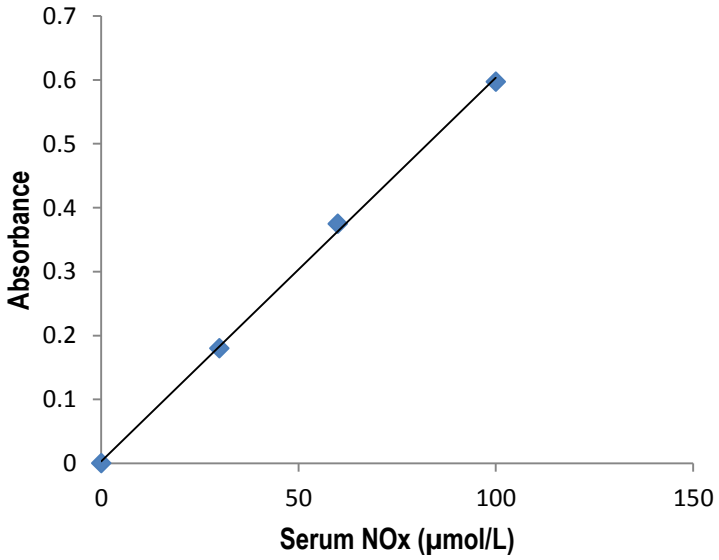
i. Working reagent (WR) for all samples and standards was prepared by mixing per reaction tube
   a. 400 µL - Sulfanilamide
   b. 400 µL - N-Naphthylethylene diamine
   c. 200 µL - Vanadium chloride III

ii. 400 µL of deproteinated sample and calibrators were added in a separate labeled eppendorf tubes.

iii. Then 800 µL of working reagent was added to each tubes.

iv. Incubated for 10 min at 60^0C.

Standard graph



Measurement

Optical density (OD) was read at 540 nm (UV-1700, UV-visible spectrophotometer, Scimadzu).

Calculation

i. Standard graph was plotted using OD against standard concentrations.
ii. Slope was determined using linear regression fitting.
iii. The NO concentration of sample was calculated as

$$Serum\ NO\ (\mu M) = \frac{ODsample - OD\ blank}{\text{Slope}}$$

b. Augmentation index (AIx)

Procedure of measurement of augmentation index is described in section 10.iii.b.

v. Evaluation of oxidative stress and antioxidant status

The blood sample was collected in the morning with overnight fasting for estimation of biochemical parameters.

a. Estimation of Serum malondialdehyde (MDA)

Serum malondialdehyde (MDA), a marker of oxidative stress was estimated by Kei Satoh method (Satoh K., 1978).

Principle

Auto-oxidation of unsaturated fatty acids involves the formation of semi-stable peroxides, which then undergo a series of reactions to form malondialdehyde. Malondialdehyde reacts with Thiobarbituric acid to form pink colored chromogen. The resulting chromogen was extracted with 4.0 ml of n-butyl alcohol and the absorbance of which was measured at 530 nm.

Reagents

1. Trichloroacetic acid (TCA) reagent: 20g/dl TCA in 100 ml distilled water to prepare 20% TCA.

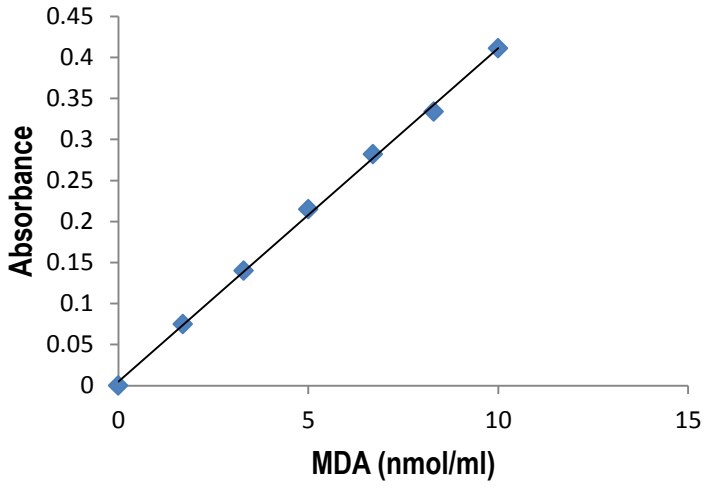
2. Sodium sulphate solution (2M): 28.4 gm of anhydrous sodium sulfate was mixed in 90 ml of distilled water by heating and stirring. Then distilled water was added to make final volume of 100 ml.
3. Thiobarbituric acid (TBA) reagent: 670 mg of TBA in 100ml of 2M sodium sulphate solution.
4. Sulphuric acid (0.05M)
5. N-butyl alcohol

Standards

Following calibrators were prepared from the working standard (10nmol/ml).

No.	Working standard	Distilled water	MDA (nmol/ml)
1	3.0 ml	----	10
2	2.5 ml	0.5 ml	8.3
3	2.0 ml	1.0 ml	6.7
4	1.5 ml	1.5 ml	5
5	1.0 ml	2.0 ml	3.3
6	0.5 ml	2.5 ml	1.7
7	0 ml	3.0 ml	0

Standard Graph



Procedure

1. 300 µl of serum and 1.5 mL of TCA was taken in a test tube and kept for 10 min at room temperature.
2. Centrifugation at 3500 rpm for 10 min was done.
3. The supernatant was decanted and the precipitate obtained was washed with 0.05M Sulphuric acid.
4. 1.5 mL of 0.05M Sulphuric acid and 3 mL of TBA reagent were added to the precipitate.
5. The test tube containing the mixture was kept in a boiling water bath for 30 min.
6. Then the tube was cooled in cold water followed by addition of 2.4 mL of n-butyl alcohol with vigorous shaking to extract the chromogen.
7. Separation of organic phase was facilitated by centrifugation at 3000 rpm for 10 min.
8. The absorbance (OD) was read at the 530 nm wavelength using spectrophotometer.

Calculation

Concentration of serum MDA (nmol/ml)

$$= \frac{\text{OD of Test}}{\text{Nano-molar Extinction Co-efficient}} \text{ X } \frac{\text{Total volume of solution in cuvette}}{\text{sample volume}}$$

$$= \frac{\text{OD of Test}}{1.56 \text{ x } 105} \text{ X } \frac{109}{1000} \text{ X} \frac{2.4}{0.3}$$

= OD of the Test x 51.28 nmol/ml.

b. Estimation of Reduced glutathione (GSH)

Blood reduced glutathione (GSH) was estimated by Earnest Beutler method (Beutler E et al. 1963).

Principle

Non-protein sulphydryl groups of red blood cells (RBC) are present in the form of reduced glutathione (GSH). 5, 5'–dithiobis-2-nitrobenzonic acid (DTNB) is a

disulphide compound which is readily reduced by sulphydryl compounds, forming a highly colored yellow compound. Optical density was measured at 412 nm and it is directly proportional to GSH concentration.

Reagent

1. Precipitating solution: 1.67gm of glacial metaphosphoric acid, 0.2gm of disodium or dipotassium ethylene diamine tetra acetic acid (EDTA) and 30 gm of sodium chloride was dissolved in 100ml of distilled water.
2. Phosphate solution: 0.3M Na_2HPO_4 (di-sodium hydrogen phosphate) was prepared by dissolving 4.68gm in 100 mL distilled water.
3. 1% Sodium citrate: 1gm of sodium citrate was dissolved in 100ml distilled water.
4. DTNB reagent: 40mg 5, 5'dithiobis– (2-nitrobenzonic acid) was dissolved in 100ml of 1% sodium citrate.
5. Reduced glutathione standard (0.5 mg/ml): Take 5 mg of reduced glutathione and dissolved in 10 ml of distilled water.

Procedure

Three test tubes were taken and labeled as blank, standard and test. The procedure of the assay was as given below.

	Blank	Standard	Test
Whole blood	--	--	0.2 mL
Standard	--	0.4 mL	--
Distilled water	2 mL	1.6Ml	1.8 mL
		Mixed well	
Precipitating Solution	3.0 mL	3.0 mL	3.0 mL

Kept for five minutes, centrifuged and 1 ml supernatant was added in a separate labeled test tubes			
Phosphate solution	4.0 mL	4.0 mL	4.0 mL
DTNB Reagent	0.5 mL	0.5 mL	0.5 mL
Mixed and absorbance was read at 412 nm against the blank within 5 minutes			

Calculation

Concentration of Erythrocyte reduced glutathione

$$= \frac{OD\ of\ test}{OD\ of\ Std} X \frac{Conc\ of\ Std}{Volume\ of\ test} X\ 100$$

$$= \frac{OD\ of\ test}{OD\ of\ Std} X \frac{0.04}{0.08} X\ 100$$

$$= \frac{OD\ of\ Test}{OD\ of\ Std} X\ 50$$

= ……. mg/dl

c. Estimation of superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was measured by Marklund and Marklund method (Marklund S & Marklund G., 1998).

Principle

Superoxide anion is involved in auto-oxidation of pyrogallol at alkalike pH (8.5). The superoxide dismutase inhibits auto-oxidation of pyrogallol which can be determined as an increase in absorbance at 420 nm.

Reagents

1. Tris buffer (0.05M): 50 mM of Tris buffer and 1 mM of EDTA was mixed with distilled water and HCL was added to adjust the pH at 8.5. A final volume of 100 ml solution at pH 8.5 was prepared.

2. Pyrogallol (20mM): 25 mg pyrogallol was dissolved in 10 mL distilled water.

Procedure

1. Control: 2.9 ml of Tris buffer was taken in a cuvette to which 0.1 ml of Pyrogallol was added. Then absorbance (OD) was read at 420 nm after 1min 30 sec and 3 min 30 sec.
2. Test: 2.8 ml of Tris buffer and 0.1 ml of serum was taken in a cuvette to which 0.1 ml of Pyrogallol was added. Then absorbance (OD) was read at 420 nm after 1min 30 sec and 3 min 30 sec.
3. Difference in absorbance (ΔA/min) was calculated as

$$\Delta A/\text{min} = \frac{\text{OD at 3 min 30 sec} - \text{OD at 1 min 30 sec}}{2}$$

Calculation

$$\text{Serum SOD activity} = \frac{\Delta A/\text{min of control} - \Delta A/\text{min of Test}}{\Delta A/\text{min of control x 50}} \text{ x 100 x } \frac{1}{\text{volume of sample}}$$

$$= \frac{C-T}{C \text{ x } 50} \text{ x 100 x } \frac{1}{0.1}$$

$$= \frac{C-T}{C \text{ x } 50} \text{ x 1000}$$

= ---------- U/ml.

One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation.

d. Estimation of serum vitamin C

Serum vitamin C was estimated by 2, 4-dinitrophenylhydrazine method (Roe JH et al., 1943; Brewster MA., 1996)

Principle

Ascorbic acid was oxidized by copper to form dehydroascorbic acid and diketogluconic acid. These products were treated with 2,4-dinitrophenyl

hydrazine (DNPH) to form the derivative bis-2,4-dinitrophenyl hydrazone. This compound in strong sulfuric acid undergoes rearrangement to form a colored product which was measured at 520 nm. The reaction was run in the presence of thiourea to provide a mildly reducing medium which helps to prevent interference from non-ascorbic acid chromogen.

Reagents

1. 10 % Trichloroacetic acid: 10 gm of Trichloroacetic acid (TCA) was dissolved in distilled water to prepare a final volume of 100 ml.
2. DTC reagent: 3.0 gm of 2, 4-dinitrophenyl hydrazine (DNPH), 0.4 gm Thiourea and 0.05 gm copper sulphate were added to 9N sulfuric acid. The final volume of 100 ml was prepared.
3. 65 % sulfuric acid: 65 ml of sulfuric acid was dissolved in 35 mL distilled water.
4. Stock standard: 100 mg ascorbic acid was dissolved in 100 mL of 5 % TCA.
5. Working standard (10µg/mL): 1mL of stock standard was dissolved in 100 mL of 5 % TCA.

Procedure

1. Deproteination

   500 µl of sample was mixed with 500 µl of 10% TCA in an eppendorf tube. Vortexed and then centrifuged. The clear supernatant protein free filtrate was used.
2. 500 µl of sample and standards were taken in a test tube separately to which 100 µl DTC reagent was added.
3. Incubated at 37^0C for 3 hours.
4. 750 µl of 65% sulfuric acid was added to all the test tubes.
5. Vortexed and kept for 30 minutes at room temperature.
6. Absorbance was read at 520 nm.

Calculation

Concentration of Serum Vitamin C

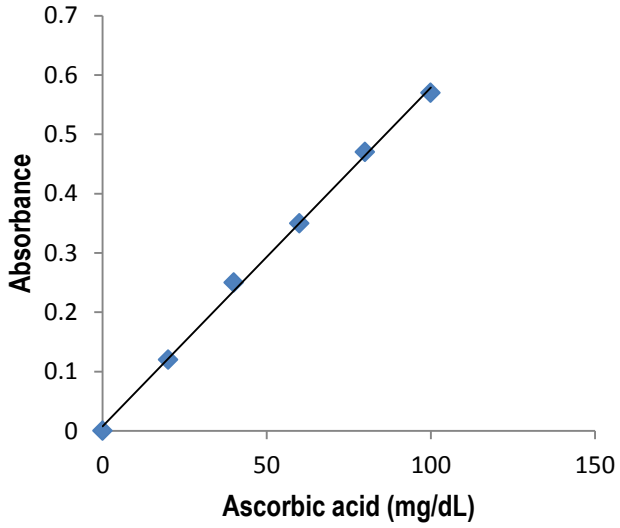
$$= \frac{OD\ of\ test}{OD\ of\ Std} X \frac{Conc\ of\ Std}{Volume\ of\ test} X\ 100$$

$$= \frac{OD\ of\ test}{OD\ of\ Std} X \frac{0.005}{0.25} X\ 100$$

$$= \frac{OD\ of\ Test}{OD\ of\ Std} X\ 2$$

= ……. mg/dl

Standard Graph



vi. Estimation of blood glucose

Fasting blood glucose was estimated by Trinder's method (Trinder P., 1969).

(Erba diagnostics Mannheim)

Principle

Glucose in sample was oxidized to yield gluconic acid and hydrogen peroxide in the presence of Glucose oxidase. The enzyme peroxidase catalyses the oxidative coupling

of 4-aminoantipyrine with phenol to yield a colored quinoneimine complex, the absorbance was proportional to the concentration of glucose in sample.

Reagent

1. Enzyme reagent

Active ingredients	Concentration
Glucose oxidase	≥ 2000 U/L
Peroxidase	≥ 2000 U/L
Phenol	10 mmol/L
Phosphate buffer	200 mmol/L

2. Glucose standard: 100mg/dl

Procedure

1. Three test tubes were taken and labeled as blank, standard and test. The procedure of the assay was as follows.

	Blank	Standard	Test
Sample	--	--	10 µl
Standard	--	10 µl	--
Enzyme reagent	1.0 ml	1.0 ml	1.0 ml

2. Mixed well and incubated at 37^0C for 5 minutes.
3. Absorbance of test and standard was read against blank at 505/670 nm.

Calculation

Glucose (mg/dl)

$$= \frac{\text{OD of test}}{\text{OD of standard}} \text{x Concentration of standard (100mg/dl)}$$

Precision of the assay

1. Inter-assay co-efficient of variability (CV): 2.34%

	Level 1	Level 2
No. of samples	10	10
Mean (mg/dl)	68	185
SD	1.9	3.5
CV %	2.79	1.89

2. Intra-assay co-efficient of variability (CV): 2.47 %

	Level 1	Level 2
No. of samples	10	10
Mean (mg/dl)	72	165
SD	1.26	5.24
CV %	1.75	3.18

vii. Estimation of lipid profile

The blood sample was collected in the morning with overnight fasting for estimation of lipid profile (Erba diagnostics Mannheim).

a. Estimation of Serum triglyceride

Serum triglyceride was estimated by glycerol phosphatase-oxidase (GPO-PAP) method (Bucolo G & David H., 1973; Fossati P & Prencipe L., 1982; McGowan MW et al., 1983).

Principle

Triglycerides were enzymatically hydrolyzed by lipase to glycerol and free fatty acids. The glycerol was subsequently measured by a coupled enzymatic reaction system. The glycerol released was phosphorylated to glycerol-3-phosphate by glycerol kinase. The glycerol-3-phosphate was oxidized by glycerol phosphate

oxidase to produce dihydroxyacetone phosphate and hydrogen peroxide. Peroxidase catalyzed the reaction of hydrogen peroxide with 4-aminoantipyrine and 3, 5-Dichloro-2-hydroxybenzene sulfonate. The absorbance of chromogen formed was measured at 505 nm. The intensity of the chromogen (Quinoneimine) formed was proportional to the triglycerides concentration in the sample.

Reagents

1. Triglyceride reagent: ATP (2.5 mmol/L), Mg^{2+} (2.5 mmol/L), 4-aminoantipyrine (0.8 mmol/L), 3, 5-Dichloro-2-hydroxybenzene sulfonate (1 mmol/L), Peroxidase (>2000U/L), Glycerol Kinase (>550 U/L), Glycerol phosphate oxidase (>8000U/L), Lipoprotein Lipase (>3500 U/L), Buffer (53mmol/L, pH 7.0 ± 0.1 at 20^0C).

2. Triglyceride standard (200mg/100ml).

Procedure

1. Three test tubes were taken and labeled as blank, standard and test. The procedure of the assay was as follows.

	Blank	Standard	Test
Sample	--	--	10 µl
Standard	--	10 µl	--
Distilled water	10 µl	--	--
Working Reagent	1000 µl	1000 µl	1000 µl

2. Mixed well and incubated at 37^0C for 10 minutes.
3. Absorbance of test and standard was read against blank at 505nm.

Calculation

Triglycerides (mg/dl)

$$= \frac{\text{OD of test}}{\text{OD of standard}} \text{ x Concentration of standard (200mg/dl)}$$

Precision of the assay

a. Inter-assay co-efficient of variability (CV): 4.15%

	Level 1	Level 2
No. of samples	10	10
Mean (mg/dl)	81	140
SD	3.2	6.1
CV %	3.95	4.35

b. Intra-assay co-efficient of variability (CV): 4.15 %

	Level 1	Level 2
No. of samples	10	10
Mean (mg/dl)	82.1	139.5
SD	3.4	5.8
CV %	4.14	4.16

b. Estimation of Serum cholesterol

Cholesterol was estimated by cholesterol oxidase-peroxidase (CHOD-PAP) enzymatic method (Allian CC et al., 1974; Roeschlau P et al., 1974)

Principle

Cholesterol esters were hydrolyzed by Cholesterol esterase to cholesterol and free fatty acids. Free cholesterol was oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. This hydrogen peroxide combined with 4-aminoantipyrine to form a chromophore (quinoneimine dye) which was measured at 505 nm.

Reagents

1. Reagent
    - Good's buffer (50mmol/L)
    - Phenol (5 mmol/L)

- 4-aminoantipyrine (0.3 mmol/L)
- Cholesterol esterase (≥200 U/L)
- Cholesterol oxidase (≥ 50 U/L)
- Peroxidase (≥ 3 kU/L)

2. Standard
   - Cholesterol (200mg/100ml)

Procedure

1. Three test tubes were taken and labeled as blank, standard and test. The procedure of the assay was as follows.

	Blank	Standard	Test
Sample	--	--	10 µl
Standard	--	10 µl	--
Reagent	1.0 ml	1.0 ml	1.0 ml
Distilled water	10 µl	--	--

2. Mixed well and incubated at 37^0C for 10 minutes.
3. Absorbance of test and standard was read against blank at 505nm.

Calculation

$$\text{Cholesterol (mg/dl)} = \frac{\text{OD of test}}{\text{OD of standard}} \text{x Concentration of standard (200mg/dl)}$$

Precision of the assay

a. Inter-assay co-efficient of variability (CV): 2.38%

	Level 1	Level 2
No. of samples	10	10
Mean (mg/dl)	122.2	216.02
SD	3.1	4.82
CV %	2.53	2.23

b. Intra-assay co-efficient of variability (CV): 2.44 %

	Level 1	Level 2
No. of samples	10	10
Mean (mg/dl)	116.25	196.83
SD	2.61	4.69
CV %	2.5	2.38

c. **Estimation of HDL cholesterol**

High density lipoprotein (HDL) cholesterol was estimated by phosphotungstic acid (PTA) method (Burstein M et al., 1970).

Principle

Phosphotungstic acid precipitates low and very low density lipoproteins (LDL & VLDL) in the presence of divalent cations such as magnesium. The high density lipoprotein (HDL) cholesterol which remains unaffected in the supernatant was estimated using cholesterol reagent.

Reagents

1. Precipitating reagent: Phosphotungstic acid (0.77 mmol/l) & Magnesium chloride (17.46 mmol/l)
2. Cholesterol working reagent
    - Good's buffer (50mmol/L)
    - Phenol (5 mmol/L)
    - 4-aminoantipyrine (0.3 mmol/L)
    - Cholesterol esterase (≥200 U/L)
    - Cholesterol oxidase (≥ 50 U/L)
    - Peroxidase (≥ 3 kU/L)
3. HDL cholesterol standard (50mg/dl)

Procedure

1. Precipitation: 500 µl of precipitating reagent was added to 250 µl serum and standard. Mixed well and kept for 10 minutes at room temperature to allow reaction, and centrifuged at 4000 rpm for 10 minutes. The clear supernatant was used for further reaction.
2. Three test tubes were taken and labeled as blank, standard and test. The procedure of the assay was as follows.

	Blank	Standard	Test
Supernatant	--	--	50 µl
Standard	--	50 µl	--
Distilled water	50 µl	--	--
Cholesterol working reagent	1.0 ml	1.0 ml	1.0 ml

3. Mixed well and incubated at 37^0C for 10 minutes.
4. Absorbance of test and standard was read against blank at 500nm.

Calculations

HDL Cholesterol (mg/dl)

$$= \frac{\text{OD of test}}{\text{OD of standard}} \text{ x Concentration of standard (50mg/dl)}$$

Precision of the assay

a. Inter-assay co-efficient of variability (CV): 5.76%

	Level 1	Level 2
No. of samples	10	10
Mean (mg/dl)	36.8	62.08
SD	1.86	4.02
CV %	5.05	6.47

b. Intra-assay co-efficient of variability (CV): 5.3%

	Level 1	Level 2
No. of samples	10	10
Mean (mg/dl)	41.2	68.2
SD	2.01	3.9
CV %	4.88	5.71

11. STATISTICAL ANALYSIS

- The obtained data was expressed in mean and standard deviation.
- Level of significance: Statistical significance was established at $p< 0.05$.
- An Unpaired‘t’ test was used to find the difference between pre-intervention or baseline values of study and control groups.
- Paired ‘t’ test (normal distribution data) and Wilcoxon signed rank test (non-normal distribution data) were applied to determine the significant difference between pre-intervention and post-intervention values within the group.
- Analysis of Covariance (ANCOVA) was applied to find the differences in the post-intervention values between study and control groups while controlling the pre-test values. It evaluates whether there is any difference in post-intervention or dependent variable (DV) between groups by reducing the within group error variance (while controlling for the effects of other continuous variables that are not of primary interest, known as covariates). ANCOVA is strongly recommended for randomized controlled studies to find the effect of treatment (Van Breukelen GJ et al., 2006). In ANCOVA, the pre-intervention or pre-test values are used as a covariate and the post-intervention values of study and control group are used as dependent variables. The purpose of using the pre-intervention values as a covariate in randomized controlled study design is to reduce the error variance and to correct the baseline imbalances (eliminate systematic bias). However, the random assignment of subject to groups guards against baseline imbalances (systematic bias), so the purpose of the ANCOVA in randomized studies is mainly to reduce error variance (Dimitrov DM & Rumrill PD Jr., 2003). ANCOVA informs whether there is an overall statistically significant difference in post-intervention values between the different interventions once their means had been adjusted for pre-intervention or baseline values.
- Data were analyzed by using SPSS software version 20.

12. REFERENCES

- Acton A. Alloys-Advances in research and application. 2013 edn. Scholarly Editions publisher, Atlanta, Georgia. 2013: pp-521.
- Allian CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974;20(4):470-5.
- Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963;61:882-8.
- Blacher J, Staessem JA, Girerd X,Gasowski J, Thijs L,Liu L, Wang JG, Fagard RH, Safar ME. Pulse pressure not mean pressure determines cardiovascular risk in older hypertensive patients. *Arch Intern Med* 2000;160(8):1085-9.
- Brewster MA. Vitamins. In Kaplan LA, Pesce AJ, Kazmierczak SC eds. Clinical chemistry theory, analysis and correlation. New York, USA: Mosby publisher; 1996; 786-7.
- Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* 1973;19(5):476-82.
- Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J Lipid Res* 1970;11(6):583-95.
- Choi JW, Pai SH, Kim SK, Ito M, Park CS, Cha YN. Increases in nitric oxide concentrations correlate strongly with body fat in obese humans. *Clin Chem* 2001;47: 1106-9.
- Dimitrov DM, Rumrill PD Jr. Pretest-posttest designs and measurement of change. Work 2003;20(2):159-65.
- Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 1982;28(10):2077-80.
- Ghiadoni L, Bruno RM, Stea F, Virdis A, Taddei S. Central blood pressure, arterial stiffness, and wave reflection: new targets of treatment in essential hypertension. *Curr Hypertens Rep* 2009;11(3):190-6.

- Indian Council for Medical Research. ICMR ethical guidelines for biomedical research on human participants 2006. Available from: http://icmr.nic.in/ethical_guidlines.pdf. Accessed 10 July 2013.
- Laurent S, Boutouyrie P. Recent advances in arterial stiffness and wave reflection in human hypertension. *Hypertension* 2007;49(6):1202-6.
- Lim MA, Townsend RR. Arterial compliance in the elderly: its effect on blood pressure measurement and cardiovascular outcomes. *Clin Geriatr Med* 2009;25(2):191-205.
- Marklund S, Marklund G. Assay of SOD activity in tissue. *J Biochem* 1998;13:305-15.
- McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 1983;29(3):538-42.
- Naidu MU, Reddy BM, Yashmaina S, Patnaik AN, Rani PU. Validity and reproducibility of arterial pulse wave velocity measurement using new device with Oscillometric technique: A pilot study. *Biomed Eng Online* 2005;4: 49.
- Naidu MUR, Reddy CP. Non-Invasive measurement of aortic pressure in patients: Comparing pulse wave analysis and applanation tonometry. *Indian J Pharmacol* 2012;44: 230-3.
- Patil SG, Dhanakshirur G, Aithala MR, Das KK. Comparison of the effects of yoga and lifestyle modification on grade-I hypertension in elderly males: A preliminary study. *Int J Clin Exp Physiol* 2014;1(1):68-72.
- Pailoor S, Telles S. A review of the scientific studies on cyclic meditation. *Int J Yoga* 2009;2: 46-8.
- Pickering TG, Hall JE, Appel LJ et al. Recommendations for blood pressure measurement in human and experimental animals: Part 1: Blood pressure measurement in humans: A statement for professionals from the subcommittee of professional and public education of the American heart association council on high blood pressure research. *Hypertension* 2005;45: 142-61.
- Roe JH, Kuether CA. Determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J Biol Chem* 1943;147:399–407.
- Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. *Z Klin Chem Klin Biochem* 1974 May;12(5):226.

- Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* 1978;90:37-43.
- Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-Aho PO, Karjalainen PA. Kubios HRV-Heart rate variability analysis software. *Comput Methods Programs Biomed* 2014;113(1):210-20.
- Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: Standards of measurement, Physiological interpretation and Clinical use. *Circulation* 1996;93: 1043-65.
- Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann. *Clin. Biochem* 1969;6:24–27.
- The Task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) 2007 Guidelines for the management of arterial hypertension. *Eur Heart J* 2007;28: 1462-536.
- Van Breukelen GJ. ANCOVA versus change from baseline: more power in randomized studies, more bias in nonrandomized studies [corrected]. *J Clin Epidemiol* 2006;59(9):920-5.
- Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol* 2000;525: 263-70.
- Yamashina A, Tomiyama H, Takeda K et al. Validity, reproducibility, and clinical significance of noninvasive brachial ankle pulse wave velocity measurement. *Hypertens Res* 2002;25: 359-64.

CHAPTER IV

PARTICIPANT FLOW & BASELINE CHARACTERISTICS

1. PARTICIPANT FLOW

The subjects were screened from 15th October 2012 to 15th December 2012. An intervention was given from 15th January to 17th April 2013. Most of the elderly participants were retired employees. Almost all the participants were routine to walking and stretching exercise before enrolling in the present study. It was surprising that there were no drop outs. The attendance of the participants for the respective trainings/practices (yoga or walking intervention) in yoga group was 91% while in the control group was 89%. The details of participant flow through the study are shown in CONSORT diagram (Fig 4.1).

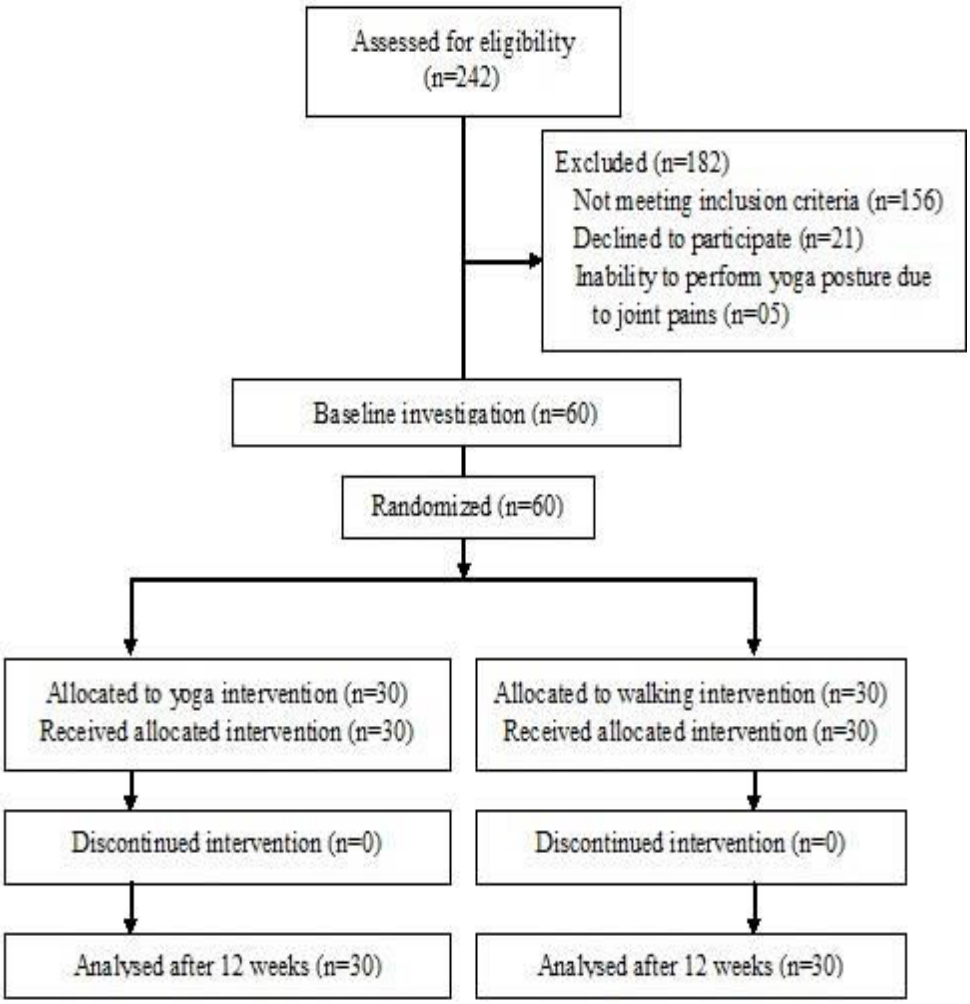


Figure 4.1 CONSORT Flow Diagram

2. BASELINE CHARACTERISTICS OF PARTICIPANTS

The baseline characteristics of the subjects of both yoga and control group were shown below in Table 4.1. As there was no significant difference in age, BMI, HR and BP parameters between the two groups, it implies an equal distribution of samples. Fasting blood glucose, serum triglyceride, total cholesterol and HDL cholesterol levels were within the normal range in both the yoga and control group.

Table 4.1 Baseline characteristics of participants

Variable	Yoga group (n=30)		Control group (n=30)		t-value	p-Value
	Mean	SD	Mean	SD		
Age (Years)	68.50	4.85	69.30	5.93	-0.572	0.57
BMI (kg/m^2)	24.64	3.65	25.17	3.90	-0.546	0.587
Heart Rate (bpm)	70.77	9.08	73.08	11.40	-0.867	0.389
Systolic BP (mmHg)	146.87	5.72	145.83	6.33	0.663	0.51
Diastolic BP (mmHg)	74.2	4.60	75.57	5.69	-1.023	0.31
Pulse Pressure (mmHg)	72.17	6.02	70.33	6.32	1.151	0.255
MAP (mmHg)	98.27	4.525	97.37	8.36	0.518	0.606
Fasting Blood Glucose (mg/dl)	93.83	11.63	91.73	11.94	0.690	0.493
Serum Triglyceride (mg/dl)	93.96	25.45	99.00	23.15	-0.801	0.426
Total Cholesterol (mg/dl)	152.5	24.32	154.23	19.79	-0.303	0.763
HDL Cholesterol (mg/dl)	46.8	4.21	46.16	4.29	0.576	0.567

Unpaired T test was applied to determine the difference between the pre-intervention values of Yoga & control group; BMI- Body mass index; MAP – Mean arterial pressure; Values are expressed in Mean ± SD.

CHAPTER V

FINDINGS, INTERPRETATION OF DATA & DISCUSSION

1. INFLUENCE OF YOGA ON CARDIAC AUTONOMIC NERVOUS SYSTEM

1.1. Results

1.1.1. Within group analysis

In a frequency domain analysis of HRV, we found a significant decrease in LF component from 78.82 nu to 76.38 nu (p=0.012) and LF/HF ratio from 4.01 to 3.47 (p<0.001) and significant increase in HF component from 21.17 nu to 23.85 nu (p=0.008) in yoga practitioners (Table 12). There was no significant effect of walking exercise on HRV in the control group participants (Table 13).

Table 12 Heart rate variability: Baseline and post-intervention values of Yoga group participants

Variable	Baseline		After 3 months		t / z value	p Value
	Mean	SD	Mean	SD		
LF (nu)	78.82	6.10	76.38	6.58	-2.509	0.012**
HF (nu)	21.17	6.10	23.85	6.64	-2.830	0.008**
LF/HF ratio	4.01	1.12	3.47	0.90	-2.487	0.000***

*p<0.05, **p<0.01, ***p<0.001

Table 13 Heart rate variability: Baseline and post-intervention values of control group participants.

Variable	Baseline		After 3 months		t / z value	p Value
	Mean	SD	Mean	SD		
LF (nu)	77.99	9.20	80.36	4.31	-1.782	0.085
HF (nu)	21.07	9.59	19.62	4.34	1.012	0.32
LF/HF ratio	4.13	1.58	4.33	1.22	-0.910	0.37

*p<0.05, **p<0.01, ***p<0.001

1.1.2. Between group analysis

To determine the difference between the effects of yoga and walking on post-intervention HRV, Analysis of Covariance (ANCOVA) was analyzed by considering pre-intervention values as covariate. ANCOVA informs whether there is an overall statistically significant difference in post-intervention values between the different interventions once their means had been adjusted for pre-intervention or baseline values.

Table 14 Results of Analysis of covariance on post-intervention low frequency component (nu) of heart rate variability spectrum between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	881.467[a]	2	440.733	21.748	.000	.433
Intercept	1149.192	1	1149.192	56.707	.000	.499
LF1	644.259	1	644.259	31.791	.000	.358
GROUP	280.710	1	280.710	13.852	.000	.196
Error	1155.135	57	20.266			
Total	370563.690	60				
Corrected Total	2036.602	59				

Dependent variable: Post-intervention LF. LF1: Pre-intervention/baseline low frequency (nu) component of heart rate variability.

Table 14 shows a statistically significant difference in post-intervention LF component of HRV between the yoga and walking interventions (F ratio-13.852, $p<0.001$), after adjusting their means for pre-intervention LF values (R Squared = 0.433; adjusted R squared = 0.413).

Table 15 shows a statistically significant difference in post-intervention HF component of HRV between the yoga and walking interventions (F ratio=12.25, $p<0.001$), after adjusting their means for pre-intervention LF values (R Squared = 0.416; adjusted R squared = 0.395).

Table 15 Results of Analysis of covariance on post-intervention high frequency component (nu) of heart rate variability spectrum between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	871.790[a]	2	435.895	20.289	.000	.416
Intercept	1296.809	1	1296.809	60.360	.000	.514
HF1	603.397	1	603.397	28.085	.000	.330
GROUP	263.149	1	263.149	12.248	.001	.177
Error	1224.626	57	21.485			
Total	30441.030	60				
Corrected Total	2096.417	59				

Dependent variable: Post-intervention HF; HF1: Pre-intervention/baseline low frequency (nu) component of heart rate variability.

Table 16 Analysis of covariance results on post-intervention LF/HF ratio between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	41.333[a]	2	20.667	34.500	.000	.548
Intercept	19.760	1	19.760	32.986	.000	.367
LFHF1	27.361	1	27.361	45.676	.000	.445
GROUP	12.176	1	12.176	20.327	.000	.263
Error	34.145	57	.599			
Total	966.338	60				
Corrected Total	75.478	59				

Dependent variable: Post-intervention LF/HFratio; LF/HF1:Pre-intervention/baseline low frequency (nu) component of heart rate variability.

Table 17 Summary of ANCOVA on post-intervention Heart rate variability between yoga and control groups.

Variable	Yoga group		Control group		F-value	p Value
	Mean	SD	Mean	SD		
LF (nu)	76.38	6.58	80.36	4.31	13.852	0.000***
HF (nu)	23.85	6.64	19.62	4.34	12.248	0.001***
LF/HF ratio	3.47	0.90	4.33	1.22	20.327	0.000***

*p<0.05, **p<0.01, ***p<0.001

Table 16 shows a statistically significant difference in post-intervention LF component of HRV between the yoga and walking interventions (F ratio-20.327, p<0.001), after adjusting their means for pre-intervention LF values (R Squared = 0.548; adjusted R squared = 0.532).

The ANCOVA on HRV has been summarized in Table 17 Above statistical findings suggest that Yoga had high significant beneficial effect on cardiac autonomic nervous system. Thus, the null hypothesis was rejected and the alternate hypothesis that stated "Yoga intervention will be more effective than walking and significantly induces beneficial changes in cardiac autonomic activity in elderly with increased PP" was accepted.

1.2. Discussion

Heart rate variability (HRV) is an established non invasive tool to study cardiac autonomic activity. HRV expresses the balance between the regulation of sympathetic and parasympathetic nervous system. LF measure reflects mainly sympathetic nervous system activity. HF measure reflects an estimate of parasympathetic activity. LF/HF ratio expresses the balance between the regulation of sympathetic and parasympathetic nervous system.

In our study, a significant decrease in LF by 3.07% (p=0.012), LF/HF ratio by 13.46% (p<0.001) and significant increase in HF by 12.65% (p=0.008) were observed in yoga group participants after yoga practice for three months. A decrease in LF component of HRV implies reduction in sympathetic activity whereas elevation in the HF component indicates an increase in parasympathetic activity. As LF/HF ratio is an index of autonomic balance, its decrease suggests a shift in the sympathovagal balance towards the parasympathetic dominance.

Yoga induced beneficial changes in HRV have been reported in different age-groups (Patil SG et al., 2013; Pal GK et al., 2013). Yoga based meditation was also shown to reduce sympathetic activity and increase vagal tone (Pailoor S & Telles S., 2009). The precise mechanism of action of yoga on autonomic nervous system has not been determined. Innes KE et al., have hypothesized that yoga may cause a shift toward parasympathetic nervous system dominance via direct vagal stimulation (Innes KE et al., 2005). It is evident from other studies that slow and regular breathing elicits various beneficial changes through CV reflex control system (Pitzalis MV et al., 1998; Stark R et al., 2000; Grossman E et al., 2001). Breath control is a vital tool of yoga. As we have included a slow and paced breathing in synchronization with all the components of the present yoga module, we presume that beneficial changes in HRV in subjects of yoga group might have elicited through the respiratory and CV reflex control systems. Meditation or relaxation techniques may modify cardiac autonomic nervous system activity through hypothalamus (Pailoor S & Telles S., 2009). Schimdt et al., found a reduction in urinary excretion of adrenaline, nor adrenaline, dopamine and aldosterone during a comprehensive residential three months of yoga training (Schimdt et al., 1997). It is evident from these findings that yoga helps in optimization of autonomic functions.

There was no significant difference in HRV measures in the control group. Audette JF et al., also did not find any change in HRV measures following brisk-walk exercise in older individuals (Audette JF et al., 2006). In a meta-analysis of 13 studies on effect of exercise training on HRV measures, Sandercock GR et al., found that studies of elderly subjects in general showed an attenuated response to training or smaller effect size than those of middle-aged or young subjects (Sandercock GR et al., 2005). Another systematic

review on comparison studies on effect of exercise and yoga on health benefits found that Yoga is more effective than exercise at improving the variety of health benefits including HRV (Ross A & Thomas S., 2010).

It can be concluded from the above findings that yoga is better than walking for beneficial modulation of age-related changes in autonomic nervous system.

2. INFLUENCE OF YOGA ON OXIDATIVE STRESS

2.1. Results

2.1.1. Oxidative stress: Within group analysis

Yoga practice for three months has significantly reduced serum MDA concentration from 1.85 to 1.47 nmol/mL ($p<0.001$) in elderly participants. In contrast, serum MDA concentration was significantly increased from 1.70 to 1.78 nmol/mL ($p=0.04$) (Figure 16).

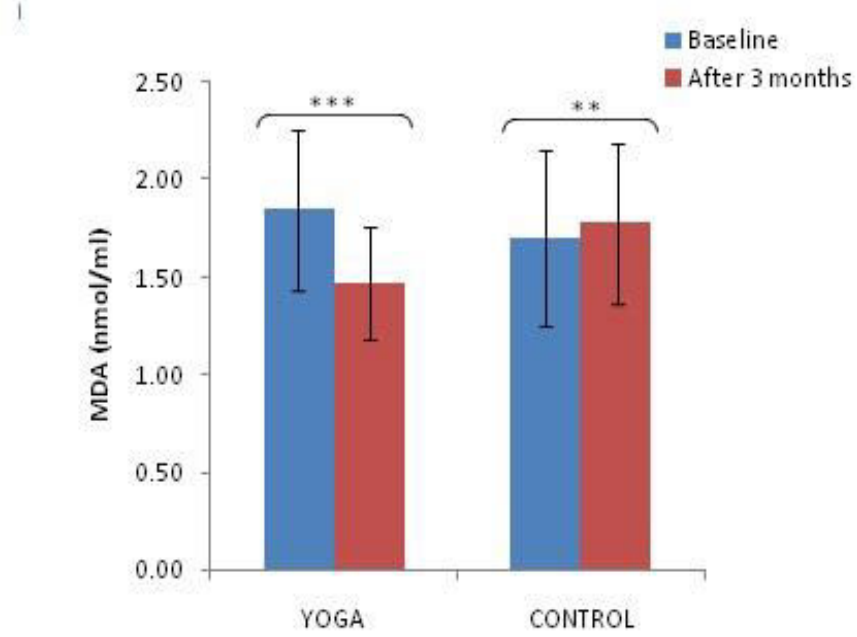


Figure 16 Serum malondialdehyde level: Baseline and post-intervention values in yoga and control groups

2.1.2. Oxidative stress: Between group analysis

The difference between the effects of yoga and walking on post-intervention serum MDA concentration was determined by using Analysis of Covariance (ANCOVA) after adjusting their means for pre-intervention values.

Table 18 shows a statistically significant difference in post-intervention serum MDA concentration between the yoga and walking interventions (F ratio= 57.85, $p<0.001$), after adjusting their means for pre-intervention MDA values (R Squared = 0.739; adjusted R squared = 0.729).

Above findings suggest that Yoga had significantly reduced oxidative stress. Thus, the null hypothesis was rejected and alternate hypothesis that stated "Yoga intervention will be more effective than walking and significantly reduces oxidative stress in elderly with increased PP" was accepted.

Table 18 Results of Analysis of covariance on post-intervention serum malondialdehyde concentration between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	6.193[a]	2	3.096	76.366	.000	.739
Intercept	.500	1	.500	12.327	.001	.186
MDA1	4.822	1	4.822	118.933	.000	.688
GROUP	2.346	1	2.346	57.850	.000	.517
Error	2.190	54	.041			
Total	158.557	57				
Corrected Total	8.382	56				

Dependent variable: Post-intervention malondialdehyde (MDA) concentration; MDA1: Pre-intervention serum malondialdehyde concentration.

1.1.3 Antioxidant capacity: within group analysis

The change in the antioxidant capacity following Yoga and walking intervention is depicted in Figure 17 to 19. Figure 17 shows that Yoga practice for three months has significantly enhanced serum SOD activity from 2.36 to 3.10 U/mL (p=0.007) in elderly participants. While in the control group there was no change in serum SOD activity (Figure 17).

Figure 18 shows an enhancement in erythrocyte reduced glutathione level from 17.01 to 20.49 mg/dL (p=0.002) in Yoga group participants following yoga practice for three months whereas no such change was noticed in the control group.

We have also noticed a significant increase in serum vitamin C level from 0.91 to 1.0 mg/dL (p=0.002) in Yoga group participants (Figure 19). In contrast, serum vitamin C

concentration was significantly decreased from 0.82 to 0.73 mg/dL (p=0.015) in the control group participants (Figure 19).

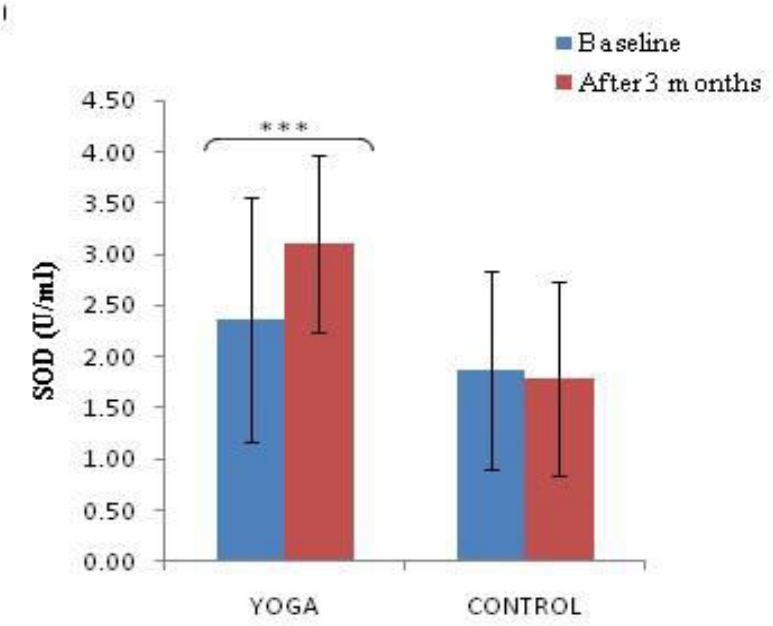


Figure 17 Serum superoxide dismutase activity: Baseline and post-intervention values in yoga and control group

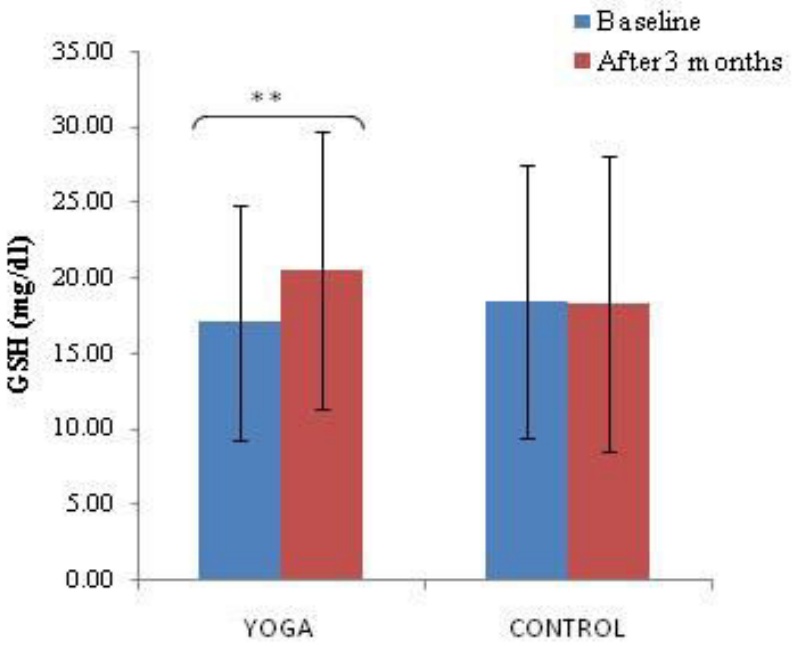


Figure 18 Erythrocyte reduced glutathione: Baseline and post-intervention values in yoga and control group

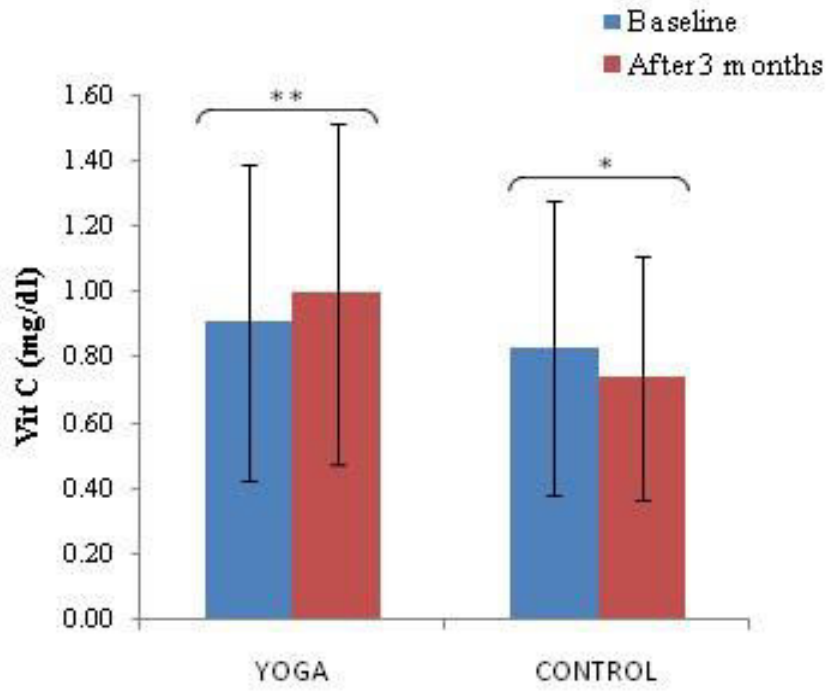


Figure 19 Serum ascorbic acid (vitamin C) level: Baseline and post-intervention values in yoga and control group

2.1.4. Antioxidant capacity: Between group analysis

The difference between the influence of yoga and walking on post-intervention serum SOD activity was analyzed using Analysis of Covariance (ANCOVA) by controlling their pre-intervention values (Table 19 to 22).

Table 19 shows a statistically significant difference in post-intervention serum MDA concentration between the yoga and walking interventions (F ratio= 27.607, $p<0.001$), after adjusting their means for pre-intervention value (R Squared = 0.547; adjusted R squared = 0.530).

Table 20 shows a statistically significant difference in post-intervention erythrocyte reduced glutathione concentration between the yoga and walking interventions (F ratio= 10.44, $p=0.002$), after adjusting their means for pre-intervention value (R Squared = 0.692; adjusted R squared = 0.680).

Table 19 Results of Analysis of covariance on post-intervention superoxide dismutase activity between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	38.530[a]	2	19.265	32.621	.000	.547
Intercept	24.923	1	24.923	42.201	.000	.439
SOD1	13.360	1	13.360	22.622	.000	.295
GROUP	16.304	1	16.304	27.607	.000	.338
Error	31.891	54	.591			
Total	404.672	57				
Corrected Total	70.422	56				

Dependent variable: Post- intervention superoxide dismutase level; SOD1: Pre-intervention serum superoxide dismutase level.

Table 20 Analysis of covariance on post-intervention erythrocyte reduced glutathione between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1709.295[a]	2	854.647	60.560	.000	.692
Intercept	297.882	1	297.882	21.108	.000	.281
GSH1	1640.283	1	1640.283	116.229	.000	.683
GROUP	147.303	1	147.303	10.438	.002	.162
Error	762.076	54	14.113			
Total	23855.657	57				
Corrected Total	2471.371	56				

Dependent variable: Post- intervention erythrocyte reduced glutathione (GSH); GSH1: Pre-intervention erythrocyte reduced glutathione.

Table 21 Results of Analysis of covariance on post-intervention serum vitamin C between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	10.006[a]	2	5.003	236.637	.000	.898
Intercept	.304	1	.304	14.378	.000	.210
VITC1	9.007	1	9.007	426.039	.000	.888
GROUP	.547	1	.547	25.882	.000	.324
Error	1.142	54	.021			
Total	53.649	57				
Corrected Total	11.147	56				

Dependent variable: Post- intervention vitamin C concentration; VITC1: Pre-intervention serum vitamin C concentration.

Table 22 Summary of ANCOVA on post-intervention oxidative stress & antioxidant capacity between yoga and control groups

Variable	Yoga group		Control group		F-value	p Value
	Mean	SD	Mean	SD		
MDA (nmol/ml)	1.47	0.29	1.78	0.41	57.850	0.000***
SOD (U/ml)	3.09	0.86	1.77	0.95	27.607	0.000***
GSH (mg/dl)	20.49	6.09	18.29	7.07	10.438	0.002***
Vit C (mg/dl)	1.00	0.48	0.73	0.37	25.882	0.000***

*p<0.05, **p<0.01, ***p<0.001

Table 21 shows a statistically significant difference in post-intervention serum vitamin C concentration between the yoga and walking interventions (F ratio= 25.88, p<0.001), after adjusting their means for pre-intervention value (R Squared = 0.898; adjusted R squared = 0.894). The ANCOVA on post-intervention oxidative stress and antioxidant capacity has been summarized in Table 22.

Above statistical findings indicate a significant enhancement in antioxidant defense in yoga participants following Yoga practice for three months. Thus, the null hypothesis was rejected and the alternate hypothesis that stated "Yoga intervention will be more effective than walking and significantly enhances antioxidant defense in elderly with increased PP" was accepted.

2.2. Discussion

Reactive oxygen species induced oxidative stress damage the membrane polyunsaturated fatty acids resulting in generation of MDA. Elevation in serum MDA level in hypertensive subjects has been demonstrated (Rodrigo R et al., 2007). In the present investigation, we found a significant reduction in serum MDA level, an indicator of oxidative stress by 20.54% in yoga practitioners, which is nearly similar to the findings of Hegde et al. (20%) and Gordon et al. (19.9%) in type 2 diabetic subjects (Hegde SV et al., 2011; Gordon LA et al., 2008). Conversely, we found an increase in the serum MDA in the control group. These results in control group are consistent with results reported by other studies (Rosado-Perez J et al., 2013; Balci SS et al., 2010). Gordon et al found reduction in oxidant level following moderate-intensity exercise for six months in individuals with type 2 diabetes (Gordon LA et al., 2008). According to Park J et al, mild-intensity exercises such as low-volume walking programme (30-60 minute walking session twice in a week) induce more beneficial changes in the oxidative stress in older adults (Park J et al., 2013). It may be noted that in the yoga module of the present study, we have incorporated 45 minutes for slow-breathing practices and meditation, while for asanas (maintaining postures) only 15 minutes was given.

Growing evidence indicates a strong association between oxidative stress and BP (Rodrigo R et al., 2007). Reactive oxygen species influences cardiovascular structure and function by modulating cell growth and inflammatory responses via reduction-oxidation-dependent signaling pathways. Increased vascular oxidative stress damage the endothelium, reduces nitric oxide production by inhibiting e-NOS pathways and impairs endothelium-dependent vasodilation with resultant enhanced vascular tone and thus hypertension (Briones AM et al., 2010; Grossman E et al., 2008; Kohen R et al., 2002;

Schulz E et al., 2011). Further, oxidative stress causes thickening of the vascular media by promoting smooth muscle cell proliferation and hypertrophy with collagen deposition resulting in narrowing of vascular lumen (Grossman E et al., 2008; Schulz E et al., 2011). These evidences suggest that oxidative stress may play an important role in the development of hypertension. In the present study, yoga has been found effective in reducing BP and oxidative stress in elderly individuals.

A decrease in the activity of antioxidants such as SOD, catalase, glutathione, vitamins C and E may also contribute to oxidative stress (Ceriello A ., 2008). Antioxidants such as SOD, catalase and glutathione act as a primary line of defense against the toxic effects of ROS. Superoxide radicals are detoxified by SOD to produce hydrogen peroxide (H_2O_2) which is further converted to water by catalase and glutathione peroxidase (GSPx). Glutathione peroxidase requires GSH as a coenzyme to convert H_2O_2 to water (Li H et al., 2013). A negative correlation between antioxidants such as SOD, GSH and vitamin C and hypertension has already been reported (Rodrigo R et al., 2007). In our study, evaluation of antioxidant status demonstrated significant increase in SOD activity by 31.35%, GSH level by 20.45% and vitamin C by 9.89% in yoga practitioners (wide Figure 17 to 19). Yoga induced enhancement in endogenous antioxidants like SOD and GSH may be due to increase in their upregulation (Koida G & Hambrecht R., 2005) and decreased rate of utilization due to lowering of oxidative stress. Similarly, an increased level of serum vitamin C, an exogenous antioxidant, in yoga practitioners may also be due to lowering rate of utilization. This yoga induced achievement in antioxidant capacity may help to cope up with deleterious effects of oxidative stress and prevents further damage to cardiovascular cells. Superoxide radicals combine with nitric oxide to form peroxynitrite leading to nitrosative stress. Yoga induced elevated SOD level may also prevent formation of peroxynitrite and thus reduces possibility of nitrosative stress.

Regular exercise has been shown to improve both exogenous and endogenous antioxidant status in animal and human studies (Gordon LA et al., 2008; Kim JD et al., 1996). However, we could not find any significant improvement in the antioxidant status of control group participants (wide Figure 17 to 19). Our results are in accordance with Rosado-Perez et al study (Rosado-Perez J et al., 2013). Some studies have reported

beneficial effects of moderate-intensity exercise on antioxidant system in elderly (Gordon LA et al., 2008; Radak Z et al., 2005). A significant decrease in serum vitamin C level by 10.84% was noticed in the subjects of control group (wide Figure 19). Reduction in vitamin C level might be due to its excessive utilization for detoxification of high levels of ROS generated in the control group subjects.

3. INFLUENCE OF YOGA ON ENDOTHELIAL FUNCTION

3.1. Results

1.1.1. Within group analysis

We have found a significant increase in total serum NOx from 32.38 to 39.9 µmol/L ($p<0.001$) in yoga group where as no such alterations were noticed in the control group participants (Figure 20).

Figure 21 shows a statistically significant decrease in aortic augmentation index from 32.93 to 30.06 ($p=0.005$) in Yoga practitioners while in the control group no significant difference was observed.

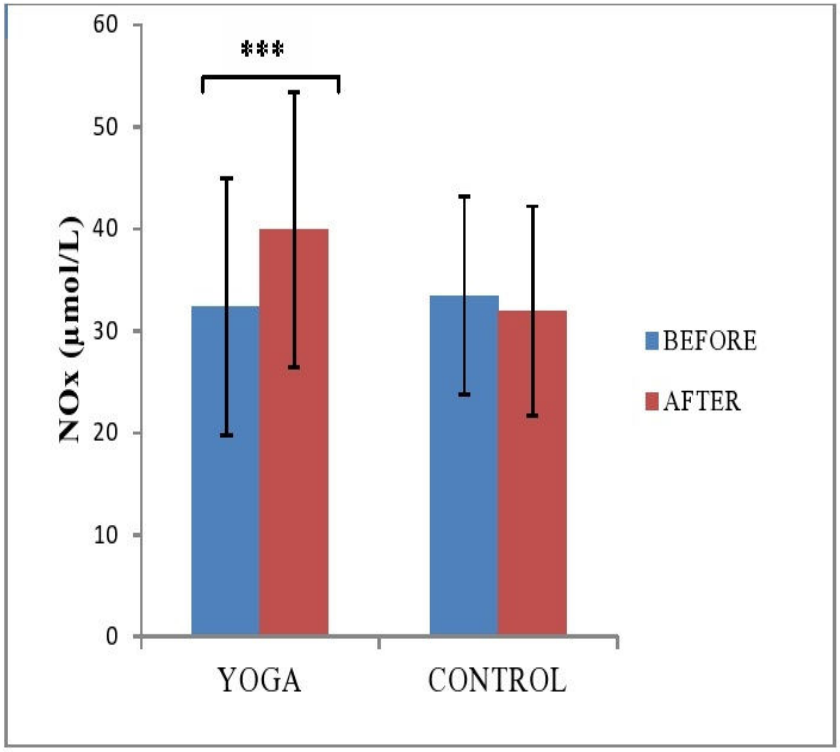


Figure 20 Serum total nitric oxide concentration: Baseline and post-intervention values in yoga and control groups

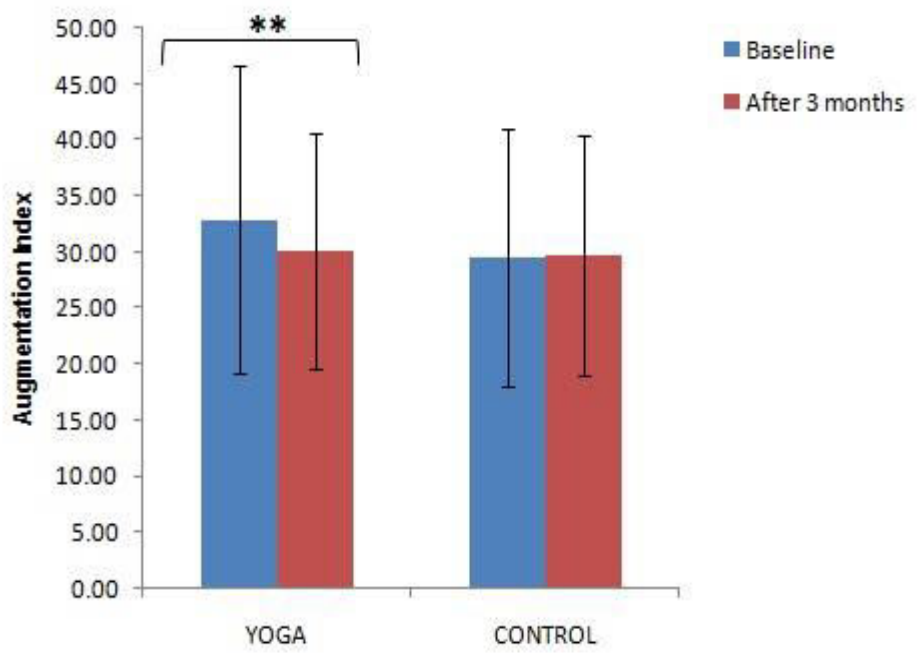


Figure 21 Aortic augmentation index: Baseline and post-intervention values in yoga and control groups

1.1.2. Between group analysis

The statistically significant difference between the effects of yoga and walking on serum NOx and AIx were analyzed using Analysis of Covariance (ANCOVA) (Table 23 & 24).

Table 23 shows a statistically significant difference in post-intervention serum NOx between the yoga and walking interventions (F ratio= 11.14, p=0.001), after adjusting their means for pre-intervention value (R Squared = 0.390; adjusted R squared = 0.368).

Within Yoga group, there was a statistically significant difference in AIx after Yoga practice. However in ANCOVA on post-intervention AIx, there was a difference at the borderline between the yoga and walking interventions (F ratio = 3.52, p=0.066), after adjusting their means for pre-intervention value (R Squared = 0.818; adjusted R squared = 0.811) (Table 24). In Table 25, ANCOVA on post-intervention endothelial function measures has been summarized.

Table 23 Results of Analysis of covariance on post-intervention serum total nitric oxide concentration between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3617.238[a]	2	1808.619	18.188	.000	.390
Intercept	1423.454	1	1423.454	14.315	.000	.201
NO1	2673.250	1	2673.250	26.883	.000	.320
GROUP	1107.711	1	1107.711	11.139	.001	.163
Error	5668.160	57	99.441			
Total	86758.383	60				
Corrected Total	9285.397	59				

Dependent variable: Post-intervention serum total nitric oxide concentration (NOx), NO1: pre-intervention serum total nitric oxide concentration.

Table 24 Analysis of covariance results on post-intervention aortic augmentation index between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	5365.065[a]	2	2682.532	127.941	.000	.818
Intercept	316.048	1	316.048	15.074	.000	.209
AIx1	5363.048	1	5363.048	255.785	.000	.818
GROUP	73.794	1	73.794	3.520	.066	.058
Error	1195.119	57	20.967			
Total	60141.000	60				
Corrected Total	6560.183	59				

Dependent variable: Post- intervention aortic augmentation index, AIx1: pre-intervention aortic augmentation index.

Table 25 Summary of ANCOVA on post-intervention endothelial function between yoga and control groups.

Variable	Yoga group		Control group		F-value	p Value
	Mean	SD	Mean	SD		
NOx (µmol/L)	39.90	13.50	31.97	10.27	11.14	0.001**
AIx (%)	30.06	10.53	29.7	10.73	3.52	0.066

*p<0.05, **p<0.01, ***p<0.001

Above findings indicate a significant improvement in endothelial function in yoga group participants after Yoga practice for three months. Thus, the null hypothesis was rejected and the alternate hypothesis that stated "Yoga intervention will be more effective than walking and significantly improves endothelial function in elderly with increased PP" was accepted.

1.2. Discussion

Age-related endothelial dysfunction results in a decreased bioavailability of NO with resultant enhanced vascular tone (Matz RL et al., 2000; Torregrossa AC et al., 2011). Nitric oxide, a potent vasodilator produced by the vascular endothelial cells is a simple molecule that regulates vascular tone, vascular permeability and antithrombotic properties (Jin RC & Loscalzo J., 2010). The endothelial-dependent vasodilator function is reduced with aging and this impaired NO-mediated vasodilatation is a potential contributor to the age-related increase in arterial stiffness (Wilkinson IB et al., 2002; Fitch RM et al., 2001). A gold standard among the non-invasive methods for the assessment of endothelial function is measurement of endothelial-dependent FMD. In a study by Tsunekawa et al., an increase in endothelial-dependent FMD (about -4%) has been observed along with a rise in serum NOx (23.18%) in elderly subjects in an experimental protocol (Tsunekawa T et al., 2001). Hence, an enhancement in serum NOx implies an improvement in endothelial function. Similarly, in our study, we have found a significant increase in serum NOx by 23.26% (p=0.001) in subjects of yoga group which

demonstrate an improvement in endothelial function in them. Shivshankaran et al. also shown that yoga improves endothelial function in subjects with coronary artery disease (Sivasankaran S et al., 2006). Further, reduction in AIx supports and demonstrates an improvement in endothelial function and its dependent vasorelaxation.

The precise benefits of mechanism of yoga on endothelial function remain unclear. Studies have also shown an association between increased sympathetic activity and endothelial dysfunction (Hijmering ML et al., 2002; Thijssen DHJ et al., 2006). Thijssen et al. demonstrated that sympathetic activation results in decrease in endothelial-dependent FMD in superficial femoral artery in older persons and attenuation of this sympathetic activity restores the FMD (Thijssen DHJ et al., 2006). We hypothesize that yoga induced beneficial changes in endothelial function may be in part by a decrease in sympathetic activity and a shift of autonomic balance towards vagal dominance.

There is a strong association between oxidative stress and endothelial dysfunction. Age-associated increase in vascular oxidative stress damages the endothelium and reduces its NO production. It also contributes to inactivation of NO. Thus, finally resulting in reduction in bioavailability of NO and endothelial dysfunction (Schulz E et al., 2011; Silva BR et al., 2012). Endothelial dysfunction associated with decreased nitric oxide production results in impaired vasodilation and increased blood pressure. It has been mentioned above in section 2 of this chapter that yoga has significantly reduced oxidative stress and improved antioxidant defense in the participants of Yoga group. Yoga induced reduction in oxidative stress may be another possible mechanism for an improvement in endothelial function in yoga practitioners.

4. INFLUENCE OF YOGA ON ARTERIAL STIFFNESS

4.1. Results

4.1.4. Within group analysis

A significant decrease in baPWV from 17.56 to 16.2 m/s ($p<0.001$), c-f PWV from 11.65 to 10.73 m/s ($p=0.001$), AIx@75 from 32.93 to 30.06 ($p=0.005$) and aASI from 45.9 to 38.96 ($p<0.001$) were observed in the yoga group following yoga practice while there was no change in bASI (Table 26). Table 27 shows no significant differences in arterial stiffness indices in control group participants after walking intervention.

Table 26 Vascular stiffness: Baseline and post-intervention values in Yoga group.

Variable	Baseline		After 3 months		t / z value	p Value
	Mean	SD	Mean	SD		
baPWV (m/s)	17.56	4.38	16.20	3.54	-3.507	0.000***
c-f PWV (m/s)	11.65	2.48	10.73	2.10	4.501	0.000***
AIx@75 (%)	32.93	13.78	30.06	10.53	-2.80	0.005**
bASI	30.33	9.82	28.33	6.40	1.723	0.096
aASI	45.9	10.86	38.96	10.41	4.064	0.000***

*p<0.05, **p<0.01, ***p<0.001

Table 27 Vascular stiffness: Baseline and post-intervention values in control group

Variable	Baseline		After 3 months		t / z value	p Value
	Mean	SD	Mean	SD		
baPWV (m/s)	16.91	3.15	17.5	4.05	-1.307	0.202
c-f PWV (m/s)	11.39	2.08	11.71	2.64	-1.277	0.212
AIx@75 (%)	29.5	11.44	29.7	10.73	-0.185	0.854
bASI	31.4	8.54	31.6	8.91	-0.293	0.771
aASI	44.23	10.75	45.6	10.68	-1.093	0.283

*p<0.05, **p<0.01, ***p<0.001

4.1.5. Between group analysis

The difference between the influence of yoga and walking on arterial stiffness measures was analyzed using Analysis of Covariance (ANCOVA) by controlling their pre-intervention values (Table 28 to 32).

Table 28 shows a statistically significant difference in post-intervention baPWV between the yoga and walking interventions (F ratio= 10.16, p=0.002), after adjusting their means for pre-intervention value (R Squared = 0.674; adjusted R squared = 0.663).

A significant difference in post-intervention c-f PWV between the yoga and walking interventions (F ratio= 14.36, p<0.001), after adjusting their means for pre-intervention value (R Squared = 0.746; adjusted R squared = 0.738) was observed (Table 29).

ANCOVA on post-intervention AIx between the yoga and walking interventions (F ratio= 3.52, p=0.066), after adjusting their means for pre-intervention value (R Squared = 0.818; adjusted R squared = 0.811) shown a difference between the groups at the borderline (Table 30).

Though within the group analysis there was no significant difference in bASI, ANCOVA on post-intervention values shows significant difference between the yoga and walking interventions (F ratio= 4.85, p=0.032), after adjusting their means for pre-intervention value (R Squared = 0.694; adjusted R squared = 0.683) (Table 31).

Table 32 shows a statistically significant difference in post-intervention aASI between the yoga and walking interventions (F ratio= 15.898, p<0.001), after adjusting their means for pre-intervention value (R Squared = 0.545; adjusted R squared = 0.529). ANCOVA on all the post-intervention arterial stiffness measures has been summarized in Table 33.

Above findings indicate a significant reduction in arterial stiffness in yoga group participants after Yoga practice for three months. Thus, the null hypothesis was rejected and the alternate hypothesis that stated “Yoga intervention will be more effective than

walking and significantly reduces arterial stiffness in elderly with increased PP" was accepted.

Table 28 Analysis of covariance results on post-intervention brachial-ankle pulse wave velocity between Yoga and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	5845201.35[a]	2	2922600.676	58.982	.000	.674
Intercept	220952.237	1	220952.237	4.459	.039	.073
baPWV1	5589355.953	1	5589355.953	112.800	.000	.664
GROUP	503236.631	1	503236.631	10.156	.002	.151
Error	2824405.980	57	49550.982			
Total	179090514.0	60				
Corrected Total	8669607.333	59				

Dependent variable: Post- intervention brachial-ankle pulse wave velocity; baPWV1: pre-intervention brachial-ankle pulse wave velocity.

Table 29 Results of Analysis of covariance on post-intervention carotid-femoral pulse wave velocity between Yoga and control group.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	2583599.63[a]	2	1291799.814	83.888	.000	.746
Intercept	19259.526	1	19259.526	1.251	.268	.021
cfPWV1	2439953.870	1	2439953.870	158.448	.000	.735
GROUP	221152.356	1	221152.356	14.361	.000	.201
Error	877748.712	57	15399.100			
Total	79014249.05	60				
Corrected Total	3461348.340	59				

Dependent variable: Post- intervention carotid-femoral pulse wave velocity; baPWV1: pre-intervention carotid-femoral pulse wave velocity.

Table 30 Results of Analysis of covariance on post-intervention aortic augmentation index between Yoga and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	5365.065[a]	2	2682.532	127.941	.000	.818
Intercept	316.048	1	316.048	15.074	.000	.209
AIx1	5363.048	1	5363.048	255.785	.000	.818
GROUP	73.794	1	73.794	3.520	.066	.058
Error	1195.119	57	20.967			
Total	60141.000	60				
Corrected Total	6560.183	59				

Dependent variable: Post- intervention values of aortic augmentation index; AIx1: pre-intervention aortic augmentation index.

Table 31 Results of Analysis of covariance on post-intervention brachial arterial stiffness index between Yoga and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2537.250[a]	2	1268.625	64.525	.000
Intercept	343.440	1	343.440	17.468	.000
bASI1	2377.183	1	2377.183	120.908	.000
GROUP	95.297	1	95.297	4.847	.032
Error	1120.684	57	19.661		
Total	57538.000	60			
Corrected Total	3657.933	59			

Dependent variable: Post- intervention brachial arterial stiffness index; bASI1: Pre-intervention brachial arterial stiffness index.

Table 32 Results of Analysis of covariance on post-intervention Tibial arterial stiffness index between Yoga and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3880.879[a]	2	1940.440	34.166	.000	.545
Intercept	397.348	1	397.348	6.996	.011	.109
aASI1	3220.863	1	3220.863	56.711	.000	.499
GROUP	902.935	1	902.935	15.898	.000	.218
Error	3237.304	57	56.795			
Total	114391.000	60				
Corrected Total	7118.183	59				

Dependent variable: Post- intervention Arterial stiffness index at Tibial artery, aASI1: Pre-intervention Arterial stiffness index at Tibial artery.

Table 33 Summary of ANCOVA on post-intervention vascular stiffness between Yoga and control groups.

Variable	Yoga group		Control group		F-value	p Value
	Mean	SD	Mean	SD		
baPWV (m/s)	16.20	3.54	17.5	4.05	10.17	0.002
c-f PWV (m/s)	10.73	2.10	11.71	2.64	14.36	0.001***
AIx@75 (%)	30.06	10.53	29.7	10.73	3.52	0.066
bASI	28.33	6.40	31.6	8.91	4.85	0.032*
aASI	38.96	10.41	45.6	10.68	15.9	0.000***

*p<0.05, **p<0.01, ***p<0.001

4.2. Discussion

Arterial stiffness has become an increasingly important biomarker in the evaluation of CV risk. The baPWV is a measure of central elastic and muscular arterial stiffness and is strongly correlated with c-f PWV, a measure of aortic stiffness (Yamashina A et al., 2002). Brachial-ankle PWV was also shown as an independent

predictor of carotid atherosclerosis in the elderly (Li JY & Zhao YS., 2010). We found a significant decrease in baPWV by 7.74% ($p<0.001$), c-f PWV by 7.89% ($p<0.001$) and AIx@75 by 8.68% ($p=0.005$) following yoga practice for three months. ANCOVA results showed a significant influence of yoga intervention on baPWV and c-f PWV. These findings imply a significant reduction in arterial stiffness in yoga practitioners. A decrease in aASI by 15.09% ($p<0.001$) in yoga group suggests a decrease in peripheral arterial stiffness at tibial artery. We believe that this may be the first randomized controlled study that assessed the influence of yoga on vascular function.

As such, we could not find any significant change in arterial stiffness in the subjects of control group. But, it is noteworthy even that there was no age-associated increase in arterial stiffness in the subjects of control group. Unlike our results in control group, other studies on moderate exercise training on older adults did not find any changes in arterial stiffness (Madden KM et al., 2013; Aizawa K et al., 2008). Gando et al., studied the effect of high-light and low-light physical activity on arterial stiffness in elderly subjects. They found that longer time-spent in light physical activity like household tasks and unstructured activities are associated with attenuation of arterial stiffening in elderly unfit people (Gando Y et al., 2010).

Age-related arterial stiffness was shown to be associated with increased sympathetic activity in hypertensive (Mancia G et al., 1999) and also in healthy subjects (Dinenno FA et al., 2000). It is speculated that reduction in sympathetic activity decreases vascular tone and thus may reduce arterial stiffness. It has been mentioned above (Chapter V, section 1) that yoga practice had significantly decreased sympathetic activity and caused a shift in the sympathovagal balance towards the parasympathetic dominance in yoga group participants. This shift in the autonomic balance towards the parasympathetic dominance and reduction in sympathetic activity may explain at least in part the possible mechanism of reduction in arterial stiffness in the subjects of yoga group.

Oxidative stress influences vascular structure and function by modulating cell growth and inflammatory responses via reduction-oxidation-dependent signaling pathways. It causes thickening of the vascular media by promoting smooth muscle cell proliferation

and hypertrophy with collagen deposition resulting in narrowing of vascular lumen and arterial stiffness (Grossman E ., 2008; Schulz E et al., 2011). Yoga induced reduction in oxidative stress, possibly may leads to beneficial changes in vascular structure and function. However, an intervention for only three months may be very less to predict beneficial changes in vascular structure, but an improvement in the vascular function (through restoration of endothelial function) and its compliance can be predicted through reduction in oxidative stress mechanism.

5. INFLUENCE OF YOGA ON BLOOD PRESSURE

5.1. Results

5.1.4. Within group analysis

A significant reduction in SBP from mean 146.96 to 133.73 mmHg ($p<0.001$), PP from 72.83 to 60.66 ($p<0.001$) and MAP from 98.33 to 93.33 ($p<0.001$) were observed in the yoga group participants following yoga intervention, but there was no significant change in the DBP ($p=0.309$) (Table 34). As such, no significant change was noticed in the control group participants after walking intervention for three months (Table 35).

Table 34 Blood pressure: Baseline and post-intervention values in Yoga participants

Variable	Baseline		After 3 months		t / z value	p Value
	Mean	SD	Mean	SD		
SBP (mmHg)	146.96	5.70	133.73	6.85	-4.72	0.000***
DBP (mmHg)	74.13	4.58	73.13	4.02	-1.02	0.309
PP (mmHg)	72.83	5.68	60.66	6.57	11.36	0.000***
MAP(mmHg)	98.33	4.27	93.33	4.17	7.43	0.000***

*p<0.05, **p<0.01, ***p<0.001

Table 35 Blood pressure: Baseline and post-intervention values in control group participants

Variable	Baseline		After 3 months		t / z value	p Value
	Mean	SD	Mean	SD		
SBP (mmHg)	145.86	6.3	146.86	6.32	-0.75	0.45
DBP (mmHg)	75.53	5.50	74.63	4.39	1.56	0.13
PP (mmHg)	70.33	5.80	72.23	6.50	-1.78	0.08
MAP(mmHg)	98.90	5.18	98.60	4.03	0.67	0.51

*p<0.05, **p<0.01, ***p<0.001

5.1.5. Between group analysis

The difference between effects of Yoga and walking intervention for three months on post-intervention BP was analyzed by ANCOVA by controlling pre-intervention values (Table 36 to 39).

Table 36 shows a statistically significant difference in post-intervention SBP between the yoga and walking interventions (F ratio= 166.14, p<0.001), after adjusting their means for pre-intervention value (R Squared = 0.802; adjusted R squared = 0.795).

Table 37 shows no significant difference between the effects of Yoga and walking on post-intervention DBP.

A significant difference between the effects of Yoga and walking on post-intervention PP (F ratio= 90.53, p<0.001), after adjusting their means for pre-intervention value (R Squared = 0.646; adjusted R squared = 0.634) was observed (Table 38).

ANCOVA on post-intervention MAP shown a significant difference between the yoga and walking interventions (F ratio= 49.33, p<0.001), after adjusting their means for pre-intervention value (R Squared = 0.703; adjusted R squared = 0.693) (Table 39). Table 40 summarizes the results of ANCOVA on post-intervention BP of both groups.

Table 36 Results of Analysis of covariance on post-intervention systolic blood pressure between Yoga and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	4098.935[a]	2	2049.468	115.701	.000	.802
Intercept	24.898	1	24.898	1.406	.241	.024
SBP1	1511.668	1	1511.668	85.340	.000	.600
GROUP	2942.900	1	2942.900	166.140	.000	.745
Error	1009.665	57	17.713			
Total	1186154.000	60				
Corrected Total	5108.600	59				

Dependent variable: Post- intervention systolic blood pressure; SBP1: Pre-intervention systolic blood pressure.

Table 37 Analysis of covariance: Intervention effect on diastolic blood pressure with differences between study and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	463.946[a]	2	231.973	22.102	.000	.437
Intercept	299.891	1	299.891	28.574	.000	.334
DBP1	430.196	1	430.196	40.989	.000	.418
GROUP	8.218	1	8.218	.783	.380	.014
Error	598.237	57	10.495			
Total	328587.000	60				
Corrected Total	1062.183	59				

Dependent variable: Post- intervention diastolic blood pressure, DBP1: Pre-intervention diastolic blood pressure.

Table 38 Results of Analysis of covariance on post-intervention pulse pressure between Yoga and control groups.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	2900.296[a]	2	1450.148	52.099	.000	.646
Intercept	114.414	1	114.414	4.111	.047	.067
PP1	893.479	1	893.479	32.100	.000	.360
GROUP	2519.773	1	2519.773	90.528	.000	.614
Error	1586.554	57	27.834			
Total	269423.000	60				
Corrected Total	4486.850	59				

Dependent variable: Post-intervention pulse pressure, PP1: Pre-intervention pulse pressure.

Table 39 Results of Analysis of covariance on post-intervention mean arterial pressure between Yoga and control group.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	980.220[a]	2	490.110	67.526	.000	.703
Intercept	131.058	1	131.058	18.057	.000	.241
MAP1	564.153	1	564.153	77.727	.000	.577
GROUP	358.055	1	358.055	49.332	.000	.464
Error	413.714	57	7.258			
Total	553970.000	60				
Corrected Total	1393.933	59				

Dependent variable: Post-intervention mean arterial pressure, MAP1: Pre-intervention mean arterial pressure.

Table 40 Summary of ANCOVA on post-intervention blood pressure between study and control group.

Variable	Yoga group		Control group		F-value	p Value
	Mean	SD	Mean	SD		
SBP (mmHg)	133.73	6.85	146.86	6.32	166.14	0.000***
DBP (mmHg)	73.13	4.02	74.63	4.39	0.783	0.38
PP (mmHg)	60.66	6.57	72.23	6.50	90.53	0.000***
MAP(mmHg)	93.33	4.17	98.60	4.03	49.33	0.000***

*p<0.05, **p<0.01, ***p<0.001

Above findings indicate a significant reduction in SBP, PP and MAP in yoga group participants after Yoga practice for three months. Thus, the null hypothesis was rejected and the alternate hypothesis that stated "Yoga intervention is more effective than walking in reducing SBP and PP in elderly" was accepted.

5.2. Discussion

In an investigation on effect of yoga on BP, a significant reduction in SBP by 9% ($p<0.001$), PP by 16.71% ($p<0.001$) and MAP by 5.08% ($p<0.001$) was observed in subjects of yoga group. It is noteworthy that no significant reduction in DBP was noticed following yoga practice (Wide Table 34). These findings are in accordance with our earlier preliminary study (Patil SG et al., 2013). While walking exercise could not induce any beneficial changes in the BP in elderly participants.

As mentioned in Chapter IV, that most of the participants are accustomed to mild walking and exercises regularly. The physical activity level in participants of both groups may be already quite high before the intervention. The benefits of daily physical activity might have reached to threshold level. So, further walking exercise may not have induced any beneficial changes in the participants of control group. As most of the elderly are unfit for exercise or brisk-walking, yoga based breathing practices, relaxation techniques and meditation may be beneficial in improving their vascular function and controlling BP. Moreover, Yoga is spiritually based, which most of the older individuals enjoy and love to perform. They are found to be involved both physically and mentally while performing Yoga, which is most important for getting beneficial changes in overall health. There are sufficient supporting evidences (already mentioned) that light physical activity are beneficial to them (Gando Y et al., 2010; Park J et al., 2013). These evidences support our findings in participants of both groups and explain why only yoga group participants were benefitted.

The yoga induced reduction in SBP and PP may be attributed to decrease in arterial stiffness, sympathetic activity, oxidative stress and increase in NO-mediated vasodilation. The exact precise mechanism of yoga induced reduction in blood pressure remains unclear. We presume that Yoga induced reduction in sympathetic activity might be playing a central role through improvement in arterial compliance and NO bioavailability in the control of hypertension.

5.3. Possible mechanism of yoga induced control of blood pressure in elderly

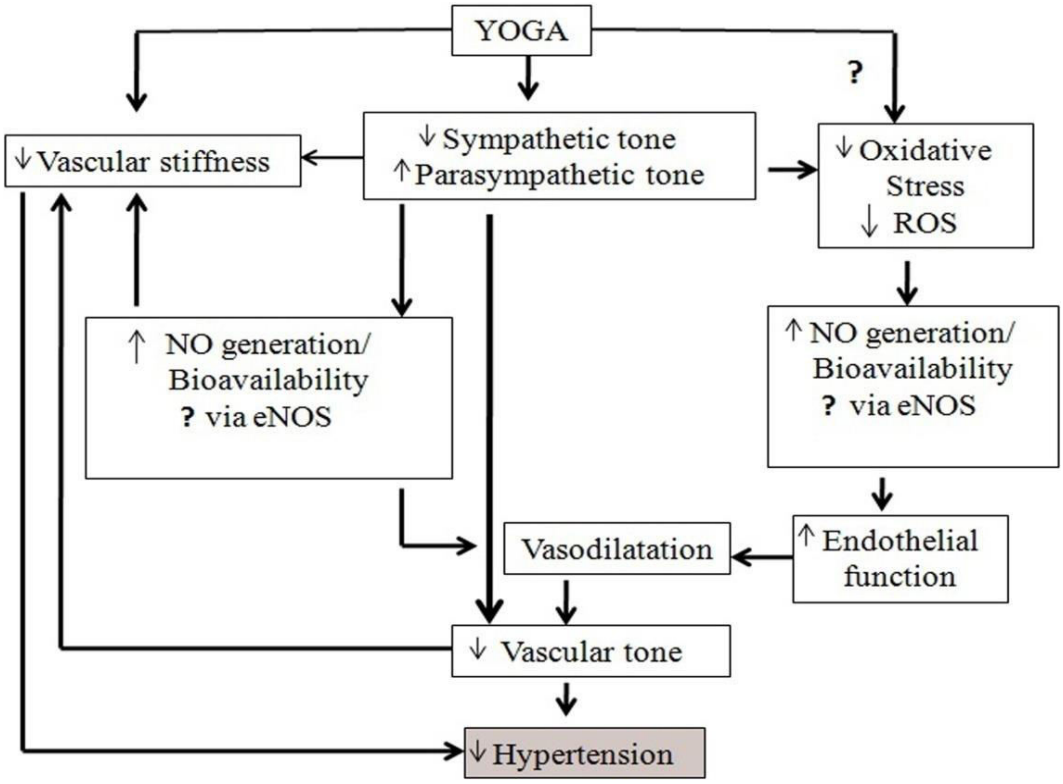


Figure 22 Possible mechanism of yoga induced control of blood pressure in elderly

We propose the possible mechanism of yoga induced control of BP in elderly on the basis of findings of this thesis. Figure 22 shows the possible pathways of yoga induced regulation of BP. There are diverse mechanisms for hypertension in elderly and thus it is presumed that yoga may control BP through diverse possible pathways in elderly. Increase in peripheral vascular resistance is a pathognomonic characteristic of hypertension in the elderly. Possibly, Yoga induced beneficial modulation in the autonomic nervous system with restoration of vagal dominance may be playing a central role in the regulation of BP. Yoga decreases sympathetic nervous system activity resulting in attenuation of vascular tone and peripheral vascular resistance leading to decrease in BP. Reduction in sympathetic activity may also reduce arterial stiffness by attenuating the vascular tone. A shift in sympathovagal balance towards vagal dominance

may improve endothelial-dependent vasodilatation resulting in reduction in peripheral vascular resistance and thus BP. However, the relationship between the sympathetic nervous system overactivity and endothelial dysfunction remains unclear. We believe that Yoga might have direct influence on oxidative stress. Yoga induced attenuation of oxidative stress leads to enhancement in bioavailability of NO which inturn reduces arterial stiffness and BP. Yoga based maintenance of posture (asanas) might induce beneficial changes in arterial stiffness directly by stretching the arteries.

6. REFERENCES

- Aizawa K, Patrella RJ. Acute and chronic impact of dynamic exercise on arterial stiffness in older hypertensives. *Open Cardiovasc Med J* 2008;2;3-8.
- Audette JF, Jin YS, Newcomer R, Stein L, Duncan G, Frontera WR. Tai Chi versus brisk walking in elderly women. *Age Ageing* 2006;35:388-93.
- Balci SS, Okudan N, Pepe H, Gokbel H, Revan S, Kurtoglu F et al. Changes in lipid peroxidation and antioxidant capacity during walking and running of the same and different intensities. *J Strength Cond Res* 2010;24:2545-50.
- Briones AM, Touyz RM. Oxidative stress and hypertension: current concepts. *Curr Hypertens Rep* 2010;12:135-42.
- Ceriello A. Possible role of oxidative stress in the pathogenesis of hypertension. *Diabetes Care* 2008;2:S181-4.
- Dinenno FA, Jones PP, Seals DR, Tanaka H. Age-associated arterial wall thickening is related to elevations in sympathetic activity in healthy humans. *Am J Physiol Heart Circ Physiol* 2000;278: 1205-10.
- Fitch RM, Vergona R, Sullivan ME, Wang YX. Nitric oxide synthase inhibition increases aortic stiffness measured by pulse wave velocity in rats. *Cardiovasc Res* 2001; 51: 351-8.
- Gando Y, Yamamoto K, Murakami H, Ohmori Y, Kawakami Y, Kawakami R, Sanada K et al., Longer time spent in light physical activity is associated with reduced arterial stiffness in older adults. Hypertension 2010;56(3):540-6.
- Gordon LA, Morrison EY, McGrowder DA, Young R, Fraser YT, Zamora EM et al. Effect of exercise therapy on lipid profile and oxidative stress indicators in patients with type 2 diabetes. *BMC Complement Altern Med* 2008;8:21.
- Grossman E. Does increased oxidative stress cause hypertension? *Diabetes Care* 2008;31:S185-9.
- Grossman E, Grossman A, Schein MH, Zimlichman R, Gavish B. Breathing-control lowers blood pressure. *J Hum Hypertens* 2001;15: 263-9.

- Hegde SV, Adhikari P, Kotian S, Pinto VJ, D'Souza S, D'Souza V. Effect of 3-month yoga on oxidative stress in type 2 diabetes with or without complications. *Diabetes Care* 2011;34:2208-10.
- Hijmering ML, Stroes ES, Olijhock J, Hutten BA, Blankestijn PJ. Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilation. *J Am Coll Cardiol* 2002;39: 683-8.
- Innes KE, Bourguignon C, Taylor AG. Risk indices associated with the insulin resistance syndrome, cardiovascular disease, and possible protection with yoga: a systematic review. *J Am Board Fam Pract* 2005;18(6):491-519.
- Jin RC, Loscalzo J Vascular nitric oxide: formation and function. *J Blood Med* 2010;2010(1): 147-62.
- Kim JD, Yu BP, McCarter RJ, Lee SY, Herlihy JT. Exercise and diet modulate cardiac lipid peroxidation and antioxidant defenses. *Free Radic Biol Med* 1996;20:83-8.
- Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* 2002;30:620-50.
- Koida G, Hambrecht R. Molecular mechanism of vascular adaptations to exercise. Physical activity as an effective antioxidant therapy? *Cardiovasc Res* 2005;67:187-97.
- Li JY, Zhao YS. Brachial-ankle pulse wave velocity is an independent predictor of carotid artery atherosclerosis in the elderly. *J Geriatr Cardiol* 2010;7: 157-60.
- Li H, Horke S, Forstermann U. Oxidative stress in vascular disease and its pharmacological prevention. *Trends Pharmacol Sci* 2013;34:313-9.
- Madden KM, Lockhart C, Cuff D, Potter TF, Meneilly GS. Aerobic training-induced improvements in arterial stiffness are not sustained in older adults with multiple cardiovascular risk factors. *J Hum Hypertens* 2013;27(5);335-9.
- Mancia G, Grassi G, Giannattasio C, Seravalle G. Sympathetic activation in the pathogenesis of hypertension and progression of organ damage. *Hypertension* 1999;34: 724-8.

- Matz RL, Schott C, Stoclet JC, Andriantsitohaina R. Age-related endothelial dysfunction with respect to nitric oxide, endothelium-derived hyperpolarizing factor and cyclooxygenase products. *Physiol Res* 2000;49: 11-8.
- Mateos-Caceres PJ, Zamorano-Leon JJ, Rodriquez-Sierra P, Macaya C, Lopez-Farre AJ. New and old mechanism associated with hypertension in the elderly. *Int J Hypertens* 2012;2012:150107.
- Pailoor S, Telles S. A review of the scientific studies on cyclic meditation. *Int J Yoga* 2009;2: 46-8.
- Park J, Miyashita M, Takahashi M, Kawanishi N, Bae S, Kim H et al. Effects of low-volume walking programme and vitamin E supplementation on oxidative damage and health-related variables in healthy older adults. *Nutr Metab (Lond)* 2013; 10:38.
- Patil SG, Mullur L, Khodnapur J, Dhanakshirur GB, Aithala MR. Effect of yoga on short-term heart rate variability measure as an index of stress in subjunior cyclists: A pilot study. *Indian J Physiol Pharmacol* 2013;57:81-6.
- Pal GK, Ganesh V, Karthik S, Nanda N, Pal P. The effects of short-term relaxation therapy on indices of heart rate variability and blood pressure in young adults. *Am J Health Promot* 2013 [Epub ahead of print].
- Patil SG, Dhanakshirur G, Aithala MR, Das KK. Comparison of the effects of yoga and lifestyle modification on grade-I hypertension in elderly males: A preliminary study. *Int J Clin Exp Physiol* 2014;1:68-72.
- Pitzalis MV, Mastropasqua F, Massari F et al. Effect of respiratory rate on the relationships between RR interval and systolic blood pressure fluctuations: a frequency-dependent phenomenon. *Cardiovasc Res* 1998;38: 332-9.
- Radak Z, Chung HY, Goto S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology* 2005;6:71-5.
- Rodrigo R, Prat H, Passalacqua W, Araya J, Guichard C, Bachler JP. Relationship between oxidative stress and essential hypertension. *Hypertens Res* 2007;30:1159-67.
- Ross A, Thomas S. The health benefits of yoga and exercise: a review of comparison studies. *J Altern Complement Med* 2010;16(1):3-12

- Rosado-Perez J, Ortiz R, Santiago-Osorio E, Mendoza-Nunez VM. Effect of Tai Chi versus walking on oxidative stress in Mexican older adults. *Oxid Med Cell Longev* 2013; 2013:298590.
- Sandercock GR, Bromley PD, Brodie DA. Effects of exercise on heart rate variability: inferences from meta-analysis. *Med Sci Sports Exerc* 2005;37:433-9.
- Schimdt T, Wijga A, Von Zur Muhlen A, Brabant G, Wagner TO. Changes in cardiovascular risk factors and hormones during a comprehensive residential three month kriya yoga training and vegetarian nutrition. *Acta Physiol Scand Suppl* 1997; 640: 158–62.
- Schulz E, Gori T, Munzel T. Oxidative stress and endothelial dysfunction in hypertension. *Hypertens Res* 2011;34:665-73.
- Sivasankaran S, Pollard-Quintner S, Sachdeva R, Pugeda J, Hoq SM. The effect of a six-week program of yoga and meditation on brachial artery reactivity: Do Psychosocial interventions affect vascular tone? *Clin Cardiol* 2006;29: 393-8.
- Stark R, Schienle A,Walter B, Vaitl D. Effects of paced respiration on heart period and heart period variability. *Psychophysiology* 2000;37: 302–9.
- Torregrossa AC, Aranke M, Bryan NS. Nitric oxide and geriatrics: Implications in diagnostics and treatment of the elderly. *J Geriatr Cardiol* 2011;8: 230-42.
- Tsunekawa T, Hayashi T, Kano H et al. Cerivastatin, a hydroxymethylglutaryl coenzyme A reductase inhibitor, improves endothelial function in elderly diabetic patients within 3 days. *Circulation* 2001;104: 376-9.
- Thijssen DHJ, Groot PD, Kooijiman M, Smits P, Hopman MTE. Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. *Am J Physiol Heart Circ Physiol* 2006;291: H3122-9.
- Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolio AP, Cockroft JR. Nitric oxide regulates local arterial distensibility in vivo. *Circulation* 2002;105: 213-7.
- Yamashina A, Tomiyama H, Takeda K et al. Validity, reproducibility, and clinical significance of noninvasive brachial ankle pulse wave velocity measurement. *Hypertens Res* 2002;25: 359-64.

CHAPTER VI

SUMMARY & CONCLUSION

1. LIMITATIONS OF THE STUDY

1.1. Though the CV risks in males are equal to females after menopause, only males have participated in the present study, which is the major limitation of the study. The female participants declined to participate in the study because of inconvenience to attend the training in the morning continuously for three months. Therefore, future studies should address the effect of yoga on both genders with hypertension.

1.2. Initially, we have incorporated pulse wave analysis (RIx) method to evaluate endothelial function including endothelial-dependent and independent vasodilation along with serum NOx. But due to technical variation in the instrument (Physiopac, Medicaid systems Ltd), we were compelled to drop pulse wave analysis method. Hence, the endothelial function was assessed by only estimation of serum NOx and AIx. Therefore, future studies should thoroughly investigate the endothelial function.

1.3. The sample size may be sufficient for an experimental design, but to infer the clinical outcomes by RCT seems to require large sample size.

2. SUMMARY AND CONCLUSION

2.1. The purpose of the study was to determine the effect of yoga on vascular function in elderly with increased PP and to explore the yoga induced benefits of mechanism on hypertension in elderly.

2.2. We hypothesized that Yoga intervention will be more effective than walking on vascular stiffness, endothelial function, cardiac autonomic nervous system activity, oxidative stress and antioxidant capacity.

2.3. The objectives of the study were evaluated in an open parallel arm randomized controlled study design. The participants were elderly subjects aged between 60 to 75 years with PP>60mmHg (n=60). All the selected participants were mild hypertensives (Grade I hypertension). Subjects with SBP > 159mmHg and DBP > 99mmHg and subjects with CV risk factors such as diabetes mellitus, hypercholesterolemia and high triglyceride level were the major exclusion criteria.

2.4. Yoga group (n=30) was assigned for yoga training and control group (n=30) for brisk-walk with stretching exercise for one hour in the morning for 6 days in a week for three months.

2.5. The following parameters were tested before and after 3 months of intervention: Arterial stiffness measures: Brachial-ankle pulse wave velocity (baPWV), Carotid-femoral pulse wave velocity (c-f PWV), augmentation index (AIx@75), arterial stiffness index at brachial (bASI) and tibial arteries (aASI); Endothelial function indices: Total serum nitric oxide concentration (NOx), augmentation index (AIx@75); Heart rate variability (HRV) measures: Low frequency (LF), high frequency (HF) and LF/HF ratio; Oxidative stress measure: serum malondialdehyde (MDA) concentration; and antioxidant capacity: serum superoxide dismutase (SOD) activity, erythrocyte reduced glutathione (GSH), serum ascorbic acid or vitamin C.

2.6. We believe that this may be the first randomized controlled study that assessed the influence of yoga on vascular function and oxidative stress in elderly with hypertension.

2.7. We found a significant decrease in c-f PWV by 7.89% ($p<0.001$), baPWV by 7.74% ($p<0.001$), AIx@75 by 8.68% ($p=0.005$) and aASI@75 by 15.09% ($p<0.001$) in yoga group participants. These findings suggest attenuation in arterial stiffening in elderly who practiced yoga. This was the novel finding of the thesis.

2.8. A reduction in LF by 3.07% ($p=0.012$), LF/HF ratio by 13.46% ($p<0.001$) and significant increase in HF by 12.65% ($p=0.008$) in yoga group suggesting a shift in the autonomic balance towards the vagal dominance.

2.9. Yoga had also significantly reduced serum MDA level ($p<0.001$), and enhanced SOD activity ($p=0.007$), serum GSH ($p=0.002$) and vitamin C ($p=0.002$). While in control group, we observed a significant increase in serum MDA level ($p=0.04$) and reduction in serum vitamin C level ($p=0.015$) with no significant difference in the SOD activity and GSH level. This was also the novel finding of the study.

2.10. A significant increase in serum NOx by 23.26% ($p<0.001$) and AIx@75 by 8.68% ($p=0.005$) in the Yoga group was noticed, which is the novel finding of the study. These findings imply an increase in bioavailability of NO and an improvement in endothelial dependent vasodilation in Yoga practitioners.

2.11. A significant decrease in SBP by 9% ($p<0.001$), PP by 16.71% ($p<0.001$) and MAP by 5.08% ($p=0.000$), was observed in the Yoga group participants.

2.12. There were no significant changes in vascular function, oxidative stress, cardiac autonomic nervous system and BP in the participants of control group following walking exercise intervention.

2.13. Most of the participants (both group) used to do mild walking and exercise regularly before enrolment for the study. The benefits of daily physical activity might have reached to threshold level, so further walking exercise may not have induced

any beneficial changes in the participants of control group. The difference in the effect of yoga therapy and brisk-walk exercise may be also due to the fact that the elderly people usually suffer from osteoarthritis and could not exercise or walk effectively.

2.14. Yoga is spiritually based, which most of the older individuals enjoys and love to perform. They were found to be involved both physically and mentally while performing Yoga, which is most important for getting beneficial changes in overall health.

2.15. These findings suggest that yoga module tested in the present study is an effective physiological means to control hypertension along with arterial stiffness in elderly. Yoga has also induced beneficial changes in endothelial function, cardiac autonomic nervous system, oxidative stress and antioxidant defense.

3. FUTURE DIRECTIONS

3.1. Future studies should address the effect of yoga on vascular function in both genders with hypertension with large sample size.

3.2. Further studies are required to make definitive conclusions on influence of yoga on endothelial function and its regulatory genes expression.

3.3. We look forward to study the effect of different components of yoga separately on vascular function in elderly.

3.4. Further research is warranted to find out the pattern of physical activity which is most beneficial to elderly.

APPENDICES

APPENDIX-1

SAMPLE WRITTEN INFORMED CONSENT FORM

BLDEU's SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE BIJAPUR
DEPARTMENT OF PHYSIOLOGY

CONSENT FORM

Title of the Project

Effect of Yoga on endothelial function, vascular compliance and sympathetic tone in elderly subjects with increased pulse pressure: A Randomized clinical study

Principal investigator's name: Satish G Patil

1. PURPOSE OF RESEARCH: I have been informed that this study will assess the effect of Yoga on vascular function in elderly subjects with hypertension. This study will be also useful to understand the benefits of mechanism of Yoga on hypertension.
2. PROCEDURE: I understand that, the procedure of the study will involve recording of various physiological and biochemical parameters. The procedure will not interfere with any of my physiological parameters and they are non invasive.
3. RISK AND DISCOMFORTS: I understand determination of above mentioned tests will not cause any discomfort to me and do not involve any risk to my health.
4. BENEFITS: I understand that my participation in the study may have a direct benefit to me and also to the field of cardiovascular research.
5. CONFIDENTIALITY: I understand that medical information produced by this study will become part of institutional records and will be subject to the confidentiality and privacy regulation of the said institute. Information of a sensitive personal nature will not be a part of medical record, but will be stored in investigators research file and identified only by a code number. The code key connecting name to numbers will be kept in a separate secured location.

If the data are used for publication in the medical literature and for teaching purposes no names will be used and other identities such as photographs, audio and video tapes

will be used only with my special written permission. I understand I may see the photographs and the video tapes and have the audio tapes before giving this permission.

6. REQUEST FOR MORE INFORMATION: I understand that I may ask more questions about the study at any time. Concerned researcher is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study which might influence my continued participation. If during the study or later, I wish to discuss my participation in all concerns regarding this study with a person not directly involved, I am aware that the social worker of the Institute is available to talk with me. A copy of this consent form will be given to me to keep for careful re-reading.
7. REFUSAL OR WITHDRAWAL OF PARTICIPATION: I understand that my participation is voluntary and may refuse to participate or may withdraw my consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that researcher may terminate my participation in this study at any time after she/he has explained the reasons for doing so and had helped arrange for my continued care by my physician or physical therapist if this is appropriate.
8. INJURY STATEMENT: I understand that in unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, then medical treatment will be available to me, but no further compensation would be provided. I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

I have explained to _____ (Patient/Relevant guardian) the purpose of the research, procedures required and the possible risk and benefits to the best of my ability.

Investigator Date

I confirm that _____ (Name of the Principal Investigator) has explained to me the purpose of research, the study procedure that I will undergo, and the possible risk and discomforts as well as benefits that I may experience. Alternative to my participation in the study have also been to give my consent from. Therefore I agree to give consent to participate as a subject and this research project.

Participant Date:

Witness to signature Date:

(Modified from Portney L.G, Watkins M.P., in Foundation of Clinical Research, Second Edition, New Jersey, Prentice Hall Health 2000.)

APPENDIX-II

AWARDS

a. **Young Research Award** (2013) for best scientific poster communication at 9th European Congress Geriatric Medicine Society held in Venice, Italy from 2-4th October 2013.

b. **Travel fellowship award** (2013) from Centre for International Co-operation in Science (CICS), Chennai, to present research findings of PhD Thesis at 9th European Congress Geriatric Medicine Society held in Venice, Italy from 2-4th October 2013.

c. **Foreign Travel Grant** (2013) from Council for Scientific and Industrial Research (CSIR) to present research findings of PhD Thesis at 9th European Congress Geriatric Medicine Society held in Venice, Italy from 2-4th October 2013.

d. ***INSPIRE Research Fellowship award*** from Department of Science & Technology (DST), New Delhi (2011) to pursue full-time PhD in BLDE University, Bijapur, Karnataka

APPENDIX-III

PUBLICATIONS

1. **Patil SG**, Dhanakshirur GB, Aithala MR, Naregal G, Das KK. Effect of yoga on oxidative stress in elderly with grade-I hypertension: A randomized controlled study. *J Clin Diagn Res* 2014;8(7):BC04-07. DOI:10.7860/JCDR/2014/9498.0000

2. **Patil SG,** Dhanakshirur G, Aithala MR, Das KK. Comparison of the effects of yoga and lifestyle modification on grade-I hypertension in elderly males: A preliminary study. *Int J Clin Exp Physiol* 2014;1:68-72.

3. **Patil SG,** Aithala MR, Das KK. Effect of Yoga on Arterial Stiffness in Elderly Subjects with Increased Pulse Pressure: A Randomized Controlled Study. *Complement Ther Med* (Communication).

Original Article

DOI: 10.7860/JCDR/2014/9498.4586

Effect of Yoga on Oxidative Stress in Elderly with Grade-I Hypertension: A Randomized Controlled Study

Physiology Section

Satish G Patil[1], Gopal B Dhanakshirur[2], Manjunatha R Aithala[3], Govindanagouda Naregal[4], Kusal K Das[5]

ABSTRACT

Background and Objectives: Hypertension, especially in elderly is a strong risk factor for cardiovascular mortality and morbidity. Oxidative stress has been implicated as one of the underlying cause of hypertension. Yoga has been found to control hypertension in the elderly, but the underlying benefits of mechanism in relation to oxidative stress regulation remains unclear. The purpose of the study was to investigate the effect of yoga on oxidative stress in elderly with Grade-I hypertension.

Methods: An open parallel-arm randomised controlled study was conducted at BLDE University's Shri B.M.Patil Medical College, Hospital and Research Centre, India on elderly male individuals with Grade-I hypertension (n=57, age 60-80 years). Study (Yoga) group was assigned for yoga intervention and control group for walking for one hour in the morning for six days in a week for three months under the supervision of yoga instructor and physical training instructor respectively. Serum malondialdehyde (MDA) as an indicator of oxidative stress and antioxidants such as serum superoxide dismutase (SOD), reduced glutathione (GSH) and vitamin C levels were estimated.

Results: Yoga practice for three months has significantly reduced serum MDA level ($p<0.001$), and enhanced antioxidants level such as SOD activity ($p=0.007$), serum GSH ($p=0.002$) and vitamin C ($p=0.002$). In the control group, we observed a significant increase in serum MDA level ($p=0.04$) and reduction in serum vitamin C level ($p=0.015$) with no significant difference in the SOD activity and GSH level.

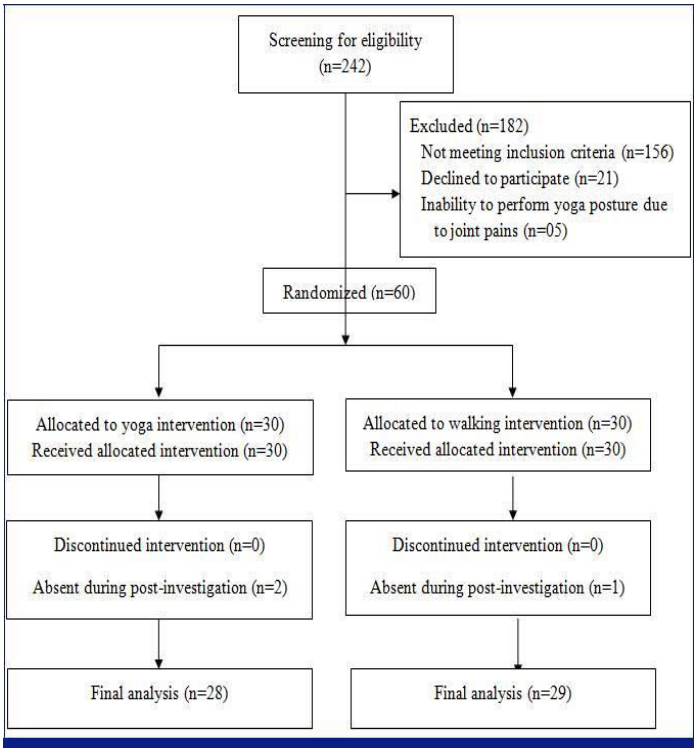
Conclusion: These findings suggest that yoga is an effective means to reduce oxidative stress and to improve antioxidant defense in elderly hypertensive individuals.

Keywords: Antioxidants, Brisk-walk, Elderly, Hypertension, Oxidative stress, Yoga

Introduction

Ageing is an established cardiovascular (CV) risk factor. Hypertension is becoming an important medical and public health problem all over the world and is found to be one of the common disorders of ageing [1]. According to World Health Organization (WHO), the most common cause of preventable death in developed countries is hypertension, which is significantly increasing in developing countries [2]. There are diverse mechanisms and age-related factors involved in the development of hypertension in older individuals. Oxidative stress has been implicated as one of the underlying cause of hypertension [3-6]. An increase in the production of reactive oxygen species (ROS) such as superoxide radicals (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and singlet oxygen causes oxidative stress. Although, ROS are generated in multiple compartments and by multiple enzymes within the cell, but the majority of ROS are produced within the mitochondria during ATP production by oxidative phosphorylation contributing to aging and age-related disorders. If ROS are not removed or neutralized, it can target various cellular constituents like lipid membranes, proteins, DNA and RNA. Our body has evolved complex antioxidant defense mechanism to prevent the deleterious effects of ROS. An imbalance between ROS and antioxidants results in oxidative stress [7]. Oxidative stress contributes to inactivation of nitric oxide, a potent vasodilator, resulting in its decreased bioavailability and endothelial dysfunction [8,9]. Endothelial dysfunction associated with decreased nitric oxide production results in impaired vasodilation and increased blood pressure (BP) [10].

Physical activity and exercise have many beneficial effects for maintaining health, preventing age-related chronic diseases and improving quality of life of older adults [11-13]. However, the optimal amount of exercise for achieving health benefits in the elderly individuals is still unknown. On the other hand, exercise when performed strenuously or even moderately by elderly individuals, is associated with increased production of ROS and oxidative stress [14]. Yoga is another life-style modality, which has well-established health benefits. Yoga has been found to control hypertension in the

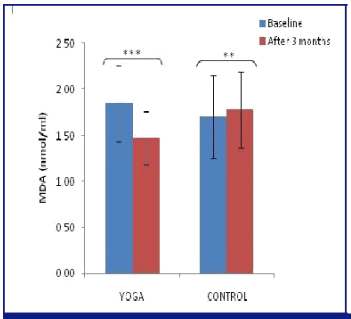


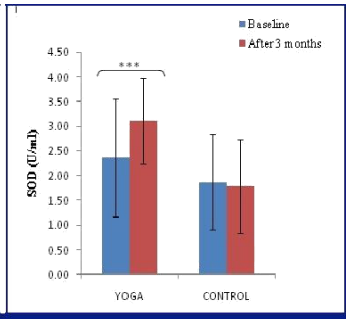
[Table/Fig-1]: Consort flow diagram

Variable	Yoga group (n=28)	Control group (n=29)	p-Value
Age (Years)	68.68 ± 4.97	69.17 ± 5.99	0.736
BMI (kg/m^2)	24.65 ± 3.76	25.54 ± 3.41	0.355
Systolic BP (mmHg)	146.07 ± 5.18	145.72 ± 5.9	0.656
Diastolic BP (mmHg)	74.25 ± 4.68	75.52 ± 5.21	0.281
Fasting Blood Glucose (mg/dl)	93.50 ± 11.94	91.27 ± 11.89	0.484
Serum Triglyceride (mg/dl)	93.89 ± 26.35	98.90 ± 23.56	0.453
Total Cholesterol (mg/dl)	151.36 ± 24.72	153.34 ± 19.52	0.737
HDL Cholesterol (mg/dl)	46.75 ± 4.09	46.07 ± 4.34	0.545

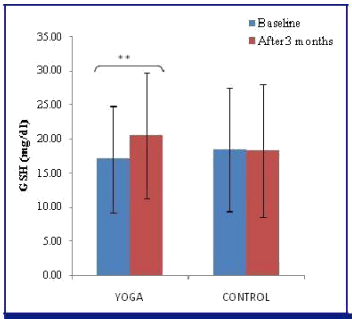
HR- Heart rate; BMI- Body mass index; MAP - Mean arterial pressure; Values are expressed in Mean ± SD. Statistical analysis was done by students unpaired t-test. P < 0.05 was considered statistically significant.

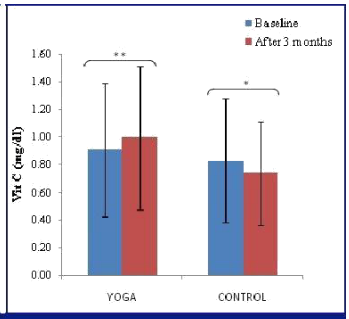
[Table/Fig-2]: Baseline characteristics of participants





[Table/Fig-3]: Comparison of serum malondialdehyde (MDA) level in yoga and control group at baseline and after 3 months of intervention. **[Table/Fig-4]:** Comparison of superoxide dismutase (SOD) activity in yoga and control group at baseline and after 3 months of intervention





[Table/Fig-5]: Comparison of reduced glutathione (GSH) level in yoga and control group at baseline and after 3 months of intervention. **[Table/Fig-6]:** Comparison of serum vitamin C level in yoga and control group at baseline and after 3 months of intervention

elderly [15], but the underlying benefits of mechanism in relation to oxidative stress regulation remains unclear. The aim of the present study was to determine the effect of yoga on oxidative stress in elderly with Grade-I hypertension.

Methods

Study design

This open parallel-group randomised controlled study was conducted on elderly male subjects with Grade-I hypertension (n=57) between 60 to 80 years, in BLDE University's Shri B.M.Patil Medical College, Hospital and Research Centre, India. Subjects with systolic blood pressure (SBP) from 140-159 mmHg and diastolic blood pressure (DBP) from 90-99 mmHg were categorised as Grade-I hypertension as per 2007 guidelines of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) [16]. Subjects on any medications and subjects with CV risk factors such as diabetes mellitus, hypercholesterolemia and high triglyceride level were excluded from the study. The study was approved by the institutional ethical committee of BLDE University's Sri B.M.Patil Medical College, Hospital and Research Centre as per the guidelines (2006) of Indian Council of Medical Research and informed written consent was obtained for participation in the study. The declaration of Helsinki has been followed during the entire study. The study has been reported as per CONSORT declaration [17].

Study protocol

Screening of elderly individuals attending geriatric clinic above 60yrs for Grade-I hypertension was done and were selected after thorough examination as per our inclusion and exclusion criteria. As BP is more variable in older people, the diagnosis of hypertension was made by taking nine BP readings on three separate visits [18]. Brachial BP was measured three times with an interval of one minute on a visit for three consecutive days in a sitting posture using mercury sphygmomanometer (Diamond, Industrial Electronic and Allied Products, India) [19]. Selected subjects were randomly divided into yoga group (n=30) and control group (n=30) by using random number table.

The yoga group was assigned for yoga practice under the supervision of yoga instructor for six days in a week for one hour daily in the morning from 06:00 to 07:00 hours for three months. The integrated yoga module for intervention includes: Opening prayer (1min); Sukshma Vyayama or loosening practices (5min); Breathing practices like Hands in and out breathing, Ankle stretch breathing, Straight leg raising breathing, Lumbar stretch breathing (5 min); Asanas or maintaining postures such as Padhastasana, Ardhachakrasana, Shashankasana, Ardha Ustrasana, Bhujangasana, Ardha Salabasana and Trikonasana (15min); Pranayama or breathing exercises such as Anuloma Viloma Pranayama and Brahmari Pranayama (5min); Cyclic meditation, a yoga based guided relaxation technique [20]; Devotional session (5min); and Closing prayer (1min). The protocol for control group includes flexibility or stretching practices for 15-20 min followed by walking for 35-40 min and rest for 5min for six days in a week, for one hour in the morning between 06:00 to 7.00 hours for three months under the supervision of an authorised instructor.

The recordings were made twice, one at baseline and another after three months of intervention in the morning between 08:00 to 11:00 hours after supine rest for 10min. On the day of investigation, no intervention was given to the participants. Person's handling data analysis were kept blinded.

The participant flow during the study is shown in [Table/Fig-1]. Two participants from the yoga group and one participant from the walking group attended the respective training in the morning

Variable	Yoga Group (n=28)				Control group (n=29)			
	Before	After	Change at 3months	p-value	Before	After	Change at 3 months	p-value
SBP (mmHg)	146.07 ± 5.18	133.86 ± 7.37	-12.21 ± 2.19	<0.001***	145.72 ± 5.9	146.82 ± 6.03	1.1 ± 0.13	0.158
DBP (mmHg)	74.25 ± 4.68	73.10 ± 4.14	-1.15 ± 0.54	0.216	75.52 ± 5.21	74.79 ± 4.37	-0.73 ± 0.84	0.61
PP (mmHg)	71.82 ± 5.37	60.75 ± 7.12	-11.07 ± 1.75	<0.001***	70.20 ± 5.91	72.03 ± 6.95	1.78 ± 1.04	0.085
MAP (mmHg)	98.07 ± 4.20	93.32 ± 4.36	-4.75 ± 0.16	<0.001***	98.86 ± 4.77	98.34 ± 3.60	-0.52 ± 1.11	0.339

Values are expressed in Mean ± SD; *p<0.05, **p<0.01, ***p<0.001. Statistical analysis was done by students paired t-test and Wilcoxon signed rank test. p < 0.05 was considered statistically significant

[Table/Fig-7]: Comparison of blood pressure changes in yoga and control group at baseline and after three months of intervention

regularly but did not appear for the post-interventional investigation due to domestic reasons. Hence, they were not included in the final analysis.

Assessment of oxidative stress and antioxidant status

The blood sample was collected through venous puncture in the morning with overnight fasting for estimation of biochemical parameters. Serum malondialdehyde (MDA), a marker of oxidative stress was estimated by Kei Satoh method [21]. Antioxidants such as reduced glutathione (GSH) was estimated by Beutler E et al., method [22]; Serum vitamin C by 2,4-dinitrophenylhydrazine method [23,24]; and superoxide dismutase (SOD) activity was measured by Marklund and Marklund method [25].

Estimation of blood glucose and lipid profile

Commercial kits from Erba-Mannheim were used for estimation of fasting blood glucose (Trinder's method), serum triglyceride (GPO-PAP method), serum cholesterol (CHOD-PAP method) and HDL cholesterol (phosphotungstic acid method).

Statistical Analysis

The obtained data was expressed as mean and standard deviation. Paired t-test for normally distributed data and Wilcoxon signed rank test for non-normally distributed data was applied for determination of statistical significance. Statistical significance was established at $p< 0.05$. SPSS software version 20 was used for data analysis.

Results

[Table/Fig-2] shows the baseline characteristics of yoga and control group participants. There was no significant difference in the characteristics of participants between two groups suggesting an equal distribution. The baseline values of fasting blood glucose, serum triglyceride, total cholesterol and HDL cholesterol were within the normal range in all the participants.

Yoga practice for three months has significantly reduced serum MDA level ($p<0.001$) in elderly participants, where as in the control group, it was significantly elevated ($p=0.04$) [Table/Fig-3].

A significant enhancement in antioxidant capacity has been observed in yoga participants. Superoxide dismutase (SOD) activity and GSH level were significantly increased ($p=0.007$ and $p=0.002$ respectively) in yoga participants where as no such change was noticed in the control group [Table/Fig-4,5]. We have also noticed a significant increase in serum vitamin C level ($p=0.002$) in yoga group, while it was significantly decreased in the control group ($p=0.015$) following intervention [Table/Fig-6].

[Table/Fig-7] shows a significant reduction in SBP ($p<0.001$), PP ($p<0.001$) and MAP ($p<0.001$) in participants of yoga group, where as in the control group no such changes were noticed.

Discussion

Reactive oxygen species induced oxidative stress, damages the membrane polyunsaturated fatty acids resulting in generation of MDA. Elevation in serum MDA level in hypertensive subjects has been demonstrated [26]. In the present investigation, we observed a significant reduction in serum MDA level, an indicator of oxidative stress by 20.54% in yoga practitioners, which is nearly similar to the findings of Hegde et al., (20%) and Gordon et al., (19.9%) in type 2 diabetic subjects [27,28]. Conversely, we found an increase in the serum MDA in the control group. These results in control group are consistent with results reported by other studies [29,30]. In another study, Gordon et al., found reduction in oxidant level following moderate-intensity exercise for six months in individuals with type 2 diabetes [28]. According to Park J et al., mild exercise such as low-volume walking programme (30-60 minute walking session twice in a week) induce more beneficial changes in the oxidative stress in older adults than moderate-intensity exercise [31]. It may be noted in the yoga module of the present study that, we have incorporated 45min for slow-breathing practices, relaxation technique and meditation, while for asanas (maintaining postures) 15min was given. It is widely accepted that increased oxygen consumption during exercise results in excess generation of ROS. Whereas, yoga based relaxation technique and meditation was found to be associated with decreased oxygen consumption [32]. Hence, we presume that low consumption of oxygen during yoga practice probably reduced serum MDA level in the yoga practitioners of the present study. To the best of our knowledge, this is the first study reporting on effect of yoga on oxidative stress and antioxidant defense in elderly hypertensives.

Growing evidence indicates a strong association between oxidative stress and BP [26]. Reactive oxygen species influences cardiovascular structure and function by modulating cell growth and inflammatory responses via reduction-oxidation-dependent signaling pathways. Increased vascular oxidative stress damage the endothelium, reduces nitric oxide production by inhibiting e-NOS pathways and impairs endothelium-dependent vasodilation with resultant enhanced vascular tone and thus hypertension [5,6,8]. Further, oxidative stress causes thickening of the vascular media by promoting smooth muscle cell proliferation and hypertrophy with collagen deposition resulting in narrowing of vascular lumen [6,8]. These evidences indicate that oxidative stress may play an important role in the development of hypertension. In the present study, yoga has been found effective in reducing BP and oxidative stress in elderly individuals.

A decrease in the activity of antioxidants such as SOD, catalase, glutathione, vitamins C and E may also contribute to oxidative stress [3]. Antioxidants such as SOD, catalase and glutathione act as a primary line of defense against the toxic effects of ROS. Superoxide radicals are detoxified by SOD to produce hydrogen peroxide (H_2O_2) which is further converted to water by catalase and glutathione peroxidase (GSPx). Glutathione peroxidase requires GSH as a coenzyme to convert H_2O_2 to water [33]. A negative correlation between antioxidants (such as SOD, GSH and vitamin C) and hypertension has already been reported [26]. In our study, evaluation of antioxidant status demonstrated significant increase in SOD activity by 31.35%, GSH level by 20.45% and vitamin C by 9.89% in yoga practitioners [Table/Fig-4-6]. Yoga induced enhancement in endogenous antioxidants like SOD and GSH may be due to increase in their upregulation [34] and decreased rate of utilization due to lowering of oxidative stress. Similarly, an increased level of serum vitamin C, an exogenous antioxidant, in yoga practitioners may also be due to lowering rate of utilization. This yoga induced achievement in antioxidant capacity may help to cope with deleterious effects of oxidative stress and prevents further damage to cardiovascular cells. Superoxide radicals combine with nitric oxide to form peroxynitrite leading to nitrosative stress. Yoga induced elevated SOD level may also prevent formation of peroxynitrite and thus reduces possibility of nitrosative stress.

Regular exercise has been shown to improve both exogenous and endogenous antioxidant status in animal and human studies [28, 35]. However, we could not find any significant improvement in the antioxidant status of control group participants [Table/Fig-4-6]. Our results are in accordance with findings of Rosado-Perez et al., study [29]. Some studies have reported beneficial effects of moderate-intensity exercise on antioxidant system in elderly [28,36]. A significant decrease in serum vitamin C level by 10.84% was noticed in the subjects of control group [Table/Fig-6]. Reduction in vitamin C level might be due to its excessive utilization for detoxification of high levels of ROS generated in the control group subjects.

Conclusion

The findings of the study suggest that yoga can be used as an effective life-style modality to reduce oxidative stress and to enhance antioxidant defense in elderly with hypertension. Further, it

is essential to develop effective physical activity strategies to reduce oxidative stress in elderly with hypertension.

Acknowledgement

We express our sincere thanks to Department of Science and Technology, Government of India and BLDE University for financial assistance. We are also thankful to all elderly volunteers for participation in the study.

References

[1] Fagard RH. Epidemiology of hypertension in the elderly. *Am J Geriatr Cardiol*. 2002;11:23-8.
[2] Ezzati M, Lopez AD, Rodgers A, Van der Hoorn S, Murray CJ. Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. *Lancet*. 2002; 360:1347-60.
[3] Ceriello A. Possible role of oxidative stress in the pathogenesis of hypertension. *Diabetes Care*. 2008;2:S181-84.
[4] Mateos-Caceres PJ, Zamorano-Leon JJ, Rodriquez-Sierra P, Macaya C, Lopez-Farre AJ. New and old mechanism associated with hypertension in the elderly. *Int J Hypertens*. 2012;2012:150107.
[5] Briones AM, Touyz RM. Oxidative stress and hypertension: current concepts. *Curr Hypertens Rep*. 2010;12:135-42.
[6] Grossman E. Does increased oxidative stress cause hypertension? *Diabetes Care*. 2008;31:S185-89.
[7] Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol*. 2002;30:620-50.
[8] Schulz E, Gori T, Munzel T. Oxidative stress and endothelial dysfunction in hypertension. *Hypertens Res*. 2011;34:665-73.
[9] Silva BR, Pernomian L, Bendhack LM. Contribution of oxidative stress to endothelial dysfunction in hypertension. *Front Physiol*. 2012;3:441.
[10] Jin RC, Loscalzo J. Vascular nitric oxide: Formation and function. *J Blood Med*. 2010;2010:147-62.
[11] Nelson ME, Rejeski WJ, Blair SN, Duncan PW, Judge JO, King AC, et al. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc*. 2007;39:1435-45.
[12] Cornelissen VA, Arnout J, Holvoet P, Fagard RH. Influence of exercise at lower and higher intensity on blood pressure and cardiovascular risk factors at older age. *J Hypertens*. 2009;27:753-62.
[13] Heckman GA, McKelvie RS. Cardiovascular aging and exercise in healthy older adults. *Clin J Sport Med*. 2008;18:479-85.
[14] Ji LL. Exercise at old age: does it increase or alleviate oxidative stress? *Ann NY Acad Sci*. 2001;928:236-47.
[15] Patil SG, Dhanakshirur G, Aithala MR, Das KK. Comparison of the effects of yoga and lifestyle modification on grade-I hypertension in elderly males: A preliminary study. *Int J Clin Exp Physiol*. 2014;1:68-72.
[16] The Task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). 2007 Guidelines for the management of arterial hypertension. *Eur Heart J*. 2007; 28:1462-536.
[17] Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomised Trials. *PloS Med*. 2010;7: e1000251.
[18] Supiano MA. Hypertension. In: Halter JB, Ouslander JG, Tinetti ME, Studenski S, High KP, Asthana S, eds. Hazard's Geriatric medicine and Gerontology. 6th edition, New Delhi: McGraw Hill; 2009: 975-82.
[19] Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, et al. Recommendations for blood pressure measurement in human and experimental animals: Part 1: Blood pressure measurement in humans: A statement for professionals from the subcommittee of professional and public education of the American heart association council on high blood pressure research. *Hypertension*. 2005;45:142-61.
[20] Pailoor S, Telles S. A review of the scientific studies on cyclic meditation. *Int J Yoga*. 2009;2:46-8.
[21] Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chimica Acta*. 1978;90:37-43.
[22] Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med*. 1963;61:882-88.
[23] Roe JH, Kuether CA. Determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J *Biol Chem*. 1943;147:399–407.
[24] Brewster MA. Vitamins. In Kaplan LA, Pesce AJ, Kazmierczak SC eds. Clinical chemistry theory, analysis and correlation. New York, USA: Mosby publisher; 1996;786-87.
[25] Marklund S, Marklund G. Assay of SOD activity in tissue. *J Biochem*. 1998;13:305-15.
[26] Rodrigo R, Prat H, Passalacqua W, Araya J, Guichard C, Bachler JP. Relationship between oxidative stress and essential hypertension. *Hypertens Res*. 2007;30:1159-67.
[27] Hegde SV, Adhikari P, Kotian S, Pinto VJ, D'Souza S, D'Souza V. Effect of 3-month yoga on oxidative stress in type 2 diabetes with or without complications. *Diabetes Care*. 2011;34:2208-10.
[28] Gordon LA, Morrison EY, McGrowder DA, Young R, Fraser YT, Zamora EM, et al. Effect of exercise therapy on lipid profile and oxidative stress indicators in patients with type 2 diabetes. *BMC Complement Altern Med*. 2008;8:21.
[29] Rosado-Perez J, Ortiz R, Santiago-Osorio E, Mendoza-Nunez VM. Effect of Tai Chi versus walking on oxidative stress in Mexican older adults. *Oxid Med Cell Longev*. 2013; 2013:2985-90.
[30] Balci SS, Okudan N, Pepe H, Gokbel H, Revan S, Kurtoglu F, et al. Changes in lipid peroxidation and antioxidant capacity during walking and running of the same and different intensities. *J Strength Cond Res*. 2010;24:2545-50.
[31] Park J, Miyashita M, Takahashi M, Kawanishi N, Bae S, Kim H, et al. Effects of low-volume walking programme and vitamin E supplementation on oxidative damage and health-related variables in healthy older adults. *Nutr Metab* (Lond). 2013; 10:38.
[32] Telles S, Reddy SK, Nagendra HR. Oxygen consumption and respiration following two yoga relaxation techniques. *Appl Psychophysiol Biofeedback*. 2000;25:221-27.
[33] Li H, Horke S, Forstermann U. Oxidative stress in vascular disease and its pharmacological prevention. *Trends Pharmacol Sci*. 2013;34:313-19.
[34] Kojda G, Hambrecht R. Molecular mechanism of vascular adaptations to exercise. Physical activity as an effective antioxidant therapy? *Cardiovasc Res*. 2005 ;67:187-97
[35] Kim JD, Yu BP, McCarter RJ, Lee SY, Herlihy JT. Exercise and diet modulate cardiac lipid peroxidation and antioxidant defenses. *Free Radic Biol Med*. 1996;20:83-8.
[36] Radak Z, Chung HY, Goto S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology*. 2005;6:71-5.

PARTICULARS OF CONTRIBUTORS:
1. PhD Fellow, Department of Physiology, BLDE University's Shri B.M.Patil Medical College, Hospital & Research Centre, Karnataka, India.
2. Professor, Department of Physiology, BLDE University's Shri B.M.Patil Medical College, Hospital & Research Centre, Karnataka, India.
3. Professor, Department of Physiology, BLDE University's Shri B.M.Patil Medical College, Hospital & Research Centre, Karnataka, India.
4. Postgraudate, Department of Biochemistry, BLDE University's Shri B.M.Patil Medical College, Hospital & Research Centre, Karnataka, India.
5. Professor, Department of Physiology, BLDE University's Shri B.M.Patil Medical College, Hospital & Research Centre, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Satish G Patil,
PhD Fellow, Department of Physiology, BLDE University's Shri B.M.Patil Medical College,
Hospital & Research Centre, Karnataka, India.
Phone: 91-9986789583, E-mail: sathupatil@yahoo.co.in

Financial OR OTHER COMPETING INTERESTS: None.

Date of Submission: Apr 01, 2014
Date of Peer Review: May 12, 2014
Date of Acceptance: May 23, 2014
Month of Publishing: July, 2014

Original Article

Comparison of the effects of yoga and lifestyle modification on grade-I hypertension in elderly males: A preliminary study

Satish Gurunathrao Patil, Gopal Dhanakshirur, Manjunatha Ramakrishna Aithala, Kusal Kanti Das

Department of Physiology, BLDE University's Shri B. M. Patil Medical College, Hospital and Research Centre, Bijapur, Karnataka, India

Abstract

Background and Aim: Aging along with hypertension is a major risk factor for cardiovascular (CV) morbidity and mortality. It is noticed that systolic hypertension in elderly is often associated with increased CV risks and is resistant to pharmacological treatment. Hence, we aimed to assess the difference between practice of yoga and lifestyle modifications (LSM) in elderly grade-I hypertensive males.

Methods: A randomized control study was conducted on age and body mass index (BMI)-matched elderly male subjects (n = 42) between 60–80 years with grade-I hypertension. They were equally divided into yoga group (n = 21) and LSM group (n = 21). Their fasting blood glucose and lipid profile were recorded before the intervention period, and both the groups were matched for these biochemical parameters. The yoga group was assigned for practice of a yoga module and the LSM group (n = 21) was assigned for stretching exercises and brisk walk, for 6 days in a week, for 1 h in the morning for 6 weeks. Their CV parameters including heart rate and blood pressure (BP) were recorded before and after the intervention period.

Results: We found a significant decrease in systolic BP (P < 0.001), pulse pressure (P < 0.001), mean arterial pressure (P < 0.001), and rate pressure product (P < 0.001) in elderly hypertensives following yoga therapy for 6 weeks, whereas no statistically significant change was noticed in the LSM group practicing stretching exercise and brisk walk for the same duration.

Conclusion: Yoga intervention for 6 weeks could be an effective non-pharmacological means for better management than the LSM for control of BP in elderly subjects having grade-I hypertension.

Key words: Elderly, grade-I hypertension, lifestyle modification, males, yoga

Received: 28th November, 2013; *Revised:* 14th January, 2014; *Accepted:* 8th February, 2014

INTRODUCTION

Increased age is an established cardiovascular (CV) risk factor. Aging along with hypertension is a major risk factor for CV morbidity and mortality.[1] The prevalence of hypertension in elderly ranges from 60 to 80%, and it is estimated that two of three individuals over 75 years of age suffer from hypertension.[1,2] A change in the patterns of hypertension with age has been observed. In elderly, systolic blood pressure (SBP) increases without much change in diastolic blood pressure (DBP), which is categorized as isolated systolic hypertension (ISH). Systolic hypertension may lead to stroke, myocardial infarction, dementia, renal failure, and death.[3] These clinical complications affect the quality and longevity of life in elderly. According to World Health Organization, the most common cause of preventable death in developed countries is hypertension, which is significantly increasing in developing countries.[4]

Reduction of systolic hypertension in elderly subjects could reduce clinical complications, extend lifespan, and improve

Access this article online	
Quick Response Code:	Website: www.ijcep.org
	DOI: 10.4103/2348-8093.129747

Address for correspondence: Dr. Kusal Kanti Das, Department of Physiology, BLDE University's Shri B. M. Patil Medical College, Hospital and Research Centre, Bijapur - 586 103, Karnataka, India. E-mail: research@bldeuniversity.ac.in

quality of life.[5] However, the elderly individuals suffering from ISH are often resistant to pharmacological treatment and attempts to reduce the SBP aggressively also lowers DBP to a greater extent that compromises coronary blood flow.[6] Among the non-pharmacological approaches, yoga has emerged as the most effective therapy to control hypertension and improve CV function.[7-10] Though practice of lifestyle modification (LSM) such as morning walk and stretching exercises is known to reduce blood pressure (BP), its impact in elderly patients may not be effective as many of them invariably suffer from osteoarthritic joint diseases that prevent them from fruitful participation in such LSM program. Yoga is an ancient system of spiritual practice having a psychosomatic discipline comprising physical and mental techniques, that help to achieve a harmony between the mind and body. However, no study has been conducted till date to compare the benefits of yoga with LSM practice in elderly mild hypertensives. Therefore, the present study was conducted to assess the difference in the effects of practice of yoga and LSM in elderly grade-I hypertensive males.

MATERIALS AND METHODS

Participants and study design

It is a randomized control study conducted on elderly male subjects between 60 to 80 years with grade-I hypertension. Subjects with SBP from 140 to 159 mmHg and DBP from 90 to 99 mmHg were included for the study. Subjects on any medications, suffering from diabetes mellitus or CV diseases, hypercholesterolemia, and high triglyceride level were excluded from the study. The task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) in its 2007 guidelines, classified hypertension/ISH into three categories: Grade-I (SBP/DBP: 140–159/90–99 mmHg), grade-II (SBP/DBP: 160–179/100–109 mmHg), and grade-III (SBP ≥ 180 or DBP ≥ 100 mmHg).[11] In the same guidelines, they recommended for lifestyle changes intervention for few months for grade-I hypertension without any CV risk factors, before drug therapy. The present study was approved by the institutional ethical committee as per the guidelines (2006) of Indian Council of Medical Research. We followed the declaration of Helsinki and the study was reported as per the recommendations of the CONSORT group.[12] Informed written consent was obtained for participation in the study.

Randomization and intervention

Subjects were randomly divided into yoga group (n = 21) and LSM group (n = 21) by using random number table. However, it was ensured that subjects of both the groups matched for age, body mass index (BMI), fasting blood glucose, and lipid profile [Table 1]. The yoga group was assigned to yoga practice by an authorized yoga instructor for 6 days in a week for 1 h daily in the morning from 06:00 to 07:00 h for 6 weeks. The integrated yoga module for intervention includes: Opening prayer (1 min); Sukshma Vyayama or loosening practices (5 min); breathing practices like hands in and out breathing, ankle stretch breathing, straight leg raising breathing, lumbar stretch breathing (5 min); asanas or maintaining postures such as Padhastasana, Ardhachakrasana, Shashankasana, Ardha Ustrasana, Bhujangasana, Ardha Salabasana, and Trikonasana (15 min); Pranayama or breathing exercises such as Anuloma-Viloma Pranayama and Brahmari Pranayama (5 min); cyclic meditation, a yoga-based guided relaxation technique;[13] devotional session (5 min); and closing prayer (1 min). The protocol for the LSM group consisted of flexibility or stretching practices for 20 min followed by brisk walk for 35 min and rest for 5 min for 6 days in a week, for 1 h in the morning between 06:00-07:00 h for 6 weeks under the supervision of an authorized instructor.

Table 1: Baseline characteristics of participants in both yoga and LSM groups

Parameters	Yoga (n=21)	LSM (n=21)	P value
Age (years)	69.42±5.32	69.52±6.59	0.959
BMI (kg/m^2)	24.18±3.39	24.64±3.67	0.676
HR (bpm)	70.33±8.30	72.09±8.82	0.509
Systolic BP (mmHg)	147.23±5.62	147.00±5.82	0.894
Diastolic BP (mmHg)	74.95±3.8	75.52±5.43	0.695
Pulse pressure (mmHg)	72.28±6.03	71.47±6.09	0.668
MAP (mmHg)	98.8±3.53	98.8±4.94	1.000
Fasting blood glucose (mg/dl)	95.09±10.79	91.52±12.51	0.328
Serum triglyceride (mg/dl)	97.85±27.14	105.76±23.29	0.317
Total cholesterol (mg/dl)	149.19±24.98	152.33±21.84	0.667
HDL cholesterol (mg/dl)	46.66±4.37	46.61±4.63	0.973

Values are expressed in mean±SD. Statistical analysis was done by student's unpaired 't' test. P<0.05 was considered statistically significant. LSM: Lifestyle modification, HR: Heart rate, BMI: Body mass index, MAP: Mean arterial pressure, HDL: High-density lipoprotein, BP: Blood pressure, SD: Standard deviation

Measurement of heart rate and BP

Heart rate (HR) was derived from RR interval of electrocardiogram (ECG) recordings (Physiopac, Medicaid systems, India). Brachial BP recordings were made twice, one at baseline and another after 6 weeks of intervention in the morning between 08:00-11:00 h after supine rest for 10 min. BP was measured thrice with an interval of 1 min for 3 consecutive days using mercury sphygmomanometer (Diamond, Industrial electronic, and allied products, India) and the average of nine measurements was considered.[5,14] Rate pressure product (RPP), a determinant of myocardial oxygen consumption and work load was calculated using the formula, RPP = (BHR × SBP) ×10^{-2}.[15] Pulse pressure (PP) was calculated as the difference between SBP and DBP.

Mean arterial pressure (MAP) was obtained by adding one-third of the PP and DBP. No intervention has been given on the day of investigation to both the yoga and LSM group. Persons handling data analysis were kept blinded.

Statistical analysis

The obtained data were expressed in mean and standard deviation. To determine the statistical significance, paired '*t*' test and Wilcoxon signed rank test for normally and non-normally distributed data were applied respectively, using software Statistical Package for Social Sciences (SPSS) version 20 (SPSS Software Inc, Chicago, IL, USA). Statistical significance was established at $P < 0.05$.

RESULTS

The baseline characteristics of the participants were shown in Table 1. As there was no significant difference in age, BMI, and BP parameters between the yoga and LSM groups, it implies that samples were equally distributed. Table 1 also shows that DBP was within the normal range where as SBP was high indicating ISH in both the groups. Fasting blood glucose, serum triglyceride, total cholesterol, and high-density lipoprotein (HDL) cholesterol levels of the participants were summarized in Table 1.

Yoga practice for 6 weeks has significantly lowered HR ($P < 0.01$), SBP ($P < 0.001$), PP ($P < 0.001$), and MAP ($P < 0.001$) in elderly individuals, whereas no significant change was noticed in the LSM group subjects practicing brisk walk and stretching exercise. There was no significant difference in DBP of both yoga and LSM groups following intervention [Table 2]. The results further revealed reductions in SBP by 2.72% and in PP by 6.53% following yoga practice for 6 weeks. RPP was significantly reduced in yoga group when compared with the LSM group [Figure 1].

DISCUSSION

There are diverse etiologies and mechanisms involved in the development of hypertension in elderly. The major age-related physiological changes attributed for the development of hypertension in elderly are vascular stiffness, endothelial dysfunction, and sympathetic overactivity.[5] Two major age-related structural changes that take place in elastic arteries are stiffness and dilatation. These changes result in decline in expansion of aorta (due to stiffness) during ventricular systole leading to elevation in SBP (ISH), and failure in recoiling (due to decreased elasticity) of the arterial wall results in decrease in DBP, thus causing widening of PP. The PP is a good indicator and independent predictor of arterial stiffness.[16] RPP is an established marker of CV risks, especially in hypertensives.[15] In the present study, there was a significant decrease in SBP and PP [Table 1], though there was no significant change in DBP following 6 week practice of yoga therapy in yoga group. Thus, reduction in PP implies improvement in vascular compliance in these subjects. Further, decreased RPP in these subjects [Figure 1] indicates decreased myocardial work stress and reduced CV risk. These findings suggest that practice of yoga for six weeks could be beneficial in reducing the SBP (arterial stiffness) and CV risks in elderly mild hypertensives. However, changes in BP parameters were not significant in LSM group, indicating that 6 week practice of LSM was not effective in these mild

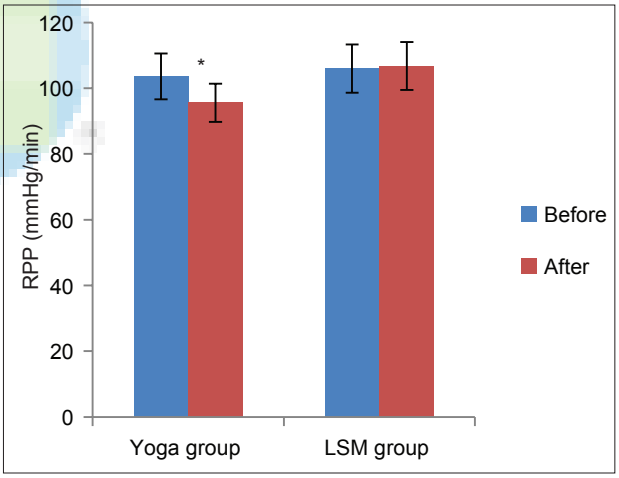


Figure 1: Change in RPP (mmHg/min) in yoga and LSM group following intervention Asterisk (*) indicates $P = 0.002$. RPP = Rate pressure product, LSM = Lifestyle modification.

Table 2: HR and BP changes in yoga and LSM group

Parameters	Yoga group			LSM group		
	Before	After	*P* value	Before	After	*P* value
HR (bpm)	70.33±8.30	66.8±5.95	0.006**	72.09±8.82	72.95±9.16	0.247
SBP (mmHg)	147.23±5.62	143.09±5.67	0.000***	147.0±5.82	146.28±5.41	0.105
DBP (mmHg)	74.95±3.8	75.33±3.54	0.214	75.52±5.43	75.09±5.43	0.358
PP (mmHg)	72.28±6.03	67.76±5.11	0.000***	71.47±6.09	71.19±5.16	0.642
MAP (mmHg)	98.8±3.53	97.61±3.12	0.000***	98.80±4.94	98.28±4.07	0.349

Values are expressed in mean±SD; *P<0.05, **P<0.01, ***P<0.001. Statistical analysis was done by student's paired '*t*' test. P<0.05 was considered statistically significant. LSM: Lifestyle modification, HR: Heart rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, PP: Pulse pressure, MAP: Mean arterial pressure, SD: Standard deviation

hypertensive elderly patients. The decrease in MAP and PP was secondary to the decrease in SBP. The present study is the first of its kind comparing the effects of yoga with LSM in elderly hypertensive patients. Another novelty of the present study is assessment of RPP, the indicator of myocardial work load and stress,[15] which was significantly less in yoga practice group, compared with the LSM practice group. Also, there was significant reduction in basal HR following practice of yoga therapy in yoga group subjects, indicating further reduction in CV risk in these subjects, as decrease in resting HR *per se* has been reported to reduce CV risk.[15]

The yoga module advocated in the present study included slow pranayamic breathing as a major component of yoga practice. It was reported in previous studies that practice of slow and regular breathing lowers BP and maintains sympathovagal balance through stabilization of CV reflex control system.[17-19] As slow and paced breathing was part of the yoga module in the present study, we presume that the reduction in BP in yoga group might be through the improvement in respiratory and CV reflex control systems. Evidences suggest that yoga reduces sympathetic activity and stabilizes the sympathovagal balance by optimizing the autonomic function.[20,21] In the present study, the practice of relaxation technique such as meditation in addition to pranayama might have contributed to the sympathovagal balance and reduction of BP, as recently a study by Pal *et al*. has reported improvement in autonomic balance and CV function following practice of such relaxation therapy.[22] Therefore, studies should be conducted to assess if reduction in BP in elderly hypertensives could be due to improvement in sympathovagal balance. Age-related endothelial dysfunction results in a decreased bioavailability of nitric oxide, a potent vasodilator, with resultant enhanced vascular tone leading to hypertension.[3,5] A study conducted by Sivasankaran *et al*. has demonstrated that the yoga practice enhances endothelial-dependent vasodilation in elderly subjects with coronary artery disease.[23] The findings of this preliminary study has not only demonstrated reduction in BP in elderly group-I hypertensives but also the reduction in CV risks in this elderly population that is at higher risk of CV diseases. Therefore, future studies should address the biochemical mechanisms, especially the level of endothelial inflammatory markers in reduction in CV risks in these highly vulnerable subjects.

The difference in the effect of yoga therapy and LSM (mainly brisk morning walk and stretching exercises) could be due to the fact that the elderly people usually suffer from osteoarthritis and do not exercise or walk effectively. Nevertheless, they adapt to yoga practice (breathing, asana, pranayama, and meditation) effectively because of their attitude towards a yoga life, which is usually observed in old age. Present study is the first of its kind to assess the difference between practice of yoga and LSM in elderly group-I hypertensive males. The novelty of the study was that we had two groups of apparently healthy subjects matched for age, BMI, blood glucose, and lipid profile, which is difficult to get in elderly population. However, this study is a preliminary one that suggests further clinical research in establishing the efficacy of yoga therapy in the management of mild hypertension in elderly population.

Limitations of the study

In the present study, the major limitation was small sample size, which was mainly due to less availability of elderly male grade-I hypertensives not on any medications and not suffering from diabetes mellitus, CV diseases, and hypercholesterolemia. It is difficult to get a larger sample size of elderly subjects aged between 60-80 years with grade-I hypertension without having diabetes and CV risks. Another limitation was that we could not do correlation and regression analysis for establishing the relationship between BP status and RPP (CV risk) due to the less sample size. The CV risks in males are equal to females after menopause. But, in the present study, we did not include females. Therefore, future studies in larger sample size should address the effect of gender on benefits of yoga, in treatment of group-I hypertension in elderly population.

CONCLUSION

In the present study, yoga practice for 6 weeks in elderly grade-I hypertensive subjects not only reduced BP but also the CV risks compared with the subjects practicing LSM. As elderly people cannot effectively perform regular physical exercises and may not be able to tolerate chronic antihypertensive medication, the yoga therapy could be a non-pharmacological alternative for management of hypertension, at least during its early phase. Yet, as the sample size was less in the present study, the results of this study cannot be directly extrapolated to application in general population. Therefore, future studies are warranted to address the effect of such yoga therapy in a larger sample size in both male and female elderly hypertensives.

ACKNOWLEDGEMENTS

The authors sincerely thank BLDE University for financial support. They are also thankful to the entire elderly participants who volunteered to be subjects in this study.

REFERENCES

1. Fagard RH. Epidemiology of hypertension in the elderly. Am J Geriatr Cardiol 2002;11:23-8.
2. Radhakrishnan S, Balamurugan S. Prevalence of diabetes

and hypertension among geriatric population in a rural community of Tamilnadu. Indian J Med Sci 2013;67:130-6.
3. Zeiman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. Arterioscler Thromb Vasc Biol 2005;25:932-43.
4. Ezzati M, Lopez AD, Rodgers A, Van der Hoorn S, Murray CJ, Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. Lancet 2002;360:1347-60.
5. Supiano MA. Hypertension. In: Halter JB, Ouslander JG, Tinetti ME, Studenski S, High KP, Asthana S, editors. Hazard's geriatric medicine and gerontology. New Delhi: McGraw Hill; 2009. p. 975-82.
6. Satoshkar RS, Bhandarkar SD, Rege NN. Pharmacology and pharmacotherapeutics. Mumbai: Popular prakashan private limited; 2005. p. 421-2.
7. Selvamurthy W, Sridharan K, Ray US, Tiwary RS, Hegde KS, Radhakrishan U, *et al*. A new physiological approach to control essential hypertension. Indian J Physiol Pharmacol 1998;42:205-13.
8. Murugesan R, Govindarajalu N, Bera TK. Effect of selected yogic practices in the management of hypertension. Indian J Physiol Pharmacol 2000;44:207-10.
9. Anand MP. Non-pharmacological management of essential hypertension. J Indian Med Assoc 1999;97:220-5.
10. Bharshankar JR, Bharshankar RN, Deshpande VN, Kaore SB, Gosavi GB. Effect of yoga on cardiovascular system in subjects above 40 years. Indian J Physiol Pharmacol 2003;42:202-6.
11. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, *et al*., The task force for the management of arterial hypertension of the European Society of Hypertension, The task force for the management of arterial hypertension of the European Society of Cardiology. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J 2007;28:1462-536.
12. Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. PloS Med 2010;7:e1000251.
13. Subramanya P, TelA review of the scientific studies on cyclic meditation. Int J Yoga 2009;2:46-8.
14. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, *et al*., Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Recommendations for blood pressure measurement in human and experimental animals: Part 1: blood pressure measurement in humans: A statement for professionals from the subcommittee of professional and public education of the American Heart Association Council on high Blood Pressure Research. Hypertension 2005;45:142-61.
15. White WB. Heart rate and the rate-pressure product as determinants of cardiovascular risk in patients with hypertension. Am J Hypertens 1999;12:S50-5.
16. Safar ME. Pulse pressure, arterial stiffness and cardiovascular risk. Curr Opin Cardiol 2000;15:258-63.
17. Grossman E, Grossman A, Schein MH, Zimlichman R, Gavish B. Breathing-control lowers blood pressure. J Hum Hypertens 2001;15:263-9.
18. Pitzalis MV, Mastropasqua F, Massari F, Passantino A, Colombo R, Mannarini A, *et al*. Effect of respiratory rate on the relationships between RR interval and systolic blood pressure fluctuations: A frequency-dependent phenomenon. Cardiovasc Res 1998;38:332-9.
19. Stark R, Schienle A, Walter B, Vaitl D. Effects of paced respiration on heart period and heart period variability. Psychophysiology 2000;37:302-9.
20. Vempati RP, Telles S. Yoga-based guided relaxation reduces sympathetic activity judged from baseline levels. Psychol Rep 2002;90:487-94.
21. Patil SG, Mullur L, Khodnapur J, Dhanakshirur GB, Aithala MR. Effect of yoga on short-term heart rate variability measure as an index of stress in subjunior cyclists: A pilot study. Indian J Physiol Pharmacol 2013;57:81-6.
22. Pal GK, Ganesh V, Karthik S, Nanda N, Pal P. The effects of short-term relaxation therapy on indices of heart rate variability and blood pressure in young adults. Am J Health Promot 2013 [Epub ahead of print].
23. Sivasankaran S, Pollard-Quintner S, Sachdeva R, Pugeda J, Hoq SM. The effect of a six-week program of yoga and meditation on brachial artery reactivity: Do psychosocial interventions affect vascular tone? Clin Cardiol 2006;29:393-8.

How to cite this article: Patil SG, Dhanakshirur G, Aithala MR, Das KK. Comparison of the effects of yoga and lifestyle modification on grade-I hypertension in elderly males: A preliminary study. Int J Clin Exp Physiol 2014;1:68-72.

Source of Support: Nil, **Conflict of Interest:** Nil.