Full Length Research Paper

Paraoxnase1 (*PON 1*) gene polymorphism in Kuwaiti Arab children with idiopathic nephrotic syndrome

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Paraoxonase1 (PON1) is a serum enzyme bound to high density lipoproteins and has antioxidant as well as anti-atherogenic effects. Molecular studies of PON1 revealed two polymorphic sites at amino acids 55 and 192 resulting in two different allozymes. These allozymes result from L- genotype (leucine/high activity) and M-genotype (Methionine /low activity) at residue 55 and A- (arginine/high activity) and B- (glutamine/low activity) at site 192 respectively. We have studied the association of Paraoxonase1 (*PON1*) gene polymorphisms with the histopathological types of idiopathic nephrotic syndrome (INS) in Kuwaiti Arab children. The *PON1* gene, 55 and 192 polymorphisms were analyzed in 50 Kuwaiti children with INS and 50 healthy controls of a similar age, sex and ethnic background. The patients included 32 children with minimal change nephrotic syndrome (MCNS) and 18 with focal segmental glomerulosclerosis (FSGS). The *PON1* gene polymorphisms were detected by using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) methods. The incidence of LL genotype (Leucine residue) was detected in 25/50 (50%) of the INS patients group compared to 24/50 (48%) in the controls (p = 0.84). The heterozygous genotype LM was detected in 21/50 (42%) in the INS patients compared to18/50 (36%) in the controls (p = 0.68). The homozygous MM-genotype (methionine residue) was detected in 4/50 (8%) INS patients compared to 8/50 (16%) in the controls (p = 0.35). The combined L-allele frequency in homozygous LL and the heterozygous subjects was 71% in INS patients compared to 66% in the controls (p = 0.54). The L-allele in both its homozygous LL and heterozygous LM genotype was found to be significantly more common in FSGS patients compared to MCNS patients (p = 0.0001) and also when compared to the controls (p = 0.0007). *PON-1* gene, 192 polymorphism, the AA-genotype (glutamine residue) was uniformly detected in all patients and controls. Our data demonstrate a strong association between the L-allele of *PON1* gene '55' polymorphism and pathogenesis of FSGS in Kuwaiti Arab children with idiopathic nephrotic syndrome.

Key words: Idiopathic nephrotic syndrome(INS); Genotype; Glomerulosclerosis; Paraoxonase1(PON1); Polymorphism

INTRODUCTION

The paraoxonase1 (PON1) is a serum enzyme involved in lipid metabolism [1,2]. It is bound to high-density lipoprotein (HDL) and acts as an antioxidant for the low-density lipoproteins (LDL) by hydrolyzing lipid peroxides thus preventing LDL oxidation and subsequently leading to anti-atherogenic effect [1-4]. Studies of the molecular actions of PON1 enzyme revealed that it has a high and a low activity form (allozymes) which are controlled by two common polymorphic sites at amino acid positions 55 and 192 [5-10]. The L- and M- allozymes represent the

presence of leucine and methionine at position 55 of the amino acid sequence. On the other hand, the A- and B- allozymes at position 192 represent the presence of arginine (high activity) and glutamine (low activity) at this position. A number of previous reports have highlighted the role of PON1 in various diseases associated with hyperlipidemia such as coronary heart disease [11], Insulin dependent Diabetes Mellitus [12], strokes [13] and atherosclerosis [14]. Hyperlipidemia is a well-known biochemical component of the idiopathic nephrotic syndrome (INS) both during relapses and remissions of the disease [15-16]. Abnormal lipid profile is believed to be a precursor for glomerulosclerosis with subsequent kidney failure [17-24]. The role of PON1 enzyme and its

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genetic polymorphisms in nephrotic children has been reported previously in different ethnic and racial groups [5,25-32] with divergent results. In this report, we have studied the association of *PON1* gene polymorphisms with INS and its histological subtypes in Kuwaiti Arab children.

METHODS

Study population

This study included 50 Kuwaiti children with idiopathic nephrotic syndrome (INS) and 50 healthy Kuwaiti age and sex-matched controls. The patients were evaluated and treated in the Pediatric Nephrology unit at Mubarak Al-Kabeer Teaching hospital of the Kuwait University. All the patients had complete biochemical tests done including blood urea, serum creatinine, serum electrolytes, total proteins and albumin level, lipid profile and urine protein: creatinine ratio. Lipid profiles included serum cholesterol, Low-density lipoprotein (LDL), High-density lipoprotein (HDL), Very low-density lipoprotein (VLDL) and triglycerides (TG). The control subjects were evaluated by a trained Pediatric Nephrologist to ensure the absence of any renal disease. All the patients underwent a kidney biopsy for either steroid dependency or resistance.

DNA analysis

The total genomic DNA was isolated from blood from INS patients and controls by using a standard method [33]. The blood was anti-coagulated with EDTA prior to DNA isolation. The genotypes for *PON1* gene polymorphisms 192 and 55 were determined by using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method described earlier [22]. For the *PON1* gene 192 polymorphism, sense primer 5'-TAT TGT TGC TGT GGG ACC TGA G-3' and anti-sense primer 5'-CAC GCT AAA CCC AAA TAC ATC TC-3' which encompass the 192 polymorphic region were used. For the 55 polymorphism, sense primer 5'-GAA GAG TGA TGT ATA GCC CCA G-3' and antisense primer 5'-TTT ATT CCA GAG CTA ATG AAA GCC -3' were used. The PCR mixture contained 200 ng genomic DNA template, 0.5 ug of each primer, 1.5 mM $MgCl_2$, 200 uM dNTPs and 1 U AmpliTaq DNA polymerase (Applied BioSystems/ABI, Foster City, USA). The PCR conditions included a denaturation step for 5 min at 94^0C, followed by 35 cycles of denaturation for 1 min, annealing at 60^0C for 1 min, extension at 72^0C for the 192 polymorphism genotyping. The resulting PCR product was digested with 8 U *Bsp*I restriction endonuclease (New England BioLabs, Boston, USA) overnight at 55^0C. The digested products were separated by electrophoresis on 2% Metaphore agarose (FMC BioProducts, USA) and

visualized under UV light after staining with ethidium bromide. In cases which have only A-alleles, the restriction site was absent (Figure 1) while in subjects carrying B-allele a *Bsp*1 cleavage site should be present. The *PON1* gene 55 polymorphism was determined using the primers and cycling conditions described above. The PCR product (170 bp) was digested with *Hsp*19211 (New England BioLabs, Boston, USA) in the presence of bovine serum albumin (BSA, 0.1 ug/ul) at 37^0C overnight. The products of restriction enzyme cleavage were analyzed and visualized as described above. Allele L-(leucine) did not contain the *Hsp*19211 site while allele-M (methionine) contained the *Hsp*19211 site resulting in 126- and 44-bp products (Figure 2). The genotypes were determined by allele counting.

Statistical analysis

Statistical analysis was done using SPSS software version 21. Man-Whitney test was used to compare medians and Fisher's exact test was used for comparing the frequency of *PON1* genotypes.

RESULTS

This study included 50 Kuwaiti children with idiopathic nephrotic syndrome seen over a period of four years. Their median age at diagnosis was 6.8 years (range 13 months-11 years). There were 37 male and 13 female. Biopsy-proven histo-pathological diagnosis of minimal change nephrotic syndrome (MCNS) was found in 32 patient, while 18 patients had a diagnosis of focal segmental glomerulosclerosis on the biopsy. The clinical and laboratory data of patients and controls is presented in Table 1. The *PON1* gene, 55 polymorphism was found to be informative in our study groups. The LL genotype (Leucine residue) was detected in 24/50 (48%) of the INS patients compared to 25/50 (50%) in the controls (Figure 3 and Table 2). The incidence of heterozygous (LM) genotype was 18/50 (36%) in INS patients group compared to 21/50 (42%) in the controls. The homozygous MM- genotype (methionine residue) was detected in 8/50 (16%) INS patients compared to 4/50 (8%) in the controls. Amongst the control subjects irrespective of their genotype, the L-allele was detected in 71% subjects while the M-allele was found in 29% subjects respectively. However, in the INS patients, the L-allele was detected in 66% patients and the M-allele was found in 34% patients irrespective of their genotypes (Table 2). The L-allele in both its homozygous LL and heterozygous LM genotype was found to be significantly higher in FSGS patients compared to MCNS patients (p = 0.0001) as well as when compared with the controls (p = 0.0007; Figure 4). In contrast, for the *PON1* gene 192 polymorphism, only the AA-genotype (glutamine residue)
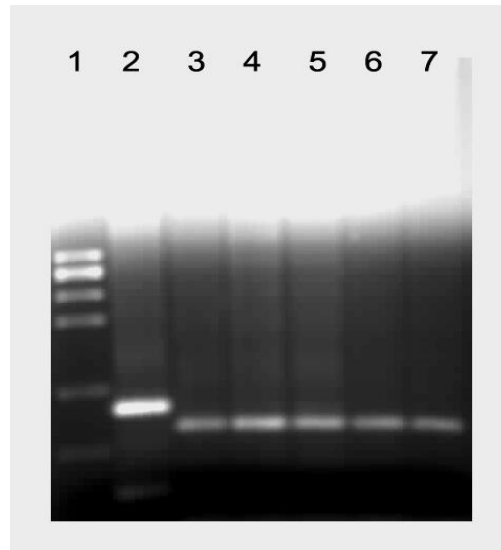
Figure 1. Detection of *PON1* gene 192 polymorphism by using a PCR-RFLP method (for details, please see the Methods section). Lane 1, *Hae*III cleaved ☐ X174 M_r size markers; lane 2, un-cleaved PCR product; lanes 3-6, *Bsp*1 cleaved PCR products from subjects with AA-genotypes. The cleavage products were analyzed using 2% agarose gel and visualized under UV light after staining with ethidium bromide.
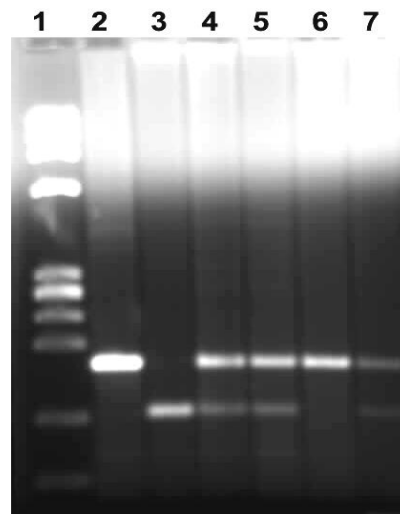

Figure 2. Detection of *PON1* gene 55 polymorphism by using a PCR-RFLP method (for details, please see the Methods section). Lane 1, *Hae*III cleaved ϕX174 M_r size markers; lane 2, un-cleaved PCR product; lanes 3, *Hsp*19211 cleaved PCR product from a subject with MM-genotype; lanes 4-5 & 7, *Hsp*19211 cleaved PCR products from subjects with heterozygous LM-genotype; lane 6, *Hsp*19211 cleaved PCR product from a subject with LL-genotype. The cleavage products were analyzed using 2% agarose gel and visualized under UV light after staining with ethidium bromide.

was detected uniformly in all the study subjects (INS patients and controls) and the BB or AB genotypes were not detected at all.

DISCUSSION

A variety of evidence suggests that hyperlipidemia is associated with renal diseases [18]. It leads to increased risk of atherosclerosis, cardiovascular complications and progression of kidney damage due to glomerulosclerosis [11-14,19-21]. A classic example of kidney disease in which hyperlipidemia is a prominent feature, even in early stages of the disease, is the idiopathic nephrotic syndrome (INS). It is present during both phases of the disease, i.e. relapses and less commonly remissions [15-16]. Many previous reports have shed some light on the pathogenesis of hyperlipidemia in INS. It is becoming
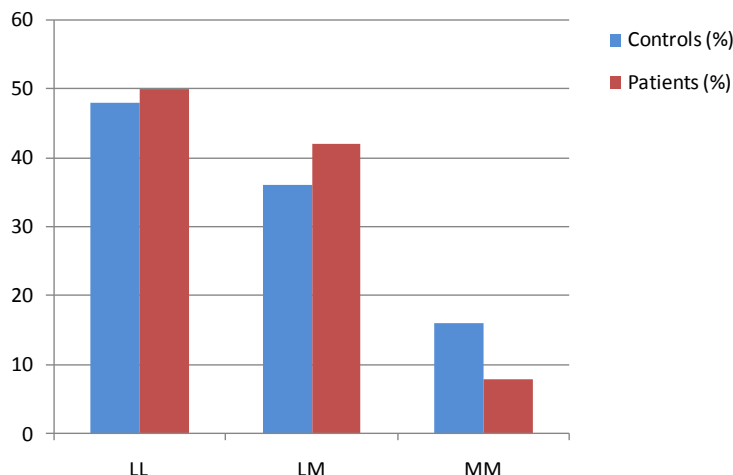
Figure 3. Incidence of *PON1* gene 55 polymorphism genotypes in Kuwaiti Arab children with nephrotic syndrome and matched control subjects. The incidence has been shown as percentage of the total number in each study group (NS patients and controls).
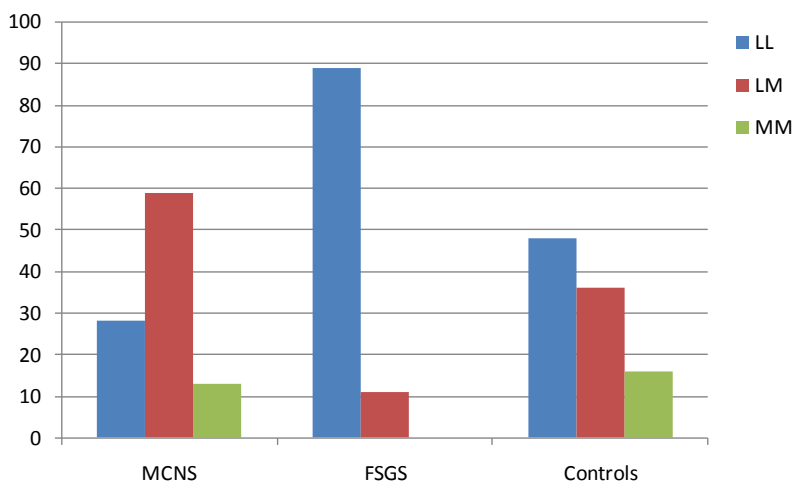

Figure 4. The incidence of *PON1* gene polymorphism genotypes in Kuwaiti patients with different clinical subtypes of idiopathic nephrotic syndrome and controls. MCNS, minimal change nephrotic syndrome; FSGS, focal segmental glomerulosclerosus.

increasingly clear that both proteinuria and low serum albumin in INS contribute to multiple abnormalities in the lipid metabolism [17,24]. Studies with radio- labelled lipoprotein suggest that hyperlipidemia in INS result more from delayed lipoprotein catabolism than increased production [7-8]. In response to lipoprotein stimulation mesangial cells might act as an important mediators of injury by producing cytokines and growth factors [8]. These inflammatory cytokines can cause mesangial cells to take up LDL in an up-regulated fashion leading to foam cell formation in FSGS. The important role of paraoxonase1 enzyme (PON1) as a lipid antioxidant of LDL has been widely reported [5]. Its action is in hydrolyzing lipid peroxides and therefore protects LDL from oxidation modification which is an important early step in the development of atherosclerosis [8,27]. Determination of PON1 states which reveals PON1 functional genotype is required for meaningful evaluations of PON1 role in risk of disease. It was reported that 10-40 times differences in the serum activity of the PON1 enzyme in humans is genetically determined and varies among different populations and races [5,30-31]. This genetic variability is determined by the gene polymorphisms of PON1 enzyme. Populations of European ancestry have been reported to have 50% homozygotes for the 192 allozyme with low PON1

activity, while 10% were found to be homozygotes for the high activity allozyme and 40% were heterozygotes [9]. Frisberg had reported a high frequency of L- allele in its heterozygous and homozygous forms in nephrotic children from an Arab race having FSGS histopathology [32]. There has been no other report from the Gulf region of the Middlle East on *PON1* gene polymorphism and its association with INS. Despite the small number of FSGS patients in our study, our results support the role of L-allele of the *PON1* gene as a risk factor for FSGS in INS children of an Arab racial background. This finding is consistent with Frisberg's findings on nephrotic children of Arab origin compared to others patients from a different racial background [32].

CONCLUSION

Our results demonstrate that the L- allele of *PON1* gene polymorphism is likely to be a risk factor for glomerulosclerosis and subsequently steroid resistance and renal failure in Kuwaiti Arab children with INS.

Ethical Approval

This study was approved by the Institutional Ethics Committee for the protection of human subjects in research.

Conflict of interest

All authors certify that there are no competing financial interests related to this publication.

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