



A STUDY ON “ENDOTHELIAL DYSFUNCTION IN PRE DIABETES AS EVIDENCED BY FLOW MEDIATED VASODILATATION”

KEYWORDS

CHD → Coronary Heart Disease, DM → Diabetes Mellitus, ED → Endothelial Dysfunction, EDRF → Endothelin Derived Releasing Factor, FBS → Fasting Blood Sugar, FMD → Flow Mediated Dilatation, NO → Nitric Oxide.

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ABSTRACT

BACKGROUND: Endothelial dysfunction is an early event in atherosclerosis and is known to appear long before the formation of structural atherosclerotic changes. Assessment of endothelial function, thus, can provide valuable insight into pre-invasive phase of atherosclerosis and can be used as an early marker of future Atherosclerotic disease. Flow mediated dilation (FMD) is known to depend on ability of the endothelium to release NO in response to shear stress and can be used reliably as an estimate of endothelial function in various disease states. Pre diabetic state characterized by impaired glucose tolerance leads to endothelial dysfunction through insulin resistance state, implicating the necessity for identification of endothelial dysfunction at an early stage and treat it.

AIM OF STUDY: To study the occurrence of endothelial dysfunction in pre diabetics and to correlate various risk factor in pre diabetics with endothelial dysfunction.

METHODOLOGY: Endothelial function was assessed non-invasively by high resolution Duplex Doppler Ultrasound of Brachial Artery in fifty pre diabetics cases who were included based on impaired fasting glucose (IFG). FMD was calculated as percentage increase in brachial artery diameter in response to increase in brachial artery flow.

RESULTS: In this study endothelial dysfunction is observed in 3(6%) out of 50 pre diabetics, out of them 2(4.65%) being male and 1(14.28%) being female. Among these pre diabetic individuals endothelial dysfunction occurred in individuals who have greater BMI ($p < 0.017$); HbA1c $> 6\%$ ($p < 0.001$) and males with greater waist/hip ratio ($p < 0.001$).

CONCLUSION: Endothelial dysfunction as assessed by FMD is also documented in pre diabetic state. Its occurrence is in correlation with risk factors such as obesity, higher HbA1c at time of diagnosis.

INTRODUCTION

Insulin resistance is a central pathogenic feature of pre diabetes, the incidence of which is rising substantially. The principal cause of end organ damage in pre diabetes premature cardiovascular atherosclerosis and patients with pre diabetes have a similar risk of a fatal cardiovascular events as non diabetic patients who have sustained a myocardial infarction. It was recently demonstrated that despite contemporary therapies, patients with pre diabetes who have sustained an acute myocardial infarction have not benefited from the improvements in outcome seen in non-diabetic patients over the last ten years¹. This finding highlights the need for better understanding of the pathophysiological mechanisms underlying the progressive vasculopathy seen in insulin resistant individuals.

Pre diabetes is characterized by a long period of insulin resistance during which a compensatory increase in pancreatic cell function maintains normglycaemia at the expense of fasting and post-prandial hyperinsulinaemia² which later advances in to increased fasting plasma glucose state. Insulin resistance per se is now well established as an independent risk factor for the development of cardiovascular atherosclerosis^{3; 4} and pre diabetes⁵. This suggests that future therapies should be targeted earlier at the mild end of the spectrum of insulin resistance. The importance of unravelling the mechanistic link between early insulin resistance and accelerated atherosclerosis is underscored by the recent finding that even in adolescence, mild insulin resistance is associated with increased cardiovascular risk⁶ to date, studies have predominantly examined endothelial dysfunction in type 2 diabetics of severe insulin resistance or type present study, was targeted against endothelial dysfunction in pre diabetics.

A hallmark and key pathophysiological step in the development of atherosclerosis is endothelial cell dysfunction⁷. The term endothelial dysfunction encompasses a range of abnormalities among which a reduction in the bioavailability of the signalling molecule nitric oxide (NO) is of particular relevance to insulin resistance/pre diabetes. Longitudinal studies have established endothelial dysfunction as an independent predictor of progressive

coronary disease⁷. The bioavailability of NO is dependent upon its production by the endothelial isoform of nitric oxide synthase (eNOS) and its inactivation by reactive oxygen species (ROS).

PREDIABETES

DEFINITION:

Prediabetic state is characterized by blood sugar level that is higher than normal, but it's not yet high enough to be classified as type 2 diabetes.

DIAGNOSTIC CRITERIA:

ADA (2011). Diabetes Care 34(Supplement 1):S11-S61.

Diagnostic method	Criteria
Impaired fasting glucose(IFG)	100-125mg/dl
IFG WHO/European Guide lines	110-125 mg/dl
Impaired glucose tolerance, 2h post (IGT)	140-199 mg/dl
HbA1c	5.7-6.4%

MATERIAL & METHODS

METHODOLOGY:

This is a cross sectional study done at Osmania General hospital, Hyderabad. Includes 50 subjects presenting to medicine and endocrinology OP with IFG(impaired fasting glucose).

Method of collection of data

Method of collection of data is by evaluation, which is done by taking detailed history, clinical examination and laboratory investigations through proforma specially designed for this study.

Inclusion criteria

1. Age 15-65 years

- 2. Both sexes.
- 3. subjects with impaired fasting glucose(IFG) i.e FPG BETWEEN 100-125 mg/dl to be diagnosed as Pre diabetes.

Exclusion criteria

- 1. Age 15 years and 65 years.
- 2. Patients who did not give consent for the study.
- 3. Diagnosed Type I and Type II Diabetes Mellitus.
- 4. Subjects with established micro and macrovascular complications.
- 5. Febrile illness
- 6. Subjects on drugs known to alter flow mediated dilatation of endothelium.

Colour Doppler ultrasonography of the brachial artery, by HELWLETT-PACKARD Image point machine using 7.5 and 10 MHz Linear probe was performed to assess FMD, which provide information regarding endothelial function.

All the patients were subjected to the following investigations before entering the study. Hb, CBP, ESR, FPG, complete urine examination, Blood urea and Serum creatinine, Lipid profile, ECG, Colour Doppler ultrasonography of the brachial artery, by HELWLETT-PACKARD Image point machine with colour Doppler using 7.5 and 10 MHz linear probe.

Observations & Results

Table 1: DISTRIBUTION OF SUBJECTS BY AGE AND SEX

Age	Sex		Total
	Male	Female	
15-25years	1	0	1
25-35years	11	2	13
35-45years	19	3	22
45-55years	12	2	14
55-65years	0	0	0
Total	43	7	50

- In this study it is observed that majority of males(19) (44.18%) are in the age group of 35-45 years.
- females (3)(42.85%) are in the age group 35-45 years.
- Mean age in males is (39.88±7.58) and mean age among female subjects is (38.428±6.28).

Graph 1: Distribution of subjects by age and sex

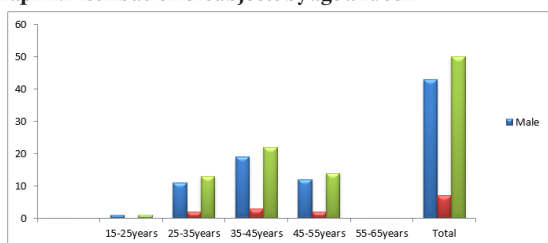
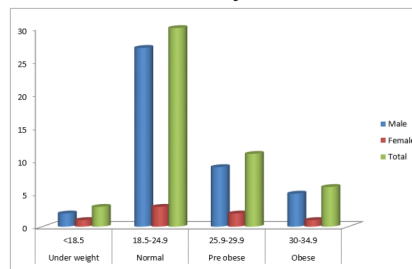


Table 2: DISTRIBUTION OF SUBJECTS BASED ON BMI

Weight categories	BMI	Male	Female	Total
Under weight	<18.5	2	1	3
Normal	18.5-24.9	27	3	30
Pre obese	25.9-29.9	9	2	11
Obese	30-34.9	5	1	6

Graph 2: DISTRIBUTION OF SUBJECTS BASED ON BMI



In this study both males 27 (62.7%) and females 3 (42.85%) have their major subject distribution in normal BMI range.

- Among males 9(20.93 %) and females 2(28.57%) are pre obese
- 5(11.63%) males and one female (14.28%) are obese
- Mean BMI and S.D(24.3±4.23)

STATISTICAL SIGNIFICANCE:

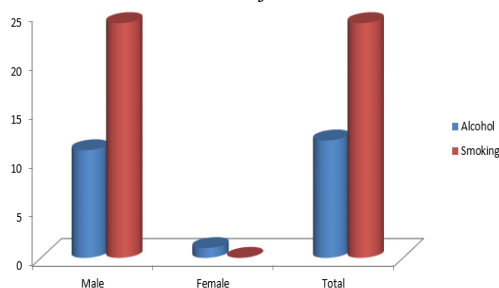
BMI between (25.9 -34.9) is a strong predictor for occurrence of endothelial dysfunction

P value 0.017%

Table 3: DISTRIBUTION OF SUBJECTS BY PERSONAL HABITS

	Male	Female	Total
Alcohol	11	1	12
Smoking	24	0	24

Table 3: DISTRIBUTION OF SUBJECTS BY PERSONAL HABITS



- In this study 24(55.81%)are smokers and all are males.
- Among alcoholics 11 are males one is female.

STATISTICAL SIGNIFICANCE:

- Smoking is associated with significant endothelial dysfunction with

P value 0.0504

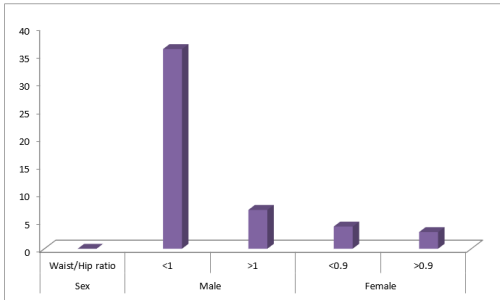
- Alcohol intake is not associated with endothelial dysfunction

P value 0.124

Table 4: DISTRIBUTION BASED ON WAIST/HIP RATIO

Sex	Waist/Hip ratio	Total
Male	<1	36
	>1	7
Female	<0.9	4
	>0.9	3

Graph 4: DISTRIBUTION BASED ON WAIST/HIP RATIO



- In this study 16.2% of males have waist/hip ratio >1
- 42.87% females have waist/hip ratio >0.9
- STATISTICAL SIGNIFICANCE:
- Waist / hip ratio >1 among MALES is a significant contributor to endothelial dysfunction. THIS ASSOCIATION IS NOT SEEN IN FEMALES

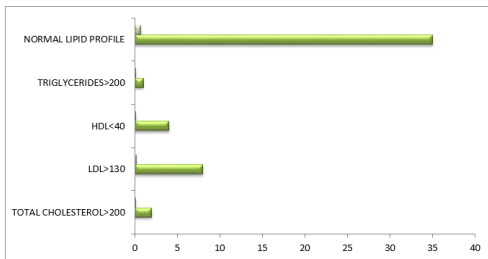
P value 0.001 in males
P value 0.212 in females

Hence pre diabetic MALES with WAIST/HIP>1 has HIGHER RISK of developing ENDOTHELIAL DYSFUNCTION

Table 5: DISTURBUTION BASED ON ABNORMAL LIPID PROFILE

Abnormal lipid profile	no.of subjects	%
TOTAL CHOLESTEROL>200	2	4%
LDL>130	8	16%
HDL<40	4	8%
TRIGLYCERIDES>200	1	2%
NORMAL LIPID PROFILE	35	70%

Graph 5: DISTRIBUTION OF SUBJECTS BASED ON LIPID PROFILE



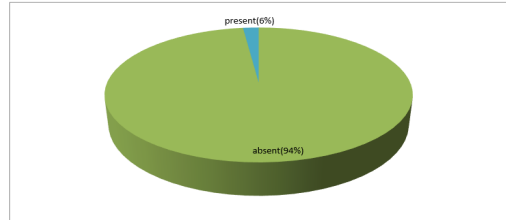
- In this study total cholesterol > 200mg/dl is seen in 2(4%)
- LDL > 130mg/dl is seen in 8(16%) with MEAN±S.D(106.8±22.3)
- HDL < 40 mg/dl is seen in 4(8%) with MEAN±S.D(51.68±)
- Triglycerides > 200mg/dl is seen in one subject with MEAN ±S.D(2%)
- Majority have their lipid profile normal 35(70%).
- STATISTICAL SIGNIFICANCE:

LDL >130 mg/dl ; P value <0.0023
HDL <40 mg/dl ;P value <0.014
TRIGLYCERIDES .200mg/dl;P value<0.213

Table 6 :DISTRIBUTION OF SUBJECTS BASE ON ENDOTHELIAL DYSFUNCTION

ENDOTHELIAL DYSFUNCTION FMD>4.5%	No.of subjects	total %
absent >4.5	47	94%
present<4.5	3	6%
Total	50	100%

Graph 6: DISTRIBUTION OF SUBJECTS BASED ON ENDOTHELIAL

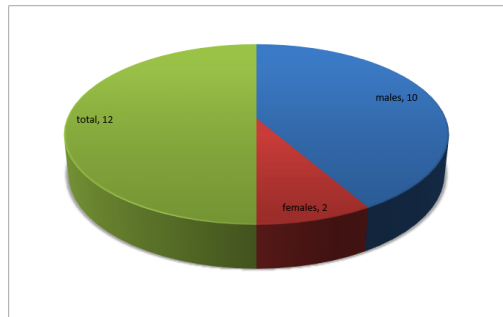


- Endothelial dysfunction is observed in 6% subjects

Table 7: DISTRIBUTION OF SUBJECTS BASED ON HbA1c

HbA1c	males	females	Total
>6%	10	2	12
<6%	33	5	38

Graph 7: DISTRIBUTION OF SUBJECTS BASED ON HbA1c



- Out of 12 subjects with HbA1c >6% endothelial dysfunction is noted in 3(25%)
- 33 subjects have their HbA1c <6%
- STATISTICAL SIGNIFICANCE:
- HbA1c >6% is significantly associated with endothelial dysfunction with

P value 0.001

TABLE 8 : DISTRIBUTION OF SUBJECTS BY ECG

Abnormal ECG	No.of subjects	Total
ISCHEMIA	2	2
INFARCTION	0	0

- Out of 50 pre diabetics ischemia is noted in 2(4%) and out of this one has endothelial dysfunction.
- STATISTICAL SIGNIFICANCE:

P value<0.078

Endothelial dysfunction is not significantly correlated with ischemic changes on ECG

TABLE 9: DISTRIBUTION OF ENDOTHELIAL DYSFUNCTION BY VARIOUS RISK FACTORS

Risk factors	FMD<4.5%(n=3)	FMD>4.5%(n=43)	p value
1.Age	40.3±6.72	39.7±7.13	<0.217
2.Sex	males(4.65%) females(14.85%)	males(81.3%) females(85.71%)	<0.017
3.Smoking	2(8.33%)	22(<0.050
4.Alcohol	0	0	<0.124
5.BMI			
Pre obese	27.5	27.64	<0.067
Obese	(33.15±3.16)	(30.8±3.9)	<0.001
6.Abnormal lipid profile			
High LDL	(133.99±01.09)	(102.4±38.07)	<0.023
Low HDL	(38±10.13)	(51.58±7.98)	<0.014
High triglycerides	(169±28.9)	(155.4±27.03)	<0.213
7.Severity by ECG	1	0	<0.078
8.Abnormal waist/hip ratio	males(1.25±0.32) females(1.0±0.28)	males(0.82±0.45) females(0.76±0.32)	males<0.001 females<0.212

TABLE 10: MEASURED PARAMETERS OF FMD IN PRE DIABETIC SUBJECTS

STUDY VARIABLES	MEAN	STANDARD DEVIATION	NORMAL VALUES
Base line diameter	3.8	0.51	3.76±0.54
Base line flow	645	218.2	631.62±222.41
Reactive hyperemic flow	890.1	366.7	780.58±356.57
Hyperemic flow %	81.65	74.94	122.39±55.39
FMD %	10.38	10.53	17.12±10.13

- Hyperemic flow % and FMD% were significantly altered in pre diabetics

DISCUSSION

Age and sex:

Richard P. Donahue et al⁸(The Western New York Study;study done on sex difference in correlation with endothelial dysfunction), they have noticed that females pre diabetes have higher chances of having endothelial dysfunction than their male counter parts ,this results are consistent with present study with more females(14.85%) with endothelial dysfunction than males(4.8%)⁸.

Alok k Gupta et al ;in their study on endothelial dysfunction with pre diabetes the mean age distribution of subjects population is(36.8±19.7) comparable with the present study with its subjects having a mean age of (39.88±7.58)⁹

Smoking and alcohol intake:

Vita et al found no significant association between smoking and endothelial dysfunction but in this study pre diabetics who are smokers have significant risk of developing endothelial dysfunction in future.

HbA1c:

Fathima Eliana et al in their study has shown that endothelial dysfunction is significantly related to HbA1c >6% ,in this study HbA1c >6% has significant correlation with endothelial dysfunction with p value <0.001¹⁰

BMI(BODY MASS INDEX):

Obese individuals with mean±S.D (33.15±.16) in this study is similar with the distribution of obese subjects in Alok k Gupta et al study

with mean(35.3±4.3) and occurrence of endothelial dysfunction was more noted in pre diabetics with obesity (p value<0.001) consistent with the present study (p value<0.001)

Waist /Hip ratio:

In the study by Alok k et al pre diabetics with endothelial dysfunction has higher waist circumference with waist /hip ratio>1 and in this study endothelial dysfunction is noted in men with higher waist /hip ratio with p value<0.0019.

Lipid profile:

A case control study by Maciej et al(2009) in Poland with male subjects aged less than 45 years and Fathima Eliana et al¹⁰ observed that FMD value correlated with pre diabetes but had no association with, hypercholesterolemia and smoking, this finding is not consistent with this study were LDL >130mg/dl and HDL<40 mg/dl has significant correlation with occurrence of endothelial dysfunction(p value<0.001).

CONCLUSION

In this study out of 50 pre diabetics, endothelial dysfunction was seen in 6% subjects. Endothelial dysfunction was equal among both males (2) and females (1). With comparable mean age group between, in male (39.88±7.58) and in female (38.72±6.12). Risk factors associated with endothelial dysfunction are:

- BMI in PRE OBESE and OBESE RANGE is a strong predictors of endothelial dysfunction both in males and females.
- Waist/hip ratio > 1 among males is a risk factor for pre diabetics to have complications in future.
- Abnormal lipid profile with LDL >130 mg/dl and HDL<40 mg/dl is also correlated with FMD <4.5%
- HbA1c > 6% strongly associated with occurrence of endothelial dysfunction.
- Smoking has significant correlation.
- Alcohol intake is not related to the development of endothelial dysfunction.

SUMMARY

This study is conducted in Osmania General Hospital. This is a cross sectional study, 50 pre diabetic individuals based on impaired fasting glucose are included in the study. Various risk factors of atherosclerosis in pre diabetics with endothelial dysfunction were correlated. In this study endothelial dysfunction was assessed non invasively (by high resolution Duplex Doppler Ultrasound of Brachial Artery) in 50 pre diabetics 43 male and 7 female.

Pre Diabetics who presented had 24 were smokers and 11 were alcoholics. Majority of pre diabetics have normal body weight and 9 pre-obese and 5 obese . Among pre diabetics abnormal Waist/hip ratio was more common in females (3/7) compared to males (7/43). Lipid profile in pre diabetics showed majority with abnormal lipid profile had high LDL 8(16%) followed by Low HDL 4(8%).

In this study it is observed that among the 50 pre diabetics that endothelial dysfunction was (FMD <4.5%) was present among 3(6%) subjects.

Lumen diameter (LD) of the brachial artery was measured at baseline, during reactive hyperemia (immediate after release of arterial compression) and again at rest (after 1 min) was measured. Flow mediated dilation (FMD %) was significantly decreased in pre diabetics with endothelial dysfunction when compared to normal standard values.

Hyperemic flow percentage in pre diabetics, which was found to be lower than normal. However, there was no significant difference in the brachial artery diameter and calculated brachial artery flow at baseline in pre diabetic and healthy subjects.

The other risk factors associated with endothelial dysfunction are smoking, higher HbA1c at the time of diagnosis.

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