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ORIGINAL ARTICLE

Association of *PON1* L55M Polymorphism and Type II Diabetes Mellitus in a Navi Mumbai Population

Mary David[1], Latika Keni[2], Aryan Pawashe[3], Amrita Nair[4], Mustansir Bhori[5], Simran Keshwani[6], Deepali Vidhate[7], Debjani Dasgupta[8], Kanchanlata Tungare[9]*
1-4,6,8,9 School of Biotechnology and Bioinformatics, D Y Patil Deemed to be University
5 Invenio Life Technology Pvt Ltd., Office No. 118, Grow More Tower, Plot No. 5, Sector 2 Kharghar, Navi Mumbai, Maharashtra 410210, India
7 Department of Biochemistry, School of Medicine, D Y Patil Deemed to be University, Navi Mumbai, Maharashtra, India.
*Corresponding author email: kanchanlata.singh@dypatil.edu

ABSTRACT
Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia either due to reduced insulin secretion from the pancreas or due to insulin resistance causing reduced uptake of glucose in peripheral tissues. Macrovascular and microvascular complications arising due to T2DM increase mortality and morbidity. Lipid peroxidation and oxidative stress play a role in the pathophysiology of T2DM. SNPs in the gene of the antioxidant enzyme Paraoxanase 1 (PON1) may negatively affect its antioxidant activity This may increase oxidative stress leading to development of T2DM. This study aims to evaluate the use of the Leu55Met (L55M) variant of PON1as a diagnostic tool for identifying individuals at risk for developing T2DM. Genotypes of 56 T2DM cases and 60 controls were determined using Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR-RFLP) assay. Students t test was used to compare anthropometric and biochemical parameters between the case and control groups. Odds ratio analysis revealed that the L55M variant posed no significant T2DM risk in the Navi Mumbai population. However significant differences were observed in some anthropometric (age, weight, BMI, waist and hip circumferences, waist to hip ratios) as well as biochemical parameters (Triglycerides and Triglycerides to HDL ratios).
Keywords: Type 2 Diabetes Mellitus; oxidative stress; Paraoxonase 1; *PON1*; L55M

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INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a highly prevalent metabolic disorder that is characterised by higher blood glucose levels**.** It is a global health concern, affecting millions of people worldwide. It initiates insidiously with either reduced insulin expression or increased insulin resistance and progresses over time, impairing the body's ability to regulate glucose homeostasis**.** Untreated hyperglycaemia over a longer period of time leads to macrovascular and microvascular T2DM complications, most frequently resulting in multiple-organ damage and mortality.

Excessive free radical generation results in oxidative stress in human tissues, which is frequently considered to be the major causative factor for the onset and progression of T2DM and its associated complications [1]**.** Moreover, free radical generation can result in pancreatic beta cell damage that ultimately leads to cell death as a result of oxidative stress and inflammation in T2DM [2,3]. Elevated expression of inflammatory mediators as well as oxidative stress has been reported to be a significant predisposing attribute in the pathogenesis of T2DM by disrupting insulin signalling and resulting in insulin resistance, pancreatic β cell and mitochondrial dysfunctions, as well as reduced glucose tolerance, thereby, indicating a cardinal role that these factors play in the development of T2DM and its associated complications [4]**.**

Oxidative stress is attenuated mainly by an antioxidant family of three paraoxonases, PON1, PON2, and PON3, encoded by genes *PON1, PON2,* and *PON3* on the q arm of chromosome 7 (q21.22) [5]. These enzymes exhibit differential activity [6] and are also expressed differentially due to Single Nucleotide Polymorphisms (SNPs) in these genes [7, 8]**.** This could adversely affect both the oxidative stress levels and the onset of numerous metabolic disorders including T2DM. Numerous studies have been conducted to unravel the association between the *PON1* gene polymorphisms and risk of T2DM incidence across widely distributed populations over the globe, albeit with conflicting results [9, 10, 11]**.**

PON1 gene encodes a glycoprotein enzyme with paraoxonase, lactonase and arylesterase activities [12]**.** Though the expression of the *PON1* gene is observed in several tissues within the human body, it exhibits an elevated expression in the liver and is secreted into the bloodstream, where it associates with high density lipoproteins (HDL). The protective antioxidant characteristics of HDL is largely attributed to PON1, in addition to the associations with other antioxidant players such as lecithin cholesterol acyl transferase and platelet-activation factor acetyl hydrolase. PON1 has diverse significant functional roles, such as anti-inflammatory and anti-oxidative modes of activity that are carried out through its enzymatic activities [13]**.** PON1 enzyme also prevents cytotoxic damage of pancreatic beta cells that would result from the high glucose concentrations and plays a role in activating the insulin synthesis and secretion from these cells [14].

Previous studies by various groups have revealed that the enzymatic activity of PON enzymes has been reduced considerably in the patients with T2DM. Although more than 200 SNPs of the *PON1* gene have so far been identified through previous studies, there has been a greater focus on a few SNPs especially -909G/C [rs854572], −162A/G [rs705381], and −108C/T [rs 705379] from the promoter region, as well as Q192R [rs662], and L55M [rs 854560] from the coding region [9]**.**

According to the International Diabetes Federation (IDF), China, followed by India tops the Countries with maximum number of diabetes patients (116.4 million and 77 million respectively), with men having slightly higher prevalence rates of diabetes (9.6%) than women (9.0%)**.** In India, the percentage of people with diabetes has risen from 7.1% in 2009 to 8.9% in 2019. It is estimated that 25.2 million adults today have Impaired Glucose Tolerance (IGT) and it is predicted that by 2045, the number will rise to 35.7 million [15]**.** Hence, in developing economies, including China and India, diabetes is an immense economic burden. Therefore, identifying risk genes that would make individuals susceptible to T2DM and targets that would aid in the early detection and timely treatment of T2DM is of paramount importance.

Study objective

The association of *PON1* coding variant L55M gene polymorphism has been studied with regard to T2DM incidence in several populations albeit with conflicting results [9, 10]**.** The aim of our study is to determine the association of the *PON1* L55M polymorphism in the pathogenesis of T2DM in a Navi Mumbai population. Diverse number of population-based studies across the globe would aid in conclusive identification of a prominent predisposing biomarker that could identify the potential T2DM risk population.

MATERIAL AND METHODS

Study design and eligibility criteria

A total of 116 individuals were included in this case-control study. Subjects were recruited at the D Y Patil Hospital and Research Centre, Navi Mumbai, Maharashtra, India, based on defined inclusion and exclusion criteria. Prior to commencement of the study, an ethical clearance was obtained from the Ethical Committee of the hospital and an informed consent was obtained from each participant.

Subjects of either gender who were 18 years or above were included in the study. 56 subjects having fasting blood glucose of 126 mg/dl and above diagnosed with T2DM were included as case subjects in the study. 60 healthy individuals who served as controls in the study were from a volunteer population. Individuals suffering from hypertension, diabetic ketoacidosis, and renal failure and those taking lipid lowering drugs were excluded from the study.

Information such as age, sex, and history of diabetes was collected through a questionnaire. Height, weight, as well as waist and hip circumference of the participants were obtained by direct measurements. The body mass index (BMI) and waist to hip (W: H) ratios of all 116 individuals were calculated.

Sample collection

Blood samples were collected from the recruited subjects, after an overnight fasting of 12 hours, in EDTA containing evacuated tubes for genotype testing, Serum separating tubes for Lipid profile testing and Sodium fluoride tubes for glucose testing. The blood samples were analysed for biochemical parameters such as fasting blood glucose (FBG), and lipid profile [including total cholesterol (TC), triglycerides (TG), High density lipoprotein (HDL), low density lipoprotein (LDL), and very low-density lipoprotein (VLDL)].

DNA extraction

DNA was extracted from the collected whole blood samples using commercially available QiaAmp DNA Blood kits (Qiagen, USA) according to the manufacturer's protocol and was assessed spectrophotometrically for its purity and quantity.

Genotype analysis of samples

The L55M SNP containing sequence in the genomic DNA was amplified using primers obtained from previously published articles [16]. For the amplification, a 2X PCR Mastermix (Cat. No. 786-449B, G-Biosciences, USA) containing Taq pol, dNTPs, $MgCl_2$**,** primers were used in a total volume of 25 µl. The mix was subjected to an initial denaturation at 94°C for 3 mins and cycles of denaturation 94°C for 30 seconds, annealing 59°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes in a thermal cycler to yield a 138 bp amplicon. Amplification of the correct sequence was confirmed using a 2% agarose gel, electrophoresed along with a reference GeneRuler 100 bp DNA ladder (Cat. No. SM0243, ThermoFisher Scientific, USA). The 138 bp amplicons were then subjected to RFLP analysis using the Hin1II restriction enzyme which cuts the M allele to yield digested products of 66 and 72 bp. The recommended digestion conditions for Hin1II enzyme is 37°C for 24 hours. Genotype of samples was assessed using a 10% PAGE gel along with a 100 bp ladder. PAGE gel was observed and results documented on using the Syngene software system.

Statistical Analysis

Statistical analysis included comparison of anthropometric and biochemical parameters of the 56 T2DM cases with that of 60 individuals of the control group. The results of comparison of anthropometric and biochemical data were expressed as mean ± standard deviation, and the statistical significance of their differences was assessed using the unpaired two-tailed student's t-test through GraphPad Prism Software v8. A significance level of $p < 0.05$ was used to determine statistical significance.

Genotype frequencies (manually calculated as number. of individuals of a genotype/ Total no. of individuals in sample population) and allele frequencies (manually calculated as total number of a particular allele in population/ total alleles in the sample population) of the *PON1* L55M variants in case and control populations were calculated. The goodness of fit of the sample population in this study with the general population was assessed by testing if the population obeyed Hardy-Weinberg's equilibrium (HWE) law. HWE analysis was performed using the online HWE Wpcalc calculator (https://wpcalc.com/en /equilibrium-hardy-weinberg/). Odds ratios were calculated to assess the relative risk caused by a particular genotype or allele with respect to another genotype or allele based on the distributions of genotypes and alleles in case and control groups. Calculation of odds ratios were carried out using the online Medcalc Odd's ratio calculator (https://www.medcalc. org/calc/odds_ratio.php). Differences in the genotype distribution of *PON1* gene to determine the association of genotypes/ alleles with the pathogenesis of T2DM was analysed by using the online Social Science statistics' Chi square calculator (https://www.socscistatistics. com/tests/chisquare/ default2.aspx).

RESULTS & DISCUSSION

Comparison of Anthropometric and Biochemical parameters between case and control groups

The comparison of anthropometric and biochemical parameters between T2DM cases and controls are represented in Table 1. The case group exhibited significantly elevated measurements for demographic factors such as age, BMI, waist and hip circumferences and W:H ratios as also in their biochemical parameters of TG value and calculated TG to HDL ratios.

As described in Table 1, significant difference was observed in the ages of incidence of T2DM cases over the control group, with the mean age of T2DM cases being 55.36 ± 11.35 years and that of controls being 36.28±14.75 years. A possible explanation for this finding in the Navi Mumbai population where the study was conducted is that aging brings about increased oxidation of cellular macromolecules including DNA and proteins causing mutations and malfunctioning of cells in various tissues and organs which could lead to the onset of various diseases including diabetes [17]**.**

PARAMETERS	CASES	CONTROL
	Mean ± SD	Mean ± SD
AGE (years)	55.36 ± 11.35	36.28 ±14.75***
BMI (kg/m2)	25.8 ± 4.45	22.49 ± 3.87***
WC (cm)	98.44 ± 13.30	86.55 ± 10.22***
HC (cm)	101.64 ± 12.44	93.88 ± 11.72***
W:H	0.97 ± 0.05	0.93 ± 0.06**
FBS (mg/dl)	161.49 ± 75.02	92.52 ± 10.38 ****
TC (mg/dl)	184.97 ± 37.23	174.75 ± 36.34NS
TG (mg/dl)	139.65 ± 59.74	114.15 ± 75.79**
HDL (mg/dl)	45.25 ± 29.22	43.13 ± 14.41NS
LDL (mg/dl)	109.36 ± 42.92	106.58 ± 30.14 NS
VLDL (mg/dl)	28.36 ±15.31	26.01 ± 20.75 NS
TG/HDL	3.73 ± 1.66	2.9 ± 2.35*

Table 1: Anthropometric and Biochemical parameters assessed between case and control groups.
*The symbols in the table indicate statistical significance: NS- -Nonsignificant, *p> 0.05, **p >0.01, *** p> 0.001*

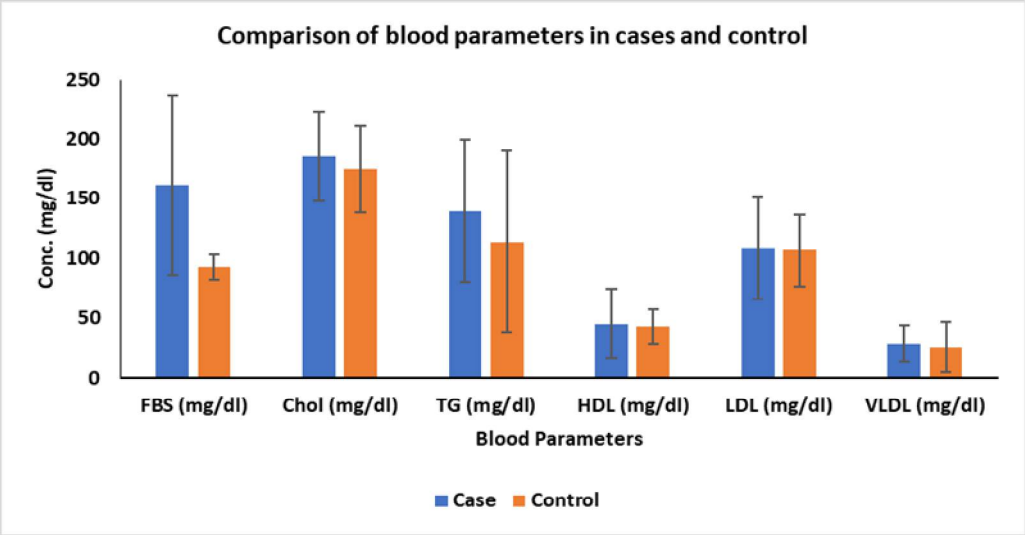

Figure 1: Comparison of blood parameters in cases and control

In addition to age, the anthropometric measurements of BMI, hip circumference, waist circumference, and W: H ratio also revealed statistical significance with notable differences between the T2DM case and control groups in the Navi Mumbai population. These parameters being indicators of obesity, could correlate with our observation that the T2DM cases were obese as compared to the controls in our study population. Several studies have shown a strong link between T2DM and obesity, with the risk of T2DM increasing with an increase in body weight. Obesity increases the chances of insulin resistance induced T2DM as absorption of glucose by cells is negatively influenced by exposure to free fatty acids and other adipocyte derived bioactive and proinflammatory compounds [18].

The biochemical parameters analysed in the current study are listed in Table 1. Among biochemical parameters, serum TG levels were higher in T2DM cases as compared to the controls in the Navi Mumbai study population. Hypertriglyceridemia is a consistent symptom associated with the T2DM conditions and is usually also observed along with low levels of HDL [19].

In our small study population, the calculated TG to HDL ratios displayed significant higher values in T2DM cases in comparison to the controls. This in all probability reinforces the important role that antioxidant enzymes such as PON1, which in association with HDL play a role in relieving oxidative stress caused by TG and its proinflammatory products.

Gender distribution in cases and controls

The percentage distribution of males and females within the T2DM case and control groups in Navi Mumbai population-based study is given in figure 2 & 3. Males were considerably higher in comparison with women in incidence of T2DM within the case subjects. On the other hand, women constituted a higher sub-group as compared to the males within the control group in the study within the Navi Mumbai

population. This is possibly due to the protective antioxidant and insulin regulatory activities of estrogen receptors and estrogen in women [20, 21].

Estrogen (E2) may have genomic and non-genomic antioxidant activities. E2 may activate the transcription of genes that control mitochondrial function. Impaired mitochondrial function due to an E2 deficiency may cause insulin resistance, reduced fatty acid oxidation leading to inhibition of insulin signalling. The non-genomic antioxidant activity of E2 may be twofold - by the activation of pathways preventing the synthesis of ROS and by scavenging already generated ROS.

Estrogen deficiency can cause a sudden decrease in metabolic rate leading to weight gain and increased central adiposity, dyslipidemia ultimately causing metabolic syndrome and diabetes.

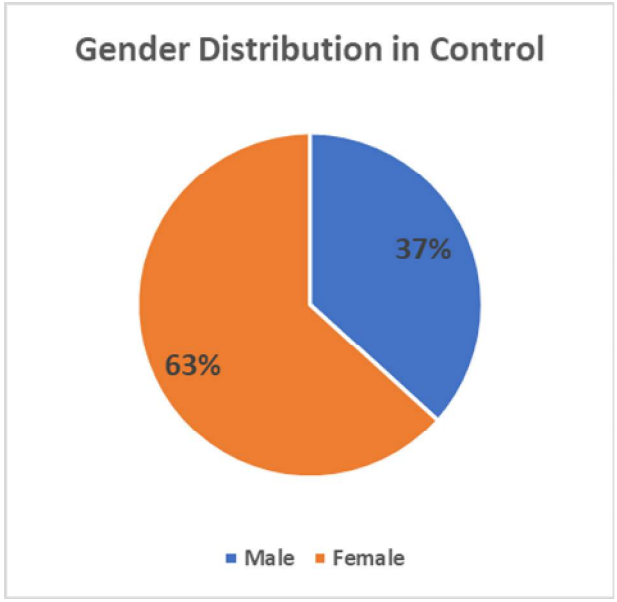

Figure 2: Gender Distribution in control

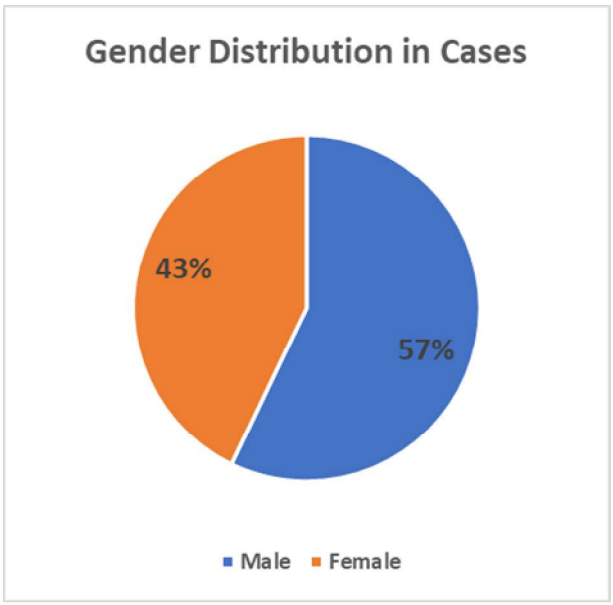

Figure 3: Gender Distribution in Cases

Comparison of genotype and allele frequencies at the L55M SNP between case and control populations

Determination of genotypes of samples

Subsequent to PCR-RFLP analysis, samples of LL genotype appeared on a 10% PAGE gel as single uncut bands of 138 bp, the LM genotype samples as having two bands - an uncut 138 bp and a cut band of 72 bp and the MM genotype as a completely cut band of about 72 bp as shown in ***Fig 4***.

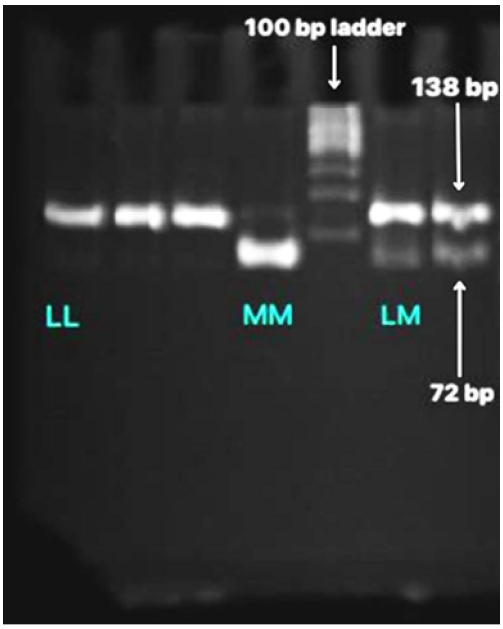

Figure 4: RFLP analysis of PCR products using Hin1 II restriction digestion. Lane 1: Sample 2-LL genotype, Lane 2: Sample 5-LL genotype, Lane 3: Sample 11-LL genotype, Lane 4: Sample 12-MM genotype, Lane 5: 100 bp ladder, Lane 6: Sample 13-LM genotype, Lane 7: Sample 14-LM genotype

Distribution of genotypes and alleles in cases and controls

Genotype frequencies and allele frequencies of the *PON1* L55M variants in case and control populations are as presented in Table 2.

***PON1* L55M**	**Cases**	**Genotype frequency (Cases)**	**Controls**	**Genotype frequency (Controls)**
n	56		60	
LL	28	0.5	30	0.5
LM	25	0.45	28	0.47
MM	03	0.05	02	0.03
Total no. of alleles	112	**Allelic frequency**	120	**Allelic frequency**
L	81	0.72	88	0.73
M	31	0.28	32	0.27
χ^2 for goodness of fit or HWE		For Cases = 0.74181		For Controls = 2.23915

Table 2: Genotype (LL, LM and MM) and allele (L & M) frequencies in cases and controls in the Navi Mumbai population

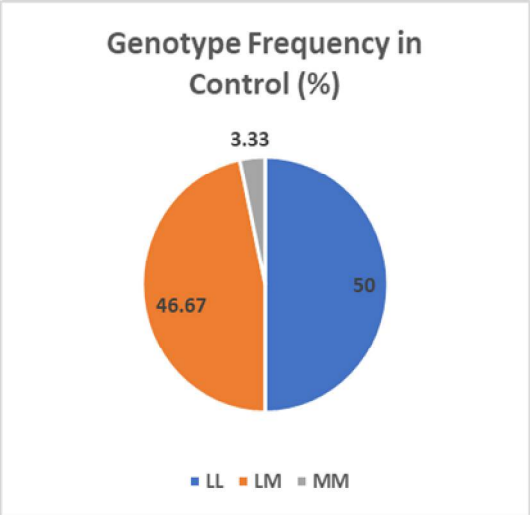

Figure 5: Genotypic Frequency in control in percent

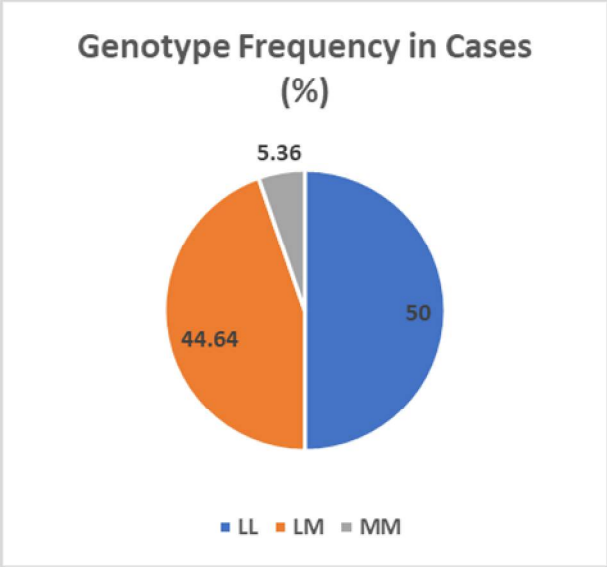

Figure 6: Genotypic Frequency in cases in percent

HWE analysis for the case-control populations

Assessment of goodness of fit of sample case, control and total populations with general population by HWE analysis using the Wpcalc calculator (https://wpcalc.com/en/equilibrium-hardy-weinberg/), displayed acceptable goodness of fit for all the groups as the Chi square value for all groups was below the critical value of 3.84 (Table 2).

χ^2 analysis to compare genotype distribution in cases and controls

Genotype distributions of LL, LM and MM genotypes were similar in both the T2DM cases and control groups in this Navi Mumbai population-based study. The χ^2 statistic for genotype distributions in cases and controls was calculated using Social Science statistics' Chi square calculator (https://www.socscistatistics.com/tests/chisquare/default2.aspx) and was found to be 0.3012 with p value being 0.86. Individuals with the MM genotype were represented slightly more in the T2DM case group as compared to the control group.

Odds Ratio analysis for the case-control populations

Results of odds ratio analysis to assess the relative risk of a particular genotype with respect to other genotypes or allele, carried out using the Medcalc Odd's ratio Calculator (https://www.medcalc.org/calc/odds_ratio.php) are as presented in Table 3. Odds ratio analysis revealed that MM genotype presented a higher relative risk of 1.68 in comparison to individuals with LM genotype and a relative risk of 1.607 with individuals having LL genotype. However, despite showing higher odds ratios the risk of MM genotype and that of M allele did not achieve statistical significance (p value) possibly due to the lower incidence numbers of the MM genotype in the small Navi Mumbai population case group involved in the study.

L55M Genotype	P- value	Odds ratio value (95% CI)
MM Vs LM	0.5864	1.68 (0.2593 to 10.8866)
LM Vs LL	0.9072	0.9566 (0.4538 to 2.0166)
MM Vs LL	0.6175	1.6071 (0.2497 - 10.3433)
Alleles		
M vs L	0.8625	1.0525 (0.5900 to 1.8776)

Table 3: Fisher's exact test values for *PON1* L55M polymorphism

Comparison of Anthropometric and Biochemical Parameters with regard to PON1 Genotypes

A comparison of anthropometric and biochemical parameters of LL, LM and MM genotypes in T2DM cases revealed that individuals with MM genotypes developed T2DM at an earlier age and despite having lower indices for obesity (BMI, WC) (Table 4). They also seemed to have a more atherogenic lipid profile, with higher cholesterol, TG, LDL and VLDL levels and also higher TG: HDL ratios. HDL levels were also found to be lower in MM cases when compared to individuals with LL genotypes. As previously reported PON1 is associated with HDL and augments its antioxidant activity. Thus, a lowered HDL level may indicate a

lower antioxidant activity of PON1, contributing to increased insulin resistance in peripheral tissues.

T2DM Cases Genotypes	LL	LM	MM
AGE (years)	54.29 ± 11.50	57.28 ± 11.30	49.33 ± 10.21
BMI (kg/m2)	26.55 ± 5.06	25.56 ± 3.66	22.33 ± 3.51
WC (cm)	98.07 ±13.58	99.42 ± 13.64	93.67 ± 9.50
HC (cm)	101.29 ± 13.07	102.4 ± 12.63	98.67 ± 3.06
W:H	0.96 ± 0.05	0.98 ± 0.04	0.97 ± 0.06
FBS (mg/dl)	167.87 ± 77.14	156.48 ± 75.78	143.66 ± 64.45
TC (mg/dl)	178.47 ± 35.66	190.88 ± 39.35	196.4 ± 33.57
TG (mg/dl)	134.30 ± 50.96	144.25 ± 63.08	151.20 ± 120.22
HDL (mg/dl)	47.22 ± 39.85	43.15 ± 11.41	44.30 ± 19.10
LDL (mg/dl)	107.35 ± 38.09	110.96 ± 49.98	114.76 ± 30.82
VLDL (mg/dl)	28.60 ± 16.35	27.83 ± 13.60	30.33 ± 23.96
TG/HDL	3.63 ± 1.67	3.75 ± 1.70	4.38 ± 1.58

Table 4: Anthropometric and Biochemical parameters of T2DM cases assessed with regard to *PON1* genotype variants

Comparison of PON1 genotype frequencies between diverse population studies

There are several studies that have tried to correlate the frequencies of LL, LM, and MM genotypes of the *PON1* gene and assess their frequencies with regard to T2DM incidence across different populations. Diverse population-based studies would promote assessing the score of a particular genotype in predicting the risk factor for T2DM occurrence in the population. We try to compare various such studies with regard to our data from the Navi Mumbai population in Table 5.

	CASES			CONTROLS			
Studies	**LL**	**LM**	**MM**	**LL**	**LM**	**MM**	**Reference**
Navi Mumbai population in current study	0.50	0.45	0.05	0. 50	0.47	0.03	
Czech population	0.34	0.48	0.18	0.48	0.42	0.10	**[10]**
Turkish population	0.54	0. 42	0.39	0.47	0.47	0.64	**[11]**
Manchester population	0.46	0.42	0.12	0.37	0.53	0. 09	**[22]**
Iranian population	0.44	0.43	0.13	0.45	0.4	0.15	**[23]**
Egyptian population	0.33	0.48	0.20	0.60	0.35	0.05	**[24]**
North-West Indian population	0.704	0.28	0.2	0.64	0.337	0.6	**[9]**
South Indian population	0.62	0.33	0.05	0.8	0.19	0.01	**[25]**

Table 5: Frequency of Genotypes in Cases and controls in different populations

A study by Flekac et al., in the Czech Republic revealed a significant increase in MM genotype frequencies in cases as compared to controls [10]. Our study however failed to show a statistically notable difference in genotype distributions between cases and controls. Agachan et al., had reported in their investigation in 2005 that the MM genotype had higher levels of lipid peroxidation products and dienes as compared to LL genotype indicating a reduced activity of PON1 in metabolizing the lipoperoxides. As per their investigations they had postulated a protective role for the LL genotype [11]. In our study, the Navi Mumbai population T2DM patients with the MM genotype had higher TG: HDL ratios and a more atherogenic lipid profile as compared to LL and LM genotypes in cases but not in controls. Studies by Mackness et al found that. PON1 activity was found to be maximal in LL genotypes and least in MM genotypes with an intermediate activity in LM genotypes in both cases as well as controls [22]. Genotypic and allelic frequencies of LL, LM and MM in cases and controls in an Iranian Population were similar and did not show any significant difference as was the case in our Navi Mumbai population. [23].

In yet another study involving an Egyptian population, the frequency of the MM genotype was significantly higher in Diabetic patients as compared to controls [24]. In the Navi Mumbai population, though individuals with MM genotype had a higher representation in cases, the disparity was statistically insignificant. However, a study among North Indians carried out by Gupta et al., revealed no significant difference between genotypes LL, LM & MM in Cases and Controls. Nevertheless, the LL genotype in controls displayed significantly higher PONase activity as compared to the other genotypes [9]. Investigations by Gomathi et al on the association of *PON1* L55M polymorphism in a Southern India population revealed significantly increased odds ratios for LM and MM genotypes and of the M allele in increasing susceptibility to T2DM [25]. In our study also, the MM genotype was found to have a higher Odds ratio increasing risk of developing T2DM. However, the OR did not achieve statistical significance as

the Navi Mumbai sample population was small. Conversely, Sampson et al., correlating L55M genotypes with lipid profile in T2DM patients, found that male individuals with the LL genotype had a more atherogenic lipid profile as compared to individuals having at least one M allele and also had higher levels of oxidized LDL. As per their investigations, individuals with LL genotype may have higher levels of oxidative stress which in turn could affect beta cell functioning and insulin sensitivity through increased lipid peroxidation leading to development of T2DM [26]. On the contrary in our Navi Mumbai T2DM cases, individuals with MM genotype had a more atherogenic lipid profile than individuals with either LL or LM genotypes.

Association studies of the L55M polymorphism have been carried out in diverse populations-based studies across the globe. Several studies had implicated the contributory role of the MM genotype and M allele to a more oxidized state in cells and tissues. This could be due to the unique locus of the L55M polymorphism in the immediate vicinity of catalytically critical residues E52 and D53 [27] identified as important in PON1 functioning by site directed mutagenesis. A variation in this site could have far reaching implications in PON1 action/ function. An investigation by Garin et al [7] had revealed that individuals with MM genotype had lower serum PON concentrations as compared to LL and LM genotypes. Liviev et al [8] had further extended their studies to include an analysis of mRNA levels of L & M isoforms of PON1 in hepatic tissues of individuals with LM genotype and discovered that L and M alleles are differentially expressed in hepatic tissues, thus explaining the discrepancy in serum levels of L and M isoforms. Investigations by Abd El Raouf et al [6] on PON1 activity in LL, LM and MM genotypes in diabetic patients had further revealed that PON1 activity was maximal in diabetic patients with LL genotype in comparison with patients of MM or LM genotype.

Since our study population is small, it has to be further extended to a larger population to unequivocally prove the liability of the L55M polymorphism in the pathogenesis of T2DM. Though statistically no association was established between *PON1* genotype variants and risk of T2DM incidence in individuals, there are probabilities of its association with further secondary complications associated with T2DM, which has to be analysed in detail across diverse populations, as well.

CONCLUSION

In our present investigation on the association of the Leu55Met (L55M) variant of PON1 in pathogenesis of T2DM in the Navi Mumbai population, we found no significant difference in genotype distributions between cases and controls though there was a slight increase in frequency of MM genotype in cases. Moreover, odds ratio analysis done for determining relative risk of the MM genotype over LL and LM genotypes revealed that the 1.6 times increased relative risk was insignificant. Therefore, our investigation confirms that L55M is not associated with pathogenesis of T2DM in Navi Mumbai population and hence is not fit for using it as diagnostic tool in early detection of T2DM. However anthropometric parameters viz. age, BMI, waist and hip circumference and W:Hip ratios as also biochemical parameters viz. Triglycerides and TG:HDL ratios were significantly increased in cases as compared to controls.

We further plan to investigate association of other variants of PON1 with pathogenesis of T2DM in Navi Mumbai population. Since oxidative stress is a key factor leading to development of diabetes, more SNPs / genotypes/ haplotypes of genes of key players that alleviate oxidative stress in humans need to be studied on a larger population in a global scale. Establishing a biomarker for early detection of diabetes will revolutionise the healthcare sector and will significantly reduce global burden caused by diabetes.

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Conflict of interest

Authors declare no conflict of interest

Abbreviations:

T2DM - Type 2 Diabetes Mellitus, IDF – International Diabetes Federation, FBG – Fasting Blood Sugar, IGT – Impaired Glucose Tolerance, SNP – Single Nucleotide Polymorphism, PON1 – Paraoxonase 1, L55M – Leucine to Methionine at position 55 of the PON1 enzyme, PCR – Polymerase Chain reaction, RFLP – RFLP – Restriction Fragment Length Polymorphism, PAGE – Polyacrylamide Gel electrophoresis, BMI – Body Mass Index, WC – Waist circumference, HC – Hip circumference, W:H – Waist to Hip ratios, HDL – High

Density Lipoprotein, LDL – Low Density Lipoprotein, VLDL – Very Low Density Lipoprotein, TC - Total Cholesterol, TG – Triglycerides, HWE – Hardy Weinberg Equilibrium.

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