A STUDY OF EFFECT OF AGE ON CARDIOVASCULAR AUTONOMIC FUNCTION TESTS IN HEALTHY SUBJECTS OF B.L.D.E.A'S SHRI B.M.PATIL MEDICAL COLLEGE, BIJAPUR

By

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DISSERTATION
Submitted to the Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore

In partial fulfillment

of the requirements for the degree of

DOCTOR OF MEDICINE

In

PHYSIOLOGY

Under the guidance of

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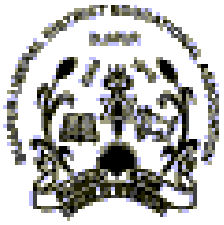

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Date: **Dr Shrilaxmi Bagali**

Place: Bijapur

LIST OF ABBREVIATIONS USED

(In alphabetical order)

ANS- Autonomic Nervous System

ACh- Acetylcholine

AV node- Atrioventricular node

BP- Blood pressure

BPV- Blood pressure variability

BMI- Body Mass Index

BSA- Body Surface Area

BRS- Baroreflex sensitivity

bpm- Beats per minute

DBP- Diastolic blood pressure

ECG- Electrocardiogram

FSH- Follicular stimulating hormone

GIT – Gastro intestinal tract

HR- Heart rate

HRV- Heart rate variability

Ht- Height

I-E – Inspiration- Expiration

LH- Luteinizing hormone

MVC- Maximum voluntary contraction

PR- Pulse rate

RR – Respiratory rate

SA node- Sinoatrial node

SBP- Systolic blood pressure

Wt- Weight

VM - Valsalva maneuver

ABSTRACT

Background & Objectives: Ageing is a physiological process. With advancement of age there is a progressive declination of almost all bodily functions including autonomic nerve function. Thus this study was designed to evaluate the effect of ageing on cardiovascular autonomic function in healthy subjects.

Methods: The autonomic function tests analyzed were Valsalva maneuver, Heart rate response to deep breathing, Heart rate response to standing, Blood pressure response to standing, Blood pressure response to Sustained Hand Grip in 152 healthy subjects in the age range of 18-65 years of BLDEA'S Shri B M Patil Medical college Bijapur. The subjects were divided into four groups according to age (Group I 18-19 years, Group II 20-34 years, Group III 35-54 years, Group IV 55-65 years). The various autonomic function tests were graded by using Ewing & Clarke scores. Unpaired Student's t-test, One Way ANOVA, Pearson correlation were used for statistical analysis.

Results: The following variations observed were statistically significant:

1) Valsalva maneuver gradually decreased from 1.50 $\pm$ 0.37 (Group I) to 1.24 $\pm$ 0.18 (Group IV)

2) HR response to deep breathing gradually decreased from 24.23 $\pm$ 5.46 bpm (Group I) to 10.40 $\pm$ 7.65 bpm (Group IV)

3) HR response to standing gradually decreased from 1.30 $\pm$ 0.18 (Group I) to 1.12 $\pm$ 0.15 (Group IV)

4) BP response to Sustained Hand Grip gradually decreased from 26.37 $\pm$ 9.24 (Group I) to 13.29 $\pm$ 4.79 (Group IV).

BP response to standing showed an insignificant increase in SBP (8.9 ± 3.04 to 13.29 ± 4.79) on standing as age advances.

All these parameters except BP response to standing were negatively correlated with age & these relationships were statistically significant. Parasympathetic, Sympathetic & Total autonomic function score showed gradual increase in the abnormal score as age advances in both sexes.

Interpretation & Conclusion:

1) Autonomic function tests showed gradual decrease in function as age advances in both sexes (18-65 years) in different age groups.

2) Heart rate variation during deep breathing (r= -0.635) & Blood pressure response to Sustained Hand Grip (r= -0. 516) appear to be more sensitive parameters amongst the three Parasympathetic function tests & the two Sympathetic function tests respectively

3) There is a gradual increase in the abnormal autonomic function score of both sexes as age advances in different age groups.

4) Parasympathetic & Sympathetic function tests showed more decline in females as compared to males of the same age group.

Key words: Parasympathetic function tests; Sympathetic function tests; effect of ageing; effect of sex; autonomic function score.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Ageing is a Physiological Process[1].

In the words of Seneca "old age is an incurable disease" but more recently, Sir James Starling Ross commented: "you do not heal old age, you protect it: you promote it: you extend it"[2].

Ageing is a privilege and a societal achievement. It is also a challenge which will impact on all aspects of 21st century society[2]. In 2000 there were 600 million people aged 60 and over. There will be 1.2 billion by 2025 and 2 billion by 2050[3].

Old age should be regarded as a normal inevitable, biological phenomenon[2].

Ageing can be defined as the time related deterioration of the physiological functions necessary for survival and fertility[4].

Chronologic & biologic ageing begin at conception, although senescent changes, defined as functional declines are usually apparent only after sexual maturation[5].

With advancement of age there is a progressive declination of almost all bodily functions, including autonomic nerve function[1]. The effects of ageing on autonomic nervous system (ANS) are multiple & vary between & within both sympathetic and parasympathetic portions[6]. Its altered function may influence almost all body systems[7]. Thus ageing may affect cardiac autonomic nerve functions, mostly heart rate (HR) and blood pressure (BP) regulations which ultimately may lead to development of many cardiovascular diseases[1].

In old age though both sympathetic and parasympathetic are affected but parasympathetic involvement appears to be more frequent than sympathetic. As age advances, the parasympathetic tone and baroreflex sensitivity are gradually reduced[1].

The ANS is vital for homeostasis and its potency is gradually reduced with increasing age[1]. Because ANS facilitates adaptation to physiological stress, autonomic insufficiency in elderly may not manifest itself under normal resting conditions, yet may occur in response to changes in homeostasis[6].

About 15% of current world population exceeds 60 yrs. Majority of them may have parasympathetic nerve impairment which may be the underlying cause of many diseases especially cardiovascular diseases[1]. Circulatory liability and orthostatic hypotension are common in the elderly subjects. In particular, cardiovascular autonomic dysfunction in elderly people is likely to contribute to the hemodynamic impairment in upright position[8]. Unfortunately most of them remain unnoticed and usually treated without knowing the underlying etiology[1].

Age-related autonomic neuropathy may produce clinical symptoms directly or result in subclinical disease, complicate therapeutic intervention in a variety of diseases (e.g. sympatholytic drugs in hypertension, aggressive insulin therapy) or decrease the safety margin upon which superimposition of additional insults (e.g. diabetes) produce symptomatic disease[6].

Since the function of ANS is for the adaptation to both internal and external milieu, this system responds to ageing itself. Autonomic dysfunction is an increasingly recognized complication of human ageing & as a result of the rising mean age of human population, has widespread manifestations for health care[9].

AGE RELATED CHANGES IN VARIOUS SYSTEMS[10]

Blood

As age advances haemopoietic marrow is gradually replaced by fatty marrow. The physiological reserve capacity for erythropoiesis & leucopoiesis is reduced in elderly.

Immune mechanisms

There is a definite decline in immune competence associated with ageing. Both cell mediated & humoral immunity are affected. The elderly are more susceptible to infections, to autoimmune diseases & there is higher incidence of malignancies in old age.

Respiratory system

The alveolar surface area decreases by 4% for every decade after the age of 30. The compliance of total respiratory system is reduced by age of 60 years due to rigidity of the chest wall. There is a reduction in pulmonary diffusing capacity and the respiratory response to hypoxia & hypercapnia is sluggish in the elderly.

Cardiovascular system

The elasticity of the aorta & other large arteries decreases with increasing age. The systolic & pulse pressure are increased, but there is little change in diastolic pressure. There is atrophy of myocardium accompanied by deposition of a brown pigment, lipofuscin. There is prolongation of the myocardial contraction & relaxation time. There is decrease in ventricular compliance. There are structural changes in the valves. **The number of pacemaker cells in the sinoatrial node is reduced, which is related to reduced heart rate response to both sympathetic & parasympathetic stimuli.**

The alimentary canal

With advancing age there is decline in the digestive & absorptive function of the GIT due to diminution of masticatory efficiency, dysphagia, and reduced absorptive surface and reduced enzyme secretion.

Excretory system

Microscopically, there is a reduction in the number & size of nephrons. **There is a 10% reduction in renal plasma flow per decade after the age of 30**. There is a progressive age related decrease in glomerular filtration rate, the secretory & reabsorptive functions of renal tubules.

Endocrines

Ageing of the endocrines has often been considered to be underlying mechanism of ageing. There may be a decrease in the blood concentration of the hormone itself or the binding protein involved in the transport of the hormone, decreased responsiveness of the target cells, and alteration in the number or sensitivity of hormone receptors or diminished response to physiological stimuli for secretion of the hormone. The response of the hypothalamo hypophyseal axis to stress is diminished with age.

In females, there is a decrease in serum estrogen & progesterone levels after menopause & an increase in FSH & LH levels. In males, there is a decrease in testosterone output & a decrease in gonadotropin levels.

Nervous system

As age advances there is atrophy of brain & neuronal loss. There is accumulation of lipofuscin in the cells & loss of synapses & dendrites. The function of various neurotransmitter systems is impaired.

Special senses

Presbyopia is a constant feature after the age of 40. The intraocular pressure also rises with age.

The ear shows diminished sensitivity after the age of 30. The sensation of smell & taste also decline with age.

Effect of ageing on autonomic nervous system[11]

There is reduced response to sympathetic nerve stimulation or infusion of catecholamine and there is an age related decrease in the β 1 & β 2 adrenoreceptor sensitivity. The observation that many old people have higher base line levels of plasma noradrenaline than the young people may be in part a compensatory mechanism. Morphological changes include reduction in the autonomic neuron cell number, higher density of lipofuscin in neuronal cytoplasm & degenerative changes in Vasa Nervorum. In both the sympathetic & parasympathetic systems there is evidence of reduced nerve conduction velocity. In the parasympathetic nervous system, cardiac muscarinic receptor responses decline, probably due to reduction in the receptor sensitivity to acetylcholine (ACh). More recently evidence has come to light which indicates that flux of Ca^{2+} ions across neuronal membranes is progressively impaired with age. The net effect of these changes is reduced efficiency in the sympathetic and the parasympathetic systems which reduce the ability to put into effect the responses required to maintain physiological homeostasis.

AUTONOMIC NERVOUS SYSTEM

History[12]

The early concepts of ANS date back to the roman period, during which Galen (A.D. 130-200) described sympathetic chain ganglion & the rami communicans. Although he hypothesized that this chain originated in the brain & provided sensory function, he thought that numerous interconnections allowed the spirits to travel between the various organs, maintaining a physiological "sympathy".

Much later in the 16th century, Bartholomeo Eustachin investigated the ganglionated nerves but did not contribute further to our understanding of this system. While still believing that this chain descended from the brain, Thomas Willis (1664) placed its origin in the posterior fossa & associated it with involuntary or autonomic motion-specifically with the motion of the heart & respiration. Francois Pourfour du Petit noted that the pupil size & the amount of the secretions from the eyes were altered when the cervical sympathetic nerves were cut & cast doubt on the sensory function of the sympathetic chain. In 1732¸Winslow introduced the term sympathetic nerve, describing great, middle & small components.

In the 19th century, a flurry research on the ANS resulted in Walter Gaskell concluding that this system was actually composed of two subsystems. At the turn of the century, the terms preganglionic, postganglionic & autonomic were first used by John Newport Langley, who theorized that this system contained both peripheral & central components. During this century, further refinements in our understanding of ANS were contributed by Thomas Elliott, Walter Dixon & Otto Loewi, among others.

Anatomical and Physiological aspects of autonomic nervous system

The essence of survival is coping up with a change. The level of activity in various organs & the level of metabolic activity need to be different at rest, during sleep & during exercise, during fasting & in postprandial state, in cold & in hot environment & so on. These adjustments are brought to a level of perfection by the central nervous system, the visceral & metabolic effects of which are mediated by the ANS. The ANS makes an important contribution to homeostasis[10].

The tem autonomic (autonomous – self governing) nervous system was introduced to describe "the system of nerves which controls the unstriated tissues, the cardiac muscles & the glandular tissue of the mammals[12]".

Originally the term applied only to neurons with axons outside the CNS. More recently, the discovery that discrete neuronal groups in the brain stem, diencephalon & the cerebral cortex are involved in the control of autonomic function has broadened the definition of the ANS to include not only peripheral afferent & efferent pathways but also complex network of neurons with in the CNS[12].

ANS controls most visceral functions of the body. This system helps control arterial pressure, gastrointestinal motility, gastrointestinal secretion, urinary bladder emptying, sweating, body temperature & many other activities some of which are controlled almost entirely & some only partially by the autonomic nervous system. One of the salient features of the ANS is the rapidity & intensity with which it can change visceral functions[13].

The autonomic nervous system is governed centrally by brain stem centers, Hypothalamus, Cerebellum, Frontal cortex and Limbic system. Hypothalamus is most

important. In fact Sherrington rightly called it as “Head Ganglion of the autonomic nervous system”[14].

Anatomic organization of the autonomic outflow[14]:

The autonomic nervous system like the somatic nervous system is organized on the basis of reflex arc which contains a visceral receptor, an afferent pathway, centre, an efferent pathway & effector organ.

The peripheral motor portions of the ANS are made up of preganglionic & postganglionic neurons. The cell bodies of preganglionic neurons are located in the visceral efferent intermediolateral gray column of the spinal cord or the homologous motor nuclei of the cranial nerves. These axons are mostly myelinated, relatively slowly conducting B fibers. The axons synapse on the cell bodies of the postganglionic neurons that are located in all cases outside the CNS. Each preganglionic axon diverges on an average of 8-9 postganglionic neurons. The axons of postganglionic neurons, mostly unmyelinated C fibers, end on the visceral effectors.

Anatomically, the autonomic outflow is divided into 2 components: the sympathetic & parasympathetic divisions of the ANS. In the GIT, these both communicate with the enteric nervous system, & this is sometimes called a third division of the ANS.

Sympathetic division:

The axons of the sympathetic preganglionic neurons leave the spinal cord with the ventral roots of the first thoracic to the third or fourth lumbar spinal nerves. They pass via white rami communicans to the paravertebral sympathetic ganglion chain, where most of them end on the cell bodies of postganglionic neurons. The axons of some postganglionic neurons pass to the viscera in various sympathetic nerves. Others reenter the spinal

nerves via grey rami communicans & are distributed to the autonomic effectors in areas supplied by these spinal nerves. The postganglionic sympathetic nerves to the head originate in the superior, middle & stellate ganglia in the cranial extension of the sympathetic ganglion chain & travels to the effectors with the blood vessels. Some preganglionic neurons pass through the paravertebral ganglion chain & end on postganglionic neurons in the collateral ganglia close to the viscera.

Parasympathetic division:

The cranial outflow of the parasympathetic division supplies the visceral structures in the head via the Occulomotor (III), facial (VII) & Glossopharygeal (IX) & in the thorax & upper abdomen via the Vagus (X) nerves. The sacral outflow supplies the pelvic viscera via the pelvic branches of the second to the fourth sacral spinal nerves. The preganglionic fibers in both outflows end on short postganglionic neurons located on or near the visceral structures.

Chemical divisions of the ANS:

On the basis of the chemical mediator released, the ANS can be divided into cholinergic & noradrenergic divisions. The neurons that are cholinergic are

1) All preganglionic neurons
2) Anatomically parasympathetic postganglionic neurons
3) The anatomically sympathetic postganglionic neurons which innervate sweat glands.
4) Anatomically sympathetic neurons which end on the blood vessels in skeletal muscles & produce vasodilatation when stimulated.

The remaining postganglionic neurons are noradrenergic.

Functions:

Sympathetic division:-

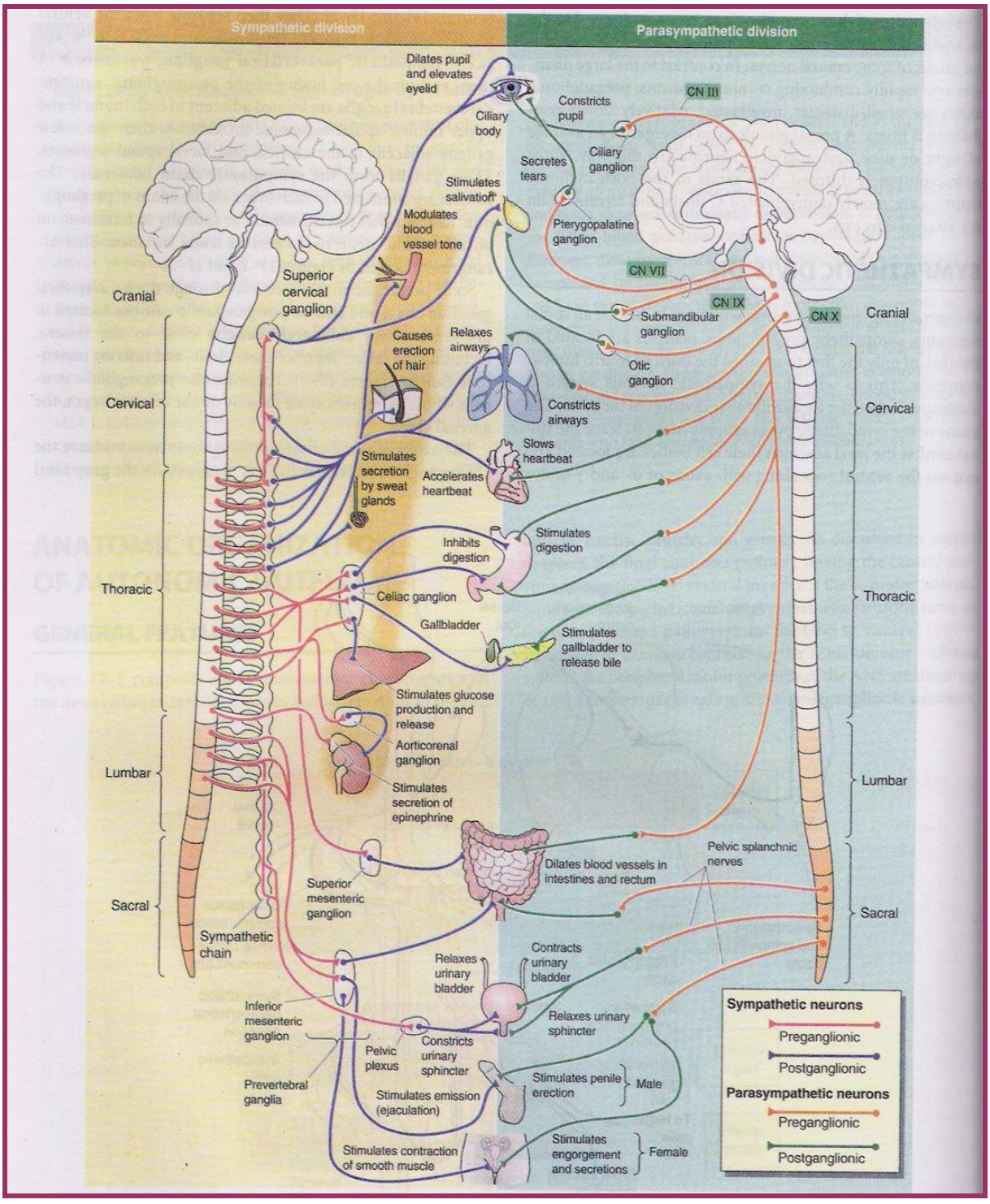
The sympathetic division in addition to sub-serving basic functions as maintenance of blood pressure & body temperature helps the individual to cope with the emergencies. Sympathetic stimulation leads to relaxation of accommodation & dilatation of the pupils, acceleration of heart beat, increase in blood pressure, increase blood flow to muscles and decreased blood flow to skin & abdominal viscera, elevated plasma glucose & free fatty acid levels. On the basis of these effects Cannon called this emergency reaction as 'preparation for flight or fight'.

Parasympathetic division:-

Generally the functions promoted by this division are those concerned with the vegetative aspects of day to day living, example digestion & absorption of food. Hence the cholinergic division is called as anabolic & the sympathetic as catabolic.

The various functions of sympathetic & parasympathetic divisions aim at maintaining homeostasis. Ageing may be accompanied by multisystem autonomic defects resulting in postural hypotension, impairment of cardiovascular & respiratory reflexes, thermoregulatory liability, bowel and bladder incontinence, pupillary and lacrimal abnormalities and impotence. The general feature of autonomic impairment can lead to an overall decline in the efficiency of homeostatic regulations and in the slower restoration of steady state conditions in the older organisms after exposure to stress[15].

Figure 1: Organization of Autonomic Nervous System



AUTONOMIC INNERVATION TO THE CARDIOVASCULAR SYSTEM[12]

The heart receives parasympathetic & sympathetic innervations. The cell bodies of parasympathetic preganglionic neurons innervating the heart are located in the medulla (Nucleus Ambigus & Dorsal Motor Nucleus of the Vagus). The axons of these neurons, which are part of Vagus nerve, join the cardiac neural plexus after entering the thorax to synapse with the neurons in the intracardiac ganglia. From these ganglia, short postganglionic parasympathetic neurons emerge to innervate myocardial tissue. Vagal innervations are particularly abundant in the nodes & the atrioventricular conducting system.

The heart draws its sympathetic innervation from the neurons in the intermediolateral columns of the spinal cord at the T_1- T_4 levels. Axons of these neurons synapse in the superior, middle & inferior (stellate) cervical ganglia which are the origin of postganglionic sympathetic neurons. Sympathetic axons innervate the sinoatrial (SA) & atrioventricular (AV) nodes, the conducting system & myocardial fibers, most prominently in the ventricles. The arteries & veins of the systemic circulation are innervated primarily by the sympathetic system.

Postganglionic parasympathetic neurons release ACh which activates muscarinic receptors in the heart, causing a decrease in heart rate as well as reductions in the conduction, excitability & contractility of myocardial cells. Postganglionic sympathetic axons release norepinephrine which primarily via β-adrenergic receptors increases the heart rate & conduction, excitability & contractility of the myocardium. Cardiac vagal neurons are activated through the baroreflex when arterial pressure increases & are

inhibited during respiration. Because ACh acts quickly & is rapidly inactivated by cholinesterase the Vagus controls the heart rate on a beat-to-beat basis.

Sympathetic outflow to the arteries, arterioles & veins of the peripheral circulation produces vasoconstriction by activating alpha adrenergic receptors. The presence of vasodilator cholinergic sympathetic fibers to skeletal muscle of humans is debatable.

Autonomic outflow to the heart & blood vessels is controlled on a moment to moment basis by a variety of reflexes, which are initiated by arterial baroreceptors & chemoreceptors & by several types of cardiac receptors. Of these reflexes, one of the best studied is the arterial baroreflex, which is a classic negative feed back mechanism that buffers fluctuations in the arterial blood pressure.

TESTS FOR CARDIOVASCULAR REFLEXES:

Autonomic function tests

More tests of autonomic function exist than for any other neurological system. Many of these tests are readily applied at bedside. Most physicians who routinely follow patients with autonomic failure develop a small armamentarium of tests they feel comfortable with and rely on. For neurologist, tests of peripheral sudomotor function may form the organizing nucleus of autonomic evaluation. For the cardiologist, it may be test of BP and heart rate. For the endocrinologist, it may be circulating catecholamine and rennin. For the ophthalmologist, it may be the pupillary tests and for the pharmacologist, it may be drug tests for evidence of stimulatable autonomic function and hypersensitivity[16].

It is imperative that any test for autonomic function has to be simple, noninvasive, and reliable and should be able to demarcate clearly normal and abnormal. The physiological basis of the test should be clearly understood. These criteria are fulfilled by tests based on cardiovascular reflexes which measure the heart rate, systolic and or diastolic blood pressure responses to a number of simple maneuvers. The cardiovascular tests: The Valsalva maneuver[17, 18, 19], Heart rate response during periods of deep breathing[20], The Heart rate response to standing up[21, 22] and the diastolic blood pressure response to sustained hand grip[23, 24], Blood pressure response to standing[20] have been employed in the present study to assess autonomic function. The first three tests evaluate the cardiac parasympathetic while the latter two tests show an altered response when there is sympathetic imbalance.

Physiological basis of the study tests:

1. Valsalva Maneuver

Nearly three centuries ago Antonio Maria Valsalva recommended forced expiration against a closed glottis for expelling pus from an infected middle ear. The procedure known thereafter as Valsalva maneuver, has been widely used in the treatment of Eustachian tube obstruction. Weber (1851) found that the Valsalva maneuver caused changes in the pulse volume. Flack (1920) modified the maneuver by having subjects blow against a column of mercury than against closed glottis[25, 26]. Results by earlier workers show that more valid assessment of Valsalva response can be made when performed in the supine or sitting position. Sitting position for the Valsalva maneuver test is used in the present study.

During Valsalva maneuver in a normal individual one can recognize four phases of change in haemodynamics[18].

Phase I:

At the onset of expiratory strain (forced expiration against closed glottis) a sudden increase in the intrathoracic pressure is transmitted to all the vessels within the thorax including the aorta and its branches producing an abrupt rise in arterial systolic and diastolic pressures.

Phase II:

As the increased intrathoracic pressure impedes venous return to the right atrium there is a progressive reduction in the left ventricular stroke volume accompanied by a fall in arterial pressure. The diminished pulse pressure acting through baroreceptors reflexly

stimulates increased sympathetic activity which manifests as tachycardia and peripheral vasoconstriction.

Phase III:

Upon release of the strain there is an abrupt increase in venous return as well as in the capacity of the pulmonary vascular bed. For several beats following the release of the strain there may be a further diminution in the arterial pressure due to transient pooling of the right ventricular output in the expanded pulmonary vascular bed.

Phase IV:

When the augmented venous return reaches the left ventricle there is a progressive increase in left ventricular stroke volume which is ejected into constricted systemic vasculature, the arterial pressure rises, exceeding the control level “Over shoot”. Finally rising pulse pressure stimulates vagal activity which manifests as bradycardia.

In the Valsalva maneuver, forced expiration against resistance (expiratory strain) causes complex reflex circulatory changes mediated by both parasympathetic and sympathetic pathways. While straining the heart rate rises. After release of strain, heart rate slows. With autonomic damage the expected sympatho-adrenal discharge fails to occur and thus there may or may not be a change in the heart rate.

The occurrence of requisite changes in the intrathoracic pressure with this procedure, integrity of the autonomic pathway and responding end organ are essential to obtain the correct response. The heart rate changes are unaffected by sympathectomy, indicating that the baroreceptors and the vagi mediate these changes. The blood pressure falls gradually during the maneuver and does not overshoot.

2. Heart rate variation (R-R interval) during deep breathing

The clinical relevance of HRV was first appreciated in 1965 when Hon and Lee noted that fetal distress was preceded by alterations in interbeat intervals before any appreciable change occurred in heart rate itself. Twenty years ago, Sayers and others focused attention on the existence of physiological rhythms embedded in the beat-to-beat heart rate signal. During the 1970s, Ewing et al devised a number of simple bedside tests of short-term RR differences to detect autonomic neuropathy in diabetic patients. The association of higher risk of post infarction mortality with reduced HRV was first shown by Wolf et al in 1977. In 1981, Akselrod et al introduced power spectral analysis of heart rate fluctuations to quantitatively evaluate beat-to-beat cardiovascular control. The clinical importance of HRV became appreciated in the late 1980s, when it was confirmed that HRV was a strong and independent predictor of mortality after an acute myocardial infarction[27].

The heart rate varies with the phases of respiration, accelerating during inspiration and decelerating during expiration. These changes are marked in younger individuals, and during deep breathing and are abolished by atropine showing the involvement of parasympathetic nerves. The impulses from stretch receptors in the lungs are relayed via afferent fibers in the vagi and inhibit the cardio-inhibitory centre in the medulla oblongata during inspiration, the tonic vagal discharge which keeps the heart rate slow decreases, thereby increasing the heart rate, withdrawal of inhibition of the cardio-inhibitory centre and excitation of vasomotor centre also contributes to the acceleration of heart rate during inspiration[14]

In presence of autonomic imbalance or autonomic neuropathy, the change in heart rate during respiration is impaired or even absent[28]. The heart rate may be fixed in patients with severe autonomic damage involving both sympathetic and parasympathetic innervations of heart.

For practical purposes the test can be best performed breathing deeply at six breathes per minute and measuring the difference between maximum and minimum heart rates over a period of one minute[20].

3. Postural stress tests:

(Heart rate and blood pressure response to standing from lying down position)

Immediate heart rate and blood pressure response to standing is used as a postural stress test. Postural stress tests are useful in assessment of cardiovascular reflex responses of normal subjects who may be involved in specialized occupations[29].

The maintenance of normal blood pressure and limitation of peripheral blood pooling postural stress is a function of cardiovascular reflexes.

Postural stress reduces intra thoracic volume and shifts to legs where it forms a pool, thereby producing fall of blood pressure and reduction in the circulating blood volume[30]. Compensation to this is brought by - 1) Reflex tachycardia 2) Arteriolar constriction – aimed at maintenance of arterial blood pressure 3) Vasoconstriction- helps to limit expansion of blood pool in legs, keeps up the preload and is helped up by the increased activity of abdomino-thoracic respiratory pump, the pumping action of calf muscles and the venous valves[31,32]

The change of posture (lying down to standing) – displaces about 600 ml of blood from thorax to the legs[33]. This deactivates baroreceptors to produce a reflex tachycardia

which is mediated mostly by vagal tone withdrawal[28]. Displacement of blood produces slight fall in systolic blood pressure and a slight increase in diastolic blood pressure. The immediate heart rate increase on standing in healthy young subjects is seen by about 15th beat and this later settles down to a steady state value at about 30th beat which is 10-15 beats per minute higher than in supine position. In patients with autonomic neuropathy the tachycardia response is markedly attenuated and may be accompanied by postural hypotension. If the 30:15 ratio is used, then the counting of beats should start when the subject starts to stand and not when he has finished the movement of standing up. The beats are counted when the subject starts to stand up in the present study.

D.J. Ewing, L. Hume et al in their study on heart rate response to standing analyzed their results by the use of time after standing rather than beats after standing but they are of opinion that counting time has no advantage and is more laborious to calculate. The maximum responses occur around 15th and the 30th beats, and the shortest and longest R-R intervals around these beats should therefore be measured.

D.J. Ewing, L. Hume, I.W.Campbell et al have studied cardiovascular reflex responses during standing up on pharmacological basis and confirmed the above mechanism[34].

In the present study Heart rate and Blood pressure changes on quiet standing from lying position was selected because of the simplicity of the test. To find postural fall blood pressure cuff is inflated above systolic blood pressure before subject stands up. Immediate heart rate response to standing (30:15 ratio) is used to study the parasympathetic pathway sensitivity and systolic blood pressure response to study the sympathetic sensitivity (or response). Values of 1.04 or more are taken as normal for

heart rate response, and 10 mm Hg or less for systolic blood pressure response to standing (D.J.Ewing and B.F.Clarke)[20].

4. Blood pressure response to sustained handgrip

Sustained handgrip is a type of isometric exercise. With the start of an isometric muscle contraction, heart rate is increased. This is largely due to a reduction in vagal tone, although increased discharge in cardiac sympathetic fibers also plays a role. Shortly thereafter, the systolic and diastolic blood pressure also rises sharply. This is brought about by increased peripheral resistance. The increase in peripheral resistance results from vasoconstriction in the inactive muscles produced by sympathetic outflow. The rise in blood pressure will be abnormally small if there is extensive peripheral sympathetic abnormality[20].

Dwain L. Eckberg and B. Gunnar Wallin in their study have found that there is an increase in heart rate and arterial pressure with the onset of isometric exercise (Brief handgrip – 30% of maximum). Their results suggest that exercise modifies, in small but significant ways, early sympathetic and vagal responses to abrupt changes of arterial baroreceptor input in humans[35].

Most young normal subjects and hypertension patients without evidence of cardiac damage (Ewing et al 1973) had increase in their blood pressure during sustained handgrip by a rate dependent increase in cardiac output. Only in older subjects and in patients with myocardial dysfunction does a second method that of increased peripheral resistance by way of vasoconstriction, come into play to raise the pressure[24].

Many workers have studied the effect of different types of isometric exercise on blood pressure and heart rate. Most of the workers are of the opinion that irrespective of the

muscle mass involved in isometric exercise there is a pressure response of the same magnitude but the pressure depends upon maximal voluntary contraction.

Assessment of heart rate variability in subjects of all ages is however complicated by changes that occur in autonomic nervous system with advancing age. The heart rate response to Valsalva maneuver and deep breathing test declines with age in normal subjects. Consequently normal ranges for such procedures that quote a single limit for all ages with or without borderline range are inappropriate and will result in errors in diagnosis, particularly in elderly[36].

There is a sizeable proportion of evidence indicating relationship between ageing and cardiovascular autonomic functions from various parts of the world. This warrants study of effect of age on human cardiovascular autonomic functions involving subjects of Indian origin.

OBJECTIVES

To evaluate the effect of age on cardiovascular autonomic function tests in healthy subjects (18-65 years) of both sexes of BLDEA'S Shri B.M.Patil Medical College, Bijapur.

REVIEW OF LITERATURE

Several studies have been performed to assess the effects of ageing on the cardiovascular autonomic function tests.

Following studies have been done on cardiovascular autonomic function tests in healthy subjects which have shown decline in function with advancing age.

Zeigler D, Laux G, Dannehl K, Spuler M, Muhlen H, Mayer P, Gries FA (1992)[37] to establish normal ranges for assessment of autonomic dysfunction performed a battery of cardiovascular reflex tests in 120 healthy subjects aged 15-67 yrs using a computer based technique. Heart rate variability was measured during deep breathing, Valsalva maneuver & standing, while blood pressure response to sustained hand grip were determined using spectral analysis. The results of all the tests except blood pressure response to sustained handgrip & blood pressure response to standing declined significantly with increasing age. They concluded that all indices of spectral & vector analysis of HRV are age dependent & were independent of sex.

Singh D, Vinod K, Saxena SC, Deepak KK (2006, Jalandhar)[38] performed spectral evaluation of ageing effects on blood pressure & heart rate variation in healthy subjects. The aim was to estimate HRV & BPV behavior to age. Eight young (21-34 years old) and eight elderly (68-85 years old) were tested. Time and frequency-domain analysis of HRV and BPV was performed on 5-minute ectopic-free recordings. BRS on the heart was estimated by frequency-domain analysis of spontaneous variability of systolic blood pressure (SBP) and RR interval. It has been observed that compared to young the elderly subjects have (i) diminished HRV; (ii) a shift in the power spectral density and median frequency to low frequency side for HRV and to higher frequency

side for BPV; and (iii) increased low-frequency alpha index and decreased high-frequency alpha index of BRS with overall alpha index augmented. The results conveyed that normal ageing in the absence of disease is associated with lesser parasympathetic regulation of heart rate. Thus it was concluded that the age is an important factor to be considered for prognosis and diagnosis by HRV and BPV.

Malvin Torsik, Amanda Haegblom, Gein Egil Eide, Erich Schmutzhard, Kaare Vetvik, Andrea Sylvia Winkler (2008)[39] performed cardiovascular autonomic function tests were in 328 participants in Africa. The test battery consisted of five autonomic function tests i.e. resting heart rate, heart rate variation to deep breathing, heart rate response to standing, postural changes in systolic blood pressure & diastolic blood pressure measured in lying position followed by blood pressure on standing. It was found that heart rate variation with deep breathing & heart rate response to standing were negatively correlated with age.

B Gautschy, P Weidmann, M P Gnadinger (1986)[40] obtain a comparative assessment of 5 diff clinical autonomic function tests as related to age & gender in normal man performed deep breathing test, orthostatic test (30/15 ratio), heart rate response to Valsalva maneuver, BP response to sustained hand grip & orthostatic blood pressure response in 120 healthy subjects (60 women & 60 men) aged 22-92yrs. Each of the functional parameters depending on the cardiac parasympathetic integrity i.e. beat to beat variation, orthostatic 30/15 R-R ratio & valsalva ratio decreased progressively with increasing age. The BP response to sustained hand grip which depends on entire reflex arc was augmented only minimally with increasing age. No significant dependence on

gender was noted, although blood pressure response to hand grip tended to be slightly greater in men than in women.

Kaijser L, Sachs C (1985)[41] studied the effect of age on autonomically mediated cardiovascular responses to certain maneuvers in 15 healthy old men & women (60-80yrs). The results were compared with groups of healthy young (about 25yrs) & middle aged (about 45 yrs) subjects. There was no significant reduction in cardiovascular responses between the young & middle aged groups. Respiratory sinus arrhythmia, heart rate & blood pressure response to isometric hand grip, heart rate decreases during a dive reflex test were significantly attenuated in the old age group. Whereas the VA & heart rate & BP changes during an orthostatic test did not differ between old & two younger age groups. They concluded that there is only a moderate attenuation of autonomic cardiovascular responses to about 60yrs, after which there is rapid decline. Also, concluded that the decreased responses are not due to an isolated impaired function of peripheral autonomic nerves but due to receptor organ or combination of defects in function of several parts of ANS in old age.

Iain A O'Brien, Paul O'Hare, Roger J M Corall (1986, Bristol) [36] studied heart rate variability in healthy subjects to determine the effect of age & derive normal ranges for tests of autonomic function. Heart rate response to single deep breath, Valsalva maneuver & heart rate & BP response to standing were performed in 310 healthy normal subjects 147 males & 163 females in the age range of 18-85yrs. Heart rate response to single deep breath, during Valsalva maneuver & standing were reduced in elderly, but the BP response to standing was found to be unrelated to age. No significant difference in response was found with respect to sex. They observed an inverse relation between

parasympathetic function & advancing age. The sympathetic function was unaffected with ageing.

Vita G, Princi P, Calabro R, Toscano A, Manna L, Messina C (1986)[42] to assess the relationship between aging and autonomic control of heart rate and blood pressure, performed cardiovascular reflex tests in 70 healthy volunteers in the age range 25-71 years. R-R interval variation, heart rate change with deep breathing, 30/15 ratio and blood pressure response to standing appeared significantly declining with age. On the other hand, Valsalva ratio and the blood pressure response to sustained handgrip appeared to be unrelated to age. These results suggest that there is an age-dependent degradation of the mechanisms involved in the cardiovascular reflexes. The assessment of age-adjusted normal values improves the criteria for delineating abnormal from normal results in individual testing of autonomic function.

Braune S, Auer S, Schulte-Monting J, Schwerbrock S, Lucking C H (1996, Germany)[43] studied cardiovascular parameters to determine their sensitivity to detect autonomic dysfunction & influence of age & sex in 137 normal subjects (18 to 85 years). BP & HR were measured continuously during active change of posture i.e. standing upright, passive tilt, Valsalva maneuver (VM), deep breathing (DB), isometric muscle exercise (IME) and a mental arithmetic test (MA). Mean heart rate activation was attenuated with increasing age in all maneuvers, but was unrelated to sex. In non orthostatic challenge procedures like MA & IME mean BP increases were independent of age & sex despite lower increases in HR in the elderly. This points to preserved sympathetic efferent activity. Following a forced fall in BP during ACT, PT & VM the initial responses & maintenance values of BP showed a significant age related decrease.

This finding was strongly related to lower BP values in males compared to females which became more pronounced with increasing age.

Gelber DA, Pfeifer M, Dawson B, Schumer M (1997, USA)[44] to determine the normative values for heart rate variation to deep breathing (VAR) & valsalva ratio (VAL) as well as effect of various confounding variables on these measures, studied 610 normal subjects in the age range of 9-79yrs. No gender effect was found for either VAR or VAL. The VAR correlated inversely with both age & MAP, while VAL correlated inversely with age & BMI. Since age is the principal confounding variable for both VAR & VAL normative values are presented stratified by age.

N R Stout, R A Kenny (1999)[45] conducted study to determine the reference ranges for a battery of autonomic function tests in 54 healthy elderly with an age range of 60-93yrs. There were 34 males. The tests performed were Max/Min 30:15 ratio, valsalva ratio, heart rate response to deep breathing, systolic blood pressure overshoot in Valsalva maneuver, and diastolic blood pressure response to cold stimulus. They concluded that autonomic function test battery is a useful clinical tool in the assessment of autonomic function in the elderly. Also, it was concluded that healthy elderly show a decline in autonomic function, but autonomic dysfunction is not a feature of normal ageing.

Yeragani VK, Pohl R, Berger R, Balon R, Srinivasan K (1994)[46]conducted a study to find the relationship between age & heart rate variability in supine & standing postures by spectral analysis of heart rate in 11 normal children (4-12yrs) and 23 normal adults (21-43yrs). It was found that there is a decrease in cholinergic & an increase of adrenergic modulation of heart rate variability with age (4-43yrs).

C Neumann, H Schmid (1997, Brasil)[47] evaluated heart rate response to deep breathing, to standing, to valsalva maneuver & blood pressure response to standing & sustained hand grip in 97 healthy (11-67 yrs) & 143 with diabetes mellitus (16 -71 yrs). Correlation was observed between age & heart rate response to standing ($r= -0.48$, $P<0.001$) & deep breathing ($r= -0.41$ $P<0.002$). No correlation was observed with Valsalva maneuver & blood pressure response to sustained hand grip & standing. Heart rate response & blood pressure response in men & women did not differ.

Low P A, Denq J, Opfer-Gehrking T. L, Dyck P J, O'Brien P C, Slezak J M (1997)[48] evaluated autonomic function tests in a total of 557 normal subjects evenly distributed by age and gender from 10 to 83 years. Heart rate (HR) response to deep breathing fell with increasing age. Valsalva ratio varied with both age and gender. QSART (quantitative sudomotor axon-reflex test) volume was consistently greater in men (approximately double) and progressively declined with age for all three lower extremity sites but not the forearm site. Orthostatic blood pressure reduction was greater with increasing age. HR at rest was significantly higher in women, and the increment with head-up tilt fell with increasing age.

Piha S J (1991, Finland)[49] performed cardiovascular autonomic reflex tests (Valsalva maneuver, deep & quiet breathing tests & active orthostatic tests) to study the normal responses & to determine the age related reference values in 143 healthy subjects aged 20-80 years. Most of the cardiovascular autonomic function tests reflecting autonomically mediated heart rate responses decline with advancing age & this leads inevitably to the need for the age related reference values. The indices are in general independent of sex.

Sega S, Jager F, Kiauta T (1993)[50] carried out a study to determine if the results of cardiovascular reflex tests & spectral analysis of heart rate variability are age dependent in 83 healthy volunteers of both genders aged 21-70 years. They found that results of all heart rate based tests & results of spectral analysis decreased with ageing, while results of blood pressure based tests did not. Parasympathetic activity predominated in younger subjects while in older subjects sympathetic activity was dominant. These findings suggest that tests based on heart rate changes may be more sensitive than tests based on blood pressure changes. Thus this study stresses the importance of age in the evaluation of the results of autonomic nervous system function.

Ingall T J, McLeod J G, O'Brien P C (1990)[51] studied the effect of ageing on autonomic nervous system function in 76 healthy subjects (5-85 years). Age related effects were found with the tests of predominantly vagal cardiovascular function. However, there were no age related effects found with the tests of predominantly sympathetic cardiovascular function. It was concluded that the age of the subject should be taken into consideration when evaluating tests of autonomic function and each laboratory should establish its own control values.

Yujiro Yamanaka, Koichi Honma (2006, Japan)[52] to determine the effect of ageing on the cardiovascular response to postural change, examined the cardiovascular sympathetic and parasympathetic response to active standing in 610 healthy Japanese subject (6–83 years) measuring the initial heart rate (HR) response for 3 min in the supine and standing position. They also measured the coefficient of variation of R–R interval (CVR–R). The cardiovascular response to active standing demonstrated a different change with aging between sympathetic and parasympathetic. Sympathetic function was

in sthenia state in young subjects, and that this function declined with age increasing. Whereas, parasympathetic function was immature enough to inhibit the sympathetic tone in young subjects and matured at 20 years of age, and had an ability to inhibit sympathetic tone. CVR–R show a linear change that decline with age increasing. These results indicated that the cardiovascular parasympathetic response to active standing shows a characteristic change with aging that differs from cardiovascular parasympathetic at rest represented by CVR–R. It was concluded that the cardiovascular response to postural change is dependent on subject's age.

Low P A, Opfer-Gehrking T L, Proper C J, Zimmerman I (1990, Rochester)[53] studied the effect of age on heart rate response to deep breathing & the Valsalva maneuver in 122 & 155 subjects respectively aged 10-83 years & quantitative sudomotor axon reflex test (QSART) in 114 subjects in same age group. The HR responses were not different between the sexes, but a consistently significant regression with age was demonstrated in response to deep breathing & Valsalva ratio. Cardiac vagal function is impaired with age, but post-ganglionic sympathetic function is little affected by age, suggesting selectivity of effects of ageing on autonomic function.

Akiko kawamoto, Kazuyuki Shimada, Kozo Mtsubayashi, Taishiro Chikamori, Osamu Kuzume, Hisakazu Ogura et al (1989, Japan)[54] studied cardiovascular regulatory functions in elderly patients with hypertension to dissociate the effects of an elevated blood pressure on the cardiovascular regulatory functions from those of ageing in the hypertensive elderly individuals. The study population consisted of 30 hypertensive patients (21 men & 9 women) aged 56-76 years, 30 male normotensive subjects aged 58-77 years & 12 young healthy male volunteers aged 16-28 years. Following examinations

were performed 1) 24 hr blood pressure monitoring 2) urinary sodium concentration.3) Urinary creatinine 4) intra arterial blood pressure 5) ECG (lead 5) 6) Cardiac index 7) Plasma norepinephrine level, plasma rennin activity, plasma aldosterone levels 8) baroreflex receptor reflex sensitivity by the change in SBP during phase II & phase IV of VM 9) Isoproterenol hydrochloride sensitivity. The variability of heart rate in 24 hr ambulatory monitoring, β-receptor responsiveness, resting vagal cardiac activity & baroreceptor reflex sensitivity were significantly depressed in elderly. It was concluded that high blood pressure although an important modulating factor in younger patients, has very limited if any influence on cardiovascular regulatory functions in older subjects whose autonomic functions have already been substantially altered by advancing age.

Chu T S, Tsai T J, Lai J S, Chen W Y (1989)[55]studied autonomic function in 76 normal adults aged 20-80 years (46 men & 30 women). The autonomic function tests included heart rate (HR) response to standing up, during Valsalva maneuver, deep breathing, diving and blood pressure (BP) response to standing up, during immersion of the hand in ice water & a sustained hand grip. The result showed 1) HR response to standing up, Valsalva maneuver & deep breathing are less variable & more suitable for follow up. 2) Women had a greater fall in systolic BP after standing up & lesser rise in diastolic pressure during sustained hand grip than did men. 3) Age correlated negatively with heart rate response to standing, deep breathing, Valsalva ratio & the cold pressure test. But age correlated positively with a fall in systolic blood pressure as women stood up, but not in men. 4) There is significant correlation between HR response to standing up & the HR response to deep breathing. They concluded that sex & age do have an

influence on some autonomic test results. The influence of the former is more related to both the sympathetic & parasympathetic system.

Following studies have been done on Parasympathetic function tests which have shown decline in function with advancing age.

Islam T, Begum N, Ferdousi S, Ali T (2008, Dhaka) [1] evaluated parasympathetic nerve function in 60 apparently healthy elderly subjects of both sexes divided into two groups. One group consisted of 30 elderly subjects with age range from 51 to 60 yrs & another group consisted of 30 elderly subjects with age range from 61-70yrs. 30 sex & BMI matched healthy adults with age range from 21-30 years were studied as control. Parasympathetic nerve function status was assessed by 1) heart rate response to deep breathing, 2) heart rate response to Valsalva maneuver, 3) heart rate response to standing (30:15). All the parameters were significantly lower in both elderly groups compared to that of control adults. Heart rate response to deep breathing was significantly lower in 61-70yrs age group compared to 51-60yrs age group. Valsalva ratio & 30:15 ratio were also lower in 61-70yrs age group than 51-60 yrs age group but the difference was not statistically significant. All the 3 parameters were negatively correlated with age which was statistically significant. From the study it was concluded that ageing process substantially impaired cardiovascular parasympathetic nerve function.

R C Melo, M D B Santos, E Silva, R J Quiterio, M A Moreno, M S Reis et al (2005, Brasil)[56]studied the effect of ageing process & an active life style on the heart rate response to deep breathing. Study group consisted of 9 young sedentary (23$\pm$2.4yrs), 16 young active (22$\pm$2.1yrs) & 8 older sedentary (63$\pm$2.4yrs) & 8 older active (61$\pm$1.1yrs) healthy men. The results suggested that ageing reduces heart rate variability. However

regular physical activity positively affects vagal activity on the heart & consequently attenuates the effects of ageing in autonomic control of heart rate.

W. Wieling, J. F. M. van Brederode, L. G. de Rijk, C. Borst and A. J. Dunning (1981)[57]studied the heart rate changes induced by standing up & forced breathing in 133 healthy subjects in the age range 10-65yrs to establish a data for studies on parasympathetic heart rate control in autonomic neuropathy. Test results declined with age. The lower limit of normal decreased from 22 to11 beats/min for forced breathing & from 26 to 16 beats/min for standing up with age increasing from 10- 65 yrs. In combination these 2 tests form a simple & reliable bed side method to establish cardiac vagal neuropathy.

Shirley A Smith (1982)[58]carried out a study to establish a normal range for use in deep breathing test for cardiac vagal integrity in diabetes mellitus in 174 healthy subjects aged 16 to 89 yrs. Results were expressed as ratio of longest R-R interval during expiration to shortest R-R interval during inspiration. The ratio declined appreciably with age but was not significantly related to resting heart rate. Measurement of this age dependent ratio which may be made with any electrocardiographic apparatus provides a simple accurate diagnostic screen for autonomic neuropathy in the clinic.

G Cybulski, W Neiwiadomski (2003)[59]studied the dynamics of heart rate changes following standing up from supine position in 41 healthy men aged 20 to 59 yrs. There was attenuation of heart rate response to standing with age.

Piha S J (1995, Finland)[19] assessed normal autonomic hemodynamic responses to the Valsalva maneuver in 158 healthy subjects aged 25-60 years. It was observed that sex had no or only marginal effect on blood pressure or heart rate responses or latencies.

Ageing was accompanied by a smaller decrease and smaller partial recovery of blood pressure during strain, with attenuation of reflectory bradycardia & lengthening of the latencies. It is concluded that age related reference values should be applied in the interpretation of the Valsalva maneuver.

Following studies have been done to study the effect of advancing age on Sympathetic function tests:

Ng AV, Callister R, Johnson DG, Seals DR.(1994)[60] studied the sympathetic neural response to stress with age in healthy humans. Sympathetic nervous system reactivity to stress is thought to increase with age in humans. This hypothesis was tested by recording postganglionic sympathetic nerve activity to skeletal muscle (MSNA) (peroneal microneurography) and by measuring plasma norepinephrine concentrations (PNE), heart rate, and arterial pressure before (pre stress control) and during cognitive challenge (mental arithmetic and colored word test), thermal stress (i.e., the cold pressor test), and exhaustive isometric handgrip exercise (40% of maximum voluntary force) / post exercise ischemia in 15 older (60-74 yr) and 15 young (19-30 yr) healthy men and women (8 males, 7 females each). The initial pre stress control level of MSNA was higher in the older subjects, but there were no significant differences for PNE, heart rate, or arterial pressure. The MSNA and PNE responses to mental stress were small and not different in the two groups. MSNA and PNE increased markedly in response to the cold pressor test and isometric handgrip exercise/post exercise ischemia in both groups. The absolute unit increases in MSNA were similar in the two groups, but the relative (percentage) increases were actually smaller in the older subjects due to their elevated baseline levels. The stress-evoked increases in arterial pressure were similar in the

groups, but the older subjects tended to demonstrate smaller increases in heart rate. In general, no gender differences were noted in either age group. These findings fail to support the long-held concept that stress-induced sympathetic nervous system stimulation becomes exaggerated with age. Thus, sympathetic neural hyper reactivity does not appear to be a fundamental property of the ageing process in humans.

Arata de Bellabarba G, Molina C, Davila Spinetti D, Villarroel V, Bellabarba S A, Torres A (2001, Venezuela)[61] studied the influence of age & gender on plasma norepinephrine changes produced by orthostatic stress. 56 men & 60 women were studied at supine & standing positions. On the basis of age of subjects they were divided into 3 groups of either men or women. Group A - young, 17-34 years. Group B-middle age, 40-60 years. Group C-senescent, 61-91 years. Senescent individuals had the highest absolute supine values of NE & significant differences between women & men were found in groups B & group C but not between young. NE increased markedly in response to orthostatic stress but the relative increases were smaller in the older subjects related to their elevated base line levels. The relative increases in NE responses were not different in men & women. It is concluded that orthostatic stress induced rise of blood NE is attenuated by age but does not appear to be dependent on gender.

To study the effect of gender on cardiovascular autonomic function tests, following studies have been done:

Ingrid Tonhajzerova, Kamil Javorka, Michal Javorka & Maria Petraskova (2002, Slovakia)[62] performed cardiovascular autonomic nervous system tests to determine reference values in young people & influence of age & gender. Deep breathing test, orthostatic test & Valsalva maneuver were performed in 206 healthy subjects (106 boys,

100 girls) in age range 15-19yrs. The most sensitive test for ascertainment of developmental changes was test of deep breathing. I/E was increased in 19yr old group compared to 15yr old group indicating an increase in vagal activity. Girls have lower values of deep breathing & orthostatic test compared to boys in same group (15-19yrs).

Piha S J[63] studied the cardiovascular responses to various autonomic tests in 224 healthy randomly selected males & females. The HR response to Valsalva maneuver was greater in females over 50yrs than in males in the same age (1.58 ± 0.34 vs. 1.44 ± 0.30, $p<0.05$). The heart rate response to beep breathing (E/I ratio) was higher in males under 50yrs than in females in same age (1.37 ± 0.17 vs. 1.34 ± 0.18, $p<0.001$). Although there were sex differences in the magnitude of responses, the effect of age was similar in males & females & accelerated attenuation of autonomic responses could not be demonstrated with increasing age.

Following studies have been done to evaluate autonomic functions in women:

Shahar Lavi, OriNevo, Israel Thaler, Rimma Rosenfeld, Lior Dayan. Nir Hirshoren (2006)[64] studied the effect of ageing on the cardiovascular homeostatic mechanisms in premenopausal & postmenopausal women with similar estrogen levels. 12 healthy post menopausal women were compared with 14 normally menstruating women during the early follicular phase to avoid as much as possible the effects of estrogen. It was concluded that in young women parasympathetic control is the main regulator of the cardiovascular system & in postmenopausal women sympathetic tone dominates. The transition from parasympathetic to sympathetic control may contribute to the increased cardiovascular morbidity with ageing.

Demetra D, Christou, Pamela Parker Jones, Jens Jordan, Andre Diedrich, David Robertson, Douglas R Seals (2005)[65] hypothesized that women have lower tonic ANS support of blood pressure & less effective baroreflex buffering of BP then men. To test these hypothesis 22 premenopausal women aged (28±1 years) & 29 men aged 27±1 years) were studied. Women had lower baseline SBP & plasma catecholamine concentrations then men. Tonic ANS support of BP was ~50% to 65% lower in women. Acute BRB of BP was 47% smaller in women. Systemic α_1- adrenergic vascular responsiveness was not different. It was concluded that women have lower tonic sympathoadrenal activity related ANS support of BP & less effective baroreflex buffering of BP then men of similar age. The lower tonic ANS support of BP could contribute to the lower chronic BP levels of premenopausal women, where as attenuated baroreflex buffering of BP may explain less effective BP regulation in women in response to vasoactive to drugs & acute stress.

MATERIALS AND METHODS

Experimental design

The cross-sectional study was carried out in 152 healthy subjects in the age range of 18 - 65 years randomly selected among the staff & students of BLDEA's Shri B M Patil Medical College, Bijapur. The ethical clearance for the study was obtained from ethical committee (Annexure: 1)

Each subject taking part was explained about the procedure to be adapted in the research. All the subjects after thoroughly understanding the procedures to be adopted signed an informed consent form provided to them (Annexure: 2). All subjects underwent thorough clinical examination.

Inclusion criteria

Only healthy subjects of Indian origin were included in the study. The subjects without signs of cardiovascular, endocrinological, neurological, hematological & inflammatory diseases were selected for the study. The apparent health status of the subject was determined through clinical examination and history taking.

Exclusion criteria

The subjects with any of the following findings were excluded from the study.

1) Evidence of hypertension (systolic blood pressure more than 150 and diastolic blood pressure more than 90 mm Hg).
2) Clinical signs of cardiac failure or ECG changes suggestive of arrhythmia, ischemia.
3) Subjects having diabetes mellitus, bronchial asthma, giddiness on standing, syncopal spells, visual disturbances, nocturnal diarrhea.

4) Subjects receiving drugs that are known to interfere with cardiac function or respiratory functions such as beta blockers, sympathomimetic drugs, vasodilators and diuretics.
5) Associated disease or conditions known to affect autonomic function like Guillean Barre syndrome, Poliomyelitis, Diphtheria, Tuberculosis, Syphilis, Amyloidosis, Chronic renal failure.
6) Subjects with history of alcohol intake.
7) Subjects with history of tobacco consumption in any form.
8) Any disease condition affecting the autonomic nervous system.

Sample size:

We have taken B.L.D.E.A'S Shri. B. M. Patil Medical College as the scope of the study and hence the data collection has been restricted to the staff and students of the college. The study was conducted on 152 healthy subjects of BLDEA's Shri B M Patil Medical College, Bijapur.

Sample size is derived from the following calculation[66]:

$$n = \frac{z^2 s^2}{E^2}$$

$z_{(0.05)} = 1.96$ (table no)

s= 5 (standard deviation) [67]

E= 0.8 (assumed value of extent of error)

∴ n= 152.

Sample size for different groups is calculated by proportional allocation technique.

The entire sample is divided into four groups according to http://medical-dictionary.thefreedictionary.com/middle+aged.[68].

Group I	18 to 19 yrs.	Adolescence
Group II	20 to 34 yrs	Early-adulthood
Group III	35 to 54 yrs.	Middle-adulthood
Group IV	55 to 65 yrs.	Late adult-hood

Method of Collection of Data

All selected subjects were asked to come to the research laboratory of Department of Physiology, Shri B M Patil Medical College at 8:30 am. The subjects were instructed to come on empty stomach with overnight abstinence from coffee and tea or any form of exercise. All the tests were conducted between 8:50 am to 11:00 am in cool and calm atmosphere at room temperature varying from 27^0 to 30^0 Celsius. The subjects were asked to relax in supine position for 30 minutes in the laboratory. The tests were performed only after complete relaxed physical and mental state of the subjects. All the subjects were subjected to recording of their physical anthropometry, various physiological parameters and autonomic function parameters.

The ECG recordings for these tests were performed on Student Physiograph Machine (Biodevices Chandigarh). ECG was recorded in lead II, at a paper speed of 5 mm/sec. The machine provided facility to record ECG simultaneously with time tracing and event marking. Blood pressure (BP) was measured with the help of mercury sphygmomanometer (Diamond)

Recording of Physical Anthropometry:

For each subject the following parameters were recorded.

Height (in cms): This was measured with the subject in standing position without his shoes, nearest to 0.1cms.

Weight (in kgs): The subjects were weighed in standardized machine with minimum of their clothing's, nearest to 0.1 kgs.

Body Surface Area (Square meters): This was calculated in each subject by using Dubois Nomogram.

Body Mass Index (Kilogram/meter^2): This was calculated for each subject from his height and weight.

Recording of Physiological Parameters:

In each subject following physiological parameters were recorded.

a) Respiratory rate (cycles/minute)
b) Heart rate (Beats/minute)
c) Systolic and Diastolic blood pressure (mm of Hg) by using mercury sphygmomanometer.

Recording of Autonomic Function Parameters

The Autonomic Nervous Function Parameters were selected as recommended by American Diabetic Association and performed as per methods described by Sir Roger Bannister[32].

A) The Parasympathetic activity was assessed by:

1) Heart Rate response to Valsalva Maneuver
2) Heart rate response to deep breathing
3) Immediate heart rate response to standing

1. Heart rate response to Valsalva maneuver:

The subject is asked to sit comfortably. A nose clip was applied and subject was asked to blow through the mouth piece attached to the mercury manometer for 15 seconds maintaining a pressure of 40mm Hg. A small air leakage in the mouth piece was done to ensure that the subject does not to blow with his cheeks (open glottis method). Throughout the maneuver ECG was recorded continuously and for 30 seconds after release of strain.

Heart rate response to Valsalva maneuver was expressed as:

$$\text{Valsalva ratio} = \frac{\text{Longest R-R interval after the maneuver}}{\text{Shortest R-R interval during the maneuver}}$$

2. Immediate heart rate response to standing (30:15 ratio):

The subject rested in supine position for 5 minutes after which he was asked to stand up unaided within 5 seconds and to remain standing for 1 minute. Continuous ECG recording was done starting from 1^{st} beat after standing up to the 60^{th} beat. The shortest R-R interval at or around the 15^{th} beat and the longest R-R interval at or around the 30^{th} beat after starting to stand are measured with a ruler. The heart rate response is expressed by the 30:15 ratio. The result was expressed as:

$$\text{Max/min} = \frac{\text{Longest R-R interval around } 30^{th} \text{ beat}}{\text{Shortest R-R interval around } 15^{th} \text{ beat}}$$

3. Heart rate response during deep breathing:

The subject sits quietly for 1 minute and after a verbal command starts to breath deeply and continuously at a rate of 6 breaths/min (5seconds inspiration and 5 seconds expiration) as trained before. ECG recorded continuously for one minute. The heart rate ratio during deep breathing was expressed as:

E/I ratio= Mean of longest R-R intervals during each expiration.
Mean of shortest R-R intervals during each inspiration

B. **The sympathetic activity was assessed by:**

1. Blood pressure response to standing

2. Blood pressure response to sustained handgrip exercise.

1. Blood pressure response to standing

The subject rested comfortably in supine position for 15 minutes. And then the subject was asked to stand up unaided and remain standing. Systolic blood pressure (SBP) was recorded in resting supine position and again immediately when he stands up. And the difference in SBP was noted.

2. Blood pressure response to sustained Hand grip exercise

The subject was asked to sit comfortably in chair. Initially the subject was asked to exert maximal hand grip strength on hand grip dynamometer with dominant hand. First the maximum voluntary contraction (MVC) is determined and then the subject was asked to exert 30% of MVC for 5 minutes (at least for 3 min) with dominant hand. Diastolic blood pressure was measured in the non-dominant hand at rest and at one minute intervals during hand grip. The maximum rise in diastolic BP during 30% of MVC over the resting diastolic blood pressure was noted.

Grading (Ewing and Clarke) [20] and autonomic function score of the results:

	Normal	Borderline	Abnormal
Score	**0**	**1**	**2**
1. H.R response to Valsalva maneuver	>1.21	1.11-1.20	<1.10
2. H.R variation during deep breathing	>15bts/min	11-14bts/min	<10bts/min
3. H.R response to standing	>1.04	1.01-1.03	<1.0
4. BP response to standing	<10mmHg	11-29mmHg	>30mmHg
5. BP response to sustained hand grip	>16mmHg	11-15mmHg	<10mmHg

Criteria for grading autonomic function as whole[69]

Scores $\leq$ 3 Normal autonomic function

> 3 & < 8 Borderline dysfunction

$\geq$ 8 to 10 abnormal function

Statistical Analysis:

All statistical analysis is done by using SPSS software version 9 under the guidance of Biostatistician of Shri B M Patil Medical College. All values are presented as Mean ± Standard Deviation (Mean ± SD). Comparison of mean values of parameters between the four different groups is done by One Way ANOVA. Comparison of mean values of parameters between male & female of the same group is done by unpaired t test. Correlation between various autonomic function parameters & ageing is done by Pearson correlation.

1. p Value >0.05 is taken as not significant[70].
2. p Value <0.05 is taken as significant[70].
3. p Value <0.01 is taken as highly significant[70].
4. p Value <0.001 is taken as very highly significant[70].

Figure 2: Showing Student Physiograph

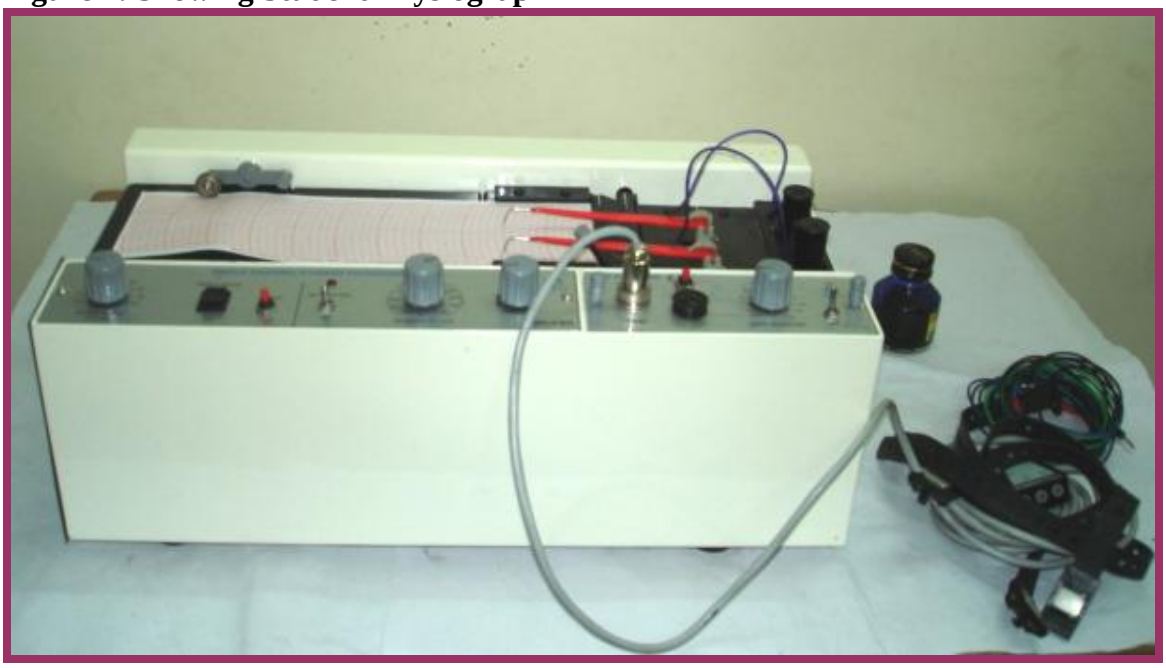


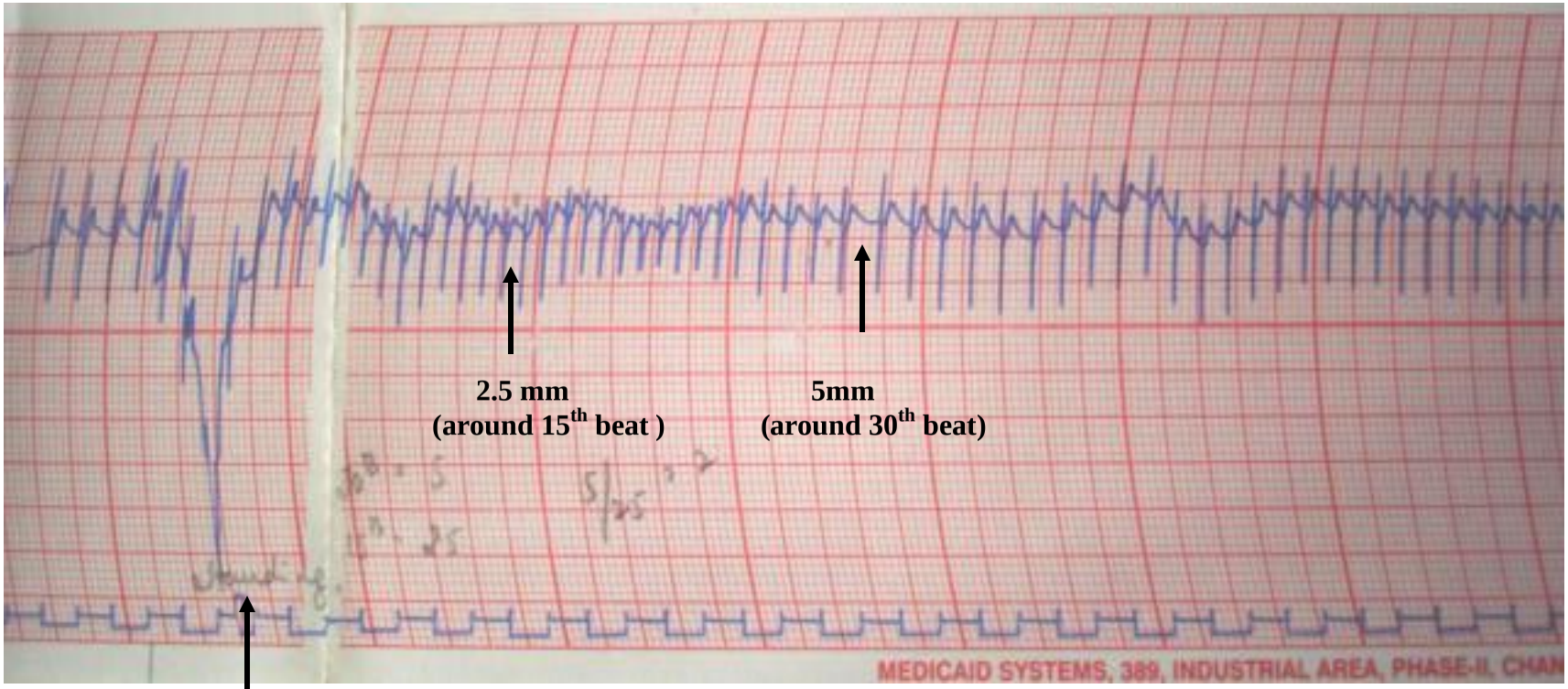
Figure 3: Showing instruments used: Hand grip Dynamometer, Stethoscope, Sphygmomanometer

Figure 4: Showing recording of Heart rate response to Valsalva maneuver

Figure 5: Showing recording of Heart rate response to standing

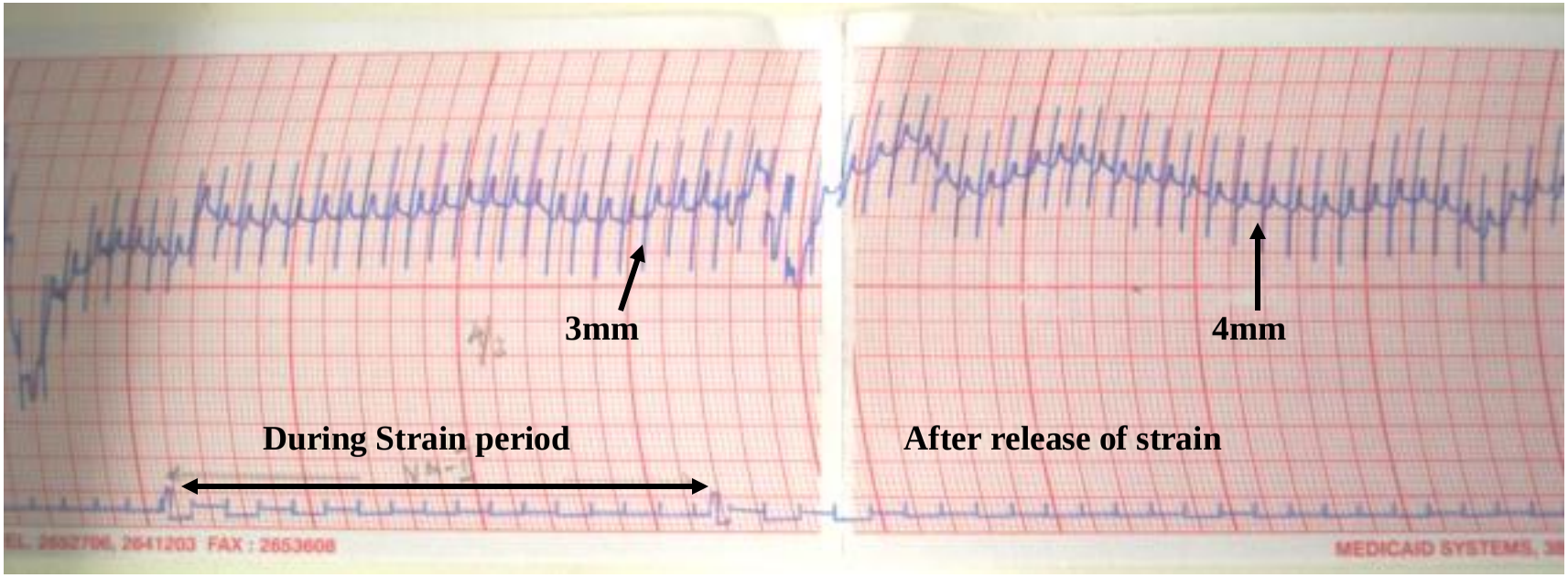
Figure 6: Showing recording of Blood pressure response to Sustained Hand Grip

Figure 7: ECG showing Heart rate response to standing **Paper speed=5mm/sec**



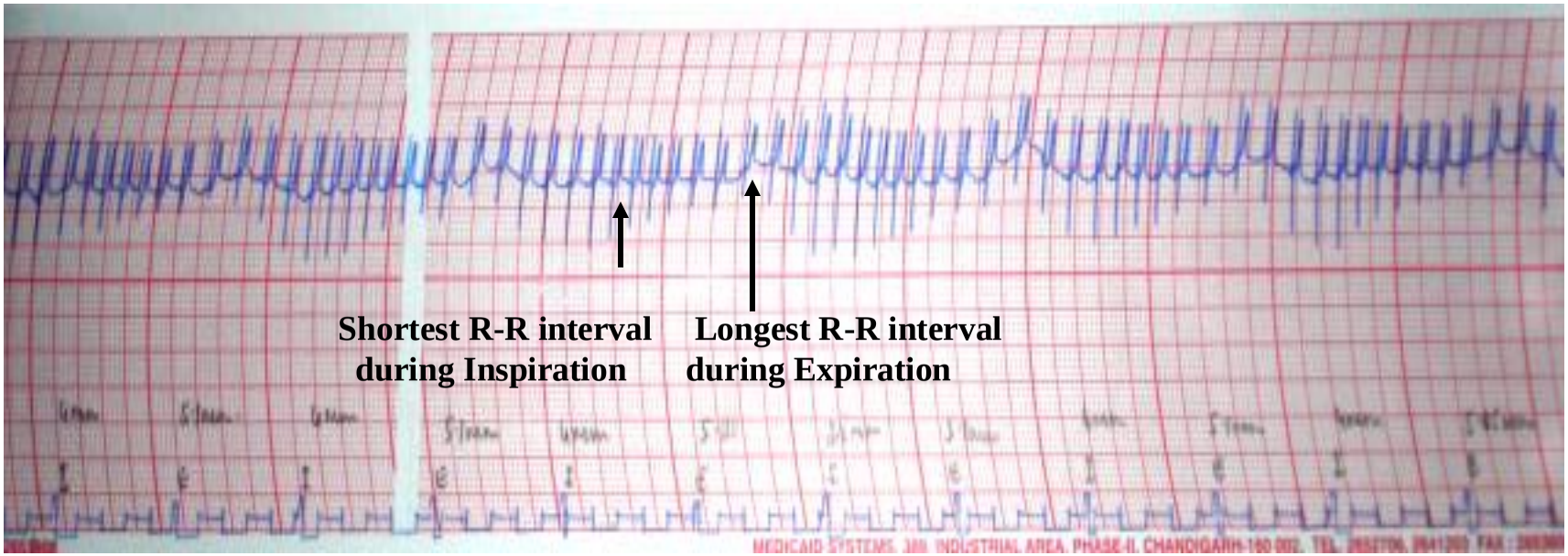
Point of Standing **30/15 ratio = 5/2.5=2**

Figure 8: ECG showing Heart rate response to Valsalva maneuver **Paper speed = 5mm/sec**



Valsalva ratio= 4/3 = 1.33

Figure 9: ECG showing Heart rate variation during deep breathing **Paper speed = 5mm/sec**



I-E = 75-60 = 15 bpm

RESULTS

Statistical Analysis:

The cross-sectional study of effect of age on cardiovascular autonomic function tests was conducted on healthy subjects in the age range of 18-65 years of B.L.D.E.A'S Shri. B. M. Patil Medical College, Bijapur.

Total number of subjects in each group is as follows:

Table no 1

Group I	18-19 years	37 (Male= 18, Female= 19)
Group II	20-34 years	40 (Male=31, Female=9)
Group III	35-54 years	38 (Male= 22, Female=16)
Group IV	55-65 years	37 (Male=20, Female=17)
Total		152 (Male=91, Female=61)

Recording of various anthropometric, Physiological and Autonomic function test parameters in Group I (18-19yrs), Group II (20-34yrs), Group III (35-54yrs), Group IV (55-65yrs) are represented in Annexure: 4a, 4b, 4c, 4d individually.

The parameters recorded include Age (years), Height (centimeters), Weight (kilograms), Body Surface Area (square meters), Body Mass Index (kilograms/meter^2).

1. Anthropometric Parameters:

The Mean Value and Standard Deviation, Level of Significance of each parameter is calculated for each group and presented in table- 2, 3, 4, 5, 6.

1. Age

Table: 2 ***Mean age (years) of subjects in different age groups***

Groups	**Males**	**Female**	**Level of Significance**
Group I (18-19yrs)	18.31± 4.77	18.37± 0.49	0.439
Group II (20-34 yrs)	22.54± 3.13	24.33± 3.07	0.09
Group III (30-54 yrs)	40.54± 4.75	40.18± 5.19	0.82
Group IV (55-65 yrs)	58.2 ± 3.65	56.529 ± 2.06	0.09

*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant.

Group I (18-19 yrs):

Group I subjects were randomly selected in the age range of 18-19yrs.

Mean Age ± SD of males 18.31 ± 4.77 years

Mean Age ± SD of females 18.37 ± 0.49 years

There is no significant variation (p=0.439) between males and females in Group I.

Group II (20-34 yrs):

Group II subjects were randomly selected in the age range of 20-34 years.

Mean Age ± SD of male subjects 22.54 ± 3.13 years

Mean Age ± SD of female subjects 24.33 ± 3.07 years

There is no significant variation (p=0.09) between males and females in Group II.

Group III (35-54 yrs):

Group III subjects were randomly selected in the age range of 35-54 years.

Mean Age ± SD of male subjects 40.54 ± 4.75 years

Mean Age ± SD of female subjects 40.18 ± 5.19 years

There is no significant variation (p=0.82) between males and females in Group III.

Group IV (55-65 years):

Group IV subjects were randomly selected in the age range of 55-65 years.

Mean Age ± SD of male subjects 58.2 ± 3.65

Mean Age ± SD of female subjects 56.529 ± 2.06

There is no significant variation (p=0.09) between males & females in Group IV.

Table 3: Anthropometric Parameters (Mean ± SD)

Parameters	Group I	Group II	Group III	Group IV	Level of significance
Height (cms)	165.35±8.27	166.47±7.94	158.84±10.52	158.59±8.46	0.000***
Weight (Kg)	62.95±13.38	58.67±9.49	60.28±11.43	59.13±11.50	0.372
BMI (kg/m^2)	23.07±4.69	21.11±2.67	23.53±4.34	23.43±3.93	0.025*
BSA (Sq m)	1.68±0.16	1.65±0.15	1.62±0.16	1.59±0.18	0.107

*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant

Height (cm):

Group I (18-19 yrs) Mean ± SD is 165.35± 8.27 cms.

Group II (20-34 yrs) Mean ± SD is 166.47± 7.94 cms

Group III (35-54 yrs) Mean ± SD is 158.84 ± 10.52 cms

Group IV (55-65 yrs) Mean ± SD is 158.59 ± 8.46 cms

There is significant (p=0.000) gradual decrease in the height of the subjects from Group I to Group IV.

Weight (Kg):

Group I (18-19 yrs): Mean ± SD 62.95±13.38 Kg.

Group II (20-34 yrs): Mean ± SD 58.67±9.49 Kg.

Group III (35-54 yrs): Mean ± SD 60.28±11.43 Kg.

Group IV (55-65 yrs): Mean ± SD 59.13±11.50 Kg.

There is no significant (p=0.372) variation in the weight of the subjects from Group I to Group IV.

BMI (kg/m^2):

Group I (18-19 yrs): Mean ± SD 23.07±4.69

Group II (20-34 yrs): Mean ± SD 21.11±2.67

Group III (35-54 yrs): Mean ± SD 23.53±4.34

Group IV (55-65 yrs): Mean ± SD 23.43±3.93

There is a significant (p=0.02) variation in the BMI of subjects from Group I to Group IV.

BSA (Square meter):

Group I (18-19 yrs): Mean ± SD 1.68±0.16

Group II (20-34 yrs): Mean ± SD 1.65±0.15

Group III (35-54 yrs): Mean ± SD 1.62±0.16

Group IV (55-65 yrs): Mean ± SD 1.59±0.18

There is no significant (p=0.107) variation in the BSA of subjects from Group I to Group IV.

II Physiological Parameters

Recording of various Physiological parameters in subjects of different age groups are represented in table 4. The parameters recorded included Resting heart rate (bpm), Respiratory rate (cycles per minute), Systolic & Diastolic blood pressure (mm Hg). The values are presented as Mean ± SD of each parameter in different groups.

Table 4: Physiological Parameters (Mean ± SD) of subjects in different age groups

Parameters	Group I	Group II	Group III	Group IV	Level of significance
Resting PR(bpm)	86.97±8.72	81.10±9.24	78.47±9.25	72.26±15.04	0.000***
Resting RR (cycles/min)	18.37±2.15	18.80±3.25	18.78±5.35	19.51±3.78	0.575
Resting SBP (mm of Hg)	117.02±13.94	109.0±11.67	115.26±12.01	121.18±13.78	0.001***
Resting DBP (mm of Hg)	77.72±8.74	74.05±8.50	80.26±9.33	80.59±9.52	0.007**

*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant

Resting Pulse Rate (beats/min):

Group I: Mean±SD 86.97±8.72

Group II: Mean±SD 81.10±9.24

Group III: Mean±SD 78.47±9.25

Group IV: Mean±SD 72.26±15.04.

There is a vey highly significant (p=0.000) gradual decrease in the resting Pulse Rate of subjects from Group I to Group IV.

Resting Respiratory Rate (cycles/min)

Group I: Mean±SD 18.37±2.15

Group II: Mean±SD 18.80±3.25

Group III: Mean±SD 18.78±5.35

Group IV: Mean±SD 19.51±3.78

There is an insignificant (p=0.575) variation in the resting respiratory rate of subjects from Group I to Group IV.

Resting Systolic Blood Pressure (mm of Hg)

Group I: Mean±SD 117.02±13.94

Group II: Mean±SD 109.0±11.67

Group III: Mean±SD 115.26±12.01

Group IV: Mean±SD 121.18±13.78

There is very highly significant (p=0.001) gradual increase in the resting SBP of subjects from Group II to Group IV.

Resting Diastolic Blood Pressure (mm of Hg)

Group I: Mean±SD 77.72±8.74

Group II: Mean±SD 74.05±8.50

Group III: Mean±SD 80.26±9.33

Group IV: Mean±SD 80.59±9.52

There is a highly significant (p=0.007) variation in the resting diastolic blood pressure of subjects from Group I to Group IV

Figure: 10 Graph showing Resting Pulse rate in different age groups

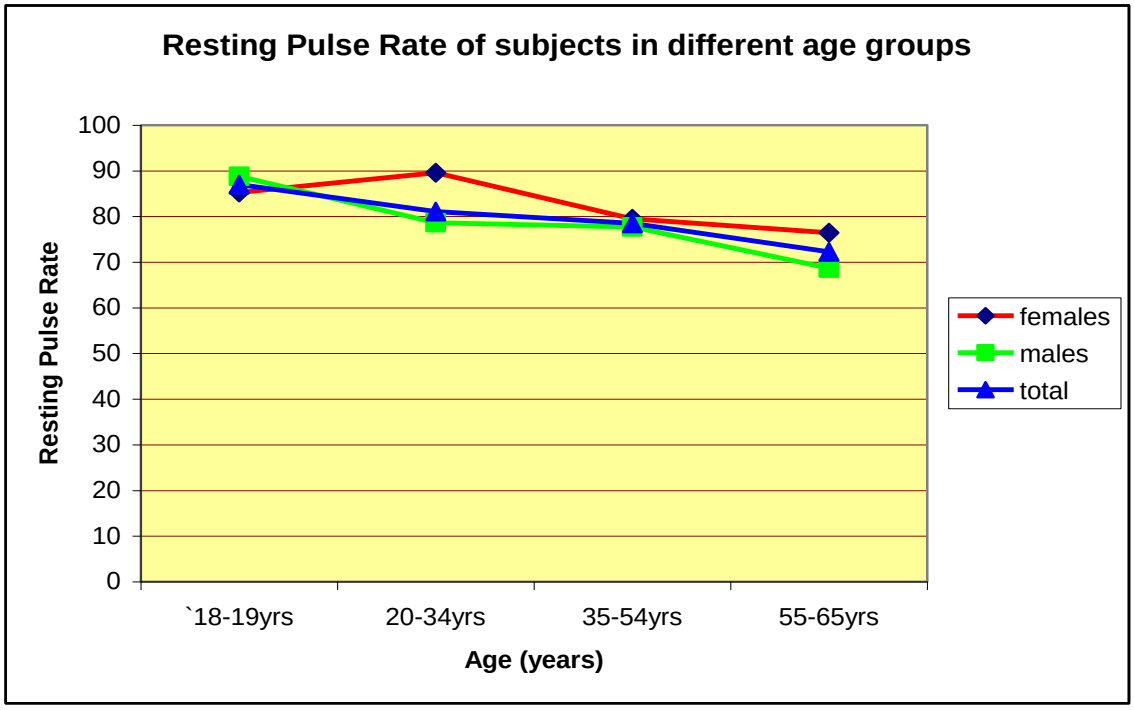
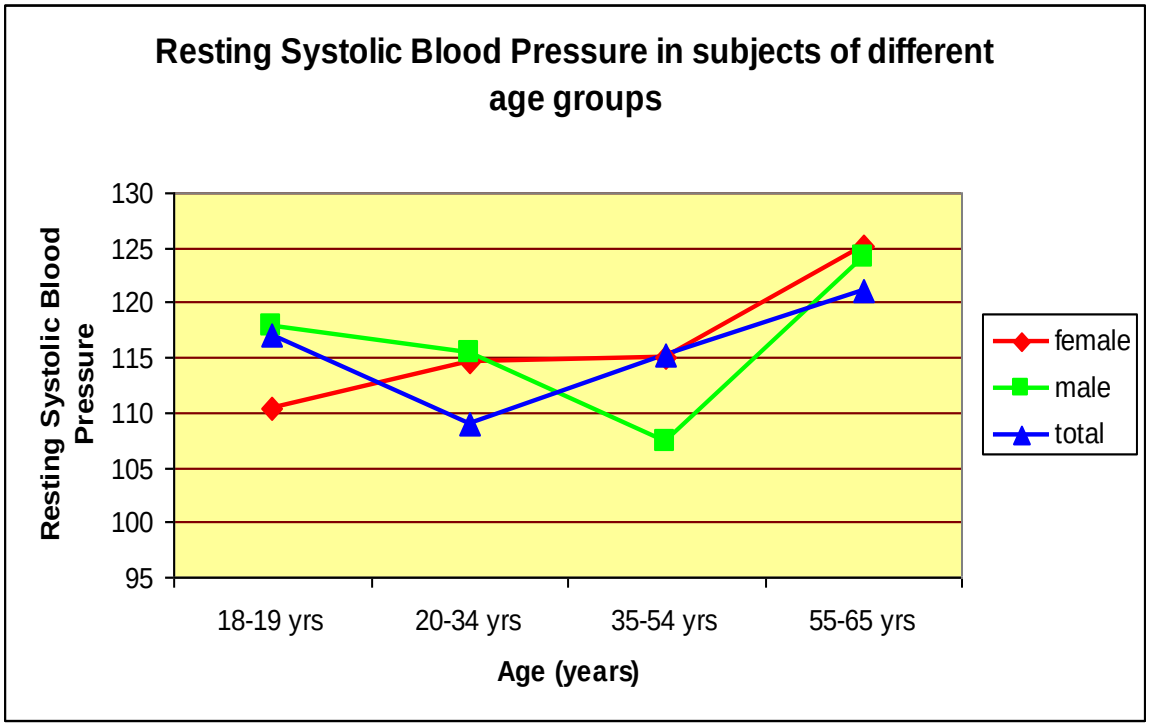


Figure: 11 **Graph showing Resting Systolic Blood Pressure in different age groups**



III AUTONOMIC FUNCTION PARAMETERS IN SUBJECTS OF DIFFERENT AGE GROUPS

Recording of various Autonomic function parameters in subjects of different age groups are represented in table 5. The parameters recorded include Heart rate response to Valsalva maneuver (Valsalva Ratio), Heart rate variation during deep breathing (I-E), Immediate heart rate response to standing, Blood pressure response to standing, Blood pressure response to sustained hand grip. The values are presented as Mean ± SD of each parameter in different age groups. By One Way ANOVA the level of significance is calculated for different age groups for each parameter.

Table: 5 Autonomic function parameters of subjects in different age groups

Autonomic function parameters	**Group I (18-19 yrs)**	**Group II (20-34 yrs)**	**Group III (35-54 yrs)**	**Group IV (55-65 yrs)**	**Level of significance**
Valsalva Ratio	1.50 ± 0.37	1.46 ± 0.33	1.42 ± 0.36	1.24 ± 0.18	0.004**
HR variation to deep breathing (Maximum-Minimum)	24.23 ± 5.46	24.65 ± 7.12	17.59 ± 7.90	10.40 ± 7.65	0.000***
Immediate HR response to standing (30:15)	1.30 ± 0.18	1.33 ± 0.20	1.21 ± 0.16	1.12 ± 0.15	0.000***
BP response to Standing (Fall in SBP)	8.9 ± 3.04	10.95 ± 5	11.21 ± 7.31	11.48 ± 5.82	0.194
BP response to sustained Hand grip (Increase in DBP)	26.37 ± 9.24	26.35 ± 7.81	20.05 ± 11.51	13.29 ± 4.79	0.000***

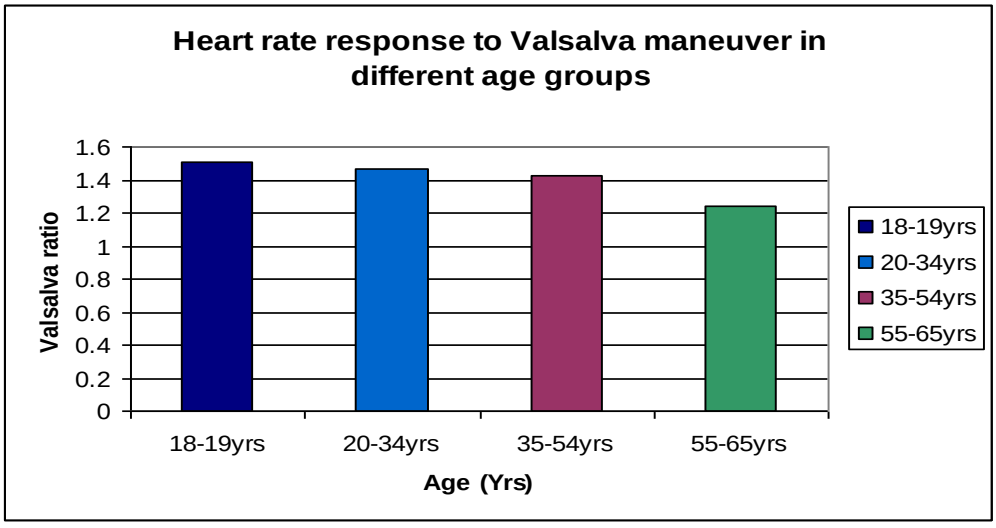
*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant.

Table 6: Mean ± SD Parasympathetic function parameters in different group (n=152)

Heart rate response			
Groups	**To Valsalva maneuver**	**To deep breathing (rate/min)**	**To standing (30:15)**
Group I (18-19 yrs)	1.50 ± 0.37	24.23 ± 5.46	1.30 ± 0.18
Group II (20-34 yrs)	1.46 ± 0.33	24.65 ± 7.12	1.33 ± 0.20
Group III (35-54 yrs)	1.42 ± 0.36	17.59 ± 7.90	1.21 ± 0.16
Group IV (55-65 yrs)	1.24 ± 0.18	10.40 ± 7.65	1.12 ± 0.15
By one way ANOVA	0.004**	0.000***	0.000***

*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant.

Figure 12:



Heart Rate response to Valsalva maneuver:

Mean VR ± SD of Group I 1.50 ± 0.37

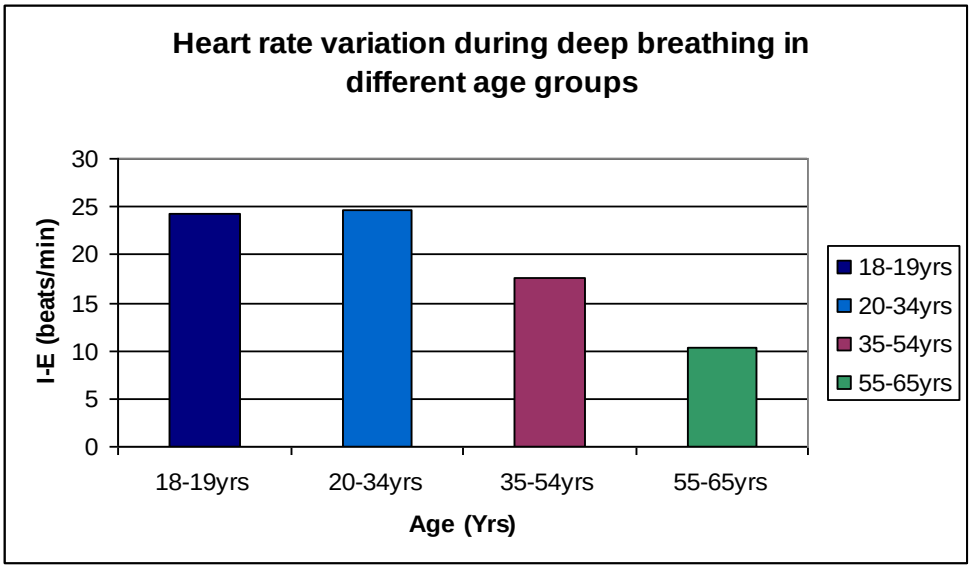
Mean VR± SD of Group II 1.46 ± 0.33

Mean VR ± SD of Group III 1.42 ± 0.36

Mean VR ± SD of Group IV 1.24 ± 0.18

There is a highly significant (p=0.004) gradual decrease in the Valsalva ratio (VR) from Group I to Group IV.

Figure 13:



Heart rate variation (HRV) during Deep Breathing:

Mean HRV $\pm$ SD of Group I (18-19 yrs) 24.23 $\pm$ 5.46

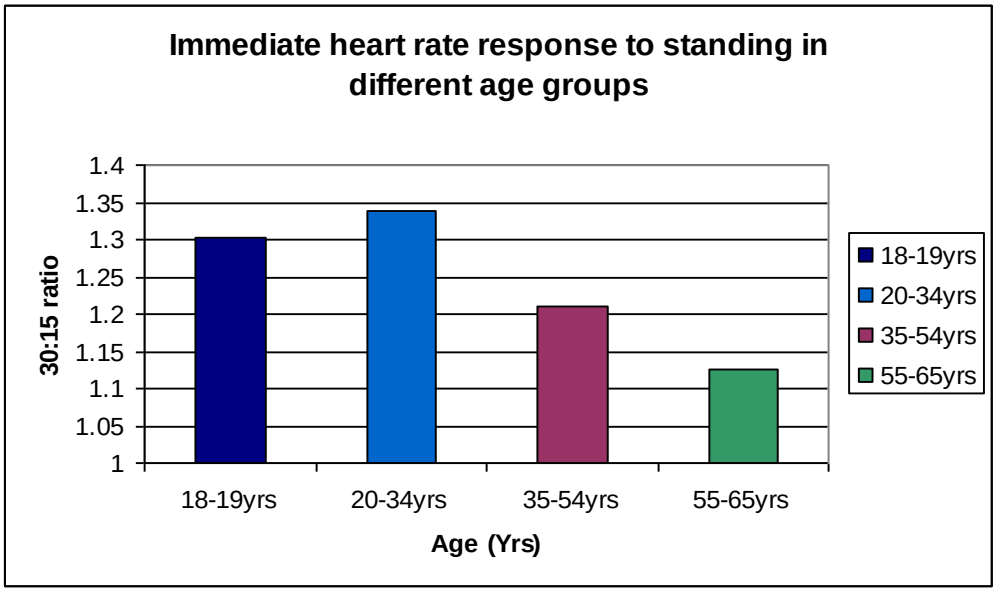
Mean HRV $\pm$ SD of Group II (20-34 yrs) 24.65 $\pm$ 7.12

Mean HRV $\pm$ SD of Group III (35-54 yrs) 17.59 $\pm$ 7.90

Mean HRV $\pm$ SD of Group IV (55-65 yrs) 10.40 $\pm$ 7.65

There is very highly significant (p=0.000) gradual decrease in the heart rate variation during deep breathing from Group I to Group IV.

Figure 14:



Immediate heart rate response to standing (30:15 ratio):

Mean ratio $\pm$ SD of Group I (18-19 yrs) 1.30 $\pm$ 0.18

Mean ratio $\pm$ SD of Group II (20-34 yrs) 1.33 $\pm$ 0.20

Mean ratio $\pm$ SD of Group III (35-54 yrs) 1.21 $\pm$ 0.16

Mean ratio $\pm$ SD of Group IV (55-65 yrs) 1.12 $\pm$ 0.15

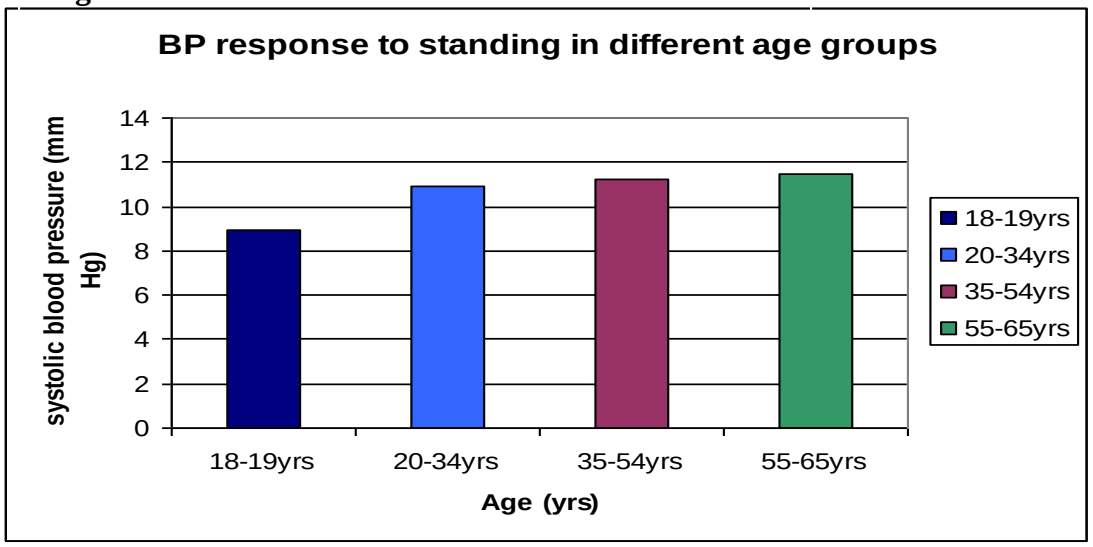
There is a very highly significant (p=0.000) gradual decrease in the immediate heart rate response to standing from Group I to Group IV.

Table 7: ***Mean ± SD sympathetic function parameters in different groups (n=152)***

Blood Pressure response		
Groups	**To standing (mm of Hg)**	**To sustained hand grip (mm of Hg)**
Group I (18-19 yrs)	8.97 ± 3.04	26.37 ± 9.24
Group II (20-34 yrs)	10.95 ± 5	26.35 ± 7.81
Group III (35-54 yrs)	11.21 ± 7.31	20.05 ± 11.51
Group IV (55-65 yrs)	11.48 ± 5.82	13.29 ± 4.79
By one way ANOVA	0.194	0.000***

*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant.

Figure 15:



Blood pressure response to standing (fall in SBP in mm of Hg):

The mean SBP ± SD in Group I (18-19 yrs) 8.9 ± 3.04

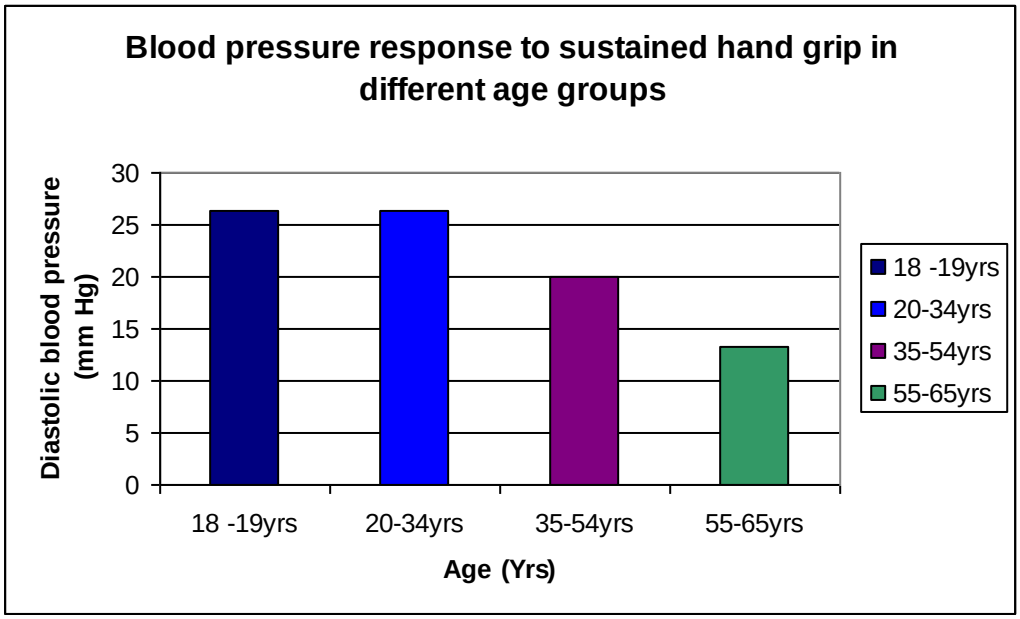
The mean SBP ± SD in Group II (20-34 yrs) 10.95 ± 5

The mean SBP ± SD in Group III (35-54 yrs) 11.21 ± 7.31

The mean SBP ± SD in Group IV (55-65 yrs) 11.48 ± 5.82

There is insignificant (P=0.194) increase in the SBP on standing from Group I to Group IV.

Figure 16:



Blood pressure response to sustained hand grip (increase in DBP in mm Hg):

Mean DBP $\pm$ SD in Group I (18-19 yrs) 26.37 $\pm$ 9.24

Mean DBP $\pm$ SD in Group II (20-34 yrs) 26.35 $\pm$ 7.81

Mean DBP $\pm$ SD in Group III (35-54) 20.05 $\pm$ 11.51

Mean DBP $\pm$ SD in Group IV (55-65 yrs) 13.29 $\pm$ 4.79

There is very highly significant (p=0.000) gradual decrease in the blood pressure response to sustained hand grip from Group I to Group IV.

IV. GENDER WISE COMPARISON OF AUTONOMIC FUNCTION PARAMETERS OF SUBJECTS IN DIFFERENT AGE GROUPS

1. Valsalva maneuver (valsalva ratio)

Table 8: Mean Valsalva ratio in males & females of different age groups.

Groups	**Males**	**Female**	**Level of Significance**
Group I (18-19yrs)	1.51± 0.36	1.50 ± 0.39	0.94
Group II (20-34 yrs)	1.49 ± 0.34	1.37 ± 0.29	0.309
Group III (35-54 yrs)	1.55 ± 0.41	1.24 ± 0.19	0.005**
Group IV (55-65 yrs)	1.31 ± 0.22	1.17 ± 0.08	0.01**
Level of significance by one way ANOVA test	0.12	0.004**	

*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant.

Group I (18-19 yrs):

Mean VM ± SD of males 1.51± 0.36

Mean VM ± SD of females 1.50 ± 0.39

There is insignificant increase (p=0.94) by 0.01 in males in comparison to females

Group II (20-34 yrs):

Mean VM ± SD of male subjects 1.49 ± 0.34

Mean VM ± SD of female subjects 1.37 ± 0.29

There is insignificant increase (p=0.30) by 0.12 in males in comparison to females

Group III (35-54 yrs):

Mean VM $\pm$ SD of male subjects 1.55 $\pm$ 0.41

Mean VM $\pm$ SD of female subjects 1.24 $\pm$ 0.19

There is a highly significant increase (p=0.005) by 0.31 in males in comparison to females.

Group IV (55-65 years):

Mean VM $\pm$ SD of male subjects 1.31 $\pm$ 0.22

Mean VM $\pm$ SD of female subjects 1.17 $\pm$ 0.08

There is a highly significant increase (p=0.01) by 0.14 in males in comparison to females.

By One-way ANOVA test

There an insignificant gradual decrease (p=0.12) of Valsalva ratio in males from Group I to Group IV.

There is a highly significant gradual decrease (p=0.004) of Valsalva ratio in females from Group I to Group IV.

2. Heart rate variation during deep breathing (I-E)

Table 9: Mean I-E (beats/min) of males & females of different age groups

Groups	**Males**	**Female**	**Level of Significance**
Group I (18-19yrs)	22.96 ± 6.38	25.43 ± 4.23	0.17
Group II (20-34 yrs)	25.85 ± 6.67	20.54 ± 7.49	0.07
Group III (35-54 yrs)	20.52 ± 7.78	13.55 ± 6.26	0.004**
Group IV (55-65 yrs)	12.78 ± 9.46	7.62 ± 3.17	0.03*
Level of significance by one way ANOVA test	7E-07***	7E-14***	

*p: <0.05: Significant, ** p: <0.01: Highly significant
*** p: <0.001: Very highly significant.

Group I (18-19 yrs):

Mean I-E ± SD of males 22.96 ± 6.38 bpm

Mean I-E ± SD of females 25.43 ± 4.23 bpm

There is insignificant decrease (p=0.17) by 3 beats/min in males in comparison to females

Group II (20-34 yrs):

Mean I-E ± SD of male subjects 25.85 ± 6.67 bpm

Mean I-E ± SD of female subjects 20.54 ± 7.49 bpm

There is an insignificant increase (p=0.07) by 5 beats/min in males in comparison to females

Group III (35-54 yrs):

Mean I-E $\pm$ SD of male subjects 20.52 $\pm$ 7.78 bpm

Mean I-E $\pm$ SD of female subjects 13.55 $\pm$ 6.26 bpm

There is a highly significant increase (p=0.004) by 7 beats/min in males in comparison to females

Group IV (55-65 years):

Mean I-E $\pm$ SD of male subjects 12.78 $\pm$ 9.46 bpm

Mean I-E $\pm$ SD of female subjects 7.62 $\pm$ 3.17 bpm

There is a significant increase (p=0.03) by 5 beats/min in males in comparison to females

By One-way ANOVA test

There is a very highly significant gradual decrease (p=7E-07) of I-E in males from Group I to Group IV except in Group II where there is increase.

There is a very highly significant gradual decrease (p=7E-14) of I-E in females from Group I to Group IV.

3. Immediate heart rate response to standing (30:15)

Table 10: Mean 30:15 ratio in males & females of different age groups

Groups	**Males**	**Female**	**Level of Significance**
Group I (18-19yrs)	1.31 ± 0.22	1.29 ± 0.13	0.81
Group II (20-34 yrs)	1.34 ± 0.15	1.31 ± 0.34	0.77
Group III (35-54 yrs)	1.23 ± 0.19	1.17 ± 0.11	0.19
Group IV (55-65 yrs)	1.15 ± 0.16	1.09 ± 0.12	0.26
Level of significance by one way ANOVA test	0.003**	0.003**	

*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant.

Group I (18-19 yrs):

Mean 30:15 ± SD of males 1.31 ± 0.22

Mean 30:15 ± SD of females 1.29 ± 0.13

There is insignificant increase (p=0.81) by 0.02 in males in comparison to females

Group II (20-34 yrs):

Mean 30:15 ± SD of males 1.34 ± 0.15

Mean 30:15 ± SD of females 1.31 ± 0.34

There is insignificant increase (p=0.77) by 0.03 in males in comparison to females

Group III (35-54 yrs):

Mean 30:15 $\pm$ SD of males 1.23 $\pm$ 0.19

Mean 30:15 $\pm$ SD of females 1.17 $\pm$ 0.11

There is insignificant increase (p=0.19) by 0.06 in males in comparison to females

Group IV (55-65 years):

Mean 30:15 $\pm$ SD of males 1.15 $\pm$ 0.16

Mean 30:15 $\pm$ SD of females 1.09 $\pm$ 0.12

There is insignificant increase (p=0.26) by 0.06 in males in comparison to females

By One-way ANOVA test

There is a highly significant gradual decrease (p=0.003) of 30:15 ratio in males from Group I to Group IV except in Group II where it has shown increase in the value.

There is a highly significant gradual decrease (p=0.003) of 30:15 in females from Group I to Group IV except in Group II where it has shown an increase in the value.

4. Blood pressure response to standing (fall in SBP in mm of Hg)

Table 11: Mean fall in SBP (mm of Hg) on standing in males & females of different age groups.

Groups	**Males**	**Female**	**Level of Significance**
Group I (18-19yrs)	9.33 ± 2.05	8.63 ± 3.77	0.48
Group II (20-34 yrs)	11.22 ± 4.83	10 ± 5.74	0.57
Group III (35-54 yrs)	10.45 ± 5.37	12.25 ± 9.46	0.50
Group IV (55-65 yrs)	11.5 ± 6.92	11.47 ± 4.47	0.98
Level of significance by one way ANOVA test	0.54	0.332	

*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant.

Group I (18-19 yrs):

Mean SBP ± SD of males 9.33 ± 2.05 mm of Hg

Mean SBP ± SD of females 8.63 ± 3.77 mm of Hg

There is insignificant, fall (p=0.48) in SBP by 1 mm of Hg more in males in comparison to females

Group II (20-34 yrs):

Mean SBP ± SD of males 11.22 ± 4.83 mm of Hg

Mean SBP ± SD of females 10 ± 5.74 mm of Hg

There is insignificant, fall (p=0.57) in SBP by 1 mm of Hg more in males in comparison to females

Group III (35-54 yrs):

Mean SBP $\pm$ SD of males 10.45 $\pm$ 5.37 mm of Hg

Mean SBP $\pm$ SD of females 12.25 $\pm$ 9.46 mm of Hg

There is insignificant fall (p=0.50) in SBP by 2 mm of Hg less in males in comparison to females

Group IV (55-65 years):

Mean SBP $\pm$ SD of males 11.5 $\pm$ 6.92 mm of Hg

Mean SBP $\pm$ SD of females 11.47 $\pm$ 4.47 mm of Hg

There is no significant variation (p=0.98) between males and females in Group IV.

By One-way ANOVA test

When comparison made between males in different age groups there is no significant variation (p=0.54) found.

When comparison made between females in different age groups there is no significant variation (p=0.332) found.

5. Blood pressure response to sustained hand grip

(Increase in DBP in mm of Hg)

Table 12: Mean increase in DBP in response to sustained hand grip in males & females of different age groups.

Groups	**Males**	**Female**	**Level of Significance**
Group I (18-19yrs)	26.11 ± 10.34	26.63 ± 8.35	0.86
Group II (20-34 yrs)	27.67 ± 7.04	21.77 ± 9.02	0.09
Group III (35-54 yrs)	22.72 ± 13.43	16.37 ± 7.05	0.06
Group IV (55-65 yrs)	14.1 ± 5.63	12.35 ± 3.25	0.24
Level of significance by one way ANOVA test	2E-05***	8E-07***	

*p: <0.05: Significant, ** p: <0.01: Highly significant,
*** p: <0.001: Very highly significant.

Group I (18-19 yrs):

Mean DBP ± SD of males 26.11 ± 10.34 mm of Hg

Mean DBP ± SD of females 26.63 ± 8.35 mm of Hg

There is no variation (p= 0.86) between males & females in Group I.

Group II (20-34 yrs):

Mean DBP ± SD of males 27.67 ± 7.04 mm of Hg

Mean DBP ± SD of females 21.77 ± 9.02 mm of Hg

There is an insignificant increase (p=0.09) in DBP by 6 mm of Hg more in males in comparison to females

Group III (35-54 yrs):

Mean DBP $\pm$ SD of males 22.72 $\pm$ 13.43 mm of Hg

Mean DBP $\pm$ SD of females 16.37 $\pm$ 7.05 mm of Hg

There is an insignificant increase (p=0.06) in DBP by 6 mm of Hg more in males in comparison to females

Group IV (55-65 years):

Mean DBP $\pm$ SD of males 14.1 $\pm$ 5.63 mm of Hg

Mean DBP $\pm$ SD of females 12.35 $\pm$ 3.25 mm of Hg

There is an insignificant increase (p=0.24) in DBP by 2 mm of Hg more in males in comparison to females

By One-way ANOVA test

There is very highly significant (p=2E-05) gradual decrease in males from Group I to Group IV.

There is very highly significant (p=8E-07) gradual decrease in females from Group I to Group IV.

Linear regression and coefficient of determination (r^2) were used to examine the strength of association between ageing and cardiovascular autonomic function parameters. A coefficient of determination with maximal possible value of 1.0 would suggest a perfect correlation between cardiovascular autonomic function tests and ageing.

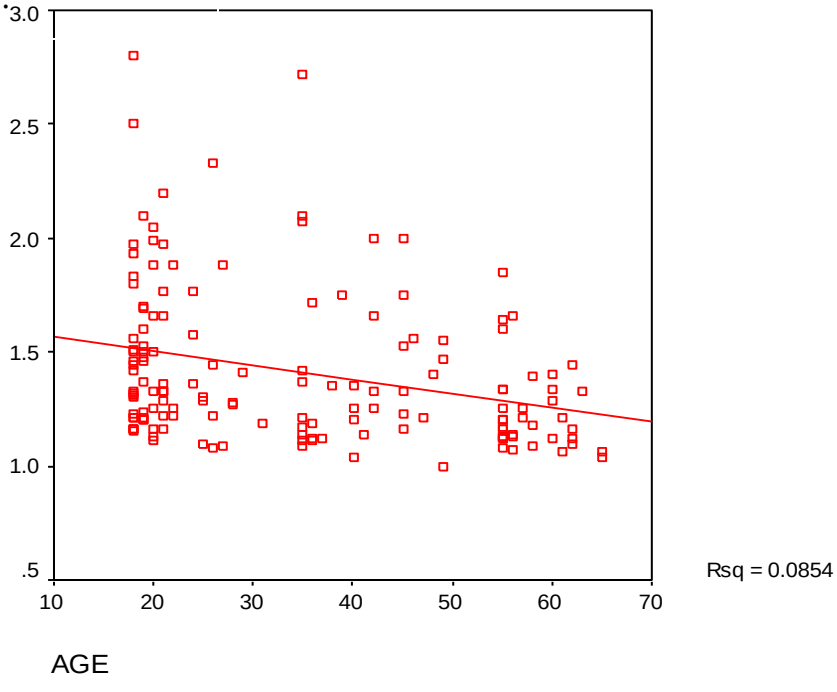
1. Correlation between ageing and Valsalva maneuver:

Table 13: Correlations

		AGE	VMMEAN
AGE	Pearson Correlation	1.000	-.292
	Sig. (2-tailed)	.	.000
	N	152	152
VMMEAN	Pearson Correlation	-.292	1.000
	Sig. (2-tailed)	.000	.
	N	152	152

** Correlation is significant at the 0.01 level (2-tailed).

Figure 17:



There is a highly significant inverse correlation between ageing and Valsalva maneuver.

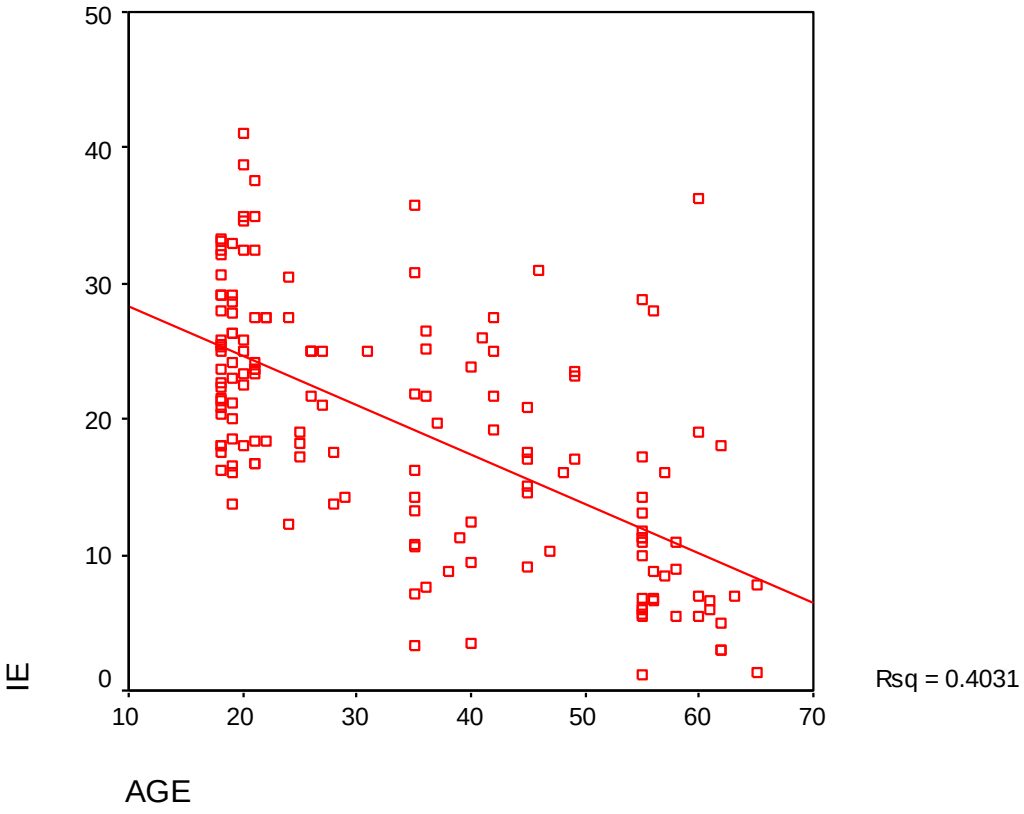
2 Correlation between ageing and I-E

Table 14: Correlations

		AGE	IE
AGE	Pearson Correlation	1.000	-.635
	Sig. (2-tailed)	.	.000
	N	152	152
IE	Pearson Correlation	-.635	1.000
	Sig. (2-tailed)	.000	.
	N	152	152

** Correlation is significant at the 0.01 level (2-tailed).

Figure 18:



There is a highly significant inverse correlation between ageing and heart rate variation during deep breathing.

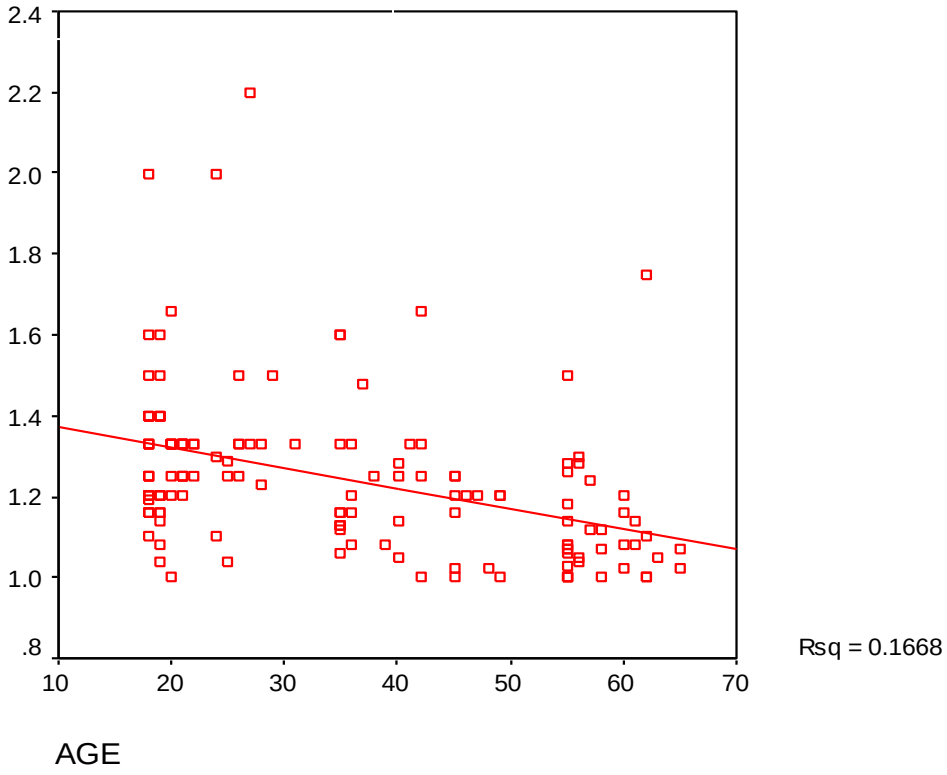
3 Correlation between ageing and immediate heart rate response to standing(30:15 ratio)

Table 15: Correlations

		AGE	HRRSTD
AGE	Pearson Correlation	1.000	-.408
	Sig. (2-tailed)	.	.000
	N	152	152
HRRSTD	Pearson Correlation	-.408	1.000
	Sig. (2-tailed)	.000	.
	N	152	152

** Correlation is significant at the 0.01 level (2-tailed).

Figure 19:



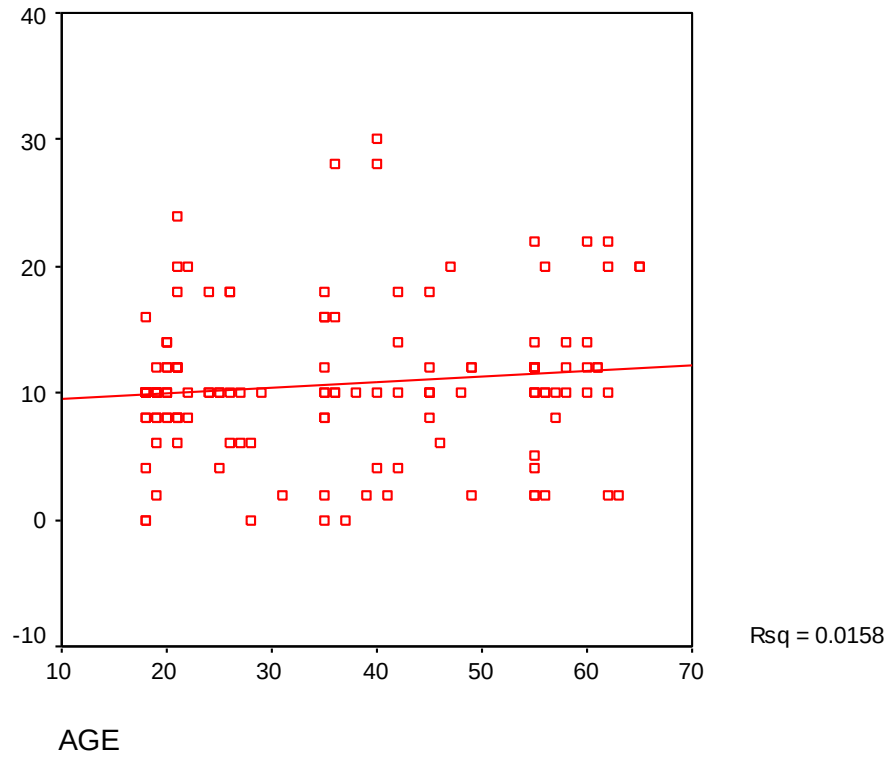
There is highly significant inverse correlation between ageing and immediate heart rate response to standing (30:15 ratio).

4 Correlation between ageing and blood pressure response to standing

Table 16: Correlations

		AGE	BPRSTD
AGE	Pearson Correlation	1.000	.126
	Sig. (2-tailed)	.	.122
	N	152	152
BPRSTD	Pearson Correlation	.126	1.000
	Sig. (2-tailed)	.122	.
	N	152	152

Figure 20:



There is no significant correlation between ageing and blood pressure response to standing.

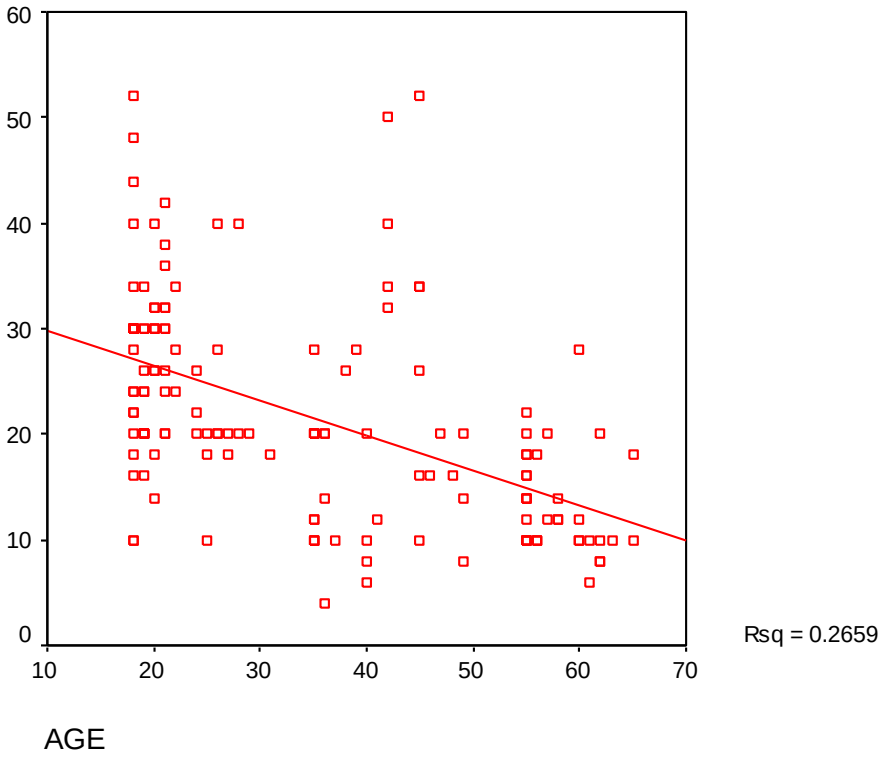
5 *Correlation between ageing & blood pressure response to sustained hand grip*

Table 17: Correlations

		AGE	BPRSHG
AGE	Pearson Correlation	1.000	-.516
	Sig. (2-tailed)	.	.000
	N	152	152
BPRSHG	Pearson Correlation	-.516	1.000
	Sig. (2-tailed)	.000	.
	N	152	152

** Correlation is significant at the 0.01 level (2-tailed).

Figure 21:



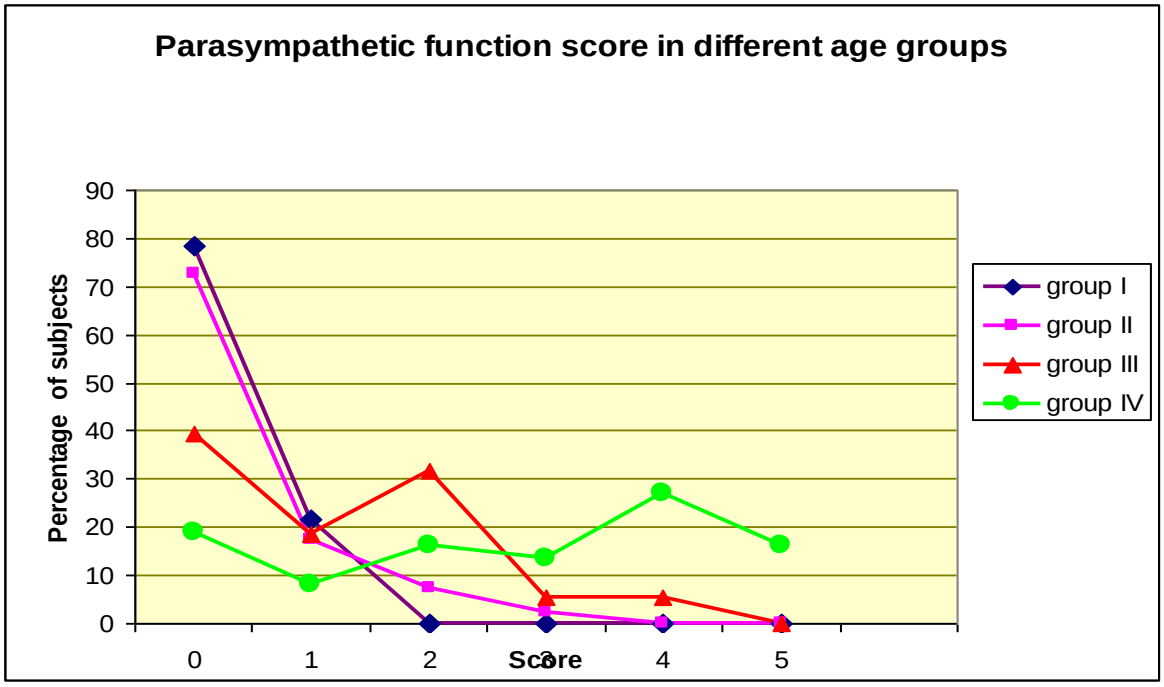
There is a highly significant inverse correlation between ageing and blood pressure response to sustained hand grip.

Table 18: Parasympathetic function score in subjects of different age groups

Parasympathetic Function Score / Groups	0	1	2	3	4	5
Group I (18-19 yrs)	**78.37**	**21.62**	**0**	**0**	**0**	**0**
Group II (20-34 yrs)	**72.5**	**17.5**	**7.5**	**2.5**	**0**	**0**
Group III (35-54 yrs)	**39.47**	**18.42**	**31.57**	**5.26**	**5.26**	**0**
Group IV (55-65 yrs)	**18.91**	**8.10**	**16.21**	**13.51**	**27.02**	**16.21**

No. of subjects expressed as the percentage of the total in each group.
Higher the score, more is the dysfunction.

Figure 22:



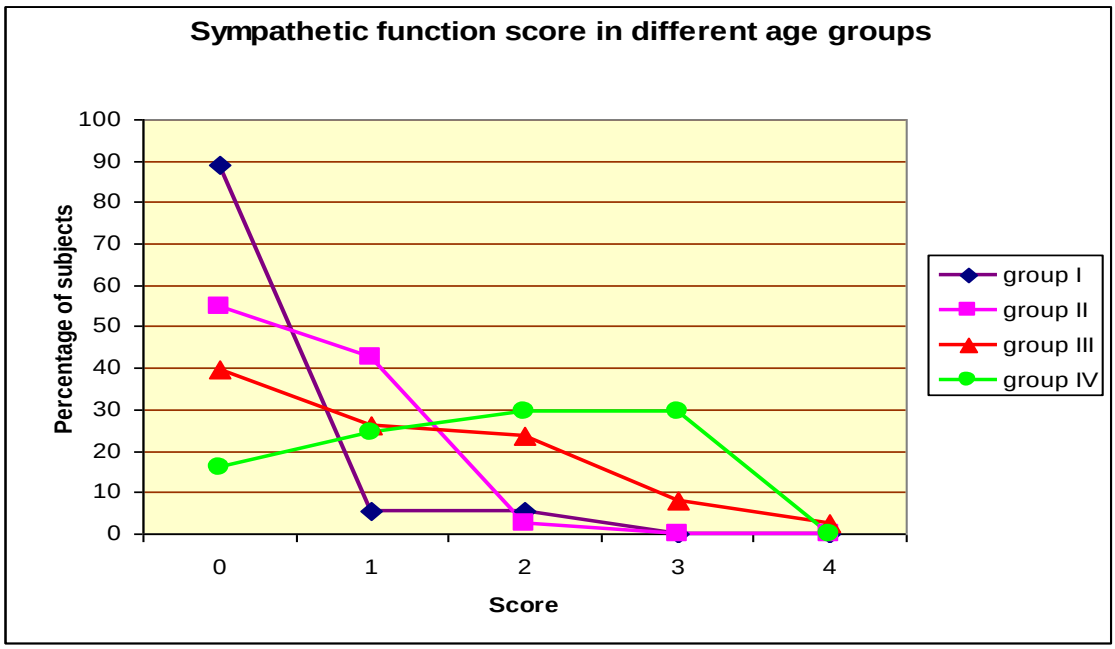
The number of subjects with normal autonomic function score decreases as age advances in different age groups with gradual increase in the number of subjects with the borderline & abnormal autonomic function score from Group I to Group IV.

Table 19: Sympathetic function score in subjects of different age groups

Sympathetic Function / *Score* / Groups	0	1	2	3	4
Group I (18-19 yrs)	**89.18**	**5.40**	**5.40**	**0**	**0**
Group II (20-34 yrs)	**55**	**42.5**	**2.5**	**0**	**0**
Group III (35-54 yrs)	**39.47**	**26.31**	**23.68**	**7.89**	**2.63**
Group IV (55-65 yrs)	**16.21**	**24.32**	**29.72**	**29.72**	**0**

No. of subjects expressed as the percentage of the total in each group.
Higher the score more is the dysfunction.

Figure 23:



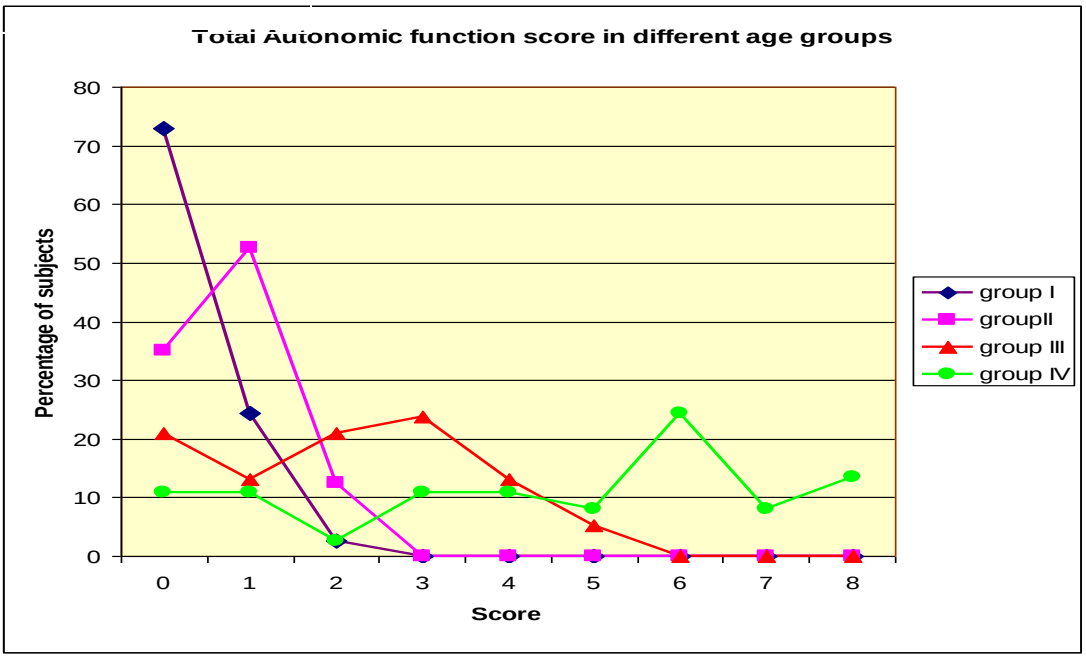
The number of subjects with normal autonomic function score decreases as age advances in different age groups with gradual increase in the number of subjects with the borderline & abnormal autonomic function score from Group I to Group IV.

Table 20: Total Autonomic function score in subjects of different age groups

Total score / Groups	0	1	2	3	4	5	6	7	8
Group I	72.92	24.32	2.70	0	0	0	0	0	0
Group II	35	52.5	12.5	0	0	0	0	0	0
Group III	21.05	13.15	21.05	23.68	13.15	5.26	0	0	0
Group IV	10.81	10.81	2.70	10.81	10.81	8.10	24.32	8.10	13.51

No. of subjects expressed as % of the total. Higher the score, more is the dysfunction

Figure 24:



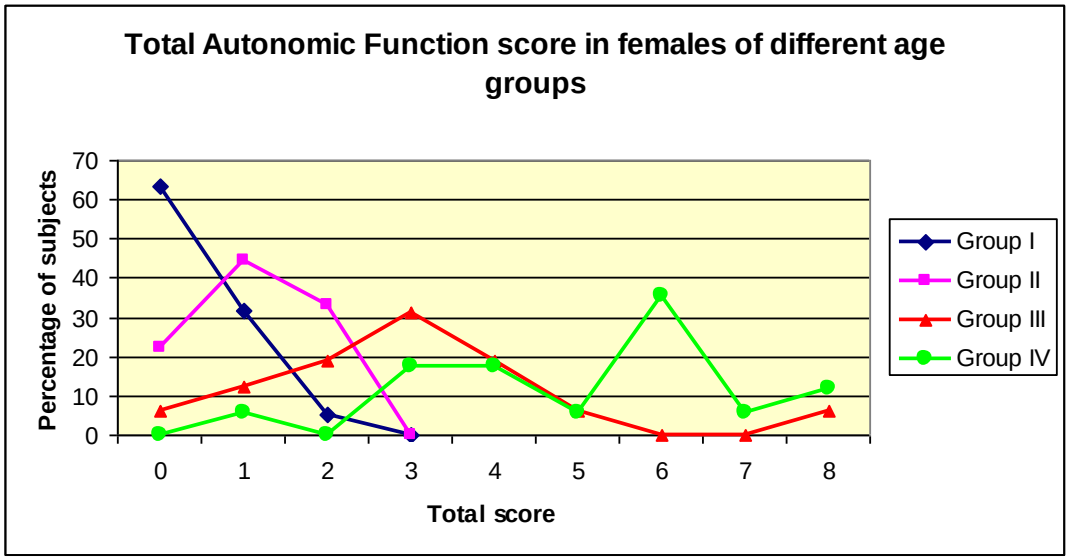
The number of subjects with normal autonomic function score decreases as age advances in different age groups with gradual increase in the number of subjects with the borderline and abnormal function score from Group I to Group IV.

Table 21: Total Autonomic function score in female subjects of different age groups.

Total score / Groups	0	1	2	3	4	5	6	7	8
Group I	63.15	31.57	5.26	0	0	0	0	0	0
Group II	22.22	44.44	33.33	0	0	0	0	0	0
Group III	6.25	12.5	18.75	31.25	18.75	6.25	0	0	6.25
Group IV	0	5.88	0	17.64	17.64	5.88	35.29	5.88	11.76

No. of subjects expressed as % of the total. Higher the score, more is the dysfunction

Figure 25:



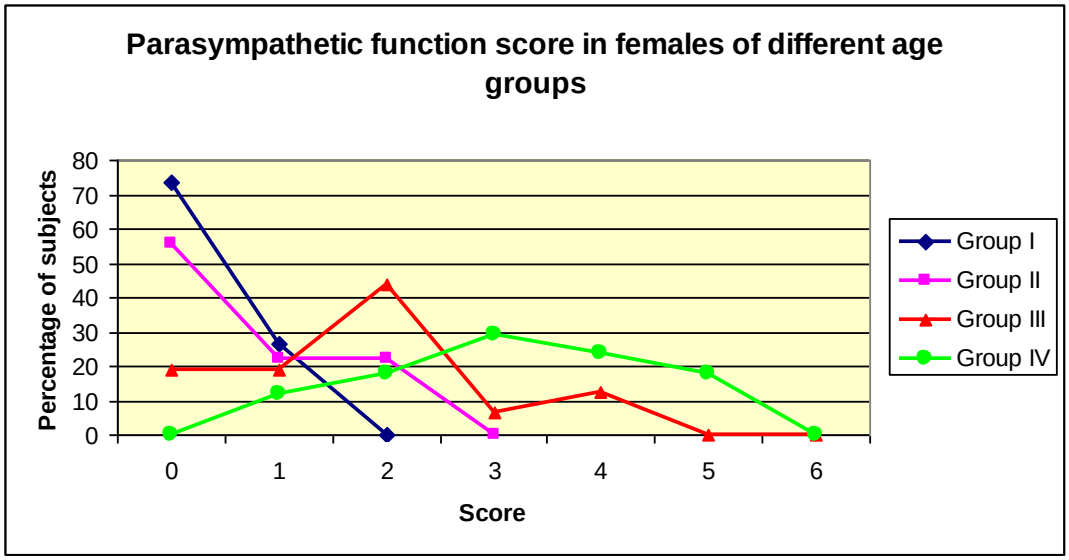
The number of subjects with normal autonomic function score decreases age advances in different age groups with gradual increase in the number of female subjects with borderline & abnormal autonomic function scores from Group I to IV.

Table 22: Parasympathetic function score in female subjects of different age groups

Parasympathetic Function Score / Groups	0	1	2	3	4	5
Group I (18-19 yrs)	**73.68**	**26.31**	**0**	**0**	**0**	**0**
Group II (20-34 yrs)	**55.55**	**22.22**	**22.22**	**0**	**0**	**0**
Group III (35-54 yrs)	**18.75**	**18.75**	**43.75**	**6.25**	**12.5**	**0**
Group IV (55-65 yrs)	**0**	**11.76**	**17.64**	**29.41**	**23.52**	**17.64**

No. of subjects are expressed as percentage of the total in each group.
Higher the score, more is the dysfunction.

Figure 26:



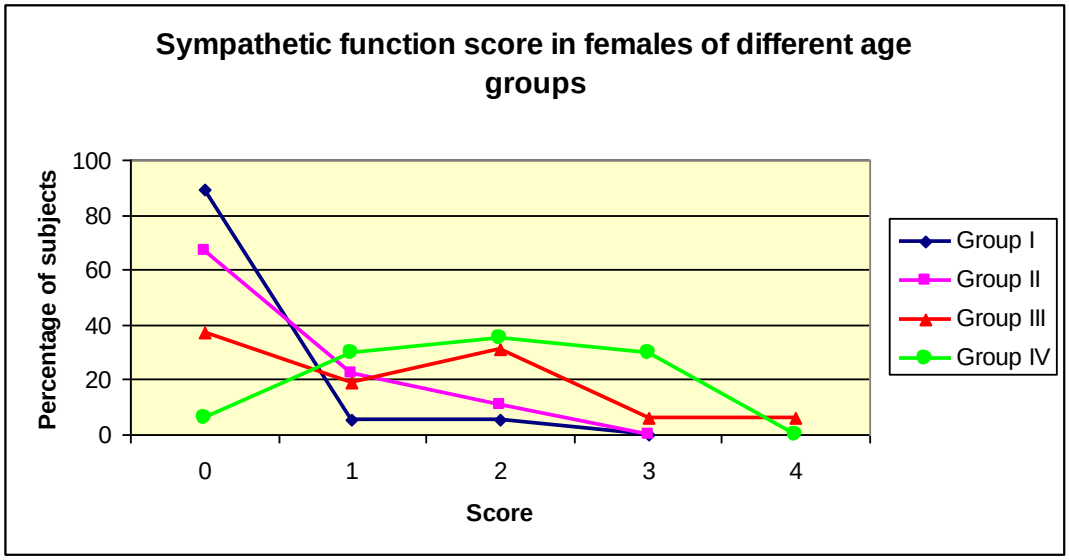
As age advances there is progressive decrease in the no. of female subjects with score zero from 73.68% in Group I, 55.55% in Group II, 18.75% in Group III, 0% in Group IV and there is a gradual increase in the number of subjects with dysfunction score from Group I to Group IV.

Table 23: Sympathetic function score in female subjects of different age groups

Sympathetic Function / *Score* / Groups	0	1	2	3	4
Group I (18-19 yrs)	**89.47**	**5.26**	**5.26**	**0**	**0**
Group II (20-34 yrs)	**66.66**	**22.22**	**11.11**	**0**	**0**
Group III (35-54 yrs)	**37.5**	**18.75**	**31.25**	**6.25**	**6.25**
Group IV (55-65 yrs)	**5.88**	**29.41**	**35.29**	**29.41**	**0**

No. of subjects are expressed as % of the total in each group.
Higher the score more is the dysfunction.

Figure 27:



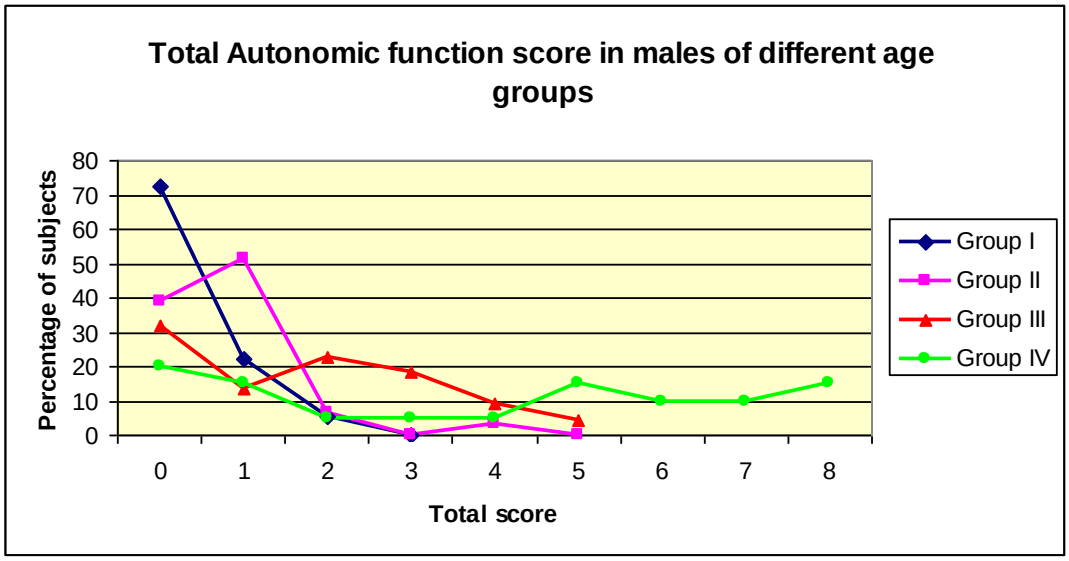
There is a progressive decrease in the no. of female subjects with score 0 as age advances from 89% in Group I, 66% in Group II, 37% in Group III and 5% in Group IVand there is gradual increase in the number of subjects with dysfunction score from Group I to Group IV.

Table 24: Total Autonomic function score in male subjects of different age groups.

Total score / Groups	0	1	2	3	4	5	6	7	8
Group I	72.22	22.22	5.55	0	0	0	0	0	0
Group II	38.70	51.61	6.45	0	3.22	0	0	0	0
Group III	31.81	13.63	22.22	18.18	9.09	4.54	0	0	0
Group IV	20	15	5	5	5	15	10	10	5

No. of subjects expressed as % of the total. Higher the score, more is the dysfunction

Figure 28:



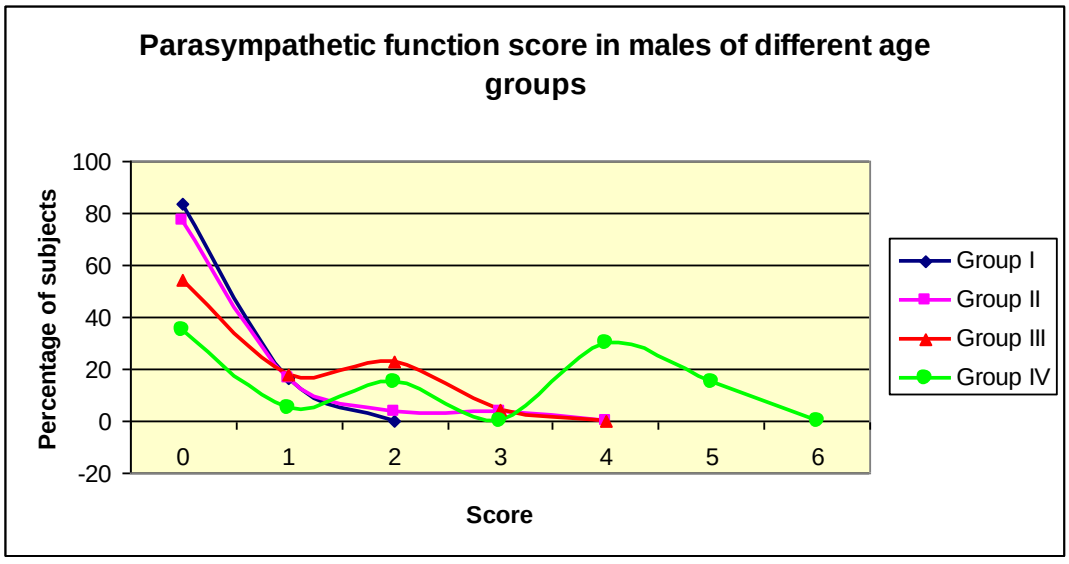
As age advances there is gradual decrease in the no. of male subjects with score zero from 72.22% in Group I, 38.70% in Group II, 31.81% in Group III, 20% in Group IV and there is a gradual increase in the number of subjects with dysfunction scores.

Table 25: Parasympathetic function score in male subjects of different age groups

Parasympathetic Function Score / Groups	0	1	2	3	4	5
Group I (18-19 yrs)	**83.33**	**16.66**	**0**	**0**	**0**	**0**
Group II (20-34 yrs)	**77.41**	**16.12**	**3.22**	**3.22**	**0**	**0**
Group III (35-54 yrs)	**54.54**	**18.18**	**22.72**	**4.54**	**0**	**0**
Group IV (55-65 yrs)	**35**	**5**	**15**	**0**	**30**	**15**

No. of subjects are expressed as % of the total in each group.
Higher the score, more is the dysfunction.

Figure 29:



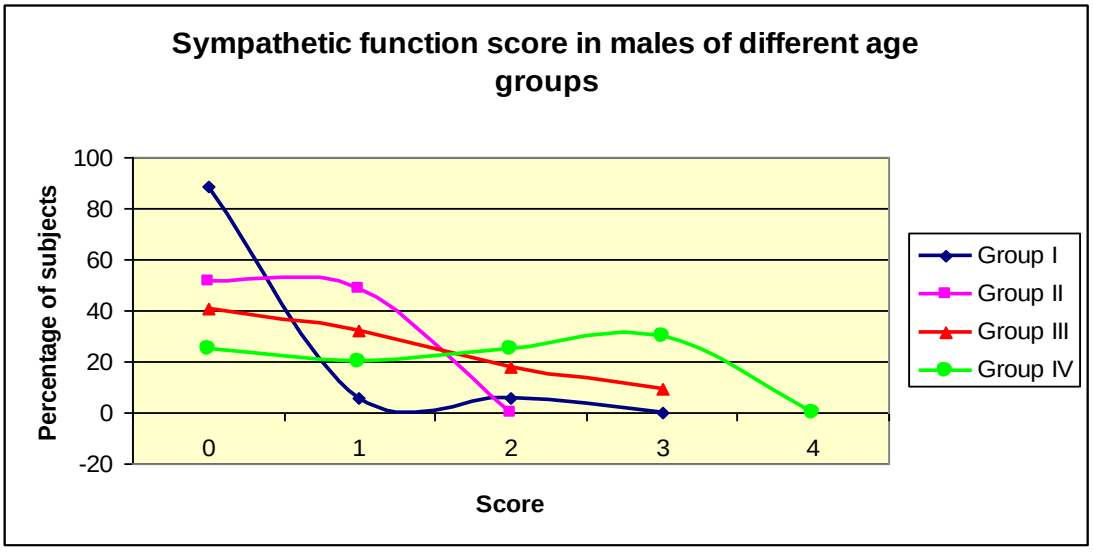
As age advances there is a gradual decrease in the no. of male subjects with score zero from 83.33% in Group I, 77.41% in Group II, 54.54% in Group III, 35% in Group IV and there is a gradual increase in the number of subjects with dysfunction scores.

Table 26: Sympathetic function score in male subjects of different age Groups

Sympathetic Function Score / Groups	0	1	2	3	4
Group I (18-19 yrs)	**88.88**	**5.55**	**5.55**	**0**	**0**
Group II (20-34 yrs)	**51.61**	**48.38**	**0**	**0**	**0**
Group III (35-54 yrs)	**40.90**	**31.81**	**18.18**	**9.09**	**0**
Group IV (55-65 yrs)	**25**	**20**	**25**	**30**	**0**

No. of subjects are expressed as % of the total in each group.
Higher the score, more is the dysfunction.

Figure 30:



As age advances there is gradual decrease in the no. of male subjects with score zero from 88.88% in Group I, 51.61% in Group II, 40.90% in Group III, 25% in Group IV & there is a gradual increase in the number of subjects with dysfunction scores.

DISCUSSION

The cross-sectional study was carried in 152 healthy subjects in the age range of 18-65 years of BLDEA's Shri B M Patil Medical College, Bijapur. The subjects were distributed in four age groups as shown in Table no 1. Evaluation of status of autonomic nervous system was done with the help of five non-invasive tests. Parasympathetic function was assessed by heart rate response to Valsalva maneuver, heart rate response to deep breathing, immediate heart rate response to standing. The sympathetic function was assessed by blood pressure response to standing & blood pressure response to sustained hand grip.

In our study we have recorded various physical & physiological parameters in all four age groups.

Physical Parameters

In our study we found a very highly significant (p=0.000) decrease in the height of subjects from Group I to Group IV except in Group II. (Table no. 3)

There is no significant (p=0.372) variation in the weight of the subjects from Group I to Group IV. (Table no. 3)

We found a significant (p=0.02) increase in the BMI of subjects from Group I to Group IV except in Group II. (Table no. 3)

There is insignificant (p=0.107) decrease in the BSA of subjects from Group I to Group IV. (Table no. 3)

Body composition changes with ageing and longitudinal decrease in height, body weight (BW), and body cell mass at older ages has been described. Such changes may be universal, but their expression and incidence may vary considerably within and between

groups of elderly adults. The smaller body size in older people is partly due to actual shrinkage over the life span (ageing) and partly due to earlier generations being physically smaller than recent ones (secular trend) or due to selective survival[71].

Variations in adult height reflected number of conditions in childhood, including economic status, psychosocial factors education, and upward social mobility. Thus, variation in height is related to the individual's lifetime exposure to certain genetic, environmental, and social factors. On the other hand, variations in body weight are more related to an individual's current health status, physical activity, smoking and dietary habits, and other factors[71].

Physiological Parameters

We found a gradual very highly significant (p=0.000) decrease in the mean resting pulse rate of subjects as age advances from Group I to Group IV. (Table no. 4)

The resting heart rate is modulated by both branches of the cardiac autonomic nervous system, with a predominance of parasympathetic influence. The vagal activity on sinus node estimated by multiple heart rate variability (HRV) indices is decreased with age. On the other hand, the literature reports that mean HR at rest does not increase with advancing age presumably due to the decrease in the intrinsic heart rate & the increase in the sympathovagal balance[56]. However in our study we did not use parasympathetic & sympathetic pharmacological blockade & consequently cannot make any statement about the intrinsic heart rate.

There was insignificant (p=0.575) variation in the resting respiratory rate of subjects in different age groups. (Table no. 4)

As age advances there is a significant (p=0.001) increase in the resting systolic blood pressure of subjects from Group II to Group IV. (Table no. 4)

There is a gradual significant (p=0.007) variation in the mean resting diastolic blood pressure of subjects in different age groups. (Table no.4)

A. PARASYMPATHETIC FUNCTION TESTS:

I. Heart rate response to Valsalva maneuver

A normal response to Valsalva maneuver is characterized by a decrease in the pulse pressure & tachycardia during strain & blood pressure overshoot & bradycardia following the strain[17]. The Valsalva maneuver tests the integrity of both parasympathetic & sympathetic divisions of autonomic nervous system. The hemodynamic changes during the maneuver are mediated via baroreceptors. With parasympathetic affection, the baroreceptor mediated reflex bradycardia response to elevated blood pressure will be reduced.

Variation due to age:

In the present study the mean value of Valsalva ratio (Table no.6) showed a significant (p=0.04) gradual decrease from Group I to Group IV still remaining in the normal range (> 1.21, according to Ewing and Clarke grading) [20].

Our findings are in accordance with earlier studies done by Ziegler D et al[37], B Gautschy & coworkers[40], Iain A D O'Brien & coworkers[36], Low P A & coworkers[48, 53], Sega S & coworkers[50], Piha S J[19].

Our results are not in agreement with Kaijser L & coworkers[41], Vita G et al[42]. They found that Valsalva ratio appeared to be independent of age.

Storm D S & coworkers[72] stated that when present, age related differences in cardiovascular responses to Valsalva maneuver were manifest by age 45. They also suggested interventions to reduce the frequency & intensity of straining which is especially important in older adults to minimize health risks associated with rapid & abrupt changes in BP.

Variation due to Gender and Age (Table no.8)

There is no variation in the mean valsalva ratio in males of different age groups. However in females there is a highly significant variation in mean Valsalva ratio from Group I to Group IV which has reduced to border line dysfunction i.e autonomic function score 1 in females of Group IV (55-65 years).

Variation due to gender in the same age groups (Table no. 8)

We observed no significant difference between males & females in Group I (18-19 years), Group II (20-34 years). Significantly lower values in females as compared to males in Group III (35-54 years) & Group IV (55-65 years).

Our study is in accordance with Low P A[48, 53] et al who stated that Valsalva ratio varied with both age & gender.

Our study is not in accordance with Zeigler D et al[37], B Gautschy & coworkers[40], Iain A D O'Brien[36], Chu T S & coworkers[55] who did not find any significant dependence on gender. Our study is not in accordance with Piha S J[63] who found HR response to VM was greater in females over 50 than in males in the same age range.

II. Heart rate response to deep breathing:

Heart rate response to deep breathing (sinus arrhythmia) is a normal phenomenon & is primarily due to fluctuation of parasympathetic output to the heart.

Variation due to age:

The difference between maximum & minimum heart rate during deep breathing (Table no. 6) showed a gradual a very highly significant (p=0.000) decrease from Group I to Group IV.

Up to Group III (35-54 years) it is in the normal range. Whereas, in Group IV (55-65 years) it has fallen to autonomic function score 1 or in other words the test has shown border line dysfunction (according to Ewing and Clarke grading)[20]

Our study is in accordance with Zeigler D et al[37], Vita G et al[42], Braune S, Auer A, Schulte-Monting J, Schwerbrock S, Lucking CH[43], Ingall T J & coworkers[51], Shirley A Smith[58] , W Weiling & coworkers[57]Low P A & coworkers[48, 53] who found that the heart rate response to deep breathing fell with increasing age.

W Weiling & coworkers[57]in their study found HRV during deep breathing decreased from 22 to11bpm. In our study also we found HRV during deep breathing decreased from to 24 to 10 beats/min.

Variation due to Gender and Age (Table no. 9)

When the heart rate response to deep breathing was compared between males of different age groups there was gradual very highly significant (p=7E-07) reduction in the response with advancing age (22.96 bpm to 12.78 bpm), with Group IV subjects the value showing borderline abnormality i.e. function score 1 (according to Ewing and Clarke)[20]. In females also we observed a similar pattern (25.43 to7.62 bpm) but the value

of heart rate response to deep breathing has reduced to borderline[20] dysfunction value i.e. score 1 (13.55 bpm) in Group III (35-54 yrs) only & definitely to abnormal[20] value i.e. score 2 (7.62 bpm) in Group IV. Therefore, in females the heart rate response to deep breathing which is a sensitive indicator of parasympathetic function has become abnormal almost a decade earlier i.e. 35-55 years when compared to males.

Variation due to gender in the same age groups (Table no. 9)

To study for any gender differences we compared the heart rate response to deep breathing in males & females of the same group. We observed no significant difference between males & females in Group I (18-19 years), Group II (20-34 years). However the values were significantly lower in females as compared to males in Group III (35-54 years) & Group IV (55-65 years).

Our results are in accordance with B Gautschy & coworkers[40], Iain A D O'Brien & coworkers[36], Braune S & coworkers[43], Gelber D A[44], Chu T S & coworkers[55]who found that gender does has an influence on heart rate response to deep breathing.

Our study is not in accordance with C Neumann & H Schmid[47], Piha S J[63] who found that the heart rate response in men & women did not differ.

Shirley A Smith[58]in his study concluded that measurement of this age dependent ratio which may be made with an electrocardiographic apparatus provides a simple accurate diagnostic screen for autonomic neuropathy in clinic.

III. Heart rate response to Standing (30: 15 ratio):

Heart rate response to standing in normal subjects consists of tachycardia maximum around 15th beat followed by relative bradycardia around 30th beat after standing[22]. These hemodynamic responses are mediated by baroreceptors.

Variation due to age:

In our study we found a gradual very highly significant (p=0.000) decrease in the 30:15 ratio (Table no. 6) from Group I to Group IV. Although the 30:15 ratio is lower in Group IV it is still in the normal range (>1.04, according to Ewing and Clarke) [20].

Our study is in accordance with Zeigler D et al[37], B Gautshy & coworkers[40], Iain A D O'Brien[36], Vita G et al[42], Ingall T J & coworkers[51], W Weiling[57].

G Cybulski & W Niewiadomski [59]observed a tendency toward attenuation of the HR response to standing with age. They linked it to the decreasing sensitivity of baroreceptors. These changes may also be caused by reduction in the number & activity of cholinergic & adrenergic receptors in the heart & stiffening of the arterial walls.

Piccirillo et al[73] concluded that cardiac baroreceptor sensitivity in normotensive individuals declines with age. It falls predominantly in middle age (from approx 48 years onwards) & remains substantially unchanged thereafter.

Variation due to Gender and Age (Table 10)

We also found a significant decrease in the 30:15 ratio in males & females of different age groups.

Variation due to gender in the same age groups (Table no. 10)

However when males & females of same age group were compared there is no significant difference found.

CORRELATION BETWEEN AGEING AND PARASYMPATHETIC FUNCTION TESTS

In this study, correlation (by Pearson correlation) of different parasympathetic nerve function parameters with age was analyzed. Here Valsalva ratio (r = -0.292), Heart rate response to deep breathing (r = -0.635) and 30:15 ratio(r = -0.408) were negatively correlated with age and all these relationships were statistically significant (p=0.01)

Our results are in accordance with Islam T & coworkers[1], Chu TS & his coworkers[55], Iain A D O'Brien & coworkers[36] who also found that age correlated negatively with heart rate response to standing, deep breathing & Valsalva ratio.

Therefore, it can be concluded that ageing process substantially impaired Cardiovascular Parasympathetic nerve function.

Heart rate response to deep breathing is very sensitive & detects the parasympathetic dysfunction at the earliest.

The results of the present study showed that impairment of parasympathetic nerve functions occurred in apparently healthy elderly subjects. Different investigators suggested that the vagal tone is reduced or loss of vagal tone occurs gradually as age advances[1]. Again, vagal damage causes reduction of heart rate to various stimuli[74].

Cardiovagal autonomic function was reported to increase during early ontogeny, to reach a peak value at adolescence, and then to decline with advancing age[75].

The baroreflex becomes functional late in gestation & its sensitivity then increases gradually, but in neonate, it is still much below the level that has been reported in young adults[76]. It has been established by a number of investigators that cardiovagal baroreflex sensitivity starts to decline from 20s & is reduced to almost zero in the 70s & 80s[77, 78].

Cardiovagal autonomic function declines with age during the adult years, which is partly because of gradual impairment of baroreflex function. Stiffening of the large elastic arteries with age has been demonstrated in a number of studies, & it is generally agreed that stiffening of the barosensory vessel wall accounts to a large extent for the age related decline in the autonomic sensitivity. Earlier reports have indicated that the carotid artery starts to stiffen already at younger ages[76].

Mechanisms underlying the reduced cardiac vagal control with ageing could occur at several levels. In humans, M_2 muscarinic receptor density is reduced with ageing & there is decreased receptor function[79]. Muscarinic receptor activity is probably reduced with ageing[80] and impaired cardiac acetylcholine release occurs in response to stimulation[81]. Whether human acetyl cholinesterase levels change with ageing is not clear[82].

But other view is that cholinergic receptor activity does not appear to be inhibited with age. Other possibilities include alterations in efferent (vagal nerve traffic), afferent (sensory) input, and/or integration of information at the cardio respiratory centre in brain[83].

B. SYMPATHETIC FUNCTION TESTS

I. Blood pressure response to standing

With change of posture from supine to standing the autonomic nervous system acts to produce a rise in heart rate & vasoconstriction in order to maintain blood pressure[84]. Vasoconstriction is mediated through sympathetic innervations to blood vessels during standing.

Variation due to age (Table no. 7)

In our study there was an insignificant (p=0.194) increase in the Systolic blood pressure on standing from Group I to Group IV. However in Group II, III, IV subjects the value showed borderline abnormality i.e. function score 1 (according to Ewing and Clarke) [20].

Variation due to Gender and Age (Table no.11)

We also compared blood pressure response to standing in males & females of different age groups. There was no significant variation found. Although there is no significant variation, as age advances the subjects have shown borderline dysfunction i.e. function score 1 beginning from Group II onwards.

Variation due to gender in the same age groups (Table no. 11)

There is no significant difference between males & females of same age group.

There is no significant (r=0.126) correlation between ageing & blood pressure response to standing.

Our study is in accordance with C Neumann& H Schmid[47] who observed no correlation between ageing & blood pressure response to standing.

Our results are not in accordance with Chu T S & coworkers[55] who observed that age correlated positively with a fall in systolic blood pressure in women not in men.

II. Blood pressure response to sustained hand grip

D.J Ewing et al (1973) first showed that during sustained hand grip, there was a sharp rise in diastolic blood pressure (DBP) due to increase in peripheral vascular resistance[23].

Variation due to age (Table no. 7)

We observed a very highly significant (p=0.000) gradual decrease in the response from Group I to IV. Although the rise in DBP is lower in Group III, it is still in the normal range. But in Group IV, the values have reduced to borderline dysfunction levels i.e. score 1 (according to Ewing and Clarke grading).

Kaijser & Sachs (1985)[41] observed a decreased blood pressure response to sustained hand grip test in older subjects, above 60 years. They attributed this decline to reduced effector organ sensitivity.

Sachs et al (1985)[85] studied the sustained hand grip response in 10 healthy old subjects (mean age 71 years) and 10 healthy younger subjects (mean age 26 years). They observed a lowered blood pressure response in older subjects than in younger subjects on sustained hand grip exercise. They attributed this to reduced capacity of older subjects to release norepinephrine upon exercise provocation.

Goldstraw P W, Warren D J (1985)[86] studied the effect of age on the cardiovascular responses to isometric exercise. They concluded that the increasing age does not affect the cardiovascular responses to isometric exercise.

Variation due to Gender and Age (Table no12)

In our study when mean increase in DBP in response to sustained hand grip was compared between males & females in different age groups there was a significant decrease observed. The values have fallen to borderline[20] dysfunction levels i.e. score 1 in Group IV (55-65yrs) in both males & females.

Variation due to gender in the same age groups (Table no. 12)

We did not find any significant difference between males and females of the same age group.

Our findings are in accordance with Braune S & his co-workers[43] who found no sex differences in the blood pressure response to isometric exercise.

Our results are not in accordance with Chu T S & coworkers[55]. They found a low rise in diastolic blood pressure during isometric exercise in women than in men.

In our study we found a significant ($r = -0.516$, $p=0.01$) inverse correlation between ageing & blood pressure response to sustained hand grip.

To date age related changes in sympathetic control of heart have been attributed primarily to down regulation of beta-adrenergic receptors & an alteration in the post-receptor activity, namely a lower G-protein activity[83].

AUTONOMIC FUNCTION SCORE IN SUBJECTS OF DIFFERENT AGE GROUPS

More is the score, more is the dysfunction.

Criteria for grading autonomic function as whole[69]

Scores ≤ 3 Normal autonomic function

> 3 & < 8 Borderline dysfunction

≥ 8 to 10 abnormal function

For grading of individual cardiovascular autonomic function, results were classified into normal, borderline, and abnormal according to Ewing & Clarke's classification[20].

	Normal 0	Borderline 1	Abnormal 2
Tests reflecting Parasympathetic function			
1. Heart rate response to Valsalva maneuver (Valsalva ratio)	>1.21	1.11-1.20	<1.10
2. Heart rate variation (R-R interval) during deep breathing (maximum-minimum heart rate)	>15 bpm	11-14 bpm	<10 bpm
3. Immediate heart rate response to standing (30:15 ratio)	>1.04	1.01-1.03	<1.00
Tests reflecting Sympathetic function			
1. Blood pressure response to standing (fall in systolic blood pressure)	<10 mm Hg	11-29 mm Hg	>30 mm Hg
2.Blood pressure response to sustained hand grip (increase in diastolic blood pressure)	>16 mm Hg	11-15 mm Hg	<10 mm Hg

An overall score $\leq$3 was considered to indicate normal autonomic function. Scores >3 and $\leq$ 8 were considered borderline and scores $\geq$ 8 were judged abnormal[69].

Parasympathetic, Sympathetic and total autonomic function score (Table no.18-20) shows a gradual increase in the number of subjects with borderline and abnormal autonomic function score from Group I to Group IV indicating autonomic dysfunction increases with age.

Parasympathetic, Sympathetic and total autonomic function scores (Table no.21-26) show a gradual increase in the number of male & female subjects with borderline and abnormal autonomic score from Group I to Group IV indicating autonomic dysfunction increases with age.

More parasympathetic & sympathetic dysfunction is observed in females when compared to males of same age group except in Group II, where males show more sympathetic dysfunction.

Our study is not in accordance with Turner MJ & coworkers who have shown a greater age-dependent decline in function in males than females[87].

Gender differences in the ANS may be present because of developmental differences or due to effects of prevailing levels of male and / or female sex hormones. Such prevailing hormone levels may also produce differences between pre- and post-menopausal women and amongst pre-menopausal women at different phases of menstrual cycle, which is characterized by estrogen secretion in the late follicular (pre-ovulatory) phase followed by a secondary phase of secretion in the luteal (post-ovulatory) phase. Progesterone secretion occurs during luteal phase. Differences in the autonomic system may be due to differences in afferent receptor stimulation, in the central reflex

transmission, in the efferent nervous system and in the post synaptic signaling. At each of these potential sites of difference, there may be effects due to different size or number of neurons, variations in receptors, differences in neurotransmitter content or metabolism as well as functional differences in the various components of the reflex arc[88].

Though the grading & function scores are accepted no reference in the literature is available following these criteria. So we have tried to present the autonomic function in the form of score which may help to grade the subjects easily rather than expressing the results as pure values of different tests.

CONCLUSION

We conducted a cross-sectional study to evaluate the effect of age on cardiovascular autonomic function tests in healthy subjects (18-65 years) of BLDEA'S Shri B M Patil Medical College, Bijapur. We performed the Parasympathetic Function tests (Valsalva maneuver, Heart rate variation during deep breathing, and Heart rate response to standing) & Sympathetic function tests (Blood pressure response to standing & Blood pressure response to sustained hand grip). We conclude from our study that

1) Autonomic function tests showed gradual decrease in function as age advances in both sexes (18-65 years).

2) Heart rate variation during deep breathing (r= -0.635) & Blood pressure response to Sustained Hand Grip (r= -0. 516) appear to be more sensitive parameters to detect age related autonomic dysfunction amongst the three Parasympathetic function tests & the two Sympathetic function tests respectively

3) There is a gradual increase in the abnormal autonomic function score of both sexes as age advances in different age groups.

4) Parasympathetic & Sympathetic function tests showed more decline in females as compared to males of the same age group.

5) Though the grading & function scores are accepted no reference in the literature is available following these criteria. So we have tried to present the autonomic function in the form of score which may help to grade the subjects easily rather than expressing the results as pure values of different tests.

To establish the relation of sex to autonomic function further studies may be undertaken by hormonal assay and study at different phases of reproductive life like pre-ovulatory, post-ovulatory, premenopausal & postmenopausal females with more number of subjects.

SUMMARY

The cross-sectional study was carried out in 152 apparently healthy subjects in age range of 18-65 years of BLDEA's Shri B M Patil Medical College to assess the effect of age on autonomic nervous system. The subjects were divided into four groups according to age (Group I 18-19 years, Group II 20-34 years, Group III 35-54 years, Group IV 55-65 years). Anthropometrical parameters like height & weight, BSA, BMI, Physiological parameters like resting pulse rate, resting respiratory rate, resting Blood pressure were recorded.

Evaluation of status of autonomic nervous system was done with the help of five non-invasive tests. Parasympathetic activity was assessed by Heart rate response to deep breathing, Heart rate response to Valsalva maneuver and Heart rate response to orthostatic test. Sympathetic function was assessed by Blood pressure response to orthostatic test and Blood pressure response to sustained hand grip. Using SPSS version 9 statistical tests were carried out. P value of 0.05 was considered as significant.

It was observed that

1) Parasympathetic function tests showed gradual decrease in function as age advances with more decrease in the function in females.
2) Heart rate variation during deep breathing (r= -0.635) appears to be more sensitive parameter amongst the three parasympathetic function tests we have studied.
3) All sympathetic tests showed gradual decrease in the function as age advances with more decrease in function in females except in Group II (20-34 yrs)

4) Blood pressure response to sustained handgrip (r= -0. 516) appears to be more sensitive parameter amongst the two sympathetic function tests we have studied.
5) Total autonomic function score (both parasympathetic & sympathetic) showed gradual increase in the abnormal score of both sexes as age advances in different age groups.
6) Though the grading & function scores are accepted no reference in the literature is available following these criteria. So we have tried to present the autonomic function in the form of score which may help to grade the subjects easily rather than expressing the results as pure values of different tests.

We have tried to present different cardiovascular autonomic function tests both as absolute values & also as function scores. It appears that presenting autonomic function as function score can be easily interpreted. Autonomic function expressed as grading as per function score may be more useful for clinical purposes.

BIBLIOGRAPHY

1. Islam T, Begum N, Ferdousi S, Ali T. Evaluation of Parasympathetic Nerve Function Status in Healthy Elderly Subjects. J Bangladesh Soc Physiol 2008; 3: 23-28.
2. Park K. Park's Textbook of Preventive and Social Medicine. 18th Edition. M/S Banarasidas Bhanot Publishers; Nov 2005. 434-435
3. http://www.who.int/topics/ageing/en.
4. Khurana Indu. Text Book Medical Physiology. 1st edition. Elsevier Publishers; 2006. 1280.
5. Christine K Cassel, Harvey J Cohen, Eric B Larson, Diane E Meier, Neil M Resnick, Laurence Z Rubenstein et al. Geriatric Medicine. 3rd edition. Springer-Verlag Publishers; 1997. 3.
6. Kuchel GA, Hof PR (eds). Autonomic Nervous System in Old Age. Interdiscipl Top Gerontol. Basel, Karger. 2004. 33, 32-44.
7. Schimazu T, Tamura N, Schimazu K. Ageing of the autonomic nervous system. Nippon Rinshaw 2005 Jun; 63(6): 973-7.
8. Laitinen Tomi, Niskanen Leo, Geelen Ghislaine, Lansimies Esko, Hartikainen Juha. Age dependency of cardiovascular autonomic responses to head-up tilt in healthy subjects. J Appl Physiol 2004 Jun; 96(6): 2333-40.
9. Akihiro Igata. Ageing and Autonomic Nervous system. Jap J Med 1986 Feb; 25 (1): 96.
10. Bijlani RL. Understanding Medical Physiology. 3rd edition. Jaypee Brothers Medical Publishers; 2004. 44-46, 819.
11. Allen S C. Medicine in Old Age. 4th edition. Churchill Livingstone Publishers; 1998.

12. Christopher G Goetz, Eric J Pappert. Textbook of Clinical Neurology. 1st edition. W B Saunders Company; 1999. 350-351, 355-356.
13. Guyton & Hall. Textbook of Medical Physiology. 11th edition. Elsevier Publishers; 2006. 748.
14. Barrett E Kim, Barman M Susan, Boitano Scott, Brooks L Heddwen. Ganong's Review of Medical Physiology. 23rd edition. McGraw hill companies; 2009. 262-65.
15. J C Brocklehurst, R C Tallis, H M Fillit. TB of Geriatric Medicine & Gerontology. 4th edition. Churchill Livingstone.
16. Bradley G Walter, Daroff B Robert, Ferrichel M Gerald, Jankovic Joseph. Neurology in Clinical Practice. 5th edition. Butlerworth Heinemann Elsevier Publishers; 2008. 2360.
17. Elisberg EI. Heart rate response to Valsalva maneuver as a test of circulating integrity. JAMA 1963; 183: 120.
18. Carol J M Porth, Virinderjit S Bamrah, Felix E, Tristani, James J Smith. The Valsalva maneuver: Mechanisms and clinical implications. Heart and Kung 1984; 13(5): 507-518.
19. Piha SJ. Autonomic responses to valsalva maneuver in healthy subjects. Clin Physiol 1995; 15(4): 339-47.
20. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. Br Med J 1982 Oct; 285: 916-918.
21. Dixit MB. Comparative cardiovascular responses to 70^{o} Head up tilt in pilots and non-pilots. Aviat Med 1983; 27: 38-42.

22. Ewing DJ, Campbell IW, Murray A, Neilson JM, Clarke BF. Immediate heart rate response to standing: Simple test for autonomic neuropathy in diabetes. Br Med J 1978 Jan 21; 1(6106): 145-7.
23. Ewing DJ, Irving JB, Kerr F, Wildsmith JAW, Clarke BF. Cardiovascular responses to sustained handgrip in normal subjects and in patients with diabetes mellitus: a test of autonomic function. Cli Sci Mol Med 1974; 46: 295-306.
24. Ewing DJ, Campbell IW, Burt AA, Clarke BF. Vascular reflexes in autonomic neuropathy. The Lancet 1973 Dec; 15: 1354-1356.
25. Bhatia SG, Dainani GS, Nayak NJ, Diwate PG. Valsalva maneuver as a test of autonomic neuropathy in diabetes mellitus. J Association Phys India 1976 Feb; 24: 89-93.
26. Editorial. Valsalva maneuver in diabetic neuropathy. J American Med Assoc. 1974 May; 228:89-93
27. Malik Marek. Heart rate variability. Standards of measurement, Physiological Interpretation and Clinical Use. Circulation 1996; 93: 1043-1065.
28. Das RK, Mahapatra K, Guha S, Padhey PK. Cardiac autonomic neuropathy in diabetes. J Assoc Phys India 1982; 30(10):740.
29. Dixit MB. Postural stress tests for the clinic-Physiological evaluation of cardiovascular reflexes. Indian J Pharmc Phys 1987; 31(1):76.
30. Rushmer RF. Cardiovascular Dynamics. 4th edition. WB Saunders.
31. Abbot FM, Halsted DD, Mark AL, Schmid PG. Reflex control of the peripheral circulation. Prog Cardiovascular Dis 1976; 18:371-403.

32. Roger Bannister. Testing autonomic reflexes in autonomic failure. Oxford Medical Publications. Oxford 1983; 52-63.
33. Sjostrend T. The regulation of Blood distribution in man. Acta Physiol Scand 1952; 26:312-327.
34. Ewing DJ, Hume L, Campbell IW, Murray A, Neilson JM, Clarke BF. Autonomic mechanisms in the control of initial heart rate response to standing. J Appl Physiol; 49:808-814.
35. Dwain L, Eckberg & B Gunnar Wallin. Isometric exercise modifies autonomic baroreflex responses in humans. J Appl Physiol 1987; 63(6): 2325-2330.
36. O'Brien IAD, O'Hare P, Corral RJM. Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. Br Heart J 1986; 55:348-54.
37. Zeigler D, Laux G, Dannehl K, Spuler M, Muhlen H, Mayer P, Gries FA. Assessment of cardiovascular autonomic function: age-related normal ranges and reproducibility of spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses. Diabet Med 1992; 9(2): 166-175
38. Singh D, Vinod K, Saxena SC, Deepak KK. Spectral evaluation of ageing effects on blood pressure and heart rate variations in healthy subjects. J Med Eng Technol 2006 May-Jun; 30(3):145-50.
39. Torsvik Malvin, Haegblom Amanda, Eide Egil Geir, Schmutzhard Erich, Vetvik Kaare, Winkler Sylvia Andrea. Cardiovascular autonomic function tests in an African population. BMC Endocrine Disorders 2008; 8: 19.
40. Gautschy B, Weidmann P, Gnadinger MP. Autonomic function tests as related to

age and gender in normal man. Journal of Medicine 1986; June 64(11): 499-505.

41. Kaijser L, Sachs C. Autonomic cardiovascular responses in old age. Clin Physiol 1985; Aug 5(4):347-57.
42. Vita G, Princi P, Calabro R, Toscano A, Manna L, Messina C. Cardiovascular reflex tests. Assessment of age-adjusted normal range. J Neurol Sci 1986; 75(3): 263-74.
43. Braune S, Auer A, Schulte-Monting J, Schwerbrock S, Lucking CH. Cardiovascular parameters: sensitivity to detect autonomic dysfunction and influence of age and sex in normal subjects. Clin Auton Res 1996; 6(1): 3-15.
44. Gelber DA, Pfeifer M, Dawson B, Schumer M. Cardiovascular autonomic nervous system tests: determination of normative values and effect of confounding variables. J Auton Nerv Syst 1997 Jan; 62(1-2): 40-4.
45. Stout NR, Kenny RA. Reference Ranges for Clinical Autonomic Function Testing In the Elderly. Age and Ageing 1999;
46. Yeragani VK, Pohl R, Berger R, Balon R, Srinivasnan K. Relationship between age and heart rate variability in supine and standing postures: a study of spectral analysis of heart rate. Pediatr Cardiol 1994 Jan-Feb; 15(1): 14-20.
47. Neumann C, Schmid H. Standardization of a computerized method for calculating autonomic function test responses in healthy subjects and patients with diabetes mellitus. Brazilian Journal of Medical and Biological Research 1997; 30: 197-205.
48. Low PA, Denq JC, Opfer – Gehrking TL, Dyck PJ, O,Brien PC, Slezak JM. Effect of age and gender on Sudomotor and cardiovagal function and blood pressure response to tilt in normal subjects. Muscle Nerve 1997; 20: 1561-1568.

49. Piha SJ. Cardiovascular autonomic reflex tests: normal responses and age related reference values. Clin Physiol 1991; 11(3): 277-90.

50. Sega S, Jager F, Kiauta T. A comparison of cardiovascular reflex tests and spectral analysis of heart rate variability in healthy subjects. Clin Auton Res 1993; 3(3): 175-82.

51. Ingall TJ, Mc Leod JG, O'Brien PC. The effect of ageing on autonomic nervous system function. Aust N Z J Med 1990; 20(4): 570-7.

52. Yamanaka, Yujiro Honma, Koichi. Cardiovascular autonomic nervous response to postural change in 610 healthy Japanese subjects in relation to age. Autonomic Neuroscience 2006; 124(1-2): 125-131.

53. Low PA, Opfer – Gehrking TL, Proper CJ, Zimmerman J. The effect of ageing on cardiac autonomic and postganglionic sudomotor function. Muscle Nerve 1990; 13(2): 152-7.

54. Kawamoto A, Shimada K, Matsubayashi, Chikamori T, Kuzume Osamu, Ogura Hisakazu, Ozawz Toshio. Cardiovascular regulatory functions in elderly patients with Hypertension. Hypertension 1989; 13: 401-407.

55. Chu TS, Tsai TJ, Lai JS, Chen WY. Evaluation of cardiovascular autonomic function tests in normal subjects. Taiwan Yi Xue Hui Za Zhi 1989; 88 (4): 404-6.

56. Melo RC, Santos MDB, Silva E, Quiterio RJ, Moreno MA, Reis MS et al. Effects of age and physical activity on the autonomic control of heart rate in healthy men. Brazilian Journal of Medical Research 2005; 38:1331-1338.

57. Weiling W, Van Brederode JF, De Rijk LG, Borst C, Dunning AJ. Reflex Control of heart rate in normal subjects in relation to age: a data base for the cardiac vagal neuropathy. Diabetologia 1982 Mar; 22(3): 163-6.
58. Smith AS. Reduced sinus arrhythmia in diabetic autonomic neuropathy: diagnostic value of an age related normal range. British Medical Journal 1982; 285:1599-1601.
59. Cybulski G, Niewiadomski W. Influence of age on the immediate heart rate response to active orthostatic test. Journal of Physiology and Pharmacology 2003; 54 (1): 65-80.
60. Ng AV, Callister R, Johnson DG, Seals DR. Sympathetic neural reactivity to stress does not increase with age in healthy humans. Am J Physiol 1994 Jul; 267: 344-53.
61. Bellabarba G, C Molina, Davila Spinetti D, Villarroel V, Bellabarba SA, Torres A. Influence of age and gender on plasma norepinephrine changes produced by orthostatic stress. Acta Client Venez 2001; 52(4): 272-7.
62. Tonhajzerova Ingrid, Javorka Kamil, Javorka Michal, Petraskova Maria. Cardiovascular autonomic nervous system tests: reference values in young people (15-19 years) and influence of age and gender. Clin Physiol Funct Imaging 2002; Nov 22(6): 398-403.
63. Piha SJ. Cardiovascular responses to various autonomic tests in males and females. Clin Auton Res 1993; 3 (1): 15-20.
64. Lavi S, Nevo O, Thaler I, Rosenfeld R, Dayan L, Hirshoren Nir, Gepstein L, Jacob G. Effect of ageing on the cardiovascular regulatory systems in healthy women. Am J Physiol Regul Integr Comp Physiol 2006; 292

65. Christou DD, Jones PP, Jordan Jens, Diedrich Andre, Robertson D, Seals RD. Women have lower Tonic Autonomic Support of Arterial Blood Pressure and less Effective Baroreflex Buffering Than Men. Circulation 2005; 111: 494-98.

66. Zar H Jerrold. Biostatistical analysis. 4th Edition. 2006; Dorling Kinder Sley. Pg No. 194.

67. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care 1985; 8: 491-8.

68. http://www.medical-dictionary.thefreedictionary.com/middle+aged

69. Nzuobontane Divine, Kathleen Blackett, Christopher. Cardiovascular autonomic dysfunction in Africans in human immunodeficiency virus. J R Soc Med 2002; 95(9): 445-447.

70. Dr. Mahajan. Methods in Biostatistics. 5th edition. Jaypee Brothers; 1989. Pg 102, 114-119, 125-39.

71. Dey Debashish Kumar, Rothenberg Elisabet, Sundh Valter, Bosaeus Ingvar, Steen Bertil. Height and Body Weight in Elderly Adults: A 21-Year Population Study on Secular Trends and Related Factors in 70-Year-Olds. Journal of Gerontology: Medical Sciences 2001; 56 (12):780-784.

72. Storm DS, Metzger BL, Therrien B. Effects of age on autonomic cardiovascular responsiveness in healthy men and women. Nurs Res 1989; 38(6): 326-30.

73. Piccirillo G, Cacciafesta M, Viola E, Santagada E, Nocco M, Lionetti M, Bucca C, Moisc A, Tarantini S, Marigliano V. Influence of aging on cardiac baroreflex sensitivity determined non-invasively by power spectral analysis. Clin Sci 2001; 100: 267-274.

74. Grubb BP. Syncope in the older patient. Hellenic J cardiol 2003; 44: 235-42.
75. Kazuma N, Otsuka K, Wakamatsu K, et al. Heart rate variability in normotensive healthy children with aging. Clin Exp Hypertens 2002; 24: 83–89.
76. Zsuzsanna Lenard, Peter Studinger, Beatrix Mersich, Laszlo Kocsis, Mark Kollai. Maturation of cardiovagal autonomic function from childhood to young adult age. Circulation 2004; 110:2307-2312.
77. Kardos A, Watterich G, de Menezes R, et al. Determinants of spontaneous baroreflex sensitivity in a healthy working population. Hypertension. 2001; 37: 911–916.
78. Tank J, Baevski RM, Fender A, et al. Reference values of indices of spontaneous baroreceptor reflex sensitivity. Am J Hypertens 2000; 13: 268–275.
79. Brodde OE, Kouschak U, Becker K. Cardiac muscarinic receptors decrease with age. In vitro & in vivo studies. J Clin Invest 1998; 101: 471-478.
80. Poller U, Nedelka G, Radke J, Ponicke K, Brodde OE. Age dependent changes in cardiac muscarinic receptor functions in healthy volunteers. J Am Coll Cardiol 1972; 29: 187-193.
81. Oberhauser V, Schwertfeger E, Rutz T, Beyersdorf F, Rump LC. Acetylcholine release in human heart atrium: influence of muscarinic auto receptors, diabetes, and age. Circulation 2001; 103: 1638-1643.
82. Stratton R John, Levy C Wayne, Jacobson Arnold, Matsuoka Dale, May Janet, Madden Ken. Effects of ageing on cardiovascular responses to parasympathetic withdrawal. J Am Coll Cardiol 2003; 41: 2077-2083.

83. Wood H Robert, Hondzinski M Jan, Lee C Mathew. Evidence of an association among age- related changes in physical, psychomotor and autonomic function. Age and Ageing 2003; 32: 415-421.

84. J Gert Van Dijk, Monique Koenderink, Aeilko H, Zwinderman Joost Hann, Cor GS, Jan C Den Heijer. Autonomic nervous system tests depend on resting heart rate and blood pressure. Journal of the Autonomic Nervous system 1991; 35: 15-24.

85. Sachs C, Hamberger B, Kaijser L. Cardiovascular responses and plasma catecholamine in old age. Clin Physiol 1985; 5(6): 553-65.

86. Goldstraw PW, Warren DJ. The effect of age on the cardiovascular responses to isometric exercise: a test of autonomic function. Gerontology 1985; 31(1): 54-8.

87. Turner MJ, Mier CM, Spina RJ, Schectman KB, Ehsani AA. Effects of age and gender on the cardiovascular responses to isoproterenol. J Gerontol A 1999; 54: 393-400.

88. Dart M Anthony, Du Xiao-Jun, Kingwell A Bronwyn. Gender, sex hormones and autonomic nervous control of the cardiovascular system. Cardiovascular Research 2002; 53: 678-687.

ANNEXURE- 1

B.L.D.E.A'S SHRI B.M.PATIL MEDICAL COLLEGE , BIJAPUR.
INSTITUTIONAL ETHICAL COMMITTEE

Ethical Committee
BIJAPUR-586103
OUT WARD
No 280/07
Date 16/11/07
B.L.D.E.A's Shri B. M. PATIL Medical College

Dr. Vijay Ganjoo
Chairperson, I.E.C.
B.L.D.E.A'S Shri B.M.Patil Medical college
Bijapur-586103

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2007

at 03-15 pm **to scrutinize the Synopsis / Research projects of post graduate student / undergraduate student / Faculty members of this college from ethical clearance point of view. After scrutiny the following original / corrected & revised version Synopsis of the Thesis/ Research project has been accorded Ethical Clearance.**

Title "A Study of effect of age on cardiovascular autonomic function tests in healthy subjects of BLDEA's Shri. B.M.Patil Medical college, Bijapur

Name of P.G / U.G student / Faculty member Dr. Shrilaxmi Bagali

Name of Guide Dr. A.R.Dhasawadkar Prof & HOD Physiology

Dr. Vijay Ganjoo
Chairperson
Institutional Ethical Committee

Date:

Following documents were placed before E.C. for scrutinization:
1)Copy of Synopsis / Research project
2) Copy of informed consent form
3) Any other relevant document/s

ANNEXURE-2

B. L. D. E. A'S SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, BIJAPUR

RESEARCH INFORMED CONSENT FORM

Title of the Project:

A STUDY OF EFFECT OF AGE ON CARDIOVASCULAR AUTONOMIC FUNCTION TESTS IN HEALTHY SUBJECTS OF BLDEA'S SHRI B M PATIL MEDICAL COLLEGE, BIJAPUR.

Principal investigator/ P.G.Guide's name: **DR.ANAND R.DHARWADKAR MD**
PROF AND HEAD, DEPARTMENT OF PHYSIOLOGY.

1: PURPOSE OF RESEARCH:

I have been informed that this study will test influence of age on cardiovascular autonomic functions. This study will be useful academically as well as for clinically to find out association between aging and cardiovascular autonomic function changes.

2: PROCEDURE:

I understand that, the procedure of the study will involve recording of various physiological physical parameters. The procedure will not interfere with any of my physiological parameters and they are noninvasive.

3: RISK AND DISCOMFORTS:

I understand determination of Autonomic functions changes 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 not have a direct benefit to me but this may have a potential beneficial effect in the field of Cardiovascular Autonomic function changes in future.

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 two 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 hospital 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/ PG (Guide) Date

I confirm that _____ (Name of the P.G. Guide /Chief researcher) 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 / Guardian 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 – E)

ANNEXURE – 3

BLDEA'S SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, BIJAPUR

DEPARTMENT OF PHYSIOLOGY

A STUDY OF EFFECT OF AGE ON CARDIOVASCULAR AUTONOMIC FUNCTION TESTS IN HEALTHY SUBJECTS OF BLDEA'S SHRI B.M.PATIL MEDICAL COLLEGE, BIJAPUR

PROFORMA

Name: Age: Sex:

Occupation: Religion/Caste

Address:

GENERAL HEALTH CONDITION

Past history;

Ischemic heart (IHD) Hypertension Surgery

Tuberculosis Syphilis Leprosy

Amyloidosis Any significant Chronic illness

Family history

Diabetes mellitus Obesity Hypertension

IHD Any other illness

Personal history:

Appetite Diet (Veg/Non-Veg) Sleep

Nourishment Bowels Bladder

Habits: Chewing pan/Gutkha, history of smoking & alcohol intake.

Symptoms of Autonomic neuropathy

- Impotence
- Giddiness (Dizziness on standing)
- Pain abdomen, diarrhea, flatulence, nausea, vomiting, constipation
- Bladder disturbances
- Sweating disturbance

Others

- Easy fatigability
- Chest pain
- Cough, expectoration, breathlessness
- Tingling, numbness
- Visual disturbances

GENERAL EXAMINATION

Built	Temperature	Pallor
Cyanosis	Edema feet	Abnormal pigmentation
JVP	Lymphadenopathy	

ANTHROPOMETRIC MEASUREMENTS

1. Height (cms):
2. Weight (Kgs):
3. Body Surface Area (Sq m):
4. Body Mass Index (Kg/m^2):

PHYSIOLOGIACAL PARAMETERS

1. Heart rate (beats/min):
2. Blood pressure (Systolic/Diastolic) mm of Hg:
3. Respiratory rate (cycles/min):

AUTONOMIC FUNCTION PARAMETERS SCORE

PARASYMPATHETIC TEST

1. Heart rate response to Valsalva maneuver:
   (R – R interval, longest: shortest ratio)

2. Heart rate variation to deep breathing:
   (Beats/min)

3. Heart rate response to standing:
   (R – R interval, 30:15)

SYMPATHETIC TESTS

1. Blood pressure response to standing (mm of Hg):

2. Blood pressure response to sustained hand grip (mm of Hg):

GRADINGS (*According to Ewing and Clarke):*

	Normal	**Borderline**	**Abnormal**
Score	0	1	2
1. HRR to Valsalva maneuver	>1.21	1.11-1.20	<1.10
2. HRR to Deep breathing	>15b/min	11-14b/min	<10b/min
3. HRR to standing	>1.04	1.01-1.03	<1.0
4. BPR to standing	<10 mm Hg	11-29mmHg	>30 mm Hg
5. BPR to sustained handgrip	>16 mm Hg	11-15 mm Hg	<10 mm Hg

KEY TO MASTER CHARTS

Ht	Height
Wt	Weight
BMI	Body mass index
BSA	Body Surface Area
F-Score	Function Score
RPR	Resting Pulse Rate
RHR	Resting Heart Rate
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
RR	Respiratory Rate
I - E	Inspiration – Expiration
VR	Valsalva Ratio
HRR	Heart rate response
BPR	Blood Pressure Response
Sus HG	Sustained Hand Grip.

Annexure-4a

Annexure-4a
Master chart (Anthropometric, Physiological And Autonomic Function Parameters in Group I 18-19 years)

Sl. No	Age	Sex	Ht	Wt	BMI	BSA	Resting PR	Resting RR	Resting SBP	Resting DBP	Resting HR	Valsalva ratio 1	Valsalva ratio 2	Valsalva ratio 3	Valsalva ratio Mean	VR function Score	I-E	I-E function score	HRR to standing	HRR to standing Function score	BPR to standing	BPR to Std function score	BPR to Sus Hand Grip	BPR to sus HG function score	Total Score
1.	14	F	155	72	29.96	1.7	76	20	110	80	84	1.14	1.33	1	1.15	1	25.87	0	1.33	0	10	0	34	0	1
2.	14	F	163	65	24.66	1.7	90	18	120	76	100	1	1.25	1.25	1.16	1	25	0	1.25	0	10	0	24	0	1
3.	14	F	153	64	27.33	1.62	96	16	106	80	81	1.28	1.33	1.5	1.37	0	23.05	0	1.16	0	8	0	20	0	0
4.	14	F	167	68.5	24.56	1.76	84	16	90	76	90	1.14	1.33	1.14	1.2	1	29.17	0	1.4	0	10	0	24	0	1
5.	14	F	160	67	26.17	1.7	76	20	120	70	81	1.28	1.33	1.33	1.31	0	29.2	0	1.4	0	10	0	24	0	0
6.	14	F	155	70	29.13	1.7	100	18	112	84	100	1	1.2	1.4	1.2	1	24.11	0	1.4	0	10	0	16	0	1
7.	14	F	157	49	19.87	1.46	90	18	120	70	90	1.6	1.6	1.4	1.53	0	26.3	0	1.2	0	10	0	26	0	0
8.	14	F	161	46	17.74	1.46	76	22	110	60	80	1.2	1.16	1.13	1.16	1	20.43	0	1.19	0	0	0	40	0	1
9.	14	F	155	42	17.48	1.36	80	22	100	70	84	2.7	2.97	2.75	2.8	0	25.5	0	1.1	0	0	0	44	0	0
10.	14	F	156	60	24.65	1.6	84	20	120	80	87	1.6	1.8	2	1.8	0	27.9	0	1.4	0	10	0	30	0	0
11.	14	F	154	62	26.14	1.6	76	16	120	90	75	1.5	1.5	1.5	1.5	0	18.57	0	1.6	0	6	0	34	0	0
12.	14	F	162	62	23.62	1.66	76	18	106	80	84	1.6	1.6	1.6	1.6	0	27.78	0	1.4	0	10	0	30	0	0
13.	18	F	163	53	19.94	1.56	80	20	90	70	87	1.6	1.33	1.33	1.42	0	29.17	0	1.2	0	10	0	30	0	0
14.	18	F	160	70	27.34	1.74	80	16	140	90	84	2	2	1.5	1.83	0	23.61	0	1.16	0	10	0	10	2	2
15.	18	F	162	54	20.83	1.56	88	22	122	70	96	1.4	1.6	1.4	1.46	0	21.43	0	1.33	0	10	0	30	0	0
16.	18	F	160	64	25	1.66	88	20	106	70	96	1.33	1.16	1.16	1.21	0	17.98	0	1.16	0	16	1	30	0	1
17.	18	F	160	52	20.11	1.52	92	20	100	68	99	1.16	1.4	1.4	1.32	0	22.73	0	1.2	0	4	0	22	0	0
18.	18	F	163	70	26.34	1.76	96	20	102	74	100	1.5	1.75	2.66	1.97	0	33.34	0	1.5	0	10	0	16	0	0
19.	18	F	158	53	21.23	1.52	92	18	102	68	100	1.5	1.6	1.6	1.56	0	32.14	0	1.25	0	10	0	22	0	0
20.	19	M	163	58	21.82	1.62	80	16	122	80	84	1.8	1.66	1.66	1.7	0	28.65	0	1.5	0	12	1	20	0	1
21.	19	M	168	65	23.03	1.74	100	22	140	90	100	1.2	1.2	1.2	1.2	1	20	0	1.2	0	2	0	20	0	1
22.	19	M	160	56	21.87	1.58	90	20	120	80	99	1.25	1.6	1.6	1.48	0	21.19	0	1.16	0	8	0	30	0	0
23.	18	M	180	57	17.59	1.74	86	18	106	70	90	1.16	1.16	1.6	1.3	0	32.37	0	1.2	0	10	0	10	2	2
24.	19	M	177	100	31.91	2.18	100	16	130	86	100	2.5	2	1.8	2.1	0	26.39	0	1.2	0	10	0	24	0	0
25.	19	M	167	55	19.72	1.62	82	18	130	70	90	1.18	1.25	1.21	1.21	0	16.49	0	1.04	0	10	0	20	0	0
26.	18	M	172	67	22.64	1.8	92	16	120	90	93	1.16	1.16	1.16	1.16	1	22.39	0	1.6	0	10	0	30	0	1
27.	18	M	168	70	24.8	1.8	98	20	118	70	100	1.6	1.33	1.6	1.51	0	21.6	0	1.4	0	10	0	28	0	0
28.	18	M	181	51	15.56	1.66	100	18	140	90	100	1.33	1.14	1.16	1.21	0	33.05	0	1.2	0	10	0	18	0	0
29.	19	M	170	58	20.06	1.66	76	16	130	80	80	1.77	1.53	1.09	1.46	0	13.67	1	1.08	0	10	0	20	0	1
30.	18	M	169	56	19.06	1.64	80	20	124	80	78	1.14	1.28	1.28	1.23	0	25.4	0	1.33	0	8	0	52	0	0
31.	18	M	169	59	20.65	1.68	72	14	100	80	84	1.45	1.44	1.44	1.44	0	17.58	0	1.33	0	10	0	30	0	0
32.	18	M	172	116	39.21	2.26	98	16	150	90	96	1.5	3	3	2.5	0	17.97	0	1.16	0	10	0	30	0	0
33.	18	M	166	66	23.95	1.74	80	16	124	80	78	1.8	2.5	1.5	1.93	0	16.19	0	2	0	8	0	30	0	0
34.	19	M	178	66	20.83	1.82	84	16	122	90	88	1.65	1.7	1.72	1.69	0	16	0	1.14	0	10	0	20	0	0
35.	18	M	178	52	16.41	1.64	84	20	112	90	63	1.25	1.75	1.5	1.5	0	20.84	0	1.25	0	10	0	48	0	0
36.	18	M	183	66	19.7	1.86	96	18	116	74	99	1	1.6	1.4	1.33	0	30.56	0	1.4	0	10	0	20	0	0
37.	19	M	173	68	22.72	1.82	100	20	130	60	100	1.4	1.16	1.16	1.24	0	32.98	0	1.4	0	10	0	20	0	0

Annexure-4b

Master chart (Anthropometric, Physiological and Autonomic function parameters in Group II 20-34 years)

Sl No	Age	Sex	Ht	Wt	BMI	BSA	RPR	R RR	Resting SBP	Resting DBP	Resting HR	Valsalva Ratio1	Valsalva Ratio2	Valsalva ratio3	VR Mean	VM F Score	I-E	I-E F score	HRR to stand	HRR to stand f score	BPR to standing	BPR to Std F score	BPR to Sus HG	BPR to sus HG Fscore	Total Score
1	26	M	172	64	21.63	1.74	78	14	110	72	66	1.25	1	1	1.08	2	21.67	0	1.25	0	10	0	40	0	2
2	20	M	169	74	25.91	1.84	74	22	112	86	72	1.25	1.5	1	1.25	0	23.33	0	1.33	0	8	0	32	0	0
3	20	M	181	56	17.09	1.74	76	20	100	68	78	1.66	2	2	1.88	0	34.53	0	1.33	0	10	0	30	0	0
4	20	M	170	49	16.95	1.56	82	16	100	68	78	1	1.25	1.25	1.16	1	22.5	0	1.33	0	14	1	40	0	2
5	21	M	179	79	24.65	1.98	67	18	110	80	66	2.33	2.33	1.25	1.97	0	23.7	0	1.25	0	12	1	38	0	1
6	21	M	168	71	25.15	1.8	82	14	104	72	78	2.3	2.3	2	2.2	0	37.5	0	1.33	0	18	1	42	0	1
7	20	M	169	63	22.05	1.72	64	18	96	78	66	1.5	1.25	1.25	1.33	0	41	0	1.25	0	12	1	30	0	1
8	21	M	177	76	24.25	1.94	68	16	130	90	60	1.5	1.4	1.2	1.36	0	23.6	0	1.25	0	8	0	26	0	0
9	22	M	170	75	25.95	1.86	72	16	110	68	60	1.25	1.25	1.25	1.25	0	18.34	0	1.25	0	8	0	34	0	0
10	27	M	177	55	17.55	1.7	80	14	120	80	84	2	2	1.66	1.88	0	25	0	1.33	0	10	0	20	0	0
11	21	M	167	65	23.3	1.72	75	18	104	76	72	1.66	1.66	1.66	1.66	0	24.17	0	1.25	0	8	0	30	0	0
12	28	M	172	72	24.33	1.82	90	16	110	80	72	1.25	1.33	1.25	1.27	0	17.5	0	1.33	0	0	0	20	0	0
13	26	M	171	62	21.2	1.72	76	16	132	94	66	2	3	2	2.33	0	25	0	1.33	0	18	1	20	0	1
14	29	M	172	72	24.34	1.82	70	14	110	70	60	1.25	1.5	1.5	1.41	0	14.17	1	1.5	0	10	0	20	0	1
15	24	M	161	60	23.14	1.64	94	20	90	62	78	2	2	1.33	1.77	0	27.5	0	2	0	18	1	22	0	1
16	26	M	164	61	22.67	1.66	82	22	98	64	84	1	1.33	1.33	1.22	0	25	0	1.5	0	18	1	20	0	1
17	22	M	160	55	21.48	1.6	78	22	108	74	84	1.66	2	2	1.88	0	27.5	0	1.33	0	10	0	28	0	0
18	20	M	164	54	20.07	1.6	76	24	102	74	84	2	2.33	1.66	1.99	0	32.5	0	1.66	0	14	1	30	0	1
19	21	M	165	58	21.3	1.64	88	22	110	80	84	1.33	1.33	1.33	1.33	0	23.33	0	1.33	0	12	1	36	0	1
20	21	M	166	54	19.59	1.6	82	18	104	76	90	1.33	1.33	1.33	1.33	0	16.66	0	1.33	0	8	0	32	0	0
21	20	M	172	49	16.56	1.58	94	20	98	72	90	1	1.33	1	1.11	1	35	0	1	2	14	1	18	0	4
22	21	M	170	50	17.3	1.58	90	18	100	72	100	1	1.33	1.33	1.22	0	27.5	0	1.33	0	20	1	24	0	1
23	22	M	170	54	18.68	1.64	78	22	108	64	78	1	1.33	1.33	1.22	0	27.5	0	1.33	0	20	1	24	0	1
24	21	M	173	58	19.37	1.68	84	20	100	64	72	1.33	1.33	1.33	1.33	0	16.66	0	1.33	0	12	1	20	0	1
25	21	M	164	54	20.07	1.6	78	20	104	64	84	1.66	2	1.66	1.77	0	32.5	0	1.33	0	12	1	20	0	1
26	20	M	169	55	19.25	1.64	84	22	110	62	84	1.66	1.66	1.66	1.66	0	25	0	1.33	0	12	1	26	0	1
27	20	M	180	73	22.53	1.94	64	18	92	64	72	1.2	1.2	1	1.13	1	38.7	0	1.33	0	8	0	32	0	1
28	20	M	164	48	17.84	1.52	70	18	110	70	66	2	1.25	1.25	1.5	0	25.83	0	1.33	0	10	0	26	0	0
29	21	M	178	68	21.46	1.86	72	18	98	72	84	1.25	1.25	1	1.16	1	18.33	0	1.33	0	6	0	32	0	1
30	26	M	158	49	19.62	1.48	94	26	110	84	84	1.66	1.33	1.33	1.44	0	25	0	1.33	0	6	0	28	0	0
31	31	M	165	58	21.3	1.62	76	20	138	90	90	1.25	1.33	1	1.19	1	25	0	1.33	0	2	0	18	0	1
32	21	F	159	44	17.4	1.42	80	14	102	70	84	1.33	1.16	1.4	1.29	0	35	0	1.2	0	24	1	30	0	1
33	25	F	154	55	23.19	1.52	80	16	120	90	76	1.33	1.28	1.26	1.29	0	19	0	1.29	0	10	0	18	0	0
34	25	F	151	59	25.87	1.54	98	20	124	80	100	1.16	1	1.16	1.1	2	18.22	0	1.25	0	10	0	20	0	2
35	25	F	153	45	19.22	1.4	98	20	102	64	100	1.2	1.5	1.2	1.3	0	17.16	0	1.04	0	4	0	10	2	2
36	24	F	152	46	19.9	1.4	92	24	122	84	93.6	1.39	1.54	1.81	1.58	0	12.33	1	1.1	0	10	0	26	0	1
37	28	F	155	49	20.39	1.46	94	26	130	70	88	1.19	1.45	1.2	1.28	0	13.66	1	1.23	0	6	0	40	0	1
38	20	F	160	55	21.48	1.56	86	18	120	72	84	1.66	2	2.5	2.05	0	18	0	1.2	0	10	0	14	1	1
39	24	F	158	50	20.02	1.48	88	16	90	70	84	1.4	1.29	1.41	1.36	0	30.5	0	1.3	0	10	0	20	0	0
40	27	F	160	53	20.7	1.54	90	16	122	72	92	1.09	1.13	1.06	1.09	2	21	0	2.2	0	6	0	18	0	2

Annexure-4c

Master chart (Anthropometric, Physiological and Autonomic function parameters in Group III 35-54 years)

Sl. No	Age	Sex	Ht	Wt	BMI	BSA	Resting PR	Resting RR	Resting SBP	Resting DBP	Resting HR	Valsalva Ratio 1	Valsalva Ratio 2	Valsalva Ratio 3	Valsalva ratio Mean	VR function Score	I-E	I-E function score	HRR to stand	HRR to std Function score	BPR to stand	BPR to Std function score	BPR to SustHand Grip	BPR to sus HG function score	Total Score
1.	42	M	157	62	25.15	1.62	74	21	104	76	66	1.25	1.25	1.25	1.25	0	19.16	0	1.66	0	18	1	34	0	1
2.	45	M	166	63	22.86	1.7	72	18	108	72	66	1.5	1.25	1.25	1.33	0	15	0	1.25	0	10	0	34	0	0
3.	45	M	156	56	23.01	1.54	78	18	110	76	78	2	2	2	2	0	17.5	0	1.25	0	10	0	34	0	0
4.	42	M	160	63	24.6	1.66	74	18	112	80	72	2	2	2	2	0	21.67	0	1.25	0	14	1	50	0	1
5.	45	M	175	82	26.77	1.98	68	16	110	70	60	1.5	1	1.2	1.23	0	20.84	0	1.2	0	10	0	52	0	0
6.	42	M	164	65	24.16	1.7	78	15	100	64	78	1.66	1.66	1.66	1.66	0	27.5	0	1.33	0	10	0	40	0	0
7.	35	M	169	62	21.7	1.7	96	17	102	74	90	1.66	4	2.5	2.72	0	35.83	0	1.33	0	8	0	20	0	0
8.	42	M	168	57	20.19	1.66	98	15	134	68	84	1.33	1.33	1.33	1.33	0	25	0	1	2	4	0	32	0	0
9.	36	M	162	50	19.05	1.52	96	13	110	60	84	1	1	1.33	1.11	1	21.66	0	1.33	0	10	0	20	0	1
10.	36	M	160	51	19.92	1.52	80	12	128	90	78	1.16	1	1.2	1.12	1	26.46	0	1.2	0	16	1	20	0	2
11.	35	M	175	70	23.12	1.84	80	14	130	80	84	1.16	1.6	1.5	1.42	0	21.79	0	1.16	0	16	1	10	2	3
12.	36	M	180	62	19.13	1.78	64	14	116	86	102	2	1.66	1.5	1.72	0	25.23	0	1.16	0	10	0	4	2	2
13.	45	M	116	73	26.49	1.82	90	20	130	90	102	1.6	1.66	1.33	1.53	0	9.09	2	1.16	0	12	1	10	2	5
14.	41	M	160	65	25.39	1.68	80	20	132	90	75	1.13	1.14	1.16	1.14	1	26.02	0	1.33	0	2	0	12	1	2
15.	45	M	158	52	20.82	1.5	68	20	132	84	72	1.66	1.8	1.8	1.75	0	14.51	1	1.02	1	18	1	26	0	3
16.	46	M	154	50	21.08	1.46	78	28	100	84	84	1.5	1.6	1.6	1.56	0	30.95	0	1.2	0	6	0	16	0	0
17.	45	M	158	39	15.62	1.34	78	18	130	100	87	1.16	1.16	1.16	1.16	1	17.06	0	1	2	8	0	16	0	3
18.	49	M	170	49	16.95	1.56	80	16	110	84	84	1.5	1.6	1.33	1.47	0	17.06	0	1	2	2	0	8	2	4
19.	35	M	160	51	19.9	1.52	62	20	120	90	86.3	2.42	1.85	1.86	2.07	0	10.83	1	1.06	0	16	1	20	0	2
20.	35	M	173	69	23.05	1.84	78	24	110	80	75	1.14	1.14	1.14	1.14	1	14.29	1	1.6	0	18	1	12	1	4
21.	35	M	166	53	19.23	1.58	68	20	100	80	93	1.8	2.25	2.25	2.1	0	30.87	0	1.6	0	0	0	10	0	2
22.	35	M	160	50	19.53	1.5	70	14	110	80	72	1.31	1.4	1.42	1.37	0	3.33	2	1.13	0	12	1	20	0	3
23.	39	F	147	52	24.06	1.44	80	16	110	70	82	1.76	1.69	1.8	1.75	0	11.34	1	1.08	0	2	0	28	0	1
24.	40	F	157	45	18.25	1.42	76	14	130	90	76	1.07	1.05	1.01	1.04	2	9.5	2	1.05	0	30	2	10	2	8
25.	36	F	161	80	30.86	1.84	76	14	128	80	76	1.2	0.98	1.39	1.19	1	7.61	2	1.08	0	28	1	14	1	5
26.	37	F	152	53	22.93	1.48	76	14	110	70	78	1.22	1.1	1.06	1.12	1	19.66	0	1.48	0	0	0	10	2	3
27.	49	F	154	69	29.09	1.68	96	13	114	90	93	1	1	1	1	2	23.1	0	1.2	0	12	1	20	0	3
28.	49	F	155	60	24.97	1.58	72	12	90	66	72	1.66	1.66	1.33	1.55	0	23.46	0	1.2	0	12	1	14	1	2
29.	40	F	150	52	23.11	1.46	80	36	132	76	69	1.14	1.6	1.33	1.35	0	23.88	0	1.14	0	28	1	6	2	3
30.	35	F	155	71	29.55	1.72	88	24	110	80	81	1.16	1.16	1.33	1.21	0	16.21	0	1.16	0	2	0	28	0	0
31.	47	F	158	90	36.05	1.99	82	20	110	90	87	1.33	1.16	1.16	1.21	0	10.34	2	1.2	0	20	1	20	0	3
32.	38	F	149	56	25.22	1.5	86	28	126	90	86.0	1.2	1.34	1.53	1.35	0	8.84	2	1.25	0	10	0	26	0	2
33.	48	F	160	70	27.34	1.74	84	24	130	90	85.7	1.46	1.28	1.46	1.4	0	16	0	1.02	1	10	0	16	0	1
34.	35	F	154	55	23.19	1.52	80	14	120	90	80	1.11	1.06	1.12	1.09	2	7.15	2	1.12	0	10	0	20	0	4
35.	40	F	160	77	30.07	1.8	78	24	108	70	84	1.33	1.14	1.14	1.2	1	12.34	1	1.28	0	4	0	20	0	2
36.	40	F	150	41	18.22	1.32	60	20	124	90	60	1.25	1.25	1.25	1.25	0	3.41	2	1.25	0	10	0	8	2	4
37.	35	F	151	68	29.82	1.68	88	22	100	80	88.5	1.17	1.26	1.08	1.17	1	10.67	1	1.13	0	8	0	12	1	3
38.	35	F	156	58	23.83	1.56	70	28	100	70	92.3	1.24	1.02	1.07	1.11	1	13.29	1	1.13	0	10	0	10	2	4

Annexure-4d

Master Chart (Anthropometric, Physiological and Autonomic Function Parameters in Group II – 55-65 yrs)

Sl. No	Age	Sex	Ht	Wt	BMI	BSA	Resting PR	Resting RR	Resting SBP	Resting DBP	Resting HR	Valsalva ratio I	Valsalva ratio 2	Valsalva ratio 3	Valsalva ratio mean	VR function Score	I-E	I-E function score	HRR to standing	HRR Standing function score	BPR to standing	BPR to standing function score	BPR to Sustained Hand Grip	BPR to sus HG function score	Total Score
1	58	F	149	44	19.81	1.24	90	24	120	60	96	1.4	1.16	1	1.18	1	8.93	2	1	2	10	0	12	1	6
2	55	F	145	54	25.68	1.44	82	20	126	80	87.3	1.08	1.14	1.16	1.12	1	5.67	2	1.03	1	10	0	10	2	6
3	55	F	151	42	18.42	1.34	80	20	140	78	80.7	1.14	1.08	1.16	1.12	1	13	1	1.08	0	12	1	14	1	4
4	55	F	145	47	22.35	1.36	80	18	118	70	88	1.32	1.3	1.4	1.34	0	6	2	1.07	0	12	1	20	0	3
5	56	F	149	79	35.58	1.72	100	20	110	90	100	1.09	1.17	1.16	1.14	1	6.84	2	1.04	0	20	1	10	2	6
6	56	F	150	46	20.44	1.38	76	20	140	90	60	1.12	1.11	1	1.07	2	8.75	2	1.28	0	2	0	10	2	6
7	55	F	156	70	28.76	1.7	60	18	120	90	66	1.1	1.2	1.2	1.16	1	6.18	2	1.5	0	14	1	10	2	6
8	55	F	150	60	26.66	1.66	74	18	108	70	72	1.13	1.13	1.13	1.13	1	1.21	2	1	2	12	1	10	2	8
9	60	F	145	47	22.35	1.36	80	24	150	84	80	1.08	1.14	1.16	1.12	1	5.4	2	1.02	1	12	1	10	2	7
10	55	F	150	49	21.77	1.42	74	24	104	76	69	1.13	1.13	1.13	1.13	1	11.68	2	1	0	12	1	16	0	5
11	62	F	155	50	20.81	1.46	72	18	132	90	70	1.22	1.1	1.06	1.12	1	3	2	1	2	20	1	8	2	8
12	58	F	153	66	28.19	1.64	70	16	140	90	70	1.37	1.27	1.53	1.39	0	10.94	1	1.12	0	12	1	14	1	3
13	58	F	158	60	24.03	1.62	66	16	128	80	60	1.11	1.06	1.12	1.09	2	5.45	2	1.07	0	14	1	12	1	6
14	55	F	160	62	24.21	1.64	74	20	128	88	66	1.14	1.33	1.14	1.2	1	10	2	1.18	0	10	0	14	1	4
15	56	F	156	68	27.92	1.68	66	14	130	90	60	1.13	1.13	1.13	1.13	1	6.7	2	1.05	0	10	0	10	2	5
16	55	F	152	40	17.31	1.32	72	22	110	86	68	1.25	1.25	1.25	1.25	0	11.34	1	1.06	0	5	0	18	0	1
17	57	F	160	70	27.34	1.74	84	16	124	84	80	1.16	1.33	1.16	1.21	0	8.45	2	1.12	0	8	0	12	1	3
18	62	M	173	77	25.72	1.92	72	14	132	80	84	1.33	1.4	1.6	1.44	0	17.97	0	1.75	0	10	0	20	0	0
19	60	M	165	54	19.83	1.58	96	14	120	82	84	1.33	1.16	1.4	1.29	0	36.31	0	1.16	0	22	1	28	0	1
20	61	M	165	65	23.87	1.72	80	28	110	80	75	1.16	1.16	1	1.06	2	6.59	2	1.08	0	12	1	6	2	7
21	60	M	168	50	17.71	1.56	74	20	90	70	72	1.4	1.4	1.4	1.4	0	19.08	0	1.2	0	10	0	10	2	2
22	55	M	168	67	23.73	1.76	72	26	110	72	68	1.04	1.9	1.85	1.6	0	14.17	1	1.07	0	2	0	22	0	1
23	65	M	161	51	19.67	1.52	76	22	140	60	68	1.05	1.08	1.07	1.06	2	7.83	2	1.07	0	20	1	18	0	5
24	55	M	159	67	26.5	1.7	80	26	124	84	71.3	1.53	1.68	1.71	1.64	0	17.16	0	1.28	0	4	0	16	0	0
25	65	M	155	60	24.97	1.58	74	26	140	80	66	1.03	1.05	1.04	1.04	2	1.33	2	1.02	1	20	1	10	2	8
26	55	M	151	42	18.42	1.34	74	20	100	60	85.7	1.67	1.89	2	1.85	0	28.83	0	1.26	0	12	1	18	0	1
27	63	M	176	65	20.98	1.8	70	16	100	64	74.3	1.35	1.24	1.41	1.33	0	7	2	1.05	0	2	0	10	2	6
28	55	M	152	40	17.31	1.32	64	18	110	90	75	1.13	1.28	1.13	1.17	1	6.72	2	1	2	12	1	10	2	8
29	62	M	166	74	26.85	1.82	60	12	132	90	54	1	1.2	1.1	1.1	2	3	2	1.1	0	2	0	10	2	6
30	55	M	162	61	23.24	1.64	54	20	112	90	63	1.13	1.42	1.5	1.34	0	5.42	2	1	2	22	2	12	1	7
31	55	M	167	50	17.92	1.56	76	16	100	72	76	1.45	1.16	1.01	1.2	1	10.85	1	1.14	0	10	0	14	1	3
32	55	M	173	80	26.72	1.94	66	20	128	76	64	1.03	1.09	1.12	1.08	2	11.34	2	1.08	0	2	0	10	2	5
33	56	M	163	70	26.34	1.76	76	18	120	86	70	1.66	1.66	1.66	1.66	0	28	0	1.3	0	10	0	18	0	0
34	57	M	166	66	23.95	1.74	72	16	124	90	70	1.25	1.25	1.25	1.25	0	16	0	1.24	0	10	0	20	0	0
35	60	M	164	68	25.28	1.74	68	20	116	88	72	1.32	1.3	1.4	1.34	0	7	2	1.08	0	14	1	12	1	4
36	61	M	170	69	23.87	1.8	64	22	118	82	72	1.16	1.16	1.33	1.21	0	6	2	1.14	0	12	1	10	2	5
37	62	M	160	58	22.65	1.6	74	20	130	90	70	1.16	1.16	1.16	1.16	1	5	2	1	2	22	1	8	2	8