P-0720 TLR4 gene variant as a risk marker of diabetic foot ulcers

*A.M. Raja¹, M.M. Zafar¹, M.A. Riaz², J. Zafar³, A. Mehmood¹, M.S. Raja¹, M. Mahmood², G.K. Raja¹
¹University Institute of Biochemistry & Biotechnology, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, 46300, Pakistan
²Department of Zoology, University of Poonch, Rawalakot, Azad Jammu & Kashmir, Pakistan
³Diabetes Center, Department of Medicine, SZABMU PIMS, Islamabad, Pakistan

Background: Diabetes and its secondary complications are the fastest growing chronic diseases worldwide (1). Diabetic foot ulcers are severe forms of diabetic complications resulting in limb amputation, severe health issues or even early death (Fig. 1). The lifetime risks of diabetes especially diabetic foot ulcers (DFU) and imputations are on rapid rise in Pakistan. Though large data exists regarding environmental and genetic risk factors of diabetes, etiology of diabetic foot ulcers genetics are yet to be identified (2). Toll Like Receptor 4 (TLR4) gene encodes a receptor with role in regeneration, immunity and tissue repair. TLR4 gene variants are found associated with infectious and other inflammatory diseases. A TLR4 gene variant, rs4986790, has been reported for impaired wound healing and thus may contribute to diabetic foot ulcers (3).

Objective: The present study was designed to identify risk allele of rs4986791 (C/T or Thr399Ile) TLR4 gene SNP and its association with DFU.

Methods: The anthropometric and clinical data were obtained from a total of 260 subjects, including 100 healthy controls, 50 diabetic patients without foot ulcer without Foot Ulcer (DFU-), and 110 Diabetics with Foot Ulcer (DFU+). For microbiota identification in DFU+ cases, pus samples from the infected foot parts were collected and cultured for characterization of frequent microbes. Allele specific For rs498679 SNP based genotyping in all three study groups, Allele Specific Polymerase Chain Reaction (AS-PCR) was performed. The allele and genotype frequencies of rs498679 SNP were calculated and their associations with DFU, anthropometric and clinical risk variables were estimated.

Results & Discussion: As presented in Table 1, the DFU+ subjects were at an advanced age and had BMI elevated as compared to the than the controls (<0.0001). Based on levels of FBG (181.8±66.8) and HbA1C (10.2±1.73), DFU+ subjects also had poor glycemic control as compared to control and DFU- (p<0.0001) (Table 1). The most frequent microbiota identified in DFU+ subjects was *Staphylococcus aureus* (26.34%) showing high sensitivity (93%) to Amikacin antibiotic (Table 2). The minor T allele frequency was raised (52%) in DFU+ compared to controls and DFU- (48%) groups. Though heterozygous C/T genotype seemed common, homozygous T/T genotype frequency was higher (14%) in DFU+ group (Table 3). The T allele lacked association with DFUs (Table 4).

Table 3: Allele and genotype frequencies of rs4986791 variant

Alleles	Frequencies (Proportions) in Study Groups		
	Control	DFU-	DFU+
C	0.52	0.52	0.48
T	0.48	0.48	0.52
Genotypes			
C/C	0.20	0.09	0.06
C/T	0.57	0.77	0.80
T/T	0.23	0.13	0.14

Figure 1: Pictures showing foot ulcer and imputation in DFU+ subjects

Table 1: Comparison of the anthropometric and clinical variables in study groups

Variables	Control	DDFU-	DDFU+	p-Value
Age	46.6±14.4	52±12.7	55.3±10.3	<0.0001
BMI	25.7±3.1	27.3±3.79	27±4.86	0.028
FBG	75.8±7.6	162.9±50.1	181.8±66.8	<0.0001
HbA1C	5.57±0.42	9.11±1.54	10.2±1.73	<0.0001
Creatinine	1.09±0.60	1.11±0.45	1.33±0.67	0.014

Table 2: Frequency of common microbiota and antibiotic sensitivity in DFU+ subjects

Microbiota	
Microbiota in DFU+ Cases	Frequency (%)
<i>Staphylococcus aureus</i>	26.34
<i>Pseudomonas aeruginosa</i>	12.52
Antibiotic Sensitivity (Amikacin)	
<i>Staphylococcus aureus</i>	93
<i>Pseudomonas aeruginosa</i>	69

Table 4: Genotype Association with Study Groups

Genotypes	Control vs DFU-		Control vs DFU+		DFU- vs DFU+	
	OR (95%CI)	p- valve	OR (95%CI)	p-value	OR (95%CI)	p- valve
C/C vs C/T	3.07 (1.07-8.77)	0.052	2.37 (1.14-4.92)	<0.0001	0.77 (0.26-2.28)	0.28
C/C vs T/T	1.61 (0.44-5.88)	0.052	0.54 (0.18-1.58)	<0.0001	0.33 (0.08-1.43)	0.28
C/C vs C/T+T/T	2.69 (0.96-7.57)	0.044	1.89 (0.92-3.87)	0.078	0.70 (0.24-2.06)	0.5

Conclusions

The prominent environmental risk factors identified in Pakistani diabetic foot ulcers were; poor glycemic control, advanced age, male gender, over-weight, serum Creatinine, and a family history of diabetes. The minor T allele frequency of rs4986791 variant of TLR4 gene was raised in DFU patients but it lacked significant association with disease. However in its heretozygous status (C/T), T allele seemed to show significant association with diabetes. Thus our results clearly indicate T allele TLR4 gene as a risk marker of diabetes which could trigger DFU+ under conditions like poor glycemic control.

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Correspondence: Asad Mehmood Raja
Email: asadscm750@gmail.com
University Institute of Biochemistry and Biotechnology
Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan
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