

*Full Length Research Paper*

# Relationship among sera lipoprotein abnormalities in healthy individuals with background of diabetic sibling

Samad Akbarzadeh<sup>1</sup>, Iraj Nabipour<sup>2</sup>, Seyed Mojtaba Jaffari<sup>3</sup>, Niloofar Motamed<sup>4</sup>, Ali Movahed<sup>1</sup>, Mostafa Mirzaei<sup>1</sup> and Ali Reza Rahbar<sup>5\*</sup>

<sup>1</sup>Department of Biochemistry, School of Medicine, Bushehr University of Medical Sciences, Iran.

<sup>2</sup>Department of Endocrinology and Metabolic Diseases, the Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Iran.

<sup>3</sup>Department of Immunology, School of Paramedicine, Bushehr University of Medical Sciences, Iran.

<sup>4</sup>Department of Social Medicine, School of Medicine, Bushehr University of Medical Sciences, Iran.

<sup>5</sup>Department of Nutrition, School of Health, Bushehr University of Medical Sciences, Iran.

Accepted 27 June, 2011

As the prevalence of lipoprotein abnormalities in adolescents is increasing dramatically, the identification of relevant risk factors is a major public health challenge. The aim of this study was to investigate whether a family history of diabetes could be a risk factor for lipid abnormalities in healthy individuals. This study is a cross-sectional case control study. 179 men and women were studied in two equal-member groups (with diabetic parents' background and without any diabetic sibling). Both groups matched in body mass index (BMI), age and sex. The serum concentration of oxidized-low density lipoprotein (LDL), Apo B100 and insulin were measured by enzyme linked immunosorbent assay technique and TG, Chol, HDL-C, FBS and GTT by enzymatic methods. The LDL-C level was calculated using the Friedewald formula. The results show that there were no significant variation in the amount of plasma FBS, GTT, Cho, TG, LDL and HDL between the two groups, whereas a significant increase was found in the amount of insulin ( $P = 0.02$ ), Apo B100 ( $P = 0.001$ ), OX-LDL ( $P = 0.001$ ) and HOMA-IR ( $P = 0.03$ ) in the case group as compared to the control group. We conclude that a family history of diabetic parents can lead to lipid parameters abnormalities and CVD risk factor via aggregation of inherited defected genes.

**Key words:** Diabetes, oxidized-low density lipoprotein (LDL), Apo B100, lipoproteins.

## INTRODUCTION

Lipoprotein abnormalities significantly contribute to the risk of developing (cardio vascular disease) CVD. The early cardiovascular risk factors precede the development of CVD in adolescence (Järvisalo et al., 2002; Krantz et al., 2004; Relimpio et al., 2002). As the prevalence of lipoprotein abnormalities in children and

adolescents is increasing dramatically, the identification of relevant risk factor is a major public health challenge.

The U.S. National Cholesterol Education Program (NCEP) recommends that to start treatment of CVD, medicine should be based on cholesterol levels and the number of risk factors. Having a male sibling, who had heart disease before 55 years old; or a female sibling, with heart disease before 65 years old is a major risk factor for CVD (Grundy et al., 2004).

Many families with familial combined hyperlipidemia (FCHL) have glucose intolerance and insulin resistance (Brouwers et al., 2010) and the expression of syndrome X appears to have a familial basis, although, the identification of gene responsible for syndrome X has proven difficult because of its etiologic heterogeneity and pleiotropic phenotype (Facchini et al., 1992; Zavaroni et

\*Corresponding author. E-mail: [rahbar\\_alireza@yahoo.com](mailto:rahbar_alireza@yahoo.com)  
Tel: 00989177724604. Fax: 00987712528.

**Abbreviations:** BMI, Body metabolic index; LDL, low density lipoprotein; FBS, fasting blood sugar; GTT, glucose tolerance test; Cho, cholesterol; TG, triglyceride; HDL, high density lipoprotein.

al., 1990; Ericksson et al., 1989; Ho et al., 1990; Xiang et al., 2001). More recently, clustering of hypertension and insulin resistance in families was accounted for, in part, by heritable factors both associated with and independent of body mass index (Xiang et al., 2001).

The association of type 2 diabetes and lipoprotein abnormalities such as elevated Apo B100 has been proved by Relimpio et al. (2002). Nakhjavani et al. (2009) have reported a significant increase of plasma oxidized-LDL in patients with diabetes as compared to normal subjects (Nakhjavani et al., 2009). Family history of diabetes as a risk factor for these lipoprotein abnormalities can be attributed to a new candidate for major gene effects.

We hypothesized that a background of diabetic parents can lead to lipid parameters abnormalities and CVD risk factor via aggregation of inherited defected genes. So for the first time we conducted this study to investigate that a family history of diabetes is a risk factor for lipid abnormalities in healthy individuals.

## MATERIALS AND METHODS

This study is a cross-sectional case control study. In general, 179 men and women were studied in two groups (treatment and the control group), 41 healthy men and 49 healthy women, 40.55 ± 9.63 years old with a first degree diabetic patient who had been referred to Abolfazl Specialized Clinic were selected for this study. Control group included 49 men and 40 women 41.37 ± 9.47 year old without any history of diabetic parents. Inclusion criteria for both groups were based on (FBS < 110 mg/dl and GTT < 140 mg/dl). Two groups matched in body mass index (BMI), age and sex and the questionnaire developed for all of them were completed by the interviewer.

They were excluded if they had a recent history (within 6 months) of myocardial infarction or stroke, significant liver or renal disease (plasma creatinine > 130 µmol L<sup>-1</sup>), microproteinuria, thyroid disorders, neoplasm, diabetes and hypertension. They had not taken contraceptives, glycocorticoids, ovulation stimulation drugs, antihypertensive, oral hypoglycemic, weight lose, estrogens or anti-androgens medications. All patients fulfilled the following criteria: Fasting blood sugar (FBS) < 110 mg/dl, BMI <30 kg/m<sup>2</sup> and GTT <140 mg/dl. The study was approved by the Ethics Committee of the Medical Sciences of Bushehr University and the reported investigations were carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000. Also, the participants in the project have given written informed consent.

Venous blood samples were taken between 8 and 9 a.m. Serum was obtained from the blood samples and was centrifuged at 3000 g for 15 min at 4°C. Immediately after centrifugation, the sera samples were frozen and stored at -80°C for a period not more than 8 weeks.

All measurements were carried out at the Research Laboratory of the Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Bushehr, I. R. Iran. FBS was measured by the glucose-oxidase method of Pars Azmoon-co, Iran (intra- and interassay coefficients of variation (CVs) were 3.5 and 3.0%, respectively). The TG, T-Chol and HDL-C levels were determined by using cholesterol oxidase phenol, Pars Azmoon-co, Iran, on an Autoanalyzer Vital Scientific Selectra 2 (Spankeren, the Netherlands) (intra- and interassay CVs were 2.0 and 2.6% for TC, and intra- and interassay CVs were 1.5 and 1.9% for HDL-C respectively). The LDL-C level was calculated using the Friedewald

formula. Apo B-100 was determined by using the enzyme linked immunosorbant assay technique (ELISA), Cayman chemical co, USA (intra- and interassay CVs were 3.5 and 4.2%, respectively). The plasma concentration of oxidized LDL-c was measured by (ELISA), Assaypro co (intra- and interassay coefficients of variation (CVs) were 3.5 and 3.0%, respectively). The plasma insulin (intra- and interassay CVs 2.1 and 3.7%) were achieved by (ELISA). The insulin kit is the brand of DRG German Company. The insulin resistance was estimated by HOMA- IR. HOMA-IR was obtained by (Song et al., 2007):

$$\text{HOMA} - \text{IR} = \frac{\text{Insulin} \left( \frac{\mu\text{U}}{\text{ml}} \right) \times \text{FBS} \left( \frac{\text{mmol}}{\text{ml}} \right)}{22.5}$$

Weight and height was measured by using stadiometer standard techniques. The body mass index (BMI) was calculated by kg weight (kg) / height (m<sup>2</sup>).

## Statistical analysis

Statistical analysis of the data was performed using SPSS statistical software version 11.5. All results were express as mean plus/minus standard deviation. The single sample Kolmogrov-Smirnov test was used to estimate the variables' distribution characteristics. Simple independent student T-test was used to compare baseline data. The differences were compared between the 2 groups with ANCOVA. With a calculated sample size of 179 persons with diabetic parents' background and without any diabetic sibling, the study will have power of 80.5% to yield a statistically significant result between these two groups. Two-tailed probability value of P < 0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION

179 assigned subjects completed the study. The characteristics of the patients confirmed that the groups were well matched for all entry criteria (Table 1). The result from this study shows that there is no significant variation in the amount of plasma FBS, GTT, Cho, TG, LDL-C and HDL-C in case and control groups. There was a significant increase in the amount of insulin (P = 0.02), Apo B100 (P = 0.001), oxidized -LDL (P = 0.001) and HOMA-IR (P =0.03) in the case group as compared to the control group (Table 2).

The findings in this study indicate a significant increase of Apo B100 in subjects with diabetic parents when compared with individuals without background of diabetes. Apo B100 is abundant in LDL and VLDL lipoprotein particles. The breakdown and reformation of these lipoproteins in liver and serum are interlinked via several steps that often require the action of enzymes, one of which is lipoprotein lipase (LPL). Several single nucleotide polymorphisms (SNP) have been found within the LPL gene (Ma et al., 2003). In a study on 386 type 2 diabetic patients, a correlation was found between the presence/severity of microalbuminuria and genetic variants of LPL (Mattu et al., 2002). Miyashita and et al. (2002) compared the levels of LPL in the blood of 40 type 2 diabetic patients and a group of healthy individuals prior to heparin injection (Miyashita et al., 2002). The findings

**Table 1.** Baseline demographic characteristics of the subjects (n=179) (means  $\pm$ SD).

Characteristic	Control group (n = 89)	Patient group (n = 90)
Age (years)	41.37 $\pm$ 9.47	40.55 $\pm$ 9.63
Women	40	49
men	49	41
Weight (kg)	74.32 $\pm$ 14.75	75.92 $\pm$ 13.43
Height (cm)	166.89 $\pm$ 8.87	165.88 $\pm$ 9.31
BMI (kg/m <sup>2</sup> )	26.52 $\pm$ 4.24	27.57 $\pm$ 4.25
WHR	0.91 $\pm$ 0.17	0.90 $\pm$ 0.14

BMI, Body mass index; WHR, waist hip ratio.

**Table 2.** Mean  $\pm$ SD concentration in variables in case and control groups.

Characteristic	Control group	Case group
Apo-B100 ( $\mu$ g/ml)	4.06 $\pm$ 3.72	7.80 $\pm$ 6.22 <sup>#</sup>
Oxidized-LDL (units/ml)	4.9 $\pm$ 4.41	5.59 $\pm$ 2.68 <sup>#</sup>
Insulin ( $\mu$ U/ml)	6.56(5.03-10.18)	8.75(5.25-16.68)*
Triglyceride (mg/dl)	155.52 $\pm$ 75.00	155.51 $\pm$ 82.3
Cholesterol (mg/dl)	196.92 $\pm$ 37.24	198.54 $\pm$ 36.85
HDL-C (mg/dl)	45.44 $\pm$ 11,65	43.00 $\pm$ 10.38
LDL-C (mg/dl)	120.04 $\pm$ 32.44	121.01 $\pm$ 31.55
FBS (mg/dl)	79.77 $\pm$ 10.46	80.50 $\pm$ 8.56
GTT (mg/dl)	92.03 $\pm$ 16.65	92.17 $\pm$ 15.68
HOMA-IR	1.29(0.98-1.93)	1.82(1.05-3.06)*

Data is presented as mean  $\pm$ SD, except for insulin and HOMA-IR [medians (interquartile ranges)]. Apo-B100: apolipoprotein B100, FBS: fasting blood sugar, GTT: glucose tolerance test, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, HOMA-IR: homeostasis model assessments for insulin resistance.\*P<0.05; #P<0.01.

of their study showed a significant decrease in the amount of LPL in the type 2 diabetes patients as compare to healthy individuals. This polymorphism was further found by Ma et al. (2003) in 785 Chinese subjects, of which about 60% had been diagnosed with type 2 diabetes (Ma et al., 2003).

Most recently, SNPs of LPL gene were investigated in Mexican Americans with insulin resistance (Goodarzi et al., 2004). These nucleotide polymorphisms were inherited together on the same chromosome (haplotypes) which is associated with insulin resistance. Insulin resistance is a pathophysiological determinant in patients with type 2 diabetes. Of note, these haplotypes were also associated with coronary artery disease, suggesting that LPL may acts as a genetic link between diabetes and atherosclerosis (Goodarzi et al., 2003). Inherited LPL abnormalities raise the levels of free fatty acids in plasma, increase the flux of free fatty acids into the liver, and enhance production of Apo B100. In addition, elevated serum insulin may promote reduced retention of free fatty acids by adipocytes. The data of our study showed a significant increase of insulin concentration in individuals with diabetic parents as compared to subjects without first degree family history of diabetes mellitus.

We hypothesized that both LPL abnormalities and insulin resistance lead to increased flux of free fatty acids into the liver, decreased proteolysis and enhanced production of Apo B100.

Other abnormalities that can aggregate in children of diabetic individuals are defect in expression of PPAR $\gamma$  gene. PPAR $\gamma$  is a member of the nuclear hormone receptor super-family of transcription factors. It is abundant in adipose tissue and plays a key role in fat cell differentiation. To date, several genes have been identified as being direct targets for PPAR $\gamma$ , including LPL, fatty acid transport protein and acetyl-CoA-synthase (Arner, 2003). The variant of the PPAR receptor inherited may affect the risk of obesity or developing type 2 diabetes (Stumvoll and Haring, 2002).

In one study conducted by Muller et al. (2003), Pima Indians of Arizona for variations in PPAR $\gamma$  were screened. Type 2 diabetes is particularly common among this population. Several new SNPs were identified, many in the promoter region of the PPAR $\gamma$  gene (Muller et al., 2003).

Our findings indicate significant elevated oxidized LDL in case group as compared to control group. Of note, the genes that regulate the expression of the major enzymes

in lipid metabolism cascade, such as LPL, CETP and hepatic lipase are defected in diabetic individuals. In nondiabetic children with diabetic parents, defected inherited gene for these enzymes can modulate the expression of small, dense LDL. This particle has a prolonged residence time in plasma, and because of decreased interaction with the LDL receptor, it is more susceptible to oxidation.

In our study, a significant increase in insulin resistance has been seen in the subjects with diabetic parents in comparison to those without family history of diabetes. In accordance with our results, Carantani et al. (1998) observed a significant relationship between insulin resistance and oxidized-LDL in a group of nondiabetic patients (Carantoni et al., 1998). Gyan et al. (1996) have recently raised the possibility of a pro-oxidant action of insulin *in vivo* by demonstrating a consistent free radical-scavenger molecule, during philological hyperinsulinemia (Galvan et al., 1996).

## Conclusion

In conclusion, we found that a background of diabetic parents was associated with a significantly increased Apo B100 and oxidized-LDL in healthy individuals. However, observational studies do not prove hypotheses and can only suggest the potential mechanisms of action. We now urgently need data from the relative contributions of genetic factors, the mode(s) of inheritance and the molecular defect(s) underlying lipoprotein abnormalities.

## ACKNOWLEDGEMENTS

This study was supported in part by a grant from Bushehr Province Technology and Research Committee, and Research Deputy of Bushehr University of Medical Science (DP/20/18/3/1237,8/9/2005). We wish to thank Safar Ghasemi, Ahmad Emadi, Najmeh Hajian, Zahra Sanjdeh and Shiva Mosadeghzadeh for coordinating the collection of samples and for laboratory management.

## REFERENCES

- Arner PC (2003). The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. *Trends Endocrinol. Metab.* 14: 137-145.
- Brouwers MC, van der Kallen CJ, Schaper NC, van Greevenbroek MM, Stehouwer CD (2010). Five-year incidence of type 2 diabetes. *Hypertens*, 5: 694-699
- Carantoni M., F. Abbasi, F. Fabienne warmerdam, M. Klebanov, P. Wang, Y. Chen, S. Azhar, G.M. Reavon, 1998. Relationship between insulin resistance and partially oxidized LDL particles in healthy, nondiabetic volunteers. *Arterioscler. Thromb. Vasc. Biol.* 18: 762-767.
- Ericksson J, Franssila-Kallunki A, Ekstrand A, Saloranta C, Widen E, Schalin C, Groop L (1989). Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 69: 343-347.
- Facchini F, Chen YD, Clinkingbeard C, Jeppesen J, Reaven GM (1992). Insulin resistance, hyperinsulinemia, and dyslipidemia in nonobese individuals with a family history of hypertension. *Am. J. Hypertens*, 5: 694-699.
- Galvan AQ, Muscelli E, Catalano C, Natali A, Sanna G, Masoni A, Bernardini B, Baracchi R, Ferranini E (1996). Insulin decreases circulating vitamin E levels in humans. *Metabolism*, 45: 998-1003.
- Goodarzi MO, Guo X, Taylor KD, Quiñones MJ, Samayoa C, Yang H, Saad MF, Palotie A, Krauss RM, Hsueh WA, Rotter JI (2003). Determination and use of haplotypes: ethnic comparison and association of the lipoprotein lipase gene and coronary artery disease in Mexican-Americans. *Genet. Med.* 5: 322-327.
- Goodarzi MO, Guo X, Taylor KD, Quiñones MJ, Saad MF, Yang H, Hsueh WA, Rotter JI (2004). Lipoprotein lipase is a gene for insulin resistance in Mexican Americans. *Diabetes*, 53: 214-220.
- Grundys S, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ (2004). Implications of recent clinical trials for the NCEP-ATP III Guidelines. *Circulation*, 110: p. 227.
- Ho LT, Chang ZY, Wang JT, Li SH, Liu YF, Che YD, Reaven GM (1990). Insulin insensitivity in offspring of parents with type 2 diabetes mellitus. *Diabet. Med.* 7: 3-34.
- Järvisalo MJ, Putto-Laurila A, Jartti L, Lehtimäki T, Solakivi T, Rönnemaa T, Raitakari OT (2002). Carotid artery intima-media thickness in children with type 1 diabetes. *Diabetes*, 51: 493-498.
- Krantz JS, Mack WJ, Hodis HN, CR Liu HN, Liu CH, Kaufman FR (2004). Early onset of subclinical atherosclerosis in young persons with type 1 diabetes. *J. Pediatrics*. 145: 452-457.
- Ma YQ, Thomas GN, Ng MC, Critchley JA, Chan JC, Tomlinson B (2003). The lipoprotein lipase gene HindIII polymorphism is associated with lipid levels in early-onset type 2 diabetic patients. *Metabolism*, 52: 338-343.
- Mattu RK, Trevelyan J, Needham EW, Khan M, Adiseshiah MA, Richter D, Murray RG, Betteridge DJ (2002). Lipoprotein lipase gene variants relate to presence and degree of microalbuminuria in Type II diabetes. *Diabetologia*, 45: 905-913.
- Miyashita Y, Shirai K, Itoh Y, Sasaki H, Totsuka M, Murano T, Watanabe H (2002). Low lipoprotein lipase mass in preheparin serum of type 2 diabetes mellitus patients and its recovery with insulin therapy. *Diabetes Res. Clin. Pract.* 56:181-187.
- Muller YL, Bogardus C, Beamer BA, Shuldiner AR, Baier LJ (2003). A functional variant in the peroxisome proliferator-activated receptor gamma2 promoter is associated with predictors of obesity and type 2 diabetes in Pima Indians. *Diabetes*, 52: 1864-1871.
- Nakhjavani M, Asgarani F, Esfahanian F, Esteghamati A (2009). Plasma Level of Oxidized Low-Density Lipoprotein in Macroalbuminuric Type 2 Diabetic Patients versus Normoalbuminuric Group. *Act. Medica Iranica*, 47: 349-352.
- Relimpio F, Losada F, Pumar A, Mangas MA, Morales F, Astorga R (2002). Relationships of apolipoprotein B(100) with the metabolic syndrome in Type 2 diabetes mellitus. *Diabetes Res. Clin. Pract.* 57:199-207.
- Song Y, Manson J, Tinker L, Howard BV, Kuller LH, Nathan L, Rifai N, Liu S (2007). Insulin sensitivity and insulin secretion determined by homeostasis model assessment (HOMA) and risk of diabetes in a multiethnic cohort of women: The women's health initiative observational stud. *Diabetes Care*, 30: 1747-1752.
- Stumvoll M, Haring H (2002). The peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism. *Diabetes*, 51: 2341-2347.
- Xiang AH, Azen S, Raffel CJ, Tan S, Cheng LS, Diaz J, Toscano E, Henderson PC, Hodis HN, Hsueh WA, Rotter JL (2001). Evidence for joint genetic control of insulin sensitivity and systolic blood pressure in Hispanic families with hypertensive probands. *Circulation*, 103: 78-83.
- Zavarani I, Mazza S, Luchetti L, Buonanno G, Bonati PA, Bergonzani M, Passeri M, Reaven GM (1990). High plasma insulin and triglyceride concentrations and blood pressure in offspring of people with impaired glucose tolerance. *Diabet. Med.* 7: 494-498.