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Single nucleotide polymorphisms in cytokine/chemokine genes are associated with severe infection, ulcer grade and amputation in diabetic foot ulcer

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ABSTRACT

Compared to other complications the genetics of diabetic foot ulcer is poorly studied. The Interleukin (IL)-6 (−174G > C/rs1800795), Tumor Necrosis Factor (TNF)-α (−308G > A/rs1800629) and (−238G > A/rs361525) and Stromal cell Derived Factor (SDF)-1 (+801G > A/rs1801157) are well characterized single nucleotide polymorphisms (SNPs) which were previously shown to be associated with Diabetic Foot Ulcer (DFU). In the present study, we looked at the association of these SNPs with foot microbial infection, Wagner's ulcer grade and treatment procedure, along with serum levels of these cytokines (intermediate phenotype) and other serum biomarkers (adiponectin, leptin, CRP and HOMA-IR) in subjects with DFU. Subjects with DFU ($n = 270$) were genotyped by PCR-RFLP and the serum levels of IL-6, TNF-α and SDF-1 were determined by ELISA. Microbial infections were determined by standard microbiological methods. Ulcer grade and treatment procedures were recorded. IL-6 (−174G > C), TNF-α (−308G > A) and SDF-1 (+801G > A) SNPs were associated with severe microbial infections. TNF-α (−308G > A) and (−238G > A) SNPs were associated with severe ulcer grades. SDF-1 (+801G > A) SNP was associated with major amputation even after adjusting for confounding variables. Identification of these SNPs in DFU subjects would help in identifying high risk individuals who need better treatment care.

1. Introduction

Global epidemiological studies have indicated pandemic increase in the incidence of diabetes, especially in the Asian countries [1]. Among the various complications, Diabetic Foot Ulcer (DFU) represents a significant and often challenging clinical entity which is mainly due to either diabetic neuropathy (DN) and/or Peripheral Arterial Disease (PAD) [2]. The etiology of DFU is multi-factorial and involves both genetic and environmental factors [3] and is characterized by a classical triad of ischemia, infection and inflammation [3]. A normal wound healing process proceeds in three phases: 1. Inflammatory phase, 2. Proliferation phase and 3. Remodeling phase. Impaired immunity, as seen in T2DM, contributes to prolonged infection causing chronic inflammation leading to reduced proliferation and impaired wound remodeling [3]. Thus, host genetic factors, microbial infections and local cytokine milieu together affect the severity and treatment outcome in DFU. However, to the best of our knowledge, studies linking these three factors in DFU are not available.

Compared to other complications of T2DM, the genetics of DFU remains poorly studied [4]. Previously SNPs in MMP-9 [5,6] and VEGF [7,8] where shown to be associated with DFU. Single nucleotide polymorphisms (SNPs) in cytokine and chemokine genes serve as valuable candidates for DFU since these proteins coordinate the three phases of wound healing [9]. Previously, we have shown strong genetic association of Interleukin-6 (IL-6: -174G > C/ rs1800795), Tumor Necrosis Factor-Alpha (TNF-α: -308G > A/rs1800629) and (−238G > A/ rs361525) and Stromal cell Derived Factor-1 (SDF-1/CXCL12: +801G > A/rs1801157) with DFU [10]. In the present study, we examined the involvement of these immunogenotypes in determining the susceptibility to foot microbial infection, disease severity (ulcer grade) and treatment-outcome (debridement vs amputation) in DFU (phenotypes). Further, the effect of these SNPs on serum cytokine levels (intermediate phenotype) and other serum biomarkers (leptin, adiponectin, C-Reactive Protein (CRP) and HOMA-IR) were also studied.

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2. Materials and methods

2.1. Study population

A total of 270 known DFU subjects were recruited for this study from a Tertiary Diabetic Foot Centre in Chennai, India. All the study subjects were of Indian origin. The study protocol was approved by the institutional ethics committee (Ref No-MVH/IHEC/002/2011/17) and written informed consent was obtained from all the study participants. The study was conducted as per Helsinki's declaration. Surgical procedures/interventions undertaken were recorded.

2.2. Diagnosis of diabetic neuropathy (DN) and peripheral arterial disease (PAD)

Vibration perception threshold (VPT) was measured using biothesiometer (Bio-medical Instruments Co., Newbury, OH, USA) [11,12]. Three readings were obtained from each foot on the first metatarsal at increasing voltage and the mean was taken. Patients with VPT >25 V were diagnosed with DN and those with 16-24 V were considered as having high risk for DN [2,11,12]. The Ankle-Brachial Index (ABI) is the ratio of the systolic blood pressure (SBP) at the ankle to that of brachial artery [11,12]. Patients with ABI ≤0.9 were diagnosed with PAD. Severity of PAD was determined, based on the ABI values (Severe ABI ≤ 0.4; Moderate ABI = 0.41–0.7; Mild ABI = 0.71–0.9; Normal ABI = 0.91–1.4; Non-compressible arteries ABI > 1.4) as per American College of Cardiology Foundation/American Heart Association Task Force (ACCF/AHA) 2011 Guidelines [2,11,12].

2.3. Sample size calculation and power of study

A pilot study was first carried out using 50 wild type and 50 mutant genotypes having DFU. Based on these preliminary results, with a confidence interval of 95%, an estimated p value < 0.05, with a power of 80%, the present sample size was derived. 10 subjects were added to each group to account for the high level of variation seen in the levels of serum biomarkers.

2.4. Inclusion and exclusion criteria

Only adult T2DM subjects with DFU were included. The exclusion criteria were patients with type-1 diabetes, gestational diabetes and T2DM subjects without DFU. Those who refused to participate or withdrew their consent were excluded.

2.5. Anthropometric measurements and biochemical parameters

Anthropometric measurements including height and weight were obtained using standardized techniques [10]. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Fasting plasma glucose (FPG) (glucose oxidase-peroxidase method), serum cholesterol (cholesterol oxidase-peroxidase-amidopyrine method), serum triglyceride lipids (TGL) (glycerol phosphate oxidase-peroxidase-amidopyrine method), high density lipoprotein cholesterol (HDL) (direct method-polyethylene glycol-pretreated enzymes), and creatinine (Jaffe's method) were measured using a Hitachi-912 autoanalyser (Hitachi, Mannheim, Germany) as described previously [10]. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald's formula (10). The intra- and inter assay coefficient of variation for the biochemical assays ranged between 4.5% and 6%. Glycated hemoglobin (HbA1c) was estimated by high pressure liquid chromatography (Bio-Rad, Hercules, CA). The plasma concentrations of high-sensitivity C-reactive protein (CRP) were measured by high sensitive nephelometric assay. The intra- and inter-assay coefficients of variation for CRP were 3% and 5.8%, respectively, and the detection limit was 0.15 mg/L.

2.6. Genotyping

Genomic DNA was extracted from peripheral blood using the standard phenol-chloroform extraction method [10]. The SNPs were determined using polymerase chain reaction followed by restriction fragment length polymorphism method (PCR-RFLP) as previously described [10]. The primer sequences, amplicon sizes and the restriction enzymes used are given in S·Table-1.

2.7. Microbial testing

The wounds were swabbed for microbiological analysis. Specimens were collected as per current clinical guidelines provided by National Institute for Health and Clinical Excellence (NICE) 2011 [13]. Pus swabs and tissue scrapings were collected under aseptic conditions and subjected to microbiological analysis. The specimen was streaked on microbial growth media, such as blood agar and MacConkey agar (Himedia Laboratories, Mumbai, India) and bacterial growth was monitored after overnight incubation at 37 °C. Initial identification was done using Gram's staining. Further identification was done using biochemical and enzymatic methods as described previously [13]. S. Tabel-2 shows the list of all the microbes identified by these methods.

2.8. Histopathology

The ulcer tissues were dissected and fixed in 10% formaldehyde. The fixed tissues were paraffin embedded and were stained with hematoxylin-eosin (HE – basic staining) and van Gieson (VG – collagen staining) stain as described previously [14]. The morphology was analyzed by Nikon Microscope Eclipse 80i (10× and 40× magnifications). S·Table-3 shows the grading of Foot ulcers using the Wagner's grading system.

2.9. Statistical analysis

Student t-test was used to compare groups for continuous variables, whereas $\chi 2$ test or Fisher exact test (as appropriate) was used to compare proportions. The genetic association of immunogenotypes with microbial infection, ulcer grade and treatment procedure was carried out using backward logistic regression analysis. First, the odds ratios were calculated without including the confounding variables (Model 0). Then the significance of the association was tested by including the confounding variables (Model 1). Serum biomarkers were stratified based on their immunogenotypes and the levels were compared using Maan-Wintneys U Test. Multiple comparisons were corrected using the Holm's correction for each set of analysis. All the analyses were done using SPSS statistical package (Version 20.0; SPSS, Chicago, IL) and p value <0.05 was considered to be significant.

3. Results

3.1. Clinical characteristics of the study cohort

The clinical and biochemical characteristics of the study subjects are shown in S·Table-4. The study subjects had significantly increased BMI (optimal <23), SBP (optimal <120 mm Hg), DBP (optimal <80 mm Hg), FPG (optimal <126 mg/dL), PPPG (optimal <200 mg/dL), HbA1c (optimal <5.6%), TGL (optimal <150 mg/dL), VLDL (optimal <30 mg/dL), urea (optimal <20 mg/dL) and creatinine (optimal<1.5 mg/dL) and decreased HDL (optimal >40 mg/dL). 71% of the study subjects had diabetic neuropathy, 49% had diabetic nephropathy, 34% had diabetic retinopathy, 29% had peripheral arterial disease, 23% had both nephropathy and retinopathy. With respect to treatment regimen, 23% were on insulin and 73% were on insulin plus oral hypoglycemic drugs.

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3.2. Genetic association of the immunogenotypes with microbial infection in DFU

The percentage of microbes present in DFU ulcers is shown in S.Fig. 1. Almost all patients had at least one bacterial infection. *Staphylococcus* sp. was the most common microbe followed by *E. coli* and other pathogens. Table 1 shows the genetic association of the immunogenotypes with microbial infections. For all genetic association studies, odds ratio was calculated individually for every SNP. With respect to IL-6 (−174G > C), compared to the wild type genotype (GG), the mutant genotypes (GC + CC) showed increased susceptibility towards *Staphylococcus sp*, *Proteus morganii* and *Citrobacter diversus* infections in DFU. The association was significant even after adjusting for the confounding factors. With respect to TNF-α (−308G > A), the mutant genotype (GA + AA) showed increased susceptibility towards *Staphylococcus sp*., *Proteus morganii* and *Citrobacter diversus*. However, the association with *Staphylococcus sp*., alone remained significant after adjusting for confounding factors. With respect to the TNF-α (−238G > A), the mutant genotype (GA + AA) showed increased susceptibility towards *Proteus morganii* and *Enterococcus* sp. However, the association was lost after adjusting for confounding factors. With respect to SDF-1 (+801G > A), the mutant genotypes (GA + AA) showed increased susceptibility towards *Staphylococcus* sp. and *Enterococcus sp.* However, the association with *Staphylococcus sp*., alone remained significant after adjusting for confounding factors. Out of the four SNPs studied, IL-6 (−174G > C), TNF-α (−308G > A) and SDF-1 (+801G > A) (but not TNF-α -238G > A) were associated with microbial infections even after adjusting for confounding factors.

3.3. Genetic association of the immunogenotypes with ulcer grade in DFU

S.Fig. 2 shows the percentage of ulcer grades among the DFU subjects. Grade-III was the most predominant one followed by Grade-II, Grade-IV and finally Grade-I. Table 2 shows the genetic association of the immunogenotypes with the ulcer grades. All the four SNPs showed some association with ulcer grades –I to III. None of the SNPs showed association with grade-IV ulcers (data not shown). Out of the four SNPs studied, only TNF-α (−308G > A) and TNF-α (−238G > A) showed strong genetic susceptibility with ulcer grades, even after adjusting for confounding variables.

Table 1
Association of immunogenotypes with pathogenic microbes in DFU.

SNPs	Organism	OR (95%CI)[#]
IL-6 (−174 G/C)	*Staphylococcus sp*	**1.5 (1.3–2.3)**
(GC + CC Vs. GG)	*Proteus morganii*	**1.8 (1.4–2.7)**
	Citrobacter diversus	**1.2 (1.1–3.9)**
TNF-α (−308 G/A)	*Staphylococcus sp*	**1.4 (1.2–1.9)**
(GA + AA Vs. GG)	*Proteus morganii*	1.9 (1.4–2.8)[a]
	Citrobacter diversus	1.2 (1.1–3.4)[b]
TNF-α (−238 G/A)	*Proteus morganii*	1.7 (1.3–2.7)[c]
(GA + AA Vs. GG)	*Enterococcus sp*	7.0 (1.1–69)[d]
SDF-1 (+801 G/A)	*Staphylococcus sp*	**1.6 (1.3–2.3)**
(GA + AA Vs. GG)	*Enterococcus sp*	1.2 (1.1–3.8)[e]

[#] Backward logistic regression analysis was carried out using genotypes as independent variables and microbes as dependent variables. All the comparisons was made between the wild-type and mutant genotypes and organisms which showed significant ($p < 0.05$) genetic association with the mutant genotype alone, after several rounds of iteration analysis were reported. Odds Ratio (95% Confidence Interval) was first calculated without confounding variables and the significance was then tested after adjusting for confounding variables (age, gender, BMI, FBS, HbA1c, LDL, HDL, TGL, leptin, adiponectin, CRP and HOMA-IR). OR which remained significant even after adjusting for confounding variables were highlighted in bold letters.

[a] The significance was lost after adjusting for FBS and HbA1c.
[b] The significance was lost after adjusting for FBS, HbA1c, TGL and LDL.
[c] The significance was lost after adjusting for BMI, FBS, TGL, HDL and LDL.
[d] The significance was lost after adjusting for BMI, FBS and HDL.
[e] The significance was lost after adjusting for age, TGL and LDL.

Table 2
Association of immunogenotypes with ulcer grade in DFU.

SNPs	OR [95%CI][#]		
	Grade 1	Grade 2	Grade 3–5
IL-6 (−174 G/C) (GC + CC Vs. GG)	2.6 (1.4–21.2)[a]	2.5 (1.4–16.3)[a]	1.8 (1.2–6.9)[a]
TNF-α (−308 G/A) (GA + AA Vs. GG)	7.1 (1.8–59.8)[b]	6.9 (1.9–43.4)[b]	**2.5 (1.3–26.5)**
TNF-α (−238 G/A) (GA + AA Vs. GG)	**3.8 (1.4–195.5)**	3.7 (1.4–146.0)[d]	4.0 (1.4–329.0)[c]
SDF-1 (+801 G/A) (GA + AA Vs. GG)	2.5 (1.3–27.4)[e]	2.5 (1.3–22.3)[e]	2.1 (1.2–17.1)[e]

[#] Backward logistic regression analysis was carried out using genotypes as independent variables and ulcer grades as dependent variables. All the comparisons was made between the wild-type and mutant genotypes and grades which showed significant ($p < 0.05$) genetic association with the mutant genotype alone, after several rounds of iteration analysis were reported. Odds Ratio (95% Confidence Interval) was first calculated without confounding variables and the significance was then tested after adjusting for confounding variables (age, gender, BMI, FBS, HbA1c, LDL, HDL, TGL, leptin, adiponectin, CRP and HOMA-IR). OR which remained significant even after adjusting for confounding variables were highlighted in bold letters.

[a] The significance was lost after adjusting for BMI and HbA1c.
[b] The significance was lost after adjusting for age, gender, BMI, FBS, HbA1c, TGL, HDL, LDL, VLDL, adiponectin, leptin, CRP, HOMA-IR.
[c] The significance was lost after adjusting for gender, BMI, FBS, HbA1c, HDL, adiponectin.
[d] The significance was lost after adjusting for gender, BMI, FBS, HbA1c, HDL, adiponectin.
[e] The significance was lost after adjusting for age, gender, BMI, FBS, HbA1c, TGL, HDL, LDL, VLDL, adiponectin, leptin, CRP, HOMAIR.

3.4. Genetic association of the immunogenotypes with treatment regimen in DFU

S.Fig. 3 shows the percentage of various treatment regimen of foot ulcer present among the DFU patients. Debridement (DB) was the most common form of treatments (43%) followed by minor amputation (Toe Amputation −25% and Trans Metatarsal Amputation −15%) and major amputation (Below Knee Amputation-13% and Above Knee Amputations −4%). Table 3 shows the genetic association of the

Table 3
Association of immunogenotypes with treatment procedure in DFU.

SNPs	OR [95%CI][#]	
	Minor amputation[*]	Major amputation[*]
IL-6 (−174 G/C) (GC + CC Vs. GG)	7.8 (2.6–83.8)[a]	15.8 (3.6–37.3)[a]
TNF-α (−308 G/A) (GA + AA Vs. GG)	1.7 (1.4–2.6)[b]	1.2 (1.1–1.3)[b]
TNF-α (−238 G/A) (GA + AA Vs. GG)	1.8 (1.3–3.7)[c]	2 (1.4–4.2)[c]
SDF-1 (801 G/A) (GA + AA Vs. GG)	1.6 (1.3–2.3)[d]	**1.5 (1.2–2.3)**

[#] Backward logistic regression analysis was carried out using genotypes as independent variables and surgical outcome as dependent variables. All the comparisons was made between the wild-type and mutant genotypes and surgical outcome which showed significant ($p < 0.05$) genetic association with the mutant genotype alone, after several rounds of iteration analysis were reported. Odds Ratio (95% Confidence Interval) was first calculated without confounding variables and the significance was then tested after adjusting for confounding variables (age, gender, BMI, FBS, HbA1c, LDL, HDL, TGL, leptin, adiponectin, CRP and HOMA-IR). OR which remained significant even after adjusting for confounding variables were highlighted in bold letters.

[*] Minor amputation included toe amputation and trans metatarsal amputation; major amputation included below knee amputation and above knee amputation.
[a] The association was lost after adjusting for gender, BMI and HbA1c.
[b] The association was lost after adjusting for leptin, HOMA-IR, HbA1c.
[c] The association was lost after adjusting for gender, adiponectin, CRP, BMI, FBS, HbA1c, TGL, HDL, LDL.
[d] The association was lost after adjusting for age, gender, leptin, CRP, HOMA-IR, BMI, FBS, HbA1c, TGL,HDL,LDL and VLDL.

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immunogenotypes with treatment outcome. The association between SDF-1 (+801G > A) with major amputation remained significant even after adjusting for confounding variables.

3.5. Genetic association of the immunogenotypes with serum biomarkers in DFU

Fig. 1 shows the serum levels of cytokines in DFU after stratification based on immunogenotypes. Out of the four SNPs studied, IL-6 (−174G > C) and TNF-α (−308G > A) were the only two SNPs which influenced their serum cytokine levels. Subjects with the mutant genotype had significantly reduced levels of serum IL-6 and TNF-α. S·Tabel-5 shows the serum levels of other serum biomarkers in DFU stratified based on immunogenotypes. TNF-α (−308G > A) mutant genotype had significantly reduced levels of hsCRP.

4. Discussion

Compared to diabetic retinopathy and diabetic nephropathy, genetic studies on diabetic foot ulcer are scant. Towards this end, in the present study, we investigated the role played by 4 SNPs in cytokine/chemokine genes in foot microbial infection, ulcer grade and treatment outcome, along with the serum levels of these proteins (intermediate phenotype) and other serum biomarkers in DFU. The current study provides four novel findings: 1) IL-6 (−174G > C), TNF-α (−174G > C) and SDF-1 (+801G > A) emerged as a major risk factor for severe wound infections; 2) TNF-α (−174G > C) and TNF-α (−238G > A) emerged as major risk factors for ulcer grade; 3) SDF-1 (+801G > A) emerged as major risk factors for amputation (foot/leg) and 4) IL-6 (−174G > C) and TNF-α (−308G > A) were the only two SNPs which influenced their serum cytokine levels.

Out of the four SNPs studied, IL-6 (−174G > C), TNF-α (−174G > C) and SDF-1 (+801G > A) showed strong association with microbial infection. Our results are well in accordance with the report of Stappers et al., which showed strong genetic association of TNF-α (−308G > A) (but not -238G > A) with skin infections in the Caucasian population [15]. In another study, the TNF-α (−308G > A) (but not TGF-β +74G > C and + 29C > T) was found to be associated with increased risk of chronic lower limb infections [16]. In that study, subjects with mutant genotype had increased expression of TNF-α in the wounds, which

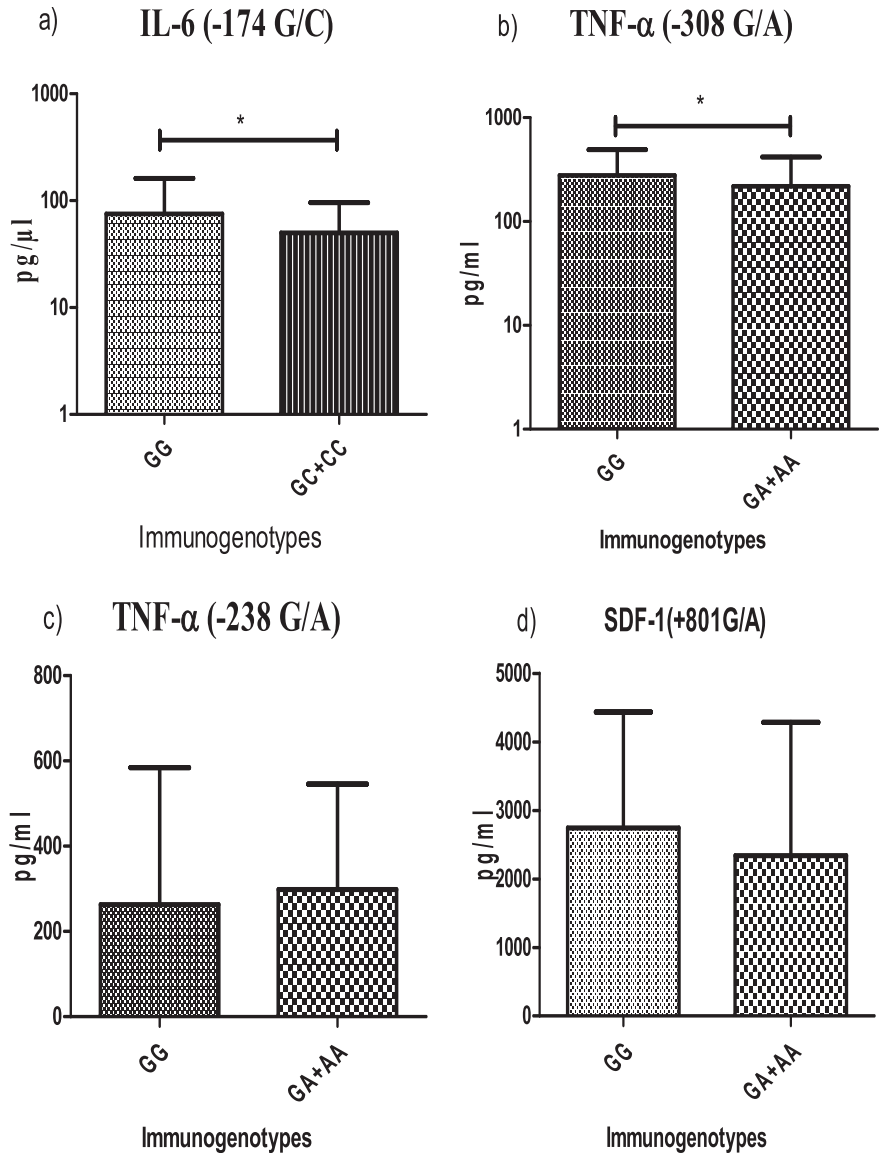

Fig. 1. Serum cytokine/chemokine levels stratified based on Immunogenotypes. Serum cytokine levels where determined by ELISA. The immunogenotypes where determined by PCR-RFLP method. The cytokine levels where then stratified based on the immunogenotypes. Wild type and mutant genotypes were compared using Mann Whitney *U* test. $p < 0.05$ was considered significant. $^{*}p < 0.05$; $^{**}p < 0.01$.

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extended the inflammatory phase and compromised wound healing [16]. Next to TNF-α, IL-6 (−174G > C) was reported to have a strong genetic association with skin infections [15]. In the present study, this SNP showed moderate association with *Staphylococcus* sp. and *Proteus morgani* infections. SDF-1 (+801G > A) showed moderate association with *Staphylococcus sp* infection. Our results are in accordance with two South Indian studies which showed strong genetic association of SDF-1 SNP with pulmonary tuberculosis [17] and HIV-TB co-infection [18].

Out of the four SNPs studied, TNF-α (both −308G > A and −238G > A) showed strong susceptibility towards the ulcer grades. Libra et al., reported genetic association of C allele of IL-6 (−174G > C) with DFU-PAD [19]. This was due to high plasma levels of IL-6 in subjects with mutant genotype [19]. IL-6 directly induces CRP and fibrinogen in the liver which were also elevated in the plasma of these individuals [19]. The high plasma concentration of CRP and fibrinogen creates a pro-inflammatory/pro-coagulant state which impairs wound healing [19]. However, in the present study subjects with IL-6 (−174 G > C) and TNF-α (−308G > A) mutant genotypes had significantly reduced levels of serum cytokine indicating that other factors might also play a role in determining genetic susceptibility. Sing et al., have also shown genetic association of TLR4 [20], MMP-9 (−1562C > T) [21] and TCF7L2 (+112998590C > T) [22] with DFU, indicating the involvement of inflammation, tissue remodeling and insulin resistance in DFU. However, in their cohort type-1 collagen (COL1A1) which is also a strong risk factor for DFU showed no association with the disease phenotype [23].

Out of the four SNPs studied, SDF-1 (+801G > A) showed increased risk for amputation (toe/ft/leg). In a previous study, the HspA1B (+1538G > A) was found to be strongly associated with severe grades of ulcers and required extended period of hospitalization and also had increased chances of amputation [24]. Amputation is an extreme treatment outcome of impaired wound healing in diabetic patients. Impaired angiogenesis leading to hypoxia and redox stress can synergize with inflammation and can lead to impaired immunity to infections [25]. This in turn can lead to poor wound healing, increased severity of ulcers and finally amputation [25]. In this regard, previously we have reported genetic association of HIF-1α (involved in hypoxia) with DFU [26]. Amoli et al., have shown genetic protection by the A allele of the VEGF (−2578C > A) gene (involved in angiogenesis) against DFU [7]. However, Corapcioglu et al., reported no genetic association between eNOS (+894G > T) and DFU [27]. Kuper et al., reported genetic association of coagulation factor XIII Val32Leu with DFU [28]. This was due to increased activation of this factor leading to a pro-coagulation state in subjects with mutant genotype which in turn affects cutaneous – microcirculation, leading to impaired wound healing [28]. Cheng et al., have reported genetic association of OPRM1 (+118A > G) of the mu-opioid receptor with severe pain in DFU [29].

Interestingly in the present study, out of the four SNPs studied, only IL-6 (−174 G > C) and TNF-α (−308G > A) mutant genotypes had significantly reduced levels of serum cytokines. However, these SNPs did showed strong association with ulcer grades and amputation. It is important to note that not all promoter/intronic SNPs affect serum cytokine levels since some of them might actually be involved in signal specific gene expression and might play an important role within the wound micro milieu.

Apart from microbial infection, ulcer grades, treatment outcome and serum levels of cytokines, no significant association was seen between these SNPs with other clinical parameters listed in S·Table-4. Previously, few studies have shown association of DFU with PAD [12,30]. Other studies have reported association of DFU with renal insufficiency [31]. The association between glycemic control and DFU is controversial [32,33]. However, in our study no association was seen between any of these clinical parameters with the tested SNPs.

In the present study, out of the four SNPs studied, TNF-α (−308G > A) and SDF-1 (+801 G > A) followed by IL-6 (−174G > C) showed increased risk for developing severe podal microbial infection, higher grades of ulcer and amputation in DFU. Thus, out of four, at least three SNPs emerged as clear risk factors for DFU. To the best of our knowledge, this is the first study to systematically compare the genetic association of SNPs with microbial infections, severity of ulcer and treatment procedure in DFU, in a high risk ethnic population. However, the major limitation of our study is its cross-sectional nature and the limited sample size, which means that no cause and effect relationship can be drawn from the present study. Screening for SNPs in TNF-α, SDF-1 and IL-6 among DFU subjects would help in identifying high risk individuals and might aid in better patient care.

Author's contribution

VA, RR and VV conceived and designed the experiment. UD performed the experiment. VA drafted the manuscript. UD and SV analyzed the data. VA, RR and VV contributed to the discussion and reviewed the manuscript.

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Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2018.07.083.

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