OLR1 Gene Polymorphism and Oxidized LDL Levels in Metabolic Syndrome in Indian Population

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Abstract

Objective: Metabolic syndrome (MetS) is associated with abnormal lipid profile and high cardiovascular risk. There is an increased prevalence of coronary artery disease and Type 2 Diabetes Mellitus in India. Oxidized Low Density Lipoprotein Receptor 1(OLR1), a cell surface endocytosis receptor recognize, internalize and degrade oxidized LDL (oxLDL) in vascular endothelium and plays a role in the pathogenesis of atherosclerosis. The aim was to explore the association of OLR1 gene polymorphism and measure the serum levels of ox-LDL in patients with MetS in Indian population. **Materials and Methods:** Forty cases fulfilling the IDF diagnostic criteria for MetS and 40 healthy controls having similar age and sex ratio were genotyped for OLR1 gene (SNP: IVS4–73C>T , rs3736234) by RFLP-PCR. Serum ox-LDL was estimated by ELISA.Their BP, BMI and waist circumference were measured. Fasting Plasma glucose, Serum Triglyceride and HDL-C were measured. **Results:** Serum oxLDL was significantly higher in MetS cases as compared to controls ($p<0.0001$). Odds ratio of T allele of above OLR1 SNP among subjects with MetS was 14.79 (95%CI: 1.80-121.2, $p<0.05$). But no association was found between the SNP and serum ox-LDL levels. People having TT allele had higher BMI compared to those having CC allele. **Conclusion:** Ox LDL, being more atherogenic might contribute in the pathogenesis of MetS. The intronic SNP: IVS4-73 C>T of OLR1 gene increases the risk of developing MetS by a yet unknown mechanism that is independent of rise in ox-LDL. This OLR1 SNP probably influences BMI.

Keywords: Metabolic syndrome, OLR gene polymorphism, restriction-fragment length polymorphism

Background

The major features of the metabolic syndrome (MetS) are central obesity, hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol, hyperglycemia, and hypertension. Worldwide prevalence of MetS can be from <10% to as much as 84%, depending on the regional and environmental conditions as well as the population studied. It can also vary according to the definition of the syndrome used.[1,2] The International Diabetes Federation (IDF) estimates that one-quarter of the world's adult population has the MetS.[3] The Chennai Urban Rural Epidemiology Study found the prevalence of the MetS to be 23%, 18%, and 26% using the World Health Organization, Adult Treatment Panel III, and IDF definitions, respectively.[4] Asian Indians show an increased prevalence of coronary artery disease (CAD) and type 2 diabetes (T2DM) as compared to other ethnic groups. The South Asian Phenotype is a special body type characterized by an increased waist circumference (WC), increased waist hip ratio, excessive body fat mass, increased plasma insulin levels, insulin resistance, and atherogenic dyslipidemia, with low levels of HDL cholesterol and increased triglyceride levels. All such factors predispose South Asians to T2DM and premature CAD. In addition, certain genetic markers could potentially make South Asians more susceptible to cardio-metabolic risks.[5]

Unmodified or native low-density lipoprotein (LDL) lacks inflammatory properties but oxidized LDL (ox-LDL) activates circulating monocytes, stimulating their ability to infiltrate the vascular wall, and induces vascular endothelial cell activation and dysfunction, resulting in pro-inflammatory responses and pro-oxidative conditions. The resulting inflammation is a primary stage in atherogenesis and is common in the MetS.

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The pathological effects of ox-LDL are mediated by its receptor LOX-1, present in the endothelial cells. LOX-1 mediates the recognition, internalization, and degradation of ox-LDL by vascular endothelial cells. LOX-1 is encoded by ox-LDL receptor 1 (*OLR1*) gene on human chromosome 12p12·3-13·1. Several *OLR1* gene functional single-nucleotide polymorphisms (SNPs) have been recently linked to cardiovascular disease (CVD) susceptibility in humans.[6]

Recently, several association studies have shown that a linkage disequilibrium (LD) block of SNPs located in the *OLR1* gene introns 4, 5, and the 3' untranslated region are associated with increased susceptibility to acute myocardial infarction (MI). Using a minigene approach, Mango *et al*.[6] showed that few particular SNPs located in the LD block modulate the retention of exon 5 of the *OLR1* gene, thus regulating the level of the new fully functional transcript. The new splice variant of the *OLR1* gene – was named LOXIN. The newly spliced mRNA has a stop codon in the open reading frame that leads to a premature termination of the translation product and is predicted to generate a protein that lacks two-third of the lectin-like domain.

One such functional intronic SNP is IVS4-73 C/T in which the C allele regulates the expression of LOXIN that encodes a truncated LOX-1 splice isoform, which lacks part of the extracellular domain, and shows a reduced binding capacity for ox-LDLs.[7] As a consequence, LOXIN expression confers increased resistance to ox-LDL induced macrophage apoptosis and atherogenesis *in vitro*, and C allele carriers are protected, whereas homozygous T allele carriers are predisposed to CVD *in vivo*.[8] Since MetS is a clinical condition characterized by an increased cardiovascular risk, it can be surmised that *OLR1* gene polymorphisms could be associated with MetS and its components.

Hence, the present study was designed (a) to study the risk of *OLR1* gene (rs 3736234) polymorphism for development of MetS, (b) to compare the serum ox-LDL level of controls with that of patients with MetS and to explore their association with the components of MetS, and (c) to find the relationship between *OLR1* gene (rs 3736234) polymorphism with ox-LDL level in selected Indian population.

Materials and Methods

A convenient sample size of forty subjects (male:female = 10:30) with clinical diagnosis of MetS based on the IDF criteria, that is, central obesity (WC: men >90 cm, women >80 cm) and any two of the following four: a) elevated triglycerides (>150 mg/dl or >1.7mmol/L), b) low HDL (<40 mg/dl or <1.03mmol/L in men, <50 mg/dl or <1.29 mmol/L in females), c) elevated blood pressure >130/85 mmHg or use of antihypertensive medication, and d) elevated fasting glucose >100 mg/dl or >5.6 mmol/L were selected. Forty healthy subjects having similar age and sex were selected as controls. Subjects with type 1 diabetes mellitus, maturity-onset diabetes or secondary diabetes, with associated major systemic diseases (cardiac, respiratory, renal, or hepatic failure) or with recent acute infection or severe diseases that could affect the oxidative stress were excluded from the study. Signature on written informed consent was taken from the cases and controls. Clinical examination with anthropometric measurements was recorded on a pre-designed proforma. About 5 ml of venous blood was collected from every case and healthy control after 12 hours of fasting. Blood (3 ml) was centrifuged at 3000 rpm for 10 minutes at room temperature and serum was separated and biochemical estimations done. Blood (2 ml) for genotyping was collected in ethylenediaminetetraacetic acid vial and stored at −80°C until the analysis.

Components of metabolic syndrome

Measurements of WC were taken with participants standing and calculated as an average of two measurements taken after inspiration and expiration at the level of widest circumference in the area between the iliac crest and lower rib, using a standard measuring tape. Blood pressure was taken three times with participants in the seated position after 5 minutes of quiet rest with the gap of 5 minutes in between the measurements using cuff of standard size with instrument at the level of individual's heart, according to standardized protocol. Average of three readings then calculated to determine the reported systolic and diastolic blood pressure. Fasting plasma glucose was assayed by glucose oxidase method using GLUC-PAP kit (Randox, UK), serum triglyceride by GPO reagent kit (Randox, UK), serum HDL cholesterol by HDL-C enzymatic clearance assay kit (Randox, UK) adapted to Olympus AU 400 (Beckman Coulter, USA).

Estimation of serum ox-LDL

Human ox-LDL enzyme-linked immunosorbent assay (ELISA) kit provided by QAYEE-BIO (Shanghai, China) that uses a double-antibody sandwich enzyme-linked immunosorbent one-step process assay to determine the level of ox-LDL in samples was used.

Gene polymorphism

The polymerase chain reaction–restriction-fragment length polymorphism (PCR–RFLP) methods were used to screen for the desired polymorphism. Total genomic DNA was extracted from whole blood using Genomic DNA Mini Kit (Genaid, Taiwan) according to the manufacturer's instructions. The presence of DNA in the extract was confirmed by agarose gel electrophoresis. PCR was performed using the primers:

- Forward: 5′-CAGTCAAGGGGATGTCAAAGA-3′ and
- Reverse: 5′-GAGGCATCAAAAAGAATGGG-3′, as previously described by Trabetti *et al*.[9] The total volume of each PCR reaction mixture was 25 µl: 0.25 µl of each primer, 3 µl of total genomic DNA, and 10 µl of the master mix and nuclease-free water to make up to total volume of 25 µl and was carried out in the thermocycler (Bioer, China).

PCR conditions were as follows: initial denaturation at 94°C for 10 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 5 minutes.

The amplified products were resolved using electrophoresis in 2% agarose gel and were 267 bp long. The amplicons were digested with restriction endonuclease BamHI (Roche Applied Sciences, Germany) and two fragments were obtained: 220 and 47 bp for the -73C allele, and a single fragment of 267 bp for the -73T allele. The conditions for digestion reaction were: PCR reaction mixture – 10 µl, 10× SuRE/Cut Buffer B – 5 µl, BamHI – 0.1 µl, and nuclease-free water – 10 µl to make a total volume of 25 µl. The mixture was incubated at 37°C for 1 hour. The products were resolved using electrophoresis in 2% agarose gel [Figure 1].

Statistical analysis

Fisher's exact test was used to examine the significance of odds ratio in the distribution of genotypes between cases and controls. Hardy–Weinberg equilibrium was tested. Mann–Whitney U test (for non-parametric data) and one-way analysis of variance followed by Tukey's post-hoc test were used to make comparisons of the parameters between the groups. *P* value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS 17 software versions.

Results

General characteristics of study population

In this study all the MetS cases fulfilled IDF criteria's for MetS. The age range of case population was 25–60 years with the mean age of 37.20 ± 9.86 years with 10 males and 30 females, and control population ranged from 18 to 64 years with the mean age of 38.18 ± 11.53 years with 12 males and 28 females. The age and sex distribution of the groups were statistically similar (*P* > 0.05). All the cases had central obesity as measured by increased WC and 70% were obese (BMI >30.0 kg/m^2). 12.5% cases in this study were hypertensive with impaired fasting glucose (77.5%), low HDL-C (80%), and hypertriglyceridemia (75%).

Serum ox-LDL

The serum levels of ox-LDL were significantly increased in cases in comparison to controls (*P* value of <0.0001 when calculated by Mann–Whitney U test).

The median and ranges of serum ox-LDL in cases were 312.20 pg/ml, 186.95–1300.64 pg/ml, and in controls were 178.11 pg/ml, 45.54–363.66 pg/ml, respectively [Figure 2].

Genotype distribution

Only 1 case had the CC genotype, 9 had the heterozygous CT genotype, and 30 had the TT genotype. Eleven healthy controls had the CC genotype, 29 had heterozygous CT genotype, and none had the TT genotype [Table 1].

Of all the components and features of MetS, there was a significant positive association of the TT genotype with obesity. Rest, including ox-LDL were not found to be significantly associated [Table 2].

Discussion

MetS has been reported to be associated with a higher prevalence of small dense LDL and among ethnic groups,

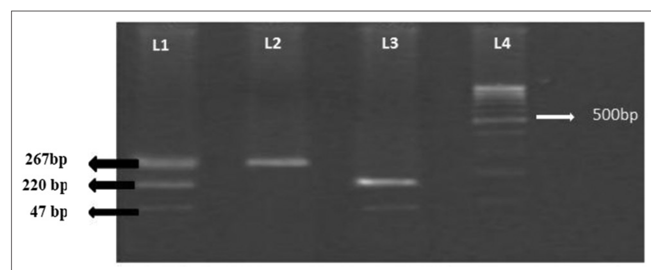

Figure 1: RFLP for detection of IVS4-73C>T polymorphism: Showing ethidium bromide stained BamHI digested products electrophoresed on 2% agarose gel. Lane 1: heterozygous CT genotype; Lane 2: homozygous TT genotype; Lane 3: homozygous CC genotype; Lane 4: 100 bp DNA Ladder

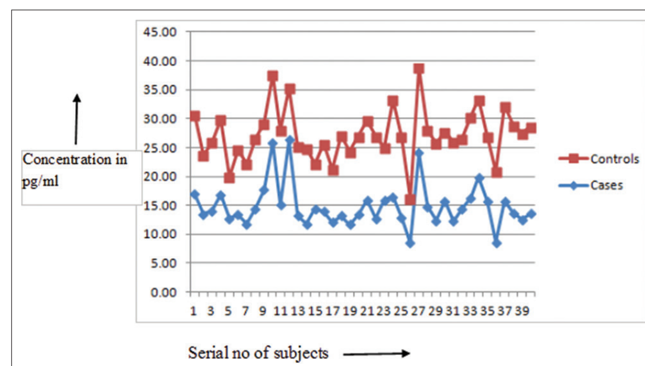

Figure 2: Line graph showing serum ox-LDL levels (pg/ml) in cases and controls

Table 1: Genotypic and allelic frequencies of OLR1 gene (rs 3736234)

	MetS cases (*n*=40)	Controls (*n*=40)	OR (95%)	*P**
Genotypes, *n* (%)				
CC	1 (2.5%)	11 (27.5%)	1	
CT	9 (22.5%)	29 (72.5%)	467.7 (17.73-12336)	<0.0001
TT + CT	39 (97.5%)	29 (72.5%)	14.79 (1.8-121.5)	0.003
Allelic frequency				
C	0.14	0.64		
T	0.86	0.36		

**P* calculated by Fisher's-exact test

Table 2: Comparison of age and other clinical characteristics among the metabolic syndrome subjects with CC, CT, and TT genotype of OLR1 gene (rs 3736234)

Characteristics	Mean+SD		
	CC	CT	TT
Age (years)	45.00	39.33+10.874	36.30+9.685
BMI (kg/m3)	31.7300	31.6832+2.60252	33.6833+3.93303*
Waist circumference (cm)	101.00	97.22+11.290	102.88+10.569
Fasting plasma glucose (mg/dl)	90.00	110.44+14.028	112.23+24.411
Systolic blood pressure (mmHg)	112.00	122.67+15.460	121.53+12.359
Diastolic blood pressure (mmHg)	68.00	77.78+8.800	76.20+7.303
Serum triglyceride (mg/dl)	170.00	172.11+6.75	239.93+13.69
Serum HDL-C (mg/dl)	31.00	40.00+7.906	42.73+6.192
Serum ox-LDL (pg/ml)	421.01	510.89+30.82	657.46+25.14

*P<0.0001 in comparison to CC genotype, by one-way analysis of variance followed by Tukey's HSD *post-hoc* test

Asian Indians are reported to show higher levels of small dense LDL particles.[10,11] As found in our study, there is a strong association of MetS with high concentrations of ox-LDL. Our findings are concurrent with studies in various populations and a study on Indian population.[11–13] As ox-LDL is taken up by scavenger receptors which are not down regulated, ox-LDL is more atherogenic than unmodified LDL. This might explain increased CAD risk in MetS cases.

To our knowledge, no data exist on the *OLR1* gene polymorphism in patients with MetS in Indian population and the only previous study done by Palmieri *et al.*[14] could not establish significant relation between *OLR1* gene polymorphism and MetS. The present study shows that Indian subjects carrying T allele of *OLR1* (IVS4-73 C > T) have nearly 15 times higher risk of developing MetS [Table 1]. Our finding is in accord with other studies on CAD patients, in particular, Mango *et al.* who showed that subjects with the TT or CT genotype at OLR1 3'UTR polymorphism are at higher risk of developing acute MI (OR: 3.74), and Chen *et al.* who showed that common genetic variation in the *OLR1* gene is associated with angiographic CAD in women.[15,16]

In this study, also the risk genotype TT of *OLR1*/IVS4-73 C > T was found to be significantly associated with high BMI, indicating the association of *OLR1* gene with obesity. LOX-1 is expressed not only in endothelial cells, but also in macrophages, vascular smooth muscle cells, platelets, and adipocytes. LOX-1 binds multiple ligands, has diverse physiological functions and plays a critical role in the signal transduction. In recent studies, LOX-1 upregulation is observed to be a vital factor in obesity and its complications and it is also claimed to contribute, at least in part, in the pathogenesis of obesity.[17] This might possibly explain the association between the *OLR1*/IVS4-73 C > T SNP and BMI in MetS cases.

OLR1 is responsible for internalization and degradation of ox-LDL. Hence a non-functional *OLR1* SNP is expected to have higher ox-LDL. But in the present study, no such association could be established [Table 2]. This might be because ox-LDL has other transporters and their role in ox-LDL catabolism might be greater than *OLR1*. How the other transporters of ox-LDL are modulated in MetS conditions and what happens to their level in cases with *OLR1* SNP are worth investigating. The limitation of small sample size should be kept in mind. The validity of the inferences is worth confirming in a wider population study.

Conclusion

The intronic SNP: IVS4-73 C → T of *OLR1* gene increases the susceptibility to develop MetS and has potential to be used as a molecular marker for the susceptibility and prognosis of MetS.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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