TO EVALUATE COGNITIVE EVOKED POTENTIAL AND SERUM ADIPONECTIN LEVEL IN PREDIABETES – A CROSS SECTIONAL STUDY

Dissertation submitted to
The Tamil Nadu Dr. MGR Medical University

In partial fulfilment of the regulations for the award of the degree of
M.D. PHYSIOLOGY
Branch V

INSTITUTE OF PHYSIOLOGY & EXPERIMENTAL MEDICINE
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THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY
CHENNAI –600032
MAY 2020

CERTIFICATE

This is to certify that the dissertation entitled **"TO EVALUATE COGNITIVE EVOKED POTENTIAL AND SERUM ADIPONECTIN LEVEL IN PREDIABETES – A CROSS SECTIONAL STUDY"** by the candidate **Dr. B. LEELA PRIYADHARSINI** for M.D Physiology is a bonafide record of the research done by her during the period of study (2017 –2020) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai –600003.

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ACKNOWLEDGEMENT

There is hardly any task than acknowledging my gratitude to all those who have helped me in so many ways during my course.

I gratefully and sincerely thank **Dr. R. JAYANTHI**, M.D., FRCP(Glasgow) the Dean of Government Madras Medical College and Hospital, Chennai for granting me permission to carry out the study at the Institute of Physiology and Experimental Medicine, Madras Medical College and Hospital.

I will forever be thankful to **Prof. Dr. C. THIRUPATHI, D.C.H, M.D.,** the Director and Head of Department of Physiology, Madras Medical College, Chennai for providing insightful discussions about the research and giving me the opportunity to develop my own individuality and allowing me to work with such independence.

I am thankful to **Prof. Dr. MAYILVAHANAM, M.D**, Former Director, Institute of Internal Medicine, Rajiv Gandhi Government General Hospital, Chennai, for granting me permission to recruit cases from the Department.

. I am extremely grateful to my guide **Prof. Dr. RATNA MANJUSHREE JAYARAMAN, M.D.,** without whom it would have been totally impossible to accomplish this work. I also sincerely thank her for her valuable guidance and motivation throughout my study.

I am thankful to my co-guide **Prof. Dr. SUNDARAMURTHY, M.D**, Former Professor, Institute of Internal Medicine, Rajiv Gandhi Government General Hospital, Chennai.

I extend my sincere thanks to **Prof. Dr. P. SATHYA, M.D., D.G.O.,** Professor, Institute of Physiology, Madras Medical College, Chennai, for her valuable suggestions and motivation throughout my study.

I extend my sincere thanks to **Dr. R. SHANTHIMALAR, M.D., D.G.O.,** Associate Professor, Institute of Physiology, Madras Medical College, Chennai, for her valuable suggestions and motivation throughout my study.

I extend my sincere thanks to **Dr. R. KANNAN, M.D.,** Associate Professor, Institute of Physiology, Madras Medical College, Chennai, for his valuable suggestions and motivation throughout my study.

I extend my sincere thanks to **Prof. Dr. A. PARIMALA, M.D., D.C.P.,** Professor of Physiology, for her valuable suggestions and motivation throughout my study.

I extend my sincere thanks to **Prof. Dr. R. VIJAYALAKSHMI, M.D,** Professor of Physiology, for her valuable suggestions and motivation throughout my study.

I extend my thanks to **Prof**. **Dr. RAMA DEVI, M.D.,** Professor and Director, Institute of Biochemistry for her kind permission to do the lab test in their department.

I would also like to express my gratitude to **Prof. Dr. PUSHKALA, M.D.,** Professor and HOD, Department of Immunology, TN Dr. MGR Medical University for permitting me to analyse my samples in the department laboratory.

I thank **Dr. S. ANBUSELVI, M.D.,** Assistant professor, Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai, who helped a lot in completing this study.

I express my sincere thanks to all Assistant Professors of Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai for their guidance and support.

I express my sincere thanks to my Colleagues in the department of Physiology, Madras Medical College, Chennai and my dear friends who readily extended their help to overcome the difficulties of my task.

I thank all the technical and non-technical staffs of IPEM, Institute of Internal Medicine, Institute of Biochemistry, Madras Medical College and Department of Immunology, The TN Dr. MGR Medical University for their timely help to complete my study.

Above all it would be unfair if I fail to mention my special gratitude to my dear parents, my lovable husband, my caring brother and my mother-in-law, who are the pillars of my career and without whom it would have been impossible to accomplish this work.

I dedicate this work to my supportive family.

Finally I thank Almighty for keeping me blessed always in all my endeavours.

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ABBREVIATIONS

1. IGT - Impaired Glucose tolerance
2. ADA - American Diabetes Association
3. IFG - Impaired fasting glucose
4. AGE - Advanced glycosylation end-products
5. BBB - Blood Brain Barrier
6. NRY - Neuropeptide Y
7. ARP - Agouti related peptide
8. POMC – Proopiomelanocortin
9. CaRT - Cocaine and amphetamine regulated transcript
10. NMDA - N-methy-D-aspartate
11. PI3K-PKC - Phosphoinositide 3 kinase-protein kinase C
12. PSD 95 - Postsynaptic density protein 95
13. βAPP - β-amyloid precursor protein
14. IDE - Insulin degrading enzyme
15. PCOS - Poly Cystic Ovarian Syndrome
16. RAGE - Receptor for AGE
17. VEGF - Vascular Endothelial Growth Factor
18. MLC - Myosin Light Chain
19. LOX-1 - Lectin-like oxidized LDL receptor-1
20. BMI – Body Mass Index
21. ROS – Reactive oxygen species
22. DPP - Diabetes Prevention Program
23. GLP-1 - Glucagon like peptide-1
24. HMW - High molecular weight
25. ERK - Extracellular signal related kinase

26. TNF-α - Tumour Necrosis Factor-α
27. PPRE - Peroxisome proliferator activated receptor γ responsive element
28. PPAR-γ - Peroxisome proliferator activated receptor γ
29. ACO - Acyl-CoA oxidase
30. UCP 2 - Uncoupling protein 2
31. AMPK - AMP activated protein kinase
32. CPT-1 - Carnitine palmitoyl transferase 1
33. ELISA – Enzyme Linked Immunosorbent Assay
34. IR – Insulin Resistance
35. PI 3K – Phoshoinositol 3 kinase
36. ras – MAPK – ras mitogen activated protein kinase
37. IRS 1 & 2 – Insulin receptor substrates 1 & 2
38. GLUT 4 – Glucose transporter 4

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Urkund Analysis Result

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Introduction

INTRODUCTION:

Diabetes mellitus is a major public health problem, the incidence of which is increasing worldwide. The prevalence of diabetes continues to increase by 49% in last decade(1). Prediabetes (Impaired Fasting Glucose and Impaired Glucose Tolerance) leads to increased risk of cerebrovascular, cardiovascular changes and overt diabetes(2).

Prediabetes is more common in young adults. The worldwide prevalence of Impaired Glucose tolerance(IGT) was found to be 343 million (7.8%)(1). This ranges from 5.8% in South East Asia to 11.4% in North American and Caribbean Countries. International Diabetes Federation says that the prevalence will increase to 471 million worldwide by 2035. In India the prevalence of Pre-diabetes is found to be 10.6%. It is found that 30 to 40% of subjects with IGT have the tendency to develop type 2 diabetes in future. Alberti (3) revealed that the term 'Prediabetes' was first used to indicate abnormalities of pregnancy (e.g., high-birth weight babies, hydramnios) or family history of type 2 diabetes mellitus. But, in 1980, the term was rejected by the World Health Organization (WHO) because many people with intermediate glucose level will not develop diabetes in future. But in 2005, American Diabetes Association (ADA) again formed this term 'Prediabetes' to collectively indicate impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). But this term does not include other risk factors for diabetes such as family history of type 2 diabetes mellitus, gestational diabetes mellitus (4). But in 2008, WHO discarded this term usage and suggested the term "intermediate hyperglycemia" to cover IGT and IFG(5). However, American Diabetes Association is using the term 'Prediabetes' (6).

Why the study group was chosen as prediabetes?

Many studies have shown that there is increased risk of developing cognitive dysfunction in diabetic individuals (7). Only few studies reveal that pre-diabetics are prone for cognitive dysfunction (8).

According to American Diabetes Association (ADA), a person is said to be pre-diabetic if he/she fulfils any one of the following criteria

1. Fasting plasma glucose of 100 to 125mg/dl

   (Or)

2. Impaired Glucose Tolerance (IGT) of 140 to 200 mg/dl after ingestion of 75g of oral glucose load.

The risk factors for developing prediabetes include increased Body Mass Index, physical inactivity, family history of diabetes mellitus, history of gestational diabetes mellitus, irregular sleep patterns, increased waist hip ratio, history of polycystic ovarian syndrome, etc. Studies have shown that 30 – 40% of these individuals may proceed to develop diabetes. The complications of prediabetes include development of overt diabetes, Hypertension, cardiovascular diseases, diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, cognitive dysfunctions, diabetic non-healing ulcers, secondary infections, diabetic ketoacidosis, fatty liver, etc. If a person is diagnosed to have prediabetes at an early stage he/she can delay the onset of diabetes by doing regular exercise, by having balanced diet, by reducing the weight and by having regular sleep. Many studies have shown that by doing these lifestyle changes, the hyperglycemic condition can to reverted back to normoglycemic condition (9).

One of the earliest neurological complications of prediabetes was found to be cognitive dysfunctions. Inspite of various reasons, Anna Marseglia et al says that the neurological complications in hyperglycemia may be due to the following reasons.

(i) Hyperglycemia in brain may lead to neuronal death which may proceed to result in cognitive dysfunctions over time (10).

(ii) Hyperglycemia may stimulate mutations in neuronal and glial cell functioning. This can lead to the production of reactive oxygen species, which results in oxidative stress, advanced glycosylation end-products (AGEs) formation, activation of advanced glycosylation end-products receptors. These changes will terminally lead to produce atherosclerosis in cerebral blood vessels.

(iii) In hyperglycemia brain atrophy and blood volume reduction is seen which may lead to cognitive decline(11).

So the objective of this study is to assess the early onset cognitive dysfunction in pre-diabetics. If diabetes is causally related to cognitive impairment, one might also expect to observe impaired cognitive performance in those with impaired fasting glucose (IFG) levels or “prediabetes.”

Therefore, we sought to determine the association between pre-diabetes and cognitive function and risk of developing cognitive impairment in pre-diabetic patients.

Event related COGNITIVE EVOKED POTENTIAL P300 as a tool for evaluating cognition:

Cognitive dysfunction in patients with diabetes mellitus was first noted in 1922, when patients with diabetes, who were “free from acidosis but usually not sugar free,” were noted to have impaired memory and attention on cognitive testing compared with controls(12). Since then, there have been many studies designed to better delineate the scope and magnitude of cognitive dysfunction in diabetes. The following are a summary of cognitive domains that have been found to be negatively affected by diabetes mellitus: Slowing of information processing psychomotor efficiency, attention, memory, learning, problem solving, motor speed, vocabulary, general intelligence, visuoconstruction, visual perception, somatosensory examination, motor strength, mental flexibility and executive function(13).

There have been controversial reports regarding effect of diabetes on cognitive functions. Most of the psychometric studies' employing variety of tests, assessing psychomotor speed, selective attention, lexical fluency, auditory verbal learning, showed that scores were lower in diabetics as compared to controls(12–14).

Cognition refers to all the mental activities involved in receiving information, comprehending it, sorting, retrieving, and using it. It is associated with goal directed behavior and helps the individual in adjusting with the changing environmental needs(15). Tomas Paus says that anterior cingulate gyrus and prefrontal cortex in brain are responsible for cognition(16). Cognitive evoked potential is one of the various tests available to test cognition.

Long latency evoked potentials are related to cognitive processing and are referred to as cognitive evoked potential (CEP). P300 is the most frequently investigated Cognitive Evoked Potential appearing at about 300 millisecond following task-related stimuli. P300 can be elicited by any stimulus, the most common being an unexpected or infrequent stimulus (oddball paradigm). This involves presentation of unexpected, infrequent stimuli randomly interspersed among frequent stimuli. The character of unexpected stimuli differs from the common stimuli in terms of frequency or intensity. Two factors

(i) stimulus infrequency or unexpectedness and

(ii) attention to task relevance operate independently.

Unexpectedness of stimuli and attention to it produce different evoked potentials. These evoked potentials are called as cognitive evoked potential. The P300 component is considered for analyzing the subject's cognition. This test reflects the subject's cognitive skill level and verifies whether disorders are present in the auditory association cortex. So cognitive evoked potential is used to assess early cognitive dysfunction in pre-diabetic individuals.

Role of serum adiponectin in diabetes:

Adiponectin is an adipocyte derived hormone that plays an important role in glucose and fatty acid metabolism. It helps in oxidation of fatty acids and also helps in reducing blood glucose level by increasing the glucose uptake by skeletal muscles. It also helps in decreasing glucose production by liver. Adiponectin also plays a role in fatty acid oxidation and glucose uptake by cardiac myocytes.

Adiponectin has a protective role against insulin resistance and inflammation. It also protects the body against metabolic diseases(17). It has anti-inflammatory and anti-atherogenic effects. It's level is correlated negatively with obesity, dyslipidemia, coronary artery disease, insulin resistance, mild cognitive impairment(18).

The normal serum level of adiponectin in non-obese individuals ranges from 4 to 30 mcg/ml. This level is slightly more in female than in male. It is less in obese individuals. Studies have shown that by losing weight the adiponectin level can be increased.

In brain, adiponectin improves insulin signaling and glucose uptake(19). The expression of inflammatory cytokines such as TNF-α and NF-κB activation can be reduced by adiponectin. Also adiponectin promotes the production of other anti-inflammatory molecules like IL-10 and IL-1 receptor antagonist(20). So when adiponectin level decreases in cognitive dysfunction this balance becomes altered and the shift of cycle occurs towards pro-inflammatory state. So decreased level of adiponectin in cognitive impairment has two effects –

- Increased expression of inflammatory cytokines and
- Decreased production of anti-inflammatory factors.

This in turn will lead to inflammation in cerebral blood vessels. This inflammation leads to cognitive dysfunction in prediabetes. So adiponectin can be taken as a marker to evaluate the cognition in pre-diabetic individuals.

The earliest changes in cognitive dysfunctions can be well reflected by evaluating patient's event related cognitive evoked potential as well as by assessing their serum

adiponectin levels. As it had been stated but yet to be considered definite, the serum adiponectin levels were found to have a direct association with changes in blood glucose levels and the changes in hippocampal formation in diabetic patients. So in this study, our objective was to evaluate the cognition in pre-diabetic individuals by assessing their cognitive evoked potential and serum adiponectin level.

Review of Literature

REVIEW OF LITERATURE

SYNTHESIS AND SECRETION OF INSULIN:

Insulin is a glucose regulating hormone. The molecular weight of insulin is about 5808. Insulin is produced in the following steps:

1. Translation of insulin RNA by ribosomes to form preproinsulin which has a molecular weight of 11500
2. Cleavage of preproinsulin to form proinsulin in endoplasmic reticulum. Proinsulin has a molecular weight of 9000
3. Cleavage of proinsulin to insulin and C-Peptide in Golgi apparatus.
4. Packaging of insulin and C-peptide in the secretory granules and its secretion into blood.

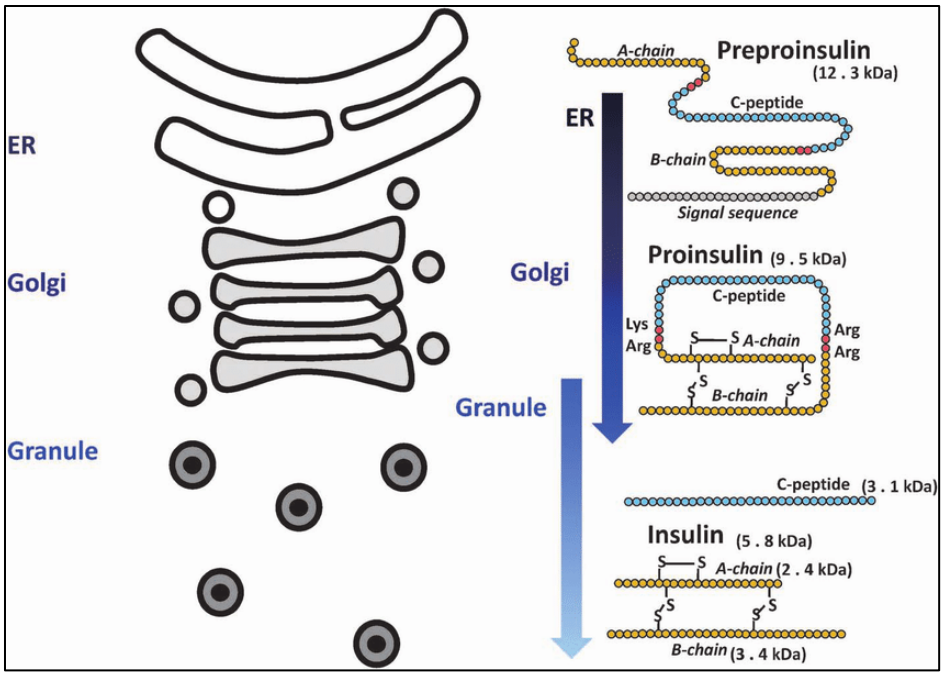


FIGURE 1: SYNTHESIS AND SECRETION OF INSULIN

MECHANISM OF ACTION OF INSULIN:

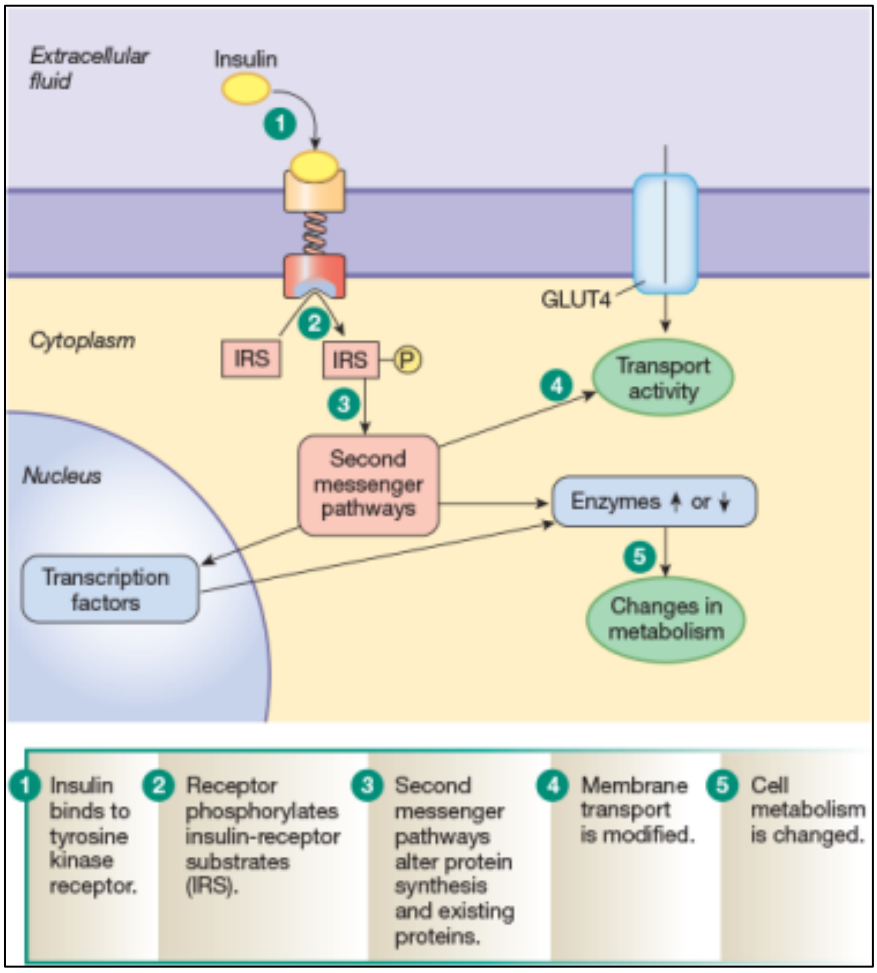


FIGURE 2: MECHANISM OF ACTION OF INSULIN

FUNCTIONS OF INSULIN:

i) Effects on Carbohydrate metabolism:

- It increases the uptake of glucose in muscles and adipose tissue by translocating the glucose transporter in cell membranes
- It increases glucose utilization by promoting glycolysis and glycogenesis
- In decreases glucose production by inhibiting gluconeogenesis and glycogenolysis

ii) Effects on lipid metabolism:

- Insulin stimulates lipogenesis in liver and helps in formation of cholesterol
- It also stimulates lipoprotein lipase and helps in deposition of circulatory fat in adipose tissue
- Insulin also inhibits lipolysis in liver and adipose tissue
- It acts as a antiketogenic hormone
- Insulin is very much important for the utilization of very low density lipoprotein and low density lipoprotein

iii) Effects on protein metabolism:

- It promotes protein synthesis by increasing the transport of amino acids in to the cells and by increasing the translation of messenger RNA on ribosomes
- It also inhibits protein degradation

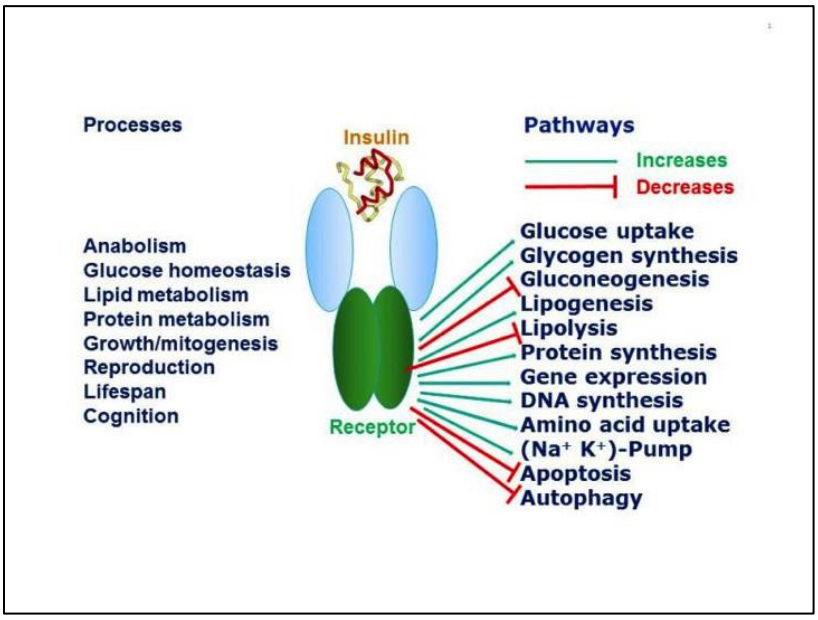


FIGURE 3: ACTIONS OF INSULIN

ROLE OF INSULIN ON CENTRAL NERVOUS SYSTEM:

Before mid-1950s, it was presumed that insulin has nothing to do with the absorption or the rate of utilization of glucose in brain. Then in late 1950s, it was found that glucose uptake by the spinal cord can be increased by insulin. After that studies have shown that injection of insulin into cerebrospinal fluid resulted in increase in the concentration of radioactive phosphorus in hypophysis, choroid plexus and epiphysis. As phosphorus is involved in glucose metabolism, it was figured indirectly that some areas' glucose metabolism in the brain could be insulin sensitive (21).

By immune-histochemical staining of the brain with anti-insulin antibodies, insulin was first discovered in the CNS. Havrankova et al in 1978 found that insulin in present in neuronal pericarya of the olfactory bulb and frontal cortex of the immature rat brain. Following him Dorn et all in 1981 proved the presence of insulin in neurons of the hypothalamus, thalamus, amygdala and hippocampus of the murine brain(22). There are two possible theories for the origin of insulin in the brain. One, brain insulin can be transported from peripheral tissues. Two, insulin can be locally synthesized in the CNS. But there is still a debate between these two theories.

Previously it was thought that glucose metabolism in brain is insensitive to insulin. But now studies have shown that brain's glucose metabolism is dependent on insulin(21). There are two glucose transporters in brain, namely GLUT1 and GLUT3 in brain, which are responsible for glucose uptake in neuronal and glial cells. The principal glucose transporter in the blood brain barrier is GLUT1. The GLUT1 mRNA levels and glucose entry in the astroglial cells of the brain is also found to be dependent on insulin. In

hypoglycemia the GLUT1 expression is increased in Blood Brain Barrier (BBB) and GLUT4 expression is decreased in skeletal muscles. So the available glucose is directed to CNS for utilization. By these mechanisms the brain is protected from hypoglycemic damage.

Woods et al demonstrated that food intake and body weight can be decreased by intracerebroventricular infusion of insulin in baboons. Insulin decreases food intake by decreasing orexigenic neuropeptides - neuropeptide Y (NPY) and Agouti related peptide (AgRP) expression and increasing anorexigenic neuropeptides proopiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CaRT) expression in the arcuate nucleus. These two collectively cause the increased activity of melanocyte stimulating hormone in neurons in the paraventricular nucleus(23).

The memory tasks including the efficiency in the passive avoidance task and Morris water maze can be enhanced by central insulin infusion in rats. The expression of dopamine neurons can be enhanced by insulin. It controls the transmission of N-methyl-D-aspartate (NMDA) receptors in hippocampal neurons. It acts through tyrosine phosphorylation of NMDA receptors NR2A and NR2B subunits. It stimulates the membrane recruitment of NMDA receptors into excitatory synapses. This mechanism plays role in the development of long term potentiation in the hippocampus, which is a significant phase in learning and memory. Insulin also controls α-amino-3-hydroxy-5-methy.-4-isoxazolepropionic acid (AMPA) receptors in cerebellum and hippocampus. Through Phosphoinositide 3 kinase-protein kinase C (PI3K-PKC) pathway it stimulates the clathrin-dependent endocytosis of AMPA receptors. This takes place in the

hippocampal CA1 neurons. This pathway has a significant place in long term depression, which is important for memory consolidation and memory flexibility(23).

In hippocampal neurons via membrane recruitment and protein synthesis, insulin regulates type A γ-aminobutyric acid (GABA) receptor activity. By this pathway it has its role in the regulation of the activity of inhibitory synapses. Insulin has its action in regulating structural plasticity in the brain, which includes dendritic plasticity, visual circuit function and synapse number. Insulin also stimulates the expression of postsynaptic density protein 95 (PSD95). PSD95 is very much important for postsynaptic junction formation. All these suggests that insulin has vital role in brain function(23).

Yang et al showed insulin's stimulation of ornithine decarboxylase activity in primary cell cultures from fetal rat brains. Ornithine carboxylase is an enzyme which is involved in the regulation of cellular metabolism and proliferation. Kappy et al in 1984 demonstrates specific insulin binding on fetal and neonatal brain membrane preparations. He also found in laboratory animals the changes in concentration and number of insulin and insulin receptors during different stages of brain development. There is increase in specific binding to plasma membranes in prenatal period. It reaches the maximum level in the early postnatal period. After that it decreases in adulthood(22). So it is said that insulin receptor number is developmentally regulated. These evidences postulate the involvement of insulin in the growth, development and metabolism of brain.

Insulin also has its control in β-amyloid precursor protein (βAPP) metabolism and thereby it balances β-amyloid (Aβ) anabolism and catabolism. Insulin degrading enzyme (IDE) is a metalloprotease involved in the degradation of various peptides, including

insulin in brain. It also helps in the catabolism of extracellular Aβ in CNS. So insulin acts as a competitive inhibitor for IDE and inhibits Aβ degradation. Thereby the extracellular concentration of Aβ will be increased. Insulin also stimulates Aβ secretion, which results in reduction in the intracellular concentration of Aβ1-40 and Aβ1-42. By these mechanisms insulin plays a key role in regulating tau protein, and Aβ and βAPP metabolism in neurons (24).

INSULIN RESISTANCE AND COGNITION:

As we have discussed already GLUT1 expression is increased during hypoglycemia to maintain glucose level in the brain. To increase GLUT1 expression in BBB vasodilation must occur to bring more endothelial cells in contact with the blood. But in hyperglycemia induced insulin resistance there are abnormalities in endothelial dependent vasodilation (the mechanism will be discussed later). So there will be failure in increasing glucose availability to brain. This mechanism plays role in cognitive decline in hyperglycemia (25).

Geert Jan Biessels et al have quoted that hippocampal insulin resistance, which is responsible for cognitive decline is due to the combined effect of impaired insulin receptor signaling and decreased transport of insulin across the BBB(26). Impaired insulin receptor signaling includes decrease in insulin stimulated phosphorylation of the insulin receptor and Akt (26).

G.J. Biessels have said that hyperglycemia is one of the risk factors for atherosclerosis of the carotid and intracranial arteries. The basement membrane

thickening can occur in cerebral capillaries because of hyperglycemia resulting in chronic and insidious ischemia in brain. This leads to the occurrence of subcortical whiter matter lesions. These changes also play a role in cognitive dysfunction development in hyperglycemia.

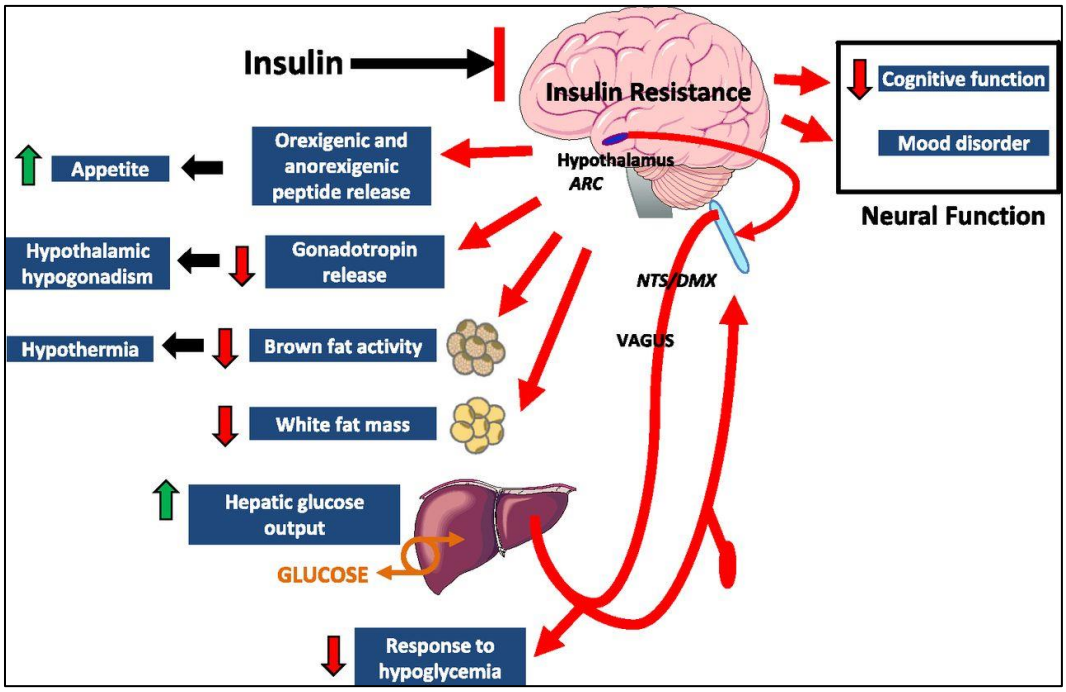


FIGURE 4: EFFECT OF INSULIN RESISTANCE ON BRAIN

NATURAL HISTORY OF DIABETES MELLITUS:

Appreciating the natural history of diabetes is very much significant to start early intervention to delay the onset of diabetes by early identification of persons prone to develop diabetes in future. Early identification is very much important in diabetes because both the macrovascular and microvascular complications in diabetes start 5 to 10 years before the onset of disease.

The normal glucose tolerant stage proceeds to develop diabetes by the following metabolic abnormalities:

1. Increase in body fat
2. Abnormalities in insulin secretion
3. Dysfunction in insulin sensitivity
4. Increased production of endogenous glucose.

Christian Weyer et all in his longitudinal study consisting of 17 Pima Indians has said that before the onset of diabetes, the individuals can undergo the various stages in between. First the increase in body weight was observed. The insulin sensitivity was decreased by 31%. This decreased insulin action was found 5 to 10 years before the onset of diabetes which suggests that insulin resistance proceeds the onset of diabetes. The acute insulin secretory response (ARI) was decreased by 57% in persons who developed diabetes. Acute insulin secretory response (ARI) is the average incremental plasma insulin concentration from the third to the fifth minute after 25g of intravenous glucose bolus. And finally the endogenous glucose output (EGO) which reflects hepatic glucose production increased by 15%. Also at this time, the insulin resistance was more pronounced (27).

The factors such as elevated free fatty acids, hyperglycemia, pregnancy, obesity, sedentary lifestyle and aging worsen insulin resistance. The intake of medications such as steroids, cis-retinoic acid, estrogens, nicotinic acid, oral contraceptives, phenothiazines, antipsychotic agents also can increase insulin resistance. Insulin

resistance is characterized by impaired responses to the physiologic effects of the hormone on glucose, lipid and protein metabolism and by affecting vascular endothelial function(28). The capacity of insulin to inhibit hepatic gluconeogenesis or to induce glucose utilization in the muscle and fat is decreased in insulin resistance. Increased hepatic glycogenolysis and gluconeogenesis leads to fasting hyperglycemia. Insulin resistance in-turn stimulates β cells to secrete more insulin which leads to β cell dysfunction. This will subsequently result in less insulin production. But in some patients with minimal insulin resistance β cell impairment can be observed. So the resultant hyperglycemia results in insulin resistance (28).

PREDIABETES:

Epidemiology:

In US it was estimated that 34% of adults aged 18 years or older have prediabetes. Nearly half of the adults (48.3%) aged 65 years or older had prediabetes. The prevalence was found to be more in men (36.6%) than women (29.3%). A meta-analysis has reported that the annual risk of progression of isolated IGT to diabetes was 4 – 6%, the annual risk for isolated IFG was 6 – 9% and the annual risk for both IGT and IFG was 15-19%. Among people belonging to different race, the prevalence of prediabetes was found to be almost similar. The prevalence of various races is shown in the following table 1

TABLE 1: PREVALENCE OF PREDIABETES IN VARIOUS RACES

Race	Prevalence (%)
Asian, non-Hispanic	35.7 (33.0–38.5)
Black, non-Hispanic	36.3 (33.3–39.4)
Hispanic	31.7 (28.4–35.2)
White, non-Hispanic	31.5 (28.3–34.9)

RISK FACTORS:

The following factors are reported as risk factors for developing prediabetes by American Diabetes Association

- Physical inactivity
- First-degree relative with diabetes
- Women with history of Gestational diabetes mellitus or history of baby delivered with birth weight more than 4 kg
- Hypertension
- High Density Lipoprotein (HDL) < 35 mg/dl and/or Triglyceride (TG) > 250 mg/dl
- $HbA_1C \geq 5.7\%$
- History of clinical conditions which are associated with insulin resistance, such as obesity, acanthosis nigricans, Poly Cystic Ovarian Syndrome(PCOS)
- History of Cardiovascular disease

ADA also recommends to begin, screening for prediabetes at the age of 10 years or at the onset of puberty in children who are overweight and have any 2 of the following risk factors

- Family history of type 2 diabetes mellitus in first or second degree relatives
- High risk race/ethnicity
- History of GDM in mother during that child's gestation
- Signs of insulin resistance or conditions associated with insulin resistance

It is found that if a child is born to a mother with GDM, there is 5.75 times increased risk of developing prediabetes.

INSULIN RESISTANCE:

Endogenous glucose production depends mainly on liver. The product of fasting insulin and endogenous glucose production is the marker of hepatic insulin resistance. it has been observed that this product has a strong relationship with fasting glycaemia. The blood glucose level after consumption of food depends on intestinal absorption of glucose, endogenous glucose production and total body glucose uptake. Endogenous glucose production is decreased in normoglycemic individuals but this is not decreased in prediabetes. If insulin secretion is increased in proportion to insulin resistance, there will be no difference in blood glucose level. But the β cells are dysfunctional at this stage. Disposition index is a constant which is the ratio of incremental insulin over incremental glucose divided by insulin resistance. This constant is higher in normal healthy individuals and lower in pre-diabetic individuals. Also it was found by autopsy studies that β-cell volume is decreased by 50% in pre-diabetic individuals.

PROGRESSION FROM PREDIABETES TO DIABETES:

The annual conversion rate from prediabetes to diabetes is about 5 – 10%. Also it was found that annualized conversion rate for isolated IGT was 4 – 6%, for isolated IFG it was 6 – 9% but for combined IFG and IGT it was around 15 – 19%. In the DPP study it was found that the annualized incidence was 11%. In the US Multi-Ethnic study of Atherosclerosis it was found that the annual incidence was 6% for participants with IFG. In a Japanese population based study it was found that the annual incidence was 7% among persons with HbA1C of 5.7 – 6.4%. ADA says that 70% of pre-diabetic individuals will get diabetes. Also it was found that the chance of developing diabetes in women with gestational diabetes mellitus is about 20 – 60%, 5 -10 years after pregnancy.

METABOLIC ABNORMALITIES DUE TO HYPERGLYCEMIA IN PREDIABETES:

In Prediabetes, insulin resistance is predominant. But the insulin secretion may be normal or even increased. The increased hepatic glycogenolysis and gluconeogenesis is not observed. But once the patient develops diabetes, the hyperglycemic state can affect many signaling pathways at tissue level. These abnormalities may be due to the direct effect of hyperglycemia or due to the metabolic substances produced due to hyperglycemia. Many of the pathways have been found out so far. They are as follows:

1. Increased aldose reductase activity
2. Increased formation of advanced glycation end products (AGE)
3. Activation of protein kinase C (PKC) isoforms
4. Increased oxidative stress due to reactive oxygen intermediates

5. Electron transport chain in the mitochondria stimulates increased production of superoxide anions which results in enhanced activation of hexosamine pathway.
6. Activation of polyol pathway.

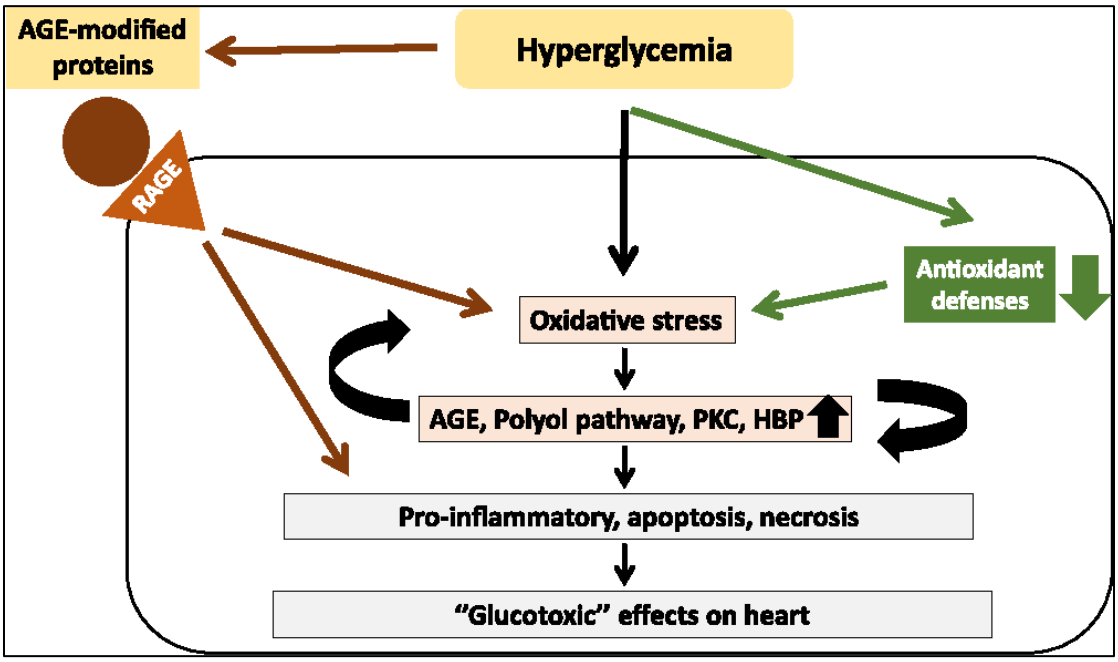


FIGURE 5: METABOLIC ABNORMALITIES OF HYPERGLYCEMIA

1. INCREASED ALDOSE REDUCTASE ACTIVITY:

Stimulation of aldose reductase enzyme produces the increased production of sorbitol at tissue level in diabetes which in-turn leads to the reduced concentration of protective organic osmolytes at tissue level. This mechanism promotes the tissue damage at cellular level which leads to macrovascular and microvascular complications. F. Kilic et all in his study has found out that the taurine level was decreased in animal model of diabetic cataract which is due to this mechanism(29). Supporting this view, Victor R. Drel et all in his study has observed that aldose reductase (fidarestat) treatment

counteracted cataract formation, retinal oxidative stress, glial activation, and apoptosis in mature streptozotocin-diabetic rats(30).

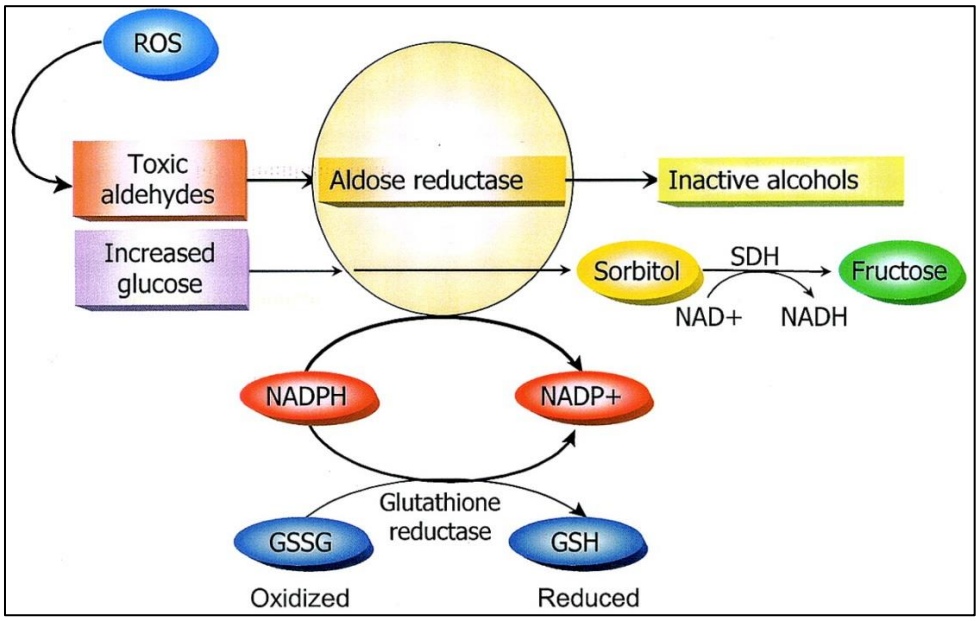
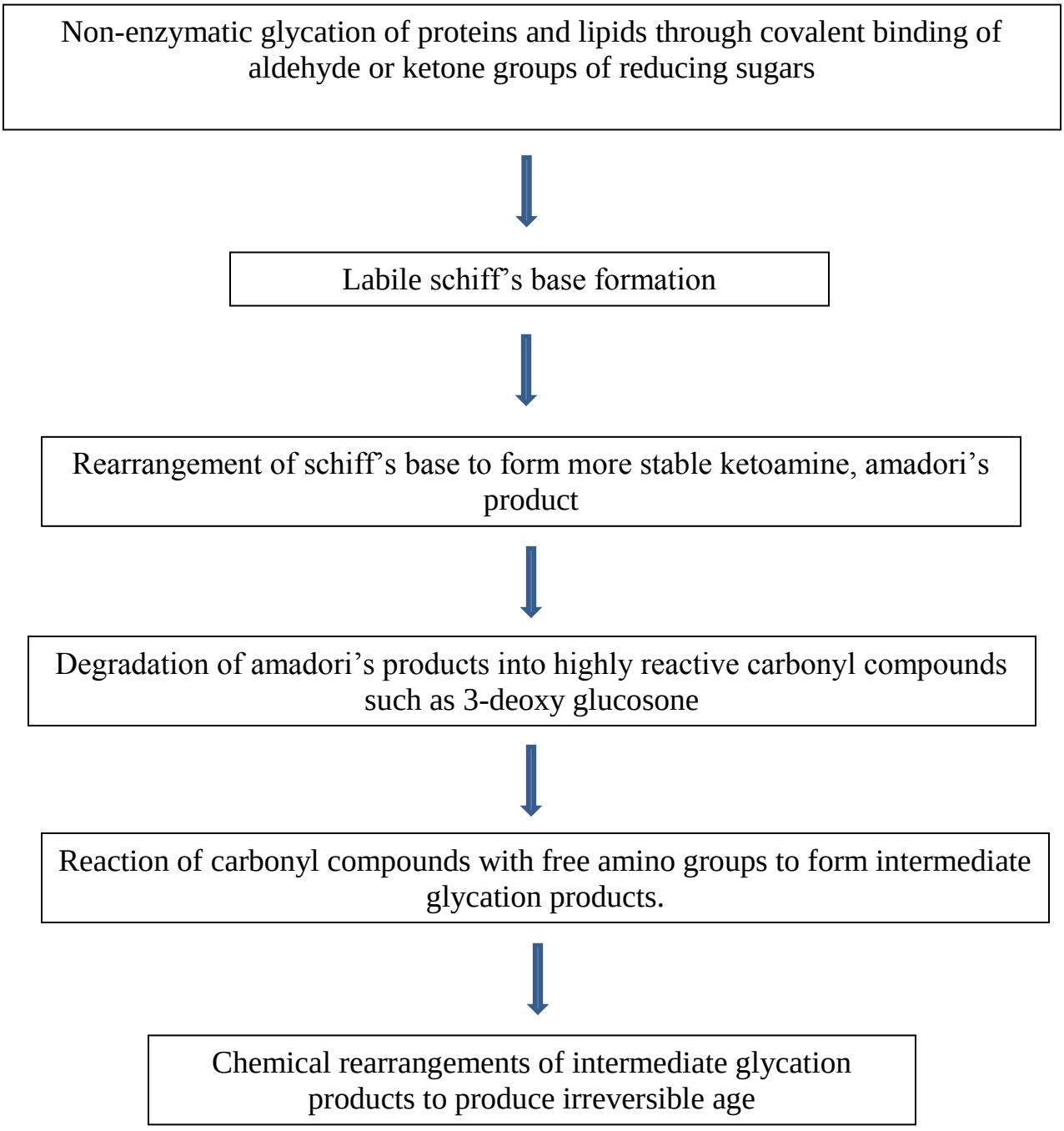


FIGURE 6: PRODUCTION OF SORBITOL

Sorbitol accumulation also leads to the decreased level of myoinositol at cellular level. This decreased myoinositol limits phosphoinositide signaling. This can lead to diabetic neuropathy. Also it was found that by supplementing myoinositol or aldose reductase inhibitors, the nerve conduction velocity was increased(31). The prostaglandin metabolism and nitric oxide synthesis is also found to be altered by deficient myoinositol. So the defects can also be counteracted by giving prostaglandin E1.

2. FORMATION OF ADVANCED GLYCATION END PRODUCTS:

The cellular properties are altered in hyperglycemia by various mechanisms. But the most significant pathway responsible for vascular changes of atherosclerosis in diabetes is the increase in non-enzymatic glycation of proteins and lipids which leads to the irreversible formation and deposition of advanced glycation end products (AGE)(32).



The intermediate glycation products which can contribute to AGE are 3-deoxy glucosone, glyoxal and methylglyoxal. AGEs consists of a large number of chemical structures such as - 2-(2-furoyl)-4(5)-furanyl- 1H-imidazole (FFI), 1-alkyl-2-formyl-3,4-diglycosyl pyrroles (AFGPs), N-q-carboxy-methyl-lysine (CML), pyrraline and pentosidine(32).

These AGEs are deposited both extracellularly and intracellularly in macrophages and vascular smooth muscle cells. These AGEs affect the integrity of vascular smooth cells in the following ways.

1. Mechanical dysfunction of the vessel wall macromolecules
2. Adherence of blood cells to the vessel wall
3. Disruption of cellular function through binding to the AGE receptors(32).

The most significant AGE that is formed in vivo is Nε-carboxymethyllysine (CML). The oxidation reaction between polyunsaturated fatty acids and protein forms Nε-carboxymethyllysine (CML). Also the oxidative breakdown of Amadori products produces CML. The reaction between derivatives of two glyoxal molecules with two lysine residues synthesizes glyoxal lysine dimer (GOLD) or methylglyoxal lysine dimer (MOLD). When two sugar molecules with one alkylamine molecule reacts alkyl formyl glycosyl pyrroles (AFGP) are formed. The reaction between Amadori dione and an arginine residue produces arginine-lysine imidazole (ALI). A dicarbonyl intermediate is formed by oxidation of sugars and lipids. This dicarbonyl intermediate binds with amino acids and forms AGEs (33).

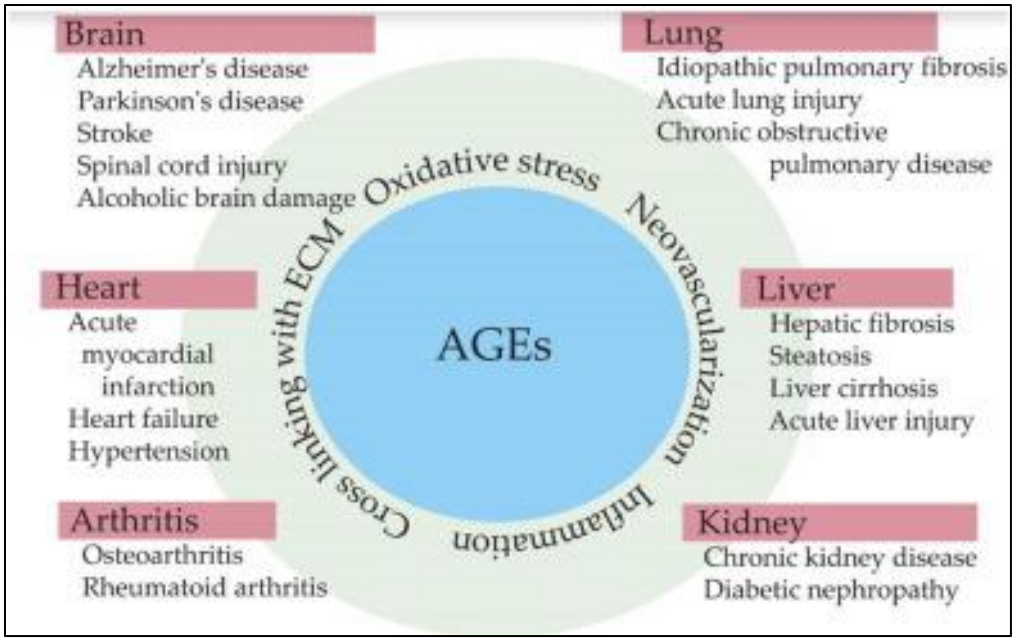


FIGURE 7: ACTIONS OF AGE ON VARIOUS ORGANS

RECEPTOR MEDIATED ACTIONS OF AGEs:

The AGEs bind with Receptor for AGE (RAGE) for exerting its actions. RAGE is 45kDa protein. It is a member of immunoglobulin superfamily. It is made up of 403 amino acids. When AGE binds with RAGE the following events occur:

Binding of AGE with RAGE

↓

Induction of intracellular reactive oxygen species (ROS)

↓

Activation of redox-sensitive transcription nuclear factor NF-κB

Transcriptional activation of various genes involved in inflammation, immunity and atherosclerosis

Quehenberger. P et all has found that AGE – RAGE interaction, which induces activation of nuclear factor NF-κB leads to the increased ET-1 antigen and increased ET-1 gene expression. This produces vasoconstriction. Also it was found that NF-κB blockage prevents ET-1 induction.

3. ACTIVATION OF PROTEIN KINASE C:

The protein kinase C is made of single polypeptide. It has two terminals – an N – terminal which is a regulatory region and a C – terminal which is a catalytic region. It has four Conserved domains – C1 to C4.

1. C1 domain has a Cysteine rich motif. The diacylglycerol/phorbol binds to this site.
2. C2 domain has the calcium binding site and the recognition site for acidic lipid
3. C3 domain
4. C4 domain

C3 and C4 domains has ATP and substrate binding sites(34)

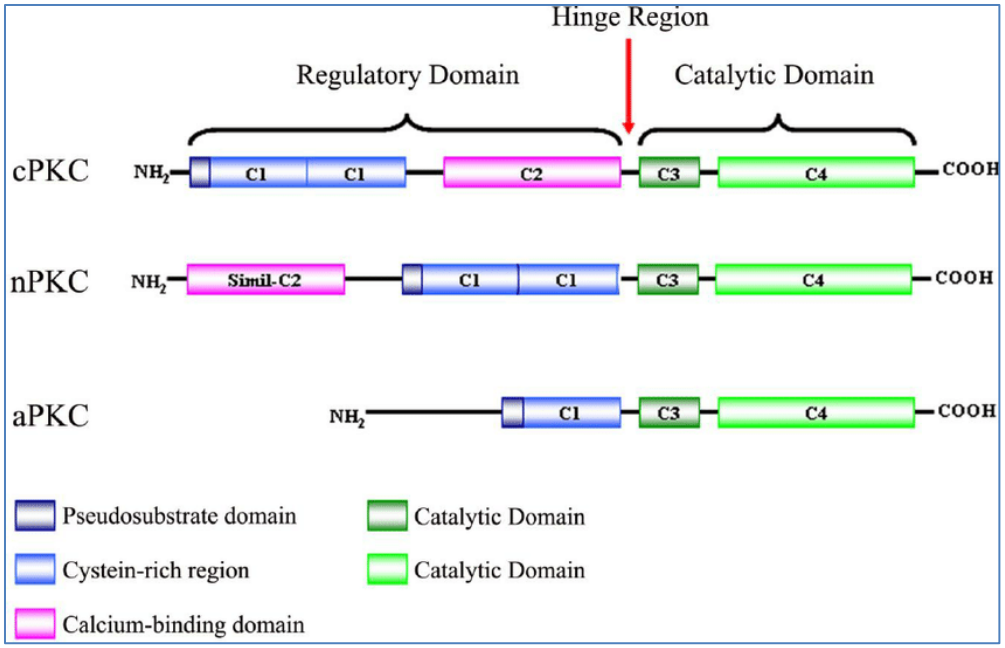


FIGURE 8: STRUCTURE OF PROTEIN KINASE C

11 isoforms of Protein kinase C was discovered so far. They are classified into three groups – conventional protein kinase C, novel protein kinase C and atypical protein kinase C

1. Conventional protein kinase C (cPKC) isoforms (PKC-α, -β1, -β2, -γ) – these isoforms are the first discovered isoforms. These are stimulated by phosphatidylserine, calcium, and DAG or phorbol esters(35).
2. Novel protein kinase C (nPKC) isoforms (PKC-δ, -ε, -θ, -η) - these are activated by phosphatidylserine, DAG or PMA, but not by calcium(35)
3. Atypical protein kinase C (aPKC) – these are not activated by calcium, DAG or PMA(35)

The glycolytic intermediate dihydroxyacetone phosphate is elevated in hyperglycemia. The dihydroxyacetone phosphate is reduced to form glycerol-3-phosphate. This in turn increases the diacylglycerol (DAG) level. The DAG activates the Protein kinase C. Hyperglycemia can result in elevated level of oxidants such as H_2O_2 and mitochondrial superoxides. These also can activate protein kinase C.

The activated protein kinase produces the following changes by acting on endothelial cells, vascular smooth muscle cells and monocytes.

ENDOTHELIAL CELLS:

Activated protein kinase C increases the permeability of albumin by disrupting endothelial cells' barrier. Inoguchi et all has found that staurosporine can prevent this effect. The activated protein kinase C alters the vasodilator effects of endothelial cells by

1. Altering nitric oxide synthase enzyme. Hence the NO synthesis pathway becomes compromised
2. Altering Vascular Endothelial Growth Factor (VEGF) expression and action
3. Reducing the Prostacyclin synthesis
4. Increasing the production of Endothelin – 1 which is a vasoconstrictor
5. Increasing the production of platelet derived growth factor – β, which results in the proliferation of blood vessel wall
6. Increasing the synthesis of transforming growth factor which helps in matrix expansion. (28,35)

VASCULAR SMOOTH MUSCLES:

The vascular tone is maintained mainly by the vascular smooth muscle cells. The activated protein kinase C (PKC) produces Vascular smooth muscle (VSM) contraction. This can happen by two mechanisms. First, CPI-17 is phosphorylated by PKC. This in turn inhibits Myosin Light Chain (MLC) phosphatase, which results in phosphorylation of Myosin Light Chain. This ultimately results in VSM contraction. Second, calponin, which is the actin binding protein, is phosphorylated by PKC. Calponin normally inhibits the actin-activated myosin ATPase. This inhibition is lost when calponin is phosphorylated. So VSM contraction is enhanced (36).

The DNA synthesis is elevated in hyperglycemia. Also in a study it was found that VSM apoptosis is reduced in hyperglycemia. These effects is blocked by PKC inhibitors such as Calphostin C(35)

Hidekatsu Nakashima et al has reported in his study that Angiotensin II induced vascular smooth muscle cell hypertrophy is mediated by activation of PKC-δ. (37)

MONOCYTES:

The activation of monocytes to form macrophages has a vital role in atherosclerosis and inflammation. The monocyte activation is mediated by protein kinase C. Lectin-like oxidized LDL receptor-1 (LOX-1) is a recently found receptor for oxidized Low Density Lipoprotein (LDL). Sawamura. T et all has found that hyperglycemia enhances LOX-1 expression. This results in enhanced macrophage mediated foam cell formation(38). Senthil Kumar Venugopal et all has done a study on α – tocopherol and

found that in hyperglycemia, α – tocopherol decreases superoxide anion release in monocytes by inhibiting protein kinase C(39).

INCREASED OXIDATIVE STRESS DUE TO REACTIVE OXYGEN INTERMEDIATES:

In hyperglycemia, both increased generation and decreased removal of reactive oxygen species (ROS) is seen. This leads to cellular oxidative stress and abnormal mitochondrial functioning. So the mitochondrial electron transport chain, yields more superoxide anions.

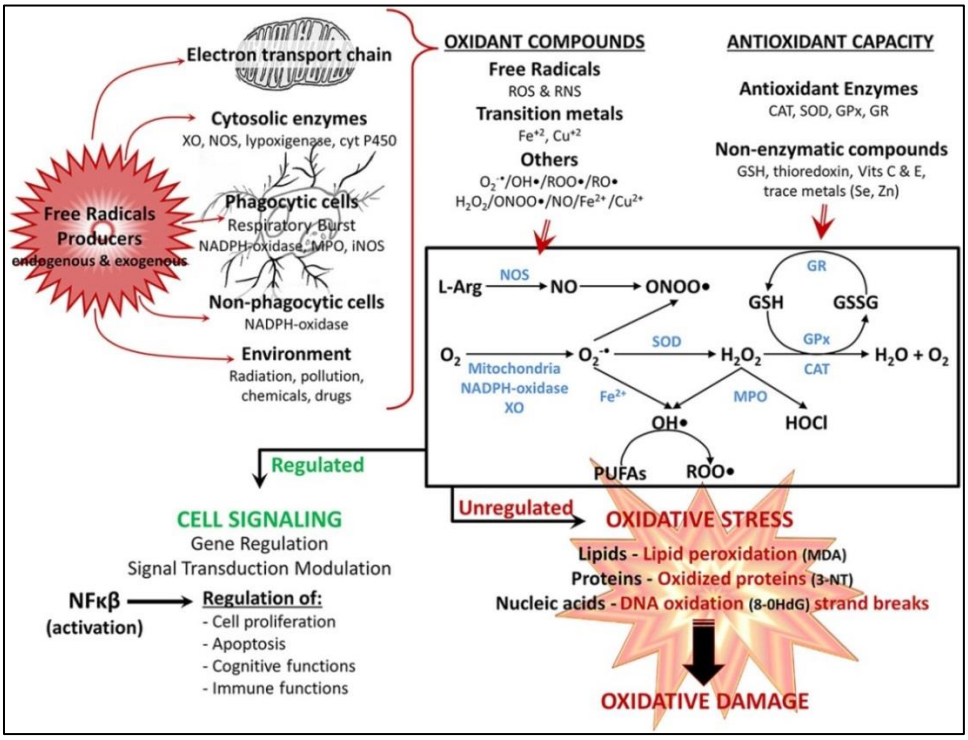


FIGURE 9: PRODUCTION OF FREE RADICALS

Many hypotheses have been formulated to explain this. They are

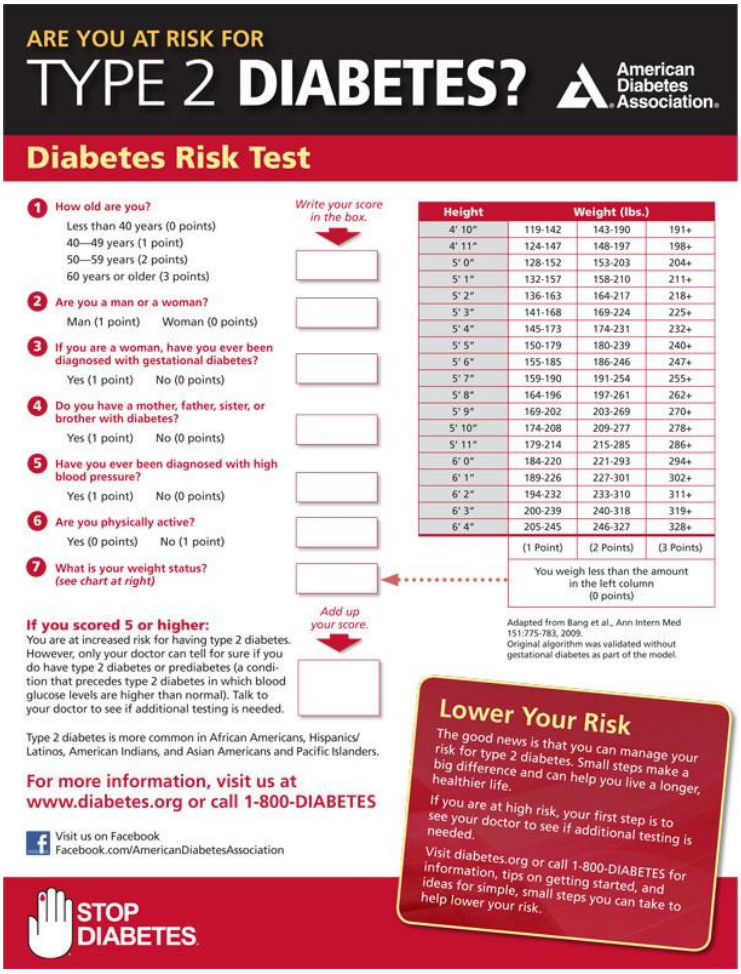
1. In hyperglycemia, there is superabundance of electrons in the mitochondrial electron transport chain. This brings about mitochondrial membrane hyperpolarization which leads to the production of ROS.
2. In hyperglycemia, glucose-6-phosphate level is increased. This will inhibit mitochondrial hexokinase enzyme, which helps in ADP recycling through inner mitochondrial membrane. So when mitochondrial hexokinase enzyme is inhibited, ADP recycling is impaired. This leads to increased mitochondrial ROS production (40).
3. In hyperglycemia, excess glucose is transformed to polyalcohol sorbitol through polyol pathway. So the intracellular concentrations of the antioxidants such as NADPH, GSH can be reduced. This leads to excess production of superoxide anions. This in turn can inhibit glucose-6-phosphate dehydrogenase, which reduces the oxidative stress, augmenting oxidative stress (41).
4. Also excess glucose produces more sorbitol, which is converted to fructose by sorbitol dehydrogenase, elevating the intracellular ratio of NADH/NAD+. This inhibits glyceraldehyde-3-phosphate dehydrogenase, which can result in elevated concentration of triose phosphate. This can sequentially lead to the production of methylglyoxal, a AGE precursor and diacylglycerol, which can activate PKC, thereby generating ROS(41).

Increase in ROS generation leads to mitochondrial and DNA damage, which can

result in cell damage / apoptosis of the cell / growth arrest. All these can produce microvascular damage in hyperglycemia(42).

SCREENING OF PREDIABETES:

If the subjects have these risk factors, then the subjects are asked to take the ADA diabetes risk test. If the subject gets a score of more than 5, then the subject must undergo Glucose Tolerance Test(GTT) to diagnose whether the patient is normoglycemic or hyperglycemic.



ADA DIABETES RISK TEST

COGNITIVE DYSFUNCTION IN PREDIABETES:

The term ‘prediabetes’ is used when, sufficient insulin is produced to prevent overt diabetes, but in the presence of Insulin resistance and it results in impaired fasting glucose and/or impaired glucose tolerance (43).

Insulin receptors are downregulated in blood brain barrier in chronic peripheral hyperinsulinemia, so it reduces insulin transport into the brain. Also in hyperglycemia, insulin signaling might be affected in the hippocampus, which is a memory processing center (44).

The brain represents about 2% of human body weight, but consumes about 25% of total body glucose. The glucose is used as energy substrate and also provides compounds for neurons. But hyperglycemia has deleterious effects in brain and can produce progressive functional and structural abnormalities in the brain (43).

The toxic effects of hyperglycemia are due to metabolic abnormalities such as increased production of AGEs, activation of protein kinase C, increased oxidative stress due to reactive oxygen species and increased flux of glucose through the polyol and hexosamine pathways, the mechanism of action of these metabolites are already discussed.

Many studies have shown that cognitive impairment is associated with hyperglycemia and the strength of association is increasing with advancing age (7)

In the prefrontal cortex, temporal cortex and cerebellar regions, impaired executive function and memory is associated with reduced gray matter density and reduced glucose metabolism(45).

OTHER COMPLICATIONS OF PREDIABETES:

Prediabetes can proceed to develop the following complications:

Pre-diabetic patients are prone to develop atherosclerosis. High blood glucose can lead to basement membrane thickening in endothelial cells. As already discussed, hyperglycemia leads to many metabolic changes such as increased ROS, formation of AGE, activation of protein Kinase C, decreased bioavailability of nitric oxide. These changes lead to vasoconstriction and endothelial damage. So these metabolic abnormalities lead to the development of atherosclerosis in hyperglycemia(46). These changes can lead to myocardial ischemia and death can also occur due to myocardial infarction. Also in diabetes, due to autonomic neuropathy the pain due to myocardial infarction will be suppressed. So the patient may not be aware of chest pain, which ultimately can lead to death of the individual.

Diabetic retinopathy is one of the most terrible microvascular complications of diabetes. It is of two types – non proliferative diabetic retinopathy and proliferative diabetic retinopathy. American diabetic association suggests to check for diabetic retinopathy at the time of diagnosis of diabetes because the microvascular changes starts 10 years before the diagnosis of diabetes mellitus. DPP study had found that about 8 percent of pre-diabetic patients presented with evidence of diabetic retinopathy(47). So it is revealed that pre-diabetic patients also have to screen for diabetic retinopathy

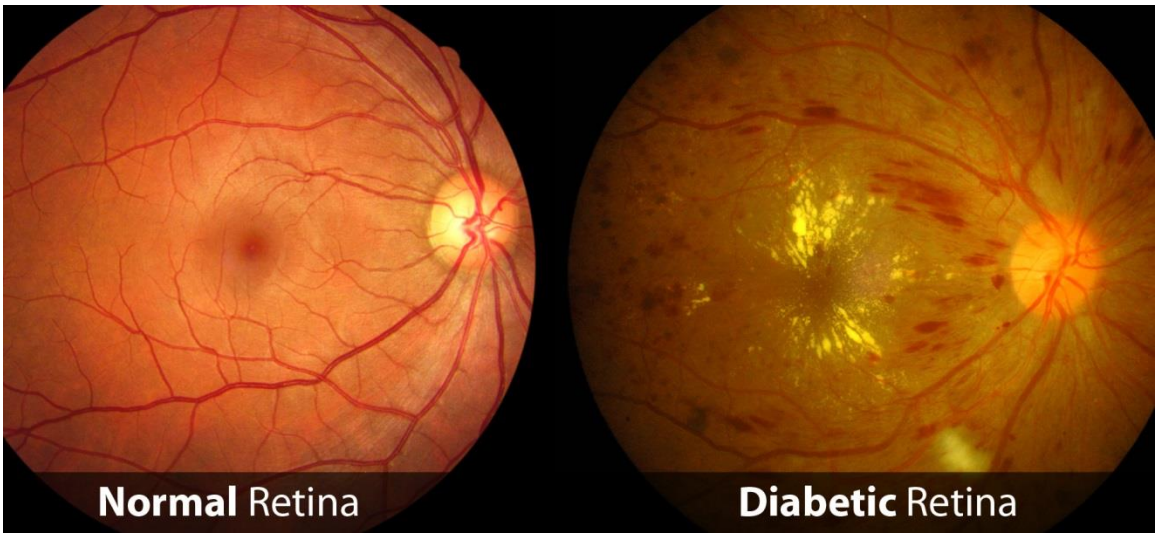


FIGURE 10: DIABETES RETINOPATHY

The permeability of blood vessels in the retina is increased which leads to the leakage of fluid or blood. Also in advanced stage, there is proliferation of new abnormal blood vessels in the retina.

National Health and Nutrition Examination Survey (NHANES) has found that the prevalence of micro albuminuria and macro albuminuria is about 10% and 1.1% respectively in IFG patients(48). So the changes in kidney start in pre-diabetic stage itself. Also glomerular filtration rate is increased in hyperglycemia. It is diagnosed by measuring albumin level in a spot urine sample. This method is recommended by ADA. In random urine specimen, when the cutoff value is taken as 17mg/l, it had a sensitivity of 100% and a specificity of 80% (49). Hyperglycemia leads to the deposition of large amounts of periodic-acid-schiff positive, mesangial matrix which is nodular shaped called Kimmelstiel-Wilson nodules.

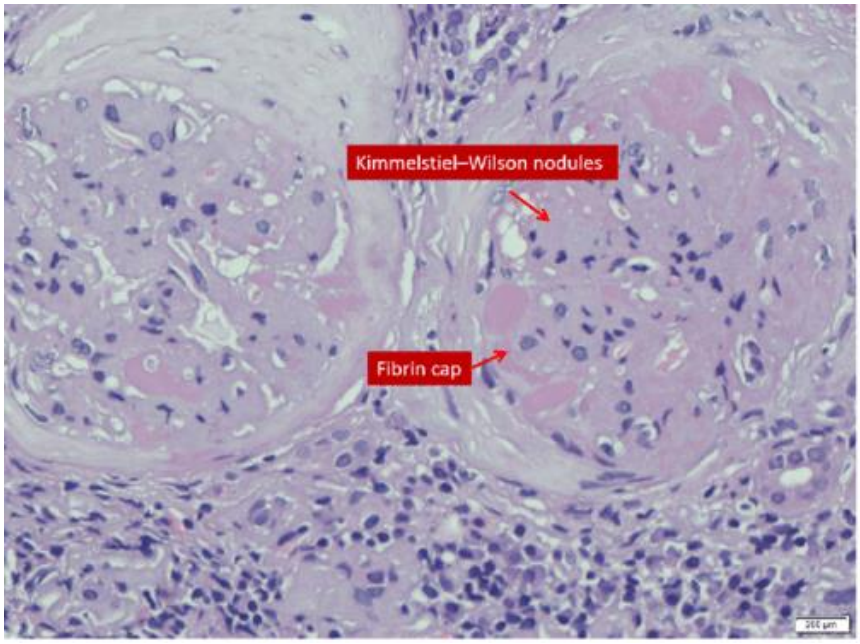


FIGURE 11: KIMMELSTIEL-WILSON NODULES

ADA defines diabetic neuropathy as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes." (50). It may present in various forms such as sensory, focal/multifocal and autonomic neuropathies. But now studies have shown that neuropathic complications appear as soon as the time of diagnosis of diabetes mellitus. It was found that about 25 – 62% of subjects with idiopathic neuropathy had prediabetes. Also about 11 – 25% of subjects with prediabetes had peripheral neuropathy(51). Pre-diabetic subjects with neuropathy may have following symptoms – neuropathy, neuropathic pain, impaired nerve conduction, reduced sweat secretion and diminished sympathetic skin response. Putz et al had said that heart rate variability is lower in IGT patients than in subjects with normoglycemia(52).

TREATMENT OF PREDIABETES:

The treatment for a pre-diabetic patient includes the following interventions

Lifestyle Modifications:

The main aim of lifestyle intervention program is to alter the modifiable risk factors of prediabetes. It is done by altering diet pattern and increasing physical activity. ADA recommends doing Aerobic exercises for ≥ 150 minutes/week. Also it recommends to avoid two consecutive non-exercise days. By doing regular exercise insulin sensitivity can be improved, dyslipidemia can be altered, blood pressure can be lowered, weight can be controlled and development of type 2 diabetes mellitus can be delayed. If weight reduction is aimed, then exercise must be done for atleast 60 minutes per day.

The balanced diet which is individualized according to the age, sex and physical activity of the patient should be taken. The diet should include 14 grams of dietary fibers/1000 kcal. The intake of sugar-sweetened beverages should be limited(53).

American diabetes association recommends planning the plate of a pre-diabetic patient. Non starchy vegetables such as broccoli, cabbage, carrots, cauliflower, green beans, salad, and zucchini should comprise of ½ of the plate. Starchy vegetables such as brown rice, bulgur, green peas, sweet potatoes, and whole wheat bread must form ¼ of the plate. The remaining ¼ plate must include proteins such as fish, chicken, eggs, and lean beef or pork, and soy products such as tofu.

Da Quig et al have done a longitudinal study on IGT patients and found out that by doing lifestyle modifications for 6 years the risk can be reduced by 34 – 69%. Also

diabetes prevention program have found out that by doing lifestyle modifications for 3 years 58% risk can be reduced (53).

Pharmacological interventions:

Metformin is advised for a patient with prediabetes to increase insulin sensitivity, to reduce Body Mass Index (BMI) and to improve lipid profile. Various studies among subjects with IGT reveal that metformin decreases risk by 45% (47). In the United States Diabetes Prevention Program (DPP), it was found that metformin was less effective than lifestyle modifications. But in the Indian DPP study, it was found that metformin was as effective as lifestyle modifications(47).

Thiazolidinediones act on peroxisome proliferator activator receptor gamma (PPAR- γ). It increases insulin sensitivity in adipose tissue, liver and also in muscles. Also the beta cells are protected by thiazolidinediones. The drugs in this group include troglitazone, pioglitazone and rosiglitazone. DPP study had shown that after 1.5 years of follow-up, the incidence of diabetes was drastically reduced in troglitazone taking groups when compared with placebo and metformin taking groups (54). But there are side effects such as weight gain, liver toxicity, increased cardiovascular risk and possible risk of bladder cancer on using these drugs.

A-glucosidase inhibitors such as acarbose and voglibose reduce the postprandial blood glucose increase by enhancing the carbohydrate digestion time and decreasing glucose absorption rate. In a study it was found that voglibose reduces the risk of incidence of diabetes by 40%. But it produces gastrointestinal side effects such as flatulence and diarrhea.

Glucagon like peptide-1 (GLP-1) increases postprandrial insulin secretion and reduces appetite. It was also found to be useful in patients with prediabetes but it has side effects such as nausea and vomiting. Also it is administered in injectable forms which hinder its use (54)

Orlistat is one of the antiobesity drugs that acts by inhibiting gastrointestinal lipase and thereby it inhibits the absorption of dietary fats by approximately 30%. In a study it was found that combined effect of orlistat and low energy diet has better outcome than low energy diet alone (6.7 kg vs 3.8kg). Also it was found that the incidence of diabetes is less when orlistat is added with low energy diet (7.6% vs 3%)(47).

ADIPONECTIN:

Adiponectin was discovered by four research groups independently in the mid 1990s. First it was named as AdipoQ by one group. Another group named it as apM1 – adipose most abundant gene transcript 1. The third and fourth group named it as GBP28 – gelatin-binding protein and Acrp 30 – adipocyte complement related protein 30 respectively. In human adiponectin is encoded by the gene ADIPOQ which was named previously as APM1 or ACDC. It is located at chromosome 3q27 and it covers 17kb. Also it was found that 3q27 carries the gene for type 2 diabetes mellitus and metabolic syndrome(55).

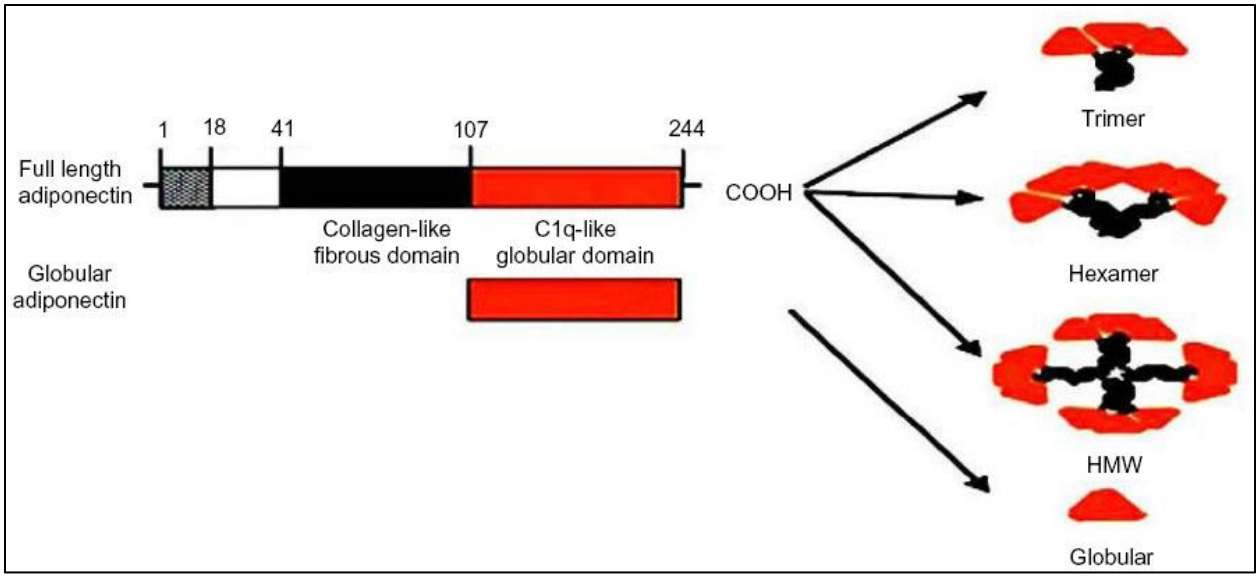


FIGURE 12: STRUCTURE OF ADIPONECTIN

Adiponectin has an N-terminal variable region which is species specific and conserved collagenous domain, which is similar to collagen VIII and collagen X. the C-terminal globular domain in adiponectin is homologous to the complement factor C1q. It circulates in various forms of multimers, such as trimers, hexamers and high molecular weight (HMW) multimers. It is found by gel electrophoresis that in human plasma HMW multimers of adiponectin are less in males than in females. These reveal that neither total adiponectin concentration nor multimer distribution is same in both genders. Impaired multimerization and impaired secretion of adiponectin due to mutation in the ADIPOQ gene results in development of insulin resistance(55)

The adipose tissue derived bioactive substances were termed as adipocytokines but some of the substances are not cytokines. Adiponectin is one of the adipocytokines(55). It is found in rabbit that by immune-histochemical examination using anti-adiponectin antibody there is no adiponectin in the normal vascular walls. But in the balloon injured

vascular walls there is existence of adiponectin(55). Adiponectin can inhibit the formation of adhesion molecules such as intracellular adhesion molecule – 1, vascular cellular adhesion molecule – 1 and E-selectin which help in adhesion of monocytes to endothelial cells during atherosclerotic changes. Adiponectin also prevents the formation of foam cells by inhibiting the expression of the scavenger receptor class A-1 (SR-A) of macrophages which leads to reduced uptake of oxidized LDL. Adiponectin inhibits the signal transduction through extracellular signal related kinase (ERK). By this mechanism adiponectin prevents the proliferation and migration of smooth muscle cells(56). So considering all these factors we can conclude that adiponectin is anti-atherogenic.

Insulin like Growth factor-1 (IGF-1) increases the expression of adiponectin gene and Tumour Necrosis Factor-α (TNF-α) and glucocorticoids downregulates adiponectin expression. Also it is found that a functional Peroxisome proliferator activated receptor γ responsive element (PPRE) is present in the promoter region of the human adiponectin gene. Peroxisome proliferator activated receptor γ (PPAR γ) upregulates adiponectin gene expression. So treatment with thiazolidinediones, which is a PPAR γ activator results in increased plasma adiponectin level(55).

Adiponectin acts via both autocrine and paracrine manner. There are two adiponectin receptor isoforms – AdipoR1 and AdipoR2. Many cell types including adipocytes express both isoforms. But in human, AdipoR1 is predominantly present in skeletal muscle and AdipoR2 is expressed mainly in the liver. AdipoR1 and AdipoR2 have different binding affinity for globular and full length adiponectin. AdipoR1 has

more affinity for globular adiponectin but less affinity for full length adiponectin. But AdipoR2 has intermediate affinity for globular and full length adiponectin(55).

Adiponectin increases fatty acid oxidation by upregulation of many genes involved in muscle lipid metabolisms. They are fatty acid translocase (FAT/CD36), acyl-CoA oxidase (ACO) and mitochondrial uncoupling protein 2 (UCP2). Also adiponectin increases AMP activated protein kinase (AMPK) phosphorylation. By this mechanism it increases glucose uptake and fatty acid oxidation by decreasing the concentration of malonyl-CoA, which is an allosteric inhibitor of carnitine palmitoyl transferase 1 (CPT-1). CPT – 1 is an enzyme which helps in transporting fatty acids into mitochondria. Fatty acid oxidation occurs inside the mitochondria. So by decreasing malonyl-CoA level, adiponectin increases fatty acid oxidation.

Adiponectin increases the expression of glucose transporter 4 (GLUT 4) in cell membrane of muscle which results in increased glucose uptake. Also AMPK phosphorylates glycogen synthase leading to its inactivation. By these mechanisms adiponectin improves glucose tolerance.

ADIPONECTIN IN BRAIN:

Adiponectin regulates food intake in brain. Intracerebroventricular administration of adiponectin was found to decrease body weight in a mouse model with type 2 diabetes mellitus. Also in brain it is involved in lipid and glucose metabolism during fasting. It helps in regulating hippocampal neural stem cells proliferation by activating p38 mitogen activated protein kinase/glycogen synthase kinase 3β/β catenin signaling cascade. In hippocampal dentate gyrus reduced level of adiponectin can result

in decreased dendritic growth and spine density. In an experimental study it was shown that adiponectin knocked out mice would show depressive like behavior. Adiponectin has its role in protecting against ischemic brain injury. 1-methyl-4-phenylpyridinium (MPP+) is a neurotoxin produced in our body as a toxic metabolite when monoamine oxidase B (MAO-B) acts on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Adiponectin is found to have protective effect in human neuroblastoma SH-SY5Y cells against MPP+ induced neurotoxicity. It also protects against amyloid β- induced neurotoxicity. Adiponectin also has its role in physical exercise induced hippocampal neurogenesis(17,57).

Many substances have been identified as involved in adiponectin receptor signaling pathway. The main role in stimulation of insulin sensitivity, mitochondrial biogenesis and oxidative phosphorylation in many cells occurs through AMP-activated protein kinase (AMPK). This in turn inhibits Insulin Receptor Substrate (IRS-1) phosphorylation at serine residues. But at tyrosine residue, insulin mediated IRS-1 phosphorylation is increased. So Akt mediated GSK3 is inhibited. When GSK3 is inhibited, phosphorylation of tau and APP metabolism is decreased. But when adiponectin is deficient, IRS-1 phosphorylation is increased at serine residues. This leads to GSK3 activation which results in increased tau phosphorylation and Aβ production in neurons which is explained in figure.

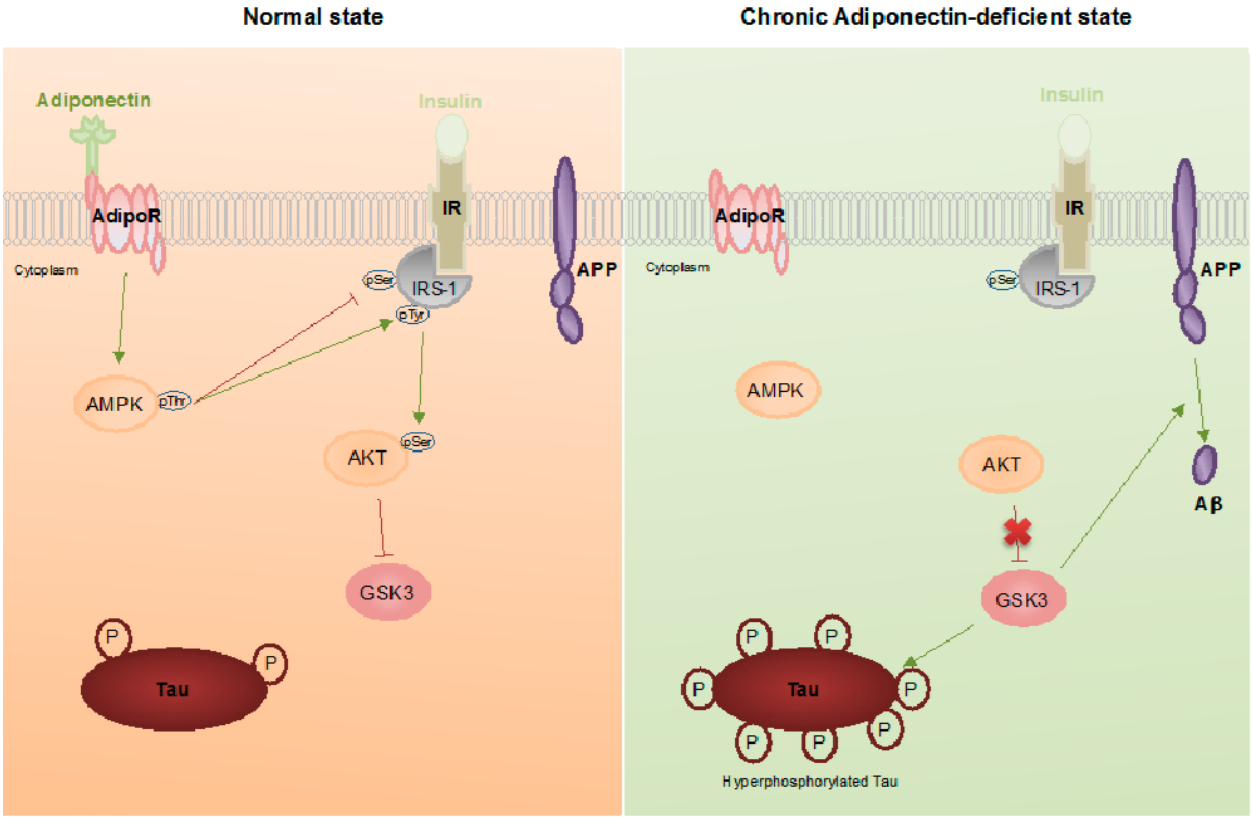


FIGURE 13: EFFECTS OF ADIPONECTIN DEFICIENCY IN BRAIN

In a mouse model of α-synucleinopathies, adiponectin acted as a remedy for protein aggregation and impaired motor activity. In the hippocampus of wild-type mice, osmotin which is a plant homologue of adiponectin reduced Aβ42-induced neurotoxicity and tau hyperphosphorylation. In adiponectin knock out mice features of brain insulin desensitization and Alzheimer's disease like pathology was observed.

ADIPONECTIN IN COGNITIVE DYSFUNCTION:

Adiponectin decreases the upregulation of inflammatory cytokines such as TNF-α and NF-κB. Also the expression of anti-inflammatory substances such as IL-10, IL-1 receptor antagonists are stimulated by adiponectin. So if adiponectin level is decreased it will lead to both increased production of inflammatory substances and decreased

synthesis of anti-inflammatory substances resulting in higher pro-inflammatory condition that is seen in mild cognitive impairment and Alzheimer's disease. Antonio L. Teixeira et al has found that serum adiponectin level is decreased in mild cognitive impairment. (57)

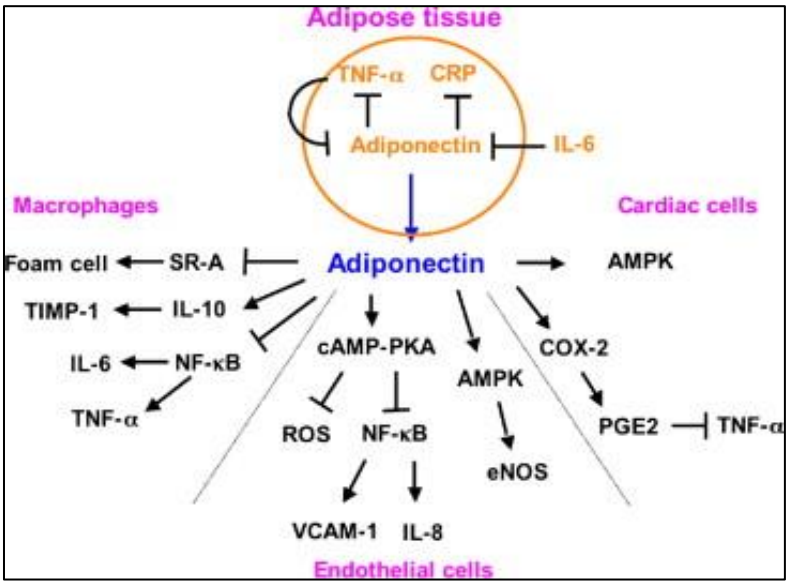


FIGURE 14: ANTI-INFLAMMATORY ACTIONS OF ADIPONECTIN

Masaaki Waragai et al say that plasma adiponectin is increased in cognitive dysfunction as compensation to reduced activity of insulin/IGF-1 signaling pathway. Like insulin resistance produced in hyperglycemia, adiponectin resistance occurs in cellular level leading to increased level of adiponectin in plasma. So either increased level or decreased level of adiponectin can be observed in cognitive impairment.

COGNITIVE EVOKED POTENTIAL:

Evoked potential is defined as an electrical potential in a specific pattern recorded from a specific part of the nervous system, especially the brain. There are two types of evoked potentials. They are short latency evoked potential and long latency evoked potential. Short latency evoked potentials are elicited by exogenous stimuli, without

patient's cooperation. They have shorter latency and lower amplitude than long latency evoked potential and are influenced by frequency and intensity of stimuli. But long latency evoked potentials are elicited by endogenous stimuli and it needs patient's cooperation. Cognitive evoked potential is a long latency evoked potential.

In 1875, Richard Caton said that "The electric currents of the grey matter appear to have a relation to its function. When the gray matter is in state of functional activity, its electric current usually exhibits negative variation."(58)

Cognitive evoked potential is also called as event related potential, P_3, P_{300} and endogenous evoked potential. It is used to assess the cognition of an individual. They are represented with a curve that has positive and negative waves. P300 is a positive wave of cognitive evoked potential which appears at about 300 ms or more following a rare task related stimulus. The amplitude which is expressed in microvolts and latency which is expressed in milliseconds are noted from the wave. P300 can be evoked by any stimulus. The most commonly used stimulus is an unexpected or infrequent stimulus (oddball paradigm). In oddball paradigm, an unexpected, infrequent stimulus is randomly interspersed with frequent stimuli. The unexpected stimulus is different from the common stimuli in terms of frequency or intensity. Any stimulus, auditory, visual, olfactory, somatosensory or pain can be given. But most commonly auditory stimulus is given through headphones. P300 is maximum in the midline, central and parietal regions. Independence of task relevance in infrequent stimulus produces a P300. It is commonly represented as P300a. This has more frontal distribution than the parietal distribution.

Attention to task relevant stimulus produces a P300, which is represented as P300b. The commonly recorded P300 shows a sum of these components (P300a and P300b).

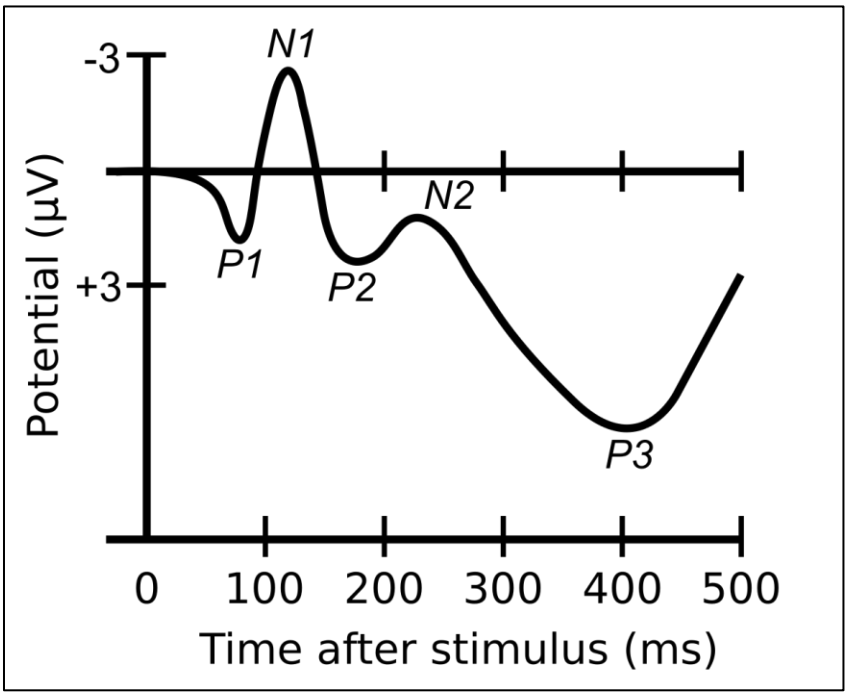


FIGURE 15: COMPONENTS OF COGNITIVE EVOKED POTENTIAL

P300 AMPLITUDE:

Amplitude is defined as the vertical distance between the pre stimulus baseline and the maximum peak in cognitive evoked potential within the time window determined by stimulus modality, task conditions and subject age. It is measured in the central electrodes. The amplitude keeps increasing from the anterior to posterior electrodes.

P300 LATENCY:

Latency is defined as the time interval between the onset of stimulus to the point of maximum peak within the same time window. It is highly reliable than P300 amplitude(59). Normally P300 amplitude decreases as the latency increases.

VARIABLES AFFECTING P300:

Age:

The amplitude for an auditory stimulus increases from the age of 5 years to 19 years but the latency decreases. But for a visual stimulus, both amplitude and latency decreases from 10 years to 21 years. After the second decade, the mean latency increases by about 1 – 1.5ms/year.

Attention:

The amplitude will be decreased when the subject is less alert in conditions such as drowsiness or inattention. In stage II sleep also P300 can be recorded. But in slow wave sleep it disappears.

Drugs:

The latency of L-DOPA will be reduced in patients with Parkinson's disease. But in normal individuals it has no effect. Tatsuro Hayakawa et al have found out that triazolam, a short half-life Benzodiazepine decreases P300 amplitude after 2hour of ingestion of drug. This decrease was found till 6 hours of ingestion(60). The latency of P300 is increased by anticholinergics and antihistamines and the amplitude will be decreased.

Technical Parameters:

Inter-stimulus interval (ISI) is the time interval between two successive frequent stimuli. The probability of occurrence of the rare stimuli (P_r) is percentage of occurrence of the rare stimuli among frequent stimuli. ISI is normally set around 2 seconds and P_r is normally set around 15%. When ISI is reduced to 0.5 seconds the amplitude will be

reduced and the latency will be increased. Donchin et al has reported that when P_r is increased, the amplitude will be decreased. (61)

Intra-individual variability:

Like any other neurophysiological test, in the same individual P300 amplitude and latency can be varied. But in a large group study of 100 subjects, the latency and amplitude variation is found to be not statistically significant in the first and second trials.

THE TRAID OF PREDIABETES, COGNITION AND ADIPONECTIN:

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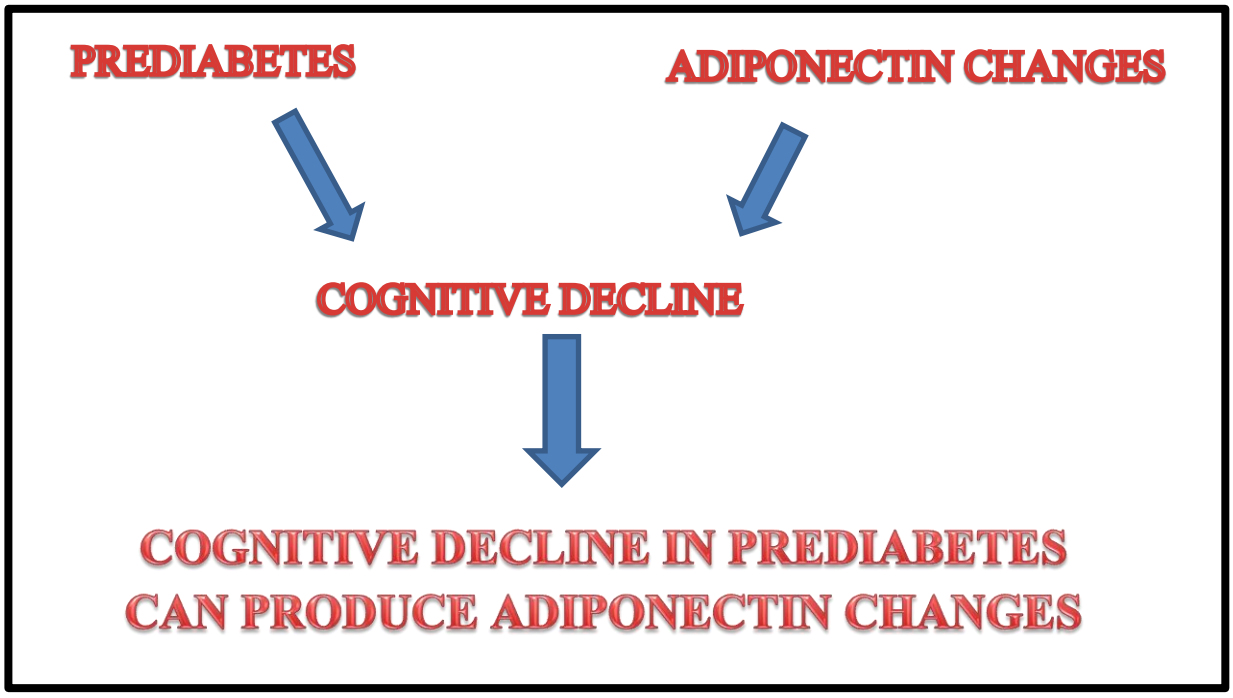


FIGURE 16. INTERRELATIONSHIP WITH PREDIABETES, ADIPONECTIN AND COGNITION

Reviewing the literature above we can know that there is relationship between prediabetes and cognition at one end and adiponectin changes in cognitive impairment at other end.

With this background we have proposed to do a study on patients with prediabetes to find out whether cognitive impairment is present and if present, whether it is correlated with adiponectin level or not.

Aim and Objectives

AIM AND OBJECTIVES

AIM:

To evaluate whether cognition is impaired in the pre-diabetic patients when compared to that of clinically healthy individuals and to find its association with serum adiponectin level in the pre-diabetic patients

OBJECTIVES:

1. To estimate the change in variables of cognitive evoked potential response in the pre-diabetic individuals and to compare the same with clinically normal healthy individuals of the study group
2. To estimate and compare the variations in serum adiponectin level between the pre-diabetic patients and clinically normal healthy individuals
3. To find if any association exists between the serum adiponectin level and parameters of the cognitive evoked potential response like latency (ms) and amplitude (mvolt) among the study group

Materials and Methods

MATERIALS AND METHODS:

This is a descriptive cross-sectional study conducted during the year 2018-19 in Rajiv Gandhi Government General Hospital (RGGGH), Madras Medical College.

The participants for this study were selected from the Non Communicable Disease (NCD) Clinic, RGGGH and from the community.

The blood samples of all the participants in the study group were tested for serum adiponectin levels in the department of Immunology, The Tamilnadu Dr. MGR Medical University.

The recording of event related cognitive evoked potential response for all the participants was recorded in the research lab - human experiments laboratory, Institute of Physiology and Experimental Medicine.

This study was started after obtaining institutional ethics clearance and written informed consent from all the participants.

Selection of subjects:

Statistically adjusted sample size of 100 participants were taken as study group and they were categorized into case study group (n=50) and control group (n= 50).

Case study group:

In the case study group, patients were clinically diagnosed as pre-diabetic based on the OGTT. In which fifty pre-diabetic patients of both the gender were selected in the age group of 25-50 years from Non Communicable Disease (NCD) clinic (cases).

Control group:

The control group was randomly selected from the community, in which fifty healthy individuals of both the gender in the age group of 25-50 years were involved in this study

Inclusion criteria:

Case study group

- Clinically diagnosed pre-diabetic patients based on OGTT
- Age group of 25-50 years of both the gender

Controls

- Clinically normal healthy individuals
- Age group of 25-50 years of both the gender

Exclusion criteria:

- People in age group of < 25 and >50 years of both the gender.
- Participants who are smokers and alcoholics
- Patients who were clinically diagnosed as type I DM and type II DM with/without treatment
- Patients with hearing loss
- Patients who were clinically diagnosed of any endocrinological diseases such as cushing's disease, acromegaly, pheochromocytoma, chronic pancreatitis, pancreatectomy, dumping syndrome, sub-optimally treated thyroid disease and currently pregnant

- Patients who are under treatment of drugs (that will alter glucose metabolism) such as glucocorticoids, pentamidine, nicotinic acid, diazoxide, beta-adrenergic agonists, thiazides, Dilantin, interferon alpha, retroviral drugs and anti-neoplastic drugs.
- Patients with any other chronic illness such as hypertension, tuberculosis, chronic kidney disease, coronary artery disease, etc.
- Patients with any other neurological, psychiatric disorders.

Procedure:

This study was started after giving clear instructions and demonstration of the procedure involved to all the participants of the study group. Any doubts of the participants were clarified. Adequate time and rest was ensured to all the participants.

After enrolling the participants in the study, a thorough clinical examination was done. It was followed by assessment of their socio-demographic details like socio-economic class, education status, height, weight, etc. Blood samples to assess the serum adiponectin levels were taken from all the participants under sterile condition in the biochemistry lab attached to the outpatient department, RGGGH. A definite date and time was given to all the participants to visit the research lab, IPEM for recording the event related cognitive evoked potential.

Patients visiting the NCD were subjected to OGTT from which the initial 50 patients diagnosed of pre-diabetic were included in the case study group.

Assessment of Body mass index:

Body mass index was calculated from the measured height and weight of the participants using the Quetlet's index, BMI = weight (in Kg)/ height (in m^2).

Measurement of weight:

The subject was asked to wear light cloths. The subjects were asked to stand straight with their arms in relaxed position at their sides. Both feet were kept closely. The subjects were asked to remove the shoes/slippers. Using a portable standard weighing machine, weight in kilograms was measured to nearest kilogram.

Measurement of height:

The height of the subject was measured using stadiometer. The subject was asked to remove foot wears. The subject was asked to stand straight in which the Frankfort horizontal plane is parallel to the ground. Frankfort horizontal plane is the line passing from the inferior border of the orbit to the superior margin of the external auditory canal. Both feet were kept closely. Both shoulders were in same level. The occiput, the butt and the heel were made to touch the wall. The flat headpiece of the stadiometer was made to form a right angle with the wall and it was lowered until it touched the vertex of the head firmly. Then the height was measured to the nearest centimeter.

Assessment of Oral Glucose Tolerance Test (OGTT):

This test was done at the central clinical laboratory at RGGGH attached to the Institute of Biochemistry, Madras Medical College. The OGTT was administered

following an overnight fast in the morning or in the afternoon after no more than a light fat-free breakfast eaten before 8:00 AM. After the initial venous blood sample, participants drank 75g anhydrous glucose over 5 minutes. A second blood sample was taken 2 hours later which was taken as the post load blood sugar level of the participant.

After disinfecting the antecubital area, 2 ml of venous blood was collected in a plain fluoride grey topped vacutainer tube. The serum is separated by centrifuging at 3000 rotations per minute for 5 to 10 minutes. The separated serum was collected in eppendorf tube. The serum was then subjected to Hexokinase Method using Roche Diagnostics Cobas GLU HK Gen.3 kit.

Impaired fasting glucose was defined as the fasting glucose values between 100 and 125 mg/dl and Impaired glucose tolerance was defined as post load glucose values between 140mg/dl to 200mg/dl by the American diabetes association (ADA). In OGTT results, the fasting blood glucose values between 100mg/dl and 125 mg/dl or post load glucose values between 140mg/dl to 200mg/dl is considered as pre-diabetic. This was the basis of selection of participants in the case study group

Principle of OGTT:

Hexokinase catalysis the phosphorylation of Glucose in presence of ATP and Mg^{2+} ions to produce glucose-6-phosphate which is oxidized to gluconate-6-phosphate by the action of glucose-6-phosphate dehydrogenase enzyme in presence of NAD^{+}. The increase in absorption is measured at 340 nm / 383 nm which is proportional to the concentration of glucose present in the sample.

Reagents used for OGTT:

- R1: MES buffer: 5.0 mmol/L, pH 6.0; Mg^{2+} : 24 mmol/L;

  ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative.

- R2: HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+}: 4 mmol/L;

  HK (yeast):≥ 300 μkat/L; G-6-PDH (E. coli): ≥ 300 μkat/L; preservative.

Analysis of OGTT:

After calibration and measurement of control, all the samples were loaded in the COBAS analyzer and the reports of fasting blood glucose and post load blood glucose were generated electronically and the results were noted.

ESTIMATION OF SERUM ADIPONECTIN LEVEL:

The study participants were asked to come in an overnight fasting state and 2ml of venous blood is withdrawn from antecubital vein under universal precaution in a red topped clot activator coated test tubes. Then it is centrifuged in 3000 rotations per minute for 5 – 10 minutes. The serum is collected in Eppendorf tubes. The serum is stored at -20^{o}C. Then the serum samples were analyzed in the Department of Immunology, The Tamilnadu Dr. MGR Medical University.

The method used was sandwich ELISA method with Human ADP ELISA kit which is used for research purpose only.

PHOTOGRAPH 1: HUMAN ADIONECTIN ELISA KIT

Principle of estimation:

The plate was pre-coated with human Adiponectin antibody. Antibody present in the sample is added and bind to antibodies coated on the wells. And then biotinylated human Adiponectin antibody is added and bind to adiponectin in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated adiponectin antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human adiponectin. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagents used:

- Standard solution – 64mg/L
- Pre-coated ELISA plate
- Standard Diluent
- Streptavidin-HRP
- Stop solution
- Substrate Solution A
- Substrate Solution B
- Wash buffer concentrate
- Biotinylated human adiponectin antibody

Other materials used:

- $37^{o}C \pm 0.5^{o}C$ incubator
- Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10 nm wavelength filter

PHOTOGRAPH 2: REAGENTS USED

Reagent preparation:

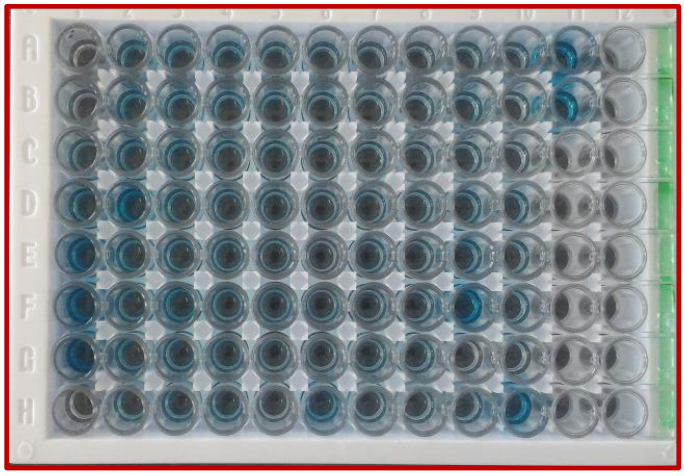
Standard – 120 µl of the standard (64mg/L) is reconstituted with 120 µl of standard diluent to generate a 32 mg/L of standard stock solution. Duplicate standard points was prepared by serially diluting the standard stock solution with standard diluent to produce 16mg/L, 8 mg/L, 4 mg/L and 2 mg/L solutions. Standard diluent serves as the zero standard (0 mg/L).

Assay Procedure:

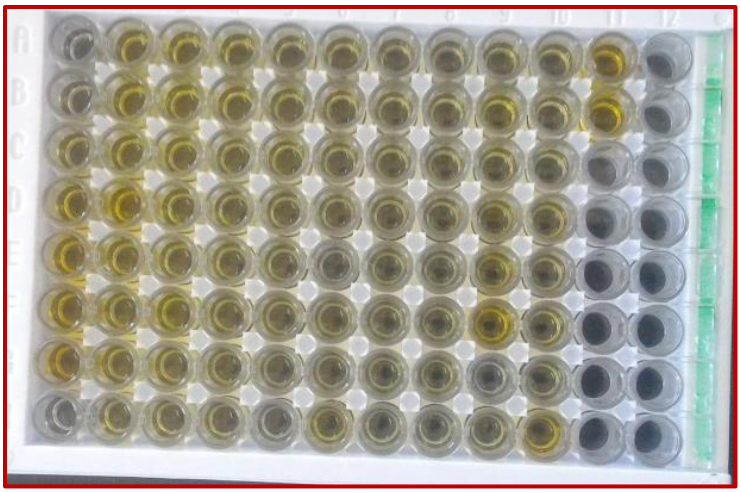
1. All reagents was brought to room temperature before use
2. 50 µl of standard was added to standard well.
3. Then 40 µl of sample was added to sample wells and then 10 µl of anti-adiponectin antibody was added to sample wells. Then 50 µl of Streptavidin-HRP

was added to standard and sample wells. They were mixed well and the plates were covered with a sealer. It was then incubated at 37 ^{o}C for 60 minutes.

4. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml of wash buffer for 30 seconds to 1 minute for each wash.
5. Then 50 µl of substrate solution A was added to each well and then 50 µl of substrate solution B was added to each well. The plate was covered with a new sealer. It was then incubated at 37^{o}C for minutes in the dark.
6. 50 µl of stop solution was then added to each well. The blue colour changed into yellow immediately
7. The optical density of each well was determined immediately using a microplate reader which was set to 450nm within 10 minutes after adding the stop solution.



PHOTOGRAPH 3: ELISA WELL BEFORE ADDING STOP SOLUTION



PHOTOGRAPH 4: ELISA WELLS AFTER ADDING STOP SOLUTION

Assessment of serum adiponectin levels:

A standard curve was constructed by plotting the average Optical density for each standard on the vertical axis against the concentration on the horizontal axis. A best fit curve was drawn through the points on the graph. Using this standard curve the OD values of each sample was extrapolated against the adiponectin standard concentrations to determine the amount of adiponectin in each sample. The unit of measurement is mg/L and the sensitivity of the kit was 0.11mg/L.

ELECTROPHYSIOLOGICAL EVALUATION:

Endogenous-event-related potentials were obtained, using the tonal P300 oddball paradigm, on all the participating subjects. The P300 wave is a late cortical neurophysiological event and is considered to reflect the speed of neuronal events underlying information processing. It appears to be strongly associated with attention and short-term memory

PROCEDURE OF RECORDING COGNITIVE EVOKED POTENTIAL (P300):

P300 was recorded in the study group at the research lab - human experiments lab, Institute of Physiology and Experimental Medicine. The procedure was explained in detail, demonstrated once and the doubts if any raised were clarified to all the participants. The recording of event related cognitive evoked potential (P300) was done in a daytime. All the participants were instructed to have a hair wash on the day of recording cognitive evoked potential (P300).

Instrument used:

The event related cognitive evoked potential (P300) was recorded using MEDICAID Neurostim machine by giving odd ball paradigm stimuli. The reliability of the values obtained was ensured by taking twice the recording for each participant at two different time points and the average of those two recordings was considered.

Placement of electrodes:

The bioelectrical signals were recorded by Ag/Agcl electrodes placed at ez, PZ according to 10-20 international system of EEG electrode placement. The electrodes were fixed using Ten20 conductive paste.

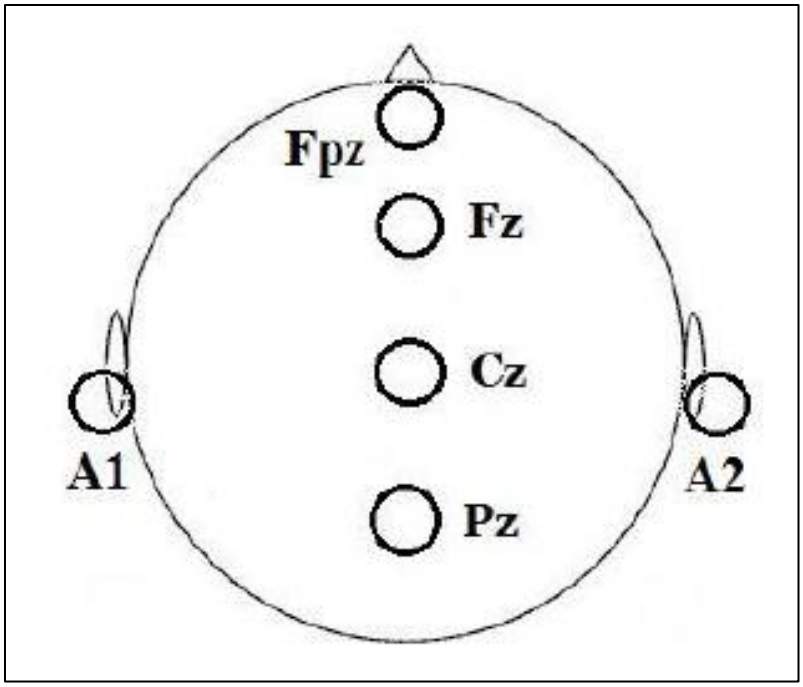


FIGURE 17: ELECTRODE PLACEMENT

The reference was placed at mastoid linked to (AI + A2), the ground electrode was placed at frontal area (Fpz) and active electrode (Cz) was kept at vertex

Auditory stimuli:

The subjects were presented with two types of auditory stimuli, a target and non-target stimuli through head phones in both the ears. The target tone (infrequent stimuli-2 kHz) and the nontarget tone (frequent stimuli - 1 kHz) used were presented over headphones at an intensity of 70 dB. They were presented with a probability of 20% to the left and right ears separately. The auditory stimuli were presented at the rate of 1.25s which of pure tone type. Totally 50 target stimuli were given. The duration of each stimulus was 100 ms. The ratio of target and non-target stimuli was 4:1.

An initial trial was given to the patient for the easy identification of the target stimuli. The subject was asked to silently count all the target tones, ignoring the non-target tones, and to report the total at the end of the test (target test).

PHOTOGRAPH 5: RECORDING OF EVENT RELATED POTENTIAL

Event Related Evoked Potential:

P300 was recorded in terms of its latency and amplitude. (P300) N2 and P3 recordings were obtained on presentation of high pitched infrequent click sound in a train of low pitched frequent and high pitched infrequent click sounds and subject counting

silently on hearing infrequent sound. Evoked responses were averaged and analysed by the Evoked Potential Recorder, MEDICAID, Neurostim. Analysis of N2, P3 (P300) components was done from ez recordings which clearly appeared during the execution of counting silently on target stimulus. These components are representative of higher cognitive level analysis of sensorial stimuli compared with the target stimuli in the memory (13).

Evoked potentials were recorded from scalp locations referred to linked mastoids, using silver lead electrodes. The target and non-target responses were averaged separately. Latency measurements were made by a rater from the peak point in the designated interval.

Results

RESULTS:

STATISTICAL ANALYSIS:

All data were evaluated and analysed using SPSS 26.0. The data obtained from our study results were categorical and continuous data. The categorical variables were represented in range, where the continuous variables were represented as mean $\pm$SD. The normality was assessed using komogrov test. Student's 't' test was used to compare the continuous and categorical variables between the case study group and control group. Spearman's correlation was used between the P300 latencies (N1, N2, P2 – in ms) and serum adiponectin levels among the study group. Pearson's correlation was used between the N2-P300 amplitude (mvolt) among the study group. P value < 0.05 was considered statistically significant.

RESULTS:

In our study we intended to compare the socio-demographic details, fasting and postprandial blood glucose levels, the latencies and amplitude of event related cognitive evoked potential and serum adiponectin level between the pre-diabetic patients(n=50) and clinically normal healthy individuals(n=50).

(i) COMPARISON OF SOCIODEMOGRAPHIC DETAILS AMONGST THE STUDY GROUP:

Both the case study group and control group were age and sex matched. The mean of age, height, weight, BMI among the study group were depicted in table 2 as shown below.

Table 2: Comparison of age, sex, height, weight, BMI between the pre-diabetic patients and the control group.

Variables	Pre-diabetic patients (n=50)		Clinically normal healthy individuals (n=50)	
	Mean	SD	Mean	SD
Age (years)	36.1	5.9	35.7	6.2
Height (cms)	162.9	6.6	164.2	9.1
Weight (kgs)	67.9	7.9*	60.1	8.3*
BMI (kg/m2)	25.7	3.1*	22.4	1.4*

SD = standard deviation, BMI = Body mass index, * $p<0.05$ considered as statistically significant

The mean age of the pre-diabetic patients and the control group were 36.1±5.9 and 35.7 ± 6.2 respectively. The mean height of the pre-diabetic patients and the control group were 162.9 ± 6.6 and 164.2 ± 9.1 respectively. The mean weight of the pre-diabetic patients and the control group were 67.9 ± 7.9 and 60.1 ± 8.3 respectively. The mean BMI of the pre-diabetic patients and the control group were 25.7 ± 3.1 and 22.4 ± 1.4 respectively.

(ii) COMPARISON OF BLOOD GLUCOSE LEVELS AMONGST THE STUDY GROUP:

The mean of fasting and post-load blood glucose between the pre-diabetic patients and the control group were depicted in table 3 as shown below.

Table 3: Comparison of fasting and post load blood glucose levels between the pre-diabeticpatients and the control group.

Variables	Pre-diabetic patients (n=50)		Clinically normal healthy individuals (n=50)	
	Mean	SD	Mean	SD
Fasting blood glucose (mg/dl)	114.0	7.5*	81.2	7.1*
Post load blood glucose (mg/dl)	166.1	14.8*	120.7	10.7*

SD = standard deviation, * $p<0.05$ considered as statistically significant

The mean fasting blood glucose of the pre-diabetic patients and the control group were 114 ± 7.5 and 81.2 ± 7.2 respectively. The mean post load blood glucose of the pre-diabetic patients and the control group were 166.1 ± 14.8 and 120.7 ± 10.7 respectively.

(iii) COMPARISON OF VARIABLES IN EVENT RELATED COGNITIVE EVOKED POTENTIAL (P300) AMONGST THE STUDY GROUP:

The mean of parameters of cognitive evoked potential (P300) – N1 latency (msec), N2 latency (msec), P2 latency (msec), P300 latency (msec) and N2 – P300 amplitude (mvolt)between the pre-diabetic patients and the control group were depicted in table 4 as shown below.

Table 4: Comparison of parameters of cognitive evoked potential (P300) – N1 latency (msec), N2 latency (msec), P2 latency (msec), P300 latency (msec) and N2 – P300 amplitude (mvolt) between the pre-diabetic patients and the control group.

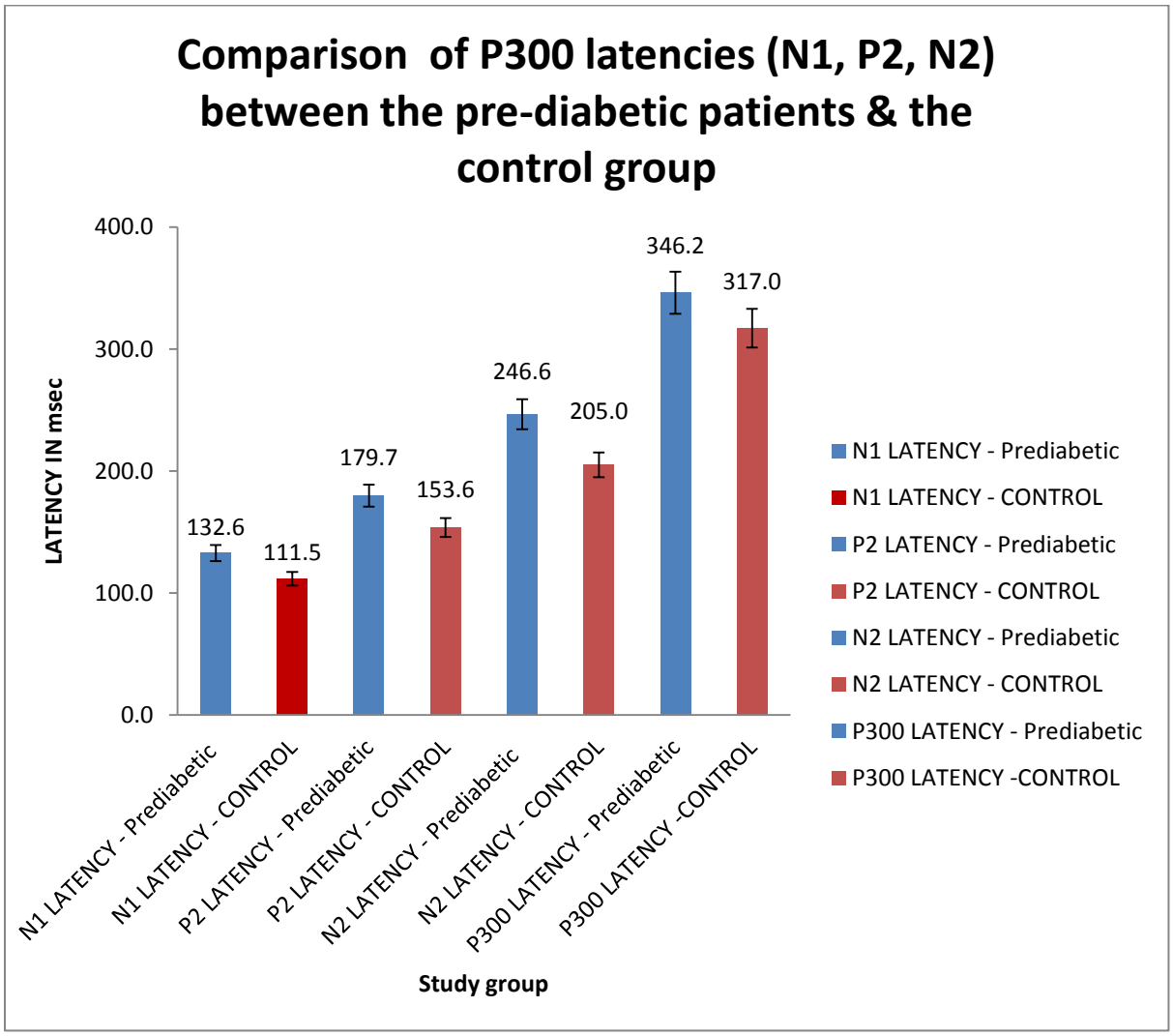
Variables	Pre-diabetic patients (n=50)		Clinically normal healthy individuals (n=50)	
	Mean	SD	Mean	SD
N1 latency (msec)	132.6	34.4*	111.5	10.2*
P2 latency (msec)	179.7	36.6*	153.6	9.5*
N2 latency (msec)	246.6	39.9*	205	15.1*
P300 latency (msec)	346.2	27.6*	317	11.3*
N2 – P300 amplitude (mvolt)	3.6	1.6*	6.1	4.2*

SD = standard deviation, * $p<0.05$ considered as statistically significant

The mean N1 latency of the pre-diabetic patients and the control group were 132.6 ± 34.4 and 111.5± 10.2 respectively. The mean P2 latency of the pre-diabetic patients and the control group were 179.7 ± 36.6 and 153.6 ± 9.5 respectively. The mean N2 latency of the pre-diabetic patients and the control group were 246.6 ± 39.9 and 205 ± 15.1 respectively. The mean P300 latency of the pre-diabetic patients and the control group were 346.2 ± 27.6 and 317 ± 11.3 respectively. The mean N2- P300 amplitude of the pre-diabetic patients and the control group were 3.6 ± 1.6 and 6.1 ± 4.2 respectively.

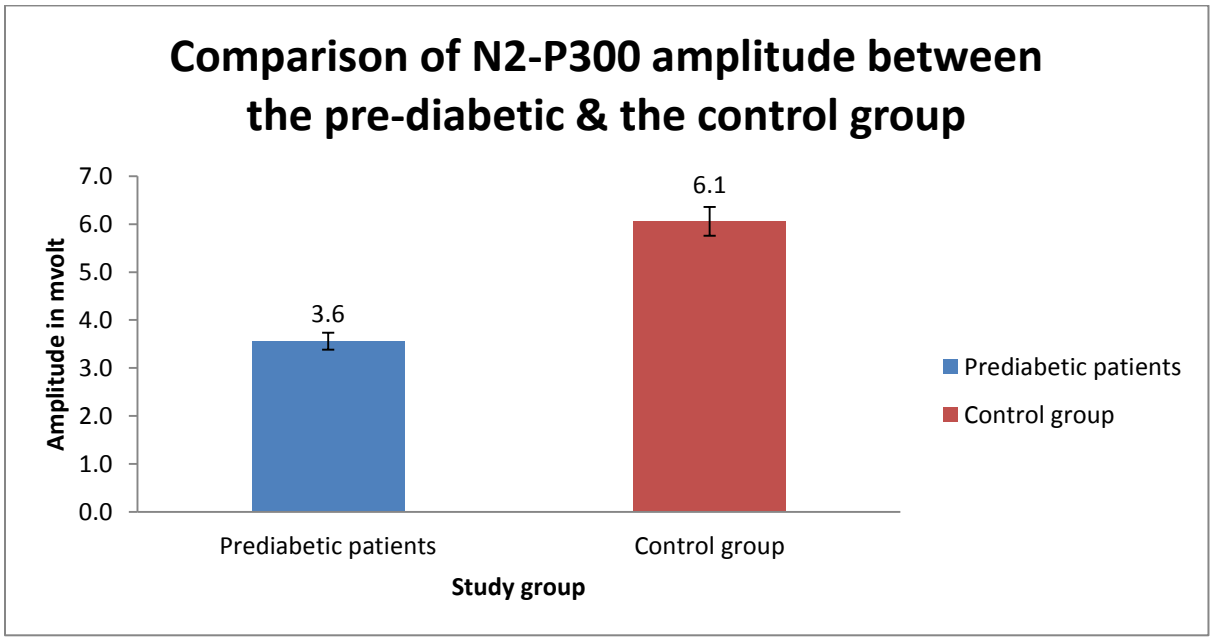
Comparison of P300 latencies in msec (N1, P2, and N2) between the pre-diabetic patients & the control group is depicted in graph 1 as shown below.

Graph 1: Comparison of P300 latencies in msec (N1, P2 and N2) between the pre-diabetic patients and the control group.



Comparison of N2-P300 amplitude in mvolt between the pre-diabetic patients & the control group is depicted in graph 2 as shown below.

Graph 2: Comparison of N2-P300 amplitude in mvolt between the pre-diabetic patients and the control group



(iv) COMPARISON OF SERUM ADIPONECTIN AMONGST THE STUDY GROUP:

The mean of serum adiponectin levels between the pre-diabetic patients and the control group were depicted in table 5 as shown below.

Table 5: Comparison of serum adiponectin levels between the pre-diabetic patients and the control group.

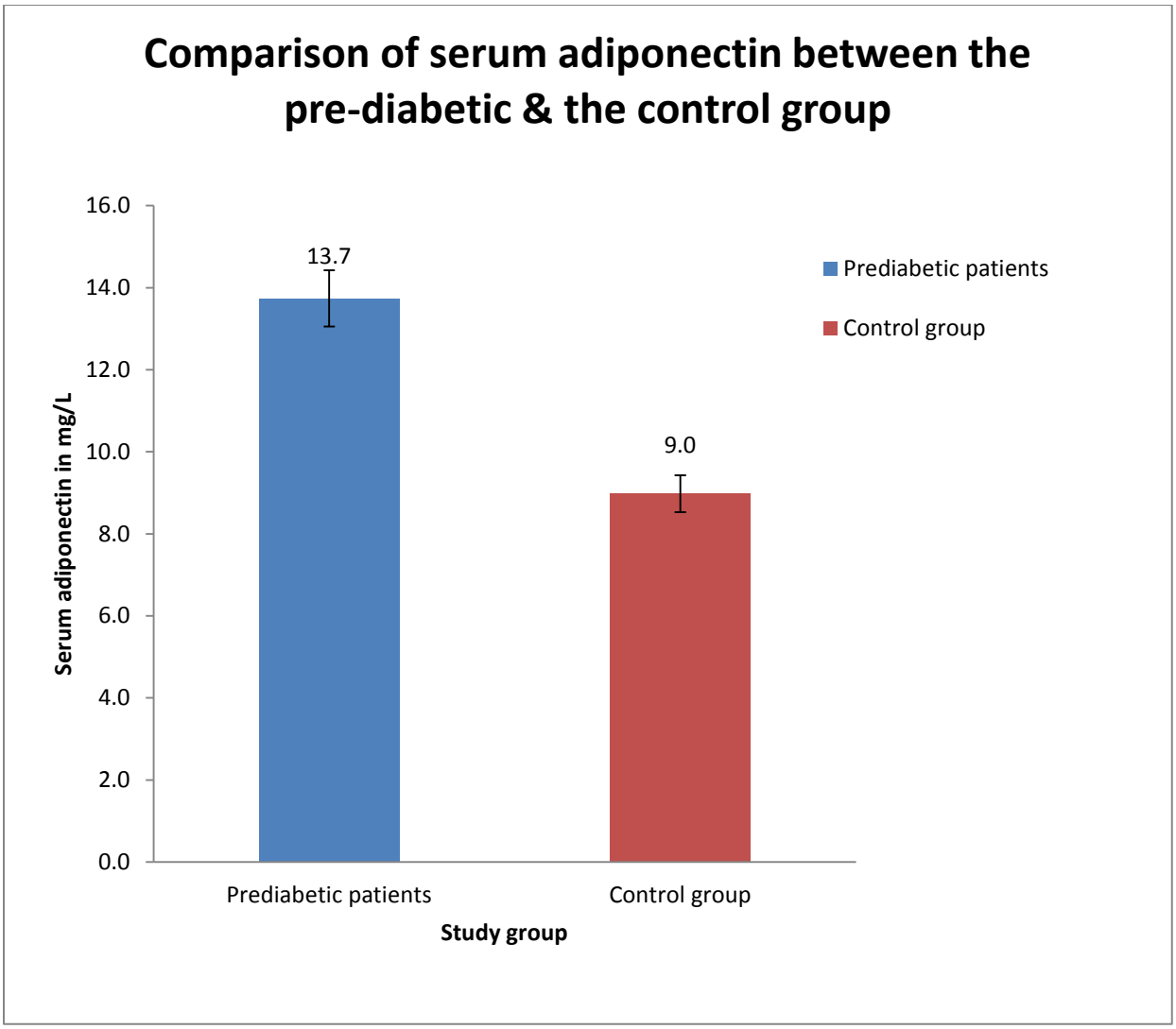
Variables	Pre-diabetic patients (n=50)		Clinically normal healthy individuals (n=50)	
	Mean	SD	Mean	SD
Serum adiponectin (mg/L)	13.7	6.3*	9	4.2*

SD = standard deviation, * $p<0.05$ considered as statistically significant

The mean serum adiponectin of the pre-diabetic patients and the control group were 13.7 ±6.3 and 9 ± 4.2 respectively.

Comparison of serum adiponectin in mg/L between the pre-diabetic patients & the control group is depicted in graph 3 as shown below.

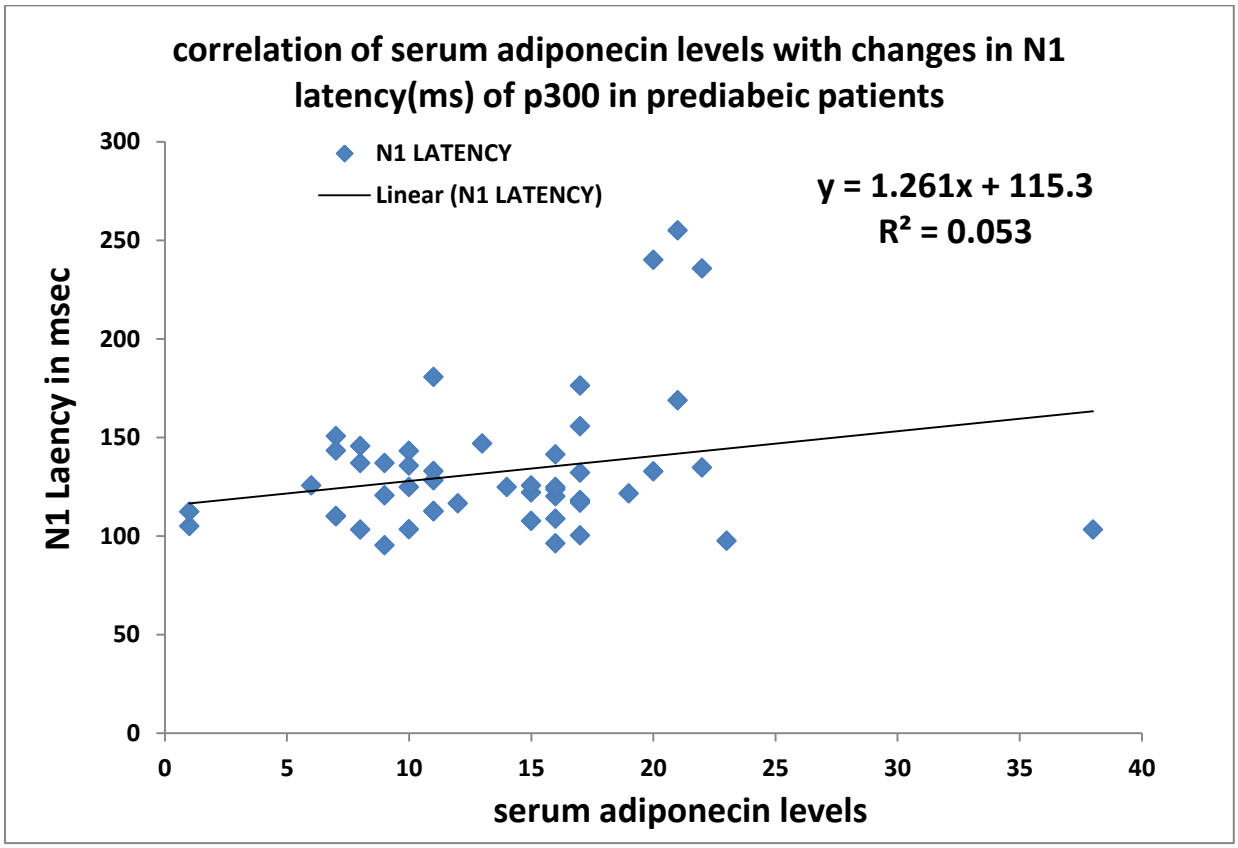
Graph 3: Comparison of serum adiponectin in mg/L between the pre-diabetic patients and the control group



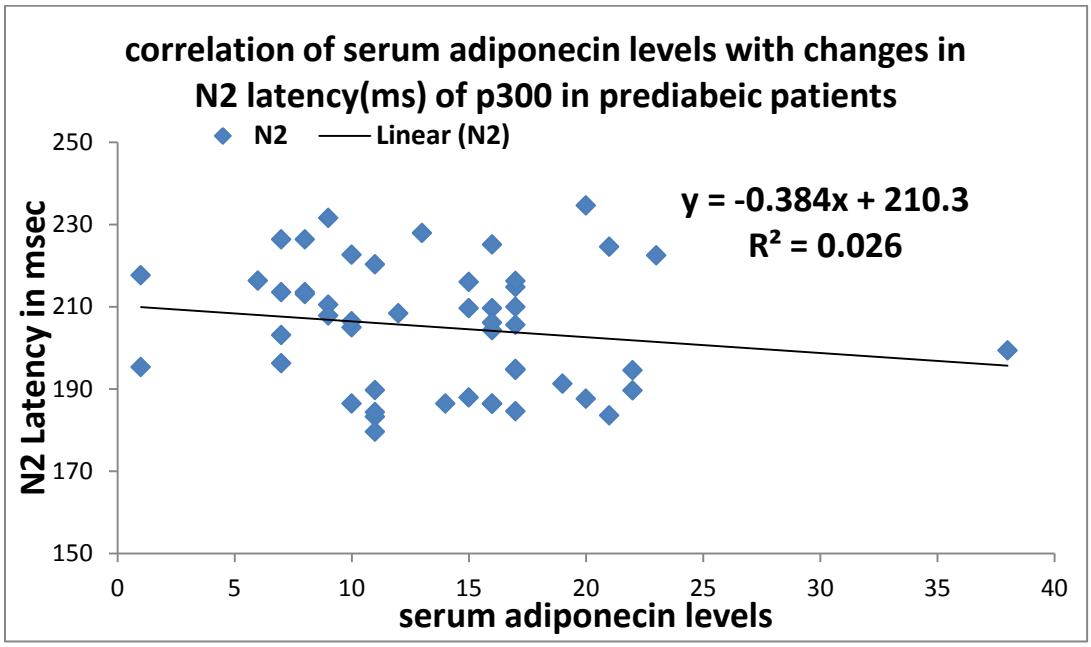
CORRELATION OF SERUM ADIPONECIN LEVELS WITH CHANGES IN N1, N2, P2 LATENCY (MS) OF P300 IN PREDIABEIC PATIENTS:

The correlation between the mean of serum adiponectin levels and the mean of N1 latency, N2 latency, and P2 latency in milliseconds amongst the study group has been shown in the following graphs. A highly significant positive correlation was found between the serum adiponectin levels with N1 latency of pre-diabetic patients. A significant negative correlation was found between the serum adiponectin levels with the N2 latency and P2 latency of pre-diabetic patients.

Graph 4: Correlation of serum adiponectin levels with changes in N1 latency

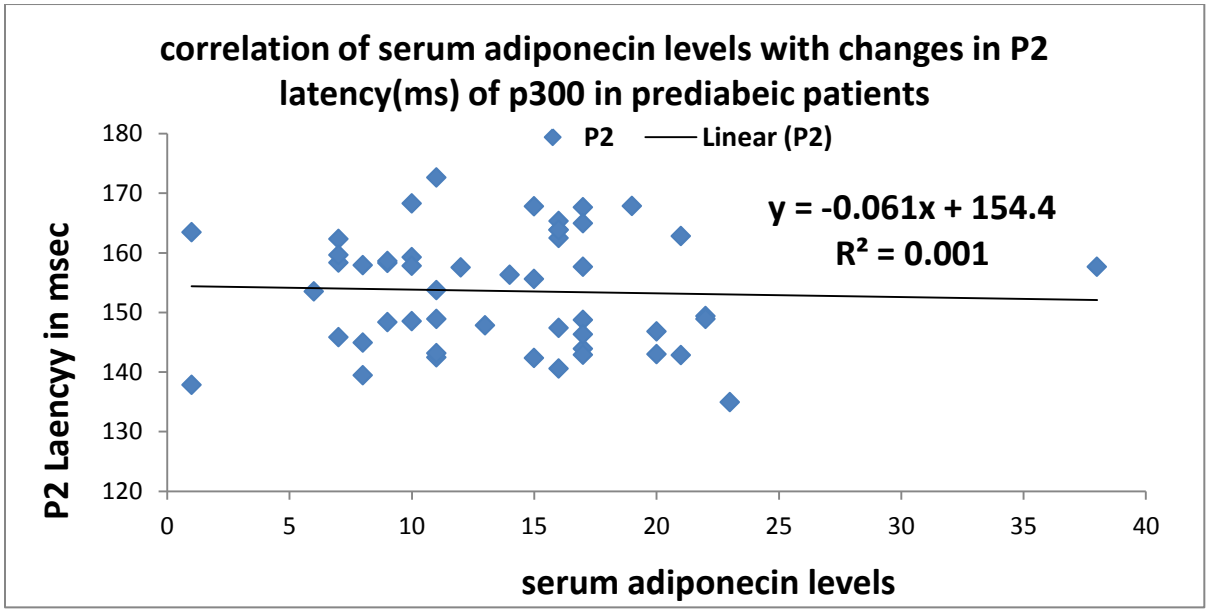


Graph 5: Correlation of serum adiponectin levels with changes in N2 latency(ms)



A significant negative correlation was found between the serum adiponectin levels with the N2 latency of pre-diabetic patients.

Graph 6: Correlation of serum adiponectin levels with changes in P2 latency (ms)



A significant negative correlation was found between the serum adiponectin levels with the P2 latency of pre-diabetic patients.

CORRELATION OF SERUM ADIPONECTIN WITH P300 LATENCY IN THE PRE-DIABETIC PATIENTS:

The correlation between the mean of serum adiponectin levels and the mean of P300 latency in milliseconds amongst the study group has been shown in table 6 and graph 7. A positive correlation was found between the serum adiponectin levels with P300 latency of pre-diabetic patients.

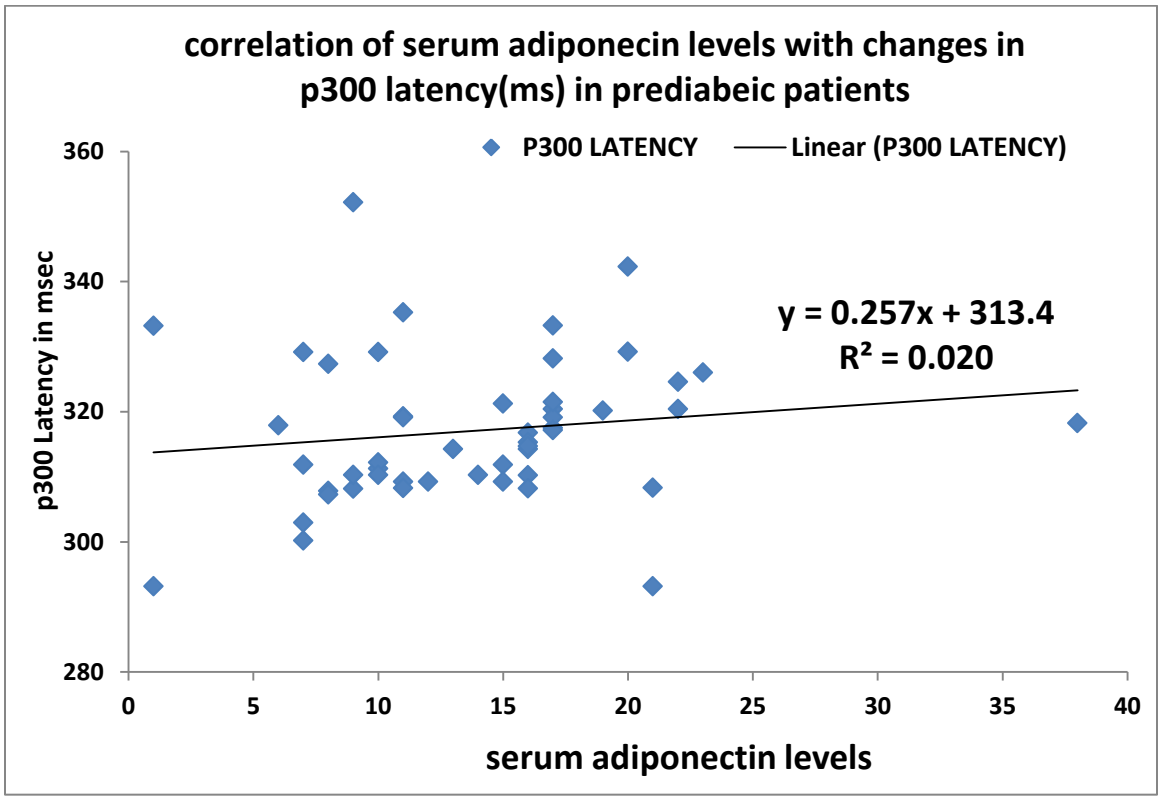
TABLE 6: Correlation of serum adiponectin (mg/L) with P300 latency (msec)

VARIABLE	**CORRELATION**	**P300 LATENCY**
SERUM ADIPONECTIN LEVEL	PEARSON'S CORRELATION (r)	0.141
	P value	0.323

* $p<0.05$ considered as statistically significant

Table 6 and Graph-7 shows the correlation between serum adiponectin with P300 latency. The Pearson's correlation (r) was 0.141. So there is positive correlation. P value is > 0.05. So it is not statistically significant.

Graph 7: Correlation of serum adiponectin levels with changes in P300 latency (ms)



CORRELATION OF SERUM ADIPONECTIN WITH N2-P300 AMPLITUDE IN THE CASES:

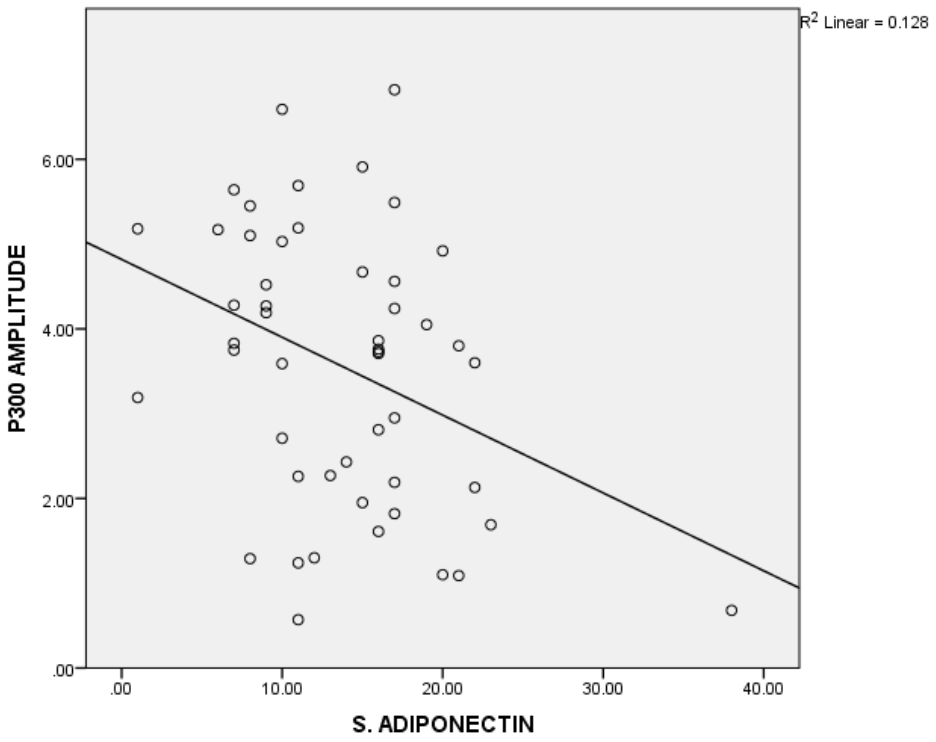
The correlation between the mean of serum adiponectin levels and the mean of N2- P300 amplitude in millivolt amongst the study group has been shown in table 7and graph 8. A negative correlation was found between the serum adiponectin levels with N2-P300 amplitude of pre-diabetic patients.

TABLE 7: Correlation of serum adiponectin with N2-P300 amplitude (mvolt)		
VARIABLE	**CORRELATION**	**N2-P300 AMPLITUDE (mvolt)**
SERUM ADIPONECTIN LEVEL	PEARSON'S CORRELATION (r)	-0.357
	P value	0.0118*

* p<0.05 considered as statistically significant

Table 7 and graph 8 shows the correlation of serum adiponectin with N2-P300 amplitude. The Pearson's correlation (r) was -0.357. There is negative correlation which indicates that when amplitude decreases, adiponectin level increases. P value is < 0.05. So it is statistically significant.

Graph 8: Correlation of serum adiponectin with N2-P300 amplitude (mvolt)



Discussion

DISCUSSION

Our aim of the study was to find out if there is any change in the event related cognitive evoked potential in the pre-diabetic patients who were diagnosed following an OGTT in this study. Also, this study intended of finding if there exist any influence of serum adiponectin levels on the latencies & amplitude of cognitive evokes potential in the same pre-diabetic patients. However, to implement this, we had chosen 50 clinically normal health individuals as control group and 50 pre-diabetic patients as case study group.

When comparing the socio-demographic details among the study group, we found that the age, body mass index, education and socioeconomic status were almost same in the control group as well as the case study group as depicted in the table 2.

In this study it was found that there was significant difference in the mean BMI between the normal subjects and the pre-diabetic patients. As per literature, this may be due to the presence of insulin resistance among the obese individuals. Steven M. Haffner et al have documented in his study that there was a significant difference in the BMI of the pre-diabetic persons and the normal individuals (62).

Saczynski JS et al have also found that compared with normal healthy individuals, pre-diabetic patients had more mean BMI which is in favour of this study(63).

Comparison of post-load glucose levels and changes in event related cognitive evoked response amongst the study group:

Our study showed that, amongst the pre-diabetic patients, the P300 latency of event related cognitive evoked potential response (P300) was prolonged and also the

amplitude was significantly reduced, when compared to that of the normal healthy control group. This implies the presence of a significant cognitive decline in the pre-diabetic patients as identified by the cognitive evoked response recording.

Satabdi Saha et al did a cross sectional study on pre-diabetic patients and found out that P300 latency was significantly increased in pre-diabetic individuals compared with normal subjects(15). This was very consistent as like our study results. It was proven by earlier studies that those individuals with impaired glucose tolerance and hyperinsulinemia were found to have decreased Mini Mental State Examination (MMSE) scores(43) and they were at increased risk for mild cognitive impairment(MCI). These results were supportive to our study.

In another study conducted by Yaffe K et al, on postmenopausal women, women in study group were classified into three groups – diabetes, prediabetes and normal glucose level. They found that women with prediabetes tended to have decreased cognitive scores and rates of cognitive decline were intermediate between those women with diabetes and those with normal plasma glucose level (64).

Antonio Convit et al had reported an association between peripheral glucose regulation and the volume of the hippocampus, which was intended to evaluate the impact of changes in glucose levels in learning and memory. They found that individuals with poorer peripheral glucose regulation were more likely to have lower memory performance and smaller head size adjusted hippocampal volumes. But these fMRI changes were present only in hippocampus. Other brain areas were not much conclusive in his study(65).

Matti Vanhanen et al had conducted a study on Finnish elderly population. They assessed the cognition levels among the study group using mini mental state examination (MMSE), Buschke Selective Reminding Test (BSRL), visual reproduction test, trial making test A and C, verbal fluency test on letters and on structured-unstructured patterns of pictures. They encountered that subjects with impaired glucose tolerance had lower MMSE and BSRL scores which was statistically significant when compared with subjects with normal glucose level. Yet another study showed a lower scoring with no significance for other neuropsychological tests in subjects with IGT(66)

Results demonstrated that reduction of glucose level in diabetic subjects takes to increase in latency and reduction of amplitude of component P300, suggesting that there is central auditory system dysfunction. Considering that nervous tissue is glucose- dependent, that is, it depends on stable glucose levels in ideal situations, episodes of hypoglycemia for prolonged periods of time may take the subject to significant neurological deficits. Thus, the investigation of P300 cognitive potential may be an important procedure for prevention and early diagnosis of neurological affections in subjects with Diabetes Mellitus

It is important to highlight that compared to those studies conducted with diabetes patients, in the present study we had included prediabetes patients and made an attempt to demonstrate the presence of any cognitive decline using a non-invasive recording of cortical activity via an auditory stimulus in these pre-diabetic patients.

ADIPONECTIN AND COGNITION:

In this study it was found that the mean serum adiponectin was increased in pre-diabetic individuals compared to normal patients. But previously many studies have reported decrease in serum adiponectin in pre-diabetic individuals. But some studies have also found that in cognitive dysfunction serum adiponectin was increased. This may be due to a phenomenon called adiponectin resistance. Similar to insulin resistance, adiponectin resistance is observed. The pathophysiological assumption might be due to the decreased sensitivity of the adipose tissue, to produce more and more adiponectin to compensate for the same (17).

Une et al has found that CSF and plasma adiponectin is decreased in mild cognitive impairment and in Alzheimer's disease compared to normal individuals. But they were not able to bring out the reason(67).

Ma J et al had also found that adiponectin was increased in cognitive dysfunction compared to normal individuals (68). This was a little contradictory to our study.

Warangai, M et al had said that serum adiponectin is increased in cognitive decline and this might be due to altered adiponectin-adiponectin receptor signaling or downregulation of adiponectin signaling in brain(69).

Lin et al did a study on insulin receptor transgenic/knockout mice and found that adiponectin is increased in these mice than normal. Also they found that adiponectin administration in these mice was not able to lower glucose level which confirmed the condition called adiponectin resistance. Also they observed decreased mRNA expression

of Adiponectin Receptor 2 in liver and muscle and there was decreased peroxisome proliferator activated receptor target gene expression in liver (70).

Supporting these studies this study showed increased adiponectin level in pre-diabetic individuals. Also P300 latency was in positive correlation with adiponectin level which indicates that adiponectin level increases with P300 latency. Also it was extremely statistically significant. P300 amplitude showed negative correlation with adiponectin level. This revealed that P300 amplitude decreases with increase in adiponectin level. Also it was statistically significant.

EFFECT OF SERUM ADIPONECIN LEVELS ON GLUCOSE IN PRE-DIABETIC CONDITION: (71–74)

Serum adiponectin enhances glucose uptake in the liver. The glucose-lowering effect of serum adiponectin was investigated by measuring glucose uptake in heart, liver and muscle tissues using a planar positron imaging system to visualize 18-fluorodeoxyglucose (FDG) after in vivo injections of synthetic adiponectin. Liver fluorodeoxyglucose levels in induced diabetic mice transfected with synthetic adiponectin were higher. These data suggests a strong association between the higher levels of adiponectin in the liver and the levels of increased hepatic glucose uptake (71-72).

Furthermore in an animal study, diabetic mice was induced to have a higher levels of gene expression for adiponectin by injecting an expressive labelled synthetic adiponectin preparation and a parallel increase in hepatic glucose uptake was observed in

those mice. This effect necessitated 4-6 weeks of treatment by periodical injection of synthetic adiponectin to all treated mice to produce a persistently high serum glucose levels. The reduction of serum adiponectin levels in animal studies of induced diabetic mice may be related to atrophy of adipocytes and/or other diseases that might be induced by diabetes mellitus (73-74).

Although circulating serum adiponectin levels were studied to be diminished in insulin-resistant clinical conditions such as obesity and type 2 diabetes, recently, it has been reported that adiponectin levels in induced diabetic wistar rats were suppressed when compared with that of non-diabetic wistar rats. Serum adiponectin has multiple biological functions and the exact mechanism on the glucose metabolism behind these differences remains unclear. Consequently, the delivery of genes for adiponectin in wistar rats has been shown to be effective in decreasing glucose levels. This study provides the first in vivo evidence that adiponectin gene expression reduces serum glucose levels in the absence of insulin. (73-74)

IMPACT OF CHANGE IN GLUCOSE LEVELS AND DEMENTIA IN DIABETIC PATIENTS:

Several studies have also found associations between diabetes and increased risk of dementia. In a large cohort of Japanese-American men, diabetes was a risk factor for both vascular dementia and Alzheimer's dementia. Whereas in another study of multi-ethnic elders, diabetes was associated with a threefold risk of stroke-associated dementia but with a 60% increase in risk of non-stroke related cognitive impairment. The probable

mechanism could be the development of diabetic complications such as renal disease, stroke, hypertension, hyperlipidemia and ischemic heart disease that may lead to impaired cognitive performance.

In addition, there are several possible mechanisms related to diabetes pathophysiology per se that may be related to impaired cognitive function. Chronic hyperglycemia could cause cognitive impairment either by direct neuronal damage, possibly by advanced glycosylated end products (AGEs) or by indirect neuronal damage from cerebral microvascular and macrovascular atherosclerotic disease. Those diabetic patients who were diagnosed to have dementia have higher brain concentrations of AGEs, such as pyraline and pentosidine when compared to normal controls and AGEs may be involved in the neurotoxic pathway in the pathogenesis of dementia.

In addition, both small and large cerebral vessel atherosclerosis have been associated with cognitive impairment and increased risk of dementia. Finally, hyperinsulinemia and insulin resistance may be independently associated with cognitive impairment. In this study, compared to normal controls, the pre-diabetic patients who were assumed to have signs of cognitive decline as recorded in event related cognitive evoked response such as a significantly decreased N2-P300 amplitude. The same pre-diabetic group showed a significantly increased serum adiponectin levels, in which we fail to exactly associate how the serum adiponectin levels reflects changes in brain structures like hippocampus .

Our study has many strengths including the inclusion of relatively high-functioning community dwelling pre-diabetic patients attending the non-communicable

disease OPD, who agreed to participate in this study. We prospectively examined the association between cognitive function and risk of cognitive impairment not just among elders with pre-diabetes but also among those with clinically normal control group. The observed increased risk of developing cognitive impairment and level of abnormal fasting glucose supports a causal association between prediabetes and cognitive decline.

Our study has its own limitations, in a way that we did not assess cognitive function with a standard battery of cognitive tests that might assesses five different domains. However, we performed an extensive clinical evaluation for all pre-diabetic patients to identify dementia using a non-invasive recording of cortical activity, an event related cognitive evoked potential. Furthermore, we also had adjusted for possible confounders such as age, education, socio-economic status and BMI in the case study group. Based on OGTT and ADA criteria, we had considered the so mentioned impaired fasting glucose limits as a selection criterion for pre-diabetes in our study. Yet the results that we derived conclusively were somewhat lower than that observed in community based studies. (75–79)

Conclusion

CONCLUSION:

In our study, results strongly suggests that there exist a prolonged latency and decrease in amplitude in pre-diabetic patients which was proved significant, in spite of slight variations in BMI that was present between the case study and the control group. However, this increase in P300 latency and decrease in N2-P300 amplitude questions the possibility of a decline in cognition present amongst the pre-diabetic patients. Also we found that the serum adiponectin levels were increased in pre-diabetic patients significantly and its levels were strongly associated with a parallel decrease in N2-P300 amplitude.

In recent scientific investigations, the glucose-lowering effect of recombinant adiponectin has been proved to be due to an increased sensitivity to insulin in pre-diabetic individuals. Though we did not prefer either a genetic evaluation or an invasive analysis, we tried to describe the impact of glucose levels and serum adiponectin through a non-invasice recording of cortical activity. Henceforth, the results obtained with the present study allowed us to primarily conclude that an investigation with P300 cognitive potential could be an important tool for early diagnosis of neurological deficits in patients diagnosed of pre-diabetics. Relying on our results we insist that the pre-diabetic patients should be screened in out-patient department on regular basis for cognitive decline to detect early cognitive impairment if any. This can lead to the possible interventions that could be made to prevent further cognitive decline.

To ensure a much more definite correlation between the serum adiponectin levels and the above said changes in N2-P300 amplitude, we lacked by a smaller sample size

which reduced the effect of our study, inspite of a moderate positive correlation shown in our results,. We could not test for all cognitive domains and are limited in the interpretation of our findings because we assessed to rule out only a minimal etiology for cognitive impairment. In addition, as we said before, we had to extend this work as more invasive studies to know it certainly how serum adiponectin levels exactly correlates to those with either cognition or glucose levels. Our findings suggest that higher serum adiponectin levels were associated with risk of cognitive decline in pre-diabetic patients. Future studies we intend to determine the impact of serum adiponectin in association with diabetic age related glycosylation as a marker for cognitive function and the neuropathological etiology underlying this association.

LIMITATIONS:

- The study sample was smaller considering the cost of the investigations
- It was a cross sectional study
- The observer was not blinded in this study. So this would have affected the study

FUTURE DIRECTIONS:

- The Pre-diabetic patients could be given treatment to keep the blood sugar level under control and the cognition can be assessed to know whether there is any improvement
- Other neurological tests could be used to assess cognition in specific domains

Summary

SUMMARY:

This study was conducted to assess the cognitive evoked potential P300 and to find its correlation with serum adiponectin level in pre-diabetic patients. Fifty patients with prediabetes and 50 controls participated in this study. The cognitive evoked potential was recorded in the study group. There was statistically significant increase in P300 latency and decrease in P300 amplitude in pre-diabetic patients compared to controls. The serum adiponectin level was increased in pre-diabetic individuals compared to control which was statistically significant. There was a statistically significant negative correlation of cognitive evoked potential amplitude with serum adiponectin level. There was a positive correlation of cognitive evoked potential latency with serum adiponectin level.

Bibliography

BIBLIOGRAPHY

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract [Internet]. 2018 Apr [cited 2019 Jun 29];138:271–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29496507
2. Marseglia A, Dahl Aslan AK, Fratiglioni L, Santoni G, Pedersen NL, Xu W. Cognitive Trajectories of Older Adults with Prediabetes and Diabetes: A Population-Based Cohort Study. Journals Gerontol - Ser A Biol Sci Med Sci. 2018;73(3):400–6.
3. Alberti KGMM. Screening and diagnosis of prediabetes: where are we headed? Diabetes, Obes Metab [Internet]. 2007 Sep [cited 2019 Jun 29];9(s1):12–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17877542
4. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care [Internet]. 2005 Jan 1 [cited 2019 Jun 29];28 Suppl 1(suppl 1):S37-42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15618111
5. definition and diagnosis of diabetes mellitus and intermediate hyperglycemia RepoRt of a WHo/IDf ConsultatIon [Internet]. 2006 [cited 2019 Jul 1]. Available from: https://apps.who.int/iris/bitstream/handle/10665/43588/9241594934_eng.pdf;jsessionid=08CD3E8B25E8EC78872F391830D58F5B?sequence=1
6. Diagnosis and Classification of Diabetes Mellitus. 2010 [cited 2019 Jul 1]; Available from: http://creativecommons.

7. Feinkohl I, Price JF, Strachan MWJ, Frier BM. The impact of diabetes on cognitive decline: Potential vascular, metabolic, and psychosocial risk factors. Vol. 7, Alzheimer's Research and Therapy. BioMed Central Ltd.; 2015.
8. S. Roriz-Filho J, Sá-Roriz TM, Rosset I, Camozzato AL, Santos AC, Chaves MLF, et al. (Pre)diabetes, brain aging, and cognition. Vol. 1792, Biochimica et Biophysica Acta - Molecular Basis of Disease. 2009. p. 432–43.
9. Pour OR, Dagogo-Jack S. Prediabetes as a therapeutic target. Clin Chem. 2011;57(2):215–20.
10. Sanz CM, Ruidavets J-B, Bongard V, Marquié J-C, Hanaire H, Ferrières J, et al. Relationship Between Markers of Insulin Resistance, Markers of Adiposity, HbA 1c , and Cognitive Functions in a Middle-Aged Population-Based Sample: the MONA LISA Study. 2013 [cited 2019 Jul 9]; Available from: https://care.diabetesjournals.org/content/diacare/36/6/1512.full.pdf
11. Jan Biessels G, Reijmer YD. Brain Changes Underlying Cognitive Dysfunction in Diabetes: What Can We Learn From MRI? Diabetes [Internet]. 2014 [cited 2019 Jul 9];63:2244–52. Available from: https://diabetes.diabetesjournals.org/content/diabetes/63/7/2244.full.pdf
12. USRDS [Internet]. [cited 2019 Oct 15]. Available from: https://www.usrds.org/atlas03.aspx
13. MILES WR. PSYCHOLOGIC TESTS APPLIED TO DIABETIC PATIENTS. Arch Intern Med [Internet]. 1922 Dec 1 [cited 2019 Oct 15];30(6):767. Available from:

http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.1922.00110120086003

14. Brands AMA, Kessels RPC, Hoogma RPLM, Henselmans JML, van der Beek Boter JW, Kappelle LJ, et al. Cognitive Performance, Psychological Well-Being, and Brain Magnetic Resonance Imaging in Older Patients With Type 1 Diabetes. Diabetes [Internet]. 2006 Jun 1 [cited 2019 Oct 15];55(6):1800–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16731845
15. Saha S, Muradi V, Bangalore P, Gowda L, Kalra P. A comparative study of event-related potential P300 between normal individuals and individuals with prediabetes. Natl J Physiol Pharm Pharmacol [Internet]. 2018 [cited 2019 Jun 25];(2). Available from: www.njppp.com
16. Paus T. Primate anterior cingulate cortex: Where motor control, drive and cognition interface. Nat Rev Neurosci [Internet]. 2001 Jun [cited 2019 Jul 1];2(6):417–24. Available from: http://www.nature.com/articles/35077500
17. Waragai M, Ho G, Takamatsu Y, Sekiyama K, Sugama S, Takenouchi T, et al. Importance of adiponectin activity in the pathogenesis of Alzheimer's disease. Vol. 4, Annals of Clinical and Translational Neurology. Wiley-Blackwell; 2017. p. 591–600.
18. Yaturu S, Bridges J, Reddy Rama Subba Reddy D. Decreased levels of plasma adiponectin in prediabetes, Type 2 Diabetes and coronary artery disease [Internet]. Available from: http://www.medscimonit.com/fulltxt.php?IDMAN=8007
19. Cezaretto A, Suemoto CK, Bensenor I, Lotufo PA, De Almeida-Pititto B, Ferreira

SRG. Association of adiponectin with cognitive function precedes overt diabetes in the Brazilian Longitudinal Study of Adult Health: ELSA. Diabetol Metab Syndr. 2018;10(1):1–8.

20. Teixeira AL, Diniz BS, Campos AC, Miranda AS, Rocha NP, Talib LL, et al. Decreased levels of circulating adiponectin in mild cognitive impairment and alzheimer's disease. NeuroMolecular Med. 2013 Mar;15(1):115–21.

21. Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A. Insulin in the Brain: Sources, Localization and Functions. [cited 2019 Jul 22]; Available from: https://umexpert.um.edu.my/file/publication/00011957_99084.pdf

22. Wozniak M, Rydzewski B, Baker S, Raizadai MK. Commentary the C E L L U L a R and Physiological Actions of Insulin in the Central Nervous System. Neurochem Int. 1993;22(I):1–10.

23. Kleinridders A, Ferris HA, Cai W, Kahn CR. Insulin Action in Brain Regulates Systemic Metabolism and Brain Function. Diabetes [Internet]. 2014 [cited 2019 Jul 22];63:2232–43. Available from: https://diabetes.diabetesjournals.org/content/diabetes/63/7/2232.full.pdf

24. Gasparini L, Netzer WJ, Greengard P, Xu H. Does insulin dysfunction play a role in Alzheimer's disease? [Internet]. Vol. 23, TRENDS in Pharmacological Sciences. 2002 [cited 2019 Jul 22]. Available from: http://tips.trends.com0165-6147/02/$-seefrontmatter

25. Convit A. Links between cognitive impairment in insulin resistance : An explanatory model. 2005;31–5.

26. Reagan LP. Hippocampal insulin resistance and cognitive dysfunction Neuroanatomical and Neurochemical Correlates of Stress View project. Artic Nat Rev Neurosci [Internet]. 2015 [cited 2019 Jul 22]; Available from: https://www.researchgate.net/publication/282872860

27. Weyer C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus-pdf. J Clin Invest [Internet]. 1999 [cited 2019 Jul 11];104(6). Available from: https://dm5migu4zj3pb.cloudfront.net/manuscripts/7000/7231/cache/7231.1-20150302104155-covered-253bed37ca4c1ab43d105aefdf7b5536.pdf

28. Pathophysiology of Type 2 Diabetes. In: Type 2 Diabetes, Pre-Diabetes, and the Metabolic Syndrome [Internet]. Totowa, NJ: Humana Press; 2005 [cited 2019 Jul 11]. p. 1–12. Available from: http://link.springer.com/10.1385/1-59259-932-X:1

29. KILIC F, BHARDWAJ R, CAULFEILD J, TREVITHICK JR. Modelling Cortical Cataractogenesis 22: Is in vitro Reduction of Damage in Model Diabetic Rat Cataract by Taurine Due to Its Antioxidant Activity? Exp Eye Res [Internet]. 1999 Sep 1 [cited 2019 Jul 12];69(3):291–300. Available from: https://www.sciencedirect.com/science/article/pii/S0014483599906978

30. Drel V, Pacher P, Ali T, Shin J, Julius U, El-Remessy A, et al. Aldose reductase inhibitor fidarestat counteracts diabetes-associated cataract formation, retinal oxidative-nitrosative stress, glial activation, and apoptosis. Int J Mol Med [Internet]. 2008 Jun 1 [cited 2019 Jul 12];21(6):667–76. Available from: http://www.spandidos-publications.com/10.3892/ijmm.21.6.667

31. Stevens MJ, Feldmanb EL, Greene DA. The Aetiology of Diabetic Neuropathy: the Combined Roles of Metabolic and Vascular Defects [Internet]. [cited 2019 Jul 12]. Available from: https://deepblue.lib.umich.edu/bitstream/handle/2027.42/75362/j.1464-5491.1995.tb00544.x.pdf?sequence=1&isAllowed=y

32. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. [cited 2019 Jul 12]; Available from: https://academic.oup.com/cardiovascres/article-abstract/63/4/582/318389

33. Salahuddin P, Rabbani G, Hasan Khan R. CELLULAR & MOLECULAR BIOLOGY LETTERS THE ROLE OF ADVANCED GLYCATION END PRODUCTS IN VARIOUS TYPES OF NEURODEGENERATIVE DISEASE: A THERAPEUTIC APPROACH. 2014 [cited 2019 Jul 16]; Available from: http://www.cmbl.org.pl

34. Newton AC. Protein Kinase C: Structure, Function, and Regulation* [Internet]. 1995 [cited 2019 Jul 17]. Available from: http://www.jbc.org/

35. Schmidt AM, Geraldes P, King GL. Activation of Protein Kinase C Isoforms and Its Impact on Diabetic Complications. 2010 [cited 2019 Jul 16]; Available from: http://circres.ahajournals.org

36. Ringvold HC, Khalil RA. Protein Kinase C as Regulator of Vascular Smooth Muscle Function and Potential Target in Vascular Disorders. In 2017 [cited 2019 Jul 17]. p. 203–301. Available from:

https://linkinghub.elsevier.com/retrieve/pii/S1054358916300485

37. Nakashima H, Frank GD, Shirai H, Hinoki A, Higuchi S, Ohtsu H, et al. Novel Role of Protein Kinase C-Tyr 311 Phosphorylation in Vascular Smooth Muscle Cell Hypertrophy by Angiotensin II. 2008 [cited 2019 Jul 17]; Available from: http://ahajournals.org

38. Li L, Sawamura T, Renier G. Glucose Enhances Human Macrophage LOX-1 Expression. Circ Res [Internet]. 2004 Apr 16 [cited 2019 Jul 17];94(7):892–901. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15001526

39. Kumar Venugopal S, Devaraj S, Yang T, Jialal I. Tocopherol Decreases Superoxide Anion Release in Human Monocytes Under Hyperglycemic Conditions Via Inhibition of Protein Kinase C [Internet]. [cited 2019 Jul 17]. Available from: https://diabetes.diabetesjournals.org/content/diabetes/51/10/3049.full.pdf

40. Evangelista Meyer L, Bender Machado L, Paula A Santiago AS, Seixas da-Silva W, De Felice FG, Holub O, et al. Mitochondrial Creatine Kinase Activity Prevents Reactive Oxygen Species Generation ANTIOXIDANT ROLE OF MITOCHONDRIAL KINASE-DEPENDENT ADP RE-CYCLING ACTIVITY * □ S Downloaded from. NUMBER 49 J Biol Chem [Internet]. 2006 [cited 2019 Jul 18];281(49):37361–71. Available from: http://www.jbc.org

41. Fiorentino TV, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced Oxidative Stress and its Role in Diabetes Mellitus Related Cardiovascular Diseases [Internet]. Vol. 19, Current Pharmaceutical Design. 2013 [cited 2019 Jul 18]. Available from: https://s3.amazonaws.com/academia.edu.documents/37871029/0005B.pdf?respons

e-content-disposition=inline%3B filename%3DHyperglycemia-induced_Oxidative_Stress_a.pdf&X-Amz-Algorithm=AWS4-HMAC-SHA256&X-Amz-Credential=AKIAIWOWYYGZ2Y53UL3A%2F20190718%2Fus-east-1

42. Michael F, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress [Internet]. Vol. 94, Cell Biology. 1997 [cited 2019 Jul 18]. Available from: www.pnas.org.

43. S. Roriz-Filho J, Sá-Roriz TM, Rosset I, Camozzato AL, Santos AC, Chaves MLF, et al. (Pre)diabetes, brain aging, and cognition. Biochim Biophys Acta - Mol Basis Dis [Internet]. 2009 May [cited 2019 Jul 31];1792(5):432–43. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0925443908002524

44. Soares E, Prediger RD, Nunes S, Castro AA, Viana SD, Lemos C, et al. Spatial memory impairments in a pre-diabetic rat model. Neuroscience [Internet]. 2013 Oct [cited 2019 Aug 2];250:565–77. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0306452213006490

45. Zilliox LA, Chadrasekaran K, Kwan JY, Russell JW. Diabetes and Cognitive Impairment. Curr Diab Rep [Internet]. 2016 [cited 2019 Jul 17];16(9):87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27491830

46. Deedwania PC, Fonseca VA. Diabetes, prediabetes, and cardiovascular risk: Shifting the paradigm. Am J Med [Internet]. 2005 Sep [cited 2019 Jul 31];118(9):939–47. Available from:

https://linkinghub.elsevier.com/retrieve/pii/S0002934305003967

47. Bansal N. Prediabetes diagnosis and treatment: A review. World J Diabetes [Internet]. 2015 Mar 15 [cited 2019 Jul 3];6(2):296–303. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25789110

48. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. Lancet [Internet]. 2012 Jun [cited 2019 Aug 1];379(9833):2279–90. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673612602839

49. Gross JL, De Azevedo MJ, Silveiro SP, Lu´is L, Canani H, Caramori ML, et al. Diabetic Nephropathy: Diagnosis, Prevention, and Treatment [Internet]. 2005 [cited 2019 Aug 1]. Available from: https://care.diabetesjournals.org/content/28/1/164.full-text.pdf

50. Fowler MJ. Microvascular and Macrovascular Complications of Diabetes. Clin Diabetes [Internet]. 2008 Apr 1 [cited 2019 Aug 1];26(2):77–82. Available from: http://clinical.diabetesjournals.org/cgi/doi/10.2337/diaclin.26.2.77

51. Papanas N, Vinik AI, Ziegler D. Neuropathy in prediabetes: does the clock start ticking early? Nat Rev Endocrinol [Internet]. 2011 Nov 12 [cited 2019 Aug 2];7(11):682–90. Available from: http://www.nature.com/articles/nrendo.2011.113

52. Putz Z, Tabak AG, Toth N, Istenes I, Nemeth N, Gandhi RA, et al. Noninvasive Evaluation of Neural Impairment in Subjects With Impaired Glucose Tolerance. Diabetes Care [Internet]. 2009 Jan 1 [cited 2019 Aug 2];32(1):181–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18835942

53. PREDIABETES PREDIABETES Strategies for Effective Screening, Intervention and Follow-up [Internet]. [cited 2019 Jul 3]. Available from: https://professional.diabetes.org/sites/professional.diabetes.org/files/media/prediabetes.pdf

54. Kanat M, DeFronzo RA, Abdul-Ghani MA. Treatment of prediabetes. World J Diabetes [Internet]. 2015 Sep 25 [cited 2019 Aug 8];6(12):1207–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26464759

55. Kong SE, Kang YE, Joung KH, Lee JH, Kim HJ, Ku BJ. Plasma Adiponectin Levels in Elderly Patients with Prediabetes. Endocrinol Metab. 2015 Oct 2;30(3):326.

56. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and Metabolic Syndrome. 2004 [cited 2018 Dec 18]; Available from: http://www.atvbaha.org

57. Ng R, Chan K-H. Potential Neuroprotective Effects of Adiponectin in Alzheimer's Disease. Int J Mol Sci [Internet]. 2017 Mar 9 [cited 2019 Aug 20];18(3):592. Available from: http://www.mdpi.com/1422-0067/18/3/592

58. Wieser HG. What are cognitive evoked potentials? Schweizer Arch fur Neurol und Psychiatr. 1998;149(6):268–72.

59. Hong JS, Lee JH, Yoon YH, Choi JH, Shin JE, Kim SM, et al. The Assessment of Reliability of Cognitive Evoked Potential in Normal Person. Ann Rehabil Med [Internet]. 2013 [cited 2019 Jun 25];37(2):263. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3660489/

60. Hayakawa T, Uchiyama M, Urata J, Enomoto T, Okubo J, Okawa M. Effects of a

small dose of triazolam on P300 [Internet]. Vol. 53, Psychiatry and Clinical Neurosciences. 1999 [cited 2019 Jul 29]. Available from: https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1440-1819.1999.00530.x

61. Jansen BH, Allam A, Kota P, Lachance K, Osho A, Sundaresan K. An exploratory study of factors affecting single trial P300 detection. IEEE Trans Biomed Eng. 2004;51(6):975–8.

62. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular Risk Factors in Confirmed Pre-diabetic Individuals. JAMA [Internet]. 1990 Jun 6 [cited 2019 Aug 30];263(21):2893. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.1990.03440210043030

63. Saczynski JS, Jonsdottir MK, Garcia ME, Jonsson P V., Peila R, Eiriksdottir G, et al. Cognitive Impairment: An Increasingly Important Complication of Type 2 Diabetes: The Age, Gene/Environment Susceptibility-Reykjavik Study. Am J Epidemiol [Internet]. 2008 Oct 9 [cited 2019 Aug 30];168(10):1132–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18836152

64. Yaffe K, Blackwell T, Kanaya AM, Davidowitz N, Barrett-Connor E, Krueger K. Diabetes, impaired fasting glucose, and development of cognitive impairment in older women. Neurology [Internet]. 2004 Aug 24 [cited 2019 Jul 31];63(4):658–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15326238

65. Convit A, Wolf OT, Tarshish C, de Leon MJ. Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among

normal elderly [Internet]. Vol. 18, PNAS February. 2003 [cited 2019 Jul 31]. Available from: www.pnas.orgcgidoi10.1073pnas.0336073100

66. Vanhanen M, Koivisto K, Kuusisto J, Mykkanen L, Helkala E-L, Hanninen T, et al. Cognitive Function in an Elderly Population With Persistent Impaired Glucose Tolerance. Diabetes Care [Internet]. 1998 Mar 1 [cited 2019 Jul 31];21(3):398–402. Available from: http://care.diabetesjournals.org/cgi/doi/10.2337/diacare.21.3.398

67. Une K, Takei YA, Tomita N, Asamura T, Ohrui T, Furukawa K, et al. Adiponectin in plasma and cerebrospinal fluid in MCI and Alzheimer's disease. Eur J Neurol [Internet]. 2011 Jul 1 [cited 2019 Aug 20];18(7):1006–9. Available from: http://doi.wiley.com/10.1111/j.1468-1331.2010.03194.x

68. Ma J, Zhang W, Wang H-F, Wang Z-X, Jiang T, Tan M-S, et al. Peripheral Blood Adipokines and Insulin Levels in Patients with Alzheimer's Disease: A Replication Study and Meta-Analysis. Curr Alzheimer Res [Internet]. 2016 [cited 2019 Aug 30];13(3):223–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26906354

69. Waragai M, Adame A, Trinh I, Sekiyama K, Takamatsu Y, Une K, et al. Possible Involvement of Adiponectin, the Anti-Diabetes Molecule, in the Pathogenesis of Alzheimer's Disease. J Alzheimer's Dis [Internet]. 2016 Jun 7 [cited 2019 Aug 30];52(4):1453–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27079710

70. Lin H V, Kim J-Y, Pocai A, Rossetti L, Shapiro L, Scherer PE, et al. Adiponectin

resistance exacerbates insulin resistance in insulin receptor transgenic/knockout mice. Diabetes [Internet]. 2007 Aug 1 [cited 2019 Aug 30];56(8):1969–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17475934

71. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in Obesity and Type 2 Diabetes: Close Association with Insulin Resistance and Hyperinsulinemia. J Clin Endocrinol Metab [Internet]. 2001 May [cited 2019 Oct 15];86(5):1930–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11344187

72. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med [Internet]. 2001 Aug 1 [cited 2019 Oct 15];7(8):947–53. Available from: http://www.nature.com/articles/nm0801_947

73. Stefan N, Stumvoll M, Vozarova B, Weyer C, Funahashi T, Matsuzawa Y, et al. Plasma Adiponectin and Endogenous Glucose Production in Humans. Diabetes Care [Internet]. 2003 Dec 1 [cited 2019 Oct 15];26(12):3315–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14633820

74. Imagawa A, Funahashi T, Nakamura T, Moriwaki M, Tanaka S, Nishizawa H, et al. Elevated serum concentration of adipose-derived factor, adiponectin, in patients with type 1 diabetes. Diabetes Care [Internet]. 2002 Sep 1 [cited 2019 Oct 15];25(9):1665–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12196453

75. MacKnight C, Rockwood K, Awalt E, McDowell I. Diabetes mellitus and the Risk

of Dementia, Alzheimer's Disease and Vascular Cognitive Impairment in the Canadian Study of Health and Aging. Dement Geriatr Cogn Disord [Internet]. 2002 [cited 2019 Oct 15];14(2):77–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12145454

76. Yan S Du, Chen X, Fu J, Chen M, Zhu H, Roher A, et al. RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. Nature [Internet]. 1996 Aug 22 [cited 2019 Oct 15];382(6593):685–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8751438

77. Kalmijn S, Feskens EJ, Launer LJ, Stijnen T, Kromhout D. Glucose intolerance, hyperinsulinaemia and cognitive function in a general population of elderly men. Diabetologia [Internet]. 1995 Sep [cited 2019 Oct 15];38(9):1096–102. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8591825

78. Cook DG, Leverenz JB, McMillan PJ, Kulstad JJ, Ericksen S, Roth RA, et al. Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. Am J Pathol [Internet]. 2003 Jan [cited 2019 Oct 15];162(1):313–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12507914

79. Ganguli M, Belle S, Ratcliff G, Seaberg E, Huff FJ, von der Porten K, et al. Sensitivity and specificity for dementia of population-based criteria for cognitive impairment: the MoVIES project. J Gerontol [Internet]. 1993 Jul [cited 2019 Oct 15];48(4):M152-61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8315228

Annexures

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.B.Leela Priyadharsini
Post Graduate in M.D. Physiology
Institute of Physiology and Experimental Medicine
Madras Medical College
Chennai

Dear Dr.B.Leela Priyadharsini,

The Institutional Ethics Committee has considered your request and approved your study titled **"TO EVALUATE COGNITIVE EVOKED POTENTIAL AND SERUM ADIPONECTIN LEVEL IN PREDIABETES – A CROSS SECTIONAL STUDY" - NO.30012018**

The following members of Ethics Committee were present in the meeting hold on **09.01.2018** conducted at Madras Medical College, Chennai 3

1. Prof.P.V.Jayashankar	:Chairperson
2. Prof.R.Narayana Babu,MD.,DCH., Dean,MMC,Ch-3	: Deputy Chairperson
3. Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4. Prof.N.Gopalakrishnan,MD,Director,Inst.of Nephrology,MMC,Ch	: Member
5. Prof.S.Mayilvahanan,MD,Director,Inst. of Int.Med,MMC, Ch-3	: Member
6. Prof.A.Pandiya Raj,Director, Inst. of Gen.Surgery,MMC	: Member
7. Prof.Shanthy Gunasingh, Director, Inst.of Social Obstetrics,KGH	: Member
8. Prof.Rema Chandramohan,Prof.of Paediatrics,ICH,Chennai	: Member
9. Prof. Susila, Director, Inst. of Pharmacology,MMC,Ch-3	: Member
10.Prof.K.Ramadevi,MD., Director, Inst. of Bio-Chemistry,MMC,Ch-3	: Member
11.Prof.Bharathi Vidya Jayanthi,Director, Inst. of Pathology,MMC,Ch-3	: Member
12.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
13.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist
14.Thiru K.Ranjith, Ch- 91	: Lay Person

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

நோயாளியின் ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு : " அறியும் ஆற்றலால் வெளிக்கொணரப்படும் ஆற்றல் வளம் மற்றும் சீரம் அடிப்போனெக்ட்டின் அளவை முன் நீரிழிவு நோயாளிகளில் மதிப்பீடு செய்தல் – ஒரு குறுக்கு வெட்டு ஆய்வு "

பெயர் :
வயது :
பால் :

தேதி :
புறநோயாளி எண்:
ஆராய்ச்சி சேர்க்கை எண் :

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும், அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன் நான். "அறியும் ஆற்றலால் **வெளிக்கொணரப்படும் ஆற்றல் வளம் மற்றும் சீரம் அடிப்போனெக்ட்டின் அளவை முன் நீரிழிவு நோயாளிகளில் மதிப்பீடு செய்தல் – ஒரு குறுக்கு வெட்டு ஆய்வு** "என்ற தலைப்பில் மேற்கொள்ளப்படும் இந்த ஆராய்ச்சியின் விபரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

இதன் மூலம் எந்த பின்விளைவும் வராது என மருத்துவர் மூலம் தெரிந்து கொண்டு என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்

தேதி :

கையொப்பம் :

ஆராய்ச்சி தகவல் தாள்,

சென்னை அரசுபொது மருத்துவமனையின் பொது மருத்துவத்துறையில் **" அறியும் ஆற்றலால் வெளிக்கொணரப்படும் ஆற்றல் வளம் மற்றும் சீரம் அடிப்போனெக்ட்டின் அளவை முன் நீரிழிவு நோயாளிகளில் மதிப்பீடு செய்தல் - ஒரு குறுக்கு வெட்டு ஆய்வு "** என்ற தலைப்பில் ஆராய்ச்சி நடைபெறுகிறது.

நமது குருதியின் சக்கரை அளவு கட்டுக்குள் இருக்க வேண்டும். சக்கரை அளவு அதிகமானால் உடம்பின் நிறைய பாதிப்புகள் உள்ளாகும். அறியும் ஆற்றலும் குறையும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்த ஆராய்ச்சியில் உங்களுக்கு வாய் வழி க்ளுகோஸ் சகிப்பு தன்மை சோதனை செய்வோம். முன் நீரிழிவு நோய் கண்டரியப்பட்டால் உங்களுடைய சீரம் அடிப்போனெக்ட்டின் அளவை அளவேடுப்போம். பின் அறியும் ஆற்றலால் வெளிக்கொணரப்படும் ஆற்றல் வளத்தை மதிப்பிடுவோம். அனைத்து தகவல்களையும் ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வரிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்கு உள்ளாகாது என்பதையும் தெரிவித்து கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக்கொள்கிறோம்

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துகொள்கிறோம்.

இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் **பங்கேற்பாளர் கையொப்பம்**

தேதி:

INFORMED CONSENT FORM

Title of the study "To Evaluate Cognitive Evoked Potential and Serum Adiponectin level in Prediabetes - A Cross Sectional Study"

Name of the Participant:

Name of the Principal Investigator: Dr. B. LEELA PRIYADHARSINI.

Name of the Institution:

Institute of Physiology and Experimental Medicine,
Madras Medical College,
Chennai - 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in

"**To Evaluate Cognitive Evoked Potential and Serum Adiponectin level in Prediabetes - A Cross Sectional Study"**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past_____ month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.

11. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
12. I have understood that my identity will be kept confidential if my data are publicly presented.
13. I have had my questions answered to my satisfaction.
14. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____

Date_____
Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____

Date_____

PRO FORMA

Name:
Age/ Sex:
Address:
OP No.:
Occupation:

History of Presenting Illness:
History of Hypertension:
History of hypothyroidism:
History of smoking/alcohol:
History of any surgery:
History of fever, injury:
History of recent infections:
History of any chronic illness (specify):
History of chronic drug intake (specify):
History of malignancy:
Family history:

Investigations:

EXAMINATION
General examination:
Pulse rate:
Blood pressure:
Height:
Weight:
Waist circumference:
Hip circumference:

Systemic examination:

Cardiovascular system:
Respiratory system:
Gastrointestinal system:
Central nervous system:

MASTER CHART FOR THE PRE-DIABETIC PATIENTS

S.NO	AGE	SEX	WT(KG)	HT(CM)	FBS	PPBS	N1 LATENCY	P2 LATENCY	N2 LATENCY	P300 LATENCY	N2 - P300 AMPLITUDE	S. ADIPONECTIN
1	42	M	65	158	102	153	255	295	310	355	3.8	21
2	31	F	53	160	102	145	235.62	268.75	310	357.6	3.6	22
3	44	M	80	166	121	176	168.75	182.5	209.37	348.75	1.09	21
4	43	F	76	161	122	161	103.12	151.87	314.37	390	0.68	38
5	32	M	62	154	106	162	240	279.37	330	380	1.1	20
6	39	F	61	158	115	153	155.62	186.87	274.37	373.12	2.95	17
7	40	F	60	156	124	152	120	199.37	287.5	386.87	1.61	16
8	44	F	83	159	109	189	116.87	167.5	296.87	375	4.56	17
9	38	M	60	182	114	168	112.5	143.75	216.87	355	5.69	11
10	41	M	75	162	101	184	121.5	162.35	215.23	351	4.05	19
11	42	M	68	160	122	185	100.23	149.3	192.36	303	1.82	17
12	37	F	80	156	124	190	112.52	158.26	201.23	303.75	5.19	11
13	40	M	65	160	110	156	95.23	112.35	198.35	285	4.27	9
14	29	M	70	168	115	149	103.26	142.32	206.32	333.12	5.03	10
15	45	M	70	165	116	168	132.02	162.32	210.84	325.62	2.19	17
16	39	M	55	152	101	158	121.98	146.82	218.65	356.87	1.95	15
17	44	F	69	160	123	167	124.82	167.39	222.36	341.25	2.43	14
18	29	M	74	170	115	145	134.65	174.26	245.65	343.75	2.13	22
19	26	M	70	165	118	182	146.82	205.67	264.92	391.87	2.27	13
20	34	F	76	156	104	175	116.49	162.42	231.56	315	1.3	12
21	30	M	80	168	109	185	96.25	136.28	186.25	265.62	3.86	16
22	32	M	52	154	108	191	180.62	210	244.37	356.87	0.57	11
23	26	F	65	158	124	155	143.12	214.37	266.25	330	2.71	10
24	40	M	68	167	121	164	132.85	199.37	249.37	314.5	1.24	11
25	27	F	73	171	124	148	128.12	204.37	301.25	375.62	2.26	11
26	35	M	58	160	115	149	110.01	141.25	215.52	323.75	5.64	7
27	44	F	66	160	104	156	135.62	168.75	295.67	353.12	3.59	10
28	34	M	63	167	118	159	124.85	185.65	215.68	335	6.59	10
29	28	M	82	173	116	173	136.87	160.62	202.52	346.51	4.52	9
30	39	F	71	161	108	159	150.62	182.5	200.24	336.72	3.83	7

S.NO	AGE	SEX	WT(KG)	HT(CM)	FBS	PPBS	N1 LATENCY	P2 LATENCY	N2 LATENCY	P300 LATENCY	N2 - P300 AMPLITUDE	S. ADIPONECTIN
31	40	F	59	154	117	184	103.12	151.87	254.37	339	5.45	8
32	28	M	63	166	100	182	105	159.37	210.62	368.24	5.18	1
33	31	M	73	168	121	172	112.25	162.35	245.65	362.48	3.19	1
34	27	M	62	165	122	148	120.52	199.65	287.25	345.19	4.19	9
35	33	M	67	164	107	167	110	141.25	215.23	299.75	3.75	7
36	42	M	75	169	108	158	136.87	260.62	308.42	348.29	1.29	8
37	43	F	57	153	107	185	125.62	172.5	188.75	315.89	5.17	6
38	29	M	66	172	114	195	125.52	182.62	215.82	327.15	4.67	15
39	30	M	69	171	124	172	141.25	178.25	215.65	349.25	2.81	16
40	41	F	62	154	120	149	118.14	185.63	277.5	341.29	5.49	17
41	43	M	58	169	115	148	117.85	155.63	271.25	337.19	6.82	17
42	38	F	72	161	118	167	108.75	133.85	256.25	383.19	3.73	16
43	35	M	68	164	117	182	132.75	186.52	285.65	362.14	4.92	20
44	30	M	79	168	108	190	145.62	183.25	278.52	372.67	5.1	8
45	29	M	69	173	109	173	123.58	184.65	212.52	329.75	3.71	16
46	33	M	57	159	122	159	143.25	195.25	259.67	375.14	4.28	7
47	34	F	75	156	105	148	97.53	165.23	242.57	339.24	1.69	23
48	38	M	66	174	114	168	124.85	162.35	218.12	351.83	3.76	16
49	45	F	69	160	119	148	176.25	209.37	250	361.24	4.24	17
50	40	F	81	159	124	154	107.52	195.62	303.75	391.27	5.91	15

MASTER CHART FOR THE CONTROL GROUP

S.NO	AGE	SEX	WT(KG)	HT(CM)	FBS	PPBS	N1 LATENCY	P2 LATENCY	N2 LATENCY	P300 LATENCY	N2 - P300 AMPLITUDE	S. ADIPONECTIN
1	35	F	58	158	70	135	102.4	142.9	224.6	308.3	5.1	18
2	38	F	55	153	75	134	114.3	149.4	194.5	320.4	6.3	15
3	41	M	70	168	88	124	95.4	162.8	183.6	293.2	5.4	14
4	26	M	57	160	90	115	124.8	157.6	199.4	318.2	7.2	15
5	45	M	65	171	69	118	137.9	143.0	187.6	342.3	5.2	6
6	36	F	63	162	84	124	98.6	143.9	205.6	317.5	5.6	19
7	26	M	66	169	86	129	96.8	163.9	209.6	310.3	6.2	7
8	38	F	50	156	92	127	112.4	157.6	214.8	320.4	6.7	9
9	39	M	60	159	94	119	107.9	148.9	183.3	335.3	5.2	6
10	27	M	72	180	76	110	122.7	167.9	191.3	320.2	5.6	5
11	29	M	71	170	74	108	111.4	142.9	209.9	333.3	6.5	10
12	30	M	66	168	73	131	119.5	153.7	220.3	309.3	6.6	12
13	36	F	51	152	80	127	108.9	158.3	231.6	352.2	5.2	7
14	44	F	48	150	94	129	107.5	159.3	186.5	311.3	6.3	19
15	43	F	53	155	73	110	98.5	148.7	194.6	319.2	5.2	25
16	40	M	67	168	95	115	99.7	167.8	216.0	321.3	5.1	9
17	29	M	63	172	81	119	104.9	156.3	186.5	310.3	4.3	7
18	44	F	48	157	74	123	110.5	148.9	189.6	324.6	5.3	10
19	33	F	44	151	76	128	100.8	147.8	227.9	314.3	7.3	9
20	37	M	70	176	73	134	108.5	157.5	208.4	309.3	1.2	6
21	41	M	64	169	86	122	117.9	162.5	186.5	316.8	6.9	9
22	26	M	72	171	84	109	114.9	142.5	189.7	319.3	5.6	9
23	30	M	75	182	76	100	109.6	157.8	205.0	312.2	5.8	9
24	42	F	46	149	84	121	127.5	143.1	184.3	319.2	8.2	8
25	44	F	58	161	73	126	97.5	172.6	179.6	308.3	6.7	8
26	36	M	69	174	81	138	104.5	158.3	196.2	300.2	9.3	9
27	39	F	65	162	82	130	118.4	148.5	206.5	329.2	5.8	7
28	40	M	68	178	79	129	117.6	168.3	222.6	310.3	5.4	8
29	37	F	53	156	78	106	120.7	148.4	210.5	308.2	8.2	6
30	36	F	56	166	87	110	108.7	145.8	203.1	303.0	5.2	7

S.NO	AGE	SEX	WT(KG)	HT(CM)	FBS	PPBS	N1 LATENCY	P2 LATENCY	N2 LATENCY	P300 LATENCY	N2 - P300 AMPLITUDE	S. ADIPONECTIN
31	28	F	55	153	85	119	107.5	144.9	213.5	307.8	6.2	9
32	29	F	60	157	86	123	92.7	137.8	217.6	333.2	4.8	7
33	27	F	59	160	84	138	113.4	163.5	195.3	293.2	5.1	8
34	26	M	66	172	77	120	118.6	158.6	207.9	310.3	6.2	5
35	38	M	66	176	88	111	117.0	162.3	213.5	311.9	5.2	7
36	33	M	67	173	84	104	107.6	157.9	226.3	327.4	7.3	9
37	43	M	60	169	72	124	103.8	153.5	216.4	317.9	6.2	9
38	26	F	66	164	83	112	121.6	142.4	209.6	311.9	6.1	9
39	42	F	48	154	85	138	98.6	140.6	225.2	315.3	5.8	8
40	29	F	56	156	71	125	107.6	167.7	194.9	328.2	6.1	7
41	45	M	61	167	86	103	124.9	146.3	184.6	317.2	8.2	6
42	38	M	63	164	91	116	116.9	165.3	204.3	314.3	5.6	4
43	35	M	68	176	90	109	117.9	146.8	234.6	329.2	9.1	8
44	36	M	66	171	80	125	100.8	139.4	213.1	307.3	6.3	5
45	29	F	45	149	81	138	127.9	147.4	206.1	308.2	4.3	9
46	40	F	59	163	68	101	116.9	159.6	226.3	329.2	5.6	7
47	43	M	69	172	77	110	134.9	134.9	222.5	326.0	7.1	5
48	41	F	55	163	74	122	104.6	163.8	186.4	314.7	6.4	6
49	40	M	73	178	85	105	109.3	165.0	216.2	321.5	7.1	8
50	28	F	48	151	84	133	112.5	155.6	187.9	309.3	5.4	5