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# Contribution of Pon1 Polymorphism in Senile Cataract among Diabetic and Non-Diabetic Egyptian Patients

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## ABSTRACT

The development of senile cataract is a multifactorial process with oxidative stress. Cataract is one of the complications of diabetes mellitus. Paraoxonase (PON) enzyme is an antioxidant high-density lipoprotein (HDL)-associated enzyme. In mammals, three genes of paraoxonase, PON1, PON2, and PON3, have been identified. This study aims to assess the contribution of PON1-55 and PON1-192 gene polymorphisms as risk factors for senile cataract formation among diabetic and non-diabetic Egyptian patients**.** 132 Egyptian cataract patients (66 without and 66 with diabetes) and 106 healthy subjects with matched age and sex were included in the present study using multiplex PCR followed by restriction fragment length polymorphism analysis. The study revealed that there was a significant difference in genotypes distribution of PON1-55; LL, LM and MM ($p$=0.0001) and in PON1-192 genotypes; QQ, QR and RR ($p$=0.0001) among cataract patients with and without diabetes and controls. Also there was a significant difference in L and M ($p$=0.003) and in Q and R frequencies ($p$=0.005) among cataract patients with and without diabetes and controls. In addition there was a significant difference in the distribution of 55 LM/192 RR combined genotypes with the highest frequency in cataract diabetic subgroup (75%), while 55LL/192RR, 55LL/192QR and 55LM/192QRcombined genotypes showed the highest frequencies among the control group (52.4%, 59.1% and 66.7% respectively). For the first time, we provide evidence that polymorphisms in the PON1 gene may influence the risk of cataract in both non-diabetic and diabetic in Egyptian populations, suggesting new clues that help to clarify the pathogenesis of cataract.

**Key words:** Paraoxonase (PON), multiplex polymerase chain reaction (MPCR), PON1-55 and PON1-192 polymorphisms, diabetic cataract.

## INTRODUCTION:

Cataract development is usually very slow or gradual process but in some cases it could occur rapidly and it generally affects both eyes **[9,12]**. Senile cataract is the commonest type of cataract affecting both sexes equally usually above the age of 50 years **[19].** Oxidative

stress is one of the major factors which may lead to the early cataract formation. As oxidative events are of great importance in diabetic complications and, particularly in the lens, they may also have a role in the pathogenesis of cataract associated with diabetes mellitus **[7].**

Paraoxonase 1 (PON1) is a high-density lipoprotein-associated enzyme that is believed to be involved in the protection against oxidative stress. There is evidence that paraoxonase activity is reduced in patients with diabetes and cataract [24]**.** Decreased PON1 activity was more pronounced in diabetic patients with cataract compared to senile cataract subjects, which may be due to glycation with increased oxidative insult [24]. Three genes, PON1, PON2, and PON3, have been identified in mammal **[20].** Only PON1 is expressed at the gene and protein levels in human lens tissues. PON1 gene is located on chromosome 7q21-22 **[8,15]**

The most common polymorphisms on the PON1 gene are in the coding regions, which include a leucine (L) to methionine (M) transition at position 55 (55L→M) and glutamine (Q) to arginine (R) transition at position 192 (192Q→R).The 55L→M-polymorphism affects the enzyme concentration in blood and 192Q→R polymorphism is responsible for a substrate specific difference in the hydrolytic activity of the enzyme i.e., affects the enzyme activity **[3].**

This study aims to assess the contribution of PON1-55 and PON1-192 polymorphisms as risk factors for senile cataract formation among diabetic and non-diabetic Egyptian patients.

## SUBJECTS AND METHODS:

### Subjects:

The study enrolled 132 unrelated Egyptian cataract patients with age ranged from 45 to 80 years. They were subdivided into 66 patients with senile cataract and 66 suffering from diabetic cataract. Patients were recruited from the Research Institute of Ophthalmology after obtaining written consent. Control subjects were 106 healthy volunteers with no cataract or any major clinical disorders and had normal blood sugar level.

### Methods:

All patients were subjected to clinical evaluation to determine the degree of lens opacity and biochemical evaluation of fasting and postprandial blood sugar. Patients with cardiovascular diseases or other systemic disorders were excluded. Genomic DNA was extracted from 5 ml of blood using salting-out procedure **[16].**

### Genotyping of PON1 gene L55M and Q192R polymorphisms:

In this study, we used a DNA-based technique of multiplex PCR with mismatch primers which introduce a recognition site for a unique restriction endonuclease ( *Hinf* I) in one allele of each PCR product, allowing the simultaneous identification of the two PON polymorphisms by one-tube amplification and subsequent restriction analysis [18].

Multiplex-Polymerase Chain Reaction (M-PCR):

The multiplex-PCR was performed in a 50 µl reaction containing 1 µg of DNA template, 0.16 µM of both PON1-192 and PON1-55 primers, 200 µM of dNTPs, 3mM $MgCl_2$, 0.8 µg/ µl final of BSA and 1U of Taq polymerase.

DNA amplification was carried out on Perkin Elmer thermal cycler (Applied Biosystem 2720) with an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for /1 min, annealing at 61°C for 45 s, and extension at 72°C for 45 s, with a final extension step of 5 min at 72°C. Primers used for multiplex PCR analysis for PON1-55 were: 5'- GAG TGA TGT ATA GCC CCA

GTT TC-3' and 5'- AG TCC ATT AGG CAG TAT CTC Cg -3'; whereas 5'-TTG AAT GAT ATT GTT GCT GTG GGA CCT GAG-3' and 5'- CGA CCA CGC TAA ACC CAA ATA CAT CTC CCA GaA-3' were the primers used for the multiplex- PCR for detection of PON1-192 polymorphism [18]. Amplified PCR products were (144 bp for PON1-55 and 111 bp of PON1-192) as shown in figure (4). Digested PCR products using *HinfI* fast digest enzyme of PON1-55 and PON1-192 polymorphisms were then electrophoresed on 3% agarose gel as shown in figure (5)**.**

**STATISTICAL ANALYSIS:**

The SPSS for Windows® Version 17.0 was used to statistically analyze the data obtained [21]. Descriptive statistics are used to analyze all variables studied. Demographic characteristics were compared by Pearson's $\chi 2$ test for categorical data. Allele frequencies were calculated with the gene counting method. Odds ratios were calculated with a 95% confidence interval. P value < 0.05 was considered significant.

**RESULTS:**

The study revealed that there was statistically significant difference in genotype distribution and allele frequencies of the PON1-55 and 192 polymorphisms between the three groups with *p* value (0.0001, 0.003) and (0.0001, 0.005) respectively. The distribution of the PON1-55, LL genotype was higher in patients having cataract with diabetes compared with cataract group without diabetes and healthy controls, (57.6% *vs*. 30.3% and 47.2% respectively) (table 1, figure 1), while RR genotype of PON1-192 was higher in diabetic patients with cataract compared with cataract group without diabetes and healthy controls, (45.5.6% *vs*. 6.1% and 22.6% respectively) (table 1, figure 2).

The OR for the M versus L allele of PON1-55 between cataract patients without diabetes and controls was (2.02, $p$ = 0.027) while the OR for the L versus M allele in cataract diabetic patients and controls was (1.75, p = 0.111) (table 2). The OR for the Q versus R allele of PON1-192 between cataract patients without diabetes and controls was (2.48, p = 0.006) while the OR for the R versus Q allele in cataract diabetic patients and controls was (1.18, p = 0.594) (table 3).

In addition, there was a significant difference in the distribution of 55LM/192RR combined genotypes with the highest frequency in cataract diabetic subgroup (75%) (Table 4, figure 3).

**DISCUSSION:**

Oxidative stress that causes oxidation of lens protein is one of the important mechanisms involved in cataract development. Decreased concentration of antioxidant enzymes such as catalase, superoxide dismutase, glutathione reductase, and glutathione peroxidase (endogenous defense mechanisms which protect the lens against oxidative damage) with increasing age in the human eye was the main factor involved in the generation of cataract [23]. Oxidative events are also of great importance in diabetic complications. Particularly in the lens, they may have a role in the pathogenesis of cataract associated with diabetes mellitus [22]. PON1 protects lipoproteins against oxidation, probably by hydrolyzing lipid peroxides such as specific oxidized cholesterol esters and phospholipids. [14].

***Hashim*** **and his colleagues** [7] found that the decrease of PON1 activity was more pronounced in diabetic patients with cataract (p< 0.001) compared to senile cataract subjects which may be due to glycation and increased oxidative insult.

There are four currently established functional common *PON1* single-nucleotide polymorphisms (SNPs) among the nearly 200 SNPs in the gene*:* two missense mutations (*PON1*$_{Q192R}$ [rs662] and *PON1*$_{M55L}$ [rs854560]) and two that alter promoter activity (*PON1*$_{-108C/T}$ [rs705379] and *PON1*$_{-162A/G}$ [rs705381]) [22]. The present study aimed to assess the contribution of PON1-55 and PON1-192 polymorphisms as risk factors for senile cataract formation among diabetic and non-diabetic Egyptian patients.

paraoxonase enzyme gene polymorphisms were studied in many diseases such as cardiovascular disorders [1], neurological disorders as Parkinson's disease [4,2], autoimmune disorders such as systemic lupus erythematosus (SLE) [5]**,** primary glomerulonephritis [6] and breast cancer [13,17]**.** However, to our knowledge this is the first study conducted to evaluate the contribution of PON1 polymorphisms as risk factors for senile cataract.

The study proved a significant difference in LL, LM and MM genotypes distribution (p=0.0001) and in QQ, QR and RR genotypes distribution (p=0.0001) between cataract patients with and without diabetes compared to controls. Also there was a significant difference in L and M (p=0.003) and in Q and R alleles frequencies (p=0.005) between cataract patients with and without diabetes in comparison to control group. The PON1 (55) LL genotype was the most frequent in healthy subjects, followed by the LM genotype, and then the MM genotype. In cataract patients without diabetes, the MM genotype was the most common, followed by the LM genotype, and then the LL genotype. In cataract patients with diabetes, the LL, LM, MM genotypes frequencies were 57.58%, 36.36%, and 6.06% respectively.

Our results may be partially consistent with that reported by recent study undertaken to evaluate the association of PON1 gene polymorphism with diabetic nephropathy. Obtained results revealed that the PON1 (55) LL genotype was the most frequent in healthy subjects, followed by the MM genotype, and then the LM genotype. In diabetic patients with nephropathy, the MM genotype was the most common, followed by the LL genotype, and then the LM genotype. In diabetic patients without nephropathy, the LL, MM, LM genotypes frequencies were 37.5%, 37.5%, and 25% respectively [10]**.**

Statistical analysis indicated that PON1-55 M allele was a significant risk factor for the development of senile cataract without diabetes with OR = 2.02 (95% CI, 1.081 to 3.776) while there was an increased risk association between diabetic cataract patients and the L allele with OR = 1.75 (95% confidence interval 0.877-3.478) and insignificant p value = 0.111. These results suggest that the L allele may have a detectable role in the development of cataract in diabetic patients, however it may probably need interaction with other genetic or environmental factors or increasing sample size in future studies to predict a more powerful significant effect of the L allele in the development of cataract in diabetic patients.

In cataract patients without diabetes, the PON1 (192) QR genotype was the most common, while in cataract patients with diabetes; the RR genotype was the most frequent. However, ***Kotani and his colleagues*** **[11]** found that the RR genotype of Q192R polymorphism was associated with a significantly lower level of oxidative stress-related markers in Japanese subjects than the QR and QQ genotypes. These may be due to ethnic variations with different genetic background.

This study showed that PON1 (192) QR genotype was the most frequent in healthy

subjects, followed by the RR genotype, and then the QQ genotype. In cataract patients without diabetes, the QR genotype was the most common, followed by the QQ genotype, and lastly the RR genotype. In cataract patients with diabetes, the RR genotype was the most common, followed by the QQ genotype, and lastly the QR genotype. *However* ***Khodeir and his colleagues [10]***, found that the PON1 (192) QQ genotype was the most frequent in healthy subjects, followed by the RR genotype, and then the QR genotype. In diabetic patients with nephropathy, the RR genotype was the most common, followed by the QR genotype, and lastly the QQ genotype. In diabetic patients without nephropathy, the RR genotype was the most common, followed by the QQ genotype, and lastly the QR genotype**.**

PON1-192 Q allele was a significant risk factor for the development of senile cataract without diabetes with OR = 2.48 (95% confidence interval 1.297- 4.745). On the other hand, there was an inconsiderable increased risk associated with R allele in senile cataract with diabetes patients as OR was 1.18 (95% confidence interval 0.638 – 2.194).

There was a significant difference in the distribution of 55LM/192RR combined genotypes with the highest frequency was in cataract diabetic subgroup (75%), while in 55LL/192RR, 55LL/192QR and 55LM/192QR combined genotypes the highest frequencies were among the control group (52.4%, 59.1% and 66.7% respectively).

In conclusion, our study suggested that PON1-55 and PON1-192 polymorphisms were significantly associated with the development of cataract with and without diabetes and that M and Q alleles are risk factors in the development of cataract without diabetes while the L allele may have a detectable role in the development of cataract in diabetic patients. On the other hand, there was an inconsiderable risk association with R allele in senile cataract in diabetic patients. Future study with larger sample size is recommended. Further studies are recommended to confirm the role of other polymorphisms of PON1 gene and their association with senile and diabetic cataract among Egyptians. Furthermore, interplay between genetic and environmental factors must be thoroughly considered in order to evaluate the etiological role of PON1 polymorphisms in other oxidative stress ophthalmic disorders.

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**Table (1):** Genotype and allele distribution of PON1-55 and PON1-192 polymorphisms in the studied groups

| | Control n=106 | Cataract without diabetes n=66 | Cataract with diabetes n=66 | p value |
|---|---|---|---|---|
| **PON1-55 genotypes, n (%)** | | | | |
| **MM** | 20 (18.9%) | 24 (36.4%) | 4 (6.1%) | |
| **LL** | 50 (47.2%) | 20 (30.3%) | 38 (57.6%) | 0.0001 |
| **LM** | 36 (34.0%) | 22 (33.3%) | 24 (36.4%) | |
| **PON1-55 allelic frequency, n (%)** | | | | |
| **M** | 38 (35.9%) | 35 (53%) | 16 (24.2%) | 0.003 |
| **L** | 68 (64.1%) | 31 (47%) | 50 (75.8%) | |
| **PON1-192 genotypes, n (%)** | | | | |
| **RR** | 24 (22.6%) | 4 (6.1%) | 30 (45.5%) | |
| **QQ** | 20 (18.9%) | 30 (45.5%) | 22 (33.3%) | 0.0001 |
| **QR** | 62 (58.5%) | 32 (48.5%) | 14 (21.2%) | |
| **PON1-192 allelic frequency, n (%)** | | | | |
| **Q** | 51 (48.1%) | 46 (69.7%) | 37 (56.1%) | |
| **R** | 55 (51.9%) | 20 (30.3%) | 29 (43.9%) | 0.005 |

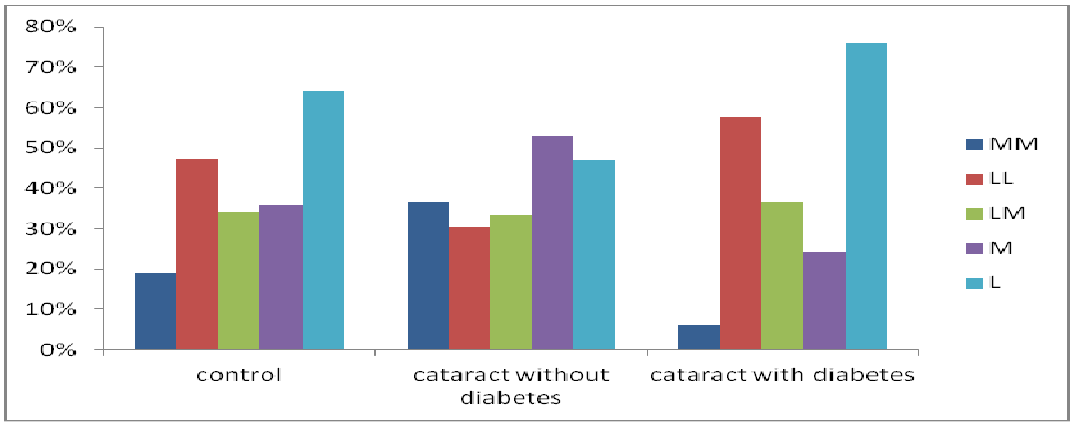


**Figure (1)** Distribution of L55M genotypes and alleles in our studied groups

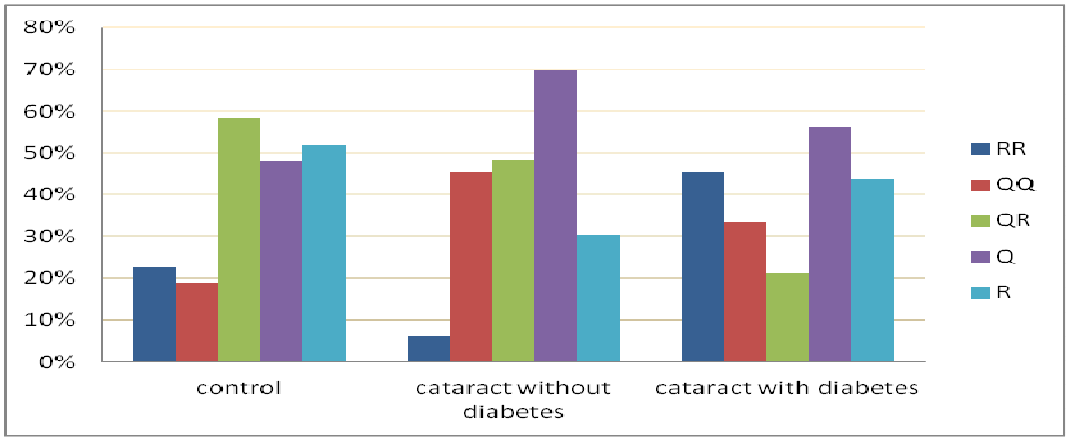


**Figure (2)** Distribution of Q192R genotypes and alleles in our studied groups

**Table (2):** Allele distribution of PON1+-55 polymorphism in cataract patients (with and without diabetes) and controls.

| PON1-55 | Control | Cataract without diabetes | *p*-value | OR | 95% CI |
|---|---|---|---|---|---|
| **M n (%)** | 38 (35.9%) | 35 (53%) | 0.027* | 2.02 | 1.081- 3.776 |
| **L n (%)** | 68 (64.1%) | 31 (47%) | | | |
| **Total n (%)** | 106 (100%) | 66 (100%) | | | |
| **PON1-55** | **Control** | **Cataract with diabetes** | ***p*-value** | **OR** | **95% CI** |
| **L n (%)** | 68 (64.1%) | 50 (75.8%) | 0.111 | 1.75 | 0.877-3.478 |
| **M n (%)** | 38 (35.9%) | 16 (24.2%) | | | |
| **Total n (%)** | 106 (100%) | 66 (100%) | | | |

**Table (3):** Allele distribution of PON1-192 polymorphism in cataract patients (with and without diabetes) and controls.

| PON1-192 | Control | Cataract without diabetes | *p*-value | OR | 95% CI |
|---|---|---|---|---|---|
| **Q n (%)** | 51 (48.1%) | 46 (69.7%) | 0.006* | 2.48 | 1.297- 4.745 |
| **R n (%)** | 55 (51.9%) | 20 (30.3%) | | | |
| **Total n (%)** | 106 (100%) | 66 (100%) | | | |
| **PON1-192** | **Control** | **Cataract with diabetes** | ***p*-value** | **OR** | **95% CI** |
| **R n (%)** | 55 (51.9%) | 29 (43.9%) | 0.594 | 1.18 | 0.638 – 2.194 |
| **Q n (%)** | 51 (48.1%) | 37 (56.1%) | | | |
| **Total n (%)** | 106 (100%) | 66(100%) | | | |

**Table (4):** The frequency of combined genotypes of L55M and Q192R polymorphisms in all studied groups

| combined genotypes1 | Control | | Cataract without diabetes | | Cataract with diabetes | | Chi-square | P value |
|---|---|---|---|---|---|---|---|---|
| | Count | Frequency | count | Frequency | Count | frequency | | |
| **55MM 192QQ** | 8 | 40% | 8 | 40% | 4 | 20% | 1.6 | 0.449 |
| **55MM 192QR** | 12 | 42.9% | 16 | 57.1% | 0 | 0 | 0.571 | 0.45 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **55LL 192RR** | 22 | 52.4% | 2 | 4.8% | 18 | 42.9% | 16 | 0.0001* |
| **55LL 192QQ** | 2 | 9.1% | 10 | 45.5% | 10 | 45.5% | 5.818 | 0.055 |
| **55LL 192QR** | 26 | 59.1% | 8 | 18.2% | 10 | 22.7% | 13.27 | 0.001* |
| **55LM 192RR** | 2 | 12.5% | 2 | 12.5% | 12 | 75% | 12.5 | 0.002* |
| **55LM 192QQ** | 10 | 33.3% | 12 | 40% | 8 | 26.7% | 0.8 | 0.67 |
| **55LM 192QR** | 24 | 66.7% | 8 | 22.2% | 4 | 11.1% | 18.67 | 0.0001* |

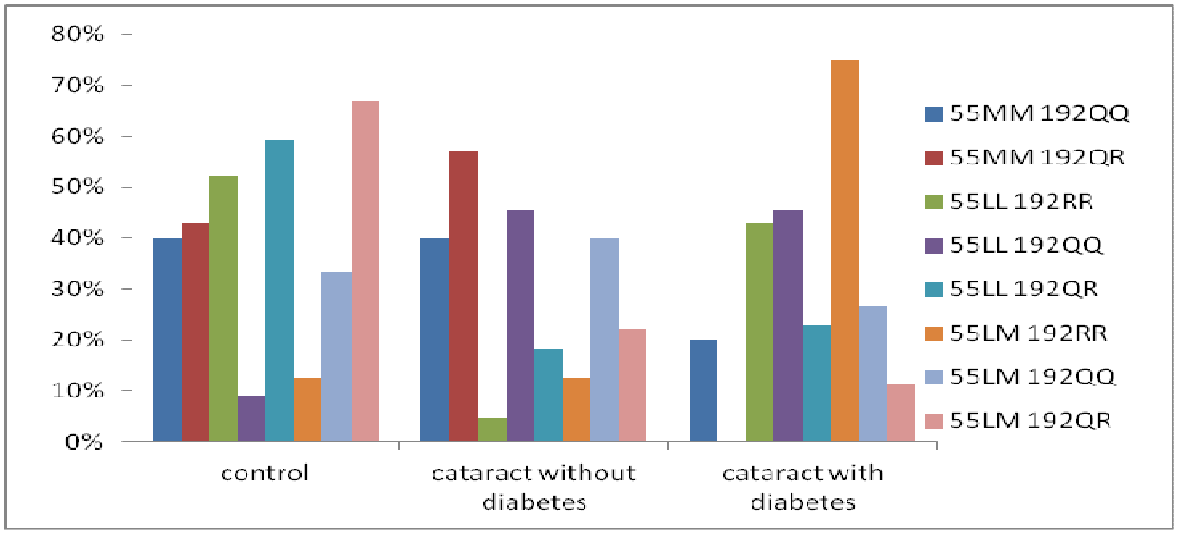


**Figure (3)** Distribution of L55M and Q192R combined genotypes in our studied groups

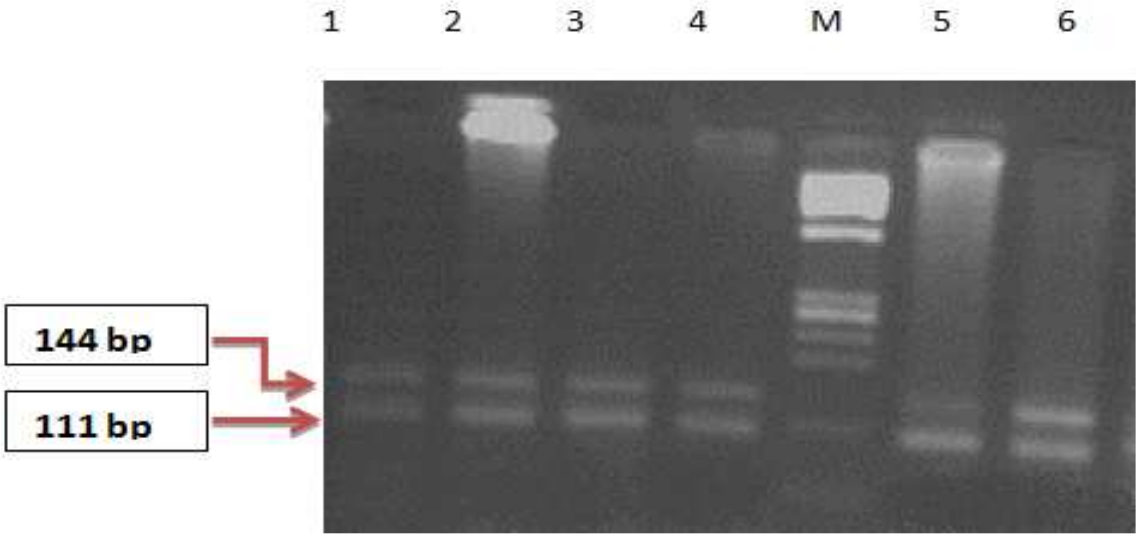


**Figure (4):** 2% agarose gel stained with ethidium bromide illustrating MPCR product. *Lanes (1- 6)*: Amplified PCR products (144 bp of PON1-55 and 111 bp of PON1-192).*M:* (PhiX174 DNA/HaeIII digest).

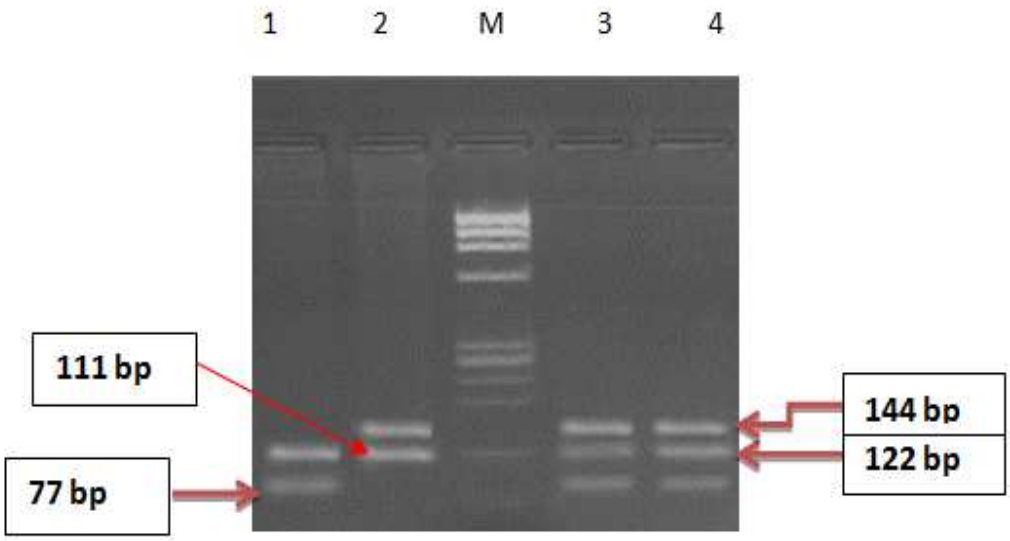


**Figure (5a)**
A 3% agarose gel showing *HinfI* digested PCR products of the PON1-55 and PON1-192 polymorphisms. Lane (1): showed 122 bp of LL genotype of PON1-55 and 77 bp of RR genotype of PON1-192. Lanes (2): showed 144 bp of MM genotype of PON1-55 and 111 bp of QQ genotype of PON1-192. Lane (3, 4): showed 144 and 122 bp of LM genotype of PON1-55 and 77 bp of RR genotype of PON1-192.M: (PhiX174 DNA/HaeIII digest)

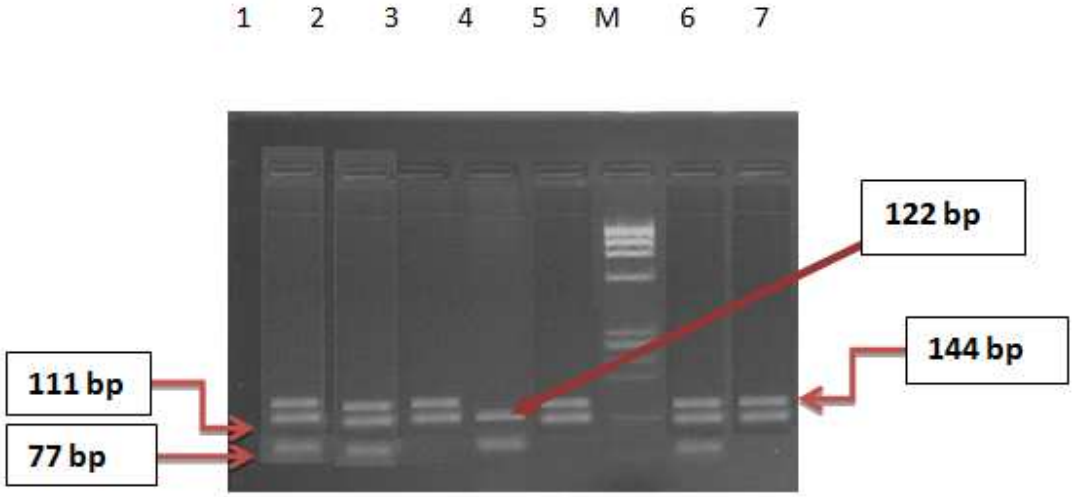


**Figure (5b)**
A 3% agarose gel showing *HinfI* digested PCR products of the PON1-55 and PON1-192 polymorphisms. Lanes (1, 2, 6): showed 144 bp band of MM genotype of PON1-55 and 111and 77 bp of QR genotype of PON1-192. Lane (3, 5, 7): showed 144 bp band of MM genotype of PON1-55 and 111 bp of QQ genotype of PON1-192.Lane (4): showed 122 bp band of LL genotype of PON1-55 and 77 bp of RR genotype of PON1-192. M: Molecular weight marker (PhiX174 DNA/HaeIII digest).