ASSESSMENT OF VASCULAR FUNCTION, INFLAMMATION, TELOMERASE ACTIVITY AND BODY COMPOSISTION IN COMPLETE GLYCEMIC SPECTRUM

SYNOPSIS

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TABLE OF CONTENTS

Title: Assessment of vascular function, inflammation, telomerase activity and body composition in complete glycemic spectrum

INTRODUCTION

Globally, diabetes is one of the major causes for mortality and morbidity. The prevalence of diabetes in adult is 8.4 % in 2017 and it is expected to rise to 9.9% in 2045 (1). Diabetes is the major important etiology for cardiovascular risk or sudden cardiac arrest (2). Many studies have reported cardio vascular disease (CVD) risk in first degree relatives of diabetes (FDRD)(3) and prediabetics (4) itself. This risk among high-risk population is often overlooked, as it not affects the day today life. Obesity in diabetes independently adds to the cardiometabolic risk. Further, inflammatory markers, insulin resistance, oxidative stress, autonomic dysfunction, vascular homeostasis alteration, reduced telomerase activity root cardiovascular risk. However, the important link between CVD and diabetes remains unclear.

Vascular dysfunction leading to micro/macro vascular complication (5) is considered the major cause of morbidity and mortality in diabetes (6). Vascular function is associated with peripheral blood mononuclear cell (PBMC) telomerase activity which synergizes with nitric oxide bioavailability to maintain vascular homeostasis (7). Nitric oxide contributes to the vascular integrity by decreasing inflammation, cellular proliferation and thrombosis (8) and decrease in its level leads to endothelial and vascular smooth muscle dysfunction culminating in atherosclerosis (9) and arterial stiffness (10). Earlier study has documented that PBMC telomerase activity is linked to the progression of diabetes and its complications especially vascular complications (11) . Both telomere length and vascular function have been reported to be reduced in prediabetes and diabetes (11, 12). However, the presence or absence of vascular dysfunction or telomere attrition in FDR (First degree relatives) of T2DM is still under debate (13, 14). Some studies have shown decrease in vascular reactivity and flow mediated dilatation in FDR of T2DM (15), whereas, there is no consensus in the literature related to vascular function in FDR of T2DM. In one recent Indian study, PBMC telomerase activity was found to be associated with insulin resistance in diabetics (11) whereas, European studies have found it to be independent of glycemic control, insulin resistance and inflammatory markers (16)

South East Asian population has been reported to have altered body composition marked by higher centripetal obesity (17, 18), body fat percentage (19) and decreased lean mass (20). They differ

from their western counterparts in terms of body composition, insulin resistance, hyperinsulinemia and dyslipidemia which increases their risk of developing diabetes and its complications one to two decades earlier. Somatotyping is an emerging technique to assess body composition (21) and has higher heritability than BMI measurement (22). While previous studies have reported that meso-endomorphic individuals are shown to be prone to develop diabetes (23), the relationship between somatotype and diabetes has not yet been studied in Indian population. Altered body composition is also associated with autonomic imbalance (24) which, in turn can affect the vascular function (25), and inflammation (26). Hence, body composition analysis plays an important role in assessing various risk factors associated with diabetes.

Further, higher body fat in T2DM contributes to the production of proinflammatory cytokines and reduced adiponectin levels which in turn increase oxidative stress, deplete nitric oxide and cause vascular dysfunction (27). Higher glycemic level can also increase oxidative stress (28) which necessitates the need to study the interrelation between body composition and glycemic status in determining the vascular complications.

Further, the relation between telomerase activity, the emerging factor to assess cardiometabolic risk in diabetes and body composition is not yet explored. In addition, the point of deterioration of these conventional risk factors in the glycemic spectrum (normoglycemic non-first-degree relatives of T2DM (control), normoglycemic first degree relatives of T2DM, prediabetes, and diabetes) is yet to be assessed. Hence, we designed a cross-sectional comparative study, to assess the interrelationship amongst vascular function, telomerase activity, inflammation, body composition and somatotyping in Indian population in the complete glycemic spectrum. Our study also got novelty in providing, the telomerase level and somatotype in complete glycemic spectrum to the research arena.

OBJECTIVES

Primary objectives:

- To assess and compare vascular function, inflammation, peripheral blood telomerase activity and body composition in complete glycemic spectrum
- To study the somatotype category in complete glycemic spectrum

Secondary objective:

- To assess and compare oxidative stress, insulin resistance and cardiac autonomic function in complete glycemic spectrum

MATERIALS AND METHODS

The present study was conducted in the department of Physiology, Biochemistry, Medicine and Radiology, JIPMER, Puducherry. This study was approved by Institute Ethics Committee (Human studies). Written informed consent was obtained from all subjects before participation.

Number of groups to be studied, their names and definitions

- Age group of 30-50yrs (Age and gender matched subjects in block of 5 years)
- Number of groups: 4

Group 1 : Healthy Normoglycemic non-FDR T2DM subjects (control group)

Group II : Normoglycemic first degree relatives of diabetics

Group III : Prediabetes (Impaired fasting glucose and impaired glucose tolerance)

Group IV : Diabetes (T2DM)

Study design: Cross sectional comparative study

Inclusion Criteria:

Group I	Fasting plasma glucose < 100mg/dL and 2hr Plasma glucose following OGTT (at least twice)
Group II	First degree relatives of diabetics (either of their parents or both parents and their sibling/s are diabetic). Fasting plasma glucose < 100mg/dL and 2hr Plasma glucose < 140mg/dl following OGTT (at least twice)
Group III	Fasting Plasma glucose level 100 mg/dL to 125 mg/dl with 2hr Plasma glucose< 140mg/dl following OGTT (at least twice) (Impaired fasting glucose group) Fasting Plasma glucose level < 100 mg/dL with 2hr Plasma glucose 140mg/dl-199mg/dl following OGTT (at least twice) (Impaired glucose tolerance group) Individuals with IFG and / or IGT will be included
Group IV	Diabetes on treatment. FPG ≥126mg/dl or 2-h plasma glucose ≥ 200mg/dl

Exclusion criteria

Group I	Subjects with any organic disorder, family history of DM, diabetes, impaired fasting glucose, smoking, pregnancy, prehypertension, hypertension, impaired glucose tolerance and morbid obesity

Group II	Diabetes, impaired fasting glucose, impaired glucose tolerance, smoking, pregnancy, prehypertension, hypertension and morbid obesity
Group III	Diabetes, smoking, pregnancy, prehypertension, hypertension and morbid obesity
Group IV	Diabetics on insulin therapy, smoking, pregnancy, hypertension and morbid obesity

Statistical analysis: Physiological and biochemical data were tested for normality. Normally distributed data were expressed as mean ± standard deviation and comparison between groups were done using One-way ANOVA followed by Post- hoc test was carried out using least significant difference (LSD) analysis. Non-normally distributed data were expressed in median with interquartile range and comparison between groups were done using Kruskal Wallis test followed by posthoc test using Mann-Whitney U test. All analyses were two-tailed and a significance level of $p<0.05$ was be used in the study.

General Biochemical Profile:

Glucose: Both Fasting and Post prandial glucose was significantly higher circulating glucose levels in Diabetes and prediabetes than control group and FDR. Further, there was significant hyperglycemia (Fasting and Post prandial) in diabetes than prediabetes

Lipid Profile: Groups were significantly difference in their lipid profile and their derived parameters on one-way ANOVA. Based on post-hoc test we found that TC, TG, LDL, VLDL, TC/HDL, TG/HDL and AIP in the following order DM>PD>FDR>Control group. However, based on all the above-mentioned parameters prediabetes and diabetes did not differ significantly.

CHAPTER 1

Objective: To assess and compare vascular function, inflammation, peripheral blood telomerase activity and body composition in complete glycemic spectrum

Methodology

Flow mediated dilation: After adequate rest, test was done in supine position. We used high frequency vascular probe (13-15 Mhz) with an axial resolution of 0.1 mm to measure brachial artery diameter of the right arm around 5 cm above the elbow joints. The brachial artery lumen diameter will be searched in cross sectional view and the recording were carried out in longitudinal section using B mode imaging. After measuring baseline brachial artery diameter, the sphygmomanometer cuff placed below the elbow is inflated to 200 mm Hg and we retained it for 5 minutes duration. Following the release of cuff the brachial artery diameter is measured again (after reactive hyperemia)

Flow mediated dilation percentage is calculated by diameter of brachial artery after reactive hyperemia divided by baseline brachial artery diameter and the value is expressed in percentage

Biochemical analysis: Endothelin-1 (ET-1), vascular endothelium growth factor (VEGF), von willebrand factor (vWF) Tumor necrosis factor-α, IL-6, leptin, adiponectin concentrations were measured using ELISA according to manufacturer guidelines.

Telomerase level measurement: Telomerase was analysed using ELISA kit. The ELISA kit was based on sandwich-ELISA principle. The ELISA wells were precoated with specific antibody. We added 100µl of standard or samples to the wells and incubated for 90 minutes at room temperature. After removing the liquid from wells, we added 100µl of biotynylated detection antibody. Followed by 1-hour incubation at room temperature. After five washes with wash buffer, we added 90µl of substrate solution to all the wells and incubated for 15 minutes. Further, we added 50µl of stop solution. We read the absorbance at 450nm using microplate reader.

Body composition:

Waist circumference and Waist-Hip ratio (WHR): Waist circumference (cm) was measured at midpoint between the top of the iliac crest and the lower margin of the last palpable rib at the end

of normal expiration. Hip circumference (cm) was measured at the wide circumference of the buttock region.We calculated WHR by dividing WC (in cm) by hip circumference (cm) (29).

Body Mass Index (BMI): We measured height using wall mounted stadiometer (VM Electronics Hardware Ltd) accurate to the nearest 0.1 cm and weight using digital weighing machine (Charder Electronic Co. Ltd. Taichung, Taiwan 2013) accurate to the nearest 0.1kg. BMI was calculating by the formula, BMI = weight (kg)/height (m^2).

Bioelectrical impedance analysis: The body composition analysis was further carried out using bioelectrical impedance analysis. The subjects were asked to lie down in supine position with the legs apart. The bodystat electrodes were placed on the right-side dorsum of hand (metacarpal and wrist joint) and feet (metatarsal and in between the two medial and lateral malleolus) with the distance of 5 cm between the injecting electrodes and reading electrodes. After placing electrodes and we fed all the anthropometric details into the machine. The subjects were not asked to move during procedure and patients with metallic implants including dental implants were excluded from this procedure. After all the precautions, we applied a current in range of 500-800 mA with 50KHz frequency. All the body composition parameters were retrieved using the software linked to the Quadscan 4000 bodystat machine.

Result (Chapter 1):

Endothelin 1: There was a trend towards statistically significant increase in Endothelin 1 as we move along the glycemic spectrum from control, FDR of T2DM, prediabetes and diabetes. Endothelin 1 value was significantly higher in all the other groups as compared to control group. The value was statistically higher in prediabetes ($p = .007$) and diabetes ($p < .001$) as compared to FDR of T2DM. Further, the value was higher in diabetes group as compared to prediabetes group ($p = .002$)

Flow mediated dilation: Baseline brachial artery diameter was comparable among the groups ($P= .602$). Brachial artery peak diameter was comparable between control and FDR of T2DM ($p =.334$), and between FDR of T2DM and prediabetes ($p = .178$) and between prediabetes and diabetes ($p =.058$). Peak diameter was significantly lower in prediabetes ($p =.007$) and diabetes ($p < .001$) as compared to control and significantly lower in diabetes as compared to FDR of T2DM ($p =.009$). FMD% was comparable between control and FDR of T2DM ($p =.926$), and between

prediabetes and diabetes (p =.079). FMD% was significantly lower in prediabetes and diabetes as compared to control (p =.008, p < .001) and FDR of T2DM (p =.009, p < .001).

VEGF: We observed a trend towards increase in VEGF values as we move along the glycemic spectrum from control, FDR of T2DM, prediabetes and diabetes.

vWF: We observed a trend towards increase in vWF values as we move along the glycemic spectrum from control, FDR of T2DM, prediabetes and diabetes.

IL 6: IL 6 was significantly higher in diabetes group as compared to control (p < .001), FDR of T2DM (p < .001) and prediabetes (p = .002) groups, while the value was comparable among control, FDR of T2DM and prediabetes and no trend was observed.

Adiponectin: There was a trend towards decrease in adiponectin values as we move along the glycaemic spectrum from control, FDR of diabetes, prediabetes and diabetes. Adiponectin was significantly lower in all the other groups as compared to control (p < .001). adiponectin value was lower in prediabetes as compared to FDR of diabetes but not statistically significant, while it was significantly lower in diabetes group. The adiponectin values were lower in diabetes group as compared to prediabetes group but not statistically significant.

Leptin: leptin was significantly higher in FDR of diabetes, prediabetes and diabetes group as compared to control group, while it was comparable between FDR of diabetes, prediabetes and diabetes group.

TNF alpha: There was a trend towards increase in TNF alpha as we move along the glycaemic spectrum from control, FDR of diabetes, prediabetes and diabetes. However, statistically the TNF value was comparable between control and FDR of diabetes (p = .411) and it was significantly higher in prediabetes and diabetes as compared to control (p = .002, p < .001) and FDR of diabetes (p = .014, p < .001). In diabetes group the value was significantly higher even as compared to prediabetes (p =. 002).

Telomerase: There was a trend towards statistically significant increase in telomerase levels as we move along the glycemic spectrum from control, FDR of diabetes, prediabetes and diabetes. However, statistically the telomerase was comparable between control and FDR of diabetes (p = .083) and between FDR of diabetes and prediabetes (p = .221) and between prediabetes and

diabetes (p = .539). Telomerase value was significantly higher in prediabetes (p =.005) and diabetes (p <.001) as compared to control, and significantly higher in diabetes (p =.037) as compared to FDR of diabetes.

Body composition: Groups were comparable based on weight, BMI, waist and hip circumference and waist/Hip ratio.

Body fat percentage: Body fat percentage was significantly higher in First degree relatives of diabetes (p = .016), prediabetes (p = .018) and diabetes group (p = .006) as compared to controls, while it was comparable among First degree relatives of diabetes, prediabetes and diabetes group.

CHAPTER 2

Objective: To study the somatotype category in complete glycemic spectrum

Methodology

Triceps skinfold: We raised a fold at back of the arm, midway between acromion and olecranon process with their arms hanging loose in anatomical position.

Subscapular skinfold: We raised the skinfold from the inferior angle of the scapula obliquely and laterally (45^0).

Supraspinale skinfold: The supraspinale skin fold was measured on a line to the anterior axillary border and on a diagonal line going downwards and medially at 45 degrees. We raised the skinfold of the subject 5-7cm (depending on the size of the subject).

Medial Calf skinfold thickness: Vertical skinfold was raised in the medial side of the calf at the maximum circumference of the calf we took the medial calf skinfold thickness.

Humerus breadth and femur breadth: The humerus breadth is obtained by measuring the distance between two epicondyles (medial and lateral) with the elbow and shoulder flexed at 90^0 using a caliper. For femur breadth, we made the participant to sit with the knees bent at a right angle, the maximum distance between the medial and lateral epicondyles of the femur is measured using the caliper

Arm girth and calf girth: The participant flexes the shoulder to 90 degrees and the elbow to 45 degrees and clenches the hand (ensuring maximal contraction of the elbow flexors and extensors). Arm girth is measured at the greatest circumference using non-stretchable measuring tape. For measuring calf girth, we asked the participants to stand with feet slightly apart, using non-stretchable measuring tape the greatest circumference of the calf.

All the measurements were taken in triplicate by the same investigator and the average of it was taken. We followed the guidelines provided by Heath-carter anthropometric somatotype instruction manual (21) for methodology and for classifying the participants into different somatotype category.

Table 1: Formulae used for calculating the somatotypes

Endomorphy	$- 0.7182 + 0.1451 X – 0.00068 X^2 + 0.0000014X^3$	
Mesomorphy	0.858 HB + 0.601 FB + 0.188AG + 0.161CG – 0.131 SH + 4.5	
Ectomorphy	0.732 HWR – 28.58	If HWR > 40.74
	0.463 HWR -17.63	If 38.25 < HWR = 40.74
	0.1	If HWR ≤ 38.25

X= (sum of triceps, subscapular and supraspinale skinfolds multiplied by (170.18/height in cm); HB = Humerus breath (cm) ; FB = femur Breadth (cm); AG = corrected arm girth (cm) (arm girth –(triceps skinfold (mm)/10); CG = corrected calf girth (cm) (calf girth - (medial calf skinfold (mm)/10)); SH = standing height (cm); HWR = height in cm over cuberoot of weight.

Table 2: Somatotype categories and its description

Central	No component differs by more than one unit from the other two
Endomorph	Endomorphy is dominant, mesomorphy and ectomorphy are more than one-half unit lower
Endomorph-mesomorph	Endomorphy and mesomorphy are equal (or do not differ by more than one- half unit and ectomorphy is smaller
Mesomorph	Mesomorphy is dominant, endomorphy and ectomorphy are more than one-half unit lower
Mesomorph-ectomorph	Ectomorphy and mesomorphy are equal (or do not differ by more than one- half unit and endomorphy is smaller
Ectomorph	Ectomorphy is dominant, mesomorphy and endomorphy are more than one-half unit lower
Ectomorph-endomorph	Ectomorphy and endomorphy are equal (or do not differ by more than one- half unit and mesomorphy is smaller

Result:

In the present study, we observed more prevalence of endomorphy somatotype category in all the groups (Control group, FDR of T2DM, Prediabetes and diabetes). We also observed 3% of ectomorphy somatotype category in control group and 2% prevalence of endomorphy mesomorph in diabetes group.

CHAPTER 3

Objective: To assess and compare oxidative stress, insulin resistance and cardiac autonomic function in complete glycemic spectrum

Methodology:

Fasting glucose and insulin: The fasting blood glucose was estimated by glucose oxidase-peroxidase method (Genuine Biosystem). Fasting insulin was measured in plasma that had been drawn after an overnight fast and frozen at −80°C until assayed. Fasting insulin (DIAsource, Belgium) concentration was measured by enzyme-linked immunosorbent assay according to manufacturer guidelines.

Insulin resistance: We used the standalone version of the Excel spreadsheet implementation of the Homeostatic model assessment calculator - HOMA **Calculator** ©The University of Oxford 2013; the calculator uses the HOMA2 model that provides insulin sensitivity (HOMA2%S) and beta cell function (HOMA2%B) as percentage, where 100% is normal. This updated model accounts for variations in peripheral glucose and hepatic resistance and considers renal glucose loss too (30). Hence can be used in hyperglycemic subjects and in subjects with high insulin section (31).

Oxidative stress: Malondialdehyde (MDA) was measured using TBARS ELISA kit according to manufacturer guidelines.

Cardiac autonomic function: After 10 minutes of rest and ensuring thermoneutral temperature (25^0C), we recorded lead II ECG of the participants using BIOPAC MP-36 in supine position for five minutes , then, we asked the participant to stand (<3 sec) and we recorded lead II ECG along with blood pressure (measured continuously) during standing. Following which, we guided the participant to take deep and paced inhalation (5 cycles) and exhalation (5 cycles). We recorded baseline blood pressure and blood pressure during isometric maneuver (using isometric handgrip, we asked the subject to grip the dynamometer for 4 minutes with 30% of their maximum voluntary contraction. The RR interval from supine lead II ECG was extracted and short-term heart rate variability was analyzed using kubios software version 2. We followed recommendations from Task force report (32) for recording and analyzing HRV. The parasympathetic indices, 30:15 ratio and expiratory: inspiratory ratio (E: I ratio) were calculated using RR interval from lying to stand

and deep breathing test respectively. The sympathetic index was calculated by subtracting the baseline diastolic blood pressure and diastolic blood pressure during the isometric grip maneuver.

Results:

MDA: We observed the following order for malondialdehyde Diabetes > Prediabetes >FDR of T2DM> control group

Insulin (ANOVA): There was a trend towards increase in insulin levels as we move along the glycaemic spectrum from control, FDR of diabetes, prediabetes and diabetes. However, statistically the insulin was comparable between control and FDR of diabetes ($p = .675$) and between prediabetes and diabetes ($p = .085$). insulin was significantly higher in prediabetes and diabetes as compared to control ($p = .003$, $p < .001$) and FDR of diabetes ($p = .012$, $p < .001$).

Insulin resistance: There was a trend towards increase in insulin resistance as we move along the glycaemic spectrum from control, FDR of diabetes, prediabetes and diabetes. However, statistically the insulin resistance was comparable between control and FDR of diabetes ($p = .431$) and between prediabetes and diabetes ($p = .388$). Insulin resistance was significantly higher in prediabetes and diabetes as compared to control ($p < .001$ $p < .001$) and FDR of diabetes ($p = .001$, $p < .001$).

Cardiac autonomic function test: We observed parasympathetic tone and reactivity test variables in the following order Control group>FDR of T2DM > Prediabetes> Diabetes. We observed sympathetic tone and reactivity test variables in the following order DM>Prediabetes> FDR of T2DM> Control group

International Physical activity questionnaire and Framingham risk score:

Almost most of the individuals across the group reported low level of physical activity.

Prevalence of the 10 year risk for developing CVD event across the complete glycemic spectrum. The risk of CVD event in 10 years were in the following order DM>PD>FDR>Control group.

DISCUSSION

In the present study, we found the presence alterations in vascular homeostasis in diabetes and prediabetes than FDR of T2DM and control group which could be due to insulin resistant state in which there is an impaired endothelial insulin signaling due down regulation of IRS-1 and IRS-2(33-35). The insulin resistance observed across the glycemic spectrum could be attributed to obesity (increased body fat percentage) (36). Though, the site of the fat would play an important role as visceral fat has implications for T2DM, than other fat depots (subcutaneous, ectopic), in the form of insulin resistance, metabolic derangements and glucose intolerance (37). Our study demonstrated that although along with age and gender distribution matching, subjects were matched for BMI, however, there was significantly higher body fat percentage in FDR of T2DM, prediabetes and diabetes than controls Our study finding corroborates with previous studies which also found that prediabetes and diabetes have lesser lean mass and higher adiposity especially centripetal in distribution (37, 38). We observed no significant difference in somatotype category between the groups and most of them were endomorphic in nature irrespective of the group might be due to the sedentary nature of the groups.

Available evidence reported that visceral fat accumulation ensues early in pathogenesis of diabetes due to environmental and genetic factors resulting in increased concentrations of NEFA (non- esterified fatty acids) or inflammatory adipokines to liver that alters reactivity in the autonomic nervous system marked by higher sympathetic nervous system (SNS) reactivity and/or reduced parasympathetic nervous system (PNS) reactivity and leads to higher insulin resistance (39). Another study documented that, diabetes progression initially causes autonomic alteration that leads to altered body fat distribution leading to Insulin resistance (40). This In our study, we observed higher body fat percentage, altered autonomic balance and insulin resistance demonstrating that they are interrelated.

Moreover, there may be functional changes in adipose tissue(41) marked by dyslipidemia and decreased adiponectin levels in FDR of T2DM, Prediabetes and Diabetes. Adiponectin is endogenous insulin sensitizer and reduced concentrations of adiponectin can initiate insulin resistance, oxidative stress (42) and increase the risk for cardiovascular event (43). Leptin is influenced predominantly by weight and not by blood glucose or insulin levels(44). We observed that leptin levels were significantly lower in control group as compared to other

groups as that of body fat percentage.

Inflammatory markers such as TNF alpha and IL6 were normal in FDR of diabetes groups. Hence, we hypothesize that hyperglycemic status is required for increase in inflammatory markers.

Vascular endothelial function as indicated by FMD started to decrease only after prediabetes in the glycemic spectrum. Similarly increase in levels of VEGF an angiogenic factor and vWF was observed only after prediabetes group. Which places prediabetes and diabetes group at higher level of risk for cardiovascular disease. However, Framingham risk score gives significant risk only for diabetes group but not for prediabetes group. Even between prediabetes and diabetes group we observed that oxidative stress (MDA) and IL6 was significantly higher in diabetes group where in prediabetes group the values were comparable with that of control group.

Our study also found increased circulating levels of telomerase level is in the following order DM>Prediabetes>FDR of T2DM>Control group which could be the significant outcome of vascular endothelial cell senescence. This derangements in the physiological level of telomerase may be due to insulin resistance (45) or hyperglycemia induced oxidative stress or vice versa decrease in telomere length may lead to early destruction of beta cells of pancreatic tissue leading to hyperglycemic status.

Conclusion:

To summarize, except for increase in body fat percentage, leptin, and endothelin 1 and decrease in adiponectin FDR of diabetes and control groups were comparable. Derangement in vascular function (FMD, VEGF, vWF), cardiac autonomic function, insulin resistance, inflammation (TNF alpha) and telomerase levels start from prediabetes group and increases further in diabetes group.

List of Publications and conference presentations:

Publications:

- **Heart rate variability non-linear analysis by Poincare plot in the complete glycemic spectrum, IJPHRD, 2018**
- **Association of TNF-α with fasting glucose, insulin and insulin resistance in complete glycemic spectrum IJPHRD, 2018**

Presentations:

1. **RSSDI, 2017Odisha, Bhubaneswar**
2. **APTCON 2018, Madhurandhagam, Chennai**
3. **ISARCON 2018, JIPMER, Puducherry**

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