Original Article

Hyperinsulinaemia Insulin Resistance and Cardiometabolic Risk Profile in Siblings of Type 2 Diabetics

Seemeen Hussain,[1] **Muhammad Aasim,**[2] **Iram Hussain,**[3] **Saira Bashir**[4]

Abstract

Objective: To compare non-diabetic siblings of Type 2 diabetics for insulin resistance, hyperinsulinaemia, dyslipidemia and biometric indices with non diabetic volunteers with no family history of diabetes.

Study Design: Cross sectional analytical study.

Place and Duration of Study: A tertiary care teaching institution during Dec.2009 to July 2010.

Methodology: Siblings of type 2 diabetics above the age of 25 years were matched as closely as possible for age, sex socioeconomic conditions with non diabetic volunteers with no family history of diabetes. Both groups were compared for fasting serum insulin, glucose, lipid levels and biometric indices using independent t-test. Insulin resistance was calculated by the Quicki (Quantitative insulin check index) method with cutoff at 0.34 on R.O.C analysis.

Results: The 106 non diabetic siblings of Type 2 diabetes mellitus (Group 1) with a mean age of 40.8 ± 12.1 years p value 0.13 were compared to 106 non diabetic volunteers (Group 2) mean age 43.5 ± 14.2 years. Out of a total of 212 participants 20 (9.4%) subjects had hyperinsulinaemia (mean serum insulin15.69 ± 6.02 µU/L $p < 0.001$) and insulin resistance (90% sensitivity CI 86 to 94, 98.96% specificity CI 97.7 to 100) and all belonged to group 1. A further comparison of the above variables was made within group 1 between 20 (9.4%) insulin resistant siblings and 86 (90.6%) non insulin resistant siblings. The insulin resistant siblings showed significant increase in HbA_1c 7.02% ± 1.5 vs 6.2% ± 1.49 $p = 0.008$, BMI 30.4 ± 6.4 kg/m^2 vs. 27.1 ± 4.7 kg / m^2 $p = 0.01$and Serum insulin levels 15.92 ± 6.11 vs. 4.57 ± 2.38 $p < 0.001$.

Conclusion: The study suggests that siblings of diabetics are a special group who have hyperinsulinemia, insulin resistance and obesity and are at high risk for developing type 2 diabetes.

Key words: Hyperinsulinaemia, insulin resistance, BMI, siblings, metabolic syndrome.

Hussain S.[1]
Associate Professor Medicine
Fatima Jinnah Medical College, Lahore

Aasim M.[2]
Statistical Officer, PMRC Research Centre, National Health Research Complex, Sheikh Zayed Hospital, Lahore

Hussain I.[3]
Bracken Ridge Hospital, Internal Medicine Department
Austin Texas 78701

Bashir S.[4]
Medical Officer PMRC
Fatima Jinnah Medical College, Lahore

Introduction

A combination of impaired insulin secretion, beta cell dysfunction and insulin resistance results in Type 2 diabetes mellitus.[1] A number of prospective and cross sectional studies have outlined the pathogenesis of type 2 diabetes mellitus. There is a suggestion that

insulin resistance is followed by compensatory hyperinsulinaemia, exhaustion of beta cells leading to impaired glucose tolerance and clinical diabetes. The interplay of genetic and environmental influences in developing Type 2 diabetes with its cluster of cardiovascular abnormalities is well recognized. Those who are genetically predisposed to developing diabetes like the Pima Indians have been well studied for specific gene mutations in comparison with people of European descent.[2] In certain populations insulin resistance is predominant, along with visceral obesity and reduced glucose tolerance. It has been demonstrated in obese Pima Indians that insulin resistance predicts the onset of Type 2 diabetes.[3] In a large prospective study of risk factors for Type 2 diabetes in middle aged British men, obesity was the single most powerful predictor of Type 2 diabetes with a 12 fold increase in relative risk between the lowest and highest BMI (< 22.9 kg/m^2 vs. > 27.9 kg/m^2).[4] In other studies hyperinsulinaemia alone may contribute to dyslipidemia, visceral adiposity, hypertension and hyperuricemia. Subjects with two or more of the above metabolic disorders constitute the metabolic syndrome as described originally by Professor Reaven.[5] In the RISC (Relationship between insulin sensitivity and cardiovascular disease) study fasting insulin has a stronger association with an adverse cardio-metabolic risk profile than insulin resistance.[6] Hyperinsulinaemia can be taken as a surrogate measure of insulin resistance.[7]

There are various methods used to measure insulin resistance. These include Hyperinsulinaemic euglycemic glucose clamp, HOMA – IR (insulin resistance homeostasis model), QUICKI (Quantitative insulin sensitivity check index method) and frequently sampled intravenous glucose tolerance test. In this study we used the QUICKI method because the results are comparable to other standard methods especially in non diabetics and it is less cumbersome to perform.[8,9]

It is established that IGT (Impaired glucose tolerance) may progress to the development of type 2 diabetes. There is an estimated conversion rate of 2 – 12% per year in different populations being roughly 10 fold higher than the incidence of NIDDM in non diabetic individuals.[10] There is very little data available on people of sub continental origin, though it is predicted that major burden of increased incidence of diabetes in future is expected in the developing world This study compared non diabetic siblings of Type 2 diabetics (Group 1) to non diabetic volunteers with no family history of diabetes mellitus (Group 2), for their insulin sensitivity and cardiometabolic risk profile. The parameters studied included fasting S. glucose; S. insulin levels lipid levels, presence of insulin resistance, glycosylated haemoglobin, blood pressure and anthropometric indices of obesity (BMI, waist, waist / hip ratio). The objective was to see the influence of family history of Type 2 diabetes on the above variables implicated in the metabolic syndrome, amongst non diabetic participants.

Subjects and Methods

A total of 246 participants were initially questioned and separated into two groups each with 106 participants on the basis of presence or absence of family history of diabetes. The initial recruitment was through patients of Type 2 diabetes mellitus attending the Diabetic outpatient clinic at Sir Ganga Ram Hospital (SGRH) Lahore They were asked to bring with them their non diabetic one male and / or female sibling above the age of 25 years to participate in the research.Group1 which included 48 (45.3%) non diabetic males and 58 (54.7%) female siblings were invited to the Pakistan Medical Research Council laboratory, located at SGRH from Dec; 2009 till July 2010. Group 2 consisted of non diabetic apparently healthy volunteers recruited through word of mouth to do the same. They were 60 (56.6%) non-diabetic males and 46 (43.4%) females matched as closely as possible with Group 1and any confounders eliminated at the onset. The researcher filled a Performa and did blood sampling at PMRC laboratory after taking written consent from all participants. The questionnaire was designed to determine age, sex, marital status, education, socioeconomic status, family history of diabetes, hypertension, ischaemic heart disease and stroke.

The inclusion criteria at the time of sampling were non-diabetic siblings of type 2 diabetics above the age of 25 years (Group 1) and non diabetic volunteers with no family history of diabetes (Group 2). The exclusion criteria were fasting S. glucose ≥ 126 mg/dl (7.0 mmol/l) and HbA_1c > 7% along with any morbid condition affecting glucose metabolism or insulin levels. Anyone with values above this criterion was considered to have Type 2 diabetes mellitus.[11] There were nine (4.2%) from amongst both groups found to have a fasting S. glucose > 126 mg/dl (7.0 mmol) and HbA_1c > 7%. They along with 25 confirmed Type 2 diabetics participated by having their blood pressure and biometric assessment along with fasting S. glucose and S. lipid analysis, but their fasting S. insulin level and

insulin sensitivity was not checked and statistical tests were not applied.

Each group underwent biometric assessment, BP measurement, blood sampling for relevant metabolic variables. The biometric variables measured included waist circumference in centimeters at the level of the umbilicus, hip at the level of greater trochanter, weight in kilograms and height in meters. These measurements were used to calculate the waist hip ratio, and the body mass index. A standard flexible measuring tape was used to measure the waist and hip to the nearest centimeter. A uniform standard weighing machine and a standiometer were used to measure the weight and height respectively. Blood pressure was measured twice and average taken using same standard mercury sphygmomanometer. Body mass index was calculated as weight in kgs divided by height in meters squared. All measurements were taken by the same person to avoid intraobservational operator error. The cut offs for obesity were taken as BMI > 25 kg/m^2, waist circumference 90 cms in men and 80 cms in females and a waist – hip ratio of 0.9 in males and 0.8 in females.[12] The mean BP was taken as < 140 / 90 mmHg.[13] The blood samples were used to measure the fasting serum glucose levels, serum insulin levels, fasting lipids and HbA_1c. Blood samples (5 ml) were collected after an overnight fast (10 – 12 hours) in all participants. The serum glucose was measured by glucose oxidase method. HbA_1c was determined using whole blood mixed with cation – exchange resin and measured calorimetrically. Serum was then isolated from the blood samples and preserved at -20°C in serum tubes for batch analysis. Serum total cholesterol concentration, serum triglycerides concentration and serum HDL – cholesterol was determined by enzymatic CHOD – PAP, (Cholesterol Oxidase phenol 4 – amino anti pyrine peroxidase) GPO – PAP and precipitation methods respectively using reagent kits from Randox, U.K. The LDL cholesterol concentration was calculated according to the Friedwald formula [LDL cholesterol (mg/l) = Total cholesterol - (Triglycerides / 5 + HDL cholesterol)]. LDL – C / HDL – C ratios were then calculated. Insulin concentrations were determined by direct immunoenzymatic colorimetric method for quantitative determination of insulin in human serum using the Eliza kit of Novo – Tec Germany.

Insulin resistance was then calculated using the quantitative insulin sensitivity check index (QUICKI method). The QUICKI method calculates the inverse of the sum of the log of fasting serum glucose levels and fasting insulin levels [1/log glucose levels + log insulin levels].[8,9]

There were no potential sources of bias. All data was entered and analyzed using SPSS 15. Data for quantitative variables was reported by mean and standard deviation and comparison between groups was made by independent sample t test, p-value less than 0.05 was significant. The same statistical test was used to compare insulin resistant siblings to non insulin resistant siblings within the same group. R.O.C. (Receiver operative characteristic curve) analysis was used to determine optimal cutoff for insulin resistance in relation to hyperinsulinaemia defined by different insulin values above and below the stated value of 9 µ units/L on the insulin kit. According to R.O.C analysis the optimal cut – off was determined at 0.34. Sensitivity and specificity was calculated and reported in percentages.

The sample size had been calculated at the start of the research to be 96 deduced per formula: n = SD^2/ SE^2 × 1-p/p where SD = standard deviation at 95% confidence interval (1.96), SE = standard error taken as 10% (0.1) p = probability of finding positive or negative variables among the sample group, presumed 80%. Slightly higher number of participants were included in case they did not come the next day for blood sampling or measurements. The attrition rate was less than five percent.

Results

A total of 246 participants included 34 confirmed diabetics, 212 non diabetic siblings and volunteers. 106 siblings of those with Type 2 diabetes (Group 1: 48 (45.3%) males and 58 (54.7%) females) with a mean age of 41 ± 12.05 years were compared with 106 non diabetic volunteers Group 2: 60 (56.6%) males and 46 (43.4%) females with a mean age of 43 ± 14.1 years and no family history of diabetes. Group 1 vs. Group 2 had a mean fasting S. glucose of 88 ± 11.8 mg/dl (4.88 mmol/l) vs. 83 ± 11mg/dl (4.59 mmol/l) and mean HbA_1c 6.4 ± 1.2% vs. 5.4 ± 0.57% p value < .001 each (Table 1).

Amongst the 106 siblings forty three (40.6%) and thirty two (30.2%) participants respectively had a HbA_1c above 6.5% and between 5.7 and 6.5%,while the figures were 21% and 29.3% for 106 non-diabetic volunteers.

The mean serum insulin level in Group 1 was significantly elevated compared to Group2 (6.60 ± 5.49 µU/L vs. 2.9 ± 2.28 p < 0.001). Siblings had higher

Table 1: General Characteristics of Diabetics, their siblings and volunteers. P value indicates comparison between Siblings and volunteers only (Diabetics excluded).

		Diabetic n = 34		Siblings n = 106		Volunteers n = 106		p-value
		Mean	SD	Mean	SD	Mean	SD	
Age		45.32	9.45	40.77	12.05	43.47	14.19	0.137
Body Mass Index		28.65	4.07	27.72	5.18	26.14	4.71	0.021
Waist (cm)	Male	102.97	9.18	94.67	13.32	91.52	10.76	0.177
	Female	101.79	10.69	96.09	13.62	89.35	19.22	0.039
Hip (cm)	Male	106.21	10.05	101.97	10.37	97.88	8.94	0.030
	Female	108.74	8.88	103.93	15.51	98.5	11.6	0.051
Waist / Hip	Male	0.97	0.06	0.93	0.07	0.93	0.06	0.528
	Female	0.94	0.07	0.94	0.22	0.91	0.16	0.308
Systolic		127.79	12.86	128.68	14.03	125.47	13.93	0.096
Diastolic		81.62	7.36	77.55	9.47	77.69	8.84	0.911
Serum Glucose		121.06	59.24	87.96	11.84	82.68	11.06	0.001
Insulin Level				6.6	5.49	2.9	2.28	< 0.001
Triglyceride		190.50	81.83	161.75	63.92	160.87	72.64	0.926
Cholesterol		195.00	34.24	188.3	42.84	181.53	39.2	0.231
HDL		37.69	3.84	37.56	4.77	37.27	4.34	0.637
LDL		117.69	30.09	115.25	26.84	114.4	26.04	0.815
HDL / LDL		0.33	0.06	0.34	0.06	0.34	0.05	0.845
Hba_1c		7.11	1.39	6.36	1.21	5.4	0.57	< 0.001

fasting serum glucose and HbA_1C levels. In both cases the p value was less than 0.001. Insulin resistance was calculated on the basis of hyperinsulinaemia where normal insulin value was taken as 9 µ units/L. The cut off for insulin resistance was taken at 0.34 according to the ROC curve (Figure 1).

Twenty (9.4%) subjects (8males and 12 females) out of a total of 212 participants were found to be insulin resistant among which Insulin resistance was predicted with a sensitivity of 90% (CI 86 to 94) and specificity of 98.96% (CI 97.7 to 100). BMI was 26.1 ± 4.7 kg/m^2 in volunteers and 27.7 ± 5.2 kg/m^2 in sib-ling's p < 0.02. Siblings of those with type 2 diabetes had a significantly higher body mass index 27.7 ± 5.2 (p = 0.02) an increased waist in women 96.1 ±

Table 2:

Item	Percent	95% Confidence Interval
Sensitivity	90.0	86.0 – 94.0
Specificity	99.0	97.7 – 100.0
Positive Predictive value	90.0	86.0 – 94.0
Negative Predictive value	99.0	97.7 – 100.0
False Positive	10.0	6.0 – 14.0
False Negative	1.0	0.0 – 2.3
Diagnostic Accuracy	98.1	96.3 – 99.9

13.6 vs. 89.4 ± 19.2 (p = 0.04) and hip circumference (p = 0.051) compared to volunteers. The waist hip ratio was above 0.9 in both male and female siblings and volunteers (p-value > 0.05). Biometric assessment and blood sampling was done in the diabetic group but p-values were not calculated. There was a progressive increase noted in waist, hip and body mass index from volunteers to siblings to those with diabetes.

A comparison of insulin resistant group of 20 (9.4%) 8 males and 12 females) with 86 (90.6%) non-insulin resistant siblings (40 male and 46 females) was made within the same group. The insulin resistant siblings had significantly elevated mean S. insulin level and BMI. Fasting serum glucose and HbA_1c were also elevated with p-values of 0.052 and 0.008 respectively (Table 3).

There was statistically no significant difference in lipid profile, i.e., serum cholesterol, triglycerides HDL and LDL levels and blood pressure between insulin resistant and non-insulin resistant siblings The

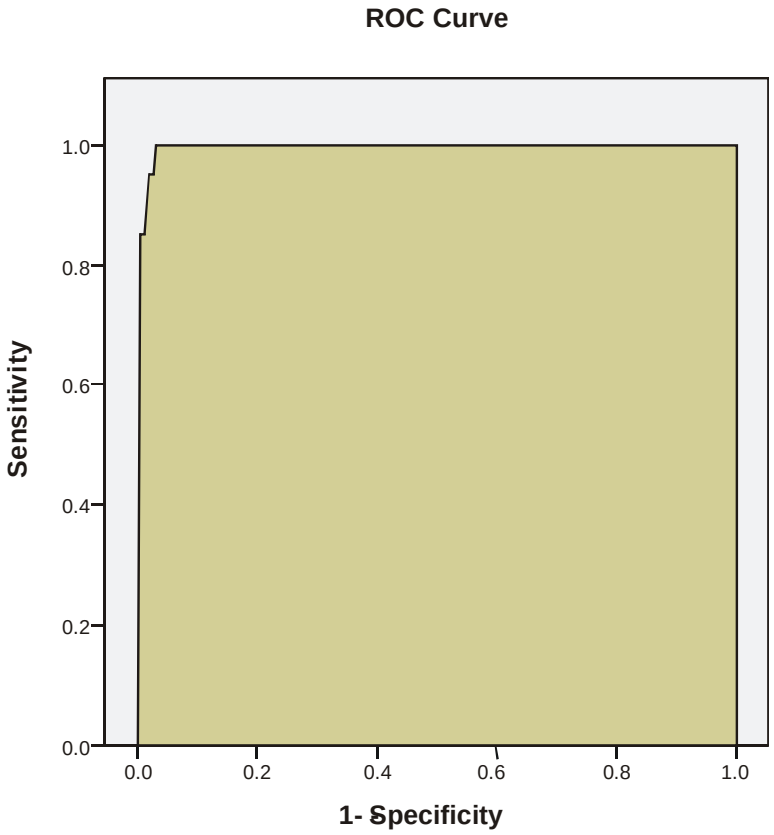

Fig. 1: ROC curve showing 99.8% area under the curve the cut – off was taken at 0.34.

Table 3: Comparison between insulin resistant and non insulin resistant siblings of diabetics.

		Non Resistant (n = 86)		Insulin Resistant (n = 20)		
		Mean	SD	Mean	SD	P-value
Age		40.33	11.93	42.79	12.69	0.423
Body Mass Index		27.14	4.72	30.37	6.40	0.013
Waist (cm)	Male	93.73	11.17	99.38	21.57	0.492
	Female	95.24	13.30	99.68	15.08	0.335
Systolic BP		128.51	14.14	129.47	13.83	0.787
Diastolic BP		77.41	9.18	78.16	10.96	0.758
Serum Glucose		86.92	11.67	92.74	11.73	0.052
Insulin Level		4.57	2.38	15.92	6.11	< 0.001
S. Triglyceride		161.37	64.46	163.47	63.08	0.897
S. Cholesterol		185.30	39.29	202.05	55.55	0.123
HDL – C		37.38	4.36	38.39	6.38	0.406
LDL – C		113.43	26.64	123.61	26.87	0.135
HDL/LDL		0.34	0.06	0.31	0.05	0.062
Hba1c		6.22	1.09	7.02	1.49	0.008

HDL cholesterol level was below the optimal level of 40 mg/dl (1.02 mmol/l) in volunteers, siblings, and those with NIDDM.

Discussion

This study focused on non-diabetic siblings in Western Punjab and confirmed findings of hyperinsulinaemia and insulin resistance occurring in those with a family history of diabetes mellitus. Other important findings were the presence of elevated body mass index and abnormal fat distribution, evident as increased waist circumference and waist / hip ratio, tending to occur in this high risk genetically predisposed group. The American Diabetes Association 2010 has recently lowered the determinants of diabetes for HbA1c from > 7% to > 6.5%.[14] Unexpectedly at analysis it was found that 43 (40.6%) participants among siblings had HbA_1c above 6.5% and 32 (30.2%) had HbA_1c between 5.7 and 6.5%. According to new guidelines this meant a 40.6% incidence of diabetes and 30.2% of pre-diabetes amongst those presumed to be non-diabetic. These individuals were unaware of being pre-diabetic or diabetic and it can be safely presumed such figures are a reflection of the general population. The majority of the participants was married, had secondary school education, belonged to middle social class and had no previous access to medical care. The limitation of the study was that it was a comparative analysis of a number of variables contributing to a cardiovascular risk but the sequence of development for each of those variables and its risk association in siblings and volunteers has not been assessed. The relative contribution of each variable to the development of metabolic syndrome has not been given weightage.

A number of studies confirm insulin resistance as well as hyperinsulinaemia independently to have strong association to cardiovascular risk profile.[15] Siblings of diabetics demonstrate significant hyperinsulinaemia and insulin resistance along with obesity. There appears to be a molecular link between obesity, insulin resistance and impaired glucose tolerance which is poorly understood.[16] Visceral adiposity one of the components of the metabolic syndrome is indirectly implicated in the development of insulin resistance, atherosclerosis and cardiovascular disease.[16,18] Studies have shown concentration of adipose – specific protein adiponectin with antiatherogenic properties to be inversely related to insulin resistance, hyperinsulinaemia and obesity. It is found in low concentration in Type 2 diabetes and obesity. Also there is genomic scan evidence to link it to insulinaemia related to a region on chromosome three.[17] This helps to explain the genetic basis of hyperinsulinaemia and insulin resistance and why siblings of diabetics maybe at a higher risk of cardiovascular disease. In the IRAS (Insulin resistance atherosclerotic study) low incidence of insulin resistance was found in African Americans, non-Hispanic and Hispanic populations whereas acute insulin response was increased in African – Americans.[19] Unfortunately we have not studied the impaired first phase acute insulin response to claim a valid comparison but only demonstrated the presence of insulin resistance and hyperinsulinemia in our population. The original selection of siblings was based on fasting S. glucose within the normal range but they were further categorized according to their HbA_1c values. Majority of siblings who were hyperinsulinaemic were also insulin resistant. A cluster of metabolic abnormalities as sequelae could well be attributed to several gene mutations, an area for separate research.

Insulin resistance correlates positively to obesity, supported by the increased waist measurements and BMI in siblings of diabetics. Waist circumference, body mass index and waist hip ratio are linked to underlying visceral adiposity.[20] Standardised cut offs for body mass index (BMI) at 25 kgm/m^2 and 30 kgm/m^2 and waist measurement action level 1 and 2 recommended internationally are derived largely from cross sectional studies of anthropometric related morbidity and mortality.[20] A BMI > 30 kg/m^2 and waist circumference action level 1 at 94cms in men, 80cms in women or action level 2 at 102cms in men and 94 cms in women may predict the incidence of metabolic syndrome at 19% and 32% respectively as stated in San Antonio heart study.[5] However according to WHO these standardized cut offs for BMI may not be applicable to South Asian population where the risk of cardiovascular morbidity and mortality may be presenting at a lower BMI of 23 kgm/m^2.[21] Interestingly in this study the BMI was 26 kgm/m^2 in non-diabetic volunteers. A previous BMI study in the same area also showed BMI > 26 kg/m^2 among otherwise healthy population.[22] It indicates we need to research to determine the optimal biometric indices specific for cardiometabolic risk in our population. Although there was a significant increase in the BMI of siblings of diabetics and further in those who are insulin resistant, 30.4 kg/m^2 being well above the cut off values, we cannot ignore the high BMI in our non diabetic volunteers.

Waist circumference is considered a surrogate

measure of insulin resistance. In a large study of 4800 middle aged Japanese men, waist circumference of 85 cms was proven to be the optimal cutoff in relation to insulin resistance.[23] The International Diabetes Federation (IDF) has adopted different cutoffs for waist circumference for different ethnicities. Cut off for Europeans are 94 cms in men and 80 cms in women and for Chinese and South Asian men and women it is 90 cms and 80 cms.[24] In this study waist measurements and waist / hip ratio are increased above the recommended cutoffs. The progressive increase in waist circumference was present in both sexes but significantly demonstrated only in female siblings. The body mass index, waist girth; insulin resistance and insulin response were independently associated with total cardiovascular load (all $P < 0.001$) in the RISC study.[6]

In another study it has been demonstrated that fasting insulin may have a stronger association with metabolic syndrome and carotid intimal thickness, a measure of systemic atherosclerosis, than insulin sensitivity.[18] Thus hyper-insulinemia may contribute towards accelerating the process of atherosclerosis. Dyslipidemia, part of the metabolic syndrome (increased triglyceride and LDL – cholesterol, decreased HDL – cholesterol) is a manifestation of the atherosclerotic process and well recognized contributor to cardiovascular risk.[25] The relevant features of metabolic syndrome present in this study are insulin resistance, hyperinsulinaemia, impaired glucose tolerance, visceral adiposity, and low HDL – C levels. The mean blood pressure recorded in siblings was 129 / 78.which was not significantly different from volunteers. All the above features were demonstrated in siblings as components of the metabolic syndrome except hypertension. However in our study sample there was no significant difference in total cholesterol, LDL cholesterol and triglyceride levels amongst diabetics, their siblings and healthy volunteers. It is noteworthy that low HDL cholesterol is contributory to the risk of IHD but unexpectedly HDL – C was <.40 mg/dl (suboptimal) not only in siblings but also in non diabetic volunteers. Volunteers showed absence of insulin resistance, hyperinsulinaemia, and less incidence of diabetes compared to siblings of diabetics. It is difficult to determine the transition from a normal to an insulin resistant state when biometric and metabolic influences are similar. In our population this distinction appears blurred because even among healthy volunteers with no family history of diabetes 29.2% were found to be pre-diabetic, nearly equal to siblings of diabetics at 30.2%. One cannot but wonder if these volunteers with no family history of diabetes will become insulin resistant or not in the course of time?

Conclusion

We have got insight about the magnitude of health challenge that diabetes poses in our population, the relatively high incidence of diabetes amongst otherwise healthy individuals and even more so in siblings of diabetics is not unexpected. There has been a dramatic change in life style in the third world promoting sedentary habits under the guise of modern comforts. We have attempted to study this population, which has had no such study in order to understand the emergence of a new health challenge. The target should be the high risk sibling population before the onset of diabetes. The focus should be on early intervention with lifestyle modifications and controlling obesity especially waist circumference. In our social environment where patient compliance to treatment is poor targeting diabetic families with a preventative view may help control the diabetic epidemic. We cannot alter our genetics but environmental influences contributing to Type 2 diabetes need to be tackled on a national level.

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