"STUDY OF GLYCEMIC STATUS AND CARDIOVASCULAR AUTONOMIC FUNCTIONS IN THE OFFSPRINGS OF TYPE 2 DIABETIC PARENTS"

By

Dr.SANGEETA TUPPAD. M.B.B.S

DISSERTATION SUBMITTED TO THE BLDE UNIVERSITY, BIJAPUR

In partial fulfillment
of the requirements for the degree of

DOCTOR OF MEDICINE

IN

PHYSIOLOGY

Under the guidance of

DR. ANAND R. DHARWADKAR M.D

Professor

DEPARTMENT OF PHYSIOLOGY

BLDEU'S SHRI. B. M. PATIL MEDICAL COLLEGE,

HOSPITAL AND RESEARCH CENTRE, BIJAPUR -KARNATAKA

2012

B.L.D.E. UNIVERSITY, BIJAPUR. KARNATAKA,

DECLARATION BY THE CANDIDATE

I **Dr.SANGEETA TUPPAD,** here by solemnly declare that this dissertation entitled "**STUDY OF GLYCEMIC STATUS AND CARDIOVASCULAR AUTONOMIC FUNCTIONS IN THE OFFSPRINGS OF TYPE 2 DIABETIC PARENTS."** is a bonafide and genuine research work carried out by me under the guidance of **Dr.ANAND R. DHARWADKAR** MD Professor , Department of Physiology, B.L.D.E.U'S Shri B. M. Patil Medical College, Hospital and Research Centre, Bijapur.

Date:

Place: Bijapur

Dr. SANGEETA. TUPPAD

Post Graduate student
Department of Physiology
B.L.D.E.U's Shri B. M. Patil
Medical College, Hospital &
Research Centre, Bijapur

CERTIFICATE BY THE GUIDE

BLDEU'S Shri B. M. Patil Medical College

Hospital & Research Centre

Bijapur

This is to certify that the dissertation titled *"***STUDY OF GLYCEMIC STATUS AND CARDIOVASCULAR AUTONOMIC FUNCTIONS IN THE OFFSPRINGS OF TYPE 2 DIABETIC PARENTS"** is a bonafide research work done by **Dr.SANGEETA TUPPAD**, in partial fulfillment of the requirement for the degree of M.D in Physiology

DR.ANAND R DHARWADKAR. M.D
Professor
Department of Physiology
B.L.D.E.U'S Shri B. M. Patil
Medical College, Hospital &
Research Centre,
Bijapur-586103

Date:

Place: Bijapur

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT AND PRINCIPAL

BLDEU'S Shri B. M. Patil Medical College

Hospital & Research Centre

Bijapur

This is to certify that the dissertation entitled **"STUDY OF GLYCEMIC STATUS AND CARDIOVASCULAR AUTONOMIC FUNCTIONS IN THE OFFSPRINGS OF TYPE 2 DIABETIC PARENTS"**, is a bonafide research work done by Dr.SANGEETA TUPPAD. under the guidance of **Dr. Anand R Dharwadkar,** MD Professor and HOD, Department of Physiology, BLDEU'S Shri B. M. Patil Medical College Hospital and Research Centre, Bijapur.

Seal & Signature of the
Head of Department of Physiology
Dr.Manjunath. A
M.D (Physiology)
BLDEU'S Shri B.M Patil
Medical College, Hospital
& Research Centre, Bijapur

Date:

Place: Bijapur

Seal & Signature of the
Principal
Dr. R.C. BIDRI
M.D (Medicine)
BLDEU'S Shri B.M Patil
Medical College, Hospital
Research Centre,
Bijapur

Date:

Place: Bijapur

COPYRIGHT

Declaration by the Candidate

I here by declare that the B.L.D.E.University, BIJAPUR shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic/research purpose.

Date: **Signature of the candidate**

Place**:** Bijapur **Dr. SANGEETA TUPPAD**

ACKNOWLEDGEMENT

I am ever grateful to the **Almighty** for His blessings on me.

It is a great honour to convey my deep sense of gratitude and sincere thanks to my respected teacher and guide Dr.Anand R Dharwadkar MD, Professor, Department of Physiology, BLDEU's Shri B M Patil Medical College, Bijapur who with his knowledge and expertise has provided able guidance and constant encouragement not only during the preparation of this dissertation but also throughout my postgraduate course.

I am also highly indebted to Dr. Mrs.Asha A Dharwadkar MD, Professor Department of Physiology for her novel orientation, encouragement and meticulous attention during the thesis work

I express my sincere gratitude to Dr. Manjunatha Aithala MD Professor & Head of the department of Physiology, B.L.D.E.U.'s Shri B. M Patil Medical College, Hospital and Research Center, Bijapur, for his advice, valuable suggestion, encouragement & support

I express my sincere gratitude to Dr G B Dhanakshirur, Professor Department of Physiology for his valuable guidance and suggestions throughout my dissertation work.

I am thankful to Dr Sumangala Patil MD Asso.Professor, Dr Lata Mullur MD Asso. Professor, for their constant encouragement & constant support throughout my work.

I am thankful to, Dr C M Kulkarni MD, Dr S A Loni MD, Dr Sujata Talikoti MD, Dr S M Patil MD, Dr.Shrilaxmi. B MD, Dr Gouhar Banu, MD,Dr Shireen Q.
Dr Jyoti MD, Dr Nandini MD, Miss Vandana Dhate, Dr Satish Patil, Dr.Pallavi and Dr Jagadish for their support during my work.

I extend my thanks to my post graduate colleagues, Dr Anita.T,.Dr Anita.D and Dr Santhosh.P

I express my deep sense of gratitude to my husband Dr.Sanganabasappa, his love, patience and understanding has helped me out through all my difficulties and has made both my study and life easier.

I am very thankful for my cute little son Pramath, who by his mischieves lightens my mood and inspires me to do the things better.

I am thankful to my parents, brother, sister, my sister in law, my niece & my in laws for their blessings & moral support in successful completion of this dissertation.

I thank technical & non teaching staff of Physiology Department for their assistance. I also thank the library staff for their kind assistance & help in providing me required books and reference materials for my study.

I am thankful to Prof & Head Department of community medicine and statistician, BLDEU'S Shri B M Patil Medical College and Mr Madagi for their assistance in statistical analysis.

I am thankful to Biochemistry staff & technicians of B.L.D.E.U.'s Shri B. M Patil Medical College, Hospital and Research Centre, Bijapur for their assistance & help provided to me during my investigations.

Date: **Dr SANGEETA TUPPAD**

Place: Bijapur

LIST OF ABBREVIATIONS USED

(In alphabetical order)

AGEs-Advanced glycosylation end products

ACh- Acetylcholine

ANS- Autonomic Nervous System

AV node- Atrioventricular node

BP- Blood pressure

BMI- Body Mass Index

BSA- Body Surface Area

BRS- Baroreflex sensitivity

bpm- Beats per minute

CNS- Central Nervous System

DAN- Diabetic Autonomic Neuropathy

DBP- Diastolic blood pressure

ECG- Electrocardiogram

FBG- Fasting blood glucose

FBG-Fasting Plasma glucose

FDR- First Degree Relative

GIT – Gastro Intestinal Tract

GDM- Gestational Diabetes Mellitus

GOD-POD- Glucose oxidase and peroxidase

HDL-High Density Lipoprotein

HF- High Frequency

HR- Heart rate

HRV- Heart rate variability

Ht- Height

I-E – Inspiration- Expiration

IFG-Impaired Fasting Glucose

IGT- Impaired Glucose Tolerance

IR- Insulin Resistant

LDL- Low density lipoprotein

LF- Low Frequency

mg -milligrams

mmHg -Millimeters of mercury

MODY - Maturity onset diabetes mellitus of young

NIR- Noninsulin Resistant

OGTT- Oral glucose Tolerance

PKC- Protein kinase C

PLBG -Post load blood glucose

PR- Pulse rate

RR – Respiratory rate

SD- Standard deviation

SA node- Sinoatrial node

SBP- Systolic blood pressure

Wt- Weight

WHO- World health organisation

VM - Valsalva maneuver

VR-Valsalva Ratio

ABSTRACT

Background & Objectives: Type 2 Diabetes Mellitus has a strong genetic component. The concordance of Type 2 Diabetes Mellitus in identical twins is between 70% to 90%. Individuals with a parent with Type 2 Diabetes Mellitus have an increased risk of Diabetes. Thus this study is designed to evaluate the Glycemic status and Cardiovascular Autonomic Functions and the Correlation between them in the offsprings of Type 2 Diabetic Parents.

Material & Methods: The autonomic function tests analyzed were Valsalva maneuver, HR response to deep breathing and standing, BP response to standing and Sustained Hand Grip and glycemic status in 30 healthy offsprings of Type 2 Diabetic Parents (Study group) and 30 healthy offsprings of Nondiabetic Parents (Control group) in the age range of 18 - 21 years among medical students of BLDEU's Shri B M Patil Medical College, Bijapur. The Study group was further divided into 3 subgroups based on history of prevalence of diabetes (S1:one parent diabetic and other parent with no family history of diabetes, n=17, S2: one parent diabetic and other parent with family history of diabetes, n=9, S3: both parents diabetic, n=4) . The various autonomic function tests were graded by using Ewing & Clarke scores. Statistical analysis expressed as Mean±SD, Correlation and its level of significance by Z test

Results and Conclusion:

There is significant increase in the height(p=0.04)*, weight(p=0.04) * and BSA (p=01)** in the of subjects in Study Group compared to Control Group and also there is gradual significant increase in these parameters from S1 to S3.

1) Autonomic function tests showed gradual decrease in function in study group compared to control.

2) Heart rate response to Valsalva maneuver & Blood pressure response to Sustained Hand Grip appear to be more sensitive parameters to detect 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 in subgroups of study group

4) There is insignificant increase in Fasting blood glucose and Postload blood glucose in study group and subgroups,compared to control group.

5) Different autonomic function parameters with glycemic status are negatively correlated.

Key words: Parasympathetic function tests; Sympathetic function tests; Offsprings of Type 2 Diabetic Parents; Glycemic status; Cardiovascular Autonomic function score, HR-Heart Rate, BP-Blood Pressure

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

INTRODUCTION

The portion of the nervous system that regulates most of the visceral functions of the body is called as Autonomic Nervous System[1]. Its main aim is to maintain the optimal internal environment (Homeostasis) of the body. It governs various body functions which are normally carried out without conscious control[2].

Diabetes Mellitus is a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of Diabetes Mellitus exist and are caused by a interaction of genetics and environmental factors[3].

Individuals with Diabetes Mellitus may develop signs of autonomic dysfunction involving cholinergic, noradrenergic and peptidergic systems. Autonomic neuropathies affecting cardiovascular system cause a resting tachycardia and orthostatic hypotension. Reports of sudden death have also been attributed to autonomic neuropathy[3].

Type 2 Diabetes Mellitus has a strong genetic component. The concordance of Type 2 Diabetes Mellitus in identical twins is between 70% to 90%. Individuals with a parent with Type 2 Diabetes Mellitus have an increased risk of Diabetes. If both parents have Type 2 Diabetes Mellitus, the risk approaches to 40%. Now a day Type 2 Diabetes Mellitus is being diagnosed more frequently in children and young adults, particularly in obese adolescents[3].

Till today, there are very few studies conducted on the young individuals with a family history of Type 2 Diabetes Mellitus in the Indian population. Indeed, its time to add autonomic function tests for monitoring Diabetes Mellitus and its important sequelae.

OBJECTIVES

To study the Glycemic status and Cardiovascular Autonomic Functions and the Correlation between glycemic status and Cardiovascular Autonomic Functions in the offsprings of Type 2 Diabetic Parents in the age group of 18-21 years of BLDEU'S Shri B.M.Patil Medical College, Bijapur.

REVIEW OF LITERATURE

DIABETES MELLITUS

Diabetes mellitus is a clinically and genetically heterogeneous group of disorders characterized by abnormally high levels of glucose in the blood. The hyperglycemia is due to deficiency of insulin secretion or to resistance of body cells to the action of insulin or to a combination of these. Often there is disturbance in fat, protein and carbohydrate metabolism[3].

It has been centuries since this syndrome was first recognized. The term **DIABETES** which is from the Greek meaning to pass through was first used by ARETAEUS of Caappadocia in the second century AD as a generic description for conditions causing increased urine output. The association of polyuria with a sweet tasting substance in the urine was first reported in Sanskrit literature dating from the 5th and 6th century AD at the time of two notable Indian physicians, Susruta and Charaka. The urine of certain polyuria patients was described as ***madhumeha*** i.e., tasting like honey, being sticky to touch and strongly attracting ant.[4]**.**

DIAGNOSIS AND CLASSIFICATION OF DIABETES MELLITUS [5]

Etiological classification of diabetes mellitus

I.Type1 Diabetes --- Beta Cell destruction usually leading to absolute insulin deficiency.

a. Immune mediated

b. Idiopathic

II. Type 2 Diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretary defect with insulin resistance).

III. Other specific types.

A. Genetic defect of Beta cell function

a. Chr12 HNF –α (MODY3)

b. Chr7 glucokinase (MODY2)

c. Chr20 HNF -4α (MODY1)

d. Chr13 insulin promoter factor1 (IPF1, MODY4)

e. Chr17 HNF -1β (MODY5)

f. Chr2 neuroD, (MODY6)

g. Mitochondrial DNA

h. Others

B. Genetic defect in insulin action

a. TypeA insulin resistance

b. Leprechaunism

c. Rabson Mendenhall syndrome

d. Lipoatropic diabetes

e.Others

C. Diseases of the exocrine pancreas

a. Pancreatitis

b. Trauma/ Pancraetectomy

c. Neoplasia

d. Cystic fibrosis

e. Hemochromatosis

f. Fibrocalculous pancreatopathy

D. Endocrinopathies

a. Acromegaly

b. Cushing's syndrome

c. Glucagonoma

d. Pheochromocytoma

e. Hyperthyroidism

f. Somatostatinoma

g. Aldosteronism

h. Others

E. Drugs or chemical induced

a. Vacor

b. Pentamidine

c. Nicotinic acid

d. Glucocorticoids

e. Thyroid hormones

f. Diazoxide

g. β Adrenergic agonists

h. Thiazides

i. Dilantin

j. α-Interferon

F. Infections

a. Congenital rubella

b. Cytomegalovirus

c. Others

G. Uncommon forms of immune mediated diabetes

a. Stiff man syndrome

b. Anti insulin receptor antibodies

c. Others

H. Other genetic syndromes associated with diabetes

a. Down's syndrome

b. Klinefelter's syndrome

c. Turner's syndrome

d. Wolfram's syndrome

e. Friedreich's chorea

f. Huntington's chorea

g. Laurence moon Biedl syndrome

h. Myotonic dystrophy

i. Porphyria

j. Prader Willi syndrome

IV. Gestational diabetes mellitus

DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS [5]

Three ways to diagnose diabetes are possible & each in the absence of unequivocal hyperglycemia, must be confirmed on a subsequent day, by any one of the three methods given in the Table I.

The use of the glycosylated hemoglobin (HbA1C) for the diagnosis of diabetes is not recommended at this time.

Table I: Diagnostic criteria for diabetes mellitus

1. Symptoms of diabetes plus casual plasma glucose concentration ≥200mg/dl(11.1mmol/l). Casual is defined as any time of the day without regard to time since last meal .The classic symptoms of diabetes include polyuria, polydipsia , & unexplained weight loss.

OR

2. FPG ≥126mg/dl(7mmol/l). Fasting is defined as no caloric intake for at least 8hours.

OR

3. Two hours post load glucose ≥200mg/dl(11.1mmol/l) during an OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.

In the absence of unequivocal hyperglycemia these criteria should be confirmed by repeat testing on a different day.

The third measure (OGTT) is not recommended for routine clinical use .The American Diabetes Association. recognizes both tests FPG and OGTT as valid but continue to recommend the FPG as the preferred diagnostic tool because it is more convenient, acceptable to patients and less expensive.

IMPAIRED FASTING GLUCOSE (IFG) is defined as having FPG levels >100mg/dl (5.6mmol/l) but <126mg/dl (7 mmol/l). The categories of FPG values are as follows

Normal FG – FPG <100mg/dl (5.6mmol/l)

Impaired FG-- FPG 100-125mg/dl (5.6mmol/l-6.9mmol/l)

Diagnostic FG-- FPG ≥ 126mg/dl (7mmol/l)

IMPAIRED GLUCOSE TOLERENCE (IGT) is defined as having two hour glucose values in the oral glucose tolerance test of >140mg/dl(7.8mmol/l) but <200mg/dl (11.1mmol/l).

Categories when the OGTT is used are as follows.

Normal OGTT-- 2 h post load glucose <140mg/dl(7.8mmol/dl)

Impaired GT -- 2 h post load glucose 140-199mg/dl(7.8-11.1mmol/l)

Diagnostic post load glucose-- 2 h post load glucose >200mg/dl (11.1mmol/l)

Epidemiology of Diabetes Mellitus.

Global prevalence-- The prevalence of diabetes for all age groups worldwide was 2.8% in 2000 and is estimated to reach 4.4% by 2030.The total number of diabetics is projected to rise from 171million in 2000 to 366million in 2030[6].

Prevalence of diabetes in India.

20% of the current global population resides in South East Asia regions .India comprises 85% of the adult population of South East Asia and therefore the major contribution to diabetic in South East Asia is from India. The prevalence of diabetes in India is 2.4% in rural and 4 to 11.6% in urban dwellers[7].

PATHOGENESIS OF TYPE 2 DIABETES.

Type 2 diabetes mellitus is characterized by three pathophysiologic abnormalities.

1. Impaired insulin secretion

2. Peripheral insulin resistance

3 .Excessive hepatic glucose production

Obesity, particularly visceral or central as evidenced by the waist-hip ratio is very common in Type 2 diabetes mellitus. Insulin resistance associated with obesity augments the genetically determined insulin resistance in Type 2 diabetes mellitus. Adipocytes secrete a number of biologic products (Leptin, tumour necrosis factor A, free fatty acids) that modulate processes such as insulin secretion. Insulin action and body weight may contribute to the insulin resistance[3].

In the early stages of disorder glucose tolerance remains normal despite insulin resistance, because pancreatic β cells compensate by increasing insulin output. As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets become unable to sustain the hyperinsulinemic state, developing impaired glucose tolerance marked by elevation in post prandial glucose. Further decline in insulin secretion and increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia. Ultimately beta cell failure may ensure[3].

Metabolic abnormalities

A. Insulin resistance

This is caused by the decreased ability of insulin to act effectively on peripheral target tissues (muscle, liver) and is a prominent feature of Type 2 diabetes. Resistance to action of insulin impairs glucose utilization by insulin sensitive tissues and increases hepatic glucose output both effects contributing to the hyperglycemia of diabetes[3].

Increased hepatic glucose output predominantly accounts for increased FPG levels, where as decreased peripheral glucose usage results in post prandial hyperglycemia[3].

The precise molecular mechanism of insulin resistance in Type 2 diabetes mellitus has yet to be elucidated. Insulin receptor levels and tyrosine kinase activity in skeletal muscle are reduced, but these alterations are most likely secondary to hyperinsulinemia and are not a primary defects. Therefore post receptor defects are believed to play the predominant role in insulin resistance. A current focus for the pathogenesis of insulin

resistance focuses on a PI-3 kinase signaling defect, which causes reduced traslocation of GLUT4 to the plasma membrane, among other abnormalities[3].

Another emerging theory proposes that elevated level of free fatty acid, a common feature of obesity may contribute to the pathogenesis of Type 2 diabetes in several different ways. Free fatty acid can impair glucose utilization in skeletal muscle, promote glucose production by the liver, and impair beta cell function[3].

B. Impaired insulin secretion

Insulin secretion and sensitivity are interrelated in Type 2 diabetes. Insulin secretion initially increases in response to insulin resistance in order to maintain normal glucose tolerance. Initially insulin secretory defect is mild and selectively involves glucose stimulated insulin secretion[3].

The reason for the decline in insulin secretory capacity in Type 2 Diabetes Mellitus is unclear. Despite the assumption that a second genetic defect super imposed upon insulin resistance leads to beta cells failure, intense genetic investigation has so far excluded mutation in islet candidate genes. Islet amyloid polypeptide or amylin is cosecreted by beta cells and likely forms the amyloid fibrillar deposit found in the islet of individuals with longstanding Type 2 Diabetes Mellitus. Whether such islet amyloid deposits are a primary or secondary event is not known. The metabolic environment may also impact islet function negatively, for example, chronic hyperglycemia paradoxically impairs islet function (glucose toxicity) and leads to a worsening of hyperglycemia. Improvement in

glycemic control is often associated with improved islet function. In addition, elevation of free fatty acid level (lipotoxicity) also worsens islet function[3].

C. Increased hepatic glucose production

In Type 2 diabetes mellitus, insulin resistance in the liver arises from the failure of hyperinsulinemia to suppress gluconeogenesis, which results in fasting hyperglycemia and decreased glucose storage by the liver in the post prandial state. How changes in hepatic glucose flux lead to insulin resistance is not clearly defined[3].

The mechanisms responsible for the increasing hepatic gluconeogenesis include Hyperglucagonemia, increased circulating levels of glucogenic precursors (lactate, alanine and glycerol), increased FFA oxidation, enhanced sensitivity to glucagon and sensitivity to insulin. Although majority of evidence indicates that increased gluconeogenesis is the major cause of hepatic glucose production in Type 2 diabetes mellitus, it is likely that accelerated glycogenolysis may also contribute[3].

Table II: Major risk factors for Type 2 diabetes mellitus[3.]

1. Family history of Diabetes, (parents or siblings with diabetes)
2. Overweight (>BMI 25kg/m^2)
3. Habitual physical Inactivity
4. Race/ ethnicity
5. Previously identified IFG or IGT
6. Hypertension (>140/90mmHg in adults)
7. HDL cholesterol <35mg/dl(0.90mmol/L) and triglyceride level >250mg/dl (2.83mmol/L)
8. History of GDM or delivery of a baby weight >9lb
9. Polycystic ovary syndrome.

PATHOGENESIS OF THE COMPLICATION OF DIABETES MELLITUS

The morbidity associated with long standing diabetes of either type result from a number of serious complications namely neuropathy, retinopathy, and nephropathy. Hence the basis of these complications is the subject of a great deal of research. Most of the available experimental and clinical evidence suggests that the complications are a consequence of the metabolic derangements mainly hyperglycemia. Multicentric clinical trials clearly show delayed progression of microvasculature diabetic complications by strict control of the hyperglycemia[8].

It is well known that Diabetes Mellitus may damage the autonomic nervous system of virtually all organs. In Type 2 diabetic populations there is evidence that the expression of neural damage may be more complex due to over lapping hormonal, metabolic and circulatory effects associated with ageing[9].

MECHANISM OF VASCULAR ABNORMALITIES IN DIABETES MELLITUS [10]

1. Hyperglycemia

Increased diacylglycerol

Protein kinase C activation

Increased sorbitol

2. Hyperinsulinemia

3. Oxidative stress

Reactive oxygen species

Carbonyl overload

4. Advanced glycation end products (AGEs)

Activation of nuclear factors kappa B (NF kappa B)

Overproduction of inflammatory cytokines

5. Dyslipidemia

Small dense LDL

Low HDL

Hypertriglyceridemia

6. Procoagulant antifibrinolytic state

Elevated fibrinogen

Increased plasminogen activator inhibitor (PAI)

Heightened platelet function

7. Genetic abnormalities

Peroxisomal proliferation activating receptors-gamma mutation.

ACTIVATION OF PROTEIN KINASE C IN DIABETES

In diabetes hyperglycemia can lead to an increased concentration of the metabolite diacylglycerol in the cell. Diacylglycerol is a classic activator of a family of enzymes that performs key regulatory functions by phosphorylating proteins important in metabolic control. This family of enzymes known as protein kinase C has some dozen members. A great deal of recent work has implicated activation of the PKC family in the vascular complication of diabetes. Activation of PKC can inhibit expression of the endothelial forms of nitric oxide synthase and thus promote impaired endothelial vasodilatation function. PKC can also augment cytokine induced tissue factor gene expression and procoagulant activity in human endothelial cell[10].

Glucose induced activation of PKC can augment the production of extracellular matrix macromalecules that accumulate during atherosclerotic lesion formation. PKC activation can also increase the production of proinflammatory cytokines and the proliferation of vascular wall cell. In vivo evidence supports a role of PKC activation in the pathogenesis of various aspects of vascular dysfunction[10].

NON ENZYMATIC GLYCOSYLATION

This refers the process by which glucose chemically attaches to the amino group of protein without the aid of enzymes. Glucose forms chemically reversible glycosylation products with protein (named Schiff bases) that may rearrange to form more stable Amadori-type early glycosylation products, which are also chemically reversible. The degree of enzymatic glycosylation is directly related to the level of blood glucose[8].

The early glycosylation products on collagen and other long lived proteins in interstitial tissues & blood vessel walls, rather than dissociating, undergo a slow series of rearrangements to form irreversible advanced glycosylation end products which accumulate over the lifetime of vessel wall. AGEs have number of chemical and biologic properties that are potentially pathogenic[8].

Chemical and Biologic properties of Advanced glycation end products (AGEs)

Chemical properties

Cross-link polypeptides of same protein Eg: Collagen

Trap nonglycosylated proteins Eg: LDL, Ig Complement

Confer resistance to proteolytic digestion

Induce lipid oxidation

Inactivate nitric oxide

Bind nucleic acid

Biological properties

Bind to AGE receptors on monocytes and mesenchymal cells--**Induce**

Monocyte emigration

Cytokines and growth factor secretion

Increase vascular permeability

Procoagulant activity

Enhanced cellular proliferation

Enhanced extracellular matrix production

AGE formation occurs on proteins, lipids, nucleic acids. On proteins such as collagen, they cause cross links between polypeptides of the collagen molecules and also trap nonglycosylated plasma or interstitial proteins.

In large vessels, trapping low density lipoproteins (LDL), retard its efflux from the vessel wall and enhances the deposition of cholesterol in the intima thus accelerating atherogenesis. In capillaries including those of renal glomeruli, plasma proteins such as

albumin bind to the glycosylated basement membrane, accounting in part for the increased basement membrane thickening characteristic of diabetic microangiopathy. AGE cross linked proteins are resistant to proteolytic digestion. Thus cross-linking decreases protein removal while enhancing protein deposition. AGE induced cross linking in collagen type IV in basement membrane may also impair the interaction of collagen with other matrix component (laminin, proteoglycan), resulting in structural and functional defects in the basement membrane.

AGE binds to receptors on many cell types, endothelium, monocytes, macrophages, lymphocytes, and mesangial cells. Binding induces a variety of biological activities, including monocyte emigration, release of cytokines and growth factors from macrophages, increased endothelial permeability, increased procoagulant activity on endothelial cells and macrophages and enhanced proliferation of and synthesis of extracellular matrix by fibroblasts and smooth muscle cells. All these effects can potentially contribute to diabetic complications[10].

AUTONOMIC NERVOUS SYSTEM

History

The early concepts of ANS date back to the roman period, during which Galen (A.D. 130-200) described sympathetic chain ganglion & the rami communicantes. 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" [11].

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[11].

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[11].

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[12].

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" [11].

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[11].

ANS controls most visceral functions of the body. This system helps to 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[1].

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"[13].

Anatomic organization of the autonomic outflow:

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[13].

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[13].

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[13].

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 communicantes 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 communicantes & 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[13].

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[13].

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[13].

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 & abd ominal 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'[13].

Parasympathetic division:-

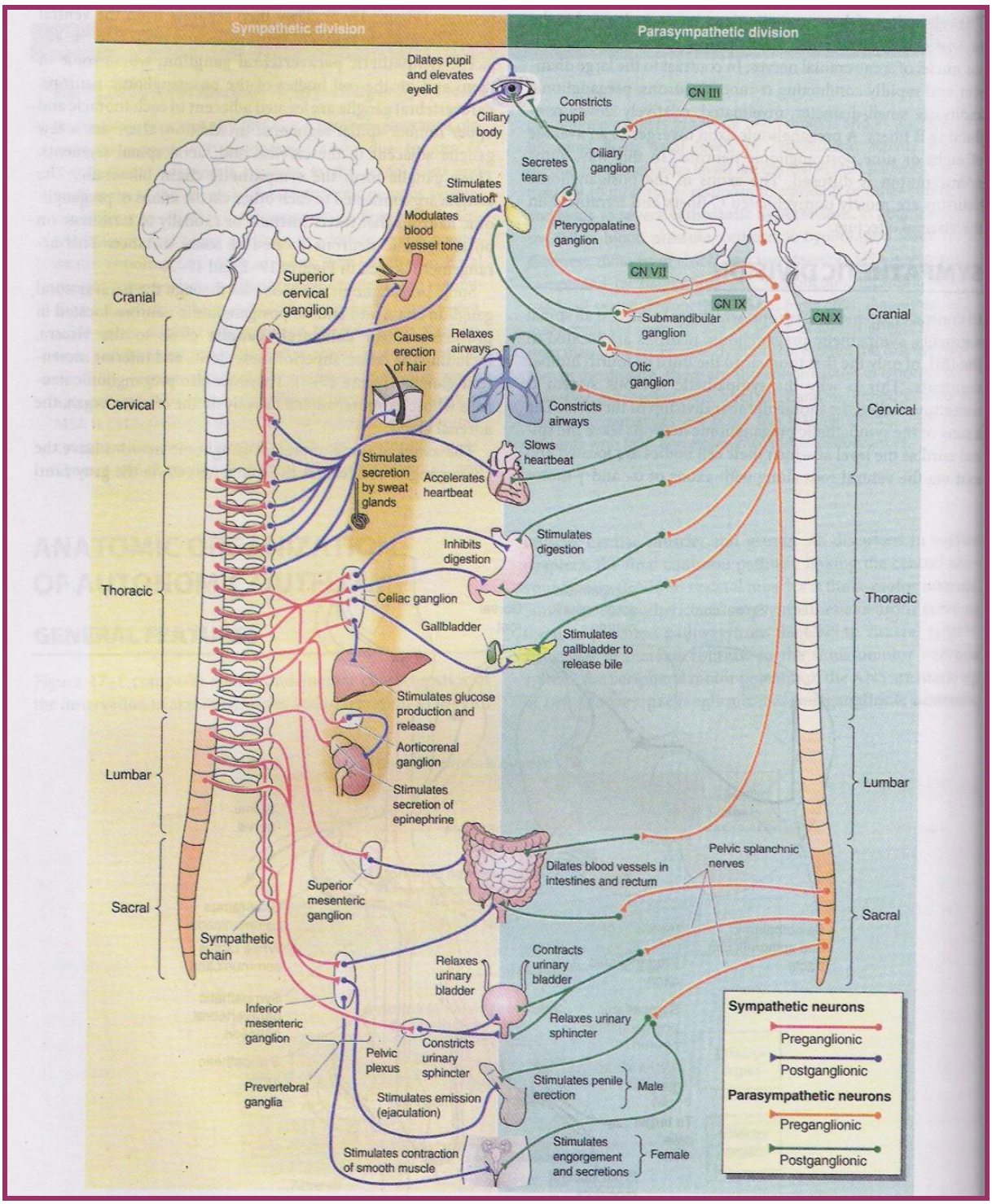
Generally the functions promoted by this division are those concerned with the vegetative aspects of day to day living, example digestion & absorption of food. Hence the cholinergic division is called as anabolic & the sympathetic as catabolic.

The various functions of sympathetic & parasympathetic divisions aim at maintaining homeostasis. 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[14].

Both sympathetic and parasympathetic nerve may be affected, with a parasympathetic dysfunction preceding sympathetic dysfunction. Although symptomatic somatosensorimotor neuropathy usually preceeds the development of symptomatic autonomic neuropathy , sometimes signs of parasympathetic neuropathy appears before other signs of neuropathy[15].

Pathological studies of diabetic nerves have shown segmental demyelination, in addition to axonal loss, vasculopathy, and inflammatory infiltrates, which finally results in neuropathy[16].

Figure 1: Organization of Autonomic Nervous System



AUTONOMIC INNERVATION TO THE CARDIOVASCULAR SYSTEM

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[11].

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[11].

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[11].

Sympathetic outflow to the arteries, arterioles & ve ins 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[11].

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[11].

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[17].

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[18, 19, 20], Heart rate response during periods of deep breathing[21], The Heart rate response to standing up[22, 23] and the diastolic blood pressure response to sustained hand grip[24,25], Blood pressure response to standing[21] 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[26,27]. 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[19].

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[28].

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[13]

In presence of autonomic imbalance or autonomic neuropathy, the change in heart rate during respiration is impaired or even absent[29]. 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[21].

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[30].

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[31]. 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[32,33,]

The change of posture (lying down to standing) – displaces about 600 ml of blood from thorax to the legs[34]. This deactivates baroreceptors to produce a reflex tachycardia which is mediated mostly by vagal tone withdrawal[29]. Displacement of blood produces s light 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[35].

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 in 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. Values of 1.04 or more are taken as normal for heart rate response, and fall of 10 mm Hg or less for systolic blood pressure response to standing (D.J.Ewing and B.F.Clarke)[21].

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[21].

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[36].

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.

DIABETIC AUTONOMIC NEUROPATHY (DAN)

Although diabetic autonomic neuropathy (DAN) is a commonly encountered complication in diabetes, it is underdiagnosed and ignored despite its impact on the survival and quality of patients with diabetes[37].

Symptomatic autonomic complications are life-threatening. Estimates of mortality range from 25%-50% within 5-10 years of diagnosis. The 5 year mortality of patients with diabetic autonomic neuropathy is three times higher than in diabetic patients without autonomic involvement[37].

PATHOGENESIS

Factors that contribute to the pathogenesis of somatic neuropathy like hyperglycemia, activation of polyol pathway, accumulation of sorbitol, and changes in NAD/NADH ratio also lead to Diabetic Autonomic Neuropathy. There is a compromise of endoneural blood flow and vasoconstriction in microvascular blood flow due to activation of PKC. Increase in free radical species causes damage to the endothelium and reduces nitric oxide bioavailability. Nitric oxide combines with superoxide anions to form peroxynitrite radicals that damage neurons and endothelium. Other mechanisms of damage include

reduction in neurotrophic growth factors, formation of AGEs, and deficiency of essential fatty acids. These compromise endoneural blood flow and alter nerve function[37].

CLINICAL FEATURES

DAN can go unrecognized because it involves multiple systems and often has confusing, nonspecific features. Although patients often present with signs and symptoms confined to the specific organ, there is generally widespread subclinical involvement, and it is essential that all other organs be evaluated[37].

The most commonly involved organs systems are the cardiac, adrenomedullary, genitourinary, gastrointestinal systems, as well as sweat glands and pupils.

CARDIAC AUTONOMIC NEUROPATHY

Cardiac autonomic neuropathy is the life threatening complication, which results from damage to the autonomic nerves that supply the heart, causing abnormal heart rates and rhythms. The earliest sign of cardiac autonomic neuropathy is loss of heart rate variability. Other symptoms include orthostatic hypotension, Exercise intolerance, and silent myocardial ischemia[37].

Following studies have been done on glycemic status and cardiovascular autonomic function tests in healthy offsprings of Type 2 Diabetic Parents.

A Fiorentini, A Perciaccante, A Paris, P Serra, and L Tubani studied heart rate variability (HRV) with 24-hours ECG Holter (HRV), the circadian autonomic activity in offspring of type 2 diabetic subjects and the relation with insulin-resistance. 50 Caucasian offsprings of type 2 diabetic subjects were divided in two groups: insulin-resistant offsprings (IR) and non insulin-resistant offsprings (NIR). The results indicated that familiarity of Type 2 Diabetes Mellitus is related to a global reduction and alteration of circadian rhythm of autonomic activity and insulin resistance in the offsprings of Type 2 Diabetic subjects[38].

C. Hauerslev Foss , E. Vestbo, A. Frøland, H.J. Gjessing, C.E. Mogensen and E.M.Damsgaard conducted study on Cardiovascular heart rate reflex tests, heart rate variation to deep breathing, valsalva manoeuvre and heart rate response to standing up on 223 nondiabetic offsprings of Type 2 Diabetic subjects and control group of 258 offsprings of nondiabetic subjects. The results indicated reduced heart rate variation, lower mean values for Cardiovascular heart rate reflexs and reduced diurnal blood pressure variation in nondiabetic offsprings of Type 2 Diabetic subjects. This study indicates that parental type 2 diabetes has an impact on the cardiac autonomic function in nondiabetic subjects**[39].**

A study was conducted on autonomic balance, which was assessed by spectral analysis of heart rate variability(SA-HRV) on 22 offsprings of Non-insulin dependent Diabetes Mellitus parents and 20 controls. The results showed significantly higher values of LF/HF ratio in offsprings of Non-insulin dependent Diabetes Mellitus parents[40].

Ferdinando Iellamo; Manfredi Tesauro; Stefano Rizza; Stefano Aquilani; Carmine Cardillo; Micaela Iantorno conducted study on Cardiovascular Autonomic regulation in 27 healthy first degree relatives(FDRs) of parents with Type 2 Diabetes Mellitus, and 15 age and gender matched control subjects. In 27 healthy first degree relatives(FDRs) of Diabetic parents, there was an impairment in the endothelial and Autonomic Nervous System functioning, which manifested with increase in the vascular sympathetic outflow and depressed baroreflex vagal control of heart rate[41].

Simona Frontoni; Daniela Bracaglia; Alessandra Baroni; Fabio Pellegrini; Michela Perna; Elena Cicconetti; conducted study on 24 hour blood pressure monitoring and assessment of heart rate variability on 69 offsprings of Type 2 Diabetic subjects and 11 control subjects with a negative family history for type 2 Diabetes. The results indicated that offsprings of Diabetic subjects had insulin resistance, an abnormal circadian rhythm of blood pressure, with reduced fall in nocturnal blood pressure and abnormalities in sympathetic activation[42].

Frontoni S, Pellegrinotti M, Bracaglia D, Farrace S, Caselli A, Baroni A, conducted study on Low to High frequency ratio(LF/HF), which was an index of sympatho-vagal balance. It was calculated by heart rate spectral analysis on 9 offsprings of Type 2 Diabetic Patients compared with 18 subjects without family history of Diabetes. The results showed significantly increased LF/HF ratio and decreased diastolic blood pressure in offsprings of Type 2 Diabetic Parents. This, in turn, may determine a chronic sympathetic activation, which could be involved in the pathogenesis of Type 2 diabetes mellitus.[43].

Laitinen T, Vauhkonen IK, Niskanen LK, Hartikainen JE, Lansimies EA, Uusitupa MI, studied Heart Rate variability during euglycemic –hyperinsulinemic clamp in 35 nondiabetic offsprings of Type 2 Diabetic Parents and 19 control subjects. Results showed increased total power of Heart Rate variability and LF/HF ratio and decreased power of the high frequency spectral component during clamp study in nondiabetic offsprings of Type 2 Diabetic Parents. We conclude that the HRV response to acute hyperinsulinemia in the offspring of type 2 diabetic probands was likely to be modulated by the type 2 diabetic phenotype of the parent[44].

MATERIALS AND METHODS

Experimental design

The cross-sectional study was carried out in 30 healthy offsprings of Type 2 Diabetic Parents (Study group) and 30 healthy offsprings of Nondiabetic Parents (Control group) in the age range of 18 - 21 years randomly selected among 1st MBBS students of BLDEU'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) Subjects having diabetes mellitus, bronchial asthma, giddiness on standing, syncopal spells, visual disturbances, nocturnal diarrhea.
3) Subjects receiving drugs that are known to interfere with cardiac function or respiratory functions such as beta blockers, sympathomimetic drugs, vasodilators and diuretics.
4) Associated disease or conditions known to affect autonomic function like Guillean Barre syndrome, Poliomyelitis, Diphtheria, Tuberculosis, Syphilis, Amyloidosis, Chronic renal failure.
5) Subjects with history of alcohol intake.
6) Subjects with history of tobacco consumption in any form.
7) Any disease condition affecting the autonomic nervous system.

Study group: This group consists of 30 normal healthy male medical students (Offsprings of Type 2 Diabetic Parents) of BLDEU'S Shri B.M.Patil Medical College, Bijapur.

Duration of the study: December 2009 to November 2010.

Control group: This group consists of 30 age matched normal healthy male medical students (Offsprings of Nondiabetic Parents) of BLDEU'S Shri B.M.Patil Medical College, Bijapur.

Age of the subjects: Male subjects in the age group of 18-21 years are included

Sample size:

As per the literature and reports of WHO, the prevelance of Diabetes Mellitus in the urban Indian population ranges from 4% to 11%, therefore an average rate of 8% is chosen for the study[45].

As per the previous studies[40], the allowable error is 10%. Hence, sample size "n" is 30.

The statistical formula used is as follows:

$$n = \frac{4pq}{L^2}$$ where p=8%, q= 1-p =92%, L=10%

Thus,

$$n = \frac{4 \times 0.92 \times 0.08}{(0.1)^2} = 30$$

As all the distributions will merge into normal distribution, sample size i.e., 30 is enough because inference that can be drawn based on 30 observations will more or less remain the same inspite of any increase in sample size.[46]

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 Computerized Physiopac(Medicaid). 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 Cardiovascular Autonomic Nervous System Function Parameters are selected as recommended by American Diabetic Association and performed as per methods described by Sir Roger Bannister[32].

A) The Parasympathetic activity is 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 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 is asked to stand up unaided within 5 seconds and to remain standing for 1 minute. Continuous ECG recording was done during and 1 minute after standing.. 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. 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. Difference between average heart rate during inspiration and expiration is taken as heart rate response (beats/min) during deep breathing.

B. **The sympathetic activity is 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 is asked to stand up unaided and remain standing. Systolic blood pressure (SBP) is recorded in resting supine position, then cuff is inflated above systolic blood pressure before subject stands up, and SBP is recorded again immediately when he stands up. And the difference in SBP in mm of Hg was noted.

2. Blood pressure response to sustained Hand grip exercise

The subject was asked to sit comfortably in chair. Initially the subject is asked to exert maximal hand grip strength on hand grip dynamometer with dominant hand. Then the subject is asked to exert 30% of maximal hand grip strength for 5 minutes (at least for 3 min) with dominant hand. Diastolic blood pressure is measured in the non-dominant

hand at rest and at one minute intervals during hand grip. The maximum rise in diastolic BP (mm of Hg) during hand grip is noted.

Grading (Ewing and Clarke) [21] 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(30:15)	>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[47]

Scores ≤ 3-- Normal autonomic function

Scores > 3 & < 8 -- Borderline dysfunction

Scores ≥ 8 to 10 --Abnormal function.

Glycemic status of an individual.

Glycemic status of an individual is determined by Oral Glucose Tolerance Test[38].

1. Fasting blood glucose.
2. Two hours After Glucose Load (Consisting of 75g glucose anhydrate in 300ml of water ingested over the course of 5 minutes).

Statistical Analysis

Statistical Analysis is done in consultation with statistician

All values are presented as Mean ± Standard Deviation (Mean ± SD). Comparison of mean values of parameters between Control and Study group is done by Z test. Correlation between various autonomic function parameters & glycemic status is done by correlation.

1. p Value >0.05 is taken as not significant[48].
2. p Value <0.05 is taken as significant[48].
3. p Value <0.01 is taken as highly significant[48].
4. p Value <0.001 is taken as very highly significant[48].

Figure 2: Showing Computerized 4-channel Physiopac.

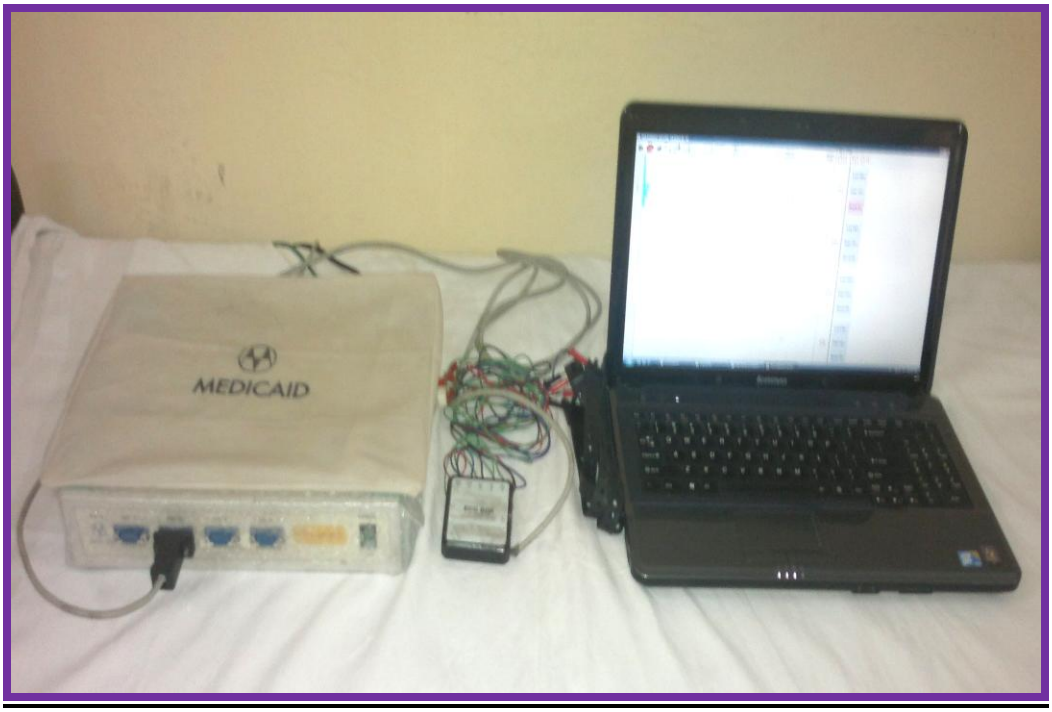


Figure 3: Showing instruments used: Hand grip Dynamometer, Stethoscope, Sphygmomanometer

Figure 4: Recording of ECG at rest.

Figure 5: Recording of Heart rate response to Valsalva maneuver

Figure 6: Recording of Heart rate response to standing

Figure 7: Recording of Blood pressure response to Sustained Handgrip

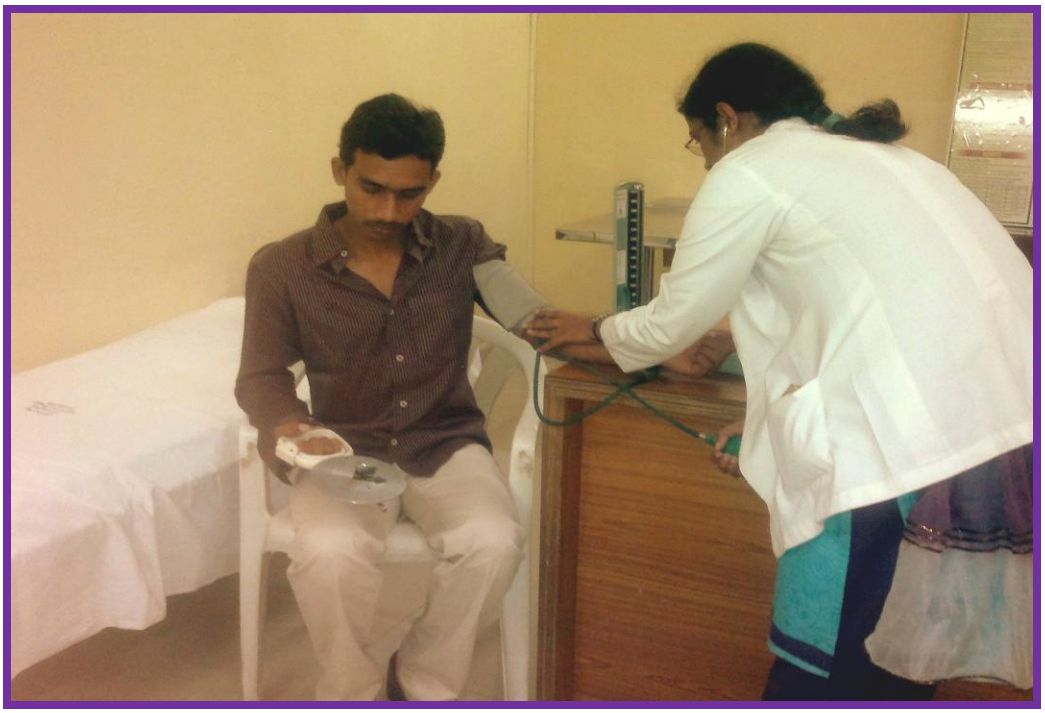


Figure 8: ECG showing Heart rate response to standing

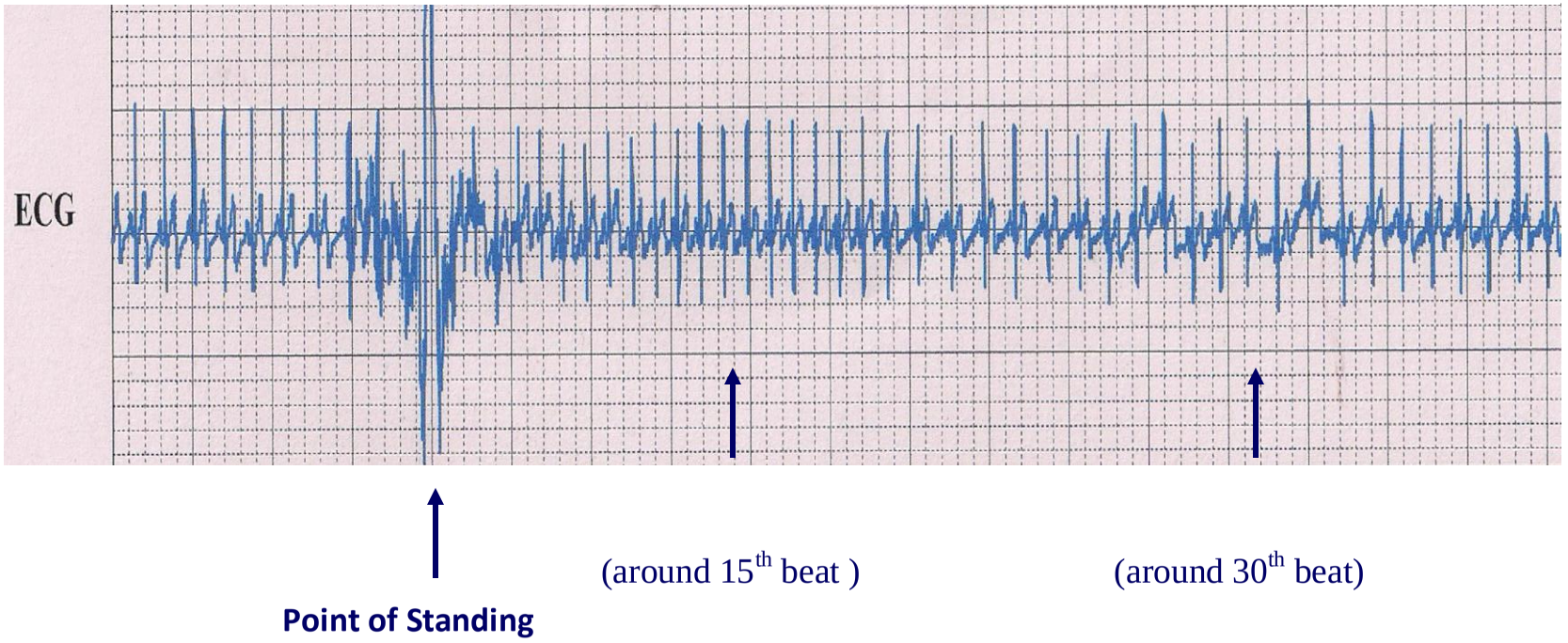


Figure 9: ECG showing Heart rate response to Valsalva maneuver

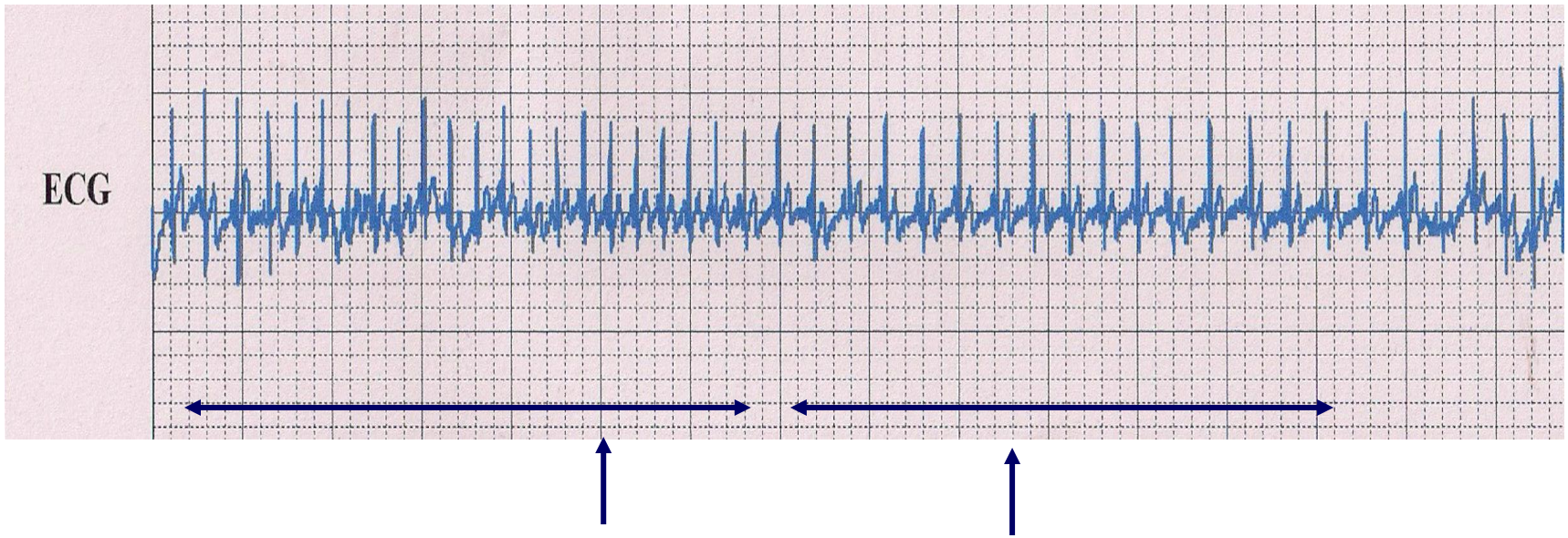
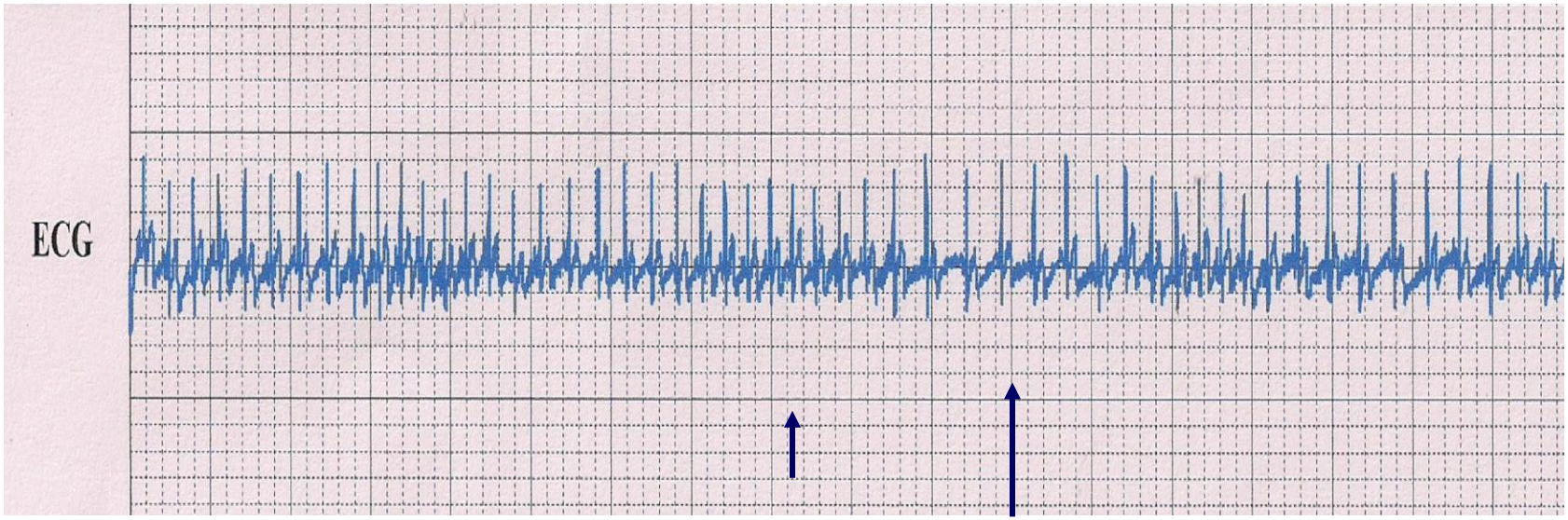


Figure 10: ECG showing Heart rate variation during deep breathing



RESULTS

Statistical Analysis:

The cross-sectional study of Glycemic status and Cardiovascular Autonomic Functions and the Correlation between glycemic status and Cardiovascular Autonomic Functions was conducted in the offsprings of Type 2 diabetic parents and offsprings of nondiabetic parents in the age group of 18-21 years of B.L.D.E.U'S Shri. B. M. Patil Medical College, Bijapur.

Total number of subjects in each group is as follows.

I] Control (Offsprings of Nondiabetic Parents), n=30

II] Study (Offsprings of Type 2 Diabetic Parents), n=30

Study Group is further divided into 3 subgroups based on history of diabetes in the family.

Study Group1(S1):one parent diabetic and other parent with no family history of diabetes, n=17.

Study Group 2 (S2): one parent diabetic and other parent with family history of diabetes, n=9

Study Group 3(S3): both parents diabetic, n=4

Results of individual Anthropometric, Physiological and Autonomic function test parameters in control and study is represented in Annexure 4a, 4b, individually:

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

I. Anthropometric Parameters:

The Mean Value and Standard Deviation, Level of Significance of each parameter is calculated for each group and presented in Table-1A and for each subgroup is presented in Table-1B and Graph 1 & 2.

Table 1A: Anthropometric Parameters (Mean ± SD) of Control and Study Group:

Parameters	**Control Group**	**Study Group**	**Level of significance**
Age (Years)	18.86 ± 1.04	19.0 ± 0.78	0.293
Height (cms)	170.16± 5.14	172.73±6.51	0.04*
Weight (Kg)	65.2 ± 10.82	70.16 ± 11.01	0. 04*
BMI (*kg/m^2*)	22.52 ± 3.55	23.51 ± 3.33	0.13
BSA (Sq m)	1.76 ±0.13	1.83 ± 0.15	0. 01**

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

Table 1B:

Anthropometric Parameters (Mean ± SD) of Control and Subgroups of Study Group:

Parameters	**Control Group**	**Study Group-1**	**Study Group-2**	**Study Group-3**
Age (Years)	18.86 ± 1.04	19.23 ± 0.83 (p =0.02) *	18.77± 0.66 (p =0.38)	18.5± 0.7 (p =0.14)
Height (cms)	170.16± 5.14	170.35 ± 6.08 (p =0.46)	176.66± 5.89 (p =0.001) ***	174.0± 6.27 (p =0.12)
Weight (Kg)	65.2 ± 10.82	65.58 ± 8.32 (p =0.45)	74.11± 11.53 (p =0.02) *	80.75± 11.52 (p =0.005) **
BMI (kg/m^2)	22.52 ± 3.55	22.60 ± 2.77 (p =0.46)	23.87± 3.92 (p =0.18)	26.57± 2.79 (p =0.004) **
BSA (Sq m)	1.76 ±0.13	1.77 ± 0.12 (p =0.38)	1.91± 0.13 (p =0.002) **	1.95± 0.16 (p =0.01) **

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

1A] Age (Years) of Control and Study Group:

Mean ± SD of Control Group- 18.86 ± 1.04

Mean ± SD of Study Group - 19.0 ± 0.78

There is no variation in age of both Groups.

1B] Age (Years) of Control and Subgroups of Study Group:

Mean ± SD of Control Group- 18.86 ± 1.04

Mean ± SD of Study group 1(S1)- 19.23 ± 0.83

Mean ± SD of Study group 2(S2)- 18.77± 0.66

Mean ± SD of Study group 3(S3)- 18.5 ± 0.57

There is no variation in age of the subjects in Subgroups of Study Group compared to Control Group.

2A] Height (cms) of Control and Study Group:

Mean ± SD of Control Group- 170.17 ± 5.14 cms.

Mean ± SD of Study Group - 172.73 ± 6.51 cms

There is significant (p=0.04)* increase in the height of the subjects in Study Group by 2.56 cms compared to Control Group.

2B] Height (cms)of Control and Subgroups of Study Group:

Mean ± SD of Control Group- 170.17 ± 5.14

Mean ± SD of Study group 1(S1)- 170.35 ± 6.08

Mean ± SD of Study group 2(S2)- 176.66± 5.89

Mean± SD of Study group 3(S3)- 174.0± 6.27

There is significant (p value=0.001) *** increase in the height of the subjects in Subgroup-2 of Study Group by 6.5cms compared to Control Group.

3A] Weight (Kg) of Control and Study Group:

Mean ± SD of Control Group- 65.2±10.82

Mean ± SD of Study Group - 70.17±11.01.

There is significant (p=0.04) * increase in the weight of the subjects in Study Group by 5kg compared to Control Group.

3B] Weight (Kg) of Control and Subgroups of Study Group:

Mean ± SD of Control Group- 65.2±10.82

Mean ± SD of Study group 1(S1) - 65.58 ± 8.32

Mean ± SD of Study group 2(S2) - 74.11 ± 11.53

Mean± SD of Study group 3(S3) - 80.75 ± 11.52

There is significant increase in the weight of the subjects in Subgroup-2 (p =0.02) * by 8.5kg & Subgroup-3 (p =0.005) ** of Study Group by 15kg compared to Control Group.

4A] BMI (kg/m^2) of Control and Study Group:

Mean ± SD of Control Group- 22.52±3.55

Mean ± SD of Study Group - 23.51±3.33

There is insignificant (p=0.13) increase in the BMI of subjects in Study Group compared to Control Group.

4B] BMI (kg/m^2) of Control and Subgroups of Study Group:

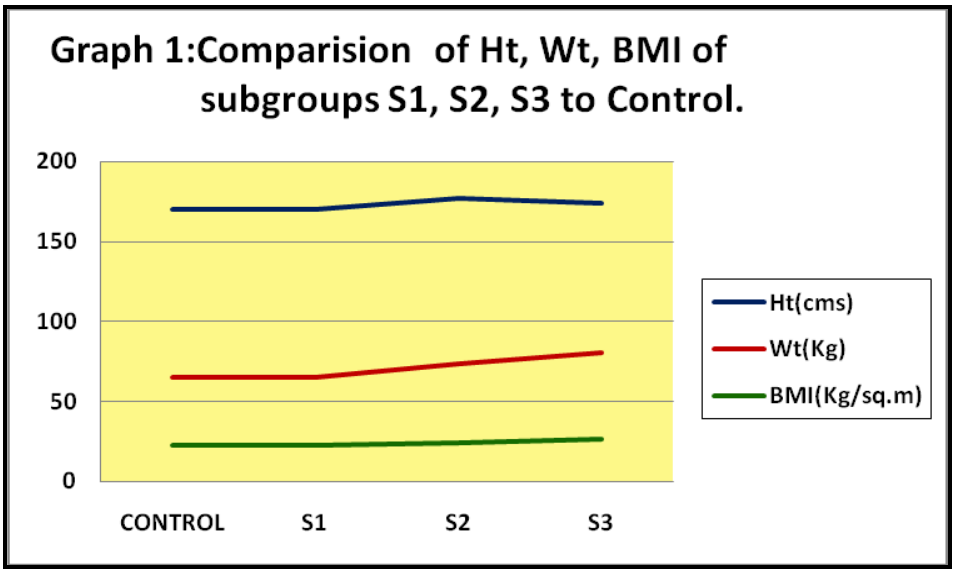
Mean ± SD of Control Group- 22.52±3.55

Mean ± SD of Study group 1(S1) - 22.60 ± 2.77

Mean ± SD of Study group 2(S2) - 23.87± 3.92

Mean± SD of Study group 3(S3) - 26.57± 2.79

There is significant (p=0.004) ** increase in the BMI of subjects in Subgroup-3 of Study Group compared to Control Group.



5A] <u>BSA (Square meter) of Control and Study Group:</u>

Mean ± SD of Control Group- 1.76±0.13

Mean ± SD of Study Group - 1.83±0.15

There is significant (p=01) ** increase in the BSA of subjects in Study Group compared to Control Group.

5B] <u>BSA (Square meter) of Control and Subgroups of Study Group:</u>

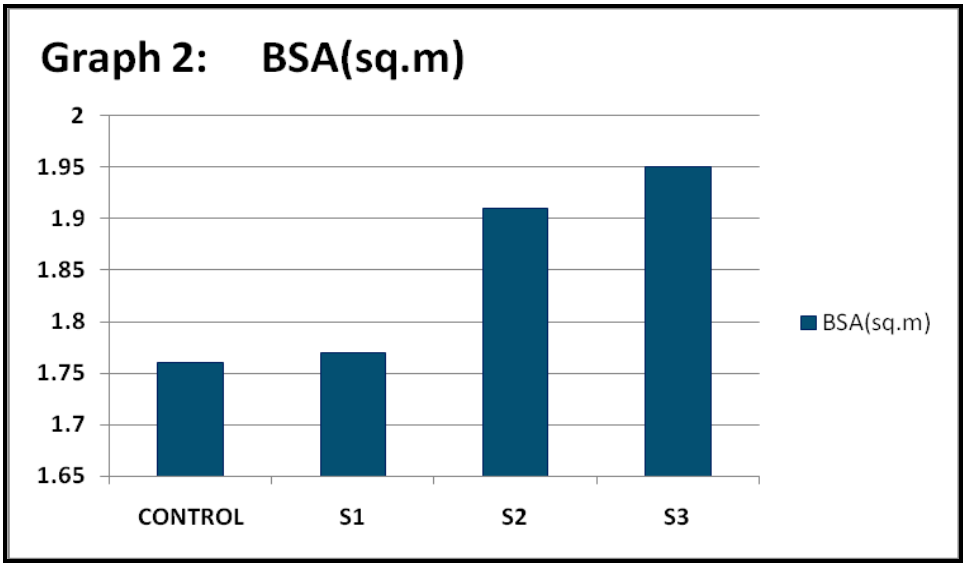
Mean ± SD of Control Group- 1.76±0.13

Mean ± SD of Study group 1(S1)- 1.77 ± 0.12

Mean ± SD of Study group 2(S2)- 1.91± 0.13

Mean± SD of Study group 3(S3)- 1.95± 0.16

There is significant increase in the BSA of subjects in Subgroup-2 (p =0.002) ** & Subgroup-3 (p =0.01) ** of Study Group compared to Control Group.



II Physiological Parameters

The Mean Value and Standard Deviation, Level of Significance of each parameters like, Resting pulse rate (bpm), Respiratory rate (cycles per minute), Systolic & Diastolic blood pressure (mm Hg) are calculated for each group and presented in Table-2A and for each subgroup is presented in Table-2B and Graph 3.

Table 2A: Physiological Parameters (Mean ± SD) of subjects in control and study group.

Parameters	**Control Group**	**Study Group**	**Level of significance**
Resting PR (bpm)	78.8±6.31	77.86±5.75	0.279
Resting RR (cycles/min)	14.03±2.51	15.50±1.57	0.003***
Resting SBP (mm of Hg)	119.53±11.9	124.13±12.80	0.07
Resting DBP (mm of Hg)	77.06±5.29	77.66±6.74	0.35

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

Table 2B:Physiological Parameters (Mean ± SD) of subjects in control and subgroups of Study group.

Parameters	Control Group	Study Group-1	Study Group-2	Study Group-3
Resting PR (bpm)	78.8±6.31	76.58±4.62 (p =0.08)	77.77±6.43 (p =0.34)	83.5±6.6 (p =0.09)
Resting RR (cycles/min)	14.03±2.51	15.05±1.51 (p =0.04) *	16.22±1.3 (p =0.001) ***	15.75±2.06 (p =0.06)
Resting SBP (mm of Hg)	119.53±11.9	122.23±12.46 (p =0.23)	125.55±13.59 (p =0.11)	129.0±14.28 (p =0.10)
Resting DBP (mm of Hg)	77.06±5.29	76.58±6.77 (p=0.40)	78.66±6.92 (p =0.26)	80.0±7.11 (p =0.21)

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

1A] Resting Pulse Rate (beats/min) of Control and Study Group:

Mean±SD of Control Group- 78.80±6.31

Mean ± SD of Study group- 77.86±5.75

There is insignificant (p=0.27) decrease in the Resting Pulse Rate of subjects in Study Group compared to Control Group.

1B] Resting Pulse Rate (beats/min) of Control and Subgroups of Study Group:

Mean±SD of Control Group- 78.80±6.31

Mean ± SD of Study group 1(S1)- 76.58±4.62

Mean ± SD of Study group 2(S2)- 77.77±6.43

Mean± SD of Study group 3(S3)- 83.5 ± 6.6

There is insignificant variation in the Resting Pulse Rate of subjects in Subgroups of Study Group compared to Control Group .

2A]Resting Respiratory Rate (cycles/min) of Control and Study Group:

Mean±SD of Control Group- 14.03±2.51

Mean ± SD of Study group - 15.50±1.57

There is a highly significant (p=0.003) ** increase in the Resting Respiratory Rate of subjects in Study Group compared to Control Group.

2B]Resting Respiratory Rate (cycles/min) of Control and Subgroups of Study Group:

Mean±SD of Control Group- 14.03±2.51

Mean ± SD of Study group 1(S1)- 15.05±1.51

Mean ± SD of Study group 2(S2)- 16.22±1.3

Mean± SD of Study group 3(S3)- 15.75±2.06

There is significant increase in the Resting Respiratory Rate of subjects in Subgroup-1(p value=0.04) *and Subgroup-2(p value=0.001) *** of Study Group compared to Control Group .

3A]Resting Systolic Blood Pressure (mm of Hg) of Control and Study Group:

Mean±SD of Control Group- 119.53±11.90

Mean ± SD of Study group - 124.13±12.80

There is insignificant (p=0.076) increase in the Resting SBP of subjects in Study Group compared to Control Group.

3B]Resting Systolic Blood Pressure (mm of Hg)of Control and Subgroups of Study Group:

Mean±SD of Control Group- 119.53±11.90

Mean ± SD of Study group 1(S1)- 122.23±12.46

Mean ± SD of Study group 2(S2)- 125.55±13.59

Mean± SD of Study group 3(S3)- 129.0±14.28

There is insignificant increase in the Resting SBP of subjects in Subgroups of Study Group compared to Control Group .

4A]Resting Diastolic Blood Pressure (mm of Hg) of Control and Study Group:

Mean±SD of Control Group- 77.06±5.29

Mean ± SD of Study group- 77.66±6.74

There is insignificant (p=0.354) increase in the Resting Diastolic Blood Pressure of subjects in Study Group compared to Control Group.

4B]Resting Diastolic Blood Pressure(mm of Hg) of Control and Subgroups of Study Group:

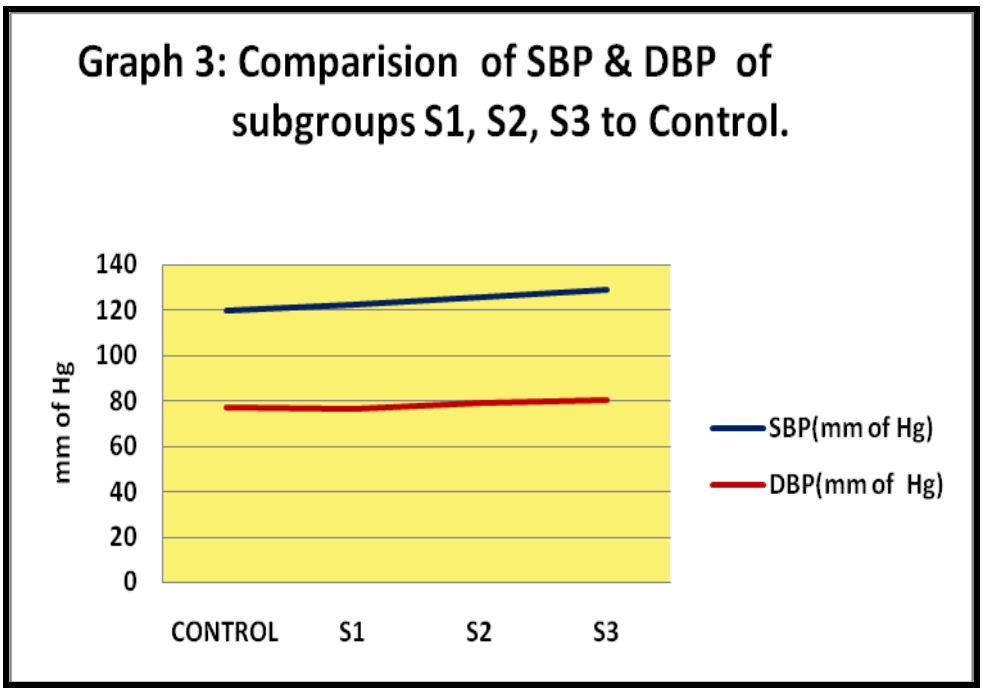
Mean±SD of Control Group- 77.06±5.29

Mean ± SD of Study group 1(S1)- 76.58±6.77

Mean ± SD of Study group 2(S2)- 78.66±6.92

Mean± SD of Study group 3(S3)- 80.0±7.11

There is insignificant increase in the Resting Diastolic Blood Pressure of subjects in Subgroups of Study Group compared to Control Group .



III AUTONOMIC FUNCTION PARAMETERS IN SUBJECTS OF CONTROL AND STUDY GROUP

The Mean Value and Standard Deviation, Level of Significance of various Autonomic function parameters like 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 are calculated for each group and presented in Table-3A and for each subgroup is presented in Table-3B and Graph 4 & 5.

Table 3A: Autonomic function parameters of subjects in Study and Control Groups.

Autonomic function parameters	Control Group	Study Group	Level of significance
Valsalva Ratio	1.33 ± 0.20	1.29 ± 0.24	0.222
HR variation to deep breathing (Maximum-Minimum)	28.56 ± 7.44	26.25 ± 8.47	0.132
Immediate HR response to standing (30:15)	1.34 ± 0.20	1.31 ± 0.20	0.335
BP response to Standing (Fall in SBP)	4.66 ± 1.76	4.86 ± 2.33	0.358
BP response to sustained Hand grip (Increase in DBP)	21.0 ± 3.95	19.4 ± 5.68	0.105

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

Table 3B: Autonomic function parameters of subjects in Control and subgroups of Study Group.

Autonomic function parameters	**Control Group**	**Study Group-1**	**Study Group-2**	**Study Group-3**
Valsalva Ratio	1.33 ± 0.20	1.34± 0.26 (p =0.42)	1.25 ± 0.20 (p =0.16)	1.11 ± 0.13 (p =0.002) **
HR variation to deep breathing (Maximum-Minimum)	28.56 ± 7.44	27.18 ± 7.31 (p =0.26)	25.40 ± 7.15 (p =0.12)	24.22 ± 16.13 (p =0.3)
Immediate HR response to standing (30:15)	1.34 ± 0.20	1.35 ± 0.24 (p =0.43)	1.29 ± 0.16 (p =0.22)	1.2 3 ± 0.12 (p =0.05) *
BP response to Standing (Fall in SBP)	4.66 ± 1.76	4.94 ± 2.56 (p =0.35)	4.66 ± 2.0 (p =0.5)	5.0 ± 2.58 (p =0.4)
BP response to sustained Hand grip (Increase in DBP)	21.0 ± 3.95	20.94 ± 5.83 (p =0.49)	17.11 ± 4.48 (p =0.01) *	18.0 ± 6.73 (p =0.19)

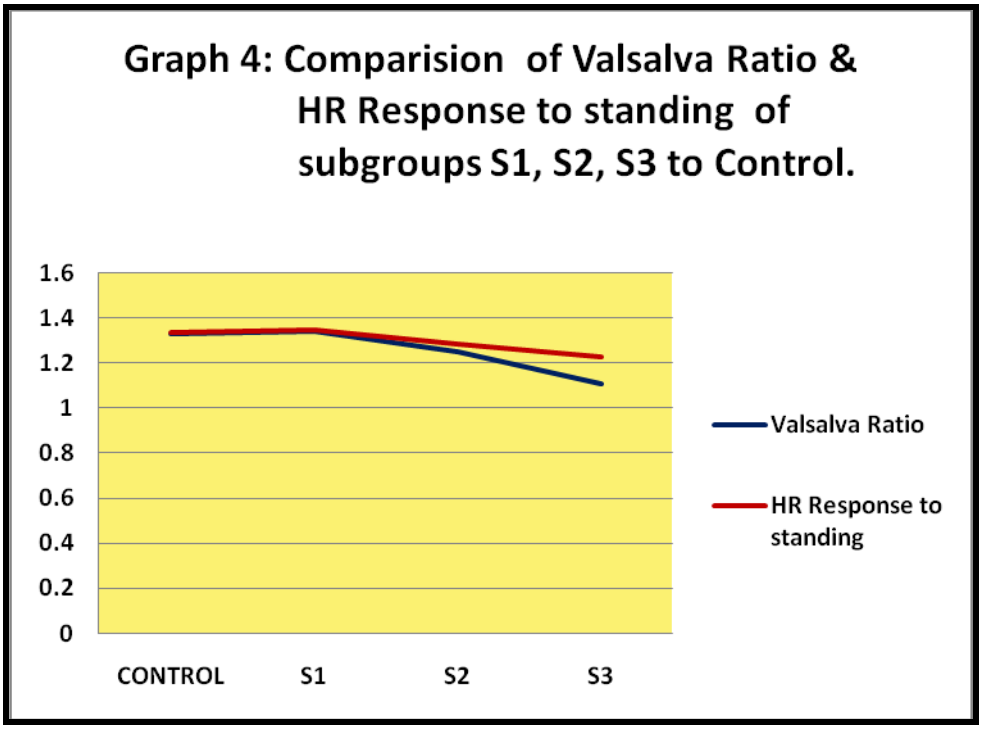
*p <0.05: Significant, ** p <0.01: Highly significant, *** p <0.001: Very highly significant.

1A]Heart Rate response to Valsalva maneuver in Control and Study Group:

Mean VR ± SD of Control Group- 1.33 ± 0.20

Mean VR± SD of Study Group - 1.29 ± 0.24

There is insignificant (p=0.222) decrease in the Valsalva ratio (VR) of subjects in Study Group compared to Control Group.



1B] Heart Rate response to Valsalva maneuver of Control and Subgroups of Study Group:

Mean VR ± SD of Control Group- 1.33 ± 0.20

Mean VR ± SD of Study group 1(S1)- 1.34± 0.26

Mean VR ± SD of Study group 2(S2)- 1.25 ± 0.20

Mean VR ± SD of Study group 3(S3)- 1.11 ± 0.13

There is significant (p=0.002) ** decrease in the Valsalva ratio (VR) of subjects in Subgroup-3 of Study Group compared to Control Group.

2A] Heart rate variation (HRV) during Deep Breathing in Control and Study Group:

Mean HRV ± SD of Control Group- 28.56 ± 7.44

Mean HRV ± SD of Study Group- 26.25 ± 8.47

There is insignificant (p=0.132) decrease in the heart rate variation during deep breathing in Study Group compared to Control Group.

2B] Heart rate variation (HRV), in beats per minute during Deep Breathing in Control and Subgroups of Study Group:

Mean HRV ± SD of Control Group- 28.56 ± 7.44

Mean HRV ± SD of Study group 1(S1)- 27.18 ± 7.31

Mean HRV ± SD of Study group 2(S2)- 25.40 ± 7.15

Mean HRV ± SD of Study group 3(S3)- 24.22 ± 16.13

There is insignificant decrease in the heart rate variation during deep breathing in Subgroups of Study Group compared to Control Group.

3A] Immediate heart rate response to standing (30:15 ratio) in Control and Study Group:

Mean Ratio ± SD of Control Group- 1.34 ± 0.20

Mean Ratio ± SD of Study Group- 1.31 ± 0.20

There is insignificant (p=0.335) decrease in the immediate heart rate response to standing in Study Group compared to Control Group.

3B] Immediate heart rate response to standing (30:15 ratio) in Control and Subgroups of Study Group:

Mean Ratio ± SD of Control Group- 1.34 ± 0.20

Mean Ratio ± SD of Study group 1(S1)- 1.35 ± 0.24

Mean Ratio ± SD of Study group 2(S2)- 1.29 ± 0.16

Mean Ratio ± SD of Study group 3(S3)- 1.23 ± 0.12

There is significant (p=0.05)* decrease in the immediate heart rate response to standing in Subgroup-3 of Study Group compared to Control Group though the values are within normal limit.

4A] Blood pressure response to standing (fall in SBP in mm of Hg) in Control and Study Group:

Mean SBP ± SD of Control Group 4.66 ± 1.76

Mean SBP ± SD of Study Group 4.86 ± 2.33

There is insignificant (P=0.358) increase in the SBP on standing in Study Group compared to Control Group.

4B]Blood pressure response to standing (fall in SBP in mm of Hg) in Control and Subgroups of Study Group:

Mean SBP ± SD in Control Group - 4.66 ± 1.76

Mean SBP ± SD of Study group 1(S1)- 4.94 ± 2.56

Mean SBP ± SD of Study group 2(S2)- 4.66 ± 2.0

Mean SBP ± SD of Study group 3(S3)- 5.0 ± 2.58

There is insignificant increase in the SBP on standing in Subgroups of Study Group compared to Control Group.

5A]Blood pressure response to sustained hand grip (increase in DBP in mm Hg) in Control and Study Group:

Mean DBP ± SD of Control Group- 21.00 ± 3.95

Mean DBP ± SD of Study Group- 19.40 ± 5.68

There is insignificant (p=0.105) decrease in the blood pressure response to sustained hand grip in Study Group compared to Control Group.

5B]Blood pressure response to sustained hand grip (increase in DBP in mm Hg) in Control and Subgroups of Study Group:

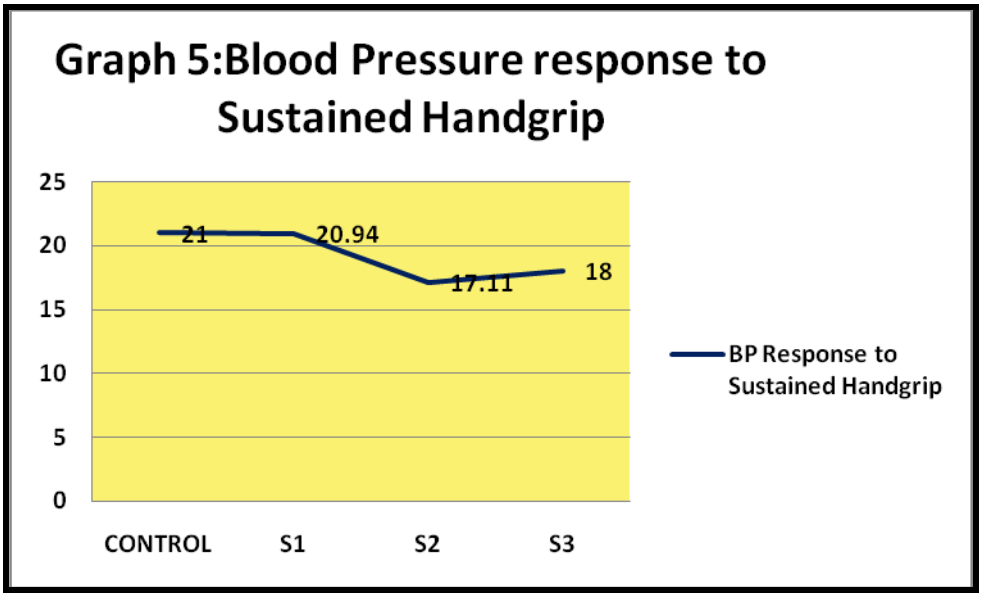
Mean DBP ± SD of Control Group- 21.00 ± 3.95

Mean DBP ± SD of Study group 1(S1)- 20.94 ± 5.83

Mean DBP ± SD of Study group 2(S2)- 17.11 ± 4.48

Mean DBP ± SD of Study group 3(S3)- 18.0 ± 6.73

There is insignificant (p=0.105) decrease in the blood pressure response to sustained hand grip in Subgroups of Study Group compared to Control Group.



AUTONOMIC FUNCTION SCORE

Total scores are done according to D.J.Ewing and B.F.Clarke[21]. No. of subjects expressed as percentage of the total number of the subjects in the groups and compared. Higher the score, more is the dysfunction.

Autonomic function scores are done separately for Parasympathetic and Sympathetic functions.

Total Autonomic function scores are calculated for each group and presented in Table-4A and for each subgroup is presented in Table-4B

Table 4A : Total Autonomic function score in Control and Study Group.

Total score / Groups	0	1	2	3	4	5	6	7	8
Control Group	**76.66%**	**3.33%**	**16.66%**	**3.33%**	**0**	**0**	**0**	**0**	**0**
Study Group	**40%**	**20%**	**23.33%**	**10%**	**6.6%**	**0**	**0**	**0**	**0**

Table 4B: Total Autonomic function score in Control and subgroups of Study Group.

Total score / Groups	0	1	2	3	4	5	6	7	8
Control Group	**76.66%**	**3.33%**	**16.66%**	**3.33%**	**0**	**0**	**0**	**0**	**0**
Study Group-1	**58.82%**	**17.64%**	**17.64%**	**5.88%**	**0**	**0**	**0**	**0**	**0**
Study Group-2	**22.22%**	**22.22%**	**22.22%**	**22.22%**	**11.11%**	**0**	**0**	**0**	**0**
Study Group-3	**0%**	**25%**	**50%**	**25%**	**0**	**0**	**0**	**0**	**0**

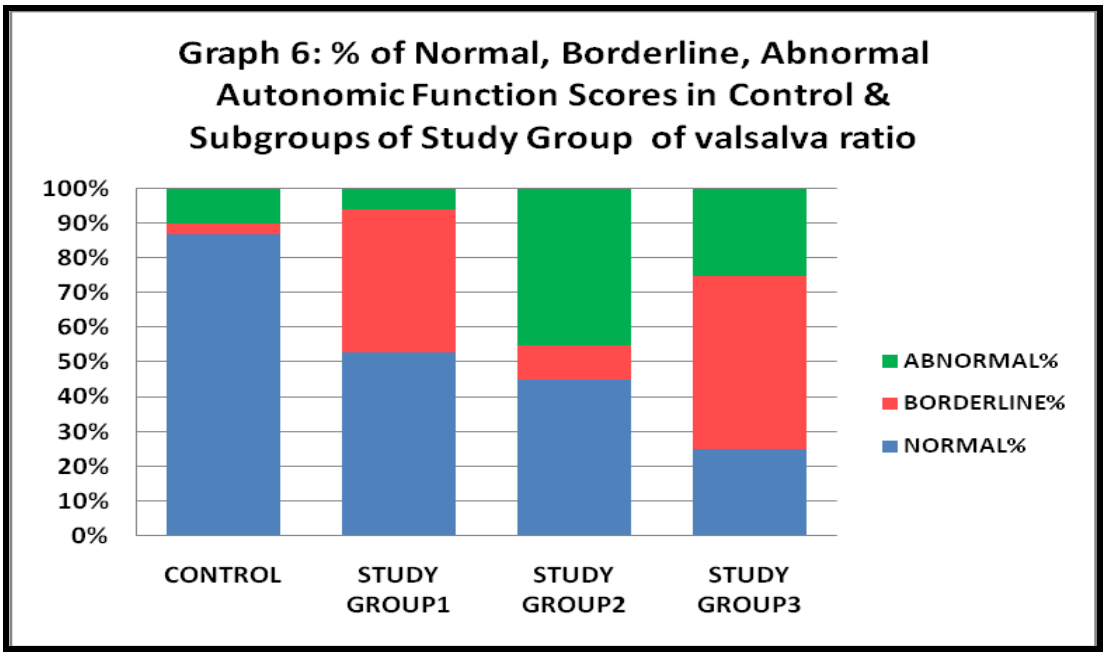
Parasympathetic function scores are calculated for each group and presented in Table-5A and for each subgroup is presented in Table-5B, and Graph 6.

Table 5A: Parasympathetic function score in Control and Study Group

Parasympathetic Function Score Groups	0	1	2	3	4	5
Control Group	**83.3%**	**6.6%**	**10%**	**0**	**0**	**0**
Study Group	**43.3%**	**33.3%**	**16.6%**	**6.6%**	**0**	**0**

Table 5B: Parasympathetic function score in Control and subgroups of Study Group

Parasympathetic Function / *Score* Groups	0	1	2	3	4	5
Control Group	**83.3%**	**6.6%**	**10%**	**0**	**0**	**0**
Study Group-1	**52.94%**	**35.29%**	**11.7%**	**0**	**0**	**0**
Study Group-2	**44.66%**	**11.1%**	**33.33%**	**0**	**11.1%**	**0**
Study Group-3	**0**	**75%**	**0**	**0**	**25%**	**0**



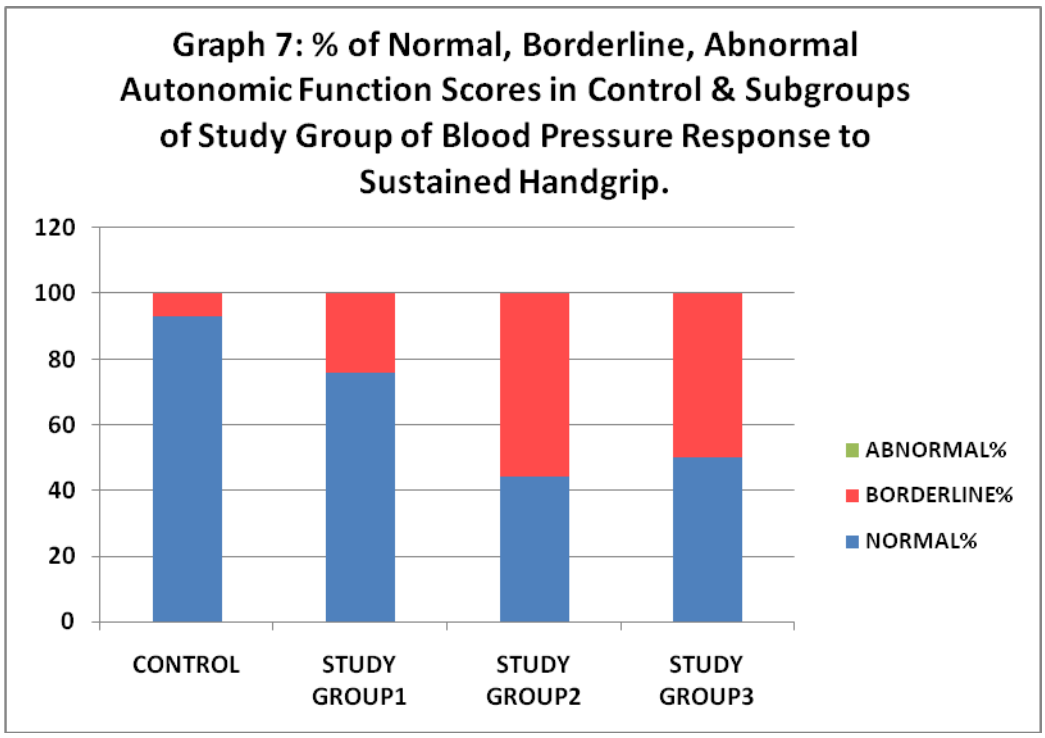
Sympathetic function scores are calculated for each group and presented in Table-6A and for each subgroup is presented in Table-6B, and Graph 7.

Table 6A: Sympathetic function score in Control and Study Group.

Sympathetic Function Score / **Groups**	**0**	**1**	**2**	**3**	**4**
Control Group	**93.66%**	**6.66%**	**0**	**0**	**0**
Study Group	**63.33%**	**36.66%**	**0**	**0**	**0**

Table 6B : Sympathetic function score in Control and subgroups of Study Group.

Sympathetic Function Score / **Groups**	**0**	**1**	**2**	**3**	**4**
Control Group	**93.66%**	**6.66%**	**0**	**0**	**0**
Study Group-1	**76.47%**	**23.52%**	**0**	**0**	**0**
Study Group-2	**44.44%**	**55.55%**	**0**	**0**	**0**
Study Group-3	**50%**	**50%**	**0**	**0**	**0**



VII] GLYCEMIC STATUS

Parameters like Fasting Blood Glucose and Postload Blood Glucose are used for assessing glycemic status of individual.

The Mean Value and Standard Deviation, Level of Significance of Fasting Blood Glucose and Postload Blood Glucose are calculated for each group and presented in Table-7A and for each subgroup is presented in Table-7B, and in Graph 8.

Table 7A: Fasting Blood Glucose and Postload Blood Glucose in Control and Study Group.

Parameters	**Control Group**	**Study Group**	**Level of significance**
Fasting Blood Glucose(mg/dl)	85.03±7.81	85.56 ±7.03	0.395
Postload Blood Glucose(mg/dl)	96.56±12.55	99.20 ±14.92	0.234

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

Table 7B: Fasting Blood Glucose and Postload Blood Glucose in Control and Subgroups of Study Group.

Parameters	**Control Group**	**Study Group-1**	**Study Group-2**	**Study Group-3**
Fasting Blood Glucose	85.03±7.81	84.70± 6.21 (p =0.44)	84.55±7.24 (p =0.43)	91.50±8.81 (p =0.08)
Postload Blood Glucose	96.56±12.55	98.52±12.6 (p =0.30)	96.11±16.27 (p=0.47)	109.00±20.99 (p =0.12)

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

1A]Fasting Blood Glucose (mg/dl) of Control and Study Group:

Mean FBG ± SD of Control Group 85.03±7.81

Mean FBG ± SD of Study Group 85.56 ±7.03

There is insignificant (p=0.395) increase in the Fasting Blood Glucose in Study Group compared to Control Group.

1B]Fasting Blood Glucose (mg/dl) in Control and Subgroups of Study Group:

Mean FBG ± SD of Control Group 85.03±7.81

Mean FBG ± SD of Study Group-1 84.70± 6.21

Mean FBG ± SD of Study Group-2 84.55±7.24

Mean FBG ± SD of Study Group-3 91.50±8.81

There is insignificant variation in the Fasting Blood Glucose in Subgroups of Study Group compared to Control Group.

2A]Postload Blood Glucose (mg/dl) of Control and Study Group:

Mean PLBG ± SD of Control Group 96.56±12.55

Mean PLBG ± SD of Study Group 99.20 ±14.92

There is insignificant (p=0.234) increase in the Postload Blood Glucose in Study Group compared to Control Group.

2B]Postload Blood Glucose (mg/dl) in Control and Subgroups of Study Group:

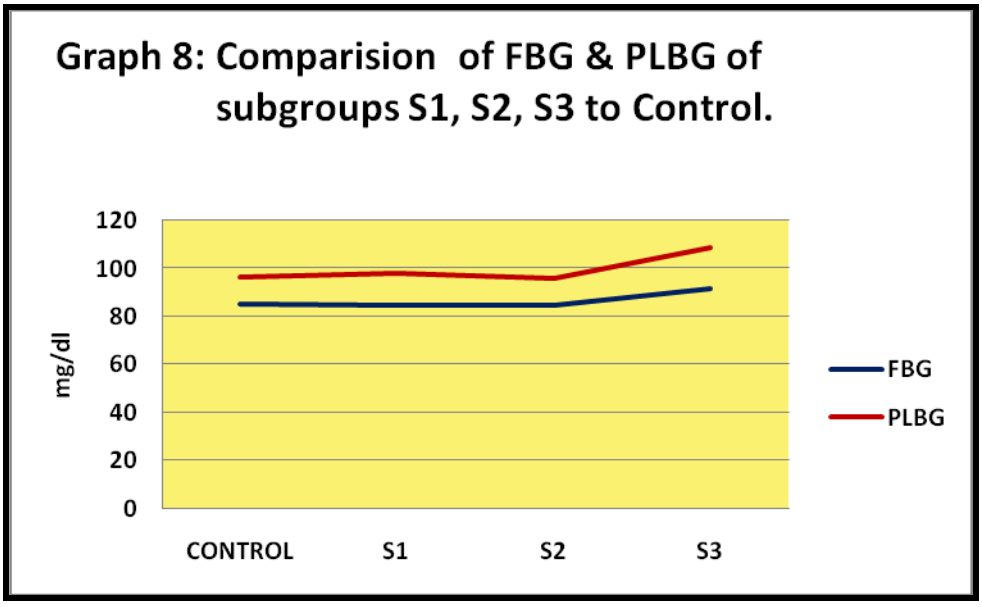
Mean PLBG ± SD of Control Group 96.56±12.55

Mean PLBG ± SD of Study Group-1 98.52±12.6

Mean PLBG ± SD of Study Group-2 96.11±16.27

Mean PLBG ± SD of Study Group-3 109.00±20.99

There is insignificant (p=0.12) increase in the Postload Blood Glucose in Subgroups of Study Group compared to Control Group.



CORRELATION BETWEEN GLYCEMIC STATUS AND AUTONOMIC FUNCTION TESTS

In this study, correlation of different autonomic function parameters with glycemic status is analyzed. Here Valsalva ratio, Blood pressure response to sustained hand grip are negatively correlated with glycemic status and all these relationships are statistically insignificant.

Table 8: Correlation Between Glycemic Status And Autonomic Function Tests

	Valsalva ratio and FBG	Valsalva ratio and PLBG	BP response to sustained hand grip and FBG	BP response to sustained hand grip and PLBG
Control	r=0.129	r=0.133	r=0.023	r=-0.138
Study	r=-0.117	r=-0.035	r=-0.220	r=-0.056

DISCUSSION

The cross-sectional study is carried in 60 normal healthy medical students (Offsprings of Type 2 Diabetic Parents n=30; and Nondiabetic Parents n=30) in the age group of 18-21years of BLDEU's Shri B M Patil Medical College, Bijapur. Evaluation of status of autonomic nervous system is done with the help of five non-invasive tests. Parasympathetic function is assessed by heart rate response to Valsalva maneuver, heart rate response to deep breathing, immediate heart rate response to standing. The sympathetic function is 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 both Control and Study groups.

Physical Parameters.

In our study, we found significant (p=0.04)* increase in the height of the subjects in Study

Group by 2.56 cms compared to Control Group (Table-1A).

There is significant (p=0.04) * increase in the weight of the subjects in Study Group by 5kg compared to Control Group (Table-1A).

We found significant (p=0.004) ** increase in the BMI of subjects in Subgroup-3 of Study Group compared to Control Group (Table-1A).

There is significant (p=01) ** increase in the BSA of subjects in Study Group compared to Control Group (Table-1A).

Our findings are in accordance with earlier studies done by Laitinen T et al[44], E. Hypponen et al[49], Jessica. E. Shill and coworkers[50.]

Both higher relative weight and height in study group are associated with an increased risk of autonomic dysfunction. The magnitude of the effect of the greater relative weight on the risk of developing automonic dysfunction/type 2 diabetes seemed to be some what stronger[49].

Obesity is a well known risk factor for type 2 diabetes. Increased adipocyte mass in obesity leads to increased levels of circulating free fatty acids and other fat cell products. The increased production of free fatty acids and some adipokines may cause insulin resistance in skeletal muscle and liver. Free fatty acids impair glucose utilization in skeletal muscle, promote glucose production by liver and impair beta cell function. The production of adipokines by adipocytes which is insulin sensitizing peptide is reduced in obesity and this may contribute to hepatic insulin resistance[3].

Insulin functions also as a permissive growth factor. Genetic tendency towards accelerated growth may be of decisive importance in inducing hyperinsulinemia. Also, hyperinsulinemia induced by overweight may be primary event in some children[49].

Physiological Parameters.

There is a highly significant (p=0.003) ** increase in the Resting Respiratory Rate of subjects in Study Group compared to Control Group(Table-2A).

There is insignificant increase in the Resting SBP and DBP in subjects of Study Group compared to Control Group (Table-2A).

Our findings are in accordance with earlier studies done by C. Hauerslev Foss et al[39], Laitinen T et al[44.]

Our results are not in agreement with Frontoni S et al[43].

A. <u>PARASYMPATHETIC FUNCTION TESTS</u>:

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.

In the present study the mean value of Valsalva ratio (Table no.3B) showed a significant ($p=0.002$) decrease in Subgroup-3 of study group compared to control group still remaining in the normal range (> 1.21, according to Ewing and Clarke grading)[21]

Our findings are in accordance with earlier studies done by C. Hauerslev Foss et al[39].

Heart rate response to Valsalva maneuver appear to be more sensitive parameters to detect autonomic dysfunction amongst the three Parasympathetic function tests.

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.

There is insignificant (p=0.132) decrease in the heart rate variation during deep breathing in Study Group compared to Control Group(Table-3A).

Our study is in accordance with studies done by C. Hauerslev Foss et al[39].

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.

In our study we found a insignificant (p=0.335) decrease in the immediate heart rate response to standing in Study Group compared to Control Group(Table-3A).

Our study is in accordance with studies done by C. Hauerslev Foss et al[39.]

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.

In our study there was an insignificant (P=0.358) increase in the SBP on standing in Study Group compared to Control Group(Table-3A).

Our study is in accordance with studies done by C. Hauerslev Foss et al[39].

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[25].

We observed a insignificant (p=0.105) decrease in the blood pressure response to sustained hand grip in Study Group compared to Control Group(Table-3A).

Our study is in accordance with studies done by C. Hauerslev Foss et al[39].

Blood pressure response to Sustained Hand Grip appear to be more sensitive parameters to detect autonomic dysfunction amongst the two Sympathetic function tests.

AUTONOMIC FUNCTION SCORE IN CONTROL AND STUDY GROUP.

More is the score, more is the dysfunction.

Criteria for grading autonomic function as whole[43]

Scores ≤ 3 Normal autonomic function

Scores > 3 & < 8 Borderline dysfunction

Scores ≥ 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[21].

	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 ≤ 3 was considered to indicate normal autonomic function. Scores >3 and ≤ 8 were considered borderline and scores ≥ 8 were judged abnormal[43].

Parasympathetic, Sympathetic and total autonomic function score (Table no.5,6,4) shows increase in the number of subjects with borderline and abnormal autonomic function score in study group(Normal,n=28, Borderline, n=2) compared to control .

Subjects in study group were divided into 3 groups based on history of prevalence of diabetes in both parents and in first degree relatives. Abnormal autonomic function score was found in subjects of subgroup-3(Normal,n=28, Borderline, n=2), whose both the parents were diabetic.

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.

GLYCEMIC STATUS:

Glycemic status of an individual is determined by Oral Glucose Tolerance Test[38].

1. Fasting blood glucose.
2. Two hours after Glucose Load (Consisting of 75g glucose anhydrate in 300ml of water ingested over the course of 5 minutes).

There is insignificant increase in the Fasting Blood Glucose and Postload Blood Glucose in Study Group compared to Control Group(Table-7A).

Our study is in accordance with studies done by I.N.Migdalis et al[51], Frontoni S et al[43]

Our results are not in agreement with C. Hauerslev Foss et al[39].

CORRELATION BETWEEN GLYCEMIC STATUS AND AUTONOMIC FUNCTION TESTS

In this study, correlation of different autonomic function parameters with glycemic status was analyzed. Here Valsalva ratio, Blood pressure response to sustained hand grip was negatively correlated with glycemic status and all these relationships were statistically insignificant.

Our results are in agreement with C. Hauerslev Foss et al[39].

We found significantly lower mean values for the three cardiovascular reflex tests in study group compared to control. The results indicate that heart rate variation is reduced in study group compared to control. We found a higher prevalence of cardiac autonomic dysfunction in study group compared to control even in presence of normoglycemia.

Our observations indicate that subclinical autonomic dysfunction may develop without the presence of long-term hyperglycemia in family members of type 2 diabetic subjects; thus, it is not simply a complication of the hyperglycemia in these patients..

An explanation could be that it is possible to inherit susceptibility genes for autonomic neuropathy, and that these genes could be expressed before—or maybe even without—the subjects developing diabetes. Different factors (including hyperglycemia) could subsequently affect the expression of the genes and influence the progression of neuropathy[43].

This cross-sectional study shows autonomic dysfunction in study group indicate that early autonomic dysfunction may be present with normoglycemia , and we suggest that autonomic dysfunction may be part of a genetic syndrome which appears earlier than appearance of dysglycemia which requires further longitudinal study in study group.

CONCLUSION

We conducted a cross-sectional study to evaluate Glycemic status and Cardiovascular Autonomic Functions in 60 normal healthy medical students (Offsprings of Type 2 Diabetic Parents and Nondiabetic Parents in the age group of 18-21years of BLDEU's Shri B M Patil Medical College, Bijapur. We performed Parasympathetic function tests (heart rate response to Valsalva maneuver, heart rate response to deep breathing, immediate heart rate response to standing). The 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 in study group compared to control.

2) Heart rate response to Valsalva maneuver & Blood pressure response to Sustained Hand Grip appear to be more sensitive parameters to detect 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 in subgroups of study group based on history of prevalence of diabetes in both parents and in first degree relatives.

4) There is insignificant increase in Fasting blood glucose and Postload blood glucose in study group compared to control 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.

Further study on insulin levels is needed to determine insulin sensitivity and insulin-resistance and its relation to autonomic dysfunction.

SUMMARY

The cross-sectional study was carried out evaluate Glycemic status and Cardiovascular Autonomic Functions in 60 normal healthy medical students (Offsprings of Type 2 Diabetic Parents n=30 and Nondiabetic Parents n=30) in the age group of 18-21years of BLDEU's Shri B M Patil Medical College, Bijapur. 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. In consultation with statistician, statistical tests were carried out. P value of 0.05 was considered as significant.

It was observed that

1) Autonomic function tests showed insignificant decrease in function in study group compared to control group.
2) Heart rate response to Valsalva maneuver & Blood pressure response to Sustained Hand Grip appear to be more sensitive parameters to detect 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 in subgroups of study group based on history of prevalence of diabetes in both parents and in first degree relatives.

4) There is insignificant increase in Fasting blood glucose and Postload blood glucose in study group compared to control 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.

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. Guyton & Hall. Textbook of medical Physiology.11th edition. Pennsylvania: Elseveir; 2006.P: 748.

2. Jain A K. Manual of practical Physiology. 3nd edition. Himachal Pradesh: Arya Publications; 2008. P: 279-280.

3. Alvin CP, Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameson LJ, editors. Harrison's principles of Internal Medicine. Vol 2.17th edn. New York: McGraw Hill Companies 2008; P:2275- 2289.

4. John CP, Gareth W. Text book of diabetes.2nd ed, London: Blackwell Science Limited 1997;1.1- 1.19.

5. The American Diabetes Association. Diagnosis and classification of Diabetes mellitus. Diabetes care 2006;29 :43-48.

6. Sarah W,Gojka R,Anders G,Richard S, King H. Global prevalence of diabetes estimates for the year 2000 and projection for 2030. Diabetic care 2004;27:1047-53.

7. Amitha S,Prabhakar S,Manoj I, Harminder and pavan T.Effect of yoga Nidra on blood glucose level on diabetic patients. Indin J physiol pharmacol 2009;53:97-101.

8. Cotron RS,Kumar V,Collins T.Robbins pathologic basis of Diseases. 6th edn Philadelphia: WBSaunders Company ;1999:919-924.

9. Melo E, Viann E.O., Gallo, Foss M.C. Pulmonary function, cholinergic Bronchomotor tone,and cardiac autonomic abnormalities in type 2 diabetic patients. Brazilian Journal ofMedical and Biological research 2003;36 : 291-299.

10. Richard WN, Peter L, Diabetic mellitus and cardiovascular system. In: Braunwald E, Douglas PZ, Peter L, Heart disease A text book of cardiovascular medicine.6th edn. Philadelphia: Elsevier Saunders ;2001:2133-2146
11. Christopher G Goetz, Eric J Pappert. Textbook of Clinical Neurology. 1st edition. W B Saunders Company; 1999. 350-351, 355-356.
12. Bijlani RL. Understanding Medical Physiology. 3rd edition. Jaypee Brothers Medical Publishers; 2004. 44-46, 819.
13. 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.
14. J C Brocklehurst, R C Tallis, H M Fillit. TB of Geriatric Medicine & Gerontology. 4th edition. Churchill Livingstone.
15. Derek LeRoith, Simeon.I.Taylor, Jerrold.M.Olefsky. Diabetes Mellitus, AFundamental &Clinical Text.Second edition, Lippincott Williams & Wilkins; 2000.912
16. Khema R. Sharma, John Cross, Oscar Farronay, D. Ram Ayyar, Robert T. Shebert, Walter G. Bradley. Demyelinating Neuropathy in Diabetes Mellitus, Arch Neurol. 2002;59:758-765.
17. Bradley G Walter, Daroff B Robert, Ferrichel M Gerald, Jankovic Joseph. Neurology in Clinical Practice. 5th edition. Butlerworth Heinemann Elsevier Publishers; 2008. 2360.
18. Elisberg EI. Heart rate response to Valsalva maneuver as a test of circulating integrity. JAMA 1963; 183: 120.

19. 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.
20. Piha SJ. Autonomic responses to valsalva maneuver in healthy subjects. Clin Physiol 1995; 15(4): 339-47.
21. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. Br Med J 1982 Oct; 285: 916-918.
22. Dixit MB. Comparative cardiovascular responses to 70^{o} Head up tilt in pilots and non-pilots. Aviat Med 1983; 27: 38-42.
23. 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.
24. 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.
25. Ewing DJ, Campbell IW, Burt AA, Clarke BF. Vascular reflexes in autonomic neuropathy. The Lancet 1973 Dec; 15: 1354-1356.
26. 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.
27. Editorial. Valsalva maneuver in diabetic neuropathy. J American Med Assoc. 1974 May; 228:89-93

28. Malik Marek. Heart rate variability. Standards of measurement, Physiological Interpretation and Clinical Use. Circulation 1996; 93: 1043-1065.
29. Das RK, Mahapatra K, Guha S, Padhey PK. Cardiac autonomic neuropathy in diabetes. J Assoc Phys India 1982; 30(10):740.
30. Dixit MB. Postural stress tests for the clinic-Physiological evaluation of cardiovascular reflexes. Indian J Pharmc Phys 1987; 31(1):76.
31. Rushmer RF. Cardiovascular Dynamics. 4th edition. WB Saunders.
32. Abbot FM, Halsted DD, Mark AL, Schmid PG. Reflex control of the peripheral circulation. Prog Cardiovascular Dis 1976; 18:371-403.
33. Roger Bannister. Testing autonomic reflexes in autonomic failure. Oxford Medical Publications. Oxford 1983; 52-63.
34. Sjostrend T. The regulation of Blood distribution in man. Acta Physiol Scand 1952; 26:312-327.
35. 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.
36. Dwain L, Eckberg & B Gunnar Wallin. Isometric exercise modifies autonomic baroreflex responses in humans. J Appl Physiol 1987; 63(6): 2325-2330.
37. Vivian A. Fonsea, Clinical Diabetes, Translating Research into Practice, 1th edition: Elseveir Publishers, Saunders Company;2006. 145.
38. Fiorentini A, Perciaccante A, Paris A, Serra P, and Tubani L. Circadian rhythm of autonomic activity in non diabetic offsprings of type 2 diabetic patients. Cardiovasc Diabetol. October 2005. 10.1186/1475-2840-4-15.

39. C. Hauerslev Foss, E. Vestbo, A. Frøland , H.J. Gjessing, C.E. Mogensen and E.M. Damsgaard. Autonomic Neuropathy in Nondiabetic Offspring of Type 2 Diabetic Subjects Is Associated With Urinary Albumin Excretion Rate and 24-h Ambulatory Blood Pressure. The Fredericia Study. Diabetes.March 2001;50(3):630- 636.

40. De Angelis C, Perelli P, Trezza R, Casagrande M, Biselli R, Pannitteri G,et al, Modified autonomic balance in offsprings of diabetics detected by spectral analysis of heart rate variability Metabolism, 2001;50(11):1270-1274.

41. Ferdinando Iellamo; Manfredi Tesauro; Stefano Rizza; Stefano Aquilani; Carmine Cardillo; Micaela Iantorno;et al. Concomitant Impairment in Endothelial Function and Neural Cardiovascular Regulation in Offspring of Type 2 Diabetic Subjects. Hypertension. 2006;48(3):418-423.

42. Frontoni S, Bracaglia D, Baroni A, Pellegrini F, Perna M, Cicconetti E,et al. Early autonomic dysfunction in glucose-tolerant but insulin-resistant offspring of type 2 diabetic patients. *Hypertension.*June 1, 2003; 41(6): 1223–1227.

43. Frontoni S, Pellegrinotti M, Bracaglia D, Farrace S, Caselli A, Baroni A, et al. Hyperinsulinaemia in offspring of Type 2 diabetic patients: impaired response Of carbohydrate metabolism, but preserved cardiovascular response. Diabet Med. 2000 Aug;17(8):606-11

44. Laitinen T, Vauhkonen IK, Niskanen LK, Hartikainen JE, Lansimies EA, Uusitupa MI, Power spectral analysis of heart rate variability during hyperinsulinemia in nondiabetic offspring of type 2 diabetic patients: evidence for possible early autonomic dysfunction in insulin-resistant subjects. Diabetes.

June 1999:48(6):1295–99

45. Park.K Park's Text Book Of Preventive and Social Medicine. 19th edition. M/S Banarasidas Bhanot Publishers, 2007 Page no. 327-328
46. Sheldon. M. Ross. Introduction to probability and statistics for scientists. Villey publication, Newyork, California, Burkley edition. 1987, P:106.
47. Nzuobontane Divine, Kathleen Blackett, Christopher. Cardiovascular autonomic dysfunction in Africans in human immunodeficiency virus. J R Soc Med 2002; 95(9): 445-447.
48. Dr. Mahajan. Methods in Biostatistics. 5th edition. Jaypee Brothers; 1989. Pg 102, 114-119, 125-39
49. Hypponen E, Virtanen S M, Kenward M G, Knip M, Akerblom H K and Childhood Diabetes Finland study group, Obesity, Increased Linear Growth, And Risk Of Type1 Diabetes In Children. Diabetes care2000; 23:17-1760,
50. Jessica E.Shill, Mark N Feinglos, Edward C Suarez, Offsprings Of Patients With Diabetes Exhibit Clustering Of Psychosocial Distress And Inflammatory And Metabolic Risk Factors. Diabetes care,2008: 31:11
51. I.N. Migdalis D. Zachariadis, K. Kalogeropoulou, C. Nounopoulos , A. Bouloukos, M. Samartzis. Metabolic Abnormalities in Offspring of NIDDM Patients with a Family History of Diabetes Mellitus. Diabetic Medicine**,** 1996 May ;13(5): 434–440,

ANNEXURE- 1

B.L.D.E.UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103

INSTITUTIONAL ETHICAL COMMITTEE

Ethical Committee BIJAPUR-586103 OUT WARD No 66109 Date 27/10/09 Shri B. M. PATIL Med.Cal College

Dr.Vijay.Ganjoo,
Chairperson. I.E.C.
B.L.D.E.University's
Sri.B.M.Patil Medical College,
BIJAPUR-586 103

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 26-10-2009 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 Study of glycemic status & cardiovascular autonomic functions in the offsprings of type-II Diabetic parents.

Name of P.G /U.G. student/ Faculty member. Dr. Sangeeta Guppad. Dept of Physiology

Name of Guide. Dr. Anand.A. Dharawadkar Prof & HOD

Dr.Vijay Ganjoo,
Chairperson,
Institutional Ethical Committee

Date:

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

ANNEXURE- 2

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

RESEARCH INFORMED CONSENT FORM

Title of the project: "**STUDY OF GLYCEMIC STATUS AND CARDIOVASCULAR AUTONOMIC FUNCTIONS IN THE OFFSPRINGS OF TYPE 2 DIABETIC PARENTS"**

Principal investigator/ P.G.Guide's name: DR.ANAND R.DHARWADKAR MD

PROFESSOR

DEPARTMENT OF PHYSIOLOGY.

1: PURPOSE OF RESEARCH:

I have been informed that this study will assess the Cardiovascular Autonomic Functions in the offsprings of Type 2 diabetic parents in the age group of 18 to 21 years. This study will be useful academically as well as for clinically to find out impairments in the Cardiovascular Autonomic Functions in the offsprings of Type 2 diabetic parents.

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.

3: RISK AND DISCOMFORTS:

I understand determination of Cardiovascular 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 _____(subject's/relevant guardian)

the purpose of the research, the procedure 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

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

DEPARTMENT OF PHYSIOLOGY

"**STUDY OF GLYCEMIC STATUS AND CARDIOVASCULAR AUTONOMIC FUNCTIONS IN THE OFFSPRINGS OF TYPE 2 DIABETIC PARENTS"**

PROFORMA

Name: **Age:** **Sex:**

Occupation: **Religion/Caste:** **Address:**

GENERAL HEALTH CONDITION:

Past history:

Ischemic Heart Disease **Hypertension** **Surgery**

Tuberculosis **Syphilis** **Leprosy**

Amyloidosis **Any Significant Chronic Illness**

Family History

Diabetes mellitus **Obesity** **Hypertension**

IHD **Any other illness.**

Detailed history of Diabetes mellitus.

MOTHER:

Age: yrs, Diabetic history--› YES/ NO, If Yes, Age at Diagnosis: yrs.

Duration of Diabetes: yrs, Diabetic history in relatives: YES/NO.

FATHER:

Age: yrs, Diabetic history--› YES/ NO, If Yes, Age at Diagnosis: yrs.

Duration of Diabetes: yrs, Diabetic history in relatives: YES/NO.

Personal history

Appetite Diet (Veg/Nonveg) Sleep

Nourishment Bowel habits Bladder habits

Habits: Chewing pan/gutkha H/o Alcohol intake, H/o Smoking.

Symptoms of Autonomic Neuropathy

- **Impotence**
- **Giddiness(Dizziness on standing)**
- **Pain in abdomen, Diarrheoa,**
- **Flatulence, Nausea, Vomiting, Constipation**
- **Bladder Disturbances**
- **Sweating Disturbances**

Others

- **Easy Fatiguability**
- **Chest Pain**
- **Cough, Expectoration, Breathlessness**
- **Tingling, Numbness**
- **Visual disturbances**

GENERAL EXAMINATION

Built	**Temperature**	**Pallor**
Cyanosis	**Edema**	**Abnormal Pigmentation**
JVP	**Lymphadenopathy**	

ANTHROPOMETRIC MEASUREMENTS

1. **Height(cms):**
2. **Weight(kgs):**
3. **Body Surface Area(/sqcms):**
4. **Body Mass Index(kg/ht):**

PHYSIOLOGICAL PARAMETERS

1. **Heart Rate(beats/min):**

2. **Blood Pressure (Systolic/Diastolic)mm of Hg:**

3. **Respiratory Rate (cycles/min):**

AUTONOMIC FUNCTION PARAMETERS

PARASYMPATHETIC TESTS **SCORE**

1. **Heart rate response to Valsalva Maneuvere:**

   (R-R interval, longest to shortest ratio)

2. **Heart Rate variation to Deep breathing:**

   (Beats/min)

3. **Heart rate response to standing:**

   (R-R interval, 30:15 ratio)

SYMPATHETIC TESTS

1. **Blood Pressure response to standing:**

   (mm of Hg)

2. **Blood Pressure response to sustained handgrip:**

   (mm of Hg)

GRADINGS (According to Ewing and Clarke)

	Normal	Borderline	Abnormal
SCORE	**0**	**1**	**2**
1. H.R to Valsalva Maneuver.	**>1.21**	**1.11-1.20**	**<1.10**
2. H.R to Deep breathing	**>15bts/min**	**11-14bts/min**	**<10bts/min**
3. H.R to standing	**>1.04**	**1.01-1.03**	**<1.0**
4. B.P to standing	**<10mm of Hg**	**11-29mm of Hg**	**>30mm of Hg**
5. B.P to sustained handgrip-	**>16mm of Hg**	**11-15mm of Hg**	**<10mm of Hg**

Glycemic status of an individual.

(Oral glucose tolerance test)

1. **Fasting Blood Glucose→ ____mg/dl**
2. **2hours After Glucose Load→ _____mg/dl**

KEY TO MASTER CHARTS

Ht	Height
Wt	Weight
BMI	Body mass index
BSA	Body Surface Area
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.
FBG	Fasting Blood Glucose
PLBG	Postload Blood Glucose

Annexure-4a

Master chart (Anthropometric, Physiological, Autonomic Function Parameters And Glycemic Status in Control Group)

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	FBG	PL BG
1	19	M	165	54	19.83	1.58	80	17	122	80	88	1.4	1.46	1.57	1.47	0	22.37	0	1.55	0	6	0	18	0	0	72	87
2	21	M	178	76	24	1.96	75	14	140	80	83	1.24	1.15	1.24	1.21	0	24.83	0	1.3	0	4	0	28	0	0	83	80
3	18	M	179	61	19.56	1.78	70	14	110	74	62	1.9	1.74	1.49	1.7	0	38.24	0	1.7	0	4	0	22	0	0	76	86
4	19	M	173	77	25.72	1.92	76	13	110	80	70	1.49	1.3	1.16	1.31	0	41.78	0	1.31	0	4	0	20	0	0	66	79
5	18	M	162	68	25.91	1.74	86	20	140	84	96	1.54	2	1.63	1.72	0	40.99	0	1.3	0	6	0	26	0	0	95	83
6	18	M	179	73	22.78	1.92	76	14	108	80	69	1.3	1.22	1.22	1.24	0	19.36	0	1.24	0	2	0	20	0	0	88	74
7	18	M	167	76	27.25	1.86	68	15	126	80	76	0.94	0.89	0.89	0.9	2	25.25	0	1.46	0	4	0	16	0	2	82	79
8	19	M	163	68	25.59	1.74	70	16	102	66	61	1.61	1.2	1.18	1.33	0	28.48	0	1.3	0	6	0	24	0	0	80	86
9	19	M	171	76	26	1.88	84	14	126	80	82	1.07	1.06	1.07	1.07	1	28.77	0	1.15	0	6	0	14	1	3	73	89
10	18	M	171	55	18.8	1.64	90	16	140	76	98	1.18	1.49	1.54	1.4	0	26.48	0	1.22	0	8	0	20	0	0	95	115
11	18	M	174	54	17.83	1.72	90	20	140	66	99	1.18	1.07	1.5	1.25	0	12.83	1	1.22	0	8	0	24	0	0	86	110
12	18	M	173	57	19	1.7	86	14	120	82	96	1.36	1.54	1.63	1.51	0	18.41	0	1.1	0	4	0	18	0	0	94	108
13	19	M	170	58	20.06	1.68	80	18	110	74	84	1.28	1.22	1.3	1.26	0	38.2	0	1.4	0	4	0	24	0	0	98	120
14	18	M	164	45	16.73	1.46	90	12	110	76	98	1.25	1.36	1.33	1.31	0	34.14	0	1.1	0	4	0	20	0	0	86	96
15	19	M	168	64	22.67	1.76	78	12	110	70	75	1.3	1.28	1.5	1.36	0	33.01	0	1.58	0	4	0	20	0	0	85	99
16	21	M	165	55	20.2	1.6	80	14	130	80	89	1.45	1.5	1.54	1.5	0	29.13	0	1.66	0	2	0	28	0	0	94	108
17	18	M	172	54	18.25	1.66	80	13	116	80	73	1.28	1.35	1.35	1.32	0	33.61	0	1.36	0	4	0	20	0	0	82	102
18	18	M	173	67	22.38	1.82	66	15	110	80	58	1.38	1.54	1.28	1.4	0	24.88	0	1.24	0	4	0	20	0	0	91	110
19	19	M	171	48	16.41	1.56	78	12	106	70	84	1.2	1.77	1.18	1.38	0	39.04	0	1.77	0	6	0	16	0	0	97	118
20	18	M	176	62	20	1.76	80	16	106	76	73	1.22	1.46	1.53	1.4	0	27.02	0	1.1	0	2	0	20	0	2	86	116
21	19	M	170	92	31.83	2.02	72	12	140	86	78	1.38	1.42	1.38	1.39	0	29.36	0	1.4	0	4	0	14	1	1	87	100
22	18	M	164	60	22.3	1.66	84	10	110	68	93	1.21	1.42	1.28	1.3	0	31.3	0	1.49	0	2	0	22	0	0	86	92
23	18	M	170	68	23.52	1.78	86	12	122	78	94	1.4	2	1.18	1.52	0	34.63	0	1.4	0	6	0	20	0	0	82	98
24	19	M	165	68	24.97	1.76	80	14	108	70	75	2	1.26	1.22	1.49	0	22.23	0	1.69	0	2	0	24	0	0	76	94
25	19	M	172	78	26.35	1.92	76	11	116	74	74	1.38	1.46	1.66	1.5	0	21.46	0	1	2	6	0	24	0	2	82	88
26	20	M	169	67	23.45	1.78	76	14	112	78	70	1.28	1.35	1.35	1.32	0	21.96	0	1.33	0	6	0	22	0	0	86	94
27	21	M	174	68	22.46	1.82	76	10	126	80	73	1.35	1.54	1.5	1.46	0	18.41	0	1.46	0	6	0	20	0	0	84	98
28	18	M	162	57	21.71	1.6	76	12	130	80	82	1.18	1.2	1.38	1.25	0	22.45	0	1.3	0	4	0	20	0	0	92	98
29	21	M	179	85	26.52	2.04	75	15	120	80	81	0.77	0.94	0.88	0.86	2	33.17	0	1.07	0	8	0	30	0	2	77	88
30	20	M	166	65	23.58	1.74	80	12	120	84	86	0.94	0.84	0.94	0.9	2	35.26	0	1.06	0	4	0	16	0	2	90	102

Annexure-4b

Master chart (Anthropometric, Physiological, Autonomic Function Parameters and Glycemic Status in Study Group)

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	FBG	PL BG
1	18	M	172	83	28	1.96	92	18	110	70	99	1	0.93	1.18	1.03	1	43.33	0	1.18	0	2	0	14	1	2	79	80
2	20	M	165	58	21.3	1.64	84	16	126	80	90	2.11	2	2.33	2.14	0	20.69	0	2	0	0	0	26	0	0	83	100
3	20	M	170	65	22.49	1.78	70	16	100	70	62	1.14	1.13	1.2	1.15	1	22.1	0	1.07	0	2	0	16	0	1	90	123
4	19	M	182	73	22	1.94	78	14	140	86	84	1.2	1.49	1.5	1.39	0	21.75	0	1.44	0	4	0	14	1	1	80	66
5	18	M	180	65	20	1.84	70	18	108	70	62	1.06	0.93	0.93	0.97	2	23.15	0	1.21	0	2	0	14	1	3	82	93
6	18	M	181	72	22	1.92	76	14	138	84	78	1.22	1.58	1.69	1.39	0	32.31	0	1.4	0	0	0	22	0	0	85	98
7	18	M	181	56	17	1.76	68	18	110	68	74	1.33	0.88	1	1.07	2	23.69	0	1.21	0	4	0	18	0	2	81	85
8	20	M	171	76	26	1.9	74	16	122	80	84	1.07	1.16	1.16	1.13	1	38.38	0	1.24	0	6	0	14	1	2	88	90
9	19	M	171	48	16.4	1.56	74	16	116	80	80	1.09	1.3	1.27	1.22	1	12	1	1.4	0	4	0	26	0	1	81	90
10	20	M	175	72	23.5	1.9	72	14	138	84	84	1.22	1.22	1.16	1.2	1	36.21	0	1.13	0	4	0	14	1	2	80	83
11	18	M	175	90	29.38	2.06	76	16	140	80	82	1.41	1.33	1.16	1.3	0	14.38	1	1.22	0	8	0	28	0	1	92	111
12	19	M	184	90	26.58	2.14	78	16	110	74	80	0.93	1.06	1.46	1.15	2	20.37	0	1	2	2	0	22	0	4	82	89
13	19	M	173	63	21	1.76	76	14	130	76	90	1.06	1.06	1.15	1.09	2	31.26	0	1.27	0	0	0	14	1	3	77	82
14	19	M	164	65	24.16	1.7	82	14	110	70	92	1.3	1.9	1.6	1.6	0	22.9	0	1.88	0	4	0	28	0	0	80	86
15	20	M	177	62	19.78	1.78	76	17	116	76	84	1.33	1.14	1.24	1.23	0	36.17	0	1.21	0	6	0	32	0	0	77	88
16	18	M	180	72	22.22	1.92	70	15	100	70	64	1.66	1.41	1.41	1.4	0	29.64	0	1.24	0	6	0	26	0	0	79	86
17	18	M	165	60	22	1.66	72	16	130	76	78	1.07	1.15	1.24	1.15	1	25.28	0	1.41	0	2	0	20	0	1	83	120
18	20	M	177	73	23.3	1.92	80	16	136	80	74	1.07	1.15	1.07	1.09	2	17.17	0	1.27	0	6	0	14	1	3	79	96
19	20	M	166	68	24.67	1.8	84	14	116	74	92	1.27	1.2	1.5	1.32	0	31.42	0	1.33	0	6	0	22	0	0	83	102
20	19	M	160	60	23.43	1.65	74	13	126	72	80	1.36	1.36	1.41	1.37	0	25.38	0	1.24	0	6	0	18	0	0	83	102
21	19	M	176	88	28.4	2.05	78	17	136	76	82	1.54	1.36	1.6	1.5	0	21.06	0	1.2	0	6	0	22	0	0	79	96
22	19	M	167	64	22.94	1.72	84	16	140	84	91	1.3	0.78	0.91	0.99	2	7.85	2	1.12	0	4	0	16	0	4	96	115
23	20	M	170	72	24.91	1.86	80	18	110	60	89	1.61	1.74	1.64	1.66	0	29.95	0	1.27	0	4	0	26	0	0	86	99
24	19	M	170	65	22.49	1.76	90	16	130	84	98	1.41	1	1.33	1.2	1	27.77	0	1.44	0	6	0	12	1	2	96	116
25	18	M	174	85	28	2	82	16	140	84	90	1.41	1.33	1.45	1.39	0	38.89	0	1.3	0	4	0	24	0	0	98	120
26	19	M	166	72	27.1	1.8	76	15	120	86	70	1.7	1.5	1.5	1.56	0	34.82	0	1.55	0	8	0	14	1	1	84	104
27	20	M	169	52	18.2	1.58	82	16	140	84	88	1.63	1.27	1.57	1.49	0	17.52	0	1.36	0	6	0	22	0	0	90	106
28	19	M	182	86	25.96	2.08	82	13	126	86	75	1.2	1.13	1.05	1.12	1	31.35	0	1.4	0	6	0	14	1	2	99	130
29	19	M	176	78	25.18	1.96	80	15	130	80	85	1.3	1	1.3	1.2	1	31.39	0	1.22	0	4	0	18	0	0	98	115
30	18	M	163	72	27.09	1.78	76	12	130	86	85	1.3	1.07	1.21	1.19	1	19.47	0	1.36	0	4	0	12	1	2	97	105

Annexure-4c

Master chart(Anthropometric, Physiological, Autonomic Function Parameters and Glycemic Status in Subgroups of Study Group)

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	FBG	PL BG
GROUP 1																											
1	20	M	165	58	21.3	1.64	84	16	126	80	90	2.11	2	2.33	2.14	0	20.69	0	2	0	0	0	26	0	0	83	100
2	20	M	170	65	22.49	1.78	70	16	100	70	62	1.14	1.13	1.2	1.15	1	22.1	0	1.07	0	2	0	16	0	1	90	123
3	18	M	181	72	22	1.92	76	14	138	84	78	1.22	1.58	1.69	1.39	0	32.31	0	1.4	0	0	0	22	0	0	85	98
4	20	M	171	76	26	1.9	74	16	122	80	84	1.07	1.16	1.16	1.13	1	38.38	0	1.24	0	6	0	14	1	2	88	90
5	19	M	171	48	16.4	1.56	74	16	116	80	80	1.09	1.3	1.27	1.22	1	12	1	1.4	0	4	0	26	0	1	81	90
6	20	M	175	72	23.5	1.9	72	14	138	84	84	1.22	1.22	1.16	1.2	1	36.21	0	1.13	0	4	0	14	1	2	80	83
7	19	M	173	63	21	1.76	76	14	130	76	90	1.06	1.06	1.15	1.09	2	31.26	0	1.27	0	0	0	14	1	3	77	82
8	19	M	164	65	24.16	1.7	82	14	110	70	92	1.3	1.9	1.6	1.6	0	22.9	0	1.88	0	4	0	28	0	0	80	86
9	20	M	177	62	19.78	1.78	76	17	116	76	84	1.33	1.14	1.24	1.23	0	36.17	0	1.21	0	6	0	32	0	0	77	88
10	18	M	180	72	22.22	1.92	70	15	100	70	64	1.66	1.41	1.41	1.4	0	29.64	0	1.24	0	6	0	26	0	0	79	86
11	18	M	165	60	22	1.66	72	16	130	76	78	1.07	1.15	1.24	1.15	1	25.28	0	1.41	0	2	0	20	0	1	83	120
12	20	M	166	68	24.67	1.8	84	14	116	74	92	1.27	1.2	1.5	1.32	0	31.42	0	1.33	0	6	0	22	0	0	83	102
13	19	M	160	60	23.43	1.65	74	13	126	72	80	1.36	1.36	1.41	1.37	0	25.38	0	1.24	0	6	0	18	0	0	83	102
14	20	M	170	72	24.91	1.86	80	18	110	60	89	1.61	1.74	1.64	1.66	0	29.95	0	1.27	0	4	0	26	0	0	86	99
15	20	M	169	52	18.2	1.58	82	16	140	84	88	1.63	1.27	1.57	1.49	0	17.52	0	1.36	0	6	0	22	0	0	90	106
16	19	M	176	78	25.18	1.96	80	15	130	80	85	1.3	1	1.3	1.2	1	31.39	0	1.22	0	4	0	18	0	0	98	115
17	18	M	163	72	27.09	1.78	76	12	130	86	85	1.3	1.07	1.21	1.19	1	19.47	0	1.36	0	4	0	12	1	2	97	105
GROUP2																											
1	19	M	182	73	22	1.94	78	14	140	86	84	1.2	1.49	1.5	1.39	0	21.75	0	1.44	0	4	0	14	1	1	80	66
2	18	M	180	65	20	1.84	70	18	108	70	62	1.06	0.93	0.93	0.97	2	23.15	0	1.21	0	2	0	14	1	3	82	93
3	18	M	181	56	17	1.76	68	18	110	68	74	1.33	0.88	1	1.07	2	23.69	0	1.21	0	4	0	18	0	2	81	85
4	19	M	184	90	26.58	2.14	78	16	110	74	80	0.93	1.06	1.46	1.15	2	20.37	0	1	2	2	0	22	0	4	82	89
5	20	M	177	73	23.3	1.92	80	16	136	80	74	1.07	1.15	1.07	1.09	2	17.17	0	1.27	0	6	0	14	1	3	79	96
6	19	M	176	88	28.4	2.05	78	17	136	76	82	1.54	1.36	1.6	1.5	0	21.06	0	1.2	0	6	0	22	0	0	79	96
7	19	M	170	65	22.49	1.76	90	16	130	84	98	1.41	1	1.33	1.2	1	27.77	0	1.44	0	6	0	12	1	2	96	116
8	18	M	174	85	28	2	82	16	140	84	90	1.41	1.33	1.45	1.39	0	38.89	0	1.3	0	4	0	24	0	0	98	120
9	19	M	166	72	27.1	1.8	76	15	120	86	70	1.7	1.5	1.5	1.56	0	34.82	0	1.55	0	8	0	14	1	1	84	104
GROUP3																											
1	18	M	172	83	28	1.96	92	18	110	70	99	1	0.93	1.18	1.03	1	43.33	0	1.18	0	2	0	14	1	2	79	80
2	18	M	175	90	29.38	2.06	76	16	140	80	82	1.41	1.33	1.16	1.3	0	14.38	1	1.22	0	8	0	28	0	1	92	111
3	19	M	167	64	22.94	1.72	84	16	140	84	91	1.3	0.78	0.91	0.99	2	7.85	2	1.12	0	4	0	16	0	4	96	115
4	19	M	182	86	25.96	2.08	82	13	126	86	75	1.2	1.13	1.05	1.12	1	31.35	0	1.4	0	6	0	14	1	2	99	130