Q192R paraoxonase1 polymorphism is a risk factor for cataract in Pakistani population

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Abstract: Cataract, the lens opacity, is among major causes of blindness in Pakistani population. In recent past, oxidative stress is suggested to play crucial role in loss of transparency. Along with other antioxidants, Paraoxonase 1 (PON1) has also shown decreased activity in patients suffering from cataract. The aim of current study was to examine the possible association of PON polymorphism with predisposition of cataract formation in local population. The study was conducted on 51 cataract patients and 50 control subjects considering all ethical issues. DNA was extracted from whole blood and PON1 polymorphism was identified using tetra primer ARMS-PCR method for both positions *L55M* and *Q192R*. Tetra primer ARMS-PCR results revealed that association between *L55M* polymorphism and cataract was insignificant while *192R* genotype PON1 frequency was higher among the people suffering from cataract (78.4%) as compared to control subjects (56%), (odds ratio=2.857, confidence interval=1.197-6.820). Hence, *R* allele is likely to be a risk factor for cataract with allele frequency (82.3%) and (odds ratio=4.552, confidence interval=1.716-12.073, p-value=0.002). PON1 *Q192R* polymorphism is likely to be a risk factor for cataract development in Pakistani population while PON1 *L55M* was not found to be associated with cataract.

Keywords*:* Cataract; Paraoxonase1 (PON1), Polymorphism, Tetra primer ARMS-PCR, Pakistan.

INTRODUCTION

Cataract is one of the most common causes of vision loss. Approximately 37 million people suffer from blindness due to cataract formation throughout the world (Resnikoff *et al*., 2002). During cataract formation, lens steadily becomes cloudy and the vision acuity of a person to distinguish objects and shapes reduces. According to the World Health Organization estimates, the average prevalence of vision loss all over the world is 0.7% and of 45 millions blind cases, 60% occurs due to loss of lens transparency. Although visual impairment varies among different age groups, 82% cases of blindness occur after 50 years of age. It has been assumed that almost 570,000 adult Pakistani populations suffer from sightlessness due to cataract (Jadoon *et al*., 2007). Generally three types of cataracts exist namely nuclear, cortical and sub capsular. Nuclear cataract is frequently found in elder population due to opacification in the center of the lens. Whereas in cortical cataract, opacity is from outside towards the center and is more prevalent in diabetic patients. Sub capsular cataract, however, develops below the capsule at the dorsum of the lens (Vinson 2006).

Cataract formation is associated with several risk factors including aging, inheritance, mutations, smoking, exposure to toxic chemicals, malnutrition, UV light exposure and diabetes (Taylor 1999). Although aging and diabetes are suggested to be the most important risk factors (Rowe *et al*., 2000), studies have highlighted the role of oxidative stress in the development of cataract (Robertson *et al*., 1989, Shui *et al*., 2006). Oxidative stress occurs due to an imbalance between generation and removal of reactive oxygen species (ROS) from the body. The abnormal production of these free radicals (ROS) can directly damage the cellular proteins, lipids and nucleic acids (Berlett *et al*., 1997). In order to maintain oxidative balance, cells and tissues are equipped with antioxidant system.

Paraoxonase (PON) is a calcium dependent antioxidant enzyme coded by a gene located on chromosome 7 (Mackness *et al*., 1996). In mammals, three types of PON have been identified namely PON1, PON2 and PON3. The PON1 enzyme is coupled with high-density lipoprotein and is widely distributed in liver, kidney, intestine, and blood circulation. Since PON2 is not present in serum and PON3 is found in very minute amount, PON1 has attained much focus amid researchers (Goswami *et al*., 2009). The physiological substrates of PON1 have not yet been understood (Primo-parmo *et al*., 1996), although its role in hydrolyzing organophosphates and aromatic acetates, commonly known as paraoxon and phenyl acetate respectively, has long been identified (Van-Himbergen *et al*., 2006). PON1 activity has been reported to decline in many diseases (Li *et al*., 2003) including cataract (Hashim *et al*., 2007).

PON1 polymorphism has been reported by many researchers and is considered to exert its direct and indirect effects on PON1 activity (Yamada *et al*., 2003). In the coding region, PON1 gene has two common missense mutations resulting in substitution of leucine

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with methionine at position 55 (*L→M* 55) and glutamine with arginine at position 192 (*Q→R* 192). The *L55M* and *Q192R* polymorphism seems to be the major determinant for the change in concentration and enzymatic activity of PON1 among different individuals (Hashemi *et al*., 2010, Garin *et al*., 1997). Various investigators have identified the detrimental effects of PON1 polymorphism in different diseases. Individuals with PON1 *R* alloenzyme are reported to be more susceptible to suffer from atherosclerosis because of poor effectiveness against lipid per oxidation (Imai *et al*., 2000) Another study revealed the risk of developing lungs cancer in people having PON1 *Q* than the people with *R* alloenzyme (Lee *et al*., 2005) while M alloenzyme is associated to be the risk factor for Parkinson's disease (Zinzaras *et al*., 2004). In contrast, people with *192R* are likely to be protected from breast cancer but with L55M associated with development of breast cancer (Antognelli *et al*., 2009).Recent study reported significant association of L55M and Q192R allele in cataract subjects with or without diabetes(Ali *et al*., 2014). The objective of the current study was to evaluate the influence of PON1 *L55M* and *Q192R* PON1 polymorphisms on cataract formation in Pakistani population.

MATERIALS AND METHODS

Subjects

The studyincluded 51 cataract patients (28 males and 23 females), with average age of 58.25 years visiting eye ward of Liaquat National Hospital, Karachi, for cataract surgery. Fifty healthy participants were selected for the control group (48 males and 2 females) with average age of 40.34 years. Participants were excluded from the study if they experienced any other metabolic disorder.

Ethical approval

The study proposal was approved by the Institutional Review Board, National Center for Proteomics, University of Karachi. All subjects were recruited after receiving informed written consent form.

Sample collection

A day proceeding to surgery of cataract patient, 2 ml venous blood samples were collected in K_2-EDTA coated vials.

DNA extraction

DNA was extracted from whole blood within 24 hours of collection as per previously described protocols (Chomczynski *et al*., 1993, Kleines *et al*., 2003, Green *et al*., 2012). Briefly 300µl of blood was transferred to the micro centrifuge tube containing 900µl of red cell lysis buffer (sucrose 0.31M, Tris 0.01M of pH 7.6, MgCl 0.002M, sodium azide 0.003M in deionized distilled water), and centrifuged for 1 minute at 13000 rpm after which supernatant was discarded. The procedure was repeated with 300ul red cell lysis buffer. Afterwards 300 µl of cell lysis buffer (Tris 0.064M of pH 8.0, Na_2 EDTA 0.017M, SDS 0.069M in deionize distilled water) was added and vortexed to break the pellet. Following 30 minutes of incubation (37˚C) 120µl protein precipitation solution, (Promega Corporation, USA) was added, vortexed, and centrifuged at 13000 rpm for 5 minutes. Supernatant was transferred to another tube containing 300µl iso-propanol and was gently shaken 15-20 times to visualize the DNA threads and then centrifuged for 5 minutes. The supernatant was discarded and subsequently 500µl ethanol was added and centrifuged for 1 minute to wash the DNA pellet. Finally, the supernatant was discarded, DNA was air dried for 20 minutes and rehydrated using 50µl of nuclease free water and stored at -20˚C till further use.

Tetra primer amplification refractory mutation system pcr

For this study, tetra primer ARMS PCR was used In this method, combination of four primers is used in which, two outer primers (FO and RO) bind to the flanking locus of interest giving non allele specific control bands while two inner allele specific primers assemble in opposite orientation in such a way that along with two outer primers it gives concurrent mutant and wild type amplicons having different base pair length. We used four sets of primers, two outer and two inner for both positions as described previously (Hashemi *et al*., 2010 a & b).

Polymerase chain reaction was performed using PCR master mix DreamTaq Green PCR Master Mix 2X, (Thermo Scientific Company EU, Lithuania) containing 25ul PCR master mix, 5µl DNA template (>150ng/µl), 1µl of each forward and reverse primer (10µM) and 16µl of nuclease free water giving a total reaction volume of 50ul. For L55M position, PCR cycling conditions were as follows: Denaturation at 94˚C, annealing at 58˚C, extension at 74˚C and total number of cycles was 35 PCR. For Q192R position, PCR cycling conditions were as follows: denaturation at 94˚C, annealing at 55˚C, extension at 74˚C and total number of cycles was 35. Amplicons were analyzed by running at 2% agarose gel, for leu55Met and Gln192Arg.

STATSTICAL ANALYSIS

Data analysis was done using statistics software SPSS version 20.0, utilizing gene counting method and Pearson's Chi square test to determine the allelic frequencies and differences in the genotypes among control and cataract subjects.

RESULTS

Figs. 1 and 2 represent results of agarose gel electrophoresis indicating product size and polymorphism

for control and cataract subjects for L55M and Q192Rpositions, respectively. Genotype and allele frequency of PON1 polymorphism in control and cataract patients is stated in fig. 3a and fig. 3b respectively. The frequency of homozygous TT (*LL*) allele in control subjects and cataract patients was 29/50 (58%) and 30/51 (58.8%) respectively. The frequency for allele group AT (*LM*) in control and cataract groups was found to be 15/50 (30%) and 07/51 (13.7%) respectively, while the frequency of AA (*MM*) was 06/50 (12%) and 14/51 (27.4%) in the above mentioned groups, respectively. Therefore, the results show that there is no significant association in between the genotype and frequency of *L55M* PON1 polymorphism (p>0.05) in the control and cataract subjects of the current study as presented in table 1.

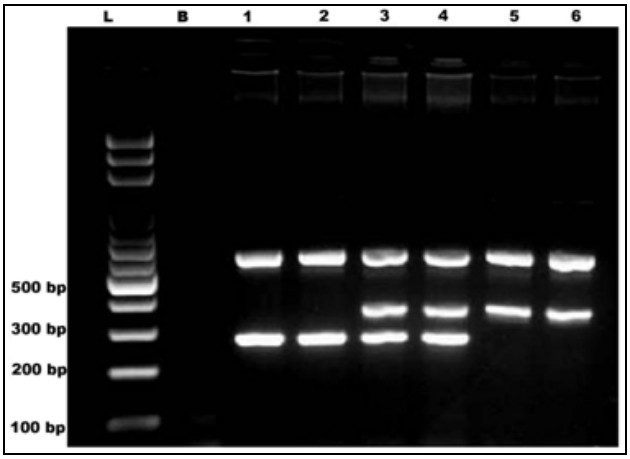

Fig. 1: Agarose gel electrophoresis of tetra primer ARMS PCR product, lanes represents 1, 2: *MM*, 3, 4: *LM*, 5, 6:*LL*. Odd and even numbers indicate samples from control and cataract while L and B stand for ladder and blank, respectively.

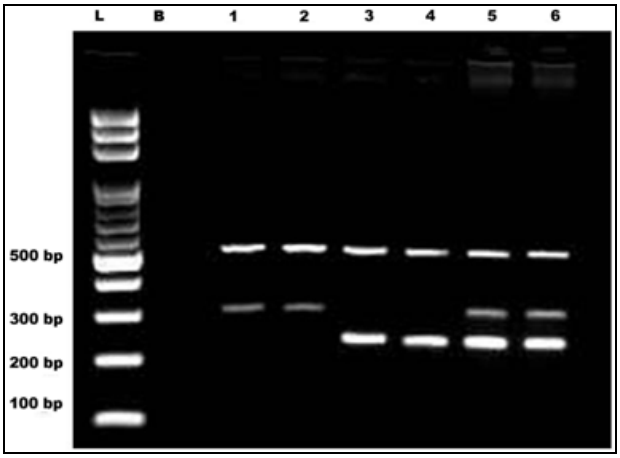

Fig. 2: Agarose gel electrophoresis of tetra primer ARMS PCR product, lanes represents 1, 2: *QQ*, 3, 4: *RR*, 5, 6:*QR*. Odd and even numbers indicate samples from control and cataract while L and B stand for ladder and blank, respectively.

The Genotype and allele frequency of *Q192R* polymorphism in PON1 is shown in fig. 4a and fig. 4b respectively. The genotype AA (*QQ*) allele in control and cataract patients was 21/50 (42%) and 07/51 (13.7%) respectively. Our results establish that the frequency for AG (*QR*) allele genotype of the same groups was 01/50 (2%) and 4/51 (7.8%). On the other hand, the GG (*RR*) allele frequency was found to be 28/50 (56%) and 40/51 (78.4%) in control and cataract participants.

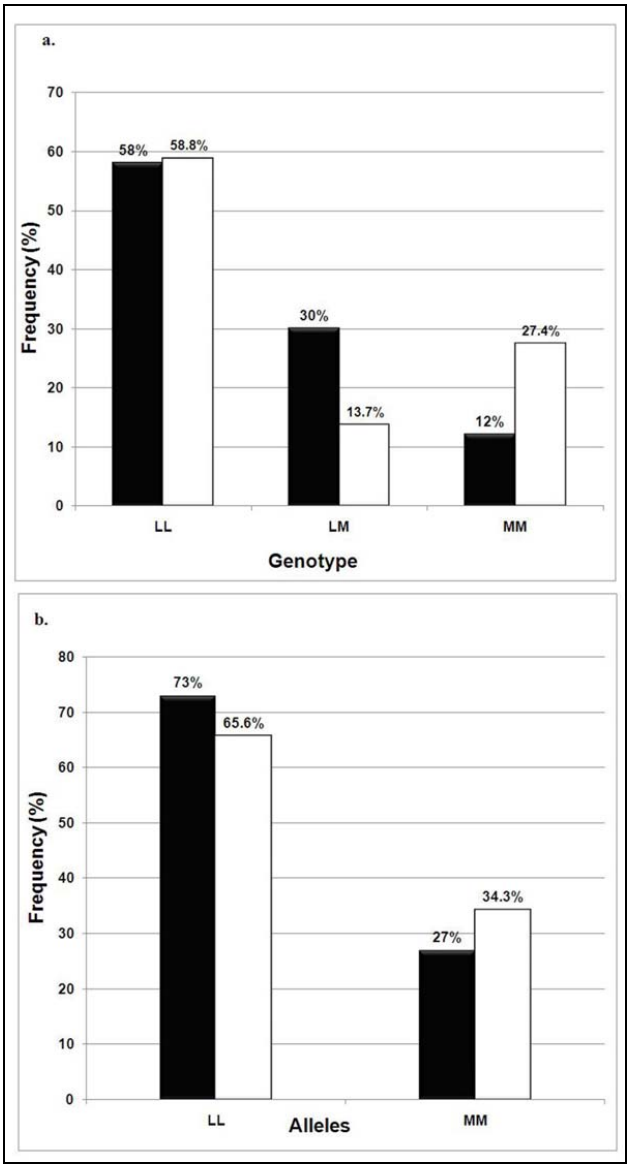

Fig. 3: Genotype (a) and Allele (b) frequency of PON1 in control (■) and cataract subjects (□) in *L55M* polymorphism.

A significant difference was found among the control and cataract subjects (p<0.05) among the GG (*RR*) genotype individuals. As mentioned in the table 2, the prevalence of GG (*RR*) allele was significantly higher in the cataract group (82.3%) as compared to control subjects (57%). odd ratio and confidence interval show that allele

combination of GG (*RR*) indicates a highly significant risk factor for the development of cataract.

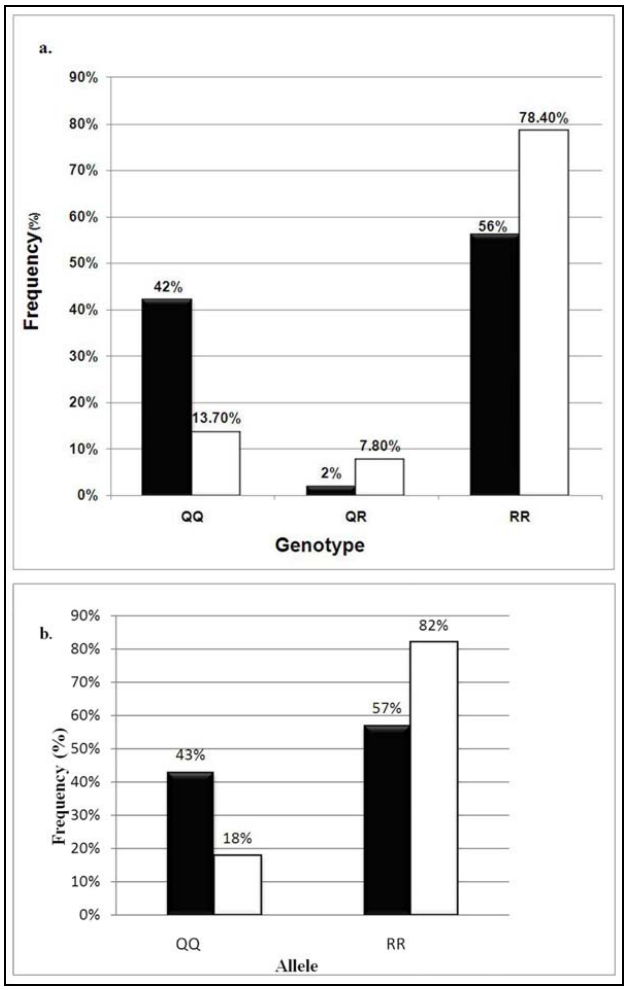

Fig. 4: Genotype (a) and Allele (b) frequency of PON1 in control (■) and cataract subjects (□) in *Q192R* polymorphism.

DISCUSSION

Cataract formation is a multi-factorial and complex process that leads to the impairment and ultimate loss of vision. Cataract is not just an outcome of metabolic reactions occurring in an eye lens but is also a consequence of several pathological factors leading to its development and progressive maturity (Harding 2002). These factors include dietary habits, UV light exposure, genetic susceptibility and dehydration of eye to name a few. All of these factors, however, eventually follow a common passage that causes oxidation of the lens proteins and lipid peroxidation. Antioxidants, therefore, could possibly be prophylactic agents that may act as a protective shield against cataract formation. A family of enzyme proteins, Paraoxonases (PON) is responsible for preventing the accumulation of LDL while remaining associated with HDL (Aviram *et al*., 1998). Although the exact physiological role has not yet been identified (Mackness *et al*., 1996), PON is reported to be decreased in numerous diseases like Alzheimer's (Erlich *et al*., 2012), rheumatoid arthritis (Baskol *et al*., 2005), coronary heart disease (CHD) (Imai *et al*., 2000), Parkinson's (Erlich *et al*., 2012) and different types of cancers (Bulbuller *et al*., 2013, Malik *et al*., 2014). In an earlier study, a significantly reduced activity of PON1 in cataract patients (Hashim and Zarina, 2007) has been reported but so far, association between PON1 polymorphism and incidence of cataract has not been examined.

A potential association between PON1 gene polymorphism and several diseases has been suggested by many researchers (Imai *et al*., 2000). PON1 gene is located in chromosome 7 (q 21.3-22.1) and has several polymorphisms in the promoter and coding regions that are identified and known to influence on PON1 enzyme levels and activity. Our study indicated that PON1 *192R* allele is linked to the cataract formation. Interestingly, although presence of *R* allele at 192 positions is found to be responsible for high PON1 activity, it seems ineffective in protecting LDL from oxidation (Ruiz *et al*., 1995). Presence of *R* allele has been suggested to be a risk factor for developing obesity in Portuguese women (Veiga *et al*., 2011), preeclampsia (Yaghmaei *et al*., 2011) and arterial ischemic stroke among young adults (Voetsch *et al*., 2002). An evidence for an effective association between PON1 polymorphism and age related macular degeneration (Ikeda *et al*., 2001) reported high frequency of allelic forms *L* at *L55M* and *R* at *Q192R* in patients as compared to the healthy controls. The association of PON1 *192R* polymorphism with coronary heart disease has been controversial as no relationship was found in Finland population (Antikainen *et al*., 1996), while the same polymorphic position was reported to be associated with coronary heart disease among Japanese (Odawara *et al*., 1997) and caucasian population suffering from non insulin dependent diabetes mellitus (*NIDDM*). This contradictory relationship has been attributed to the fact that PON1 192R polymorphism may not directly be associated with CHD and is likely to be a result of linkage disequilibrium with in PON1 gene or nearby loci. Furthermore, inter-individual and inter-ethnic groups variation may also be responsible (Sanghera *et al*., 1998). In spite of high activity, the detrimental effect of *R* allele is likely to be due to insufficient lipid per oxidation and modification of LDL (Ruiz *et al*., 1995).

PON1 polymorphism at position 55, on the other hand, is linked with PON1 concentration. Plasma concentration of PON1 gradually decreases with changes in alleles from *L* to *M* (*L*→*M*) reflecting enzyme activity and serum concentration are independent of each other (Hasemi *et al*., 2010). In *CHD* studies, M allele is seldom linked with *192R* allele, most likely due to linkage disequilibrium; the same reason has been given for the infrequent *192R* allele

Table 1: Statistical analysis of PON1 in control and cataract patients for position L55M.

Position 55 T------> A		Control	Cataract	Odds ratio	95% CI	χ^2
Genotypes	TT (LL)	29/50	30/51	1.034	0.469-2.283	0.933
	AT (LM)	15/50	07/51	0.371	0.136-1.010	0 .048
	AA (MM)	6/50	14/51	2.775	0.969-7.942	0.051
Alleles	TT (LL)	73/100	67/102	0.360	0.126-1.031	0.051
	AA (MM)	27/100	35/102	0.967	0.438-2.133	0.933

CI = Confidence Interval

Table 2: Statistical analysis of PON1 in control and cataract patients for position Q192R.

Position 192 A------ >G		Control	Cataract	Odds ratio	95% CI	χ^2
Genotype	AA (*QQ*)	21/50	07/51	0.220	0.083-0.583	0.002*
	AG (*QR*)	1/50	4/51	4.170	0.450-38.687	0.176
	GG (*RR*)	28/50	40/51	2.857	1.197-6.820	0.016*
Allele	AA (*QQ*)	43/100	18/102	0.350	0.147-0.835	0.016*
	GG (*RR*)	57/100	84/102	4.552	1.716-12.073	0.002*

***** =Statistically significant (p<0.05), CI = Confidence Interval

combination with the frequent *55L* allele conferring haplotype of both risk associated alleles (Voetsch *et al*., 2002). Study on Egyptian population indicated that incidence of combined genotype LM55/192R was a risk factor for development of diabetic cataract (Ali *et al*., 2014). In the current study, we report the greater incident of PON1 *192R* allele is likely to be a risk factor for cataract development (p<0.05), whereas *L55M* polymorphism gave no association in cataract patients.

In conclusion, our study examined the relationship between PON1 polymorphism and cataract formation. Our data suggested a noteworthy polymorphic association between PON1 *Q192R* and cataract incidence indicating RR allele to be more prevalent in cataract patients as compared to control subjects. No marked relationship, however, was observed between PON1 *L55M* polymorphism and cataract susceptibility. It can be suggested that the presence of PON1 *192R* genotype in studied population is likely to be attributed as an additional risk for cataract development. Further studies are needed to ascertain the relationship between cataract formation and PON1 polymorphism since demographic, ethnic and environmental factors may also contribute in etiology of a disease.

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