PARAOXONASE-1 (PON-1) L/M 55 GENE POLYMORPHISM IN THE CASES OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

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OBJECTIVES. Viral infections cause to trigger the body to produce antibody destroy the thrombocytes in the pathogenesis of ITP. That antibody depended hydrogen peroxide production cause to cellular damage, which is an oxidant yield, is an another reason for occurring ITP. Oxidative stress and free-radicals could be responsible the pathogenesis and prognosis of ITP. PON-1 is an antioxidative feature. In this study, it had investigated that the role of PON-1 Leu (L)/Met (M) 55 gene polymorphism in etiopathogenesis of ITP, course of disorder and its effects of the therapy.

METHODS. 51 patients with acute, 15 patients with chronic ITP and 60 healthy controls were investigated (Table I, II). Treatment to be used was selected randomly among higher doses of methylprednisolone (HDMP, 30 mg/kg/d for 3, and then 20 mg/kg/d for 4 days), standard dose methylprednisolone (SDMP, 2 mg/kg/d), intravenous immune globulin (IVIG, 0.5 mg/kg for 5 days), and anti-D (50 µg/kg). Approval from the Ethics Committee (# 1600), and informed consent of the parents were obtained.

DNA purification was realized using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The primer used (oligonucleotide) in the analysis was purchased from Bio Basic firm (Bio Basic Inc., Ontario, Canada). The fragment Hsp192 II belonging to the locus PON-1 55 was amplified by PCR, cleaved by restriction endonuclease, subjected to 2% agarose gel electrophoresis, and genotyped. PON-1 L/M 55 gene polymorphisms are shown in Figure I. As seen in Figure II, L allele was detected at 170 bc, and M allele at 126 bc, and 44 bc bands, respectively.

For intra-, and intergroup analyses, one way variance analysis (ANOVA), and for post-ANOVA tests LSD, and Tukey B tests were used. Differences in genotype distribution was evaluated using *chi*-square test. The significance of G, and T allele frequencies was evaluated using Fisher's Exact Test.

RESULTS. Most common genotype in all patients with ITP, acute and chronic ITP group, control group was LM genotype, while MM genotype was found lower in all groups. The frequency of L allel in chronic ITP group were higher. For acute ITP group, the frequency of M allel were higher than the frequency of chronic group. Individuals with a LM genotype were found to be more resistive to anti-D therapy. It is recommended to use HDMP or IVIG for patients with LL and LM genotype and only IVIG for patients with MM genotype (Table 1-3, Figure 1-3).

CONCLUSION. PON-1 L/M 55 gene polymorphism in the cases of ITP displayed differences when compared those of controls. It could effect course of disorder, even might change response of treatment. In cases with ITP, evaluation of PON-1 Q/R 192, and L/M 55 polymorphism in conjunction with PON-1 activity will yield more illuminating results.

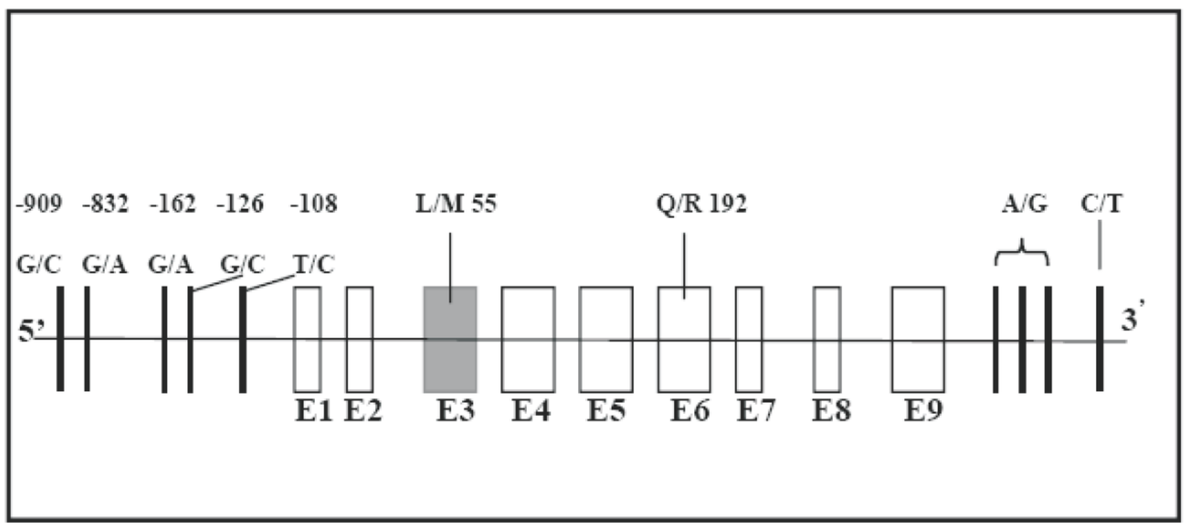

Figure I. PON-1 L/M 55 gene polymorphisms
The structure of PON-1 gene is shown with 9 exon (E1-E9) boxes. Five polymorphisms at 5'regulatory end,
2 polymorphisms in the coding region., and 4 polymorphisms at 3' untranslated end are seen.

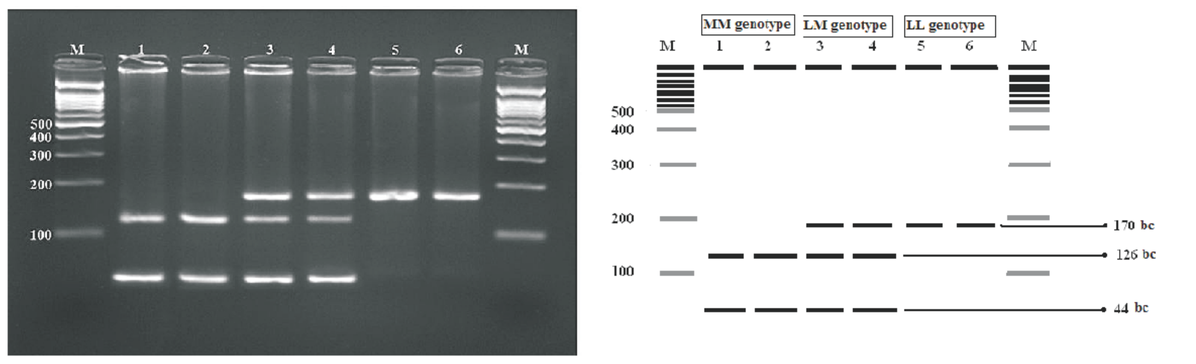

Figure IIa, IIb: PCR products cut by Hsp92 II enzyme related to PON-1 L/M 55 polymorphisms in our cases as seen in agarose gel electrophoresis.
Case 1 and 2: MM genotype, Case 3 and 4: LM genotype,
Case 5 and 6: LL genotype. L allel 170 base couple (bc), M allel 126 and 44 bc. M: 100 bc DNA dimension marker

Table I. Demographic characteristics in ITP and control groups

	Total ITP (a)	Acute ITP (b)	Chronic ITP (c)	Control (d)	p<0.05
n	66	51	15	60	
Age (mean±SD,year) (min-max)	7.24±3.90 (1.5-16)	6.33±3.62 (1.5-14)	10.33±3.26 (4-16)	7.45±3.76 (1.5-16)	b-c, c-d
Gender (%)	34M/32 F (52/48)	29 M/22 F (57/43)	5 M/10 F (33/67)	39 M/21 F (65/35)	b-c, c-d

Table II. Dispersion of genotype of PON-1 L/M 55 gene polymorphism and frequency of L/M allel in the patient and control groups

	Total ITP n=66 (1)	Acute ITP n=51 (2)	Chronic ITP n=15 (3)	Control n=60 (4)	p<0.05
L/M 55 dispersion of genotype					
LL (n, %)	16 (24.2)	10 (19.6)	6 (40)	16 (26.7)	2-3, 2-4, 3-4
LM (n, %)	38 (57.6)	31 (60.8)	7 (46.7)	39 (65)	2-3, 3-4
MM (n, %)	12 (18.2)	10 (19.6)	2 (13.3)	5 (8.3)	1-4, 2-3, 2-4, 3-4
Frequency of allel					
L (n, %)	70 (53)	51 (50)	19 (63.3)	71 (59.2)	1-4, 2-3, 2-4
M (n, %)	62 (47)	51 (50)	11 (36.7)	49 (40.8)	1-4, 2-3

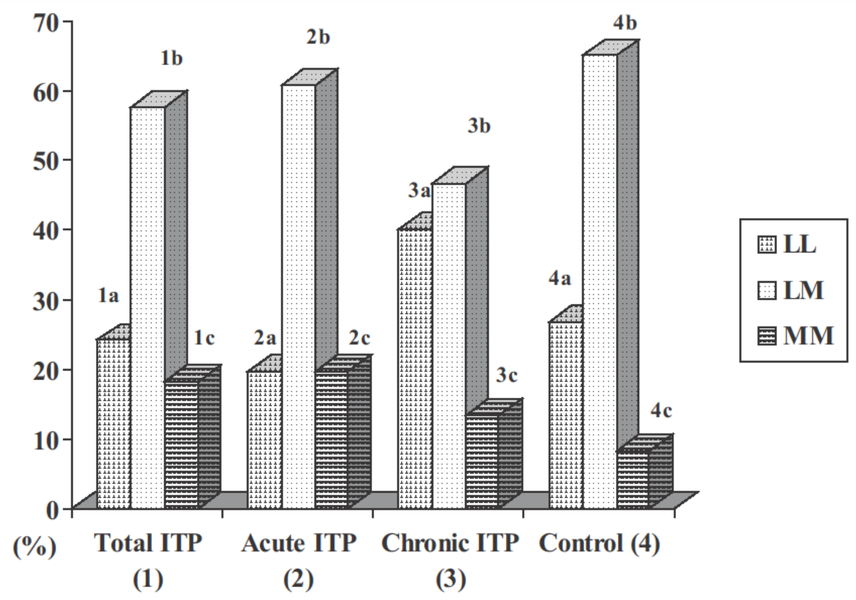

Figure III. Dispersion of PON-1 L/M 55 genotype in the patient and control group

2a-3a= p<0.05 2b-3b= p<0.05 1c-4c= p<0.05
2a-4a= p<0.05 3b-4b= p<0.05 2c-3c= p<0.05
3a-4a= p<0.05 2c-4c= p<0.05
3c-4c= p<0.05

Table III. Distribution of PON-1 L/M 55 genotypes among groups, and its relation with age, and thrombocytic parameters.

	LL n=10 (a)	LM n=31 (b)	MM n=10 (c)	LL n=6 (a)	LM n=7 (b)	MM n=2 (c)	LL n=16 (a)	LM n=39(b)	MM n=5 (c)
Age (year) (mean±SD) (min-max)	6.25±3.77 (1.5-12)	5.53±3.13 (1.5-14)	8.9±4.04 (2.5-14)	10.91±3.72 (5.5-16)	10.42±2.33 (7.5-14)	8.25±6.01 (4-12.5)	7.53±4.15 (2-16)	7.12±3.51 (1.5-14)	9.7±4.38 (2-13)
p	1b-1c= p<0.05			2a-2c= p<0.05, 2b-2c= p<0.05			3a-3c= p<0.05, 3b-3c= p<0.05		
Platelet (10^3/mm^3) (mean±SD) (min-max)	15.10±9.93 (2.0-36.0)	12.86±13.58 (2.0-56.0)	17.50±18.75 (2.0-55.0)	30.33±33.7 (6.0-89.0)	12.14±12.5 (3.0-39.0)	5.50±0.70 (5.0-6.0)	352.50±103.70 (211.0-586.0)	326.00±71.02 (201.0-468.0)	279.80±40.27 (231.0-331.0)
p	1b-1c= p<0.05			2a-2b= p<0.05, 2a-2c= p<0.05, 2b-2c= p<0.05			AD		
MPV (fL) (mean±SD) (min-max)	11.05±2.44 (8-15.3)	11.57±3.86 (4.7-20.3)	9.69±3.44 (3.8-16.4)	12.51±1.50 (10.4-14.5)	12.28±1.32 (10.4-14.6)	15.75±6.29 (11.3-20.2)	10.61±1.92 (8.3-13)	10.52±1.53 (8.4-13.0)	10.10±1.90 (9.1-13.5)
p	AD			2a-2c= p<0.05, 2b-2c= p<0.05			AD		
PDW (mean±SD) (min-max)	34.45±21.91 (14.5-73.4)	33.69±18.24 (12.4-78.0)	33.53±17.83 (11.2-67.3)	34.23±8.92 (22.0-42.0)	33.78±6.32 (26.8-45.0)	46.0±4.24 (43.0-49.0)	28.66±6.33 (19.4-38.7)	31.0±6.60 (18.5-44.0)	35.56±5.49 (26.3-40.5)
p	AD			2a-2c= p<0.05, 2b-2c= p<0.05			3a-3c= p<0.05		

AD: p>0.05

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