Atherogenic Response in Diabetic Patients Due to Increased BMI in Haryana Population - A Randomized Trial.
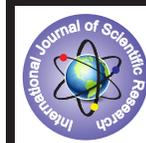
Parveen Kumar Vats	Department of Zoology, Pt. C.L.S. Government College, Karnal-132001, Haryana, India
*Jitendra Kumar Sharma	Department of Biotechnology, Pt. C.L.S. Government College, Karnal-132001, Haryana, India. * Corresponding author
Ranjeet Singh	Department of Botany, Pt. C.L.S. Government College, Karnal-132001, Haryana, India
Mohan Lal Bansal	Department of Zoology, Pt. C.L.S. Government College, Karnal-132001, Haryana, India

ABSTRACT
Diabetes is a major health problem worldwide which contributes to physical health and economic burden on society. Obesity is positively associated with diabetes, as a small increase in body fat leads to development of diabetes. In the present study, we aimed to examine the correlation between level of obesity and their effects on plasma lipids, lipoproteins and fasting plasma glucose (FPG) in diabetic patients. A total 187 subjects, out of which 88 were diabetic patients, aged between 30-95 years, and 99 healthy subjects, aged between 27-88 years, from the north Indian state Haryana were included in this study. They were classified into non-obese, overweight and obese groups on the basis of BMI. FPG, TC, HDL-C, LDL, VLDL-C and triglycerides were assessed. Biochemical changes between the DM-II and control groups were compared by using independent sample t-test (2-tailed) and to check their association among themselves, Pearson's correlation test was performed. The levels of FPG and BMI of DM-II and control non-obese, overweight and obese subjects were compared and highly significant difference was observed among them. Significant positive correlation was found between BMI and triglyceride(r= 0.396) in non-obese DM-II patients, while in control non-obese subjects, it was positively correlated (r=0.159) but not significant. From our study, we concluded that obesity alone cannot be considered as the predictor for severity or risk of developing diabetes. Level of lipid components and FPG were also found to be independent of BMI.*

Introduction:

Diabetic mellitus type 2 (DM-II) is a major health problem globally, and contributes to a major portion of DALYs (Disability Adjusted Life Years) in humans (Kumar D., and Mukherjee, K 2014). DM-II has direct clinical effect on patients and is a great burden on society, both physically and economically. Manifestation of DM-II and other metabolic disorders such as coronary heart disease, ischemic stroke, and certain type of cancers are positively associated with obesity (Tesfaye *et al.*, 2007, Sharma J.K. *et al. 2014*). A small increase in body fat can increase the risk of developing DM-II up to many folds and impairment of overall health. It subsequently leads to insulin resistance and elevation of several other toxic metabolites in the body (Arora *et al.* 2007). Based on WHO report (1997), central obesity is more common in South Asians in terms of waist circumference and elevated Body Mass Index (BMI). For measurement of obesity, different methods have been used such as body mass index, size of fat cells and their number, waist-hip ratio or skin fold thickness, and body density (Mukhyaprana *et al.*, 2004, Brunzell J. D. *et al.*, 1983, Bray G.A. *et al.*, 1985, Kraemer H. *et al.*, 1990). Among all these measures, Body mass index has gained popularity as a better measure for adiposity due to its simplicity and convenience (Must *et al.*, 1991).

In the present study, we aimed to examine the correlation between level of obesity and their effects on plasma lipid, lipoproteins and fasting plasma glucose (FPG) in diabetic patients.

Subjects and Methods:

A total 88 diabetic patients, aged between 30-95 years, and 99 healthy subjects, aged between 27-88 years, from the north Indian state Haryana were included in this study. In the present study, diabetic patients (previously diagnosed) with FPG level ≥110mg/dL were classified as DM-II group. Patients who were dependent on insulin and suffered from other diseases were excluded from this study.

A Participant Information Sheet (PIS) was explained to subjects and Informed Consent Form (ICF) duly signed by subjects were collected. Information regarding age, sex, history of diabetes were recorded. Height and weight of all subjects were measured by standard technique. BMI was calculated as the ratio of weight (Kg) divided by height (m^2)

$$BMI = \frac{Weight\ (in\ kg)}{Height\ (in\ square\ meter)}$$

On the basis of BMI, DM-II and control subjects were categorized into 3 categories (Mukhyaprana *et al.*, 2004):

1) Non- Obese (BMI<25 Kg/m²),
2) Overweight (BMI 25-29.9 Kg/m²) and
3) Obese (BMI> 30 Kg/m²).

Based on the above categories, the number in DM-II patient group was non-obese (n=34), overweight (n=39) and obese (n=15). Similarly, in control subjects, non-obese (n= 38), overweight (n=44), and obese (n=17). Overnight fasting blood sample from each subject was collected next morning in EDTA containing vial for estimation of plasma glucose and lipid profile. FPG was estimated by using glucose oxidase peroxidase method (Raabo *et al.*, 1960). Total cholesterol (TC) and Triglycerides were determined by using standard enzymatic methods. High density lipoprotein cholesterol (HDL-C) was determined in supernatant by precipitating apolipoprotein B (LDL and VLDL) containing lipoproteins using phosphotungstate in presence of magnesium ion. Low density lipoprotein cholesterol (LDL-C) was calculated by using Friedewald formula for those with triglycerides <400 mg/L (Friedewald *et al.*, 1972). For triglycerides values less than 400 mg/L, following equation was used:

$$LDLC\ (\frac{mg}{dL}) = TC - (HDLC + TGL/5)$$

The data generated was analyzed statistically using SPSS statistical software Version 20. To compare the biochemical changes between the DM-II and control groups, independent sample t-test (2-tailed) was used. To evaluate various correlations, Pearson's correlation test was performed and P value at <0.05 confidence

level was considered as significant.

Results:
Data for total of 88 DM-II patients and 99 control subjects were analyzed. DM-II group had 51 males and 37 female patients, while control group included 60 males and 39 female subjects.

The mean ± SEM age of non-obese DM-II patients and control subjects was 54.76 ± 2.39 and 51.97± 2.54 respectively. In overweight and obese DM-II group, mean age was 53.00 ± 1.50 and 52.07 ± 2.32 respectively. In control group, overweight and obese subjects, mean age was 48.73 ± 1.79 and 50.53 ± 3.28 respectively (Table1).

The mean ± SEM BMI of non-obese DM-II patients and control subjects was 22.52 ± 0.33 and 22.71 ± 0.26 respectively. In overweight and obese DM-II patients mean BMI was 27.68 ± 0.22 and 33.31 ± 1.25 respectively. In control overweight and obese subjects mean BMI was 26.93 ± 0.18 and 33.90 ±1.01 respectively (Table1).

In DM-II non-obese, overweight and obese patients, mean ± SEM of FPG were 188.49±16.55, 153.04±7.74 and 182.91 ± 11.71 mg/dL respectively. While in control non-obese, overweight and obese subjects FPG were 91.70 ± 2.86, 87.68 ± 1.85, and 105.11 ± 11.25 respectively (Table1).

The level of total cholesterol (mean ± SEM) in non-obese, overweight and obese DM-II patients was found to be 214.93 ± 10.68, 190.88 ± 8.04 and 201.31 ± 7.36 respectively. In non-obese, overweight and obese subjects, the level was found to be 187.95 ± 8.06, 184.22 ± 5.98 and, 199.38 ± 8.16 respectively (Table 1).

The mean ± SEM of triglycerides in non-obese DM-II patients and control subjects was 216.03 ± 10.52 and, 151.06 ± 10.89 respectively. In overweight and obese DM-II patients, mean triglycerides was 190.58 ± 12.19 and 214.28 ± 19.45 mg/dL respectively. In control overweight and obese subjects, mean triglycerides was 183.24 ± 9.97 and 160.11 ± 13.71 respectively (Table 1).

In DM-II group, non-obese, overweight and obese patients, mean ± SEM of HDL-C, was found to be 42.06 ± 0.81, 41.64 ± 0.893 and 43.60 ± 1.41 mg/dL respectively. While in control non-obese, overweight and obese subjects HDL-C were 44.45 ± 0.68, 44.07 ± 0.74, and 43.76 ± 1.18 respectively (Table1).

The mean ± SEM values of LDL-C in non-obese DM-II and control, overweight DM-II and control, obese DM-II and control subjects were 133.90 ± 8.47 and 112.99 ± 7.37, 114.34 ± 7.30 and 100.87 ± 5.74, and 116.68 ± 9.22 and 123.59± 7.72 respectively (Table 1).

The mean values of BMI for non-obese, overweight and obese DM-II patients were compared with that of non-obese, overweight and obese DM-II control subjects; significant difference was observed between overweight DM-II and control subjects (Table1).

The levels of FPG of DM-II and control non-obese, overweight and obese subjects were compared and highly significant difference was observed among them (Table 1).

It was observed that the mean level of total cholesterol in non-obese DM-II patients was significantly higher than non-obese controls. Level of triglycerides in overweight and obese diabetic patients was observed to be slightly higher (190.58 ± 12.19) than overweight and obese control, but these differences were not statically significant (Table 1).

The levels of HDL-C in non-obese, overweight and obese DM-II

were slightly lower than non-obese, overweight and obese controls. Statically significant difference was observed between diabetic non-obese and control non-obese, diabetic overweight and control overweight subjects (Table 1).

In diabetic non-obese and overweight subjects, mean values for LDL-C were slightly higher than non-obese and overweight controls, while mean values for LDL-C were lower in obese diabetic patients than obese control subjects, but these difference were not statically significant (Table 1).

Significant difference was observed in mean level of VLDL-C of non-obese DM-II and non-obese controls, although mean values of obese DM-II were slightly higher than control obese subjects, but difference were not statically significant.

Table 2 shows the significant positive correlation between BMI and triglycerides (r= 0.396) in non-obese DM-II patients while in control non-obese subjects it was positively correlated (r = 0.159), although not significant. FPG was significantly correlated with BMI (r = 0.640**) in obese control subjects. Non-significant positive correlation between BMI and TC were found in overweight (r = 0.089) and control subjects (r = 0.119). HDL-C is non-significantly, negatively correlated with BMI in non-obese, overweight and obese DM-II subjects, while in controls, except obese subjects, non-obese and overweight subjects, HDL-C was positively correlated with BMI. In obese control subjects, BMI has been found to be positively correlated with HDL-C.

Discussion:
The present study has demonstrated comparative correlation between level of obesity (BMI) and their effects on plasma lipid, lipoproteins and FPG in non-obese, overweight and obese diabetic patients. Hypertriglyceridemia is the most common lipid abnormalities in diabetes regardless of cholesterol level (Al-Nuaim AR *et al.* 1995). In our study, when we compared the lipid profile in non-obese overweight and obese subjects, they showed significant increase in triglycerides and total cholesterol in DM-II than of controls, while level of HDL-C were found to be decreased in DM-II. These results were consistent with other studies (El Hazmi MA 1999, Khoja A 1999, Naheed T 2004, Lee WRW 2001, Sindelka G 2002). Some earlier studies have been shown that increased triglycerides and low HDL-C in DM-II are independent on the degree of obesity (Ronnemaa T. *et al.,* 1989, Laakso M. *et al.,* 1985), but our study is not consistent with their results, which might be due to biological variations between the sample populations and environmental effects. However, in an earlier study (Laakso M & Pyrola, 1990), it has been stated that obesity effects on serum lipid and lipoproteins were more pronounced in diabetics than the same in non-diabetics, which is consistent with our present results.

Interestingly, according to Dudekula A.B. & coworkers (2012), obesity is uncommon among the DM-II patients in Indian population as compared with western population. In our study FPG was significantly correlated with BMI (r = 0.640**) in obese control subjects, but in diabetic non-obese, overweight and obese subjects no correlation was found, which might be due to intake of hypoglycemic agents or diet management by patients.

Conclusions:
From our study we conclude that obesity cannot be considered as the sole predictor for severity or risk of developing diabetes. Also, the level of lipid components and FPG have been found to be independent of BMI.

Acknowledgements:

Table: 1 Distribution of mean values and standard error of mean of age, BMI, FBG and four lipid components in non-obese, overweight and obese diabetic mellitus 2 and control subjects.

	NON OBESE			OVER WEIGHT			OBESE		
	DM-II	Control	t-value	DM-II	Control	t-value	DM-II	Control	t-value
Age (Years)	54.76±2.39	51.97±2.54	0.794	53.00±1.50	48.73±1.79	1.799	52.07±2.32	50.53±3.28	0.373
Body Mass Index (Kg/m²)	22.52±0.33	22.71±0.26	-0.466	27.68±0.22	26.93±0.18	2.661**	33.31±1.25	33.90±1.01	-0.373
Fasting Blood Glucose (mg/dL)	188.49±16.55	91.70±2.86	6.076**	153.04±7.74	87.68±1.85	8.669**	182.91±11.71	105.11±11.25	4.782**
Cholesterol (mg/dL)	214.93±10.68	187.95±8.06	2.041*	190.88±8.04	184.22±5.98	0.674	201.31±7.36	199.38±8.16	0.174
Triglycerides (mg/dL)	216.03±10.52	151.06±10.89	4.268**	190.58±12.19	183.24±9.97	0.470	214.28±19.45	160.11±13.71	2.333*
HDL cholesterol (mg/dL)	42.06±0.81	44.45±0.68	-2.268*	41.64±0.893	44.07±0.74	-2.109*	43.60±1.41	43.76±1.18	-0.090
LDL cholesterol (mg/dL)	133.90±8.47	112.99±7.37	1.870	114.34±7.30	100.87±5.74	1.465	116.68±9.22	123.59±7.72	-0.579
VLDL cholesterol (mg/dL)	38.96±3.22	30.21±2.18	2.284**	34.90±2.34	41.75±4.11	-1.400	41.03±6.09	32.02±2.74	1.403

*p≤ 0.05 and, **p≤ 0.01

Table 2: Analysis of correlation coefficient between BMI, FBG and Lipid Profile.

	Non-Obese		Overweight		Obese	
	DM-II	Control	DM-II	Control	DM-II	Control
Fasting Blood Glucose (mg/dL)	-0.018	-0.028	-0.134	0.251	-0.030	0.640**
Cholesterol (mg/dL)	-0.127	-0.051	-0.015	0.089	-0.251	0.119
Triglycerides (mg/dL)	0.396*	0.159	-0.275	0.077	-0.098	0.315
HDL cholesterol (mg/dL)	-0.289	0.204	-0.081	0.061	-0.060	-0.075
LDL cholesterol (mg/dL)	-0.166	-0.127	0.119	0.037	-0.137	0.025
VLDL cholesterol (mg/dL)	0.329	0.159	-0.393*	0.004	-0.082	0.315

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

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